From: Dan Baker
To: Griffin, Paul C
Subject: [EXTERNAL] Accepted: NPS IAA formation: GonaCon
Attachments: invite.ics

---

danbaker@colostate.edu <danbaker@colostate.edu> has accepted your event invitation.

From: Dan Baker
To: Griffin, Paul C
Subject: [EXTERNAL] BLM Final Report
Date: Saturday, January 9, 2021 3:59:50 PM
Attachments: BLM Final Report 2015-2020.1.8.docx
Revised Proposal_SOW_Tech_Approach_BLM_Wild_Horse 5.18.16.docx
Reimmunization with GonaCon.pdf
Standard Operating Procedures for Remote Delivery of GonaCon Equine.docx
Kathleen.Eddy.11.9.20 Thesis.docx

Hi Paul,

I apologize for the delay in getting this report to you and I appreciate your patience and that of the BLM. Attached are several supporting documents including: 1) this final report, 2) the original BLM Project Proposal - 2015, 3) PLoS ONE 2018 publication, 4) Kathleen Eddy's MS thesis, and 5) Standard Operating Procedures for dart loading and field application for GonaCon-Equine.

I regret not being able to spend more time with our statistician analyzing these data but he had limited time and I wanted to get this already over-due report to you. In our next session, we will test for differences among treatment intervals. My cursory examination of the data suggest that there may well be a treatment x time interaction that may not be statistically significant but biologically important to resource manages deciding upon a treatment interval. If so, I will belatedly send this information to you and we will, assuredly, include this in the publication.

Please feel free to offer comments and send anything back to me for further clarification. It's been my great pleasure to work with you and the BLM on this project. I just wish we could have had a couple of more seasons of data. I greatly value the support you have shown me during all phases of this project. I consider you a valuable colleague and friend. Hopefully, we will get the opportunity to work together again.

Best to you,

Dan

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email:danbaker@colostate.edu

From: Dan Baker
To: Griffin, Paul C
Subject: [EXTERNAL] Fwd: Our FA folks advise that the timeline is possible
Date: Monday, December 16, 2019 3:12:05 PM

Hi Paul,

I just got this from Blake.

Dan

-------- Forwarded Message --------
Subject: Our FA folks advise that the timeline is possible
Date: Mon, 16 Dec 2019 15:09:53 -0700
From: McCann, Blake <blake_mccann@nps.gov>
To: Dan Baker <danbaker@colostate.edu>

We just need to get things going ASAP.

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From:	Dan Baker
To:	rhart@wildlifepm.com; "Jeff Kemp"; "Doug Eckery"; Baker,Danny; Nett,Terry; Jason Bruemmer; McCann, Blake E; Griffin, Paul C
Subject:	[EXTERNAL] Re: BLM Report to Congress
Date:	Wednesday, May 13, 2020 9:07:51 AM

Hi Roch,

No, I haven't seen this. Thanks for keeping us informed.

Pages 10-12 are encouraging relative to the large scale application of GonaCon. Also, I think that page 22 under Research offers a unique research opportunity to determine if long-term infertility related to GonaCon reimmunization results in permanent sterilization of treated mares and argues for continued monitoring of these mares at THRO, as well as, continued assessment of duration of effectiveness of current experimental booster intervals.

Dan

On 5/12/2020 9:20 PM, rhart@wildlifepm.com wrote:

> Hello Everyone
> I'm assuming you all have seen this? If not, read page 10.
> Roch

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Dan Baker
To: Parker, Leona B; Griffin, Paul C; Link,Maura
Cc: Shepherd, Alan B
Subject: [EXTERNAL] Re: Domestic horse per diem appears to be within scope of budget, for L15AC00145
Date: Tuesday, July 7, 2020 4:34:53 PM

Dear Paul and Leona,

Thank you for your understanding and assistance with this request. Your decision will allow us to complete a very timely and significant aspect of our overall research project, as well as, provide financial support to a well-deserving graduate student.

Kind regards,

Dan Baker

On 7/7/2020 3:15 PM, Parker, Leona B wrote:

> Good afternoon, reviewing your request I see nothing wrong with your request. If you feel that you need to move funds you can do that between categories if its NO more than 10%..
>
> Thanks
> L

Leona B. Parker,
California Grants Management Officer
Bureau of Land Management, California
REMOTE Duty Station: Eagle Lake Field Office
Department of the Interior, Region 8/10
Office: (530) 252-5338 I Fax: (530-257-4831

Attention DOI Financial Assistance Recipients: DOI is moving to GrantSolutions in October 2020,
please visit the following website: https://www.doi.gov/grants/grantsolutions

Grant and Agreement Regulations - Code of Federal Regulations (CFR) Link:
http://www.ecfr.gov/cgi-bin/text-idx?SID=ed90f54836feb6a994f657188eb05e33&node=2:1.1.2.2.1&rgn=div5
PROGRAM OFFICERS WEBSITE: https://doimspp.sharepoint.com/sites/blm-wo-800/blmfa/SitePages/Home.aspx

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From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Tuesday, July 7, 2020 1:23 PM
To: Link,Maura <Maura.Link@colostate.edu>; Dan Baker <danbaker@colostate.edu>
Cc: Parker, Leona B <lparker@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>
Subject: Domestic horse per diem appears to be within scope of budget, for L15AC00145

Dan and Maura,

Thank you for calling to my attention your concern about spending $800 on about a month of domestic horse per diem, for the purposes of sampling blood and feces from several mares throughout their estrus cycles. This $800 is a relatively small expense, but I would ask Leona Parker (grants management officer, cc'd here) to please email me or everyone cc'd here guidance if my interpretation of the existing agreement (below) is incorrect.

Confirming that fecal estrogen samples are a reliable indicator of pregnancy status is identified as a performance measure in the proposal that led to this agreement (L15AC00145), so it makes sense that CSU paying to temporarily feed and house domestic horses that you collect samples from should be covered under the agreement. Note: Personally, I pay more than that per month, to board just 2 ponies here in Fort Collins, so it sounds to me like the costs are reasonable.

On the phone today Dr. Baker asked me which category of expense the $800 should be associated with. I would expect that the most straightforward choice is to use the 'other' category that you identified in your budget (on page 35 of the attached, base award). You have $5,000 budgeted for 'other' expenses in year 5 of the award, which is this year. Alternately, you might choose to allocate this expense in the 'supplies' category, where the supply being used is the cost of feed for the animals while they are housed at the CSU barn, which is needed as part of the fecal estrogen assay work. Supplies are typically defined items with a cost of less than $5,000, and often with a one-time use, and hay seems to fit that description. From what Dr. Baker said, you are not retaining possession of these horses, you are borrowing them from a private owner, and you are only paying for some hay, water, and use of some shelter (fenced lot). Your base award shows you have $1,950 budgeted for supplies in year 5 of this agreement, which is more than double the $800 you are asking about.

I cannot authorize any changes in your budget -- that is something only Ms. Parker can do, as the grants management officer. However, given what I know about this agreement and the agreed-upon budget, it does not appear to me that you need to request any change in your budget to process this expense.

Again, thank you for asking. It is always better to be cautious about this type of question.

Sincerely,

Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From:	Nett,Terry
To:	Baker,Danny; Griffin, Paul C; Powers, Jenny; McCann, Blake E; Bruemmer,Jason; Doug Eckery; Galloway, Nathan L
Subject:	[EXTERNAL] RE: Foaling data - 2020
Date:	Friday, September 18, 2020 10:46:40 AM

Thanks Dan,

Good to know. The two year interval still looks great. Should be a good management tool.

Terry

Terry M. Nett, PhD
Professor Emeritus
Animal Reproduction &
Biotechnology Laboratory
Fort Collins, CO 80523
Office: (970)491-1307
Cell: (970)420-0233

-----Original Message-----
From: Baker,Danny <Dan2.Baker@ColoState.EDU>
Sent: Friday, September 18, 2020 10:34 AM
To: Paul Griffin <pgriffin@blm.gov>; Jenny Powers <jenny_powers@nps.gov>; Blake McCann <blake_mccann@nps.gov>; Bruemmer,Jason <Jason.Bruemmer@ColoState.EDU>; Nett,Terry <Terry.Nett@ColoState.EDU>; Doug Eckery <douglas.c.eckery@aphis.usda.gov>; Nathan Galloway <nathan_galloway@nps.gov>
Subject: Foaling data - 2020

All,

Attached are "quick and dirty" preliminary results from the 2020 foaling season and a brief comparative summary of data for foaling proportions and vaccine effectiveness from previous years. Clearly, a more sophisticated statistical analysis will be forthcoming as we move into the data analysis and writing phases of this research. Your input will be most appreciated. Let me know if you have questions or comments. Thanks.

Dan

Dan L. Baker, PhD
Affiliate Faculty Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory Colorado State University Fort Collins, Colorado 80535 USA Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Eckery, Douglas C - APHIS
To: Dan Baker
Cc: Griffin, Paul C
Subject: [EXTERNAL] RE: GonaCon Bulletin for Review
Date: Tuesday, April 28, 2020 9:49:02 AM
Attachments: USDA_bw_transparent.png

Hi Dan,
Thanks for forwarding the bulletin. I don't have any additional changes to the content. In the acknowledgements, one of the two attached logos should be used.
I hope you and your family are doing well and staying healthy.
Best regards,
Doug

Douglas C. Eckery, PhD
Assistant Director
National Wildlife Research Center
USDA APHIS Wildlife Services
4101 LaPorte Avenue
Fort Collins, CO 80521
Office: 970-266-6164
Mobile: 970-692-7387

From: Dan Baker [mailto:danbaker@colostate.edu]
Sent: Monday, April 27, 2020 9:47 AM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>; Paul Griffin <pgriffin@blm.gov>
Subject: Fwd: GonaCon Bulletin for Review

Hi Doug and Paul,
I just received this daft from TR and wanted to get your comments. I've offered some comments on content but I also I think that it would be appropriate to include acknowledgements to NWRC, BLM, and Morris Animal Foundation.
Thanks,
Dan

-------- Forwarded Message --------
Subject: GonaCon Bulletin for Review
Date: Mon, 27 Apr 2020 06:47:53 -0800
From: Klosterman, Megan E <megan_klosterman@nps.gov>
To: McCann, Blake E <blake_mccann@nps.gov>, McCann, Amy J <Amy_McCann@nps.gov>, Baker,Danny <Dan2.Baker@ColoState.EDU>
CC: Sedlacek, Katherine M <Katherine_Sedlacek@nps.gov>, Lincoln Eddy <eddylinc@gmail.com>, Eddy, Lincoln R <Lincoln_Eddy@nps.gov>

Hi everyone,
We have put together a draft bulletin for your review. We would like to hear all your thoughts, comments, and concerns pertaining to the content and the layout. If you have any references that you think would be good to make available to the public (we already have the two that Dan shared with us previously), please let us know about that as well. We plan to include one link on the bulletin that will bring people to our website where we will have a list of further

references. We were not sure if NWRC would want to be mentioned on this bulletin, so if anyone has any insight on that, please let us know.

Thank you Kate and Lincoln for all your hard work on this!!

Megan E. Klosterman | Resource Management Specialist
NPS · Theodore Roosevelt National Park
☎ (701) 623-4730 ext.1407 | ✉ Megan_Klosterman@nps.gov (she/her)
*☎ (during telework) (937) 974-1245

From: Dan Baker
To: Griffin, Paul C
Subject: [EXTERNAL] T. Roosevelt Horse Project
Date: Tuesday, May 21, 2019 2:50:36 PM
Importance: High

Hi Paul,

Since I haven't heard from you in a while, I'm worried that I might be in trouble. Maybe I should give you call instead of vice-versa. I do have a couple of updates that I should let you know about. No rush. I hope all is well with you.

Dan

--
Dan L Baker, PhD
Research Biologist
Faculty Affiliate
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, CO 80535
(970) 556-8518
danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker; Powers, Jenny; Galloway, Nathan L
Cc: Parker, Leona B; Healey, Sherlyn K
Subject: 1-month extension for BLM Final Report, L15AC00145
Date: Friday, December 4, 2020 4:32:51 PM

Hi Dan,
Despite it being Friday afternoon, GMO Leona Parker let me know that she has approved a 1-month extension for CSU to return the final performance report to BLM (i.e., January 7). We already received the final financial report (form SF-425) from your university.
So, good luck with your final analyses. If you would still like to talk next week, I will be available.
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 631-4808 (mobile / pandemic)
(970) 226-9358 (office)

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, December 4, 2020 4:20 PM
To: Dan Baker <danbaker@colostate.edu>; Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
Subject: Re: [EXTERNAL] BLM Final Report

Hi Dan,
Thank you for your message today. My family and I are fine, and I hope the same is true of you, Jenny, Dr. Nett, and Kathleen.
I don't have the authority to grant the request for a delay on the final report, but I have forwarded it to the grants management officers (currently Sherry Healey or Leona Parker, because Brandon Riley left the BLM), along with reasons I think it is a reasonable request. Hopefully, they will agree, and let us know early next week.
Really good to see those three papers that you are working on. Please be sure to include funding from BLM agreement L15AC00145 in the acknowledgements. As with the papers, when Kathleen's thesis is approved, please do send me a pdf copy for our records.
Most important of all, stay safe out there. Common sense says: don't hurry anything along if it elevates risk of exposure to coronavirus.
Looking forward to communicating more next week.
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 631-4808 (mobile / pandemic)
(970) 226-9358 (office)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, December 4, 2020 10:11 AM
To: Griffin, Paul C <pgriffin@blm.gov>; Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
Subject: [EXTERNAL] BLM Final Report

Hi Paul,
I hope all is well with you and your family during these most challenging times.
I'm writing to give you an update on the status of our final report that is due on December 6. Unfortunately, we will not be able to provide a complete and meaningful final report by this date and are requesting an extension of this deadline. We apologize for this inconvenience and are diligently working to provide this information as soon as possible. We are planning to submit three manuscripts for potential publication in peer-reviewed journals. The information from these papers will provide the basis for the final report and we should be able to provide a meaningful document in the next few weeks that should be acceptable to the BLM. These manuscripts are in different stages of preparation and include the following:

Manuscript 1. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine in free-ranging horses *(Equus caballus):* Limitations and side effects - Update 2018-2020

This paper will update our previous 2018 publication in PLoS ONE and will include data from the 2018, 2019, and 2020 foaling seasons. Our thoughts now are to again submit this manuscript to PLoS ONE for publication. This paper will not be nearly as long as the previous one since we will be able to reference the Methods from the previous paper. To date, I've written a partial draft for this paper and only need final statistical analysis of foaling proportions and effectiveness to update previous results. With this information, we should be able to provide reliable summary of these results for the BLM report and well-before submitting the manuscript to PLoS ONE for the review process.

Manuscript 2. Optimum reimmunization interval for delivery of GnRH immunocontraceptive vaccine (GonaCon-Equine) to feral horses (*Equus caballus)* using prototype syringe darts.

This manuscript will combine data on remote dart delivery of GonaCon and an assessment of the optimum reimmunization interval . We have a partial draft of this paper and are currently conducting a comprehensive statistical analysis comparing foaling proportions and

effectiveness for the four treatment intervals across 2017, 2018, 2019, and 2020. Similar to Manuscript 1, once we have a preliminary analysis of these data, I can include this information in the final report. We have not yet decided on the ultimate outlet for publication of the results from study, possibly Wildlife Society Bulletin. Any suggestions?

Manuscript 3. Pregnancy diagnosis in captive and free-ranging horses (*Equus caballus)* using serum and fecal estradiol analysis.

I would like to include this information in the final report because, as you know, it was funded in large part by the BLM. Except for re-running a few samples, the laboratory phase of this study has been completed the results are reported in Kathleen Eddy's MS thesis, which she successfully defended last month. Dr. Terry Nett and I are assisting her in converting her thesis into a publishable manuscript. However, she also has teaching responsibilities until the end of this semester and at present has limited time to devote to this effort. We will make a concerted effort to provide, at least, a preliminary summary of this research as soon as possible. We are considering Animal Reproduction Science and Wildlife Society Bulletin as possible journals for this publication.

Again, I apologize for having to request an extension to the current deadline but as you can see, the funding from the BLM for these studies as resulted in plethora of novel and exciting information that should be invaluable to the management of free-ranging horses. We just need a little more time to complete our data analysis, interpret results, and discuss the significance of these results in a well-written final report or publication. Thank you for your consideration of this request. Please give me a call if you would like to discuss this matter.

Kind regards,

Dan

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

RE: Phone call re: BLM funds

McCann, Blake E <blake_mccann@nps.gov>
Fri 4/24/2020 8:45 AM

To: Griffin, Paul C <pgriffin@blm.gov>
Cc: Reiland, Michael J <mreiland@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>; Rittenhouse, Bruce H <brittenh@blm.gov>; Melzo, Kevin A <kevin_melzo@nps.gov>; Klosterman, Megan E <megan_klosterman@nps.gov>

Thank you Paul;

We will expend funds as requested for extension of field season and/or collateral duty GS-05 staff time to complete research operations.

Blake

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, April 24, 2020 8:21 AM
To: McCann, Blake E <blake_mccann@nps.gov>
Cc: Reiland, Michael J <mreiland@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>; Rittenhouse, Bruce H <brittenh@blm.gov>
Subject: Re: Phone call re: BLM funds

Good morning Blake,
BLM is glad to hear that the research into GonaCon's effects is continuing this summer. Thank you for your request yesterday to spend what you projected to be a relative surplus of about $4,000, to be used on further costs in support of the research outlined in agreement L20PG00022. The initial obligation from BLM for the agreement was $19,000. Let this email serve as a written approval for NPS to spend up to that full amount for related expenses such as those you suggested. Here, I am cc-ing the BLM budget contact on the agreement (Michael Reiland), who has let me know this approval can be given to NPS, and the technical contact on the agreement (Bruce Rittenhouse).
Of the costs that you suggested, BLM would be most supportive of items 1 or 3 (continuing to pay for more field work through the period of performance of the agreement, which extend through February 2022), or tasking a GS-05 Bio-Tech with collateral duty horse research.
Please call or write me any time if you have further questions.
Stay well,
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: McCann, Blake E <blake_mccann@nps.gov>
Sent: Thursday, April 23, 2020 8:33 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: Phone call re: BLM funds

Hey Paul;

With our pandemic response, we have had to scale back on seasonal staff, and this has affected how I have hired for horse research. Luckily, I have two staff that have worked on the project who are currently in ND. I am bringing both on as GS-06 Bio-Techs and feel that their salary fits the IAA description reasonably well. It looks, however, like we may have around $4000 remaining from the IAA by August 15. Options are to 1) continue to pay for field work beyond that date, 2) cover housing for CSU techs currently in the park, 3) task a GS-05 Bio-Tech (primary role weed control) with collateral duty horse research, 4) send the remainder back, or 5) some other solution that I am not envisioning here?

Regardless, the good news is that we will be able to staff field work for the research project this summer. I thought we could talk by phone to identify the most responsible method to manage funds. Are you available for a call?

Thank you.

Blake

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

Re: Domestic horse per diem appears to be within scope of budget, for L15AC00145

Parker, Leona B <lparker@blm.gov>

Tue 7/7/2020 3:15 PM

To: Griffin, Paul C <pgriffin@blm.gov>; Link,Maura <Maura.Link@colostate.edu>; Dan Baker <danbaker@colostate.edu>
Cc: Shepherd, Alan B <ashepher@blm.gov>

Good afternoon, reviewing your request I see nothing wrong with your request. If you feel that you need to move funds you can do that between categories if its NO more than 10%

Thanks
L

Leona B. Parker,
California Grants Management Officer
Bureau of Land Management, California
REMOTE Duty Station: Eagle Lake Field Office
Department of the Interior, Region 8/10
Office (530) 252 5338 I Fax (530 257 4831

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Grant and Agreement Regulations - Code of Federal Regulations (CFR) Link:
http://www.ecfr.gov/cgi-bin/text-idx?SID=ed90f54836feb6a994f657188eb05e33&node=2:1.1.2.2.1&rgn=div5
PROGRAM OFFICERS WEBSITE: https://doimspp.sharepoint.com/sites/blm-wo-800/blmfa/SitePages/Home.aspx

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From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Tuesday, July 7, 2020 1:23 PM
To: Link,Maura <Maura.Link@colostate.edu>; Dan Baker <danbaker@colostate.edu>
Cc: Parker, Leona B <lparker@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>
Subject: Domestic horse per diem appears to be within scope of budget, for L15AC00145

Dan and Maura,
Thank you for calling to my attention your concern about spending $800 on about a month of domestic horse per diem, for the purposes of sampling blood and feces from several mares throughout their estrus cycles This $800 is a relatively small expense, but I would ask Leona Parker (grants management officer, cc'd here) to please email me or everyone cc'd here guidance if my interpretation of the existing agreement (below) is incorrect.
Confirming that fecal estrogen samples are a reliable indicator of pregnancy status is identified as a performance measure in the proposal that led to this agreement (L15AC00145), so it makes sense that CSU paying to temporarily feed and house domestic horses that you collect samples from should be covered under the agreement. Note: Personally, I pay more than that per month, to board just 2 ponies here in Fort Collins, so it sounds to me like the costs are reasonable.
On the phone today Dr Baker asked me which category of expense the $800 should be associated with I would expect that the most straightforward choice is to use the 'other' category that you identified in your budget (on page 35 of the attached, base award). You have $5,000 budgeted for 'other' expenses in year 5 of the award, which is this year. Alternately, you might choose to allocate this expense in the 'supplies' category, where the supply being used is the cost of feed for the animals while they are housed at the CSU barn, which is needed as part of the fecal estrogen assay work. Supplies are typically defined items with a cost of less than $5,000, and often with a one-time

use, and hay seems to fit that description. From what Dr. Baker said, you are not retaining possession of these horses, you are borrowing them from a private owner, and you are only paying for some hay, water, and use of some shelter (fenced lot). Your base award shows you have $1,950 budgeted for supplies in year 5 of this agreement, which is more than double the $800 you are asking about.

I cannot authorize any changes in your budget that is something only Ms Parker can do, as the grants management officer. However, given what I know about this agreement and the agreed-upon budget, it does not appear to me that you need to request any change in your budget to process this expense.

Again, thank you for asking It is always better to be cautious about this type of question
Sincerely,
Paul

Paul Griffin, Ph D
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Google Calendar on behalf of McCann, Blake E
To: Griffin, Paul C
Subject: Accepted: NPS IAA formation: GonaCon @ Wed Dec 18, 2019 1pm - 1:30pm (MST) (pgriffin@blm.gov)
Attachments: invite.ics

Blake McCann
has accepted this invitation.

NPS IAA formation: GonaCon

When
Wed Dec 18, 2019 1pm – 1:30pm Mountain Time - Denver
Video call
https://hangouts.google.com/hangouts/_/doi.gov/pgriffin <https://hangouts.google.com/hangouts/_/doi.gov/pgriffin?hceid=cGdyaWZmaW5AYmxtLmdvdg.66adv0iqsihjhao6uh11d6a4lb>
Calendar
pgriffin@blm.gov
Who
• pgriffin@blm.gov
- organizer
• McCann, Blake
• ashepher@blm.gov
• danbaker@colostate edu
• dan2.baker@colostate.edu
Invitation from Google Calendar <https://www.google.com/calendar/>

Print Cancel

Seeking Dave's help for new IAA formation (BLM-NPS, $19,000)

Griffin, Paul C <pgriffin@blm.gov>
Tue 1/14/2020 11:15 AM

To: Appold, David W <dappold@blm.gov>
Cc: Shepherd, Alan B <ashepher@blm.gov>; Rittenhouse, Bruce H <brittenh@blm.gov>; Reiland, Michael J <mreiland@blm.gov>; McCann, Blake E <blake_mccann@nps.gov>; Anderson, Jacky L <j1anders@blm.gov>

1 attachments (36 KB)
BLM NPS Interagency agreement articles SOW_13Jan2019.docx;

Good morning Dave,
I'm writing to ask for your help in forming a new Interagency Agreement with Theodore Roosevelt National Park (National Park Service).
IAA articles / statement of work are attached here, for your review to make sure they are in order.
PR # 40473695 has been created to pay for this $19,000 obligation, and has supervisory and certifying funds approvals completed.
Please call me if there is anything I can do to help prepare for formation of the IAA.
If it's all right with you, I'll try to reach you by phone during the day today, to see if there's anything more you'd need.
Thank you,
Paul

From: Griffin, Paul C
To: Dan Baker
Subject: BLM agreement document
Date: Tuesday, July 7, 2020 1:41:53 PM
Attachments: L15AC00145, Base Award, Complete.pdf

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FOR BLM USE ONLY, PROPOSAL # _____
(MM-YY-####)

WH&B FINAL REPORT

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort

Study Project Name: Reimmunization of Free-ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance

Institution: Colorado State University

Title of Project: Same as above

Principal Investigator(s): Dan L. Baker and Terry M. Nett

Co-Investigators: Jenny G. Powers, Blake E. McCann, Jason E. Ransom, Nathan L. Galloway, Jason I. Bruemmer, Douglas C. Eckery

Starting Date: 09/05/2015

Completion Date: 09/05/2020

Executive Summary:

During 2015-2020, we investigated the effects of revaccination with GonaCon vaccine on reproduction and side effects in free-ranging mares at Theodore Roosevelt National Park, North Dakota. We selected study mares on the basis of age and reproductive status and randomly assigned them to one of four reimmunization groups (4yr, 2yr, 1yr. 0.5 yr). Except for the 4yr group, which was hand-injected with GonaCon all other experimental mares received a primary and secondary immunization with GonaCon-Equine vaccine via remote dart delivery. We conducted foaling observations and documented physiological side effects on all mares during March-August 2015-2020. Foaling proportion for mares in the four-year immunization treatment group was shown to be 0.09(95% CI= 0.002-0.146) compared to 0.80 (95% CI = 0.75-0.85) for control mares over the same time period and resulted in an average vaccine effectiveness of 0.91 (0.82-0.99).The shorter booster intervals were less effective but all were lower (P <0.001) than that for control mares for all subsequent years (2015–2020). Differences among treatment intervals were not available at the time of this writing. The only detectable adverse side effect of vaccination was intramuscular swelling at the vaccination site. But, regardless of type or severity of the injection site reaction, we did not observe any clinical evidence of lameness, impaired mobility, depression, or decreased health or fitness in any treated animal. To deliver experimental booster intervals to experimental mares required developing and testing a novel prototype dart configuration for remotely delivering GonaCon-Equine in a syringe dart. We successfully accomplished this objective. Our 91% success rate (73/80) for first attempts and overall success with follow-up shots to administer the vaccine to all treatment animals is encouraging and can provide resource managers with a practical method for delivering this vaccine, at prescribed application intervals. Finally, we developed, validated, and tested a reliable, non-invasive field technique for monitoring reproductive hormones in feces that could potentially provide biologists with a useful tool for assessing the effectiveness of fertility control applications in free-ranging wild ungulate populations.

BACKGROUND

The period of performance covered in this report is focused primarily on research conducted during 2015-2020. However, the determination of the optimum reimmunization interval was initiated in 2013 and, for the sake of continuity, and comparative purposes, these results are also included in this final report. The rationale supporting the need for this research, the research question, the supporting literature, the objectives, and hypotheses to be tested are presented in detail in the attached BLM Project Proposal 2015 and in Baker et al. 2018 (see attachments). In addition to accomplishing the primary objectives of this research, we also developed and tested a novel laboratory methodology for diagnosing pregnancy in free-ranging horses using fecal steroid analysis (see Objective 4) and attachment (Eddy, 2020, MS thesis). A general summary of specific objectives and related investigations are described below. A more detailed description of each area of research is available in these supporting and attached documents.

Summary of Objectives: (Please restate, in writing, each objective and then summarize progress, were the objectives accomplished? Explain why or why not; limit to no more than three pages per objective)

Optimum reimmunization interval

1) To determine the most effective reimmunization schedule for GonaCon-Equine for suppressing reproductive rates in free-ranging horses, the duration of effectiveness, and the return to fertility following treatment.

HYPOTHESIS: (H_1) Based on extensive evidence in the immunological literature, we predicted that decreasing the interval between the primary and booster immunizations would decrease the effective duration of GonaCon-Equine in free-ranging horses.

METHODS

Four year reimmunization interval. In 2013, four years following the primary vaccination, the THRO feral horse population was similarly gathered and handled through existing corrals and chute systems to remove excess animals from the park. Given this unique opportunity and unknown effects of revaccination with GonaCon, we retained mares previously immunized with a single vaccination and revaccinated them (four years post-primary vaccination), assessed general health, pregnancy status, and body condition using techniques identical to those applied at the 2009 roundup (Baker et al. 2018, attached). Mares previously revaccinated four years post-primary were maintained as a reference point treatment interval in the following experiment.

Two, one, and 0.5 reimmunization intervals. In order to evaluate the effects of additional experimental booster intervals on foaling proportions and vaccine effectiveness required not only successful development of dart delivery of GonaCon but also additional experimental groups of horses. We selected three additional revaccination intervals as companion treatments to the already established four-year interval. Because of a limited number of reproductive age females in the THRO feral horse population, controls from 2013 were also maintained as concurrent controls in this experiment (Table 1).

Table 1. Summary of study design for primary and reimmunization intervals, sample size, and mode of vaccine application for each experimental group of horses treated with GonaCon-Equine vaccine at Theodore Roosevelt National Park, North Dakota, USA.

Revaccination Interval	Sample Size (n)	Primary Vaccination Date	Revaccination Date	Mode of Delivery
4-year	25	October 2009	September 2013	Hand injection
2-year	11	September 2013	September 2015	Dart injection
1-year	15	September 2015	September 2016	Dart injection
0.5-year	15	September 2015	March 2016	Dart injection
Control	25	October 2009	September 2013	Hand injection

RESULTS

Preliminary results relating to foaling proportions and vaccine effectiveness for reimmunization intervals are presented in Fig. 1 and Table 2. These results represent the first breeding seasons that reimmunization treatment on foaling proportions could be affected by GonaCon vaccine during 2015-2020. Averaged over this six year period, mean foaling proportions in the four-year treatment group were lower (P <0.001) than that for control mares for all subsequent years (2015–2020). Foaling proportion for mares in this treatment group was shown to be 0.09(95% CI= 0.002-0.146) compared to 0.80 (95% CI = 0.75-0.85) for control mares over the same time period and resulted in an average vaccine effectiveness of 0.91 (0.82-0.99). During this time period, 21% (5/24) of treated mares regained fertility and produced a healthy foal that was born during the normal breeding season (March-August). This study demonstrates that a booster vaccination, even four years post-primary can stimulate a highly effective immune response that can result in multiple years (≥ 6 yrs.) of contraception and possible permanent infertility in some free-ranging horses.

Foaling proportions for the first year following revaccination for 1yr and 0.5yr post-primary vaccinations were similar (0.14 (2/14) vs 0.15 (2/13), (P = 0.78) and comparable (0.08 2/24) to mares hand-injected with GonaCon-Equine (4 yr interval) (Table 2). Likewise, vaccine effectiveness for these groups tended to be similar (0.82 vs 0.81, and approached the effectiveness of the 4 yr treatment group (0.90). In contrast to 2018, however, during 2019-2020, foaling proportions in the 1 yr and 0.5 groups increased on average by 50-60%, whereas the 2 yr interval remained consistently at approximately 0.31 (SE = 0.12) over the four year period 2017-2020 (Table 2). Likewise, vaccine effectiveness in the 1 yr decreased only slightly from 0.80 to 0.70 while the 0.5 yr group decreased significantly (P = 0.0*5*) from 0.78 to 0.48 over this same time period. These results are reflected in the rate of reversibility following booster immunizations. On average 40% of the mares in the shorter intervals regained fertility over the three year period while only 25% of mares in the 4yr have reversed over the same time period. Further statistical analysis will be conducted to assess potential differences in short-term intervals over time and test the *a priori hypothesis* of this experiment. In summary, these results suggests that practical application of this vaccine in feral horses will require an initial inoculation that may provide only modest suppression of fertility followed by reimmunization that together will result in greater suppression of fertility over time in free-ranging horses.

Table 2. Comparative foaling proportions (%) and vaccine effectiveness (proportional reduction in foaling between control and treated mares) for four-post primary reimmunization intervals and a saline control group during 2015-2020 foaling seasons in free-ranging horses at Theodore Roosevelt National Park, North Dakota, USA.

Comparative Parameters	**Treatment Groups**				
	Control	4	2	1	0.5
Method of delivery:	Hand - injection	Hand-injection	Dart	Dart	Dart
Year: 2015					
Foaling proportion % (y/n):	0.84 (21/25)	0.00 (0/25)	0.45 (5/11)	N/A	N/A
Vaccine effectiveness:	N/A	1**	0.46	N/A	N/A
Year: 2016					
Foaling proportion % (y/n):	0.84 (21/25)	0.16(4/25)	N/A	N/A	N/A
Vaccine effectiveness:	N/A	0.84	N/A	N/A	N/A
Year: 2017					
Foaling proportion % (y/n):	0.76 (19/25)	0.04 (1/24)	0.36 (4/11)	N/A	N/A

Vaccine effectiveness:	N/A	0.94	0.52**	N/A	N/A
Year: 2018					
Foaling proportion % (y/n):	0.72 (18/25)	0.08 (2/24)	0.27 (3/11)	0.14 (2/14)	0.15 (2/13)
Vaccine effectiveness:	N/A	0.88	0.62**	0.8**	0.78**
Year: 2019					
Foaling proportion % (y/n):	0.77 (17/22)	0.08 (2/24)	0.36 (4/11)	0.35 (5/14)	0.31 (4/13)
Vaccine effectiveness:	N/A	0.89	0.52	0.537	0.601
Year: 2020					
Foaling proportions % (y/n):	0.90 (19/21)	0.21 (5/24)	0.27 (3/11)	0.36 (5/14)	0.46 (6/13)
Vaccine effectiveness:	N/A	0.769	0.698	0.605	0.489

**Indicate the first foaling season that GonaCon-Equine treatment could have an effect on mare fertility.

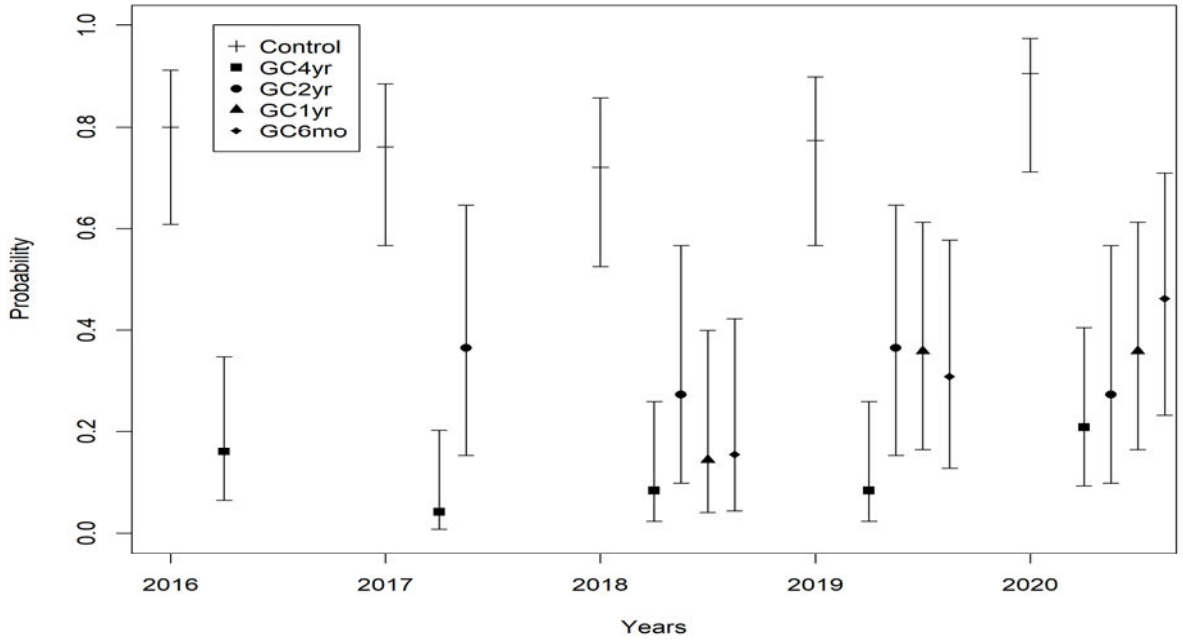

Fig 1. Comparative probability of foaling for four experimental reimmunization groups (treatments) and a control group of free-ranging feral horses (*Equus caballus)* selected for this experiment. The foaling proportions in this graph represent the first breeding seasons that a reimmunization treatment on mare fertility could be detected when using foaling observations to assess successful contraception by GonaCon- Equine vaccine. Bars represent 95%confidence intervals. Abbreviations: GC4 = four year interval; GC2 = two year interval; GC1 = one year interval; GC6mo = six month interval.

Dart development and testing

2) To develop and test a safe and effective dart configuration and injection system for remotely administering GonaCon-Equine reimmunizations to free-ranging horses by means of syringe dart.

METHODS

Dart configuration: In collaboration with Pneu-Dart (Pneu-Dart Inc., Williamsport, PA),we developed 2cc Type-P syringe darts configured with Slo-inject™ technology and a 3.81 cm, 14-gauge, Tri-Port needle with a gel barb positioned 1.27 cm forward of the ferrule. We hypothesized that slowing the rate of injection and allowing the viscous fluid to eject through multiple ports on the needle would reduce blow back, and that the gel-barb would penetrate the skin to hold the needle in place for complete intramuscular injection comparable to that achieved with hand injection. To minimize striking velocity and reduce dart rebound, we calibrated a Dan Inject JM Special (DanWild LLC, Austin, TX) dart projector to deliver syringe darts at perceived minimum velocities, based on personal experience and ad-hock experimentation. Prior to loading darts with vaccine, we weighed each dart to the nearest hundredth gram (0.01) using a digital scale. We then loaded the vaccine and reweighed darts to calculate the mass of each dose. We were unable to load a full dose (2g) of vaccine into darts, as per hand-held syringe Baker et al. (2018,attachment), instead averaging 1.85g. Prior studies have reported that 1.0-2.0g is a sufficient enough dose to invoke an effective immune response in feral horses and other wild ungulates. Standard operating procedures (SOP) for loading darts and remote delivery in the field are attached to this document.

RESULTS

Dart delivery: We fired a total of eighty-eight darts at forty individual mares at distances ranging 10-21m to administer initial and booster doses of GonaCon. Of those, seventy-three darts were known to have successfully delivered full doses at first attempt and four successfully provided follow-up doses to complete vaccinations. Given the general injection success observed in-process for most darts sticking solidly and being retained for periods >30 seconds, we felt confident that animals were successfully vaccinated. Procedural errors and loss of darts precluded evaluation of vaccine amounts administered in nine cases. The behavioral responses of horses to dart delivery were generally predictable for both the individual animal being darted and the associated band. In almost all cases the individual animal receiving the dart jumped when struck by the dart, ran in circles trying to bite the dart or ran with the band, settled, and resumed grazing or standing. The behavior of the band also followed a predictable pattern ranging from no response to being startled by the report of the dart rifle and/or the darted individual then running 100-300m away from the darting team. Following this initial reaction, the band generally settled within 5-15 min, resumed feeding and allowed the approach of the darting team for another darting opportunity. These horses were conditioned to human presence and most received no more than 2 dart applications.

Validation of dart delivery: After dart configuration proved satisfactory in trials with sham vaccine, we selected eleven previously untreated mares (2 yr. interval group) of similar age and body condition to receive the active vaccine at the 2013 roundup. We then sorted mares singly or in pairs into paddocks), as space allowed, for darting. We delivered remote injections into the right hip at distances ranging from 10-15.5m and pressures ranging from 3.0-3.25 bars. For each dart fired, we noted the behavior of the darted animal and time of dart retention (to the nearest minute). To ensure adequate velocities for dart discharge during subsequent field operations, we experimented with pressure settings. We utilized two projectors, a DanInject JM-Special and a Pneu-Dart X-Caliber (Pneu-Dart Inc., Williamsport, PA), and fired darts through a chronograph (Beta Master chronograph, Shooting Chrony Inc., Amherst, NY) placed at target to measure velocity of impact at ranges of 10 and 20 meters, the distance to which park horses can typically be approached. We used laser range finders to measure distance to the nearest meter for chronograph trials and field darting (Nikin RifleHunter 550, Nikon, Inc., Melville, NY). We observed that calibration of projectors for velocities of 46-48 meters per second at target provided adequate velocity for dart discharge without over penetration. When darting in the field we generally used the 10m pressure setting for shots ranging 10-14.5m and the 20m setting for shots

ranging 15-20m. After darts fell free of the animals, we retrieved and then reweighed each to determine the dose administered and documented retention time of the dart in the selected mare.

Treatment application: We conducted field operations in September 2015, boosting the eleven mares (2-year treatment group) that were first vaccinated at roundup in October 2013 and provided initial vaccinations to the 29 other experimental animals. We boosted the six-month treatment group in March 2016, and the 1-year treatment group in September 2016 (Table 1). In each case, we recorded the dose administered, behavioral response of the darted animal, the associated band, injections site reactions (left or right hip) and dart retention time (to the nearest minute), making every effort to observe dart drop and recover darts, searching visually and with metal detectors. If weight of darts indicated <50% dose administered, then we redarted animals with another full dose. Where weight indicated >50% but < 90% dose administered, we darted animals again with a half dose (1g) of the vaccine. Animals were boosted in the hip opposite to initial injection site for independent evaluation of both injection site reactions. This study demonstrated that GonaCon can successfully be remotely delivered to free-ranging animals via appropriately configured syringe dart. Our 91% success rate (73/80) for first attempts and overall success with follow-up shots to administer vaccine to all treatment animals is encouraging. We found that 3.81cm ferrules can provide sufficiently deep intramuscular injections of GonaCon, but that a gel barb is required, in addition to Slo-inject™ and TriPort needle dart configurations. Mean overall dart retention time of 11.3 minutes across treatments further attests to the consistency of injections achieved with this dart design. In combination with our measurements of retention time across successful darting attempts, we surmise that where darts are observed to stick for ≥1-minute, an adequate dose of vaccine has been delivered.

Physiological side effects

3) To determine the safety and physiological side-effects of GonaCon-Equine vaccine (if any) in feral horses following revaccination including visual assessment of general health, body condition, injection site reactions, effects on current pregnancy, and neonatal survival.

METHODS

Concurrently with foaling observations, we evaluated and compared potential adverse side effects of treatment on injection site reactions, body condition, success of existing pregnancy, and neonate survival in treated and control mares. Assessment of these potential side effects were made monthly during the primary foaling season and opportunistically for the remainder of the year. Each mare was observed for the presence or absence of visible lesions, swellings, or discharge at the injection site. In addition, we documented evidence of lameness (e.g. limping, gait alteration, reluctance to stand or bear weight on a limb), if present, as well as, behavioral depression, muscle tremors or other systemic reactions that could be related to neonate survival in treated and control mares. We classified injection-site reactions according to the following criteria: 1) **abscess** – an open sore usually with fluid drainage or discharge, 2) **swelling** – a raised area of tissue of variable size and shape with no visible fluid drainage, 3) **lameness** – any abnormal range of movement or stiffness in the leg where the vaccine injection was delivered, 4) **none** – no observable reaction. For these observations, we approached as near as possible to individual horses (≤ 50 m) and assessed and photographed each injection-site reaction for later evaluation. At the same time, we visually evaluated body condition of each mare and foal (if present) and scored condition as previously described. We measured neonatal survival as the proportion of foals surviving to 14 days of age and post-natal survival to 200 days.

RESULTS

The only detectable adverse side effect of vaccination was intramuscular swelling at the vaccination site. Mares treated with GonaCon consistently showed evidence of inflammatory reactions at the site of injection. Approximately, 70% of mares treated with GonaCon via remote dart delivery showed evidence of injection site reactions following both the primary injection and booster. Approximately 50% of these were classified as draining abscesses. Most of these abscesses drained within several weeks, then healed over, and were difficult to observe from 50m, thereafter. Given the designed, highly

inflammatory nature of both the adjuvant, which contains killed mycobacteria and non-biodegradable oil, as well as, the foreign protein carrier molecule, these types of reactions are predictable. In fact, they are likely necessary for optimum vaccine efficacy. It was impossible to assess the total impact of these lesions on animal welfare; however, in this investigation, these did not have a measurable effect on body condition, locomotion, or social behaviors. Therefore, until additional research suggests otherwise, we conclude that the presence of injection site lesions following GonaCon vaccination do not pose a serious contraindication associated with the application of this vaccine, and there appear to be minimal long-term effects on individual animal welfare.

Pregnancy Diagnosis

4) To develop and test a non-invasive, laboratory methodology for accurately diagnosing pregnancy in free-ranging horses using a single fecal sample collection.

Development of a reliable, non-invasive field technique for monitoring reproductive hormones in feces can potentially provide biologist with a useful tool for assessing the effectiveness of fertility control applications in free-ranging wild ungulate populations. In addition, metabolites of reproductive steroids are excreted in feces and their concentrations can be used to assess pregnancy status, estrus, conception, fetal loss, parturition, and neonatal mortality without manipulating or stressing the animal. However, for a reliable prediction of pregnancy status in animals, it is essential that fecal steroids reflect variation in blood concentrations that are physiologically relevant to the species of interest. Nevertheless, to date, few studies have demonstrated this relationship. Clearly, a validated procedure that relies on a single fecal sample protocol and provides a highly accurate discrimination between pregnant and non-pregnant animals throughout gestation is needed for application in free-ranging horses. Thus, the primary goals of this study were to develop a methodology that accurately diagnoses pregnancy in free-ranging horses using a single fecal sample. Specific objectives were:1) **validation:** to define the relationship between serum and fecal estradiol concentrations for pregnant, cycling, and feral horses, 2) **discrimination:** to determine concentrations of serum and fecal estradiol that significantly discriminate between pregnant and non-pregnant feral mares during the gestation period, and 3) **accuracy:** to determine the optimum period of gestation when fecal estradiol concentrations would most accurately distinguish between pregnant and non-pregnant feral mares.

METHODS

Study 1: Measurement of fecal and serum estradiol in the domestic mare

Daily blood and fecal samples were concurrently collected from eight, non-pregnant cycling mares, ranging in age from 9 to 11 years. While mares were not evaluated to determine day of cycle at sample initiation, the average reported length of an equine estrous cycle is 21-22 days, therefore, samples were taken from mares for a total of 23 days. Likewise, blood and feces were collected concurrently on a weekly basis throughout gestation from 8 pregnant domestic mares with known embryo transfer dates of 7-day embryos. At the time of sampling, mares ranged in age from 6-16 years. Although blood and feces were taken weekly, the mares were asynchronous in their gestational timing, and initiation of sampling was earlier in gestation in some mares than others. For both groups (non-pregnant and pregnant), fecal samples were obtained via fecal grab sampling while mares were restrained in standing stocks. Directly following fecal collection, jugular blood was drawn for each mare at each blood collection. Fecal samples were processed within 4 hours of collection, while blood samples were left at room temperature overnight. After sitting overnight at room temperature, each blood sample was centrifuged for 30 minutes at 2400xg, and serum placed into 2.0 mL cryogenic vials for storage at -20°C until extraction. Radioimmunoassay (RIA) specific for estradiol 17β was used to quantify extracted fecal and serum samples for the two groups.

Study 2: ***Measurement of fecal and serum estradiol in the feral mare for determination of pregnancy***

This study was initiated in October 2009 with a park-wide round-up of all the horses at THRO. The first round-up included 48 feral mares, while the second roundup in September 2013 included the initial 48, plus three more, for a total sample size of 51 mares. Mares in both roundups were processed the same. Each mare was positioned in the squeeze shoot, a fecal grab sample was collected, followed by ultrasound to determine whether the mare was pregnant or not. Simultaneously, blood was drawn using an 18-gauge 1.5-inch needle. All fecal samples collected at both roundups were placed in whirl-packs labeled with date and mare identification, then frozen until processing. Following the 2009 round-up, yearly fecal sample collection was initiated for the 51 mares in the study. The primary breeding season at THRO ranges from March – August (Baker et al 2018, attached), and aside from the round-up samples, the majority of the additional 272 fecal samples were collected during the month of November from 2010-2015. During each collection year, an attempt was made to obtain at least one fecal sample for each of the 51 mares on the study. Samples were frozen until extraction. We then calculated day of gestation using the foaling data from the following year and counting the days between sample collection and foaling date, with an estimated gestational length of 345 days for all mares on the study.

RESULTS

Domestic mare trial. Fecal and serum results from this study indicate that measurement of estradiol 17β is a reliable method for pregnancy determination in the domestic mare. Of the collected fecal samples that exceeded the cut-off-date 105 days of gestation, 96.6% of them remained above the cut-off-date of 10 pg/mg for the entirety of gestation. Of the serum samples collected after the cut-off-date of 128 days of gestation, 94.8% returned values above the cut-off-date of 46 pg/mL serum throughout gestation. Regarding the fecal estradiol portion of this study, the earliest calculated day of gestation that returned estradiol 17β concentrations over those of cycling mare values was 105 days of gestation.

Feral mare. For fecal estradiol, a total of the 77 samples were collected over the span of both round-ups with 62 of them surpassing the cut-off day of 105 days and a concentration of 10 pg/mg of feces. None of the samples from the non-pregnant mares were above this cut-off-concentration. Determining pregnancy status from single fecal samples taken over the course of the study was found to be possible when measuring fecal estradiol from mares in this study. From the data collected during the study, the overall percentage of samples above the cut-off value taken in September were 85.4%, 77.8%; in October, 96.2%; in November, and 100% in February. This information is potentially useful to resource managers for estimating population-level fertility rates related to contraceptive treatments or fundamental knowdledge of reproductive parameters for free-ranging horses or other wild ungulate species.

Explain Major Findings and Limitations:

Major Findings:

1. This research suggests that practical application of this vaccine in feral horses will require an initial inoculation that may provide only modest suppression of fertility followed by reimmunization over time that together could provide resource managers with a practical management tool for suppressing the growth rates of some free-ranging horse populations. Preliminary results from the reimmunization experiment clearly indicates that GonaCon revaccination using dart delivery can be effective in suppressing fertility in free-ranging horses. However, additional statistical analysis will be required to assess differences associated with different reimmunization intervals.

2. W**e** demonstrated that GonaCon could be remotely delivered to free-ranging animals via appropriately configured syringe dart. Our 91% success rate (73/80) for first attempts and overall success with follow-up shots to administer vaccine to all treatment animals provides resource managers an effective alternative method for administering GonaCon vaccine to some free-ranging horse populations or other wild ungulate species.

3. We developed and tested a novel and practical, non-invasive field methodology for diagnosing pregnancy in free-ranging horses and other wild ungulate species treated with GonaCon vaccine or other contraceptive technologies.

Limitation: Changes in the objectives and strategies for management of the free-ranging horse population at THRO prevented continuation of reproductive monitoring of the experimental animals in this study. This action resulted in the termination of this experiment following the 2020 foaling season. As a consequence, we were unable to statistically substantiate return to fertility or duration of vaccine effectiveness for experimental reimmunization intervals. However, our results strongly suggest that GonaCon administered as a primary vaccination followed by a booster four years later will result in long-term infertility and/or possible permanent infertility in free-ranging horses. For short-term revaccination intervals, resource managers should expect reduced effective duration of GonaCon vaccine when applied, via remote dart delivery, to free-ranging horses or other ungulate species.

Information of Practical Value to the WH&B Program (e.g., how could this information be used for program improvements): See above Publications and time frame for publications:

1. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine (GonaCon-Equine) in free-ranging horses (*Equus caballus)*: limitations and side effects – an update. Submit to: PLoS ONE (anticipated submission: 02/15/2021)

2. An optimum reimmunization interval for delivery of GnRH Immunocontraceptive vaccine (GonaCon-Equine) to feral horses (*Equus caballus) u*sing prototype syringe darts. Submit to: The Wildlife Society Bulletin (anticipated submission: 03/15/2021)

3. Pregnancy diagnosis in free-ranging horses using serum and fecal estradiol analysis. Submit to Animal Reproduction Science, Wildlife Society Bulletin (anticipated submission: 03/01/2021).

Colorado State University Budget Justification for FY 2020

PERSONNEL

Dr. Terry M. Nett, Ph.D. (CO-PI) (1%) is responsible for project oversight, assisting with analysis of estradiol in fecal samples for evaluation as a method for detecting pregnancy, and assisting with data analysis and preparation of publications of research findings.

Dr. Dan L. Baker, Ph.D. (CO-PI) (720 hours @hourly rate) will assume lead responsibility for conducting research to evaluate the effectiveness and side-effects of the GnRH vaccine (GonaCon) in free-ranging horses at Theodore Roosevelt National Park (THRO), North Dakota. This role will include hiring and oversight of training field technicians in collection of data, data analysis and summation, and publication/presentation of research results. Additional duties will include preparation and oversight of budget, annual progress reports, IACUC, and communication and scheduling with national park service staff at (THRO), and other cooperating agencies (e.g. BLM, USDA, CSU).

Ms. Johanna Hodge, B.S. (Crew Leader) (3 month research technician @ $14/hr) will be the daily, onsite contact person between CSU and THRO and responsible for planning, training and scheduling daily work assignments for two field research technicians, as well as, daily oversight and quality control of data collection and transfer of data to PI's. This will be Ms. Hodge's fourth field season at THRO. She is well-versed and familiar with the objectives, methods of data collection, and logistics of this research project. In addition, her knowledge of the park landmarks and terrain, identification of individual experimental horses and location identification of individual experimental horses, and their band associations is unequaled. Her knowledge and experience over the last three years make her invaluable in the training of two new technicians and, as such, we are requesting a pay increase for her from $12/hr for the last three years to $14/hr for FY2020.

Project Field Research Technicians – Two field research technicians (4.5 mo each = 9 mo total) @ $12/hr). The role of these technicians, once trained, will be daily observations and measurements on 85 experimental free-ranging mares at THRO during 1 March-1August, 2020. Measurements will include identification of individual mares and determination of presence/absence of a foal, body condition of each mare/foal, evaluation of injection site reactions from GonaCon vaccinations, and band composition. Additional roles will be daily communication and data transfer to crew leader, interaction with THRO staff, and park visitors.

Additional funds requested for personnel in the final year of this project is $16,175 direct funds and $2,825 indirect (17.5%) for a total of $19,000 ***additional funds requested***.

This would bring the project total to $306,884 ($261,183 direct and $45,701 indirect).

From:	Griffin, Paul C
To:	Link,Maura; Dan Baker
Cc:	Parker, Leona B; Shepherd, Alan B
Subject:	Domestic horse per diem appears to be within scope of budget, for L15AC00145
Date:	Tuesday, July 7, 2020 2:23:24 PM
Attachments:	L15AC00145, Base Award, Complete.pdf

Dan and Maura,

Thank you for calling to my attention your concern about spending $800 on about a month of domestic horse per diem, for the purposes of sampling blood and feces from several mares throughout their estrus cycles. This $800 is a relatively small expense, but I would ask Leona Parker (grants management officer, cc'd here) to please email me or everyone cc'd here guidance if my interpretation of the existing agreement (below) is incorrect.

Confirming that fecal estrogen samples are a reliable indicator of pregnancy status is identified as a performance measure in the proposal that led to this agreement (L15AC00145), so it makes sense that CSU paying to temporarily feed and house domestic horses that you collect samples from should be covered under the agreement. Note: Personally, I pay more than that per month, to board just 2 ponies here in Fort Collins, so it sounds to me like the costs are reasonable.

On the phone today Dr. Baker asked me which category of expense the $800 should be associated with. I would expect that the most straightforward choice is to use the 'other' category that you identified in your budget (on page 35 of the attached, base award). You have $5,000 budgeted for 'other' expenses in year 5 of the award, which is this year. Alternately, you might choose to allocate this expense in the 'supplies' category, where the supply being used is the cost of feed for the animals while they are housed at the CSU barn, which is needed as part of the fecal estrogen assay work. Supplies are typically defined items with a cost of less than $5,000, and often with a one-time use, and hay seems to fit that description. From what Dr. Baker said, you are not retaining possession of these horses, you are borrowing them from a private owner, and you are only paying for some hay, water, and use of some shelter (fenced lot). Your base award shows you have $1,950 budgeted for supplies in year 5 of this agreement, which is more than double the $800 you are asking about.

I cannot authorize any changes in your budget -- that is something only Ms. Parker can do, as the grants management officer. However, given what I know about this agreement and the agreed-upon budget, it does not appear to me that you need to request any change in your budget to process this expense.

Again, thank you for asking. It is always better to be cautious about this type of question.

Sincerely,

Paul

Paul Griffin, Ph.D.

Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From:	Griffin, Paul C
To:	McCann, Blake E
Cc:	Shepherd, Alan B; Melzo, Kevin A; Appold, David W
Subject:	draft 1, SOW for BLM-NPS IAA
Date:	Thursday, December 26, 2019 1:18:34 PM
Attachments:	draft1_BLM NPS Interagency agreement articles SOW_26Dec2019.docx

Blake,
Attached is a draft statement of work / articles for an interagency agreement in support of NPS finishing the GonaCon field work at Theodore Roosevelt National Park in 2020. Please let me know your thoughts and suggested edits. I appreciate what you said in our phone conversation about the need to move quickly on this, to allow time for finishing all aspects of agreement formation in time for hiring fieldwork staff.
One question: Is Kevin Melzo the right person to list as both a budget contact and a billing / payment contact?
Note: I've written this in a way that would have a $19K obligation, but with a maximum of up to $24K, in case some unforeseen need arises. However, if you are aware of any $20K threshold for any particular level of approvals needed, we can also back it down to just the $19K, for simplicity and speed of approvals.
I am cc-ing Contracting Officer Dave Appold, who may be involved with agreement formation in the new year, as well as BLM WHB program on-range branch chief Alan Shepherd, BLM WHB program budget advisory Michael Reiland, and BLM WHB program division chief Bruce Rittenhouse.
Thank you,
Paul

--
Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

INTRA-GOVERNMENTAL ORDER (IGO) ARTICLES

I. PROJECT TITLE:

BLM-NPS Wild Horse Research IAA

II. OBJECTIVE:

The purpose of this IGO is for the Bureau of Land Management (BLM) to secure assistance from United States National Park Service Theodore Roosevelt National Park (NPS THRO) to complete ongoing feral horse fertility control research. The NPS THRO is the management authority for Theodore Roosevelt NP, including management of a herd of feral horses where this research has been taking place since 2009. Feral horses at NPS THRO are not subject to the Wild Free-Roaming Horses and Burros Act of 1971. The NPS THRO has been involved in conducting research into the use and effects of GonaCon fertility control vaccine in that feral horse herd. BLM does not have any management authority at NPS THRO, or oversight of the feral horses in that GonaCon vaccine research. The expertise and capacities that the NPS THRO can bring to concluding the research into the effects of GonaCon vaccine on feral horses will support the overall mission of the BLM, especially in reducing fertility rates in wild horses and burros.

III. STATEMENT OF WORK:

This interagency agreement responds to BLM's needs for scientific expertise in wild horse and burro fertility control research. The BLM needs NPS THRO to conduct field work in 2020 to finish the last year of data collection for a scientific study on feral horses on NPS THRO lands. That study informs BLM's wild horse and burro management on BLM lands.

The purpose of the ongoing study is to determine the efficacy of booster doses of the GonaCon immunocontraceptive vaccine, delivered at four different times after the primer dose (6 months, 1 year, 2 years, or 4 years after the primer dose). The ongoing study has been funded by NPS THRO and by BLM, and supported in kind by NPS THRO. Preliminary results from the study indicate a high potential for booster doses of GonaCon vaccine to provide long-term fertility control effects in feral horses. The methods and preliminary results are described in a publicly available, peer-reviewed journal article by Baker et al. (2018. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine (GonaCon-Equine) in free-ranging horses (*Equus caballus*): Limitations and side effects. PLoS ONE 13(7): e0201570. https://doi.org/10.1371/journal.pone.0201570). The final year of the study is planned to take place in 2020, and will provide information about contraceptive effects up to seven years after booster dose delivery. The field work in 2020 will center around field work observations to determine what fraction of treated mares do or do not give birth to a foal. The specific services provided by NPS THRO may include, but are not limited to: field data collection; statistical analyses; office assistance for collating and interpretation of data, and associated technical advisory roles; and assistance with preparation of peer-reviewed scientific literature.

Detailed descriptions of the study design are in Baker et al. (2018). A description of the associated costs for the field work identified under this IGO in in Appendix 1.

A. The NPS THRO agrees to:

1. Provide experienced personnel, at the appropriate skill level capable of effectively achieving the research tasks identified.

2. Provide deliverables, including a summary report or memorandum outlining the progress of field observations.

3. Supply any additional equipment as necessary in order to complete specific tasks outlined in this Inter-Governmental Order (IGO).

4. Submit results of scientific studies to peer-reviewed journals or other appropriate scientific outlets for publication, with input from appropriate coauthors.

5. Manage any related data that is in NPS THRO's possession in keeping with DOI guidelines for data documentation and preservation.

Note: The responsible official for the NPS THRO is to ensure all personnel who work on this project and any expenses incurred are charged to the account classification number identified in the IGO.

B. The BLM agrees to:

1. Fund the completion of the field work observations (budget noted in Appendix 1).

2. Work with the NPS THRO on a case-by-case basis to agree to a reasonable schedule for the implementation and completion of any specific work request.

IV. REPORTING:

A final report or memorandum will outline 2020 progress on the GonaCon vaccine research project, problems encountered, as well as project-related costs. The report or memorandum will be submitted in writing to the BLM Technical Contact, and to the BLM WHB program research coordinator (pgriffin@blm.gov), in the form of two hard copies and one electronic copy by 31 March of 2021.

V. AVAILABILITY OF APPROPRIATED FUNDS

The ability of the parties to carry out their responsibilities under this IGO is subject to their respective funding procedures and the availability of appropriated funds. Should either party encounter budgetary problems in the course of its respective internal procedures which may affect the activities to be carried out under this IGO, that party will notify the other party in writing in a timely manner.

Available funding provided by the BLM under this IGO is fiscal year 2020 funding.

VI. ADVANCE FUNDING (ADVANCE PAYMENT)

There is no provision for advanced funding within this IGO.

VII. SETTLEMENT OF DISPUTES

The parties under this IGO are responsible for resolving any disputes that may arise within 30 business days of the billing date. If the dispute cannot be resolved within this period, then the matter will be referred the following business day to the Department of Interior Office of Financial Management Office (OFM).

VIII. FINANCIAL ARRANGEMENTS (PAYMENT):

This IGO is not to exceed the amount as stated herein: $24,000 inclusive of all Modifications, with an initial obligation of $19,000. The charges for goods/services will include both direct and indirect costs applicable to this IGO. The NPS THRO will submit their billing through the Intra-governmental Payment and Collection (IPAC) system or the Intra-governmental Transaction Portal – whichever is applicable. The bill will reference the BLM's Dun & Bradstreet Number, the Requesting Agency Location Code (ALC), the Treasury Account Symbol, the Accounting Classification Reference Code(s), the Obligating Document Number, a brief description of the service performed, the Accounts Payable POC name and phone number, and identify the amount of money being billed for each project on every invoice. Payments will be made monthly.

The BLM shall not be obligated to pay for, nor will the NPS THRO be obligated to perform any effort that will require the expenditure of Federal funds above the amount obligated.

IX. TERMS OF IGO:

This IGO shall become effective upon signature by both parties (BLM and NPS THRO) and shall remain in effect until one year after initiation unless terminated in accordance with paragraph VII. The effective date will be determined based upon the last party who signed and dated the IGO.

The parties agree to annually review the IAA if the agreement period is modified to exceed one year. Appropriate changes will be made by amendment to and/or modification to any affected Order(s).

X. TERMINATION:

This IGO may be terminated by either party upon 30 days written notice. If the IGO is cancelled by the BLM, the NPS THRO will be reimbursed for costs incurred prior to cancellation, plus any termination costs. All costs claimed by the NPS THRO must be itemized and furnished to the BLM. If the IGO is terminated by NPS THRO, then NPS THRO and appropriate coauthors nevertheless commit to publishing the results that have been collected up to that point in time of all studies, whether in appropriate peer-reviewed journals or in a NPS publication, in a timely manner.

XI. MODIFYING THE IGO:

Either party under this IGO may propose to make changes under this IGO by notifying the other party in writing. All changes under this IGO must be modified and agreed upon by both parties in writing.

XII. POINTS OF CONTACT:

Changes to the Points of Contact identified below may be made by written notification to each of the parties under this IGO.

REQUESTING AGENCY (BLM)

IGO Technical Contact:
Name: Bruce Rittenhouse
Title: Acting Division Chief, WHB Program
Address: 20 M Street, SE
Washington, DC 20003
Phone: (202) 912-7648
Fax: (202) 912-7182
Email: brittenh@blm.gov

Budget Contact:
Name: Michael Reiland
Title: Budget Officer
Address: 20 M Street, SE
Washington, DC 20003
Phone: (202) 912-7261
Fax: (202) 912-7182
Email: mreiland@blm.gov

Payment Contact:
Name: David Appold
Title: Contracting Officer
Address: BLM Nevada,
Division of Support Services
1340 Financial Blvd., Reno, NV 89502
Phone: (775) 861-6417
Fax: (775) 861-6634
Email: dappold@blm.gov

SERVICING AGENCY (National Park Service, Theodore Roosevelt National Park)

IGO Technical Contact:
Name: Blake McCann, Ph.D.
Title: Chief of Resource Management
Address: Theodore Roosevelt National Park
315 Second Avenue
P.O. Box 7
Medora, ND 58645
Phone: (701) 623-4730 ext. 1433
Email: blake_mccann@nps.gov

Budget Contact:
Name: Kevin Melzo
Title: Administrative Officer
Address: Theodore Roosevelt National Park
315 Second Avenue
P.O. Box 7
Medora, ND 58645
Phone: (701) 623-4730 ext. 1403
Fax: (701) 623-4840
Email: kevin_melzo@nps.gov

Billing Contact:
Name: Kevin Melzo
Title: Administrative Officer
Address: Theodore Roosevelt National Park
315 Second Avenue
P.O. Box 7
Medora, ND 58645
Phone: (701) 623-4730 ext. 1403
Email: kevin_melzo@nps.gov

Attachment 1: Budget Detail for Expenses at NPS Theodore Roosevelt National Park

The field crew staff needed to complete the 2020 observations at Theodore Roosevelt National Park (NPS THRO) are described below. However, only a portion of the total costs of 2020 field work needs to be funded by BLM, because some of the labor costs to support these field staff have already been secured from other funding sources. The total funding amount from BLM that NPS THRO needs to complete this project (personnel plus overhead) is $19,000.

Description of 2020 Field Work Staff Positions

Crew Leader The Crew Leader (3 months @ $14/hr) will be the daily, onsite contact person responsible for planning, training and scheduling daily work assignments for two field research technicians, as well as daily oversight and quality control of data collection. Any Crew Leader that will be supported by NPS THRO would be well-versed and familiar with the objectives, methods of data collection, and logistics of this research project. In addition, the Crew Leader will have knowledge of the park landmarks and terrain, identification of individual experimental horses and location identification of individual experimental horses, and their band associations. The Crew Leader will play a role in the training of two new technicians.

Project Field Research Technicians Two field research technicians (4.5 months each @ $12/hr). The role of these technicians will be daily observations and measurements on ~85 experimental free-ranging mares at NPS THRO during 1 March-1August, 2020. Measurements will include identification of individual mares and determination of presence/absence of a foal, body condition of each mare/foal, evaluation of injection site reactions from GonaCon vaccinations, and band composition. Additional roles will be daily communication and data transfer to crew leader, interactions with NPS THRO staff, and park visitors.

From: Griffin, Paul C
To: McCann, Blake E
Cc: Melzo, Kevin A
Subject: Expect IAA articles for your signature early next week
Date: Friday, January 24, 2020 10:35:19 AM

Hi Blake,

Contracting Officer Dave Appold told me today that you can expect to see the interagency agreement articles for NPS signature early next week. Those will include the changes you suggested, about GS level employees. Specifically, the text on that last page will say:

"**Description of 2020 Field Work Staff Positions**

Crew Leader The Crew Leader (i.e., GS-6) will be the daily, onsite contact person responsible for planning, training and scheduling daily work assignments for two field research technicians, as well as daily oversight and quality control of data collection. Any Crew Leader that will be supported by NPS THRO would be well-versed and familiar with the objectives, methods of data collection, and logistics of this research project. In addition, the Crew Leader will have knowledge of the park landmarks and terrain, identification of individual experimental horses and location identification of individual experimental horses, and their band associations. The Crew Leader will play a role in the training of two new technicians.

Project Field Research Technicians Two field research technicians (i.e., GS-3 or GS-4). The role of these technicians will be daily observations and measurements on ~85 experimental free-ranging mares at NPS THRO during 1 March-1August, 2020. Measurements will include identification of individual mares and determination of presence/absence of a foal, body condition of each mare/foal, evaluation of injection site reactions from GonaCon vaccinations, and band composition. Additional roles will be daily communication and data transfer to crew leader, interactions with NPS THRO staff, and park visitors."

Does that sound OK? Thank you very much,

Paul

From: McCann, Blake E
To: Griffin, Paul C; Melzo, Kevin A
Subject: IAA with BLM for horse reseach
Date: Wednesday, December 18, 2019 1:17:01 PM

Just connecting you for transacting the IAA.

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

THESIS

USE OF FECAL AND SERUM ESTRADIOL ANALYSIS FOR ESTIMATION OF PREGNANCY STATUS IN THE MARE

Submitted by

Kathleen M. Eddy

Department of Biomedical Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, CO

Fall 2020

Master's Committee:

Advisor: Terry M. Nett
Co-Advisor: Douglas C. Eckery

Dan L. Baker
Jason E. Bruemmer
Fiona K. Hollinshead

ABSTRACT

USE OF FECAL AND SERUM ESTRADIOL ANALYSIS
FOR ESTIMATION OF PREGNANCY STATUS IN THE MARE

Overabundant feral horse populations within the United States cause significant and detrimental economic and ecological impacts. Aside from helicopter roundups and long-term holding facilities, current management practices of feral horses include application of contraception in conjunction with non-invasive determination of pregnancy through the measurement of fecal steroid metabolite monitoring. Prior to this study, the earliest timing of definitive pregnancy diagnosis was between 120 – 180 days of gestation, when measuring total unconjugated fecal estrogens (Bamberg et al 1984; Kirkpatrick et al 1989), or from samples taken at least 150 days of gestation when measuring fecal estrone sulfate (Henderson et al 1998 and 1999). The studies in this thesis examined measurement of estradiol 17β, an estrogen that has yet to be quantified in the feces of domestic and feral mares. The overall objectives of the studies in this thesis were to determine the efficacy of fecal and serum estradiol measurement in the estimation of pregnancy in the mare, as well as the definitive timing within gestation when fecal and serum concentrations diverged from those of non-pregnant mares.

The first study of this thesis utilized 8 pregnant domestic mares with known embryo transfer dates, as well as 8 non-pregnant cycling mares. Weekly fecal and blood samples were collected from the pregnant mares for the entirety of gestation, while daily fecal and blood samples were taken from the cycling mares for 23 days. Radioimmunoassay (RIA) specific for estradiol 17β was used to quantify extracted fecal and serum samples for the two groups. It was found that at a mean of 105 days of gestation, fecal estradiol concentrations in pregnant mares surpassed non-pregnant mare concentrations, with a calculated cut-off value of 10 pg/mg feces. Serum estradiol concentrations of

pregnant mares surpassed those of non-pregnant mares at an average of 128 days of gestation, with a concentration of at least 46 pg/mL serum. Additionally, aside from increasing earlier in gestation, compared to serum, fecal estradiol was found to fluctuate less throughout pregnancy.

The second study of this thesis examined 77 fecal and serum samples collected from 51 feral mares during two roundups in Theodore Roosevelt National Park (THRO), as well as 272 individual fecal samples collected over a 6 year period from the same 51 mares. Using the cut-off days and concentrations affiliated with the first study, correlative comparisons were made for the feral mare samples, and pregnancy status was elucidated. Of the 62 fecal samples taken during the roundups past the cut-off day of 105 days, 60 of them surpassed the fecal cut-off concentration of 10 pg/mg feces. Thirty-four of 49 serum samples taken past the cut-off day of 128 surpassed the cut-off concentration of 46 pg/mL. While only two of the 62 fecal samples taken past the cut-off of 105 days were below the cut-off concentration, 14 of the 49 serum samples taken past cut-off day 128 were below the serum cut-off concentration of 46 pg/mL serum. This trend was similar to what was seen in domestic mares.

Although the majority of the field fecal samples were collected in November, there were also the fecal samples from the September and October roundups, as well as a few February samples. While all but 4/131 samples from November were in the estimated 152 -202 day range of gestation, 6/41 in September, and 9/36 in October were below cut-off day 105. In a population similar to THRO, this could potentially result in 14.6% and 25% of concentrations from samples taken in September and October being too low to differentiate between pregnant and non-pregnant individuals. However, when examining the estimated sample distribution range of 101-151 days, 96% of September samples, 91.7% of both October and November samples resulted in concentrations above the cut-off value of 10 pg/mg.

From the studies completed in both domestic and feral mares, it can be said with confidence that the quantification of estradiol 17β using RIA is a reliable method for indicating pregnancy status in

the mare. Mares with fecal estradiol concentrations above 10 pg/mg from samples taken at least 105 days post conception were pregnant, as were mares with measured serum estradiol concentrations above 46 pg/mL collected after 128 days post conception. Additionally, fecal samples taken from feral mares during the non-breeding season in THRO resulted in 96% of samples collected in September, and 91.7% of samples collected in October and November resulting in concentrations above the cut-off concentration of 10 pg/mg feces. This data supports the reliability of fecal estradiol measurement as a tool for pregnancy status determination in the mare.

ACKNOWLEDGEMENTS

I am lucky in that I have such an amazing support group comprised of family, friends, and committee members. While completing my Masters took longer than I ever imagined, the experience strengthened my resolve and solidified my resilience. I would like to thank everyone that played a part in this achievement, with deepest thanks to my committee who continued to work with me throughout my struggles. It is because of you that I am here, and I will be forever grateful for the opportunities you gave me.

I would like to leave this note of acknowledgement with a phrase gifted to me from my father, one that I remind myself of daily: $- = \infty$. A reminder that a single line on a page has the potential to create anything.

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INTRODUCTION

Overabundant feral horse populations within the United States cause significant and detrimental economic and ecologic impacts. The lack of natural predators of the feral horse has resulted in populations increasing as much as 20% a year (National Research Council 2013). In areas with rapidly expanding feral horse numbers comes depletion of resources, which lead to starvation, dehydration, and death (BLM 2017). Additionally, increased herd numbers result in compounded stress on native plant communities, specifically because horses are indiscriminate grazers, causing fewer plant species to remain ungrazed (Beever 2003; Zeigenfaus et al 2014). Reduction of native grasses results in increased growth of non-palatable and invasive plant species such as cheat grass and bitterbrush, lowering the nutritional value of available forage for horses, as well as other species sharing the same range (Beever and Brussard 2000; Davies and Boyd 2019). Feral horses also vie with native wildlife for accessible resources, and because of their large size and aggressive behavior (Berger 1985), they are often able to out compete smaller, native ungulates such as deer, pronghorn, and bighorn sheep from necessary resources such as water (Berger 1985; Coates and Schemnitz 1994).

The Bureau of Land Management (BLM) has been tasked with the management of feral horses on public land, which includes ensuring sufficient grazing capacity for both native and non-native species. To do this, the BLM developed Appropriate Management Levels (AMLs), which have specific Herd Management Areas (HMAs). AMLs reflect the calculated numbers of feral horses allowable within each HMA, while considering ecological factors including native wildlife, recreation, and livestock grazing (Davies and Boyd 2019). As of March 1st, 2020, the overall AML was calculated to be sustainable at 23,851 horses, while the actual estimated horse population was approximately 79,600, an excess of nearly 56,000 individuals (BLM 2020).

Due to the significant overpopulation, the BLM oversees annual roundups, but dependent on overpopulation levels within a state and associated HMA, there are oftentimes more than one yearly gather. These gathers have resulted in the roughly 46,000 horses currently being held in off range facilities, with cumulative management costs expected to exceed $1 billion in the next 20 years (BLM 2017).

Aside from populations managed by the BLM, the National Park Service (NPS) is responsible for overseeing approximately 1000 feral horses across 20 NPS units. Horses within the NPS system fall into a variety of categories, including minimal management of populations not considered to be of cultural importance, up to significant intervention and management of herds considered to be vital to the cultural landscape of the park (Powers 2014). Horses within the NPS system, as well as throughout the BLM, are managed with non-lethal fertility control, such as immunocontraceptive drugs. As managers of feral horse populations make decisions based on pregnancy status of individuals within their populations, knowledge of pregnancy status is vital.

While pregnancy determination in domestic mares is feasible through blood draws measuring reproductive hormones, such as equine chorionic gonadotropin (eCG) and estradiol, bleeding feral horses is not feasible without restraint. Accordingly, many managers have opted for the measurement of fecal steroids. Historically, the most commonly measured of the fecal estrogen metabolites is estrone sulfate, with a variety of studies (Linklater et al 2000; Henderson et al 1998 and 1999) accurately determining pregnancy status in feral mares with samples taken at least 150 days into gestation. Other studies measuring total unconjugated fecal estrogens have determined definitive pregnancy status at approximately 120-180 days of gestation. No studies in the literature have resulted in definitive pregnancy status through measurement of fecal estrogens earlier than 120 days of gestation.

The first study presented in this thesis examines the determination of a reliable cut-off day and estradiol concentration that clearly delineate between pregnant and non-pregnant domestic mares, specific to feces and serum. The second study correlates the cut-off values found in domestic mares to those in feral mares. Feral mare samples measured in this study were fecal and serum samples taken during two roundups, as well as single fecal samples collected over the course of six years.

CHAPTER 1

Review of Literature

History of Feral Horses

The diverse background of the feral horse has led to overabundance within the United States, causing significant detrimental ecological and economic impacts. Truly wild horses, or horses without domesticated ancestry, went extinct in North America at the end of the Pleistocene era between 10,000 and 14,000 years ago (Grayson 2006; Davis and Boyd 2019). In the sixteenth and seventeenth centuries, self-sustaining feral horse herds were introduced by Spanish explorers (Haines 1938), and in the proceeding years, the numbers only continued to rise. This was due in part to increased lifespan resulting from decreases in predation, increased mobility due to lack of confinement, and both accidental and intentional release of domestic horses to the range (Beever 2003). Additionally, as feral horse band structure is generally one stallion with multiple mares, ranchers took advantage of feral herds, killing band stallions and then artificially manipulating blood lines and altering genetics to their liking by releasing domestic stallions into the herds. (Bowling 1994; Hyslop 2017).

Feral herds continued to increase in the United States until the mid-nineteenth century, leading to an estimated population peak of 2-7 million animals (Ryden 1978), followed by a sharp decline throughout the mid-20th century. This was in part due to the Taylor Grazing Act, which initiated grazing districts in which land was apportioned, resulting in feral horse removal, as well as re-domestication (Wagner 1983). By the early 1970's there was an estimated 17,300 feral horses on public lands within the United States (BLM 2020). To protect declining feral horse (and burro) herds, the Wild Free-Roaming Horses and Burro Act (WHBA) was passed in 1971; stating that "wild, free-roaming horses and burros shall be protected from capture, branding, harassment or death; and to accomplish this, they are to be

considered in the area where presently found, as an integral part of the natural system of the public lands." (BLM 2020).

Feral Horse Management

At the signing of the WHBA, the Bureau of Land Management (BLM) was charged with the protection and management of these herds. To ecologically maintain public lands, ensuring sufficient grazing resources for both native and non-native wildlife, the BLM produced annual Appropriate Management Levels (AMLs), and within these, specific Herd Management Areas (HMAs), totaling 26.9 million acres across ten states (Figure 1) (BLM 2020). HMAs are areas where feral horses and burros existed at the signing of the WHBA and have since been designated as areas of continued management by the BLM (BLM 2017). AMLs reflect the calculated numbers of horses and burros allowable within each HMA, while considering ecological factors including native wildlife, recreation, and livestock grazing (Davies and Boyd 2019). As of March 1st, 2020, nearly 50 years after the signing of the WHBA, the overall AML was calculated to be 23,851 feral horses, while the actual estimated horse population was 79,600, an excess of nearly 56,000 individuals (BLM 2020); of the 177 HMAs, nearly 80% of them are over their projected AML (BLM 2020).

With the current equine overpopulation comes a myriad of problems, most of which are ecologically associated with rangeland issues and interactions. Due to a lack of natural predators, most feral horse populations have the capacity to reach mean annual increases of 20% (Nat Research Council of the National Academies 2013). With herd size increases in areas of already strained ecosystems, forage and water resources become depleted, resulting in starvation, dehydration, and death (BLM 2017). Additionally, increased herd numbers result in compounded stress on native plant communities, specifically because horses are indiscriminate grazers, causing fewer plant species to remain un-grazed compared to areas of grazing by other ungulates (Beever 2003; Zeigenfuss et al 2014).

Figure 1. Herd Management Areas (HMAs), Bureau of Land Management. Blue areas are horse HMAs, orange are burro (BLM 2020).

Due to overgrazing, areas with feral horses have reduced plant cover of native plants, as well as increases in non-palatable and invasive plant species such as cheat grass and bitterbrush (Beever and Brussard 2000; BLM 17; Davies and Boyd 2019;). Aside from botanical impacts, feral horses vie with native wildlife for resources, and due to their larger size and oftentimes aggressive behavior (Berger 1985), are able to successfully displace other ungulates such as deer, bighorn sheep and pronghorn away from necessary resources such as water (Berger 1985; Coates and Schemnitz 1994; Gooch et al 2017).

With the intention of decreasing overabundant feral horse herds in a humane manner, the BLM currently manages multiple herds across ten states, each of which has its own affiliated HMA and corresponding AML with calculated capacities. The BLM is allotted an annual monetary appropriation, and in fiscal year (FY) 2019, received $80.6 million, of which $58 million was utilized for off range handling and holding facilities of horses collected via helicopter round-ups, an additional expenditure of $4 million annually (BLM 2020). Depending on the state and HMA, there are oftentimes more than one annual gather, resulting in roughly 46,000 horses currently being held in off range facilities (BLM 2020), with expected cumulative costs to exceed $1 billion over the next 20 years (BLM 2017). Although the BLM attempts to mitigate numbers via horse and burro adoptions to the public, even in years with record-breaking numbers, such as in FY2019 with 7104 adoptions (BLM 2020), the sobering fact remains that thousands of horses and burros remain in long term holding facilities, frequently for life.

While the BLM is tasked with feral horse management on public lands, they do not care for hoses that reside within national parks, which are under the management of the National Park Service (NPS). Upon its' establishment in 1916, the NPS's primary mission has been: "…to conserve the scenery and the natural and historic objects and the wild life therein and to provide for the enjoyment of the same in such manner and by such means as will leave them unimpaired for the enjoyment of future generations." (NPS 2006). Additionally, the NPS management policies remind managers that "All exotic

[non-native] plant and animal species that are not maintained to meet an identified park purpose will be managed – up to and including eradication – if 1) control is prudent and feasible, and 2) the exotic species interferes with natural processes, native species, or natural habitats….." (NPS 2006). As feral horses are considered non-native species by the NPS, it therefore depends on each individual park, and the unique circumstances surrounding each herd as to management protocol.

Feral horses currently reside in approximately 20 NPS units across the United States, and while specific numbers are unknown, estimates are of about 1000 individuals (Powers 2014). These horses fall into one of four management categories; the first of which the animals are residents within the NPS unit, but not specifically maintained as a cultural resource. In this situation, populations have existed prior to the park establishment, and management ranges from eradication, to nothing at all, due to lack of funding (Powers 2014). The second category occurs when animals have trespassed onto park lands, resulting in roundups for removal and adoption, while the third consists of trespass livestock from privately owned property. In this case, determination of ownership is attempted, and if not established, animals are considered abandoned and removed, sometimes through lethal means (Powers 2014). The last category is both the most popular and controversial, as it includes non-native horses and burros that are included as part of the cultural landscape of the park; the herds tend to be small, highly visible, and include many interested stakeholders in the well-being and management of the animals (Powers 2014).

Areas in which horses are maintained as culturally important have made significant research contributions within the field of non-lethal fertility control as a means of population management (Powers 2014). This form of management is ideal for feral equines and could contribute to decreasing the numbers of horses sent to and housed in the BLM long term holding facilities. In addition to the use of immunocontraception, the combined utilization of pregnancy determination through non-invasive means as early as possible in gestation would be helpful in the overall management of feral horse herds.

Management measures such as these would be dependent on reproductive factors such as cyclicity of mares within the population, and relative hormone profiles.

Reproduction in the Mare: Cyclicity

The mare is a seasonally polyestrous animal, undergoing estrous cycles from April through September in the Northern Hemisphere (Nagy et al 2000). Each cycle averages 21-22 days in length, and is divided into a follicular phase (estrus), in which the mare is sexually receptive, and a luteal phase (diestrus), in which she is not. Estrus generally lasts for 4-7 days of the cycle, with diestrus comprising the rest at 10-15 days (Figure 2) (Brinsko et al 2011). This pattern of estrous cyclicity is maintained by a specific hormone balance of hypothalamic, pituitary, and gonadal hormones; the effector of which is gonadotropin releasing hormone, or GnRH.

Responsible for reproductive function, GnRH is a highly conserved decapeptide found in all vertebrate mammalian species (Ginther 1992). Produced by the neurosecretory cells of the hypothalamus, its pulsatile secretion directly impacts the release of gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) from anterior pituitary gonadotropes. The release of GnRH is directly related to photoperiod. During the anovulatory season, hypothalamic GnRH content and secretion is reduced (Bergfelt and Ginther 1991; Peltier et al 1997). The absence of reproductivity in the fall and winter is linked to an inverse relationship between shorter photoperiods and GnRH secretion stemming from inhibitory neuronal interactions of the pineal gland and hypothalamus (Ginther et al 1992; Nagy et al 2000). Although mares have been observed exhibiting estrus-like behavior during the anestrous season, they are incapable of reproduction, as ovulation does not occur (Nagy et al 2000).

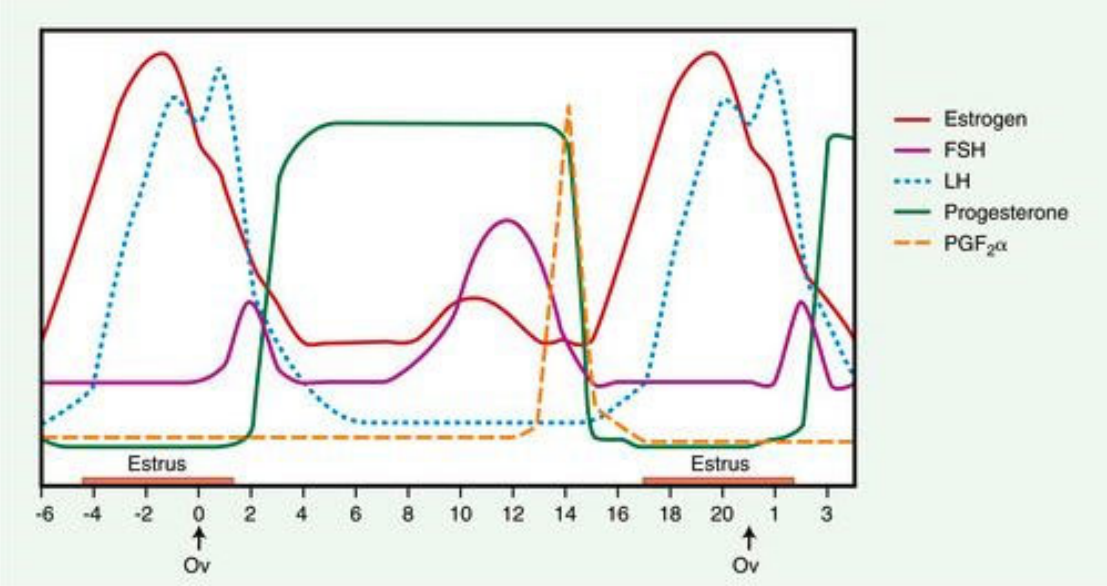


Figure 2. Estrous cycle of the mare. (Brinsko et al 2011)

In the mare, the anovulatory season has been divided into three periods: transition into the fall months, in which the hypothalamic-pituitary- gonadal (hpg) axis activity begins to decline, deep anestrus of the winter months, where there is little hpg axis activity, and transition into the spring, in which increasing day length causes a gradual recrudescence of the hpg axis, allowing for increasing follicular growth and eventual ovulation (Donadeu and Watson 2006). Follicular growth in the mare is mediated via follicular waves; classified as either minor or major, with first emergence of minor waves during the spring transition, producing non-ovulatory follicles (Donadeu and Ginther 2000; Donadeu and Watson 2007; Donadeu and Pederson 2008;). Major waves, occurring towards the end of diestrus, result in the production of the dominant, ovulatory follicle (Donadeu and Pederson 2008).

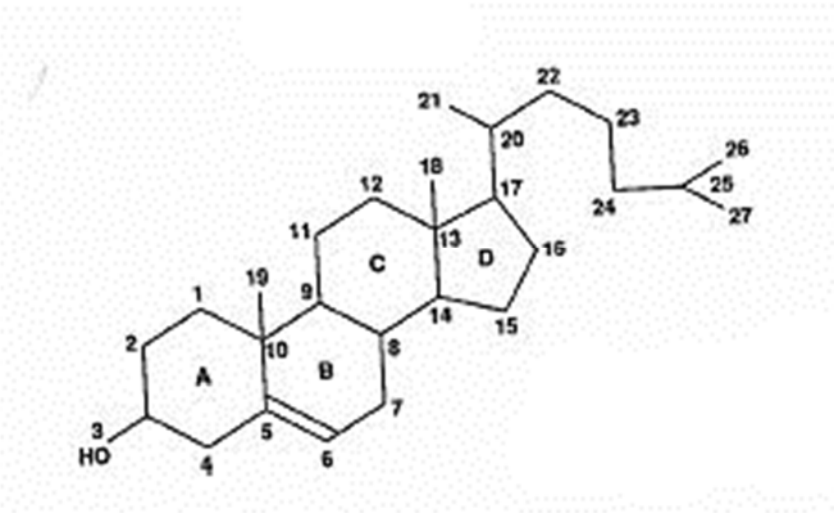
Unlike pre-antral follicles, antral follicles cannot continue maturation without adequate gonadotropin stimulation. The process of follicular growth is mediated by FSH levels and characterized by the periodic growth of cohorts, or follicular waves (Bergfelt and Ginther 1991). Preceded by increasing levels of FSH from the anterior pituitary gland, equine follicular waves involve the near simultaneous growth of follicles at a common rate until deviation, or follicular selection, which occurs when the two largest follicles of the cohort reach approximately 22 mm in diameter (Donadeu and Ginther 2002). While the wave-preceding increase of FSH is important in supplementing follicular growth, the ability of follicles to reach ovulatory diameter is dependent on LH, and without it, they subsequently lose the ability to do so (Ginther 1992). Deviation occurs roughly 7 days prior to ovulation and is indicated by continuous growth of the largest follicles, as well as concurrent increases in LH, inhibin, and estradiol. With the increasing release of inhibin from the dominant follicle, subordinate follicles no longer receive enough FSH to continue to grow, and begin to regress and undergo atresia (Donadeu and Pederson 2008). Once deviation occurs, the dominant follicle continues to grow until reaching an ovulatory diameter of 30-45 mm, after which it will either ovulate or regress (Ginther 1992; Donadeu and Pederson 2008).

Ovulation is dependent on the magnitude of the LH surge, which is regulated by the availability of sufficient levels of estradiol (Donadeu and Pederson 2008). Following ovulation, the formation of the corpus luteum (CL) and resulting progesterone production causes negative feedback on LH at the level of hypothalamus and anterior pituitary. If the mare does not become pregnant, the CL undergoes regression, and initiation of the next follicular phase occurs (Brinsko et al 2011). Knowledge of the equine's seasonality relating to estrous cyclicity is key when determining practical functionality of monitoring hormone metabolites for determination of pregnancy status in the mare.

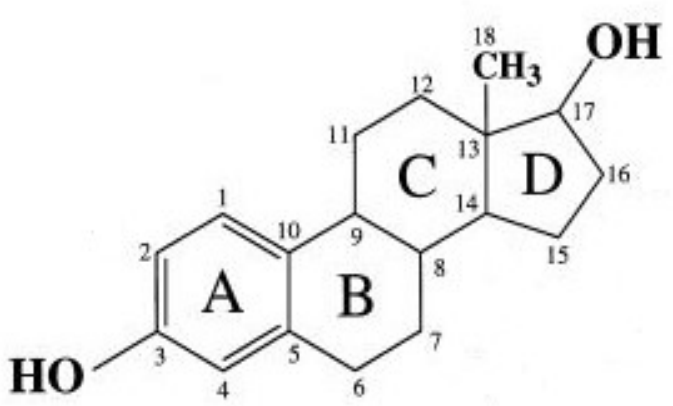
Measurement of Hormone Metabolites

Monitoring of hormone metabolites was originally developed in zoos because of the need to obtain biological samples without incurring capture and restraint stress (Lasley et al 1991). Initial work was focused on method development in monomorphic avians such as parrots. Sex and breeding potential were determined through measurement of unconjugated excrement steroids, specifically the ratio of estrogens and androgens (Lasley et al 1991).

The production of estradiol is ubiquitous among species. As shown in Figure 3, as a derivative of cholesterol, it has the same basic sterol structure of four rings (A-D), differing in the attached groups and double-bond placement (Ginther 1992). During estrogen metabolism, both estradiol and its metabolite estrone can undergo conjugation to either sulfates or glucuronides, the two most abundant circulating estrogen conjugates (Raftogianis et al 2000), a process which occurs in the liver of mammals (Kirkpatrick et al 1989). While estrogens can be measured in the urine, feces or blood in unconjugated or conjugated form, most early mammalian work focused on urinary concentrations of estrone conjugates, including estrone sulfates and glucuronides, which were measured to characterize estrous cycles, reproductive seasonality, and breeding potential in a variety of captive species (Lasley et al 1991).



A. Steric structure of cholesterol (adapted from Ginther 1992)



B. Steric structure of estradiol (adapted from Zhu et al 1998)

Figure 3. Steric structures of cholesterol and estradiol

Species included in early work were okapi (*Okapi johnstoni*), giraffe (*Girrafa Camelopardalis*), Indian rhinoceros (*Rhinoceros unicornis*), Asian elephant (*Elephas maximus*), lion-tailed macaque (*Macaca silenus*), and ruffed lemur (*Lemur variegatus*) (Lasley et al 1991).

As pregnancy detection is also vital in zoo-animal management, steroid metabolite monitoring for this purpose began to emerge. In many species, the feto-placental unit produces large quantities of estrogen, with marked increases at species specific points of gestation (Lasley et al 1991). Therefore, accurate pregnancy determination has been accomplished using urinary estrone sulfate measurement in gorilla (*Gorilla gorilla*), tapirs (*Tapirus terrestris and T.indicus*), and Hartmann's zebra (*E.zebra*) (Lasley et al 1991). Although measurement of urinary estrogens have been effective, collection of urine is not always feasible, and fecal steroid metabolites have also been measured in zoo animals and captive non-equine ungulate herds. Pregnancy has been successfully diagnosed measuring total fecal estrogens in red buffalo (*Syncerus caffer nanus*), yak (*Bos mutus*), Nubian ibex (*Capra ibex nubiana*) and hippo (*Hippopotamus amphibius*) (Lasley et al 1991). Additionally, pregnancy status in the pigtailed macaque (*M. nemestrina*) and Grevy's zebra (*Equus grevyi*) has been successfully determined through measurement of fecal estradiol (Wasser et al 1988; Asa et al 2001).

In addition to monitoring of estrogen metabolites, pregnancy diagnosis via progesterone has been utilized, albeit to a somewhat lesser degree due to fluctuation in concentrations. A study measuring progesterone from the feces of captive giraffes at Busch Gardens found that concentrations in non-pregnant giraffes ranged significantly (3,420 ± 5290 ng/ml) in 41 samples from seven individuals. Extreme variability existed not only between individuals, but within samples from the same individual taken only a few days apart (Dumonceaux 2006). Another study in captive pregnant Red Brocket deer found significant variability in fecal progesterone concentrations throughout gestation (Krepschi 1995). Additionally, a study measuring fecal progesterone in Grevy's zebra mares found that concentrations

were quite variable throughout gestation, declining to or near levels seen during the estrous cycle at 1-2-week intervals, then increasing again (Asa et al 2001).

Pregnancy Determination and Management in the Mare

Pregnancy in the domestic mare can be determined by blood draw as early as 35 to 42 days, when equine chorionic gonadotropin (eCG) reaches detectable levels prior to declining to undetectable levels at approximately 100 days (Brinsko et al 2011). As bleeding feral mares is not possible without significant restraint, other means of pregnancy diagnosis have been examined.

Aside from the obvious necessity to obtain samples for pregnancy analysis in a non-invasive manner, the end goals in feral horse management differ significantly from those in place for domestic horses. For example, most domestic horse owners have at least a rough idea as to when breeding occurred, as well as the ability to handle their horses should the need arise for samples to be obtained. Conversely, as many feral horse herds are unused to prolonged human interaction, handling is not a realistic option. Additionally, unless closely monitored, breeding dates may be completely unknown.

As many managers of feral herds are interested in either decreasing or maintaining current populations, reproductive management related to measurement of pregnancy hormones should focus on hormones that both increase early in gestation, but also definitively differentiate from non-pregnant hormone concentrations as early as possible in pregnancy. Knowing the pregnancy status of the mares within their herds allows managers to better design their course of management practices season to season.

Since the initiation of measuring steroid metabolites, research has been completed in domestic and feral mares regarding determination of pregnancy. Of these studies, the majority examine estrogen, while a few examine progesterone. Although measurable in both the blood and feces of the pregnant mare, progesterone concentrations have been found to vacillate throughout gestation, making it an

unreliable indicator of gestation. In their study of saddle domestic mares, Holtan and colleagues (1975) found that peripheral plasma progesterone concentrations fluctuated widely throughout gestation (Figure 4). Although progesterone significantly increased at approximately day 28, reaching maximum values on day 64, it began a precipitous decline at 120 days to levels less than those seen in post-partum mares (Holtan et al 1975). While progesterone did increase in the last 30 days of gestation, measured concentrations did not exceed those observed during the estrous cycle (Holtan et al 1975). Therefore, progesterone would not be suitable for use to distinguish pregnancy status after 120 days of gestation, and especially unsuitable if the gestation timing was completely unknown, as is often the case in feral mares. Another study examining fecal progesterone in Lippizzan, Trotter and Thoroughbred mares found that although progesterone levels began to increase in the last three months of gestation, maximal levels weren't reached until approximately a month prior to parturition (Schwarzenberger 1991). If sampling in a feral population, it would be challenging to obtain a single sample from which pregnancy could be definitively diagnosed, unless taken during the last month of gestation. As managers of feral herds need to know pregnancy status as early as possible, waiting until the last 30 days of an approximately 345 day gestational period is not ideal.

In the pregnant mare, the estrogens found in increasing concentrations are estrone, estrone sulfate, estradiol, equilin, and equilinen (Bamberg et al 1984). Although equilin and equilinen are found in both the blood and urine in measurable amounts, they are sterically very similar, making them difficult to differentiate between (Bhavnani 1988). Regarding the other estrogens, there have been several studies examining pregnancy status in domestic and feral mares; utilizing urine, feces or blood. While measurement in urine is possible in feral mares (Kirkpatrick et al 1988; Henderson et al 1999), it's unpractical and time-consuming, requiring extraction of urine from the soil.

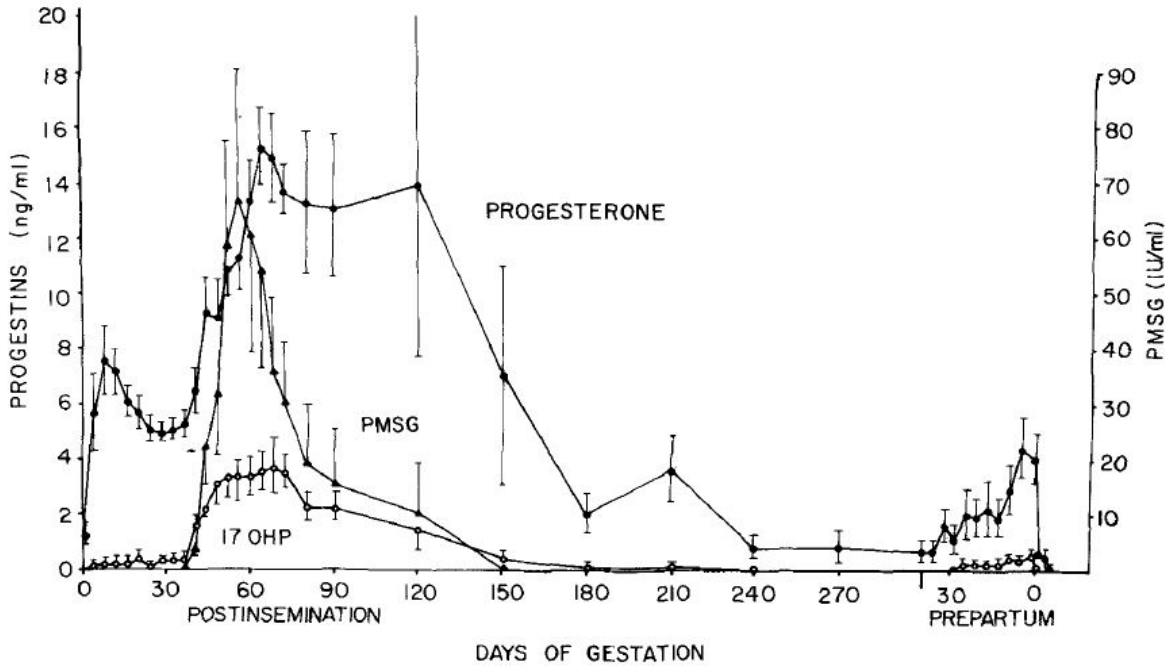


Figure 4. Plasma progesterone through gestation in the domestic mare (adapted from Holtan et al 1975)

Of the equine studies measuring fecal estrogens, the most widely measured metabolite has been estrone sulfate, which is also the most abundant in circulation (Raftogianis et al 2000). In an extensive study using 116 nonpregnant domestic mares and 39 pregnant mares, Henderson and colleagues found that of the pregnant mares sampled at least 150 days post-breeding, 93% of the collected fecal samples resulted in estrone sulfate concentrations of >80 ng/g. None of the fecal samples from non-pregnant mares returned values greater than 80 ng/g feces, with only five non-pregnant mare samples above 65 ng/g for fecal estrone sulfate (Henderson et al 1999). This study found that pregnant mares sampled at least 150 days post-mating and with fecal estrone sulfate values > 80 ng/g of fecal estrone sulfate were unequivocally pregnant (Henderson et al 1999). The following year, a similar study measuring estrone sulfate in feral mares found that while the first 100 days of gestation resulted in concentrations similar to nonpregnant mares, samples taken from mares at approximately 150 – 200 days of gestation resulted in values of >100 ng/g feces (Linklater et al 2000). Non-pregnant feral mare fecal estrone sulfate values in this study were consistently less than 57 ng/g of fecal estrone sulfate (Linklater et al 2000).

Fecal estrone sulfate is not the only estrogen that has been examined in the mare; a few studies have measured pregnancy status using total fecal unconjugated estrogens. During a feral mare study, Kirkpatrick and colleagues determined that fecal samples obtained between days 120 – 180 post-breeding resulted in mean values of 3.18 ± 0.70 ng/g of total unconjugated fecal estrogens in pregnant mares, compared to 0.552 ± 0.08 ng/g of total unconjugated fecal estrogens in non-pregnant mares (Kirkpatrick et al 1989). These results were markedly different from those found by Bamberg, in which pregnant mare concentrations ranged between 100 – 300 ng/g of total unconjugated fecal estrogens if sampled at least 120 days after breeding (Bamberg et al 1984). The mean values for nonpregnant mares in this study were 4.1± 3.4 ng/g of total unconjugated estrogens (Bamberg et al 1984).

Although it is not feasible to measure estrogens in the blood of feral mares, it is in domestic mares. Researchers measured plasma concentrations of E_1 (estrone, equilin, equilinen) and E_2 (estradiol 17β and estradiol 17α). Concentrations of E_1 significantly increased 90 days post-insemination, with means of 43 pg/mL E_1, while E_2 concentrations significantly increased at approximately 150 days, with means of 34.6 pg/mL E_2 (Nett et al 1973). Both concentrations continued to rise early in gestation, with E_1 peaking at 210 days post-insemination, and E_2 at 240 days (Nett et al 1973). Both E_1 and E_2 dropped following their respective maximum values, decreasing to concentrations seen in post-partum mares by the end of gestation (Figure 5) (Nett et al 1973).

While there have been no fecal estradiol studies specific to the equine, there has been one completed in the Grevy's Zebra (*Equus grevyi*) (Asa et al 2001). Although the gestational length of 390 to 406 days is longer than that of horses, the pattern of fecal estradiol during pregnancy was similar to that of reported for serum estradiol in domestic horses (Terqui and Palmer 1979; Henderson et al 1998), with highest levels occurring mid gestation then declining prior to parturition (Asa et al 2001). The study used 3 zebra mares, with a mean gestational length of 372 days. Fecal estradiol levels began to rise at a mean of 88 days into gestation; significantly increasing at approximately day 120 over cyclic patterns (Asa et al 2001). Although this study was completed in equids with longer gestational periods than equine, fecal estradiol concentrations still increased significantly at a timepoint similar to increases seen in equine mares.

Radioimmunoassay

RIA was initially developed to measure the distribution and clearance of insulin in diabetic and non-diabetic individuals (Yarlow and Benson 1959). Following its initial use, RIA was primarily utilized for measurement of peptide hormones, but by the late 1960's was being applied to other disciplines, including toxicology, oncology, and infectious disease (Patrono and Peskar 1987).

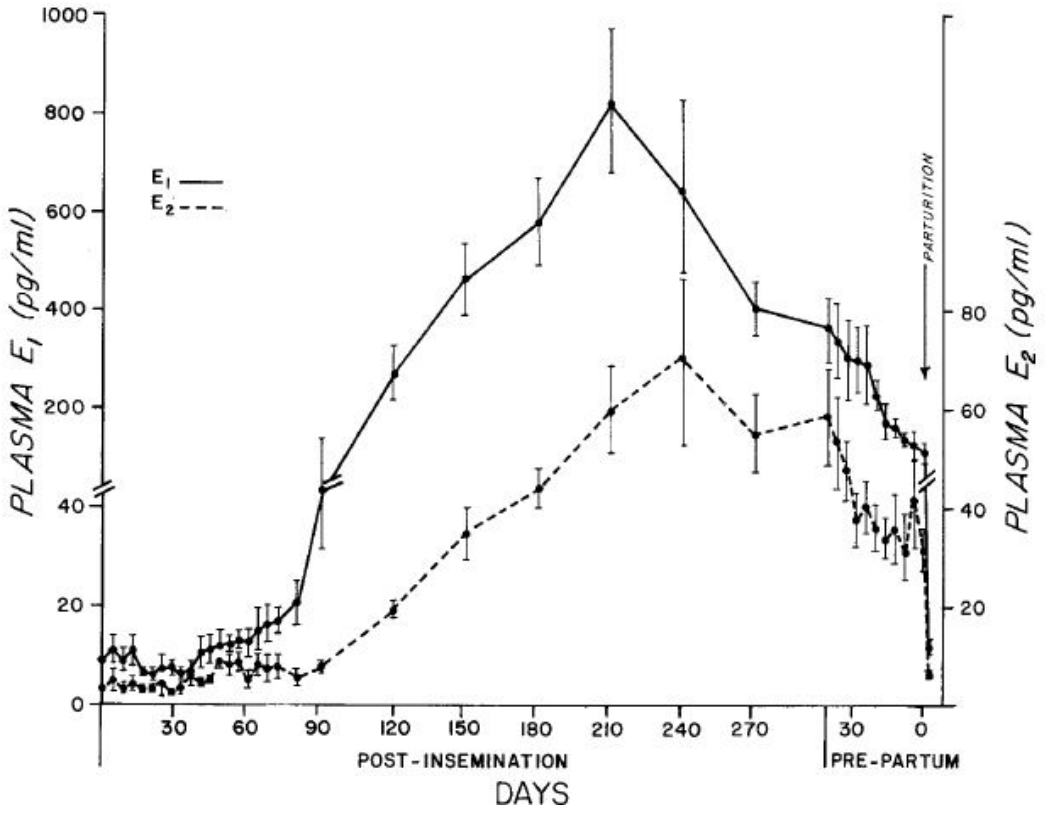


Figure 5. Plasma E_1 (estrone, equilin, equilinen) and E_2 (estradiol 17β and estradiol 17α) in pregnant mares throughout gestation (Nett et al 1973).

RIA is an immunochemical method for quantifying substances through competitive binding of ligands to antibodies of high affinity. Quantification is based on the ability of the endogenous (non-radioactive) ligand of a sample to compete in binding antibody against a radioactive form of the ligand (Nett and Malvey 1999). Prior to achieving quantification of non-radioactive ligand in a sample, the percentage of radioactive ligand bound to primary antibody, in the absence of competition, is measured. This is known as the B_0 (Nett and Malvey 1999). The B_0 is important because it dictates the basis for the calculation of the non-radioactive ligand, and B_0's with primary antibody dilutions that result in 30-50% of radioactive ligand bound to primary antibody are generally chosen, as illustrated in Figure 6 (Nett and Malvey 1999).

The percentage of antibody-bound radioactive ligand directly correlates to the ability for the non-radioactive ligand to competitively bind antibody when added to the mixture. Quantification of non-radioactive ligand is achieved through competitive binding with radioactive ligand for antibody binding sites, with both antibody dilution and radioactive ligand held constant. If a B_0 with primary antibody dilution is chosen that results in <50% binding of radioactive ligand to antibody, then more non-radioactive ligand will need to be added to ensure competitive binding and inhibition of the radioactive ligand (Nett and Malvey 1999). While less radioactive material bound to antibody results in assays with higher sensitivity, as more non-radioactive ligand is bound, there still needs to be sufficient bound radioactive ligand for the assay to be accurate. An assay that does not contain enough radioactive material results in fewer counts, more error, and more variability.

As quantification of RIA is achieved through measurement of ligand bound to antibody, it is imperative that the antibody used is specific to the ligand of interest. To produce ligand-specific antisera, the ligand needs to be capable of eliciting an immune response, the essential features of which include size larger than 3000 Daltons, rigidness, chemical complexity, and foreignness (Tizard 1982).

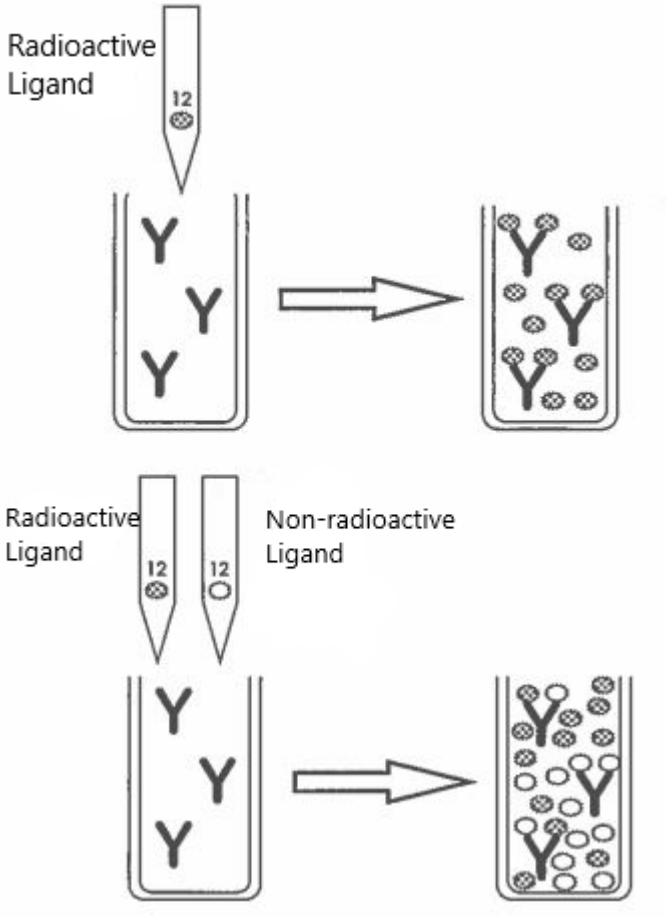


Figure 6. Antibody and radioactive ligand are initially combined in concentrations such that approximately 50% of the radioactive ligand is bound to antibody. If nonradioactive ligand is added to the mixture, and radioactive ligand and antibody concentrations held constant, the resulting binding of radioactive ligand to antibody is reduced due to competitive binding by the non-radioactive ligand (Nett and Malvey 1999).

Some ligands do not meet that criteria on their own, so must first be altered via attachment to a larger molecule, or carrier, such as bovine serum albumin (BSA), after which they are capable of antibody production, making them antigenic (Tizard 1982; Nett and Malvey 1999). Once attached to a carrier molecule, ligands are referred to as haptens. When conjugated to carriers, haptens can be mixed with adjuvant to further stimulate the immune response and injected into a host. While rabbits are often used as hosts due to both their handleable size and ability to produce relatively large amounts of antiserum with limited amounts of ligand used, other commonly used animals include sheep and goats (Nett and Malvey 1999; Leenaars and Hendriksen 2005). Immunogenic injections into a host causes the immune system to produce antibodies, or immunoglobulins, specific to the injection, over the course of several weeks (Nett and Malvey 1999). The time course of the immunogenic response varies, although administration of a booster injection timed with decreasing systemic blood antibody concentration can result in an extreme response of increased antibody titers (Nett and Malvey 1999).

The quantitative ability of an RIA to successfully measure ligands of biological samples is based assay sensitivity, which is defined as the lowest concentration of non-radioactive ligand that the assay is able to measure (Midgley et al 1969). The sensitivity of an RIA is indicated by the point of interception on the x-axis by a 95% confidence interval calculated from the B_0 (Nett and Malvey 1999).

RIA sensitivity is also linked to the type of radioisotope used, and ^{125}iodine (^{125}I) is a common choice. It can interact with and attach to a hapten linked to tyrosine methyl ester (TME), a derivative of tyrosine (Nett and Malvey 1999). Although previous equine studies have utilized ^{3}H estrogens to measure fecal estrogens (Bamberg et al 1984; Mostl et al 1984), there is a compelling reason to use ^{125}I estrogens. Radioiodinated haptens have a much higher specific activity than ^{3}H haptens and undergo disintegrations 75 times more rapidly than ^{3}H, resulting in the necessity of 75 times as many ^{3}H molecules to generate the same levels of radioactivity as ^{125}I (Nett and Malvey 1999). Therefore, significantly less mass of radioiodinated haptens are required to release a defined amount of

radioactivity, as compared to ^{3}H hapten mass (Nett and Malvey 1999). Additionally, as the sensitivity of a RIA is relative to the measurable amount of radioactive disintegrations from radiolabeled ligand bound to antibody, using ^{125}I labeled haptens results in assays two or three orders of magnitude more sensitive than those using ^{3}H labeled haptens (Nett and Malvey 1999).

To quantify ligand bound to antibody, the radioactive form of a ligand is used, with the resulting specificity of the assay directly correlated to the purity of the radioactive ligand (Nett and Malvey 1999). This necessity is related to the antiserum produced by the host. While it contains the antibodies it was produced for, it also retains others relative to any other foreign substance to which the host had been previously exposed, which can lead to cross-reactivity of the antiserum to different analytes that are structurally similar. If a nonpure radioactive ligand contains proteins to which the host has antibodies, they will bind, resulting in interference of quantification to the ligand of interest (Nett and Malvey 1999). To ensure both the purity of the radioactive ligand, as well the RIA specificity, it is crucial to confirm that other components within the measured sample do not interfere with the radioactive ligand, and is generally considered to be the most important indicator in assay reliability. If a radioactive ligand is pure, anything that inhibits its binding with antibody is most likely to either be an identical or closely related substance to the ligand (Nett and Malvey 1999).

In their study examining plasma estrogens in domestic mares, Nett and colleagues developed an RIA with specificity and sensitivity to estrone and estradiol 17β (Nett et al 1973). They utilized column chromatography to separate the estrogens, with one fraction containing estrone, equilin, and equilinen (E_1 fraction), and the other containing estradiol (E_2 fraction) (Nett et al 1973). They determined that the antiserum used for the RIA did not cross-react with non-estrogenic steroids, and had limited cross reactivity with other estrogen metabolites, which were differentiated via inhibition curves (Nett et al 1973). Inhibition curves compare a standard of a substance of interest to other similar samples, and parallelism of all curves is indicative that substances other than the one of interest are not causing

antibody binding interference, and are not interacting with the antiserum used (Nett and Malvey 1999). Pooled E_1 (estrone-equilin-equilinen) and E_2 (estradiol 17β and α) fractions were parallel to those obtained with estrone and estradiol 17β standards (Nett et al 1973), indicating a lack of binding inhibition, and an RIA with high specificity. These assays also resulted in sensitivities of 4 pg/tube for both estrone and estradiol 17β (Nett et al 1973).

The last points of interest to be considered for reliable RIA are precision and accuracy. Precision is the amount of variation noted in the estimated non-radioactive ligand (Midgley et al 1969). While variances within an assay are affected by pipetting error relative to assay preparation, another effect is the counting error produced by the gamma counter used. This error is calculated by taking the square root of the total radioactive counts, divided by the total counts (Nett and Malvey 1999). The counting error can then be used to establish estimates of variation both within an assay, and between two identical assays, cumulatively known as coefficients of variation, which should be less than 20% (Nett and Malvey 1999). Assay accuracy can be defined as the mean of an infinite number of measurements of a material, and how they align with the exact amount of the material present in a sample (Midgley et al 1969). An optimal method for examining RIA accuracy is to compare results obtained from several RIAs with a variety of preparations, such as similar antibodies (Midgley et al 1969). If relative assay results agree, it is likely that the same substance is being measured across assays. Another option is through weighing of a known amount of the ligand, and then adding varying amounts to a biological fluid of the same species that is lacking the ligand; for example, adding varying concentrations of estradiol to the serum of an ovariectomized mare. The concentration of ligand in the serum is then determined by RIA, and correlation graphed between ligand added (x-axis) and amount measured (y-axis). If the result is a line with a slope of 1, the two amounts are equal (Nett and Malvey 1999).

The final consideration to be made in RIA is how best to separate free ligand from bound, so that assay quantification can be completed. While there are a variety of techniques, the most common

method utilized is termed the ‘double-antibody procedure’, and requires that of a second antibody, generated against immunoglobulins specific to the species in which the primary antibody was produced (Nett and Malvey 1999). Secondary antibody is added to the mixture after the reaction of primary antibody and ligand has completed. The secondary antibodies then bind to the primary antibody and ligand, causing immunoprecipitation of the ligand/antibody complex from the solution. (Nett and Malvey 1999).

CHAPTER 2

Measurement of Fecal and Serum Estradiol in the Domestic Mare

Introduction

Measurement of estrogen and estrogen metabolites for pregnancy determination was originally developed in zoos as a means of accurate pregnancy diagnosis without the physical stressors of restraint (Lasley et al 1991). Since their initial use, they have also been utilized in non-exotic species, including both domestic and feral mares. There have been numerous equine specific studies that have examined fecal estrogen and metabolites as a means of pregnancy diagnosis, as well as definitive timing for optimal sample collection. Those examining total fecal unconjugated estrogens have determined pregnancy from samples collected between 120-180 days (Bamberg et al 1984; Kirkpatrick et al 1989), while those measuring fecal estrone sulfate report fecal concentrations relative to definitive diagnosis from samples taken at 150 days of gestation and beyond (Henderson et al 1998 and 1999; Linklater et al 2000). While there have not yet been any equine studies focusing on fecal estradiol measurement, plasma estradiol concentrations of pregnant mares were found to differentiate from cycling mares at approximately 150 days of gestation (Nett et al 1973).

This study was designed to examine changes in fecal estradiol concentration throughout gestation, using serum estradiol levels as a comparison tool. The objectives of this study were: 1) to determine a reliable cut-off concentration that clearly delineates between pregnant and non-pregnant mares; specific to both feces and serum and 2) determine the day of gestation in which pregnant mare fecal and serum estradiol concentrations surpass non-pregnant concentrations.

Materials and Methods

Collection and Preparation of Feces and Blood

Daily blood and fecal samples were concurrently collected from eight non-pregnant cycling mares, ranging in age from 9 to 11 years. Seven mares were grade, while the eighth was a paint. Table 1 depicts the demographics specific to the cycling mares. While mares were not ultra-sounded to determine day of cycle at sample initiation, the average reported length of an equine estrous cycle is 21-22 days (Brinsko et al 2011), so samples were taken from mares for a total of 23 days, with the exception of mare 443, who had samples taken for 26 days. Feces and serum estradiol concentrations were examined separately, and samples aligned such that the highest concentrations were the first in the sample list.

Blood and feces were also collected concurrently on a weekly basis from 8 pregnant domestic mares with known embryo transfer dates of 7-day embryos. Mares were all grade, and at the time of sampling, ranged in age from 6-16 years. Although blood and feces were taken weekly, the mares were asynchronous in their gestational timing, and initiation of sampling was earlier in gestation in some mares than others, as shown in the second portion of Table 1.

For both groups (non-pregnant and pregnant), fecal samples were obtained via fecal grabs while mares were restrained in standing stocks. Sample sleeves were inverted around sample, and then labeled with mare identification and corresponding date. Directly following fecal collection, jugular blood was drawn using 18-gauge 1.5 inch needles, filling 2 red top vacutainer tubes for each mare at each bleed.

Table 1: Domestic mare sampling demographics including age and breed of the non-pregnant cycling mares and pregnant mares, as well as the day of pregnancy the first sample was taken in pregnant mares, and the day of parturition. Asterisks indicate samples taken the day of parturition.

CYCLING MARES

Mare	Age(years)	Breed
00	11	Grade
24	15	Grade
49	9	Grade
95	10	Grade
124	10	Paint
150	11	Grade
231	11	Grade
443	10	Grade

GESTATIONAL MARES

Mare	Age (years)	Breed	First Sample	Parturition*
15029	9	Grade	d27	d356*
15024	8	Grade	d28	d331
15105	6	Grade	d53	d355*
15099	10	Grade	d54	d351*
15042	13	Grade	d59	d340*
15110	8	Grade	d88	d350*
15004	7	Grade	d89	d342
15049	16	Grade	d119	d361

* sample taken that day

Immediately following collection, labeled fecal samples were placed on ice, with tube racks on top, to be kept cool for transport. Fecal samples were processed within 4 hours of collection, while blood samples were left at room temperature overnight. After sitting overnight at room temperature, each blood sample was centrifuged for 30 minutes at 2400xg, and serum placed into 2.0 mL cryogenic vials for storage at -20°C until extraction.

Extraction

Although extraction procedures differ for feces and serum, both methods utilized quality controls (QCs), which were extracted alongside samples. For this study, the QCs were made from hypophysectomized sheep serum, with varying concentrations of estradiol added (Nett 2005). The concentration of the low QC was 15 pg/mL of estradiol, medium 60 pg/mL, and high 240 pg/mL. Additionally, there was a solvent QC, to be extracted with just diethyl ether (Nett 2005). All QC's were extracted at a volume of 250 µL.

Extraction of feces contained one pre-extraction step, and two extraction steps (Nett 2005). Prior to extraction, each raw chilled fecal sample was placed into a 94x16 petri dish in a thin layer that completely covered the bottom half of the dish. Any extra raw fecal matter was placed into a 50 mL conical vial, labeled with mare information and date, and then frozen at -20°C. Each petri dish of feces had a Kim wipe taped over the top for ventilation during lyopholization. The dishes were then frozen at -20°C for 30 minutes prior to placement into a lyopholizer for 72 hours, until dried completely. Each sample was then hand ground to a fine sand-like consistency and placed into a labeled 50 mL conical vial. Ten milligrams of feces for each sample was placed into 16x150 mm glass tubes and rehydrated with 1 mL of double-deionized water (DDH_2O). Resulting sample slurries were agitated for 1-1.5 hours, followed by centrifugation at 2400xg for 15 minutes. Five hundred microliters of supernatant was removed from each sample for use in the first extraction.

Fecal extractions one and two contained washing steps; for each 500 µL equine sample aliquot in a 16x150 mm tube, two 16x150 mm tubes were labeled with the same sample number, each containing 500 µL of DDH_2O for washing. Each 250 µL QC aliquot also had the same number of tubes prepared. For the second extraction, 5 mL of diethyl ether was added to each 500 µL equine aliquot or 250 µL QC, and then vortexed for 5 minutes. Samples were snap-frozen in a methanol-dry ice bath, and the organic phases poured into the first of the corresponding clean 16x150 mm tubes containing 500 µL DDH_2O for washing. Tubes were then vortexed again for 2 minutes, snap-frozen in a methanol-dry ice bath, and the washed organic phases poured into clean 12x75 mm glass tubes, placed into a heating block, and evaporated under nitrogen (Nett 2005). The second extraction was completed by adding 5 mL of diethyl ether to the original aliquots, and the protocol run again, using the second set of washing tubes during the wash phase (Nett 2005). All 495 domestic mare fecal samples were processed in this manner.

Following the second extraction, the 12x75 mm tubes containing samples and QC's were reconstituted with 0.1% PBS-gel at the volume they were extracted at: 500 µL for the mare samples, and 250µL for the QC's (Nett 2005). They were then vortexed for 2 minutes, incubated at 4°C overnight, vortexed again for 2 minutes and then frozen at -20°C until assay.

Serum required two extractions. From each serum sample, a 500 µL aliquot was placed into a labeled 16x150 mm glass tube, which had a corresponding labeled 12x75 mm labeled glass tube. As with fecal extractions, there were also labeled 16x150 mm and 12x75 mm labeled glass tubes for the QCs to be extracted alongside the equine samples. Five mL of diethyl ether was added to all tubes, which were then vortexed for 2 minutes, and allowed to stand for 5 minutes (Nett 2005). All tubes were then snap-frozen in a methanol-dry ice bath, then organic phases poured into the 12x75 mm tubes, which were placed into a heating block, and evaporated under nitrogen. For the second extraction, the steps from the addition of 5 mL diethyl ether on were replicated, and the same 12x75 mm tubes dried down. All

tubes were then reconstituted with 0.1% PBS-gel at the same extraction volume used, 500 µL for equine samples, and 250 µL for QCs. All tubes were then vortexed for two minutes, incubated overnight at 4°C, vortexed again for 2 minutes and then frozen at -20°C until assayed (Nett 2005). All 500 domestic mare serum samples were extracted using this method.

Assay

An RIA specific for estradiol 17β was utilized for this research, and both primary and secondary antibodies used were created prior to the study. The primary antibody was produced in rabbits from estradiol conjugated to bovine serum albumin (E26BSA), while the secondary antibody was produced in a goat.

All fecal, serum, and QC samples were assayed in duplicate, while 6 standards were assayed in triplicate (Nett 2005) and ranged in concentration from 40 pg to 409.6 fg. The amount of equine sample added to each tube was dependent on status of pregnancy, as well as sample type: feces or serum. Extracted fecal samples from pregnant mares were split at gestational day 100; prior to day 100, 100 µL of extracted sample was added to each tube, after day 100, 10 µL of extracted sample was used. Extracted serum samples collected from pregnant mares were divided at gestation day 130; prior to day 130, 200 µL of sample was added to each tube, after day 130, 50µl was added. For cycling mares, 200 µL of sample/tube was added, for both feces and serum. Eighty microliters of each extracted QC was used for QC tubes.

Tubes were numbered prior to start of RIA, with tubes 1-3 the total count tubes (TC- radioactive ligand), 4-6 Non-specific Binding (NSB) tubes, 7-14 QC's,15-17 Buffer Control (BC-0.1%PBS-gel) tubes, and 18-23 Standards (Nett 2005). Two additional sets of BC and Standard tubes were throughout the protocol, but tube number was specific to individual RIA. Total volume for each tube of sample/QC or standard, plus buffer (PBS-gel 0.1%) was 500 µL (Nett 2005). Five hundred microliters of buffer was

added to NSB tubes and BC tubes. One hundred microliters of iodinated estradiol 17β-6-TME was added to all tubes in the assay, followed by 200µL of 1:400 Normal Rabbit Serum (NRS) to the NSB tubes (Nett 2005). Two hundred microliters of diluted primary antibody (1:400,000 in 1:400 NRS) was added to all tubes except the TC and NSB tubes. All tubes in the assay were vortexed for approximately 5 seconds, then incubated at 4°C for 24 hours (Nett 2005). After 24 hours of incubation, the secondary antibody, 200 µL of diluted secondary antibody (1:00 goat anti rabbit (GAR) in PBS-EDTA), was added to all tubes except TC tubes, vortexed, and returned to the 4°C for additional incubation of 72 hours. Seventy-two hours after adding GAR, 3 mL of cold phosphate buffered saline was added to all tubes except TC. Excluding TC tubes, all others were centrifuged for 30 minutes at 2400xg, and resulting supernatant decanted into waste containers. All tubes, including TC, were then counted on a gamma counter with a counting efficiency of 85% (Nett 2005).

The RIA used had 100% cross reactivity with estradiol 17β, 12% with estrone, 7% with estradiol 17α, 9% to estriol, and 1% with both testosterone and progesterone (Niswender et al 1969). The sensitivity of the RIA was found to be an average of 228 fg/tube with the 50% dose of the theoretical curve at an average of 6 pg/tube of equine estradiol.

Results

Cycling Mares

The concentration of estradiol 17β in 185 fecal samples obtained from 8 cycling mares throughout one estrous cycle ranged from 0.2 – 9 pg/mg of feces, with an overall mean of 1.3± 0.1 (SEM) pg/mg. The cycling mare values were utilized to create a cut-off concentration (COC) delineating between non-pregnant and pregnant mares, which was calculated by taking the highest fecal estradiol concentration of the cycle for each mare and averaging them, resulting in a concentration of 5pg/mg of feces .The value 2 standard deviations (SD) above this mean was calculated to be 10 pg/mg of feces.

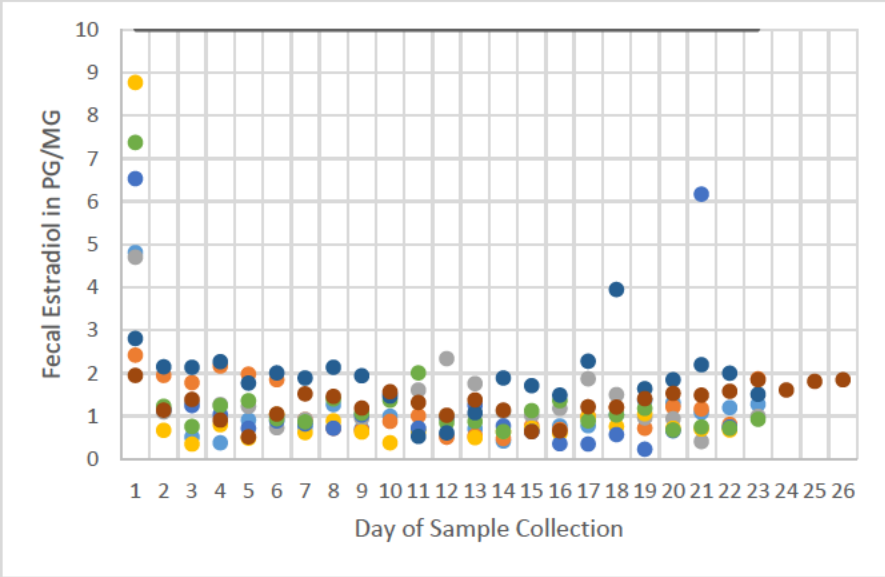
Of the 185 fecal samples collected from cycling mares, none of them were above the calculated COC, which is depicted in Figure 7.

Serum concentration of estradiol 17β in 186 samples obtained from the 8 cycling mares throughout one estrous cycle ranged from 0.6 - 43 pg/mL of serum, with an overall mean of 10 ± 0.6 (SEM) pg/mL. The COC for serum was created using the same methods as for feces; the highest serum concentration for each mare was obtained, and all values averaged, resulting in 27 pg/mL serum. The COC 2 SD of this average was calculated to be 46 pg/mL of serum, which eclipsed all individual sample concentrations for the cycling mares, as shown in Figure 8.

Gestational Mares

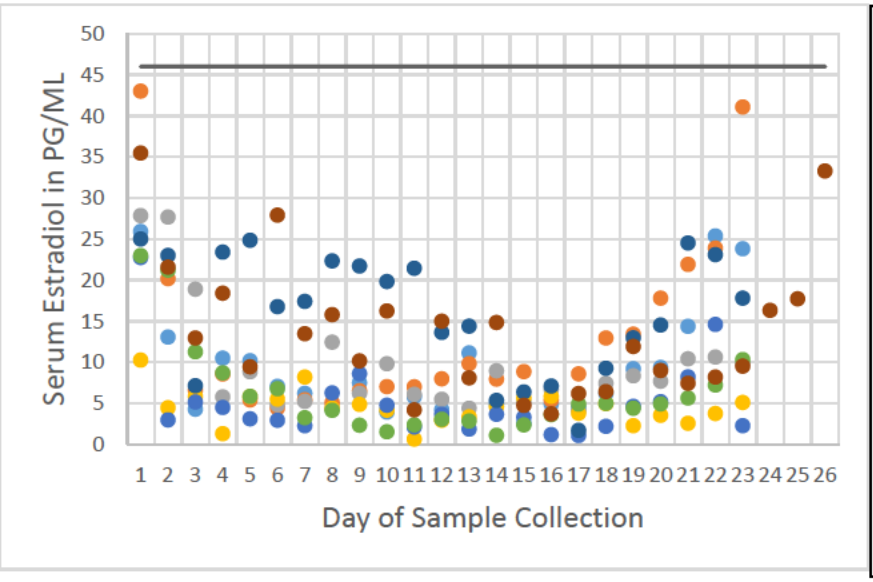
The average gestational length for mares in this study was 348 days. The range in concentrations of fecal estradiol 17β in eight gestational mares was 1 - 134 pg/mg of feces. Concentrations increased early in pregnancy as pregnancy progressed, surpassing the calculated COC of 10 pg/mg feces at an average of 105 days of gestation, before decreasing below the COC directly before parturition, as indicated in both Figure 9A and Table 2.

Of the 310 fecal samples, 43 were collected prior to the cut-off day (COD) of 105 days of gestation. Of those 43, 34 failed to surpass the COC of 10 pg/mg feces, while nine did. Of the remaining 267 samples taken past the COD, all but 9 exceeded the COC. Three of those nine were from samples taken close to the COD, which were day 108 (d108) for mare 15105, d111 for 15110, and d105 for 15099. The remaining 6 were taken close to the day of parturition, with 4 taken the day of parturition for mares 15042, 15029, 15105, and 15110, and two taken from the day prior from mares 15024 and 15105. Figure 9B illustrates the fecal concentrations of the 8 gestational mares 30 days pre-parturition, of which the 6 values that dropped below the COC at the end of gestation can easily be seen.



Cycling Mares	Highest Fecal Concentration (pg/mg)
00	5
24	2
49	5
95	9
124	7
150	7
231	4
443	2
AVG	5
SD	2
Avg +2SD (Cut-Off)	10

Figure 7: Cycling domestic mare fecal estradiol 17β concentrations throughout the estrous cycle, with included cut-off calculation of 10 pg/mg feces. This value is the mean of the highest fecal estradiol concentration of each mare + 2 standard deviations.



Cycling Mares	Highest Serum Concentration (pg/mL)
00	26
24	43
49	28
95	10
124	23
150	23
231	25
443	35
AVG	27
SD	10
Avg+2SD (Cut-Off)	46

Figure 8. Cycling domestic mare serum estradiol 17β concentrations throughout estrous cycle, with calculations for cut-off value of 46 pg/ml of serum included. This value is the mean of the highest serum estradiol concentration of each mare + 2 standard deviations.

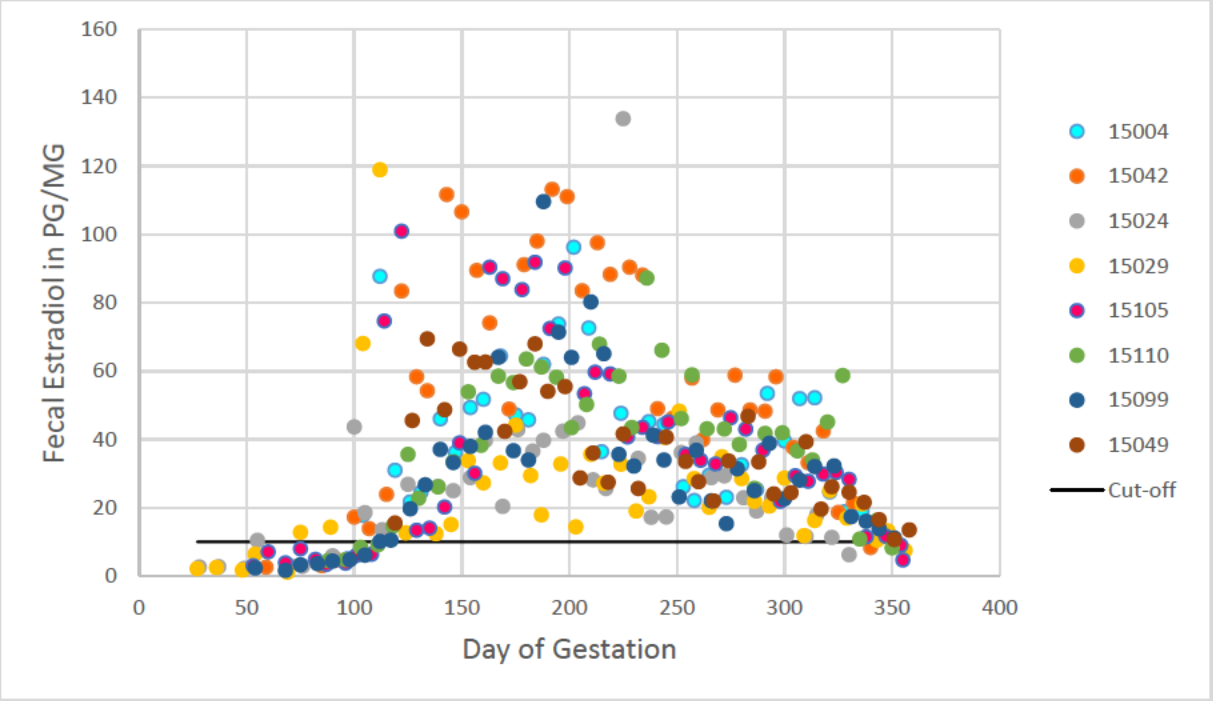


Figure 9A. Weekly fecal estradiol 17β concentrations in pg/mg of feces throughout gestation in 8 domestic mares. Cut-off value is 10 pg/mg of feces and is indicated by black horizontal line.

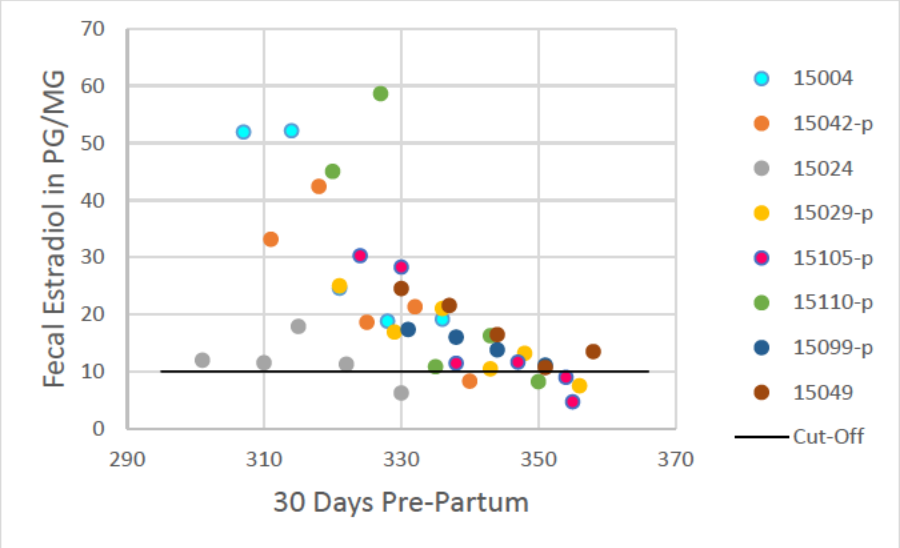


Figure 9B. Fecal estradiol 17β concentrations 30 days prior to parturition in 8 domestic mares. Cut-off value is 10 pg/mg of feces and is indicated by the black line. Mare numbers labeled with a -p indicate samples taken on the day of parturition.

Table 2. The first day of gestation for each of 8 pregnant domestic mares that surpass the calculated cut-off values for feces and serum.

Gestational Mares	Day Surpassed Avg+2SD using highest fecal value: 10 pg/mg	Day Surpassed Avg+2SD using highest serum value: 45.875 pg/mL
15004	104	140
15042	100	134
15024	100	113
15029	75	138
15105	114	129
15110	118	125
15099	112	117
15049	119	127
	Average: 105.25 = 105 days	Average: 127.88 = 128 days

Serum estradiol 17β concentrations ranged from 1.7 – 447 pg/mL, with concentrations exceeding the calculated COC of 46 pg/mL serum at an average of 128 days (Figure 10A and Table 2). Of the 317 serum samples, 65 were collected before the COD of 128 days, with 5 samples surpassing the COC of 45.87 pg/mL serum. Of the remaining 252 serum samples collected after the COD, 13 of them failed to surpass the COC. Two of those 13 were taken at gestation days 131 (mare 15004) and 129 (mare 15042), both of which were relatively early in gestation. Mare 15004 had six mid gestation samples between days 154 and 321 that dropped below the COC, while the remaining 5 were from 4 mares. Two were from the same mare, 15042, with one eight days prior to parturition, and the other the day of parturition. The remaining three, from mares 15024, 15029 and 15110 were taken the day of parturition. Figure 10B illustrates the serum estradiol concentrations from the 8 gestational mares 30 days pre-partum.

Discussion

The results of this study indicated that measurement of estradiol 17β through the use of RIA is a reliable method for pregnancy determination in the domestic mare. Of the collected fecal samples that exceeded the COD of 105 days of gestation, 96.6% of them remained above the COC of 10 pg/mg for the entirety of gestation. Of the serum samples collected after COD of 128 of gestation, 94.8% of them returned values above the COC of 46 pg/mL serum throughout gestation.

When computing the values that would delineate between non-pregnant or pregnant mares (i.e. the cut-offs), calculations were made relative to methods used in the literature. Referenced studies (Bamberg et al 1984; Henderson et al 1998 and 1999; Linklater et al 2000), utilized the overall mean from non-pregnant mares + 3 standard deviations (SD). Instead of using the overall mean of non-pregnant mares in this study, the highest concentration from each non-pregnant mare's cycle was averaged, and 2 SD's added. This decision was contingent on a few factors.

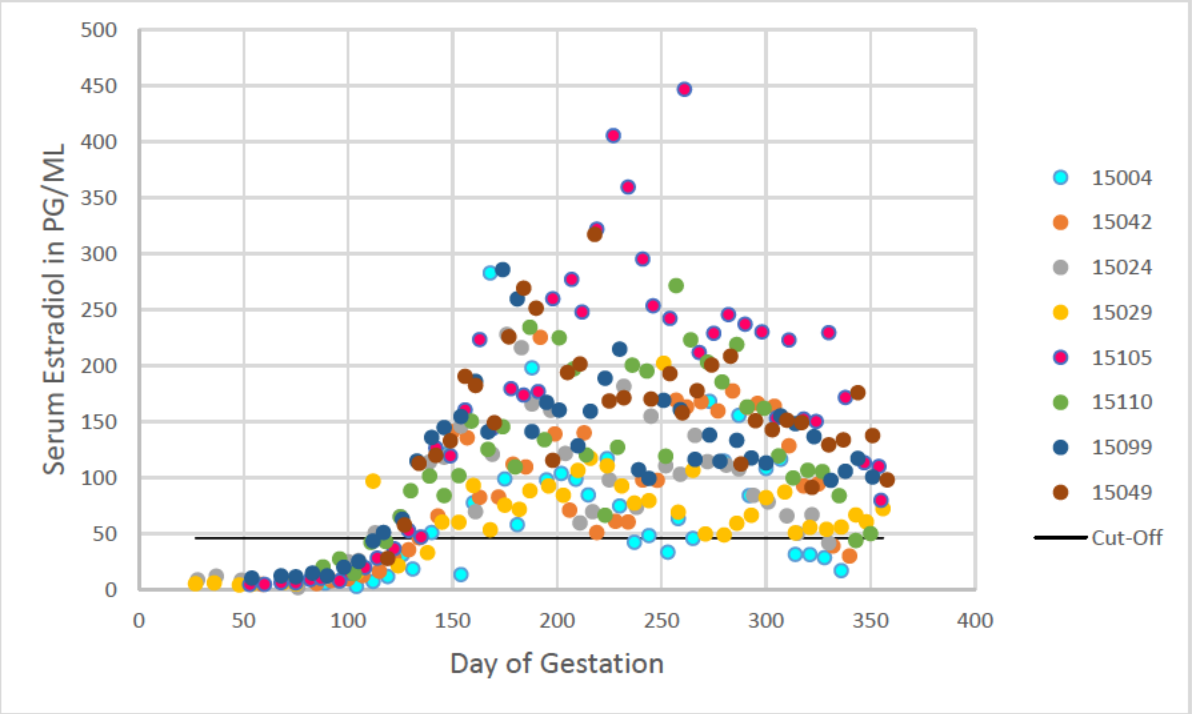


Figure 10A. Weekly serum estradiol 17β concentrations in pg/mL of serum throughout gestation in 8 domestic mares. The cut-off value is 46 pg/mL of serum and is indicated by black horizontal line.

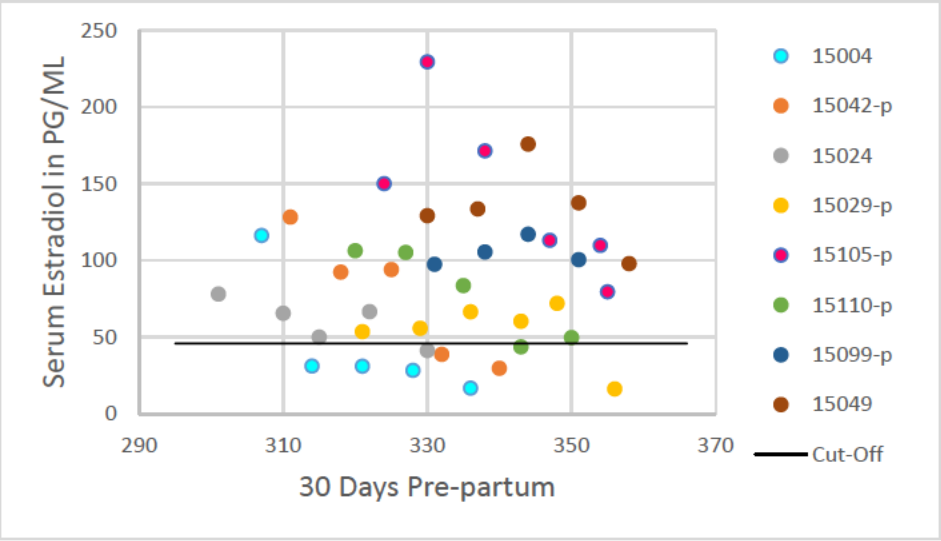


Figure 10B. Serum estradiol 17β concentrations 30 days prior to parturition in 8 domestic mares, with the 46 pg/mL cut-off indicated by black line. Mare numbers labeled with a -p indicate samples taken on the day of parturition.

The overall means of the non-pregnant mare values for both feces and serum were much lower than that of the mean from the highest concentrations (Table 3), resulting in COC of 2 pg/mg feces (mean +3SD), and 23 pg/mL serum (mean +3SD). These values were lower than some of the individual sample concentrations, and therefore not suitable for use in determination of pregnancy.

Although both of the calculated COC's using 3SD above the mean (12 pg/mg feces and 56 pg/mL serum; Table 3) surpassed all individual sample concentrations, they were quite conservative, having been calculated from the average of the highest individual concentrations of feces and serum from each mare. This would mean that determination of pregnancy relative to COD would be later in gestation, which wouldn't be ideal for feral horse managers, whose needs include the earliest possible detection of pregnancy for population management decisions. Therefore, calculations were made utilizing 2SD above the mean of the highest concentrations, which resulted in the COC's of 10 pg/mg of feces and 46 pg/mL of serum. While these concentrations surpassed all of the individual mare samples, they also had the advantage of earlier pregnancy detection and COD, compared to the more conservative calculations.

Of the 310 fecal samples collected from pregnant mares, 267 of them were obtained past COD 105. Of those 267, 258 surpassed the COC (96.6%), while nine of them did not (3.4%). Of the 9, three were near the COD: d108 (mare 15105), d111 (mare 15110) and d105 (mare 15099). While the following samples collected for each of these mares resulted in the definitive day of pregnancy determination based on the COC, a certain amount of variation is expected for timing of increases in fecal estradiol concentration. This is because the COD of 105 days was the average calculated from all of the individual mare values, as seen in Table 2. Additionally, from the 43 samples collected prior to COD 105, nine of them surpassed the COC, which again speaks to the calculation of the COD. Therefore, the failure of the 3 samples taken so closely after the COD to surpass the COC of 10 pg/mg of feces is not overly alarming.

Table 3. Calculation of cut-off values for feces and serum from domestic mares. Boxed values in the left panel were the ones chosen for use.

Cycling Mares	Highest Fecal Concentration
00	5
24	2
49	5
95	9
124	7
150	7
231	4
443	2
AVG	5
SD	2
Avg +3SD	12
Avg +2SD	**10**

Cycling Mares	Average Fecal Concentration
00	1
24	1
49	1
95	1
124	1
150	1
231	2
443	1
AVG	1
SD	0.3
Avg +3SD	2
Avg +2SD	2

Cycling Mares	Highest Serum Concentration
00	26
24	43
49	28
95	10
124	23
150	23
231	25
443	35
AVG	27
SD	10
Avg+3SD	56
Avg+2SD	**46**

Cycling Mares	Average Serum Concentration
00	10
24	13
49	10
95	5
124	5
150	6
231	16
443	14
AVG	10
SD	4
Avg +3SD	23
Avg +2SD	18

The remaining 6 samples taken after COD 105 that failed to surpass the COC were all from either the day of or directly prior to parturition. As it is known that plasma estradiol concentrations decrease during the last 30 days of gestation (Nett et al 1973), it would be expected that fecal estradiol concentrations would as well.

The resulting percentage of fecal samples that were over COC (96.6%) were similar to outcomes from previous studies examining fecal estrone sulfate, which resulted in 93% and 97.3% fecal concentrations above calculated cut-offs (Henderson et al 1998 and 1999).

Of the 317 serum samples taken from pregnant mares in the study, 252 were collected after the COD of 128 days, with 239 (94.8%) of them surpassing the COC of 46 pg/mL of serum. Of the 13 that failed to surpass the COC, two of them were near the COD: d131 (mare 15004) and d129 (mare 15042). The same rationale that was used for feces is applied here as well, which is that the COD was a calculated average determined from individual mares (Table 2), with some expected variation. Of the other 11 samples, six were from one mare, while five were taken from four mares roughly a week prior to parturition. As it is known that plasma estradiol decreases approximately 30 days prior to parturition (Nett et al 1973), the 5 samples with concentration decreases within eight days of parturition can be expected. As far as the six mid-gestational samples from mare 15004, that could just be individual variances, as no other sample resulted from fluctuations similar to hers.

Studies in the literature have reported definitive pregnancy determination through both fecal metabolite monitoring and measurement of plasma estradiol, with timing dependent on type of estrogen measured. When measuring total unconjugated fecal estrogens, pregnancy determination was made between approximately 120 and 180 days (Bamberg et al 1984; Kirkpatrick et al 1989), while fecal estrone sulfate concentrations resulted in measurable pregnancy status when samples were taken at least 150 days into gestation (Henderson et al 1998 and 1999). In the measurement of plasma estradiol,

concentrations relative to pregnant mares surpassed those of non-pregnant mares at approximately 150 days post insemination (Nett et al 1973).

In this study, fecal estradiol 17β concentrations of pregnant mares diverged from those of non-pregnant at an average of 105 days of gestation, while that of serum estradiol 17β diverged at a mean of 128 days. In the calculation for feces, one mare, 15049, was initially sampled on day 119 of gestation, which was also the first day that her fecal estradiol concentration surpassed the cut-off value (Table 2). When she is removed from the calculation for fecal estradiol, the average day for definitive pregnancy diagnosis drops to 103 days. In this instance, serum was unaffected, as the first concentration that surpassed the cut-off value was on day 127 of gestation. Using either average calculation for feces, the average day is roughly 2 weeks – 2.5 months sooner than studies measuring total unconjugated fecal estrogens (Bamberg et al 1984; Kirkpatrick et al 1989), and approximately 1.5 months earlier than those measuring fecal estrone sulfate concentrations (Henderson et al 1998 and 1999; Linklater et al 2000). As would be expected, the serum estradiol concentrations for pregnancy determination are similar to that of plasma estradiol (Nett et al 1973). Between the fecal and serum estradiol results, the fecal estradiol information is more crucial regarding feral horse management. The earlier that managers can confirm or discount pregnancy within their herds, the more time they have for management decisions.

For this method to be the most effective, while it would be ideal if managers could collect a few fecal samples throughout the breeding season, the most important sample would be one collected at least 3.5 months after the conclusion of the breeding season. This would ensure that any mares bred at the end of the season would have progressed far enough into gestation for concentrations of fecal estradiol 17β to be discernable from those of non-pregnant mares. Additionally, at that point, any concentrations returned below the cut-off could be concluded as not pregnant, as mares are not capable of ovulation during anestrus.

CHAPTER 3

Measurement of Fecal and Serum Estradiol in the Feral Mare

Introduction

While there are several feral horse populations throughout the United States (BLM 2020; NPS 2006), this study examined fecal and serum samples from feral horses in Theodore Roosevelt National Park (THRO). The 47,000-acre fenced park is located in Medora, North Dakota, and currently houses approximately 167 feral horses, as well as several hundred bison, antelope, and smaller mammals (THRO 2020). Historically, the feral horse herd at THRO was extensively managed through large roundups, but the park presently oversees equine numbers through fertility control and small gathers. Therefore, knowing pregnancy status of individual mares would be helpful when making decisions about contraceptive administration, and which horses to remove from the park.

The feral mares in this research are part of a GonaCon-Equine study in THRO. GonaCon-Equine is an immunocontraceptive approved for use in adult horses and burros by the Environmental Protection Agency (EPA) as a restricted use pesticide (Baker et al 2018). The formulation of GonaCon-Equine utilizes a non-biodegradable oil in water-based emulsion that contains immunostimulatory killed mycobacteria (Baker et al 2018). When injected into the muscle of the recipient animal, a slow release depot forms, which produces prolonged efficacy effects (Baker et al 2018).

As described in Chapter 2, the measurement of fecal and serum estradiol 17β concentrations in the mare is a reliable means of determining pregnancy status. After 105 days of gestation and until directly before parturition, fecal estradiol concentrations above 10 pg/mg are a reliable indicator of pregnancy, while after 128 days of gestation and approximately a week prior to parturition, serum estradiol concentrations above 46 pg/mL serum are indicative of pregnancy. Therefore, this study was

designed to compare fecal and serum estradiol cut-offs to pregnancy determined in domestic mares to those of fecal and serum samples from feral mares. The objectives of this study were 1) to correlate findings of measured fecal and serum estradiol concentrations compared to ultrasound results, and 2) to determine the accuracy of pregnancy status from single fecal samples from feral mares.

Materials and Methods

This study was initiated in October 2009 with a park-wide roundup of all the horses in THRO, and included feral mares born between 1992- 2007. The first roundup included 48 feral mares, while the roundup in September 2013 included the initial 48, plus three more, for a study total of 51 mares. The 2009 roundup was the initiation of the GonaCon-Equine study, with 25 mares injected with GonaCon-Equine (treatment), and 23 mares injected with saline (control). In 2013, three additional mares were added to the study and assigned to the control group, for a total of 25 GonaCon-Equine treated mares, and 26 saline treated mares. Repeat GonaCon-Equine injections were completed at this time, as well as repeat saline injections to the original control group, and three new saline injections to the added three mares.

The process for fecal and serum collection from each mare was the same for both roundups. Once a mare was positioned in the squeeze shoot, a fecal grab was taken, followed by ultrasound to determine whether the mare was pregnant. While this was occurring, two red top vacutainer tubes of jugular blood were drawn using an 18-gauge 1.5-inch needle. All fecal samples collected at both roundups were placed in whirl packs labeled with date and mare identification, then frozen at -20°C until processing. All blood samples sat at room temperature for 30 minutes and were then centrifuged for 30 minutes at 2400xg, after which resulting serum was placed into 2 mL cryogenic vials and frozen at -20°C until extraction.

Following the 2009 round-up, yearly fecal sample collection was initiated for the 51 mares in the study. The breeding season in THRO ranges from March – August (Baker et al 2018), and aside from the roundup samples, the majority of the additional 272 fecal samples were collected during the month of November from 2010-2015. A smaller proportion of samples were also collected in February from 2011 – 2015. During each collection year, an attempt was made to obtain at least one fecal sample for each of the 51 mares on the study. Due to the large size and rough terrain of THRO, it was not always possible to find all mares every year. For this study, only samples obtained during the non-breeding season were used, with the rationale that no additional mares would be bred between the months of September – February. Fecal collection was completed by locating the mare of interest, and then tracking her until she defecated. Feces was collected from 4-5 areas within the pile; samples urinated on by the band stallion were not used. Samples were placed into whirl packs labeled with date and the mare's information, then placed into a cooler with ice packs. At the conclusion of each 8-12 hour collection day, samples were frozen at -20°C until extraction.

As all fecal samples were collected from mares of unknown pregnancy status, day of gestation was estimated for each sample. This calculation was completed using the foaling data for the following year and counting the days between sample collection and foaling date, with an estimated gestational length of 345 days for all mares on the study.

Extraction

The method used for extraction of domestic mare samples was also utilized in this study. The extraction methods below have been taken from the description in Chapter 2. Although extraction procedures differ for feces and serum, both methods utilize quality controls (QCs), which are extracted alongside samples. For this study, the QCs were made from hypophysectomized sheep serum, with varying concentrations of estradiol added (Nett 2005). The concentration of the low QC was 15 pg/mL of

estradiol, medium 60 pg/mL, and high 240 pg/mL. Additionally, there was a solvent QC, to be extracted with just diethyl ether (Nett 2005). All QC's were extracted at a volume of 250 µL.

Extraction of feces contained one pre-extraction step, and two extraction steps (Nett 2005). Prior to extraction, each raw chilled fecal sample was placed into a 94x16 petri dish in a thin layer that completely covered the bottom half of the dish. Any extra raw fecal matter was placed into a 50 mL conical vial, labeled with mare information and date, and then frozen at -20°C. Each petri dish of feces had a Kim wipe taped over the top for ventilation during lyopholization. The dishes were then frozen at -20°C for 30 minutes prior to placement into a lyopholizer for 72 hours, until dried completely. Each sample was then hand ground to a fine sand-like consistency and placed into a labeled 50 mL conical vial. Ten milligrams of feces for each sample was placed into 16x150 mm glass tubes and rehydrated with 1 mL of double-deionized water (DDH_2O). Resulting sample slurries were agitated for 1-1.5 hours, followed by centrifugation at 2400xg for 15 minutes. Five hundred microliters of supernatant was removed from each sample for use in the first extraction.

Fecal extractions one and two contained washing steps; for each 500 µL equine sample aliquot in a 16x150 mm tube, two 16x150 mm tubes were labeled with the same sample number, each containing 500 µL of DDH_2O for washing. Each 250 µL QC aliquot also had the same number of tubes prepared. For the second extraction, 5 mL of diethyl ether was added to each 500 µL equine aliquot or 250 µL QC, and then vortexed for 5 minutes. Samples were snap-frozen in a methanol-dry ice bath, and the organic phases poured into the first of the corresponding clean 16x150 mm tubes containing 500 µL DDH_2O for washing. Tubes were then vortexed again for 2 minutes, snap-frozen in a methanol-dry ice bath, and the washed organic phases poured into clean 12x75 mm glass tubes, placed into a heating block, and evaporated under nitrogen (Nett 2005). The second extraction was completed by adding 5 mL of diethyl ether to the original aliquots, and the protocol run again, although using the second set of washing tubes during the wash phase (Nett 2005).

Following the second extraction, the 12x75 mm tubes containing samples and QC's were reconstituted with 0.1% PBS-gel at the volume they were extracted at 500 µL for the mare samples, and 250µL for the QC's (Nett 2005). They were then vortexed for 2 minutes, incubated at 4°C overnight, vortexed again for 2 minutes and then frozen at -20°C until assay.

Serum required two extractions. From each serum sample, a 500 µL aliquot was placed into a labeled 16x150 mm glass tube, which had a corresponding labeled 12x75 mm labeled glass tube. As with fecal extractions, there were also labeled 16x150 mm and 12x75 mm labeled glass tubes for the QCs to be extracted alongside the equine samples. Five mL of diethyl ether was added to all tubes, which were then vortexed for 2 minutes, and allowed to stand for 5 minutes (Nett 2005). All tubes were then snap-frozen in a methanol-dry ice bath, then organic phases poured into the 12x75 mm tubes, which were placed into a heating block, and evaporated under nitrogen. For the second extraction, the steps from the addition of 5 mL diethyl ether on were replicated, and the same 12x75 mm tubes dried down. All tubes were then reconstituted with 0.1% PBS-gel at the same extraction volume used, 500 µL for equine samples, and 250 µL for QCs. All tubes were then vortexed for two minutes, incubated overnight at 4°C, vortexed again for 2 minutes and then frozen at -20°C until assayed (Nett 2005).

Assay

An RIA specific for estradiol 17β was utilized for this research, and both primary and secondary antibodies used were created prior to the study. The primary antibody was produced in rabbits from estradiol conjugated to bovine serum albumin (E26BSA), while the secondary antibody was produced in a goat.

All fecal, serum, and QC samples were assayed in duplicate, while 6 standards were assayed in triplicate (Nett 2005) and ranged in concentration from 40 pg to 409.6 fg. The amount of equine sample added to each tube was dependent on status of pregnancy, as well as sample type: feces or serum.

Extracted fecal samples from pregnant mares were split at gestational day 100; prior to day 100, 100 µL of extracted sample was added to each tube, after day 100, 10 µL of extracted sample was used. Extracted serum samples collected from pregnant mares were divided at gestation day 130; prior to day 130, 200 µL of sample was added to each tube, after day 130, 50µl was added. For cycling mares, 200 µL of sample/tube was added, for both feces and serum. Eighty microliters of each extracted QC was used for QC tubes.

Tubes were numbered prior to start of RIA, with tubes 1-3 the total count tubes (TC- radioactive ligand), 4-6 Non-specific Binding (NSB) tubes, 7-14 QC's,15-17 Buffer Control (BC-0.1%PBS-gel) tubes, and 18-23 Standards (Nett 2005). Two additional sets of BC and Standard tubes were throughout the protocol, but tube number was specific to individual RIA. Total volume for each tube of sample/QC or standard, plus buffer (PBS-gel 0.1%) was 500 µL (Nett 2005). Five hundred microliters of buffer was added to NSB tubes and BC tubes. One hundred microliters of iodinated estradiol 17β-6-TME was added to all tubes in the assay, followed by 200µL of 1:400 Normal Rabbit Serum (NRS) to the NSB tubes (Nett 2005). Two hundred microliters of diluted primary antibody (1:400,000 in 1:400 NRS) was added to all tubes except the TC and NSB tubes. All tubes in the assay were vortexed for approximately 5 seconds, then incubated at 4°C for 24 hours (Nett 2005). After 24 hours of incubation, the secondary antibody, 200 µL of diluted secondary antibody (1:00 goat anti rabbit (GAR) in PBS-EDTA), was added to all tubes except TC tubes, vortexed, and returned to the 4°C for additional incubation of 72 hours.

Seventy-two hours after adding GAR, 3 mL of cold phosphate buffered saline was added to all tubes except TC. Excluding TC tubes, all others were centrifuged for 30 minutes at 2400xg, and resulting supernatant decanted into waste containers. All tubes, including TC, were then counted on a gamma counter with a counting efficiency of 85% (Nett 2005).

The RIA used had 100% cross reactivity with estradiol 17β, 12% with estrone, 7% with estradiol 17α, 9% to estriol, and 1% with both testosterone and progesterone (Niswender et al 1969). The sensitivity of the RIA was found to be an average of 228 fg/tube with the 50% dose of the theoretical curve at an average of 6 pg/tube of equine estradiol.

Results

At the 2009 and 2013 roundups, a total of 51 mares were processed for feces and blood collection, 48 in 2009 and all 51 in 2013. Of the 48 mares in 2009, 39 were determined pregnant by ultrasound, while 9 were found to not be pregnant. In 2013, ultrasound showed that 41 mares were pregnant and 10 were not. At the 2009 roundup, mares were assigned as either treatment or control mares, so there were no GonaCon-Equine treatment effects noted, as this year was the initiation of the study. In the 2013 roundup, 5 of the non-pregnant mares were treatment mares, while 5 were control mares.

While 39 mares were pregnant in the 2009 round-up, fecal and serum samples from three mares were not included in pregnancy data, as they did not have a foal the following year, making gestation estimates impossible. Figure 11A and 11B depict the fecal estradiol concentrations of pregnant mares for both roundups, for a total of 77 fecal samples. Figure 11A illustrates the entire sample set from all mares, while Figure 11B indicates fecal data starting at day 80 of gestation, or just under a month prior to the COD of 105 days, along with the fecal estradiol COC of 10 pg/mg of feces.

Figures 12A and 12B show the serum estradiol concentrations of pregnant mares for both roundups, again for a total of 77 serum samples. Figure 12A illustrates the entire sample set for all mares, while Figure 12B shows the serum data starting at day 105, or roughly a month prior to the cut-off value of 128 days, along with the serum estradiol COC of 46 pg/mg of feces.

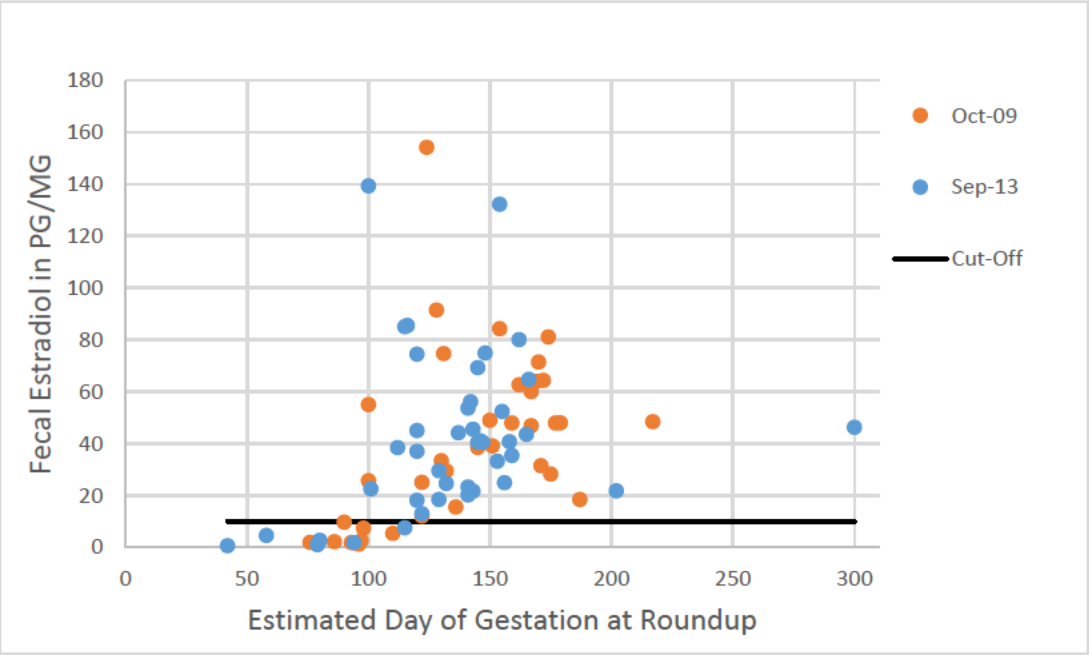


Figure 11A. Fecal estradiol 17β concentration from pregnant feral mare samples collected during the 2009 and 2013 roundups. The horizontal line indicates the cut-off concentration of 10 pg/mg feces.

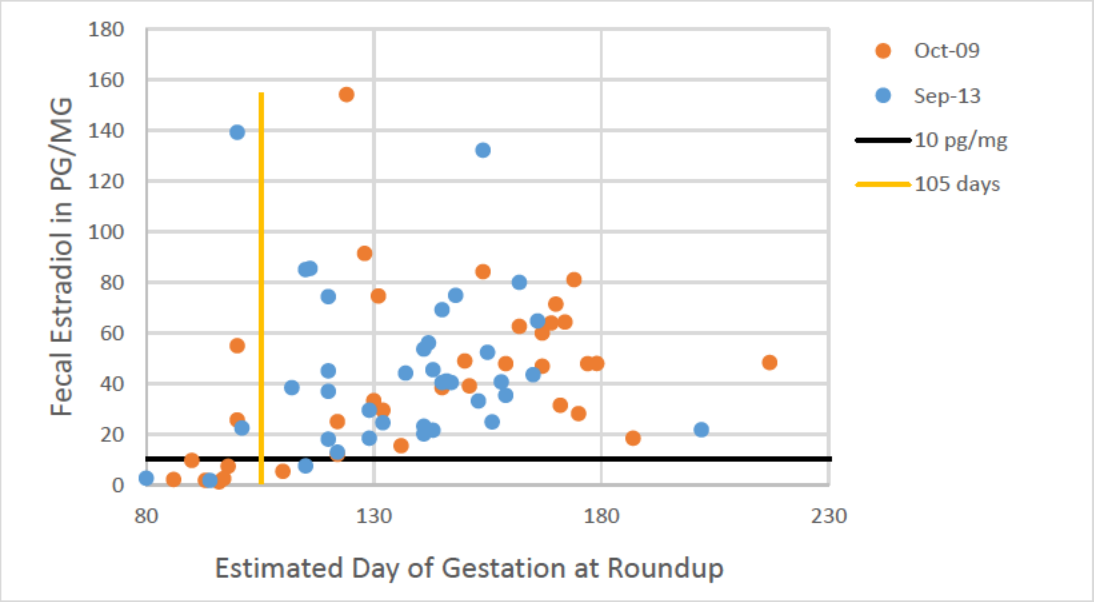


Figure 11B. Fecal estradiol 17β concentrations from pregnant feral mare samples collected after day 80 during the 2009 and 2013 roundups. The horizontal line indicates the 10 pg/mg cut-off concentration, while the vertical line indicates the cut-off day of 105.

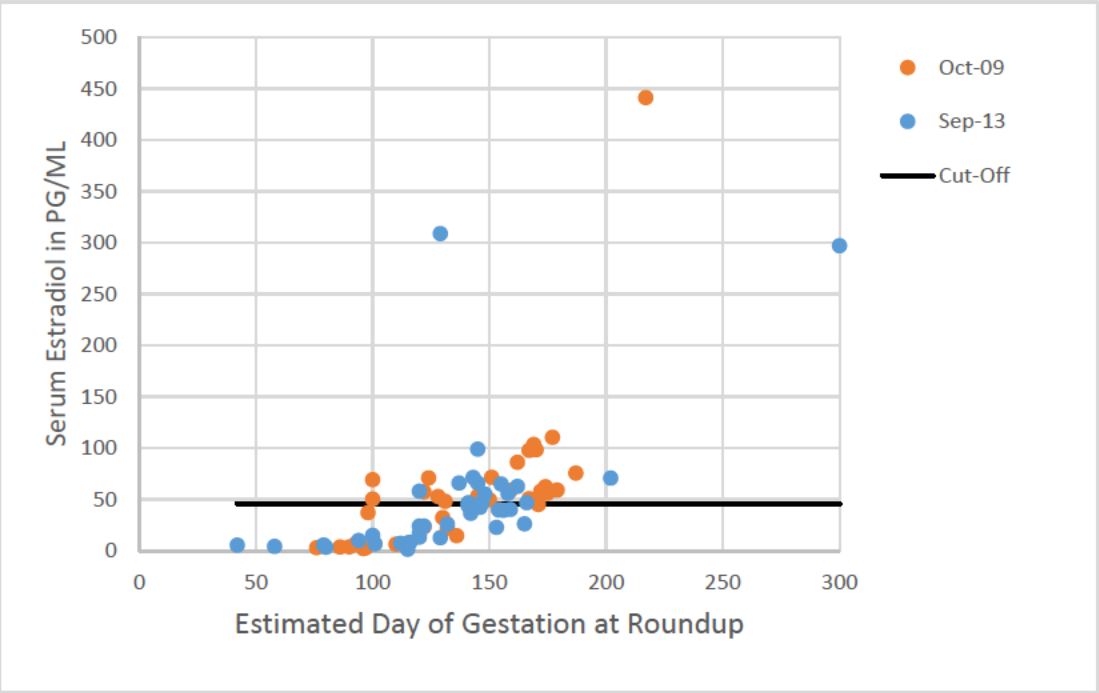


Figure 12A. Serum estradiol 17β concentration from pregnant feral mare samples collected during the 2009 and 2013 roundups. The horizontal line indicates the cut-off concentration of 46 pg/mL serum.

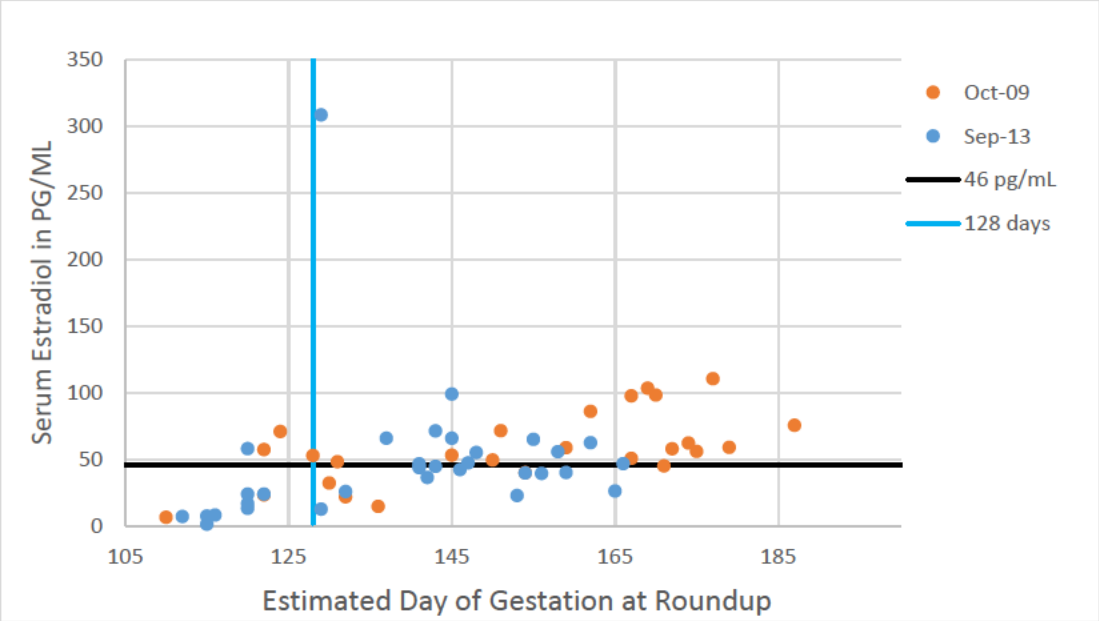


Figure 12B. Serum estradiol 17β concentrations from pregnant feral mare samples collected after day 105 during the 2009 and 2013 roundups. The horizontal line indicates the 46 pg/mL cut-off concentration, while the vertical line indicates the cut-off day of 128.

As shown in Table 4, of the 77 counted fecal samples collected between the two roundups, 62 surpassed the 105 day cut off, with 60/62 surpassing the COC of 10 pg/mg feces. The two samples that fell short of the COC are shown in Table 5. Of the 15 samples collected prior to day 105, 13 of them were below the COC of 10 pg/mg of feces. Regarding collected serum samples, 49 of the samples were collected past the serum COD of 128 days, with 34 of those samples surpassing the COC of 46 pg/mL serum. The collection day and corresponding concentration data for the 15 serum samples that surpassed the COD of 128, but not the COC is shown in Table 5. Of the 28 samples collected prior to day 128, 26 of them failed to surpass the COC, which is shown in Table 4.

Regarding non-pregnant mare data, there were a total of 19 mares during both roundups that were not pregnant. Table 6 displays both the fecal and serum concentrations obtained from these mares, none of which surpass the designated cut-offs. The samples in red indicate those from treatment mares.

Although the roundups accounted for all serum samples, they only addressed a portion of the 368 total fecal samples collected during the study. Figures 13A-B, 14 and 15A-D show samples collected during both roundups, as well as November – February across 6 years of sampling. Figure 13A indicates the 121 samples from non-pregnant mares throughout the study, none of which surpass the COC of 10 pg/mg feces. Figure 13B indicates the same samples, but grouped according to treatment. The first grouping was from the 2009 roundup, which was the study initiation. Following that year, all additional samples taken from non-pregnant mares were denoted as either from treatment or control mares. Figure 14 illustrates the fecal concentration data for 225 pregnant mare samples. As it is difficult to discern the number of fecal samples from pregnant mares that failed to surpass the fecal estradiol COC in Figure 14, Figures 15A-D display a series of graphs divided into gestation timepoints: 15A: 0-100 days of gestation ,15B: 101 – 151 days, 15C: 152 – 202, and 15D: 203 – parturition. Figure 15A depicts the first 100 days of gestation, where only 4 fecal estradiol concentrations surpassed COC of 10 pg/mg

feces, all at day 100, with concentrations of 26, 36, 55, and 139 pg/mg feces. In Figure 15B (101-151 days of gestation), 5 samples failed to surpass the COC: 110 days; 5 pg/mg of feces, 111 days; 9 pg/mg feces, 115 days; 8 pg/mg feces, 118 days; 10 pg/mg feces, and 128 days; 9 pg/mg of feces. All samples from the subsequent two figures, 15C and 15D surpassed both COD as well as COC.

As mentioned previously, in the 2009 round-up, there were three mares who were pregnant at the time of the round-up, but no foals were found the following year. Aside from these three, there were an additional 19 mares throughout the study whose fecal estradiol values indicated pregnancy at time of sampling, but no foals were found. Data for the three mares from the 2009 round-up is shown in Table 7, while Figure 16 illustrates the data from the remaining 19.

Table 4. Pregnant and non-pregnant feral mare fecal and serum estradiol samples that surpassed and missed cut-off values during the 2009 and 2013 roundups.

Roundup 2009	Roundup 2013	
Mares: 48 total Pregnant: 36 Non-pregnant: 9	Mares: 51 total Pregnant: 41 Non-pregnant: 10	
ALL MARE FECAL ESTRADIOL CONCENTRATIONS		
PREGNANT MARE TOTAL		
Fecal Estradiol	Cut-Off 105 days	Cut-Off: 10 pg/mg
Surpassed	62/77 samples	60/62 samples
Below	15/77 samples	13/15 samples
ALL NON-PREGNANT MARES	0/19 mares	0/19 mares
ALL MARE SERUM ESTRADIOL CONCENTRATIONS		
PREGNANT MARE TOTAL		
Serum Estradiol	Cut-Off 128 days	Cut-Off: 46 pg/mL
Surpassed	49/77 samples	34/49 samples
Below	28/77 samples	26/28 samples
ALL NON-PREGNANT MARES	0/19 mares	0/19 mares

Table 5. Roundup pregnant feral mare samples past the cut-off day, but not cut-off concentration.

Roundup Pregnant Mare Samples Below Concentration Cut-Off			
FECES		**SERUM**	
Estimated Day of Gestation	Fecal Estradiol in pg/mg	Estimated Day of Gestation	Serum Estradiol in pg/mL
110	5	129	13
115	8	130	32
		132	26
		132	26
		136	15
		141	44
		142	36
		143	45
		146	42
		153	23
		154	40
		156	39
		159	40
		165	26
		171	45

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Table 6. Fecal and serum estradiol concentrations collected from non-pregnant feral mares during the roundups. The 2013 samples in red are from GonaCon-Equine treated mares. No sample concentrations surpassed feces COC of 10 pg/mg or serum COC of 46 pg/mL.

2009 Round-up		
Sample Date	Feces: PG/MG	Serum: PG/ML
10/11/09	1	6
10/11/09	1	5
10/11/09	1	2
10/11/09	1	5
10/11/09	1	8
10/11/09	1	2
10/11/09	2	1
10/11/09	4	13
10/11/09	1	9

2013 Round-up		
Sample Date	Feces: PG/MG	Serum: PG/ML
9/23/13	1	14
9/24/13	2	2
9/24/13	1	7
9/24/13	1	3
9/23/13	1	9
9/23/13	7	8
9/23/13	1	7
9/23/13	7	9
9/23/13	2	9
9/24/13	1	3

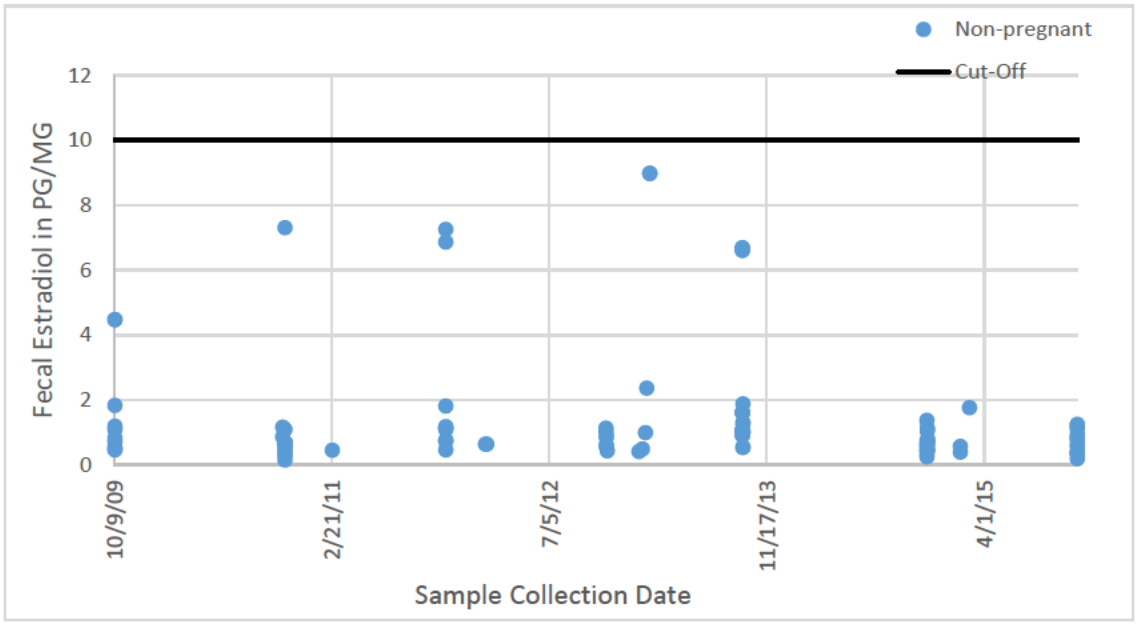

Figure 13A. Total distribution of fecal estradiol concentrations in pg/mg from collected samples of non-pregnant feral mares. The horizontal line indicates the cut-off concentration of 10 pg/mg feces.

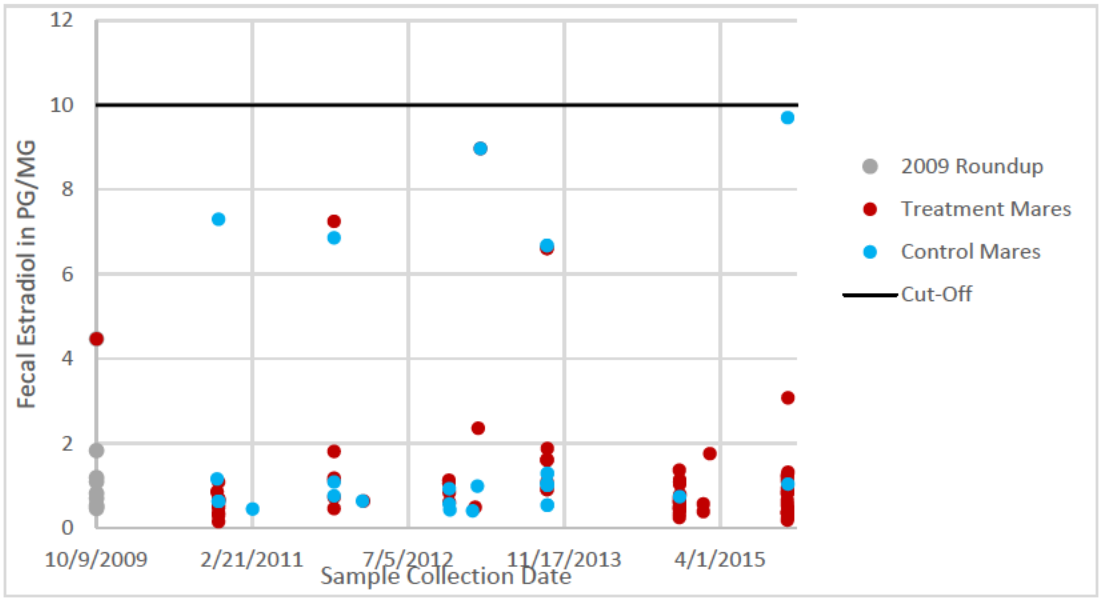

Figure 13B. Fecal estradiol concentrations in pg/mg from collected samples of non-pregnant feral mares. Samples are delineated as those collected at the 2009 roundup, or start of the study, as well as during proceeding years, labeled as treatment and control mare samples. The horizontal line represents the 10 pg/mg cut-off concentration.

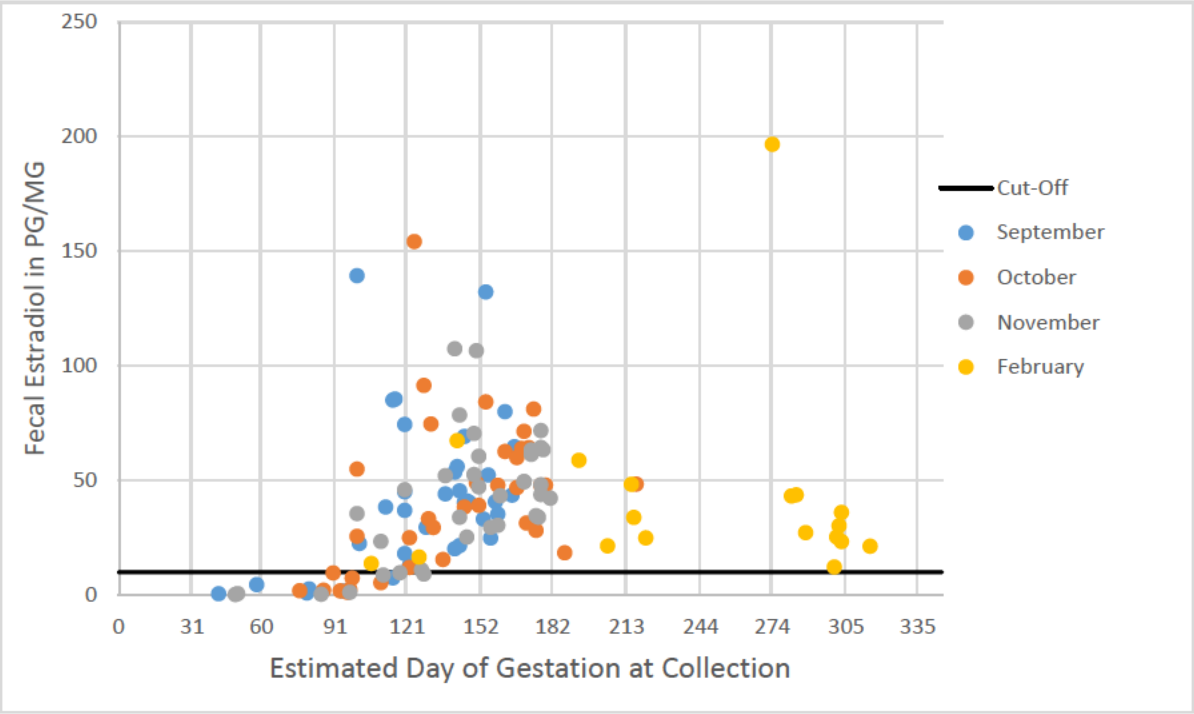

Figure 14. Fecal estradiol concentrations from samples collected from pregnant feral mares in pg/mg. The horizontal line indicates the COC of 10 pg/mg feces.

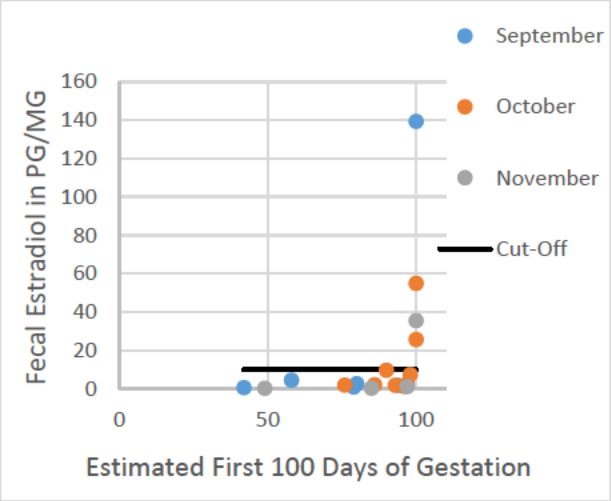

Figure 15A

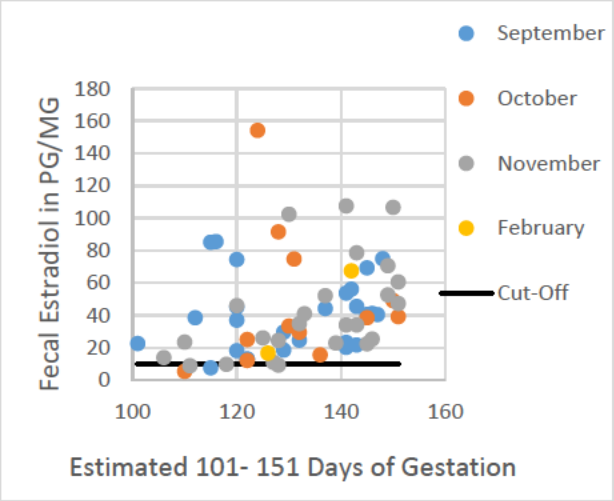

Figure 15B

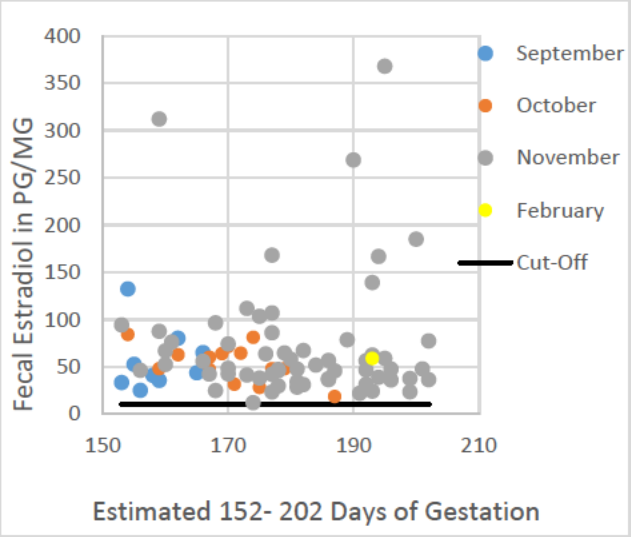

Figure 15C

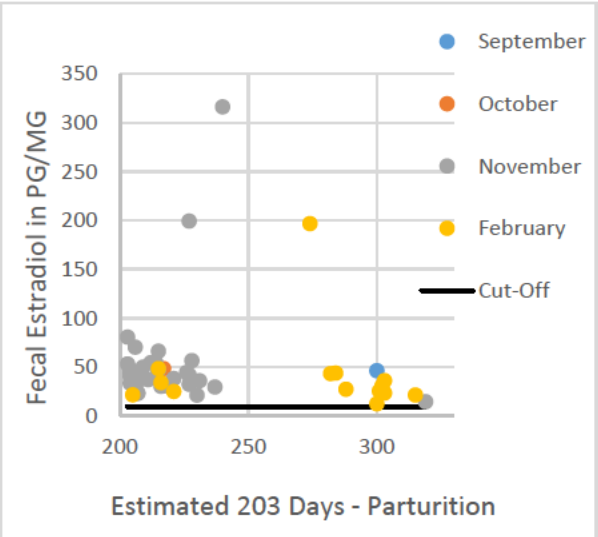

Figure 15D

Figure 15A-D. Progression of estimated day of gestation relative to fecal estradiol concentration in pg/mg. All horizontal lines indicate the cut-off concentration of 10 pg/mg feces.

Table 7. Fecal and serum concentrations taken from 3 pregnant feral mares at roundups whose foals were not found the following year.

Date of Collection	Fecal Estradiol in pg/mg	Serum Estradiol in pg/mL
10/11/09	19	52
10/11/09	38	32
10/11/09	35	63

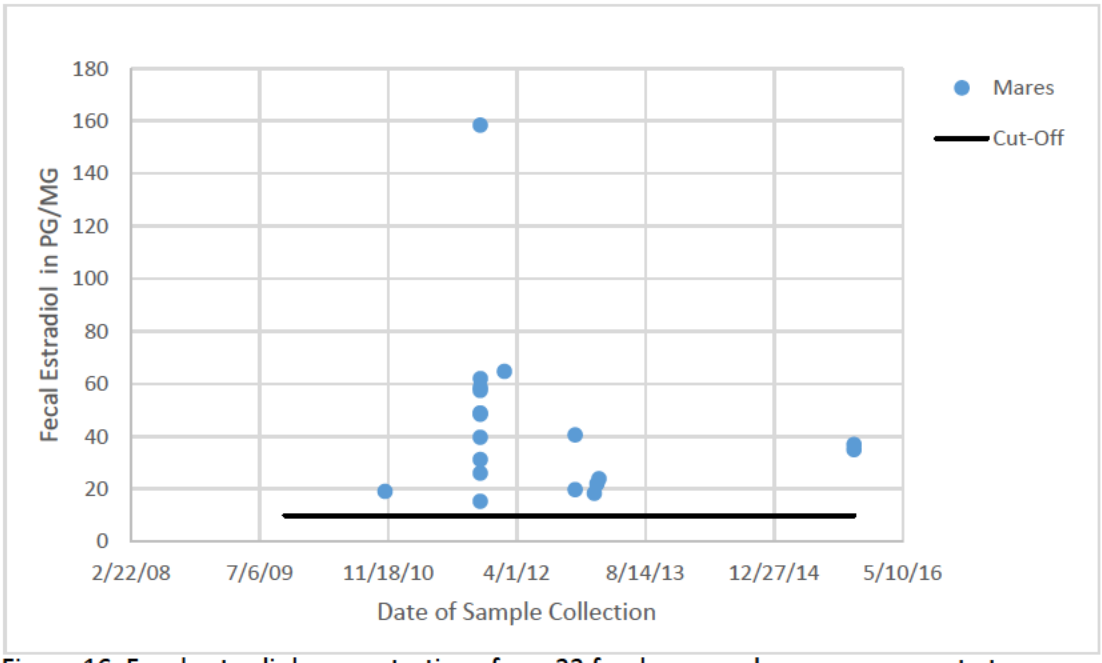

Figure 16. Fecal estradiol concentrations from 22 feral mares who were pregnant at time of collection, but whose foals were not found the following year. The horizontal line indicates the concentration cut-off of 10 pg/mg feces.

Discussion

The fecal and serum results obtained from feral mares across two roundups indicate that fecal estradiol is a reliable pregnancy determinant, while serum concentrations of estradiol fluctuate after the COD. Of the fecal samples taken from pregnant mares during the round-up past 105 days of gestation, 60/62 samples were above the cut-off concentration of 10 pg/mg feces, as noted in Table 4. Conversely, of the 49 serum samples taken post 128, 15 of them were below the designated serum cut-off of 46 pg/mL (Table 4). Additionally, all 19 fecal and serum samples taken from non-pregnant mares at the round-up were below their respective concentration cut-offs (Table 6). This trend is similar to what was seen in pregnant domestic mare fecal and serum samples, in which 258/267 of fecal estradiol concentrations after day 105 and prior to two days of parturition remained above cut-off concentrations (Figure 9A). Although 239/252 serum samples collected after the COD of 128 surpassed the COC of 46 pg/mL, the remaining 13 fluctuated below the COC during mid-gestation, as seen in Figure 10A.

As all sample gestation date estimates were based on a pre-determined estimate of 345 days of gestation length in conjunction with foaling data, some gestational data could be incorrect. This could contribute to samples that passed the COD, but not COC, such as the fecal and serum samples seen in Table 5. For example, it is possible that either of the two fecal samples in that table could have been taken earlier in gestation than calculated, and not actually beyond the COD. While this is plausible, in order to complete the calculations for the data, gestational length had to be chosen, and 345 days is the longest average gestation noted in the literature (Ginther 1992).

As shown in Figure 13B, the treatment effect of GonaCon-Equine can be seen relative to sampling timepoint. After the booster in September 2013, the overall number of samples from non-pregnant GonaCon-Equine treated mares increased. Of the total 121 samples, there were 11 collected in 2010 from non-pregnant GonaCon-Equine treated mares, while in 2015, there were 25 samples

collected. Although these results indicate a possible positive efficacy effect with repeated GonaCon-Equine injections at a four-year frequency, additional research would need to be completed.

While the roundups occurred during September and October, the majority of the fecal collections during the 6-year sampling timeframe were completed in November. While Figure15A-D shows the overall breakdown by sampling month relative to gestation, it is interesting to note the distribution of samples. As seen in Table 8, of the 41 samples taken in September, 35 of them surpassed the cut-off concentration of 10 pg/mg feces, while October resulted in 28/36, November in 126/132, and February 18/18. From this data, the overall percentage of samples over the cut-off value taken in September were 85.4%, 77.8% in October, 96.2% in November, and 100% in February. It should be noted that while these samples were taken over the course of six years, they were from 51 members of the entire population, and of the 226 samples discussed here, just under half were taken in November, resulting in an uneven distribution over the sampling timeframe.

Even with the uneven sampling distribution, the majority of samples taken in November were in the estimated 152-202 day range of gestation, with only 4/131 samples taken prior to the COD of 105. This would mean that in a population with a similar breeding timeframe to mares in THRO, samples taken in November would result in the majority being past 105 days of pregnancy, and more likely to be in the estimated 152-202 day range. When examining October samples, 9/36 were taken prior to 105 days, indicating roughly 25% of samples potentially taken too early in gestation to reliably distinguish between pregnant and non-pregnant individuals. Six samples taken in September were below the COD of 105 days, which would result in about 14.6% of samples taken too early. Although 100% of samples taken in February resulted in samples over 105 days, 14/18 samples were during days 203- parturition time point, which would be too late for making effective management decisions for feral herds. With that said, however, when only looking at the sample distribution relative to estimated 101-151 days, 96% of the September samples and 91.7% of both the October and November samples were above the

COC. Knowing this, it would therefore be up to the managers of individual feral herds to determine what level of uncertainty in sample concentration returns they would be comfortable with.

Prior to this study, foaling rates in the park were determined by the number of live foals seen on the ground. Over the course of this study, there were a total of 22 pregnant mares whose foals the following year were not seen. Of the 22, 3 of the mares were part of the 2009 round-up, so were known to be pregnant, but no foals were seen the following year. The serum and estradiol concentrations for these mares are in Table 7, and all but one surpassed respective fecal and serum concentration cut-offs. While the second serum sample in the table of 32 pg/mL failed to surpass the COC, the corresponding fecal sample concentration of 38 pg/mg feces did, indicating potential early pregnancy. Figure 16 depicts the fecal concentrations of these three mares along with the remaining 19, whose fecal samples were taken not knowing pregnancy status. As can be seen in this figure, all fecal estradiol concentrations are above the cut-off value of 10 pg/mg, indicating that all 22 mares should have been at least 105 days into gestation. While it is not known what happened to their foals following sample collection, they appeared to be pregnant at the time of collection.

Table 8. Estimated day of gestation in feral mares relative to the number of samples taken during each collection month. The first number indicates how many of the total for each set surpassed the cut-off concentration of 10 pg/mg.

Estimated Day of Gestation Relative to Sampling Month				
	Estimated Day of Gestation			
	0-100 days	*101-151 days*	*152-202 days*	*203-345 days*
Month	Number of Samples			
September	1 of 6	24 of 25	9 of 9	1 of 1
October	2 of 9	11 of 12	14 of 14	1 of 1
November	1 of 4	22 of 24	73 of 73	30 of 30
February	0 of 0	4 of 4	0 of 0	14 of 14

CHAPTER 4

Conclusions

Through completion of the two studies measuring fecal and serum estradiol, it was found that fecal estradiol measurements indicate pregnancy status both at an earlier average day of gestation (105 days), but also at a lower cut-off concentration (10 pg/mg feces), as compared to serum. Serum estradiol resulted in an average cut-off day of 128 days, and cut-off concentration of 46 pg/mL serum.

Additionally, fecal estradiol concentrations were found to fluctuate less than serum, once the initial cut-off day had passed. In pregnant domestic mares, 258/267 (96.6%) of fecal estradiol samples surpassed both the COD of 105 days, as well as COC of 10 pg/mg feces. Of the nine that failed to surpass the COC, all of them were either within a few days of the COD or parturition. In domestic mares, serum estradiol concentrations in domestic mares resulted in 239/252 (94.8%) samples exceeded COD of 128. Of the 13 that failed to surpass COC of 46 pg/mL, many were scattered throughout gestation, instead of early or late. This trend was also seen in the feral mare samples collected at the roundups in Theodore Roosevelt National Park (THRO), in which 60/62 (96.8%) of fecal samples collected at the roundups surpassed both cut-offs, while only 34/49 (69.4%) serum samples did.

Aside from the fluctuations seen in both feces and serum, the resulting timepoint for definitive diagnosis of pregnancy via fecal estradiol is earlier than what has previously been reported in the literature, with the cuf-off day being roughly two weeks – 2.5 months earlier than studies measuring total unconjugated fecal estrogens (Bamberg et al 1984; Kirkpatrick et al 1989), and approximately 1.5 months earlier than those measuring fecal estrone sulfate concentrations (Henderson et al 1998 and 1999). The results from this study therefore provide a useful, earlier alternate option to the current methods utilized for pregnancy diagnosis in the feral horse.

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APPENDICES

Appendix 1
Colorado State University
Animal Reproduction and Biotechnology Laboratory
Endocrine Laboratory

EXTRACTION OF FECAL ESTROGEN (EXT-FECAL-ESTRADIOL)

Components of Estrogen Hormone Extraction for Fecal Samples

A. Sample
   1. Weight of feces to extract: 10 mg
   2. Reconstituted Extract Dilution Factor 1.0

B. Quality Control (QC) Serum
   1. Low QC: **E_2 LO** Middle QC: **E_2 MED** High QC: **E_2 HI** Solvent Control
   2. Volume to Extract: 0.5g
   3. Reconstituted Extract Dilution Factor: 1.0

C. Solvent
   1. Solvent: **ETHYL ETHER**
   2. Volume Per Tube for Extraction **5.0 ml**

D. Buffer
   1. Buffer: **0.1% PBS-GEL PH 7.0**
   2. Volume to Reconstitute Sample: **0.5 ml** (concentration=1ml/ml)

E. First Extraction
F. Second Extraction
G. Third Extraction
H. Reconstitute

PROCEDURE

A. Extraction Preparation
   1. Freeze feces in -20°C (-22 to -18°C) freezer.
   2. Tape a Kim wipe onto the top of each container of feces.
   3. Lyophilize for 36-48 hr.
   4. Hand grind until fine.
   5. Weigh out approximately 10 mg of feces and place into 16x150mm glass tubes. Record weight for each sample.
   6. Rehydrate with 5ml double-deionized water.
   7. Agitate the slurries for 1 hr(1.0 – 1.5hrs)
   8. Collect approximately 3 ml of supernatant.
   9. Freeze supernatant at -20°C (-22 to -18°C) until second extraction.

B. First Extraction
   1. Aliquot 500 µL fecal supernatant into 16x150mm glass tube.
   2. Add 5mL ethyl ether to each tube.
   3. Vortex for 1 min (1-2 min).
   4. Snap-freeze tubes in a methanol-dry ice bath.
   5. Pour organic phases into clean 16x150mm glass tubes containing 500µL double distilled water for washing.
   6. Vortex tubes for 1 min (1-2 min).
   7. Snap-freeze tubes in a methanol-dry ice bath.
   8. Pour washed organic phases into clean 12x75mm glass tubes.
   9. Dry down under nitrogen.

C. Second Extraction
   Repeat First Extraction steps 2-9.

D. Reconstitute
   1. Reconstitute 12x75mm glass tubes to 0.5 ml with PBS gel.
   2. Vortex for 1 min.
   3. Incubate samples for 18 hr (18 – 20 hr) at 22 °C (20-24°C).
   4. Vortex again and assay.

Appendix 2
Colorado State University
Animal Reproduction and Biotechnology Laboratory
Endocrine Laboratory

EXTRACTION OF ESTRADIOL (EXT-E2)

Components of Estradiol Extraction

A. Sample
   1. Volume of Serum to Extract: **up to 1.0 ml**
   2. Reconstituted Extract Dilution Factor: **1.0**

B. Quality Control (QC) Serum
   1. Low QC: **E2 LO** Middle QC: **E2 MED** High QC: **E2 HI** Solvent Control
   2. Volume to Extract: **1.0 ml**
   3. Reconstituted Extract Dilution Factor: **1.0**

C. Solvent
   1. Solvent: **DIETHYL ETHER**
   2. Volume Per Tube for Extraction: **5.0 ml**

D. Buffer
   1. Buffer: **0.1% PBS-GEL PH 7.0**
   2. Volume to Reconstitute Sample: **1.0 ml** (concentration=1ml/ml)
E. Aliquot Sample
F. First Extraction
G. Second Extraction
H. Reconstitute

PROCEDURE

A. Aliquot Serum Sample
   1. Label 16x150mm glass tubes totaling the number of serum samples.
   2. Label 12x75mm glass tubes with the same numbers.
   3. Label 16x150mm glass tubes and 12x75mm tubes for QC and solvent control.
   4. Aliquot 1.0 ml of serum into 16x150mm glass tubes. Includes QCs.

B. First Extraction
   1. In the extraction hood, add 5.0 ml Diethyl Ether to all tubes.
   2. Vortex for 1 min (1-2 min). Let stand for 5 – 10 minutes.
   3. Freeze in dry-ice and methanol bath.
   4. Pour into 12x75mm glass tubes and dry down in the heating block under nitrogen.

C. Second Extraction
   Repeat First Extraction steps 1-4 using same 16x150mm and 12x75mm glass tubes.

D. Reconstitute
   1. Reconstitute 12x75mm glass tubes to 1.0ml (up to 1.0ml) with PBS-Gel.
   2. Vortex for 1 minute.
   3. Incubate at least 2 h at room temperature or preferably overnight at 4°C (2-6°C).
   4. Vortex again and assay at 200 µL.

Appendix 3
Colorado State University
Animal Reproduction and Biotechnology Laboratory
Endocrine Laboratory

RADIOIMMUNOASSAY FOR 17 B ESTRADIOL-6TME (RIA-E2-6)

Components of Assay
A. Antibody
   1. Antibody Batch Identification **A737**
   2. Dilution of Antibody: **1:400,000**
   3. Diluent: **1:400 Normal Rabbit Serum(NRS) in PBS-EDTA**
   4. Volume Per Tube in Assay: **200 µL**

B. Iodinated Preparation
   1. Iodinated Preparation Identification: ^{125}**I-ESTRADIOL-17BETA-6-TME**
   2. Approximate Counts Per Minute/100 µL: **30,000 IN PBS-GEL**
   3. Volume Per Tube in Assay: **100 µL**

C. Standards
   1. Standards Batch Identification: **E2-CSU-S15**
   2. Stock: **USP 1250008 Lot# R025F0**
   3. **0.2** ng/ml
   4. Number of Points in Curve: **6**
   5. Dilution Factor: **0.4**

D. Secondary Antibody
   1. Secondary Antibody Identification: **A1039** Goat Anti-Rabbit (GAR)
   2. Dilution 1:**100** in PBS-EDTA
   3. Volume to Assay: **200 µL**

E. Quality Control (QC) Sera
   1. Low QC: **E2 LO** Middle QC: **E2 MED** High QC: **E2 HI** Solvent Control
   2. Reconstituted Extract Dilution Factor: **1.0**
   3. Volume to Assay: **80 µL**

F. Sample
   1. Reconstituted Extract Dilution Factor: **1.0**
   2. Volume to Assay: Up to 200 µL

G. Buffer
   1. PBS-Gel 0.1% pH 7.0
   2. Volume to Assay: Appropriate amount to bring Sample + Buffer to 500 µL

H. Incubation: **24 – 72 hours minimum at 4°C**

PROCEDURE

A. Assay Procedure
   1. To glass 12x75mm tubes add up to 200 µL of extracted sample/QC or standard and appropriate amount of buffer to bring column of sample/QC or standard + buffer to 500 µL. Add 500 µL buffer to Non-Specific Binding (NSB) (tubes 4 – 6) and maximum binding tubes (tubes 13 – 15).
   2. Add 100 µL ^{125}I-E2-6TME to all tubes.
   3. To the NSB tubes add 200 µL NRS at 1:400 dilution.
   4. Add 200 µL E2-6TME antisera to all tubes except total count (TC) tubes (tubes 1-3) and NSB tubes.
   5. Vortex.
   6. Incubate in 4°C for 24 hours.

B. Addition of Secondary Antibody
   1. At least 24 hours after adding the radioactive tracer to the assay, add 200µL ARGG to all tubes except the TC tubes.
   2. Vortex.
   3. Return to the 4°C incubator for 72 hours.

C. Pouring off Assay
   1. At 72 (± 8) hours after adding GAR, load all of the tubes, except TC tubes, into the centrifuge carriers.
   2. Add 3 mL cold phosphate buffered saline to each tube.
   3. Balance carriers.
   4. Centrifuge at 2500 rpm for 30 minutes.
   5. Pour off the supernatant into liquid waste containers.
   6. Blot the tube rims gently.

D. Counting
   1. Determine the efficiency/background of gamma spectrometer using calibrated ^{125}I sources.
   2. Count the radioactivity associated with the pellet in a gamma spectrometer.
   3. Reduce raw counts onto 3 ½" floppy disk.

E. Process Data
   1. Using 3 ½ "floppy disk, transfer raw counts from gamma spectrometer to computer containing the program RIANAL.
   2. Save as counts file (.ct) which corresponds to protocol file (.pt).

F. Analyze Data
   1. Generate results of assay using RIANAL program.
   2. Save Analysis onto private Drive (U:)

G. Quality Control
   1. Record quality control information in the QC book.
   2. Compare results of QCs from assay with values from previous assays. If results of QCs from current assay differ more than 2 standard deviations from the mean of previous assays, the assay is to be rerun.

H. Assay Review
   1. Non-Detectable level must be determined for all assays that contain non-detectable values in the results.
   2. All completed assays must be reviewed by laboratory supervisor before sending out results.

Grant and Cooperative Agreement

CHOOSE ONE:
- [X] COOPERATIVE AGREEMENT
- [ ] GRANT

CHOOSE ONE: [ ] EDUCATION [ ] FACILITIES [ ] RESEARCH [ ] SDCR [ ] TRAINING

1. GRANT/COOPERATIVE AGREEMENT NUMBER	2. SUPPLEMENT NUMBER	3. EFFECTIVE DATE	4. COMPLETION DATE
L15AC00145		09/08/2015	09/07/2020

5. ISSUED TO
NAME/ADDRESS OF RECIPIENT (No., Street, City/County, State, Zip)
COLORADO STATE UNIVERSITY
Attn: TRACEY TRUJILLO, RSRCH ADMIN
601 S HOWES ST
FORT COLLINS CO 80521-2807

6. ISSUED BY BLM OR-ST OFC PROC MGMT BR(OR952)
Mailing Address: 1220 SW 3rd Avenue, 12th Floor
PORTLAND OR 97204

7. TAXPAYER IDENTIFICATION NO. (TIN)

8. COMMERCIAL & GOVERNMENT ENTITY (CAGE) NO.

9. PRINCIPAL INVESTIGATOR/ORGANIZATION'S PROJECT OR PROGRAM MGR. (*Name & Phone*)
Dan L. Baker, Senior Scientist
970-556-8518, danbaker@colostate.edu

10. RESEARCH, PROJECT OR PROGRAM TITLE
WILD HORSE AND BURRO CONTRACEPTIVE TECHNIQUES AND PROTOCOLS

11. PURPOSE
See Schedule.

12. PERIOD OF PERFORMANCE (*Approximately*)
09/08/2015 through 09/07/2020

13A.	AWARD HISTORY	13B.	FUNDING HISTORY
PREVIOUS	$0.00	PREVIOUS	$0.00
THIS ACTION	$159,708.00	THIS ACTION	$159,708.00
CASH SHARE	$0.00	**TOTAL**	$159,708.00
NON-CASH SHARE	$0.00		
RECIPIENT SHARE	$0.00		
TOTAL	$159,708.00		

14. ACCOUNTING AND APPROPRIATION DATA
01

PURCHASE REQUEST NO.	JOB ORDER NO.	AMOUNT	STATUS
0020082150			

15. POINTS OF CONTACT

	NAME	MAIL STOP	TELEPHONE	E-MAIL ADDRESS
TECHNICAL OFFICER	PO: Paul Griffin		970-226-9358	pgriffin@blm.gov
NEGOTIATOR				
ADMINISTRATOR	Walter Ullrey		503-808-6302	wullrey@blm.gov
PAYMENTS				

16. THIS AWARD IS MADE UNDER THE AUTHORITY OF :
The Wild Free-Roaming Horses and Burros Act of 1971, 16 USC 1336, PL 92-195, Section 6.

17. APPLICABLE STATEMENT(S), IF CHECKED:
- [ ] NO CHANGE IS MADE TO EXISTING PROVISIONS
- [ ] FDP TERMS AND CONDITIONS AND THE AGENCY-SPECIFIC REQUIREMENTS APPLY TO THIS GRANT

18. APPLICABLE ENCLOSURE(S), IF CHECKED:
- [ ] PROVISIONS
- [ ] SPECIAL CONDITIONS
- [ ] REQUIRED PUBLICATIONS AND REPORTS

UNITED STATES OF AMERICA		COOPERATIVE AGREEMENT RECIPIENT	
CONTRACTING/GRANT OFFICER	DATE	AUTHORIZED REPRESENTATIVE	DATE
Walter Ullrey	09/08/2015		

Grant and Cooperative Agreement

ITEM NO. (A)	ITEM OR SERVICE (Include Specifications and Special Instructions) (B)	QUANTITY (C)	UNIT (D)	ESTIMATED COST	
				UNIT PRICE (E)	AMOUNT (F)
	CFDA Number: 15.229 DUNS Number: 785979618 Funding Opportunity No. L14AS00048 WILD HORSE AND BURRO CONTRACEPTIVE TECHNIQUES AND PROTOCOLS Account Assignment: K G/L Account: 6100.411C0 Business Area: L000 Commitment Item: 411C00 Cost Center: LLWO260000 Functional Area: L10600000.PC0000 Fund: 15XL1109AF Fund Center: LLWO260000 Project/WBS: LX.SI.RSCH0000 PR Acct Assign Line: 01 Period of Performance: 09/08/2015 to 09/07/2020				
00010	Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance (Base Award, POP: 9/8/15-9/7/18) Obligated Amount: $159,708.00 ***IMPORTANT INFORMATION*** REPORTING FREQUENCY: Performance/Progress Reports: Semi-Annual Financial Status Reports (SF-425): Semi-Annual Semi-annual reporting periods end March 31st and September 30th of each year. Reports are due within 30 days after the end of the period. REPORT SUBMISSION: Submit reports via email to "blm_or_so_fa_reports@blm.gov" with a courtesy copy (cc) to the BLM Program Officer. Financial status reports should include documentation detailing from which budget categories funds were expended. Continued ...				159,708.00

Grant and Cooperative Agreement

ITEM NO. (A)	ITEM OR SERVICE (Include Specifications and Special Instructions) (B)	QUANTITY (C)	UNIT (D)	ESTIMATED COST UNIT PRICE (E)	ESTIMATED COST AMOUNT (F)
	BLM GRANTS MANAGEMENT OFFICER (GMO): Walter B. "Bert" Ullrey Bureau of Land Management, OR/WA State Office PO Box 2965, Portland, OR 97208 Telephone: 503-808-6302 Email: wullrey@blm.gov BLM PROGRAM OFFICER (PO): Paul Griffin, Ph.D., Research Coordinator BLM Wild Horse and Burro Program 2150 Centre Ave, Building C Fort Collins, CO 80526 Telephone: 970-226-9358 Email: pgriffin@blm.gov AWARD RECIPIENT: Tracey Trujillo, Research Administrator Colorado State University 601 South Howes Street Campus Delivery 2002 Fort Collins, CO 80523-2002 Telephone: 970-491-1560 Email: tracey.trujillo@colostate.edu RECIPIENT PROJECT MANAGER/PRINCIPAL INVESTIGATOR: Dan L. Baker, Senior Scientist Colorado State University Animal Reproduction and Biotechnology Laboratory 601 South Howes Street Fort Collins, CO 80523 Telephone: 970-556-8518 Email: danbaker@colostate.edu The total amount of award: $159,708.00. The obligation for this award is $159,708.00.				

I. STATEMENT OF JOINT OBJECTIVES

A. Purpose. This financial assistance agreement is made and entered into by the Department of the Interior, Bureau of Land Management, Oregon/Washington State Office (BLM), and Colorado State University, the recipient, for the purpose of transferring something of value to the recipient in order to carry out a public purpose of support or stimulation authorized by a law of the United States. This agreement is issued under the umbrella of the Great Plains Cooperative Ecosystem Studies Unit (CESU) Cooperative and Joint Venture Agreement, BLM No. KAA119001, the terms and conditions of which include a negotiated indirect cost rate not to exceed 17.5% of Modified Total Direct Costs (MTDC).

B. Objective. The objective of this cooperative agreement is to support both refinement of existing techniques and encourage development of new techniques and protocols in the contraception or permanent sterilization of either male or female wild horses and/or burros in the field. Projects conducted in a controlled environment will have the final goal of applying the sterilization or contraception techniques to free-roaming animals on the range.

C. Authority. **The Wild Free-Roaming Horses and Burros Act of 1971, 16 USC 1336, PL 92-195, Section 6. Section 1336.** The Secretary is authorized and directed to protect and manage wild free-roaming horses and burros as components of the public lands, and he may designate and maintain specific ranges on public lands as sanctuaries for their protection and preservation…Section 1336. The Secretary is authorized to enter into cooperative agreements with other landowners and with State and local governmental agencies and may issue such regulations as he deems necessary for the furtherance of the purpose of this chapter.

1. Public Benefit. The activity(ies) to be undertaken through this assistance provide the following public benefit(s): Overpopulation of wild horses and burros is damaging to rangelands and the health of the herds. Effective population growth suppression methods for wild horses and burros by is critical to effectively manage herd population growth rates and enable healthy herds to thrive on healthy rangelands. The public benefits from enhanced enjoyment of wild horse and burro hers and healthier, more productive rangelands. Population controls help to lessen the burden on already over-grazed range.

D. Performance Goals and Measures. The activity(ies) to be undertaken through this assistance agreement will be evaluated using the following BLM Performance Measures:

1. Goals & Estimated Timelines:
    a. Determine optimal timing of re-vaccination, to achieve reduced pregnancy rates.
    b. Determine dart efficiency in delivering vaccine.
    c. Assess body condition of animals in each treatment group.
    d. Assess effects of vaccine re-inoculation on mare and neonate health.
    e. Assess potential behavioral side-effects of vaccine re-inoculation.
    f. Estimate the foaling rate for re-vaccinated animals.
2. Measures:

a. A well-designed study of treatment groups with re-vaccination at 6 month, 1 year, 2 year, and 4-years after the initial vaccination, all comparable to control animals. The measurable outcomes for all treatment groups are pregnancy and foaling rates, as measured via fecal estrogen assay, and observations of mares and any foals.

b. Weigh pneumatic syringe darts before and after firing at subject animals. The difference is attributable to vaccine that was expelled. Other measures include dart retention rate and dart failure rate recording.

c. Body condition is scored according to the Henneke system, which has clearly defined scores from 1 to 9.

d. Observe mare and foal health from a distance, via weekly observations. Observations will include search for apparent lameness and injection site side-effects.

e. Analyze behavioral observations of re-vaccinated mares; thee observations were made in 2010 and 2013; analysis would be in year 4 of this study.

f. Analyses of foaling rate will make use of data from each treatment group, using linear mixed-models, and chi-square tests to assess statistical significance of differences between treatment groups.

II. PROJECT MANAGEMENT PLAN

A. The recipient and the BLM both agree the attached proposal entitled "Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance," and dated 08/21/15, is accepted.

B. Documents Incorporated by Reference. The following recipient documents are incorporated by reference: Standard Form (SF) 424, Application for Federal Assistance, SF-424A, Budget Information - Non-Construction Programs, SF-424B, Assurances - Non-Construction Programs, Budget Detail, and Certification Regarding Lobbying - Certification for Contracts, Grants, Loans and Cooperative Agreements.

III. TERM OF AGREEMENT

A. Term. This agreement shall become effective as of the date shown on the signed award cover page. It may remain in effect for a maximum of five (5) years. The BLM will consider continued support of the project upon; (a) the recipient showing progress satisfactory to the BLM toward program goals and the determination by the BLM that continuation of the program would be in the best interests of the Government, and/or (b) the availability of funds.

B. Modifications.

1. Recipients must request prior approvals from BLM's GMO for one or more of the following program or budget-related reasons: 1). Report deviations from budget or project scope or objective, 2). Any change in the project scope, key personnel, period of performance, budgeted costs, cost share or matching, administration or any other change to this agreement constitutes a modification of the agreement.

2. All requests for modification of the agreement shall be made in writing, provide a full description of the reason for the request, and be sent to the attention of the BLM Program Officer 30 calendar days before the expiration of the agreement and/or project/budget period. Requests involving additional support or funding will require new SF-424 Applications for Federal Assistance, including new project proposals and budgets. Any determination to modify, extend the period of performance, or provide follow-on funding for continuation of a project is solely at the discretion of the BLM.

3. All modifications to the agreement shall be in writing and signed by the GMO. No oral statements or any written statements made by any person other than the GMO, shall in any manner modify or otherwise affect the terms of the agreement. All modifications to the agreement may be signed unilaterally by the GMO, including actions to suspend or terminate the agreement in accordance with 2 CFR, Subpart D. Section 200.339, Termination.

C. Budget Revisions.

1. The budget submitted as part of the SF-424 Application for Federal Assistance and approved during the award process is the financial expression of the project scope, objective or program. Recipients are required to report deviations from the approved budget and program plans and request prior approval for revisions in accordance with 2 CFR Subpart C 200.308, Revision of budget and program plans.

2. The BLM may, at its option, restrict the transfer of funds among direct cost categories or programs, functions and activities for awards in which the federal share of the project exceeds $100,000 and the cumulative amount of such transfers exceeds, or is expected to exceed, ten percent (10%) of the total budget as last approved by the BLM. No revision or transfer of funds shall be used for purposes other than those consistent with the original intent of the award.

D. Termination. This agreement may be terminated in accordance with the provisions of 2 CFR, Subpart D. Section 200.339, Termination.

IV. FINANCIAL SUPPORT

A. Funding. This agreement may be funded each fiscal year (FY) based on the availability of BLM funding.

B. Per diem fees. Financial support is to be used for reimbursement of actual costs expended. If the recipient's estimated costs include a per diem (daily allowance) budgeted cost for animal care, the recipient is reminded that funding support awarded through financial assistance agreements is to be used for reimbursement of actual costs expended and shall not be calculated or justified using per diem rates.

C. Fiscal Year (FY) Carryover. As long as expenditures are within the approved Period of Performance, funds obligated but not expended by the recipient in a FY may be carried forward and expended in subsequent years.

D. Maximum Obligations. The total obligations, including modifications, represent the amount for which the BLM will be responsible under the terms of this agreement. The BLM shall not be responsible to pay for, nor shall the recipient be responsible to perform, any effort that will require the expenditure of Federal funds above the current obligated amount.

E. Cost Sharing or Matching.

1. Cost sharing or matching for this agreement shall be in accordance with 43 CFR, Subpart C, Section 12.64 and 2 CFR 200.306.

2. There is no cost share or match legislatively required for this award.

F. Program Income. Program income generated for this agreement shall be in accordance with 2 CFR, Subpart D, Section 200.307, Program income. Unless otherwise stated, program income shall be added to the funds committed to this agreement and be used for the purposes, and under the conditions of, the grant agreement.

G. Indirect Costs. Indirect costs are approved for reimbursement under this agreement at the Cooperative Ecosystem Studies Unit (CESU) Joint Venture Agreement participant rate of 17.5% (see Section I. A. Purpose). The indirect cost base shall be the same base identified in the recipient's Federal negotiated indirect cost rate agreement (NICRA).

V. PAYMENTS

A. Automated Standard Application for Payment (ASAP) System.

1. Payments will be made by the U.S. Department of the Treasury, Financial Management Service (FMS), ASAP system. The ASAP (https://www.asap.gov) system is an online recipient-initiated payment and information system for Financial Assistance Agreements. The recipient must register and request federal funds that are due directly from the Federal Reserve Bank on a reimbursable basis.

2. The ASAP Requestor ID, furnished by the Department of the Treasury, is used for account access and requesting reimbursement payments. The BLM will create an ASAP Account ID unique to this agreement. The first ten (10) characters will be the agreement number, and the remaining characters will identify BLM funding line items. Drawdown of funds must be taken from specific lines on the agreement.

B. Advance Payments. Payments are made by the Department of the Treasury through the ASAP system within three (3) days after request. Advance payments should not be required.

C. Drawdowns.

1. Treasury Circular 1075 (31 CFR 205) requires that drawdowns to a recipient organization shall be limited to the minimum amounts needed and shall be timed to be in accordance with the actual, immediate cash requirements of the recipient organization in carrying out the purposes of the approved program or project. The timing and amount of cash advances shall be as close as is administratively feasible to the actual disbursements by the recipient organization for direct program or project costs and the proportionate share of any allowable indirect costs.

VI. PROPERTY MANAGEMENT

A. Government-Furnished Property (GFP). Tools and equipment furnished by the BLM to the recipient shall be used for official purposes only and shall be subject to the terms of the agreement. Tools and equipment shall be returned in the same condition received except for normal wear and tear in project use.

B. Property Management Provisions. Any BLM property used or other property acquired under this agreement, including intangible property such as copyrights and patents shall be governed by the property management provisions of 2 CFR, Subpart D, Sections 200.310 to 200.316, Property Standards.

C. Defensive Driving. Recipient staff will be required to complete a BLM-approved Defensive Driving Course if driving a Government-owned vehicle (GOV).

D. All-Terrain Vehicles (ATV). Recipient staff will be required to complete a BLM-approved Four-wheel ATV safety and training program if using Government-furnished ATVs.

E. Power Equipment. Recipient staff will be required to complete a BLM-approved safety and training program if using Government-furnished power equipment, such as chainsaws, wood chippers, etc. The recipient will be responsible for meeting all protective equipment requirements if using Government-furnished equipment.

VII. LIABILITY, INSURANCE, AND INDEMNIFICATION

A. Liability. The BLM assumes no liability for any actions or activities conducted under this agreement except to the extent that recourse or remedies are provided by Congress under the Federal Tort Claims Act, 28 USC 2671.

B. Indemnification. The recipient hereby agrees:

1. To indemnify the federal government, Bureau of Land Management (BLM), from any act or omission of the recipient, its officers, employees, or (members, participants, agents, representatives, agents as appropriate) (1) against third party claims for damages arising from one or more activities carried out in connection with this financial assistance agreement and (2) for damage or loss to government property resulting from such an activity, to the extent the laws

of the State where the recipient is located permit. This obligation shall survive the termination of this agreement.

2. To pay the United States the full value for all damage to the lands or other property of the United States caused by the recipient, its officers, employees, or (members, participants, agents, representatives, agents as appropriate).

3. To provide workers' compensation protection to the recipient's officers, employees, and representatives.

4. To cooperate with the BLM in the investigation and defense of any claims that may be filed with the BLM arising out of the activities of the recipient, its agents, and employees.

5. In the event of damage to or destruction of the buildings and facilities assigned for the use of the recipient in whole or in part by any cause whatsoever, nothing herein contained shall be deemed to require the BLM to replace or repair the buildings or facilities. If the BLM determines in writing, after consultation with the recipient that damage to the buildings or portions thereof renders such buildings unsuitable for continued use by the recipient, the BLM shall assume sole control over such buildings or portions thereof. If the buildings or facilities rendered unsuitable for use are essential for conducting operations authorized under this agreement, then failure to substitute and assign other facilities acceptable to the recipient will constitute termination of this agreement by the BLM.

C. Flow-down. For the purposes of this clause, "recipient" includes such subrecipients, contractors, or subcontractors as, in the judgment of the recipient and subject to the Government's determination of sufficiency, have sufficient resources and/or maintain adequate and appropriate insurance to achieve the purposes of this clause.

D. Identified Activities. All activities carried out in connection with this financial assistance agreement.

VIII. REPORTING REQUIREMENTS

A. Periodic Reporting. Submission of periodic financial, performance/progress, and (if applicable) youth employment reports is required whether or not any work has been attempted or completed and/or whether or not any funds have been drawn down or expended.

B. Federal Financial Reports.

1. Recipients of federal financial assistance are required to submit periodic financial reports which document the financial status of their awards. The Federal Financial Report form (FFR), also known as Standard Form (SF) 425, is the standard form used to report financial status. Award expenditures and/or income may be reported either on a cash or accrual basis, whichever method is normally used by the recipient. Financial reports are reviewed to identify questionable patterns of expenditures, such as accelerated or delayed drawdowns, and to assess whether performance or financial management problems exist.

2. In addition to the SF-425, the recipient must include detailed information on the costs for which the funds were used, listed by budget category as approved on their SF-424A Budget Information form. This additional information must be cumulative and may include a brief narrative describing the grant activities supported by the funds.

3. Recipients must sign the SF-425 in Box 13b certifying that the information being reported is complete, accurate, consistent with the recipient's accounting system, and that all expenditures and obligations are for the purposes set forth in the agreement. The SF-425 represents a claim to the Federal government. Filing a false claim may result in civil or criminal penalties.

4. Blank SF-425 forms and instructions are available on the Office of Management & Budget's (OMB) web site, address: http://www.whitehouse.gov/omb/grants_forms.

5. Standard Form (SF) 425 financial reports for this cooperative agreement shall be submitted: **SEMI-ANNUALLY.**

a. The first financial report shall cover from the date of award through: **March 31, 2016.**

b. Semi-annual financial reports are due 30 calendar days past the reporting period end date and each reporting period end date thereafter for the term of the agreement. The table below shows the reporting periods and their corresponding report submission due dates:

Semi-Annual Reporting Period	Submit Reports By:
October 1 to March 31	April 30th
April 1 to September 30	October 30th

c. Email Financial Reports to: BLM_OR_SO_FA_Reports@blm.gov. In addition, send courtesy copies (cc) to the BLM Program Officer(s) and Technical Advisor(s) as listed on the award cover pages.

6. Final SF-425 financial reports shall be submitted no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement.

C. Performance/Progress Status Reports

1. The recipient shall submit periodic performance reports to the GMO. The performance report must be prepared in accordance with 2 CFR, Subpart D, Section 200.328, Monitoring and reporting program performance.

2. The performance report shall include a narrative summary both of completed activities and activities in progress, a calculation of percent of completed work based on work

identified in the Recipient's submitted proposal, Project Management Plan, the reason for slippage if objectives or milestones are not met, a prediction of future activities and how they will be accomplished, and a discussion of issues and problems which may impact the ability to complete the work on time. Recommendations to overcome problems shall also be provided. In addition, the performance report should reflect the BLM Performance Measures as listed in Section III, Paragraph B. A Performance Report Template is available at our Public Web Site, address: http://www.blm.gov/or/procurement/agreements.php.

2. Performance/progress reports for this cooperative agreement shall be submitted: **SEMI-ANNUALLY.**

a. The first performance/progress report covers from the date of award through: **March 31, 2016.**

b. Annual performance/progress reports are due 30 calendar days past the reporting period end date and each reporting period end date thereafter for the term of the agreement. The table below shows the reporting periods and their corresponding report submission due dates:

Semi-Annual Reporting Period	Submit Reports By:
October 1 to March 31	April 30th
April 1 to September 30	October 30th

c. Email Performance/Progress Reports to address: BLM_OR_SO_FA_Reports@blm.gov. In addition, send courtesy copies (cc) to the BLM Program Officer(s) and Technical Advisor(s) as listed on the award cover pages.

3. Final performance/progress reports shall be submitted no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement.

E. Non-compliance: Failure to comply with the reporting requirements contained in this agreement may be considered a material non-compliance with the terms and conditions of the award. Non-compliance may result in withholding of future payments, suspension or termination of the agreement, recovery of funds paid under the agreement, and withholding of future awards.

F. Agency Review: If a recipient has a history of poor performance, financial instability, has a management system not meeting standards prescribed by the Uniform Administrative Requirements, has not conformed to the terms and conditions of the award, and/or is not otherwise responsible in safeguarding federal funds, they may be placed on Agency Review. Agency Review limits a recipient's access to funds by requiring that all drawdowns must be requested, reviewed, and approved prior to their being released. Recipients on agency review must submit a completed Standard Form (SF) 270 Request for Advance Payment or Reimbursement for each payment requested along with a detailed explanation of how the costs correspond to the approved budget categories as listed on their Application for Federal

Assistance SF-424A Budget Information and their Detailed Budget Breakdown or Challenge Cost Share Program Commitment Document, whichever is applicable. This process does not relieve the recipient of their required SF-425 financial report or performance report submission requirements.

IX. MONITORING

A. General. The recipient is responsible for oversight of the operations of the federal award supported activities to assure compliance with applicable Federal requirements and performance expectations. The BLM conducts pre-award and post-award, programmatic and financial monitoring. Depending upon the program, monitoring activities may include desk reviews (review of the award file including discussion(s) with the recipient regarding reporting, award activities, and project status), monitoring reviews (analysis of performance/progress and financial reports), and onsite or virtual site visits (discussion(s) of specific issues related to project implementation, observation of project activity, and review of planned versus actual progress).

1. Programmatic Monitoring. Program monitoring addresses the content and substance of the program. It is a qualitative review to determine performance, innovation, and contributions to the field. The BLM may make site visits as warranted by program needs. In addition, the BLM has the right of timely and unrestricted access to any books, documents, papers, or other records of the recipient's that are pertinent to the award, in order to make audits, examinations, excerpts, transcripts and copies of such document. This right also includes timely and reasonable access to recipient personnel for the purpose of interviews and discussions related to such documents.

2. Financial Monitoring. Financial monitoring ensures compliance with financial guidelines and general accounting practices. Onsite or internal financial reviews are conducted to determine if: (1) award recipients are properly accounting for the receipt and expenditures of federal funds; (2) expenditures are in compliance with federal requirements and award special conditions; and (3) proper documentation on financial monitoring activities is prepared, maintained, and distributed as appropriate.

B. Inspection. The BLM has the right to inspect and evaluate the work performed or being performed under this agreement, and the premises where the work is being performed, at all reasonable times and in a manner that will not unduly delay the work. If BLM performs inspection or evaluation on the premises of the recipient or a sub-recipient, the recipient shall furnish and shall require sub-recipients to furnish all reasonable facilities and assistance for the safe and convenient performance of these duties.

C. Audit Requirements.

1. Non-Federal entities that expend $750,000 or more in federal funds during a year shall have a single or program-specific audit conducted for that year in accordance with the Single Audit Act Amendments of 1996 (31 U.S.C. 7501-7507) and revised OMB Circular A-133, which is available at http://www.whitehouse.gov/omb/grants/grants_circulars.html .

Federal awards are defined as federal financial assistance and federal cost-reimbursement contracts that non-federal entities receive directly from federal awarding agencies or indirectly from pass-through entities. They do not include procurement contracts under grants or contracts used to buy goods or services from vendors. Non-federal entities that expend less than $750,000 for a fiscal year in federal awards are exempt from federal audit requirements for that year, except as noted in A-133, §_215(a), but records must be available for review or audit by appropriate officials of the federal agency, pass-through entity, and General Accounting Office (GAO).

2. Audits shall be made by an independent auditor in accordance with generally accepted government auditing standards covering financial audits. Additional audit requirements applicable to this agreement are found at 2 CFR, Subpart F, Section 200.501, Audits.

3. This and any other federal financial assistance award should be reported under its appropriate Catalog of Federal Domestic Assistance (CFDA) number.

4. For more information on the Single Audit process, go to the Federal Audit Clearinghouse Web Site at https://harvester.census.gov/facweb/Default.aspx.

X. KEY OFFICIALS

The key officials on this agreement are listed on the award cover page(s) and are considered to be essential to ensure maximum coordination and communication between the parties and the work being performed. Upon written notice, either party may designate an alternate to act in the place of their designated key official.

XI. STANDARD AWARD TERMS AND CONDITIONS

A. Due to changes to Federal grant regulations on December 26, 2013, the Office of Management and Budget (OMB) issued final rules for implementation of the new Uniform Guidance for grants. The Uniform Guidance consolidates guidance previously contained in the OMB circulars governing grants administration: A-21 (2 CFR Part 220), A-87 (2 CFR Part 225), A-110 (2 CFR Part 215), A-122 (2 CFR part 230), A-89, A-102, A-133, and the guidance in Circular A-50 on Single Audit Act follow-up). The new Uniform Guidance provides a streamlined format to improve clarity and consistency and may affect how you receive, manage, expend and report Federal grant funds. Currently, guidance is posted in Title 2 of the Code of Federal Regulations (CFR).

B. The U.S. Department of the Interior agencies, including the Bureau of Land Management implemented the new regulations on December 26, 2014 in the 2 CFR, Part 200—UNIFORM ADMINISTRATIVE REQUIREMENTS, COST PRINCIPLES, AND AUDIT REQUIREMENTS FOR FEDERAL AWARDS

1. Administrative and National Policy Requirements.

a. Office of Management and Budget Circulars. By accepting Federal funding under the current Federal assistance, your organization agrees to abide by the applicable OMB Circulars in the expenditure of Federal funds and performance under this program. OMB circulars are available at the following web site: http://www.whitehouse.gov/omb/circulars/

2. Administrative Requirements.

a. 2 CFR Part 200 Subparts A through D - Uniform Administrative Requirements and Cost Principles.

b. 2 CFR, Subpart B, 200.112 - Conflict of Interest

The Recipient must establish safeguards to prohibit its employees and Subrecipients from using their positions for purposes that constitute or present the appearance of a personal or organizational conflict of interest. The Recipient is responsible for notifying the Grants Officer in writing of any actual or potential conflicts of interest that may arise during the life of this award. Conflicts of interest include any relationship or matter which might place the Recipient or its employees in a position of conflict, real or apparent, between their responsibilities under the agreement and any other outside interests. Conflicts of interest may also include, but are not limited to, direct or indirect financial interests, close personal relationships, positions of trust in outside organizations, consideration of future employment arrangements with a different organization, or decision-making affecting the award that would cause a reasonable person with knowledge of the relevant facts to question the impartiality of the Recipient and/or Recipient's employees and Sub-recipients in the matter.

The Grants Officer and the servicing Ethics Counselor will determine if a conflict of interest exists. If a conflict of interest exists, the Grants Officer will determine whether a mitigation plan is feasible. Mitigation plans must be approved by the Grants Officer in writing. Failure to resolve conflicts of interest in a manner that satisfies the government may be cause for termination of the award.

Failure to make required disclosures may result in any of the remedies described in 2 CFR § 200.338, Remedies for Noncompliance, including suspension or debarment (see also 2 CFR Part 180).

Definitions: This section incorporates by reference 2 CFR Part 200, Subpart A, Acronyms and Definitions including, but not limited to the following additional terms:

(1) Conflict of Interest is defined as any relationship or matter which might place the Recipient, its employees, and/or its Subrecipients in a position of conflict, real or apparent, between their responsibilities under the agreement and any other outside interests. Conflicts of interest may also include, but are not limited to, direct or indirect financial interests, close personal relationships, positions of trust in outside organizations, consideration of future employment arrangements with a different organization, or decision-making affecting the award that would cause a reasonable person with knowledge of the relevant facts to question the impartiality of the Recipient and/or Recipient's employees and Subrecipients in the matter.

(2) Close Personal Relationship means a Federal award program

employee's childhood or other friend, sibling, or other family relations that may compromise or impair the fairness and impartiality of the Proposal Evaluator and Advisor and Grants Officer in the review, selection, award, and management of a financial assistance award.

(3) Discretionary Federal Financial Assistance means Federal awards including grants and agreements that are awarded at the discretion of the agency.

(4) Employment means:

(a) In any capacity, even if otherwise permissible, by any applicant or potential applicant for a Federal financial assistance award;

(b) Employment within the last 12 months with a different organization applying for some portion of the award's approved project activities and funding to complete them OR expected to apply for and to receive some portion of the award; and/or

(c) Employment with a different organization of any member of the organization employee's household or a relative with whom the organization's employee has a close personal relationship who is applying for some portion of the award's approved project activities and funding to complete them OR expected to apply for and to receive some portion of the award.

(5) Non-Federal entity means a State, local government, Indian tribe, institution of higher education, or nonprofit organization that carries out a Federal award as a Recipient or Subrecipient.

(6) Recipient means a non-Federal entity that receives a Federal award directly from a Federal awarding agency to carry out an activity under a Federal program. The term Recipient does not include Subrecipients.

(7) Subrecipient means a non-Federal entity that receives a subaward from a pass-through entity to carry out part of a Federal program, but does not include an individual who is a beneficiary of such program. A Subrecipient may also be a recipient of other Federal awards directly from a Federal awarding agency.

c. 2 CFR Part 200 Subpart F - Audit Requirements. Non-Federal entities that expend $750,000.00, or more, in federal awards in a single year shall have a single or program-specific audit conducted for that year in accordance with the Single Audit Act Amendments of 1996 (31 U.S.C. 7501-7507) and revised OMB Circular A-133, available at: http://www.whitehouse.gov/omb/circulars_default.

d. Indirect Facilities and Administration (F&A) Costs.

(1) 2 CFR Part 200.414 - Indirect (F&A) Costs

(2) 2 CFR, Appendix III to Part 200 - Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs)

(3) Appendix IV to Part 200 - Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Nonprofit Organizations

(4) Appendix V to Part 200 - State/Local Government-wide Central Service Cost Allocation Plans

(a) The provisions of 2 CFR 200.414(c) require Federal agencies to accept federally negotiated indirect cost rates. The BLM has applied the following policies, procedures and general decision-making criteria for deviations from negotiated Indirect Cost Rates for financial assistance programs and agreements.

(b) Distribution Basis. For all deviations to the Federal negotiated indirect cost rate, including statutory, regulatory, programmatic, and voluntary, the basis of direct costs against which the indirect cost rate is applied must be:

i The same base identified in the recipient's negotiated indirect cost rate agreement, if the recipient has a federally negotiated indirect cost rate agreement; or

ii The Modified Total Direct Cost (MTDC) base in cases where the recipient does not have a federally negotiated indirect cost rate agreement or, with prior approval of the Awarding Agency, when the recipient's federally negotiated indirect cost rate agreement base is only a subset of the MTDC (such as salaries and wages) and the use of the MTDC still results in an overall reduction in the total indirect cost recovered. MTDC is the base defined by 2 CFR 200.68, "Modified Total Direct Cost (MTDC)."

iii In cases where the recipient does not have a federally negotiated indirect cost rate agreement, under no circumstances will the Department use a modified rate based upon Total Direct Cost or other base not identified in the federally negotiated indirect cost rate agreement or defined within 2 CFR 200.68. The purpose of this restriction is to ensure that the reduced rate is applied against a base that does not include any potentially distorting items (such as pass-through funds, subcontracts in excess of $25,000, and participant support costs) and is based on the requirements outlined in 2 CFR 200.68; 2 CFR 200.414(f); 2 CFR 200 Appendix III, Section C.2.; 2 CFR 200 Appendix IV, Section B.3.f.; and Appendix VII, Section C.2.c.

(c) Indirect Cost Rate Reductions Used as Cost-Share. Instances where the recipient elects to use a rate lower than the federally negotiated indirect cost rate, and uses the balance of the unrecovered indirect costs to meet a cost-share or matching requirement required by the program and/or statute, are not considered a deviation from 2 CFR 200.414(c) as the federally negotiated indirect cost rate is being applied under the agreement in order to meet the terms and conditions of the award.

3. Program Legislation and/or Regulations. N/A

4. Standard Award Terms and Conditions.

a. Code of Federal Regulations/Regulatory Requirements, as applicable (contact your program officer with any questions regarding the applicability of the following):

(1) 2 CFR Part 25, *Universal Identifier and System of Award Management*

(2) 2 CFR Part 170, *Reporting Subawards and Executive Compensation*

(3) 2 CFR Part 175, *Award Term for Trafficking in Persons*

(4) 2 CFR Part 1400, *Government-wide Debarment and Suspension (Non-procurement)*

(5) 2 CFR Part 1401, *Requirements for Drug-Free Workplace (Financial Assistance)*

(6) 43 CFR 18, *New Restrictions on Lobbying*: Submission of an application also represents the applicant's certification of the statements in 43 CFR Part 18, Appendix A, *Certification Regarding Lobbying*.

(7) 41 USC §4712, *Pilot Program for Enhancement of Recipient and Sub-recipient Employee Whistleblower Protection*: This requirement applies to all awards issued after July 1, 2013 and shall be in effect until January 1, 2017.

(8) 41 USC §6306, *Prohibition on Members of Congress Making Contracts with Federal Government*: No member of or delegate to the United States Congress or Resident Commissioner shall be admitted to any share or part of this award, or to any benefit that may arise therefrom; this provision shall not be construed to extend to an award made to a corporation for the public's general benefit.

(9) Executive Order 13513, *Federal Leadership on Reducing Text Messaging while Driving*: Recipients are encouraged to adopt and enforce policies that ban text messaging while driving, including conducting initiatives of the type described in section 3(a) of the order.

(10) Executive Order 13658, *Minimum Wage for Contractors*, seeks to increase the efficiency and cost savings in the work performed by parties who contract with the Federal Government by increasing the hourly minimum wage paid by those contractors (see 79 CFR 9851). The Executive Order requires agencies to include a clause in applicable contracts and Contract like instruments that specifies, as a condition of payment, that the Executive Order Minimum wage be paid to workers in the performance of the contract and any subcontracts.

(11) Executive Order 13043, *Increase Seat Belt Use in the United States* Recipients of grants/cooperative agreements and/or sub-awards are encouraged to adopt and enforce on-the-job seat belt use policies and programs for their employees when operating company-owned, rented, or personally owned vehicles. These measures include, but are not limited to, conducting education, awareness, and other appropriate programs for their employees about the importance of wearing seat belts and the consequences of not wearing them.

(12) Opposition to Any Legislation. In accordance with the Department of the Interior, Environment, and Related Agencies Act, 2006, Title IV, Section 402, no part of any appropriation contained in this Act shall be available for any activity or the publication or distribution of literature that in any way tends to promote public support or opposition to any legislative proposal on which Congressional action is not complete other than to communicate to Members of Congress as described in 18 U.S.C. 1913.

(13) Metric Conversion. All performance and final reports, other reports, or publications, produced under this agreement, shall employ the metric system of measurements to the maximum extent practicable. Both metric and inch-pound units (dual units) may be used if necessary during and transition period(s). However, the recipient may use non-metric measurements to the extent the recipient has supporting documentation that the use of metric measurements is impracticable or is likely to cause significant inefficiencies or loss of markets to the recipient, such as when foreign competitors are producing competing products in non-metric units.

(14) Reimbursable Costs and Limitations. The recipient shall not incur costs or obligate funds for any purpose pertaining to operation of the program or activities beyond the expiration date stated in the agreement. The only costs which are authorized for a period of up to 90 days following the award expiration date are those strictly associated with closeout activities for preparation of the final report.

(15) The BLM's financial participation is limited. The BLM will only fund up to its share of those amounts requested in the project proposal and as are subsequently approved and funded in the agreement. The recipient shall not be obligated to continue performance under the agreement or to incur costs in excess of the costs set forth in the proposal and subsequent agreement. However, if the Recipient chooses to expend funds in excess of the approved project budget, the Recipient will be responsible to fund the excess without funding participation by the Bureau.

(16) 2 CFR, Part 200 Procurement Standards.

(17) 200-317 Procurement by States. When procuring property and services under a Federal award, a state must follow the same policies and procedures it uses for procurements from its non-Federal funds. The state will comply with §200.322 Procurement of recovered materials and ensure that every purchase order or other contract includes any clauses required by section §200.326 Contract provisions.

(18) All other non-Federal entities, including subrecipients of a state, will follow §§200.318 General procurement standards through 200.326 Contract provisions.

(19) §200.318 General procurement standards.

(20) §200.319 Competition.

(a) All procurement transactions must be conducted in a manner providing full and open competition consistent with the standards of this section. In order to ensure

objective contractor performance and eliminate unfair competitive advantage, contractors that develop or draft specifications, requirements, statements of work, or invitations for bids or requests for proposals must be excluded from competing for such procurements.

(21) §200.320 Methods of procurement to be followed.

(a) The non-Federal entity must use one of the following methods of procurement:

i Procurement by micro-purchases.

ii Procurement by small purchase procedures.

iii Procurement by sealed bids (formal advertising).

(22) Compliance with Buy American Act. NOTICE: Pursuant to Section 307 of the Omnibus Consolidated Appropriations Act of 1997, Public Law 104-208, 110 Stat. 3009, please be advised of the following: In the case of any equipment or product that may be authorized to be purchased with financial assistance provided using funds made available in this act, it is the sense of the Congress that entities receiving the assistance should, in expending the assistance, purchase only American-made equipment and products.

(23) Endorsements.

(a) Recipient shall not publicize or otherwise circulate, promotional material (such as advertisements, sales brochures, press releases, speeches, still and motion pictures, articles, manuscripts or other publications) which states or implies governmental, Departmental, bureau, or government employee endorsement of a product, service, or position which the recipient represents. No release of information relating to this award may state or imply that the Government approves of the recipient's work products, or considers the recipient's work product to be superior to other products or services.

(b) All information submitted for publication or other public releases of information regarding this project shall carry the following disclaimer:

> *The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Government. Mention of trade names or commercial products does not constitute their endorsement by the U.S. Government.*

(c) Recipient must obtain prior Government approval for any public information releases concerning this award which refer to the Department of the Interior or any bureau or employee (by name or title). The specific text, layout photographs, etc. of the proposed release must be submitted with the request for approval.

(d) A recipient further agrees to include this provision in a subaward to and subrecipient, except for a subaward to a State government, a local government, or to a federally recognized Indian tribal government.

(e) See also XII. SPECIAL TERMS AND CONDITIONS, B. Data Management, Paragraph 6, below.

(24) Intangible Property and Rights to Data.

a. Recipients are subject to the administrative standards set forth in 2 CFR, Subpart D, Sections 200.310 to 200.316, Property Standards.

b. See also XII. SPECIAL TERMS AND CONDITIONS, B. Data Management, below.

(25) Retention and Access Requirements for Records.

a. All recipient financial and programmatic records, supporting documents, statistical records, and other grants-related records shall be maintained and available for access in accordance with 2 CFR, Subpart D, Sections 200.333 through 200.337, Record Retention and Access.

b. Inspector General's (IG's) Office Access to Records - Recipients shall provide additional access for the IG's office to examine recipient's records and to interview officers/employees of recipient.

(25) Order of Precedence. Any inconsistency in this agreement shall be resolved by giving precedence in the following order: (a) Any national policy requirements and administrative management standards; (b) 43 CFR Part 12; (c) requirements of the applicable OMB Circulars and Treasury regulations; (d) special terms and conditions; (e) all agreement sections, documents, exhibits, and attachments; and (f) the recipient's project proposal.

XII. SPECIAL TERMS AND CONDITIONS

A. Scientific Integrity. Scientific integrity is vital to Department of the Interior (DOI) activities under which scientific research, data, summaries, syntheses, interpretations, presentations, and/or publications are developed and used. Failure to uphold the highest degree of scientific integrity will result not only in potentially flawed scientific results, interpretations, and applications but will damage DOI's reputation and ability to uphold the public's trust. All work performed must comply with the DOI Scientific Integrity Policy posted to http://www.doi.gov, or its equivalent as provided by their organization or State law.

B. Data Management.

1. Recipients should follow practices and guidelines for data management that are commensurate with those required by the National Institutes of Health (NIH), and by their own

university. The following guidelines for the sharing of research results are based on NIH standards:

2. The results and accomplishments of activities funded by the BLM should be made available to the public. Principal Investigators (PI) and awardee organizations are expected to make the results and accomplishments of their activities available to the research community and to the public at large. If the outcomes of the research result in inventions, the provisions of the Bayh-Dole Act of 1980, as implemented in 37 CFR 401, apply. As long as awardees abide by the provisions of the Bayh-Dole Act, as amended by the Technology Transfer Commercialization Act of 2000 (P.L. 106-404), and 37 CFR 401, they have the right to retain title to any invention conceived or first actually reduced to practice using BLM financial assistance funds.

3. In general, awardees own the rights in data resulting from a project supported by a BLM financial assistance agreement (grant or cooperative agreement). Special terms and conditions of the award may indicate alternative rights, e.g., under a cooperative agreement or based on specific programmatic considerations as stated in the applicable Request for Applications (RFA). Except as otherwise provided in the terms and conditions of the award, any publications, data, or other copyrightable works developed under a BLM cooperative agreement may be copyrighted without BLM approval. For this purpose, "data" means recorded information, regardless of the form or media on which it may be recorded, and includes writings, films, sound recordings, pictorial reproductions, drawings, designs, or other graphic representations, procedural manuals, forms, diagrams, work flow charts, equipment descriptions, data files, data processing or computer programs (software), statistical records, and other research data.

4. Rights in data also extend to students, fellows, or trainees under awards whose primary purpose is educational, with the authors free to copyright works without BLM approval. In all cases, BLM must be given a royalty-free, nonexclusive, and irrevocable license for the Federal government to reproduce, publish, or otherwise use the material and to authorize others to do so for Federal purposes. Data developed by a consortium participant also is subject to this policy.

5. As a means of sharing knowledge, BLM encourages awardees to arrange for publication of BLM-supported original research in primary scientific journals. Awardees also should assert copyright in scientific and technical articles based on data produced under the award where necessary to effect journal publication or inclusion in proceedings associated with professional activities.

6. All awardees must acknowledge Federal funding when issuing statements, press releases, requests for proposals, bid invitations, and other documents describing projects or programs funded in whole or in part with Federal money. Each publication, press release, or other document about research supported by a BLM award must include:

1. An acknowledgment of BLM support such as:

"Research reported in this [publication, release] was supported by the Bureau of Land Management under award number [cite the proper agreement number here]."

2. A disclaimer that says:
"The content is solely the responsibility of the authors and does not necessarily represent the official views of the Bureau of Land Management."

7. If the awardee plans to issue a press release about research supported by a BLM Wild Horse and Burro (WHB) program award, it should notify the BLM WHB program in advance to allow for coordination.

C. Publications.

1. Publications resulting from work performed under a BLM financial assistance-supported project must be included as part of the semi-annual or final Performance/Progress report submitted to the BLM. When publications are available electronically, the URL or the PMCID number must be provided. If not available electronically, one copy of the publication may be provided along with the progress report.

2. In addition to any requirements listed in the Project Management Plan, two (2) copies of each applicable publication produced under this agreement shall be sent to the Natural Resources Library with a transmittal that identifies the sender and the publication, and states that the publication is intended for deposit in the Natural Resources Library. Publications shall be sent to the following address:

U.S. Department of the Interior
Natural Resources Library
Interior Service Center
Gifts and Exchanges Section
1849 C Street, N.W.
Washington, D.C. 20240

D. Recipient/Subrecipient Personnel Security and Suitability Requirements

1. As implemented by Homeland Security Presidential Directive-12 (HSPD-12), if performance of this agreement requires recipient/subrecipient personnel to have a Federal government-issued Personal Identity Verification (PIV) credential before being allowed unsupervised access to a DOI facility and/or information system, the Program Officer will be the sponsoring official and will make the arrangements through a DOI Access Card Sponsor for personal identity verification and DOI Access Card issuance.

2. At least two weeks before start of agreement performance, the recipient must identify all recipient and subrecipient personnel who will require physical and/or logical access for performance of work under this agreement. Physical Access means routine, unescorted or unmonitored access to non-public areas of a Federally-controlled facility. Logical Access means routine, unsupervised access to a Federally-controlled information system. The recipient and

subrecipient must make their personnel available at the place and time specified by the Program Officer in order to initiate screening and background investigations. The following forms, or their equivalent, may be used to initiate the credentialing process:

a. OPM Standard Form 85 or 85P

b. OF 306

c. National Criminal History Check (NCHC) (local procedures may require the fingerprinting to be done at a police station; in this case, any charges are to be borne by the recipient or subrecipient, as applicable)

d. Release to Obtain Credit Information

e. PIV card application (web-based)

3. Before starting work under this agreement, a National Criminal History Check (NCHC) will be initiated to verify the identity of the individual applying for clearance and to determine the individual's suitability for the position. If the NCHC adjudication is favorable, a DOI Access Card will be issued for that individual. If the adjudication is unfavorable, the credentials will not be issued and the recipient or subrecipient must make other arrangements for performance of the work. In the event of a disagreement between the recipient/subrecipient and the Government concerning the suitability of an individual to perform work under this agreement, DOI shall have the right of final determination.

4. Recipient and subrecipient employees must give, and authorize others to give, full, frank, and truthful answers to relevant and material questions needed to reach a suitability determination. Refusal or failure to furnish or authorize provision of information may constitute grounds for denial or revocation of credentials. Government personnel may contact the recipient or subrecipient personnel being screened or investigated in person, by telephone or in writing, and the recipient or subrecipient must ensure they are available for such contact.

5. Alternatively, if an individual has already been credentialed by another agency through the Office of Personnel Management (OPM), and that credential has not yet expired, further clearance may not be necessary. In that case, the recipient/subrecipient must provide the sponsoring office with documentation that supports the individual's credentialed status.

6. Recipient and subrecipient employees who have been successfully adjudicated will be issued DOI Access Cards, which must be activated at a USAccess Credentialing Center. Those Recipient or subrecipient employees not located within a reasonable travel time of a USAccess Credentialing Center will be screened and issued alternate credentials, such as temporary access badges.

7. During performance of this agreement, the recipient must keep the Program Officer apprised of changes in personnel to ensure that performance is not delayed by compliance with credentialing processes. Cards that have been lost, damaged, or stolen must be reported to the

Program Officer, Grants Management Officer, and Issuing Office within 24 hours. If reissuance of expired credentials is needed, it will be coordinated through the Program Officer.

8. At the end of this agreement's performance, or when a recipient/subrecipient employee is no longer working under this agreement, the recipient will ensure that all identification cards are returned to the Program Officer.

E. Federal Information Systems Security Awareness Training. Before the recipient, or any of its employees or subrecipients, are granted access to the BLM Federal computer system, they must first successfully complete the U.S. Department of the Interior's (DOI) Federal Information Systems Security Awareness Online Course. This course was designed specifically for users of Federal computer systems. The course is a Web-based training product that explains the importance of Information Systems Security and takes approximately one hour to complete. This course is mandatory for all DOI employees, contractors, recipients, and all other users of DOI computer resources. Topics covered in the course include: threats and vulnerabilities, malicious code, user responsibilities, and new developments affecting Information Systems Security.

END OF AGREEMENT

BUREAU OF LAND MANAGEMENT
OR-WA State Office
Grants & Cooperative Agreements

PROJECT PROPOSAL

Instructions: A Project Proposal must be submitted with the Standard Form (SF) 424 Application for Financial Assistance for all Financial Assistance Agreements. A new proposal must be included with any request for modification which involves a revision to funding, project scope, period of performance, or key personnel.

Agreement or Funding Opportunity No.: L14AS00048 Date: 08/21/15

Organization Name: Colorado State University

Project Title: Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance

Current Funding Estimated Period of Performance (PoP): **08/24/15** to 8/23/20

Name & Title of Person Submitting Proposal: Dan L. Baker, Senior Scientist
Terry M. Nett, Senior Scientist

* If a specific start work date is needed, contact your BLM Program Officer. Do not start work without prior approval from the Grants Management Officer.

1. Purpose, Objectives, and Relevance:

(Describe why the project is needed, the applicant's objectives, how the applicant's objectives support their mission, and how this project benefits the general public.)

BACKGROUND

1. Re-immunization

In many areas of the western United States, overabundant and rapidly expanding populations of feral horses (*Equus caballus*) pose a significant dilemma for natural resource managers. The Wild Free-Roaming Horses and Burros Act of 1971(P.L.92-195) provided protection for feral horses and burros (*Equus asinus*) on most federal lands and established guidance for their management as a wildland species (Wagner 1983). There is, however, wide-spread concern among state, federal, and private land management agencies that unregulated feral horse populations are severely altering native plant communities and limiting the abundance and diversity of habitat resources allocated for native wildlife and other domestic livestock species.

Current population control methods such as utilizing periodic roundups and adoption or sale of excess animals, or maintaining excess feral horses in long-term holding facilities are expensive, resource intensive, and unsustainable. Clearly, more efficient, cost effective, and humane approaches to reducing feral horse densities on public lands are needed. Controlling the fertility of female horses offers a potential non-lethal alternative to conventional methods (National Research Council 2013).

A promising immunological approach to contraception in feral horses involves

immunization against the neuropeptide gonadotropin releasing hormone (GnRH). Scientists at the National Wildlife Research Center (NWRC) have conjugated synthetic GnRH peptides to a highly immunogenic carrier protein that, when combined with a potent adjuvant, stimulates the host's immune system to produce antibodies that bind to endogenous GnRH. This, in turn, prevents synthesis and secretion of important downstream reproductive hormones necessary for reproduction. Animals generally return to fertility as antibodies concentrations decline (Powers et al. 2011).

Multiple years of infertility have been achieved in captive and free-ranging wild ungulates with a single inoculation with the GnRH-based vaccine, known as GonaCon. This vaccines has been experimentally tested and found to provide multiple years of infertility after a single application in white-tailed deer (*Odocoileus virginianus)(* Miller et al. 2008, Gionfriddo et al. 2011*a*), bison (*Bison bison)(* Miller et al. 2004), elk (*Cervus elaphus)(* Killian et al. 2009, Powers et al. 2011, 2014), wild pig (*Sus scrofa)(* Massei et al. 2012), and feral horses (Killian et al. 2008, Gray et al. 2010, Baker et al. 2013). However, multiple years of infertility are only experienced in a fraction of vaccinated animals. In free-ranging elk, there was approximately a 90% treatment effect the first year after vaccination but that dropped to 50% by the second year and by the third year of the study, there was no measureable response (Powers et al. 2014). Similarly, during the first 3 years of our current investigation in feral horses at THRO, we observed a 25-35% decrease in foaling in treated versus control mares for the first and second years of the study but no effect by year three (Baker et al. 2013).

Repeat vaccinations generally result in a more profound and longer-lasting antibody production due to the anamnestic response (Tizard 1982). Therefore, we expect longer- lasting contraceptive effects in re-vaccinated mares. The single-injection GonaCon vaccine is unique in that the formulation initiates high antibody titers that remain elevated in some applications; however, to our knowledge, no research has been conducted to evaluate booster doses of this vaccine in any mammalian species.

Booster immunizations using a variety of GnRH vaccines in domestic horses have been shown to improve contraceptive efficacy and to suppress behavioral and physiological estrus (Garza et al.1986, Elhay et al. 2007, Botha et al 2008). However, these GnRH vaccines differ from GonaCon in that they incorporate different protein carrier molecules and adjuvants, and are formulated for short duration (< 1 yr.) contraceptive effectiveness that is generally achieved by using a primary immunization followed 35 days later by a booster inoculation.

While a single vaccination is often preferred from a management perspective, GonaCon vaccine may prove to be more effective if repeat vaccinations are delivered on a periodic basis. Efficacy data collected from 25 mares treated with single application of GonaCon in 2009, at Theodore Roosevelt National Park (THRO) revealed a moderate 2-year decline of approximately 30% in foaling rates, with all mares regaining fertility by three years post-primary vaccination treatment (Baker et al. 2013). Surprisingly, re-vaccination of these same mares in the fall 2013 (four years post-primary vaccination) has resulted to date, in complete infertility during the 2015 foaling season (the first season to expect a re-vaccination effect on fertility). Clearly, these results are both statistically and biologically significant, as well as encouraging from a fertility control perspective.

If these results persist over time and these mares remain infertile, it would lend support to our hypothesis that re-vaccination with GonaCon, even four years post-primary vaccination produces a strong anamnestic response in horses that stimulates anti-GnRH antibodies and suppresses fertility. At present, however, it is premature to predict how many of these re-vaccinated mares failed to conceive during the 2014 breeding season and will not foal or regain fertility during 2015 and beyond. It is possible that the booster vaccination simply delayed the estrous cycle in these mares, which could result in foals being born later in the foaling season.

While these findings are tentative and inconclusive, they suggest that repeat vaccinations are likely needed to achieve high efficacy of GonaCon vaccine in free-ranging horses and these

effects have not been investigated or determined. Thus, our proposed research offers a unique opportunity to address this question at THRO and will have relevance, not only to feral horses, but also to other wild ungulates that have been treated with a single treatment of GonaCon vaccine. Our proposed research will begin to define the vaccination schedule needed to maintain infertility in free-ranging horses and whether or not long-term or permanent sterility is a possible outcome. We will investigate the safety and efficacy of a repeat vaccination under the hypothesis that this vaccine will be more efficacious and longer-lasting than the original primary immunization.

2. Remote Dart Delivery

Fundamental to practical field application of GonaCon vaccine in free-ranging horses is a safe, reliable, and effective method of administering a single dose of the vaccine to free-ranging horses by means of a syringe dart. Many contraceptive agents have been successfully applied via syringe dart or biodegradable implant to an assortment of wild ungulate species including white-tailed deer (Turner et al. 1992, Jacobsen et al. 1995, DeNicola et al. 1997), elk (Shideler et al. 2002, Baker et al. 2005), feral horses (Kirkpatrick et al.1990, Roelle and Ransom 2009), and elephants (*Loxodonta Africana*) (Delsink et al. 2002). However, to our knowledge, evaluation of remotely-delivered GonaCon vaccine is limited to one field investigation with white-tailed deer (DeNicola unpublished data). Although dart performance in this study was less than expected, it provided important basic information regarding optimum dart configuration and delivery ballistics. Using this preliminary data, technicians at Pneu-Dart, Inc. developed a prototype dart configuration for delivering this highly viscous vaccine formulation to free-ranging horses.

We tested this GonaCon-specific dart delivery system with captive feral horses at the 2013 scheduled roundup at THRO. Eleven adult mares (2-4 years of age), that had not been previously vaccinated, were held in small paddocks and remotely darted in the biceps femoris muscle with 2 ml (2000 µg) of GonaCon vaccine. All darts were weighed (± 0.01g) before and after injection to determine the precise dose delivered. Darting distance varied from 10-15 m. Nine out of 11 darts delivered, on average, 95% of the GonaCon vaccine formulation. Two darts failed to discharge possibly due to low muzzle velocity. All darts appeared to dispense the vaccine deep into the muscle mass and none of the darts were observed to bounce without penetration, partially discharge, blow-out, or show evidence of subcutaneous delivery of the vaccine. The two horses in which the darts failed to discharge were subsequently re-treated and the second darts successfully delivered a full dose. With 85% of the 2015 foaling season complete, 7/11 (63%) of these mares have not foaled. In contrast, only 16% of the untreated mares have not foaled to date. A dependable dart delivery system for administering GonCon remotely to free-ranging horses is critical to the determination of an optimum re-vaccination schedule in our proposed study. If successful, this technology will potentially provide resource managers with an alternative strategy for managing this feral horse population.

3. Biological Side-Effects

Evaluation of the biological side-effects of GonaCon vaccine treatments have been reported for numerous wild ungulate species including white-tailed deer (Curtis et al. 2008, Gionfriddo et al. 2011*b*), elk (Powers et al. 2011, 2012, 2014), bison (Miller et al. 2004) and feral horses (Baker et al. 2013). Results from these investigations generally conclude that GonaCon does not cause serious adverse effects on general health, body condition, existing pregnancy, neonatal health, major organ systems, or fertility of male and female offspring of females treated during pregnancy.

Granulomatous intramuscular injection-site lesions, that occasionally break and drain as abscesses, are the only adverse effect of vaccination consistently reported in these studies. The formation of these injection site lesions may be necessary for stimulation of a strong immune response and infertility. GonaCon vaccine contains AdjuVac; a water-in-oil based adjuvant

developed from a USDA approved Johnes disease vaccine called Myocopar™ (Fort Dodge Animal Health). AdjuVac contains killed *Mycobacterium avium,* which is needed to induce a rapid, strong, and sustained contraceptive response (Miller et al. 2008*a*, Perry et al. 2008). This combination of water - in- oil emulsion and killed mycobacteria results in a highly potent adjuvant that stimulates both humoral and cellular immunity (Warren et al. 1986).

Vaccines, like GonaCon, that contain mycobacteria may induce strong immune responses because of the formation of a repository or depot at the injection site (Fukanoki et al. 2000). In response to the presence of the depot, a granuloma forms as the immune system attempts to isolate the foreign material. The continued existence of this depot, which initiates a chronic inflammatory response, likely provides a long-term source of antigen stimulation and persistent antibody production. We speculate that this is the mechanism by which a single vaccination can provide multiple years of infertility in a portion of the population in many species that have been studied.

However, even with this prolonged antigenic stimulation, the immune response from a single vaccination does not consistently provide multiple years of infertility in all or even a high proportion of animals (Powers et al. 2014, Baker et al. 2013). In all studies, where post-mortem examinations were performed, prevalence of injection-site inflammation and granulomas were present but in some species, such as white-tailed deer and elk, they were not apparent antemortem (Curtis et al. 2008, Powers et al. 2011, Gionfriddo et al. 2011*b*).

In contrast to these species, injection site reactions in feral horses, following GonaCon vaccination at THRO, are readily observable as subcutaneous swellings. In past studies at THRO (2009-2013), all injection site reactions appeared to be confined to the general gluteus muscle where the vaccine was first hand-injected. Reactions to the vaccine were first observed 30 days post-treatment in 17.2% (5/29) of mares and by the second breeding season, 79.3% (23/29) of treated females showed some evidence of inflammation or swelling at the injection site. Saline control mares displayed no evidence of injection site reactions. Swellings of various sizes (marble to baseball size) were most common, followed by nodules, and rarely a draining abscess. Most of these reactions were observable for three years post-treatment, then began to resolve and become less visible by year 4 (many that could not be visually observed were still manually palpable at the 2013 roundup).

However, similar to other studies where injection site reactions have been evaluated, we did not observe any clinical evidence of lameness, impaired mobility, depression, or decreased health or fitness in any animal that was associated with GonaCon vaccine treatment. While results from the above investigations are generally consistent relative to the effects of GonaCon-induced injection site reactions, they are also limited to the consequences of a single vaccination usually delivered by hand-injection.

At the 2013 THRO round-up, GonaCon –treated mares were re-vaccinated, four years post-primary vaccination, with a booster dose on the opposite side in the biceps femoris muscle. This investigation is in progress but thus far, injection site reactions appear to be less apparent than those observed following the 2009 vaccination (Baker et al. unpublished). At this time, the cumulative effects of re-vaccination are unknown and the potential for more intense immune reactions with additional doses of this vaccine delivered by syringe dart is a consideration (Broderson 1989, Roelle and Ransom 2009).

4. Behavioral Side-Effects

Behavioral side-effects of GonaCon vaccination in wild ungulates have not been extensively investigated (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014). Given the physiological mechanism of action, GonaCon vaccine has the potential to suppress fertility and diminish the reproductive behaviors typically associated with estrus. However, in GonaCon-vaccinated female elk (Powers et al. 2011) and free-ranging horses (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014) such behaviors were maintained throughout the first breeding season

after immunization and were not different from untreated females.

In a previous study at THRO during 2009-2010, daily activity patterns, social interactions, and reproductive behaviors were similar for GonaCon treated and control mares (Baker et al. 2012, Ransom et al. 2014). But, since GonaCon only prevented conception in 50% of treated mares (n = 28), behavioral observations were limited to only 14 infertile females. Thus, inferences to free-ranging feral horse populations are not definitive and deserve further investigation prior to use in management applications.

In an attempt to further our understanding of the behavioral side-effects GonaCon vaccine, we conducted behavioral observations during the first breeding season following re-vaccination of these same mares at THRO in 2013. We measured the effects of this vaccine on sociosexual behavior, harem dynamics, and activity budgets of treated (n = 25) and control (n =25) horses. To date (July 20 2015), none of the re-vaccinated mares have foaled, whereas 84% (21/25) of the control mares have done so. As a result of higher vaccine efficacy in treated mares, our sample size increased by 44% and offered a more rigorous quantitative investigation into potential effects of GonaCon treatment on feral horse behaviors.

5. Population Modeling

We will integrate contraceptive efficacy and population monitoring data at THRO to estimate parameters and unobserved states in a Bayesian hierarchal model (Dulberger et al. 2010, Monello et al. 2014, Hobbs and Hooten 2015, Hobbs et al. 2015, Rahio et al. in review).We will use the model to evaluate the population-level effects of GonaCon on the free-ranging horse population at THRO. We will forecast the consequences of alternative contraceptive strategies on population performance with rigorous evaluation of uncertainty. There is an urgent need to extend studies of efficacy of individuals to populations (Ransom et at. 2014). A key extension of our experimental research is to determine the effects of different GonaCon delivery regimes on the growth rate of the THRO population.

OBJECTIVES:

The primary objectives of this research are:

a) to begin to determine the optimum and most effective re-vaccination schedule with GonaCon vaccine for suppressing reproductive rates in free-ranging horses, the duration of effectiveness, and the return to fertility following treatment.

b) to determine the safety and physiological side-effects (if any) in feral horses following re-vaccination with GonaCon including visual assessment of general health, body condition, injection site reactions, effects on current pregnancy, and neonatal health and survival.

c) to determine the effects of GonaCon vaccination on the behavioral side-effects (if any) in free-ranging horses including quantitative assessment of the effects on daily activity patterns and social interactions.

d) to develop and test a safe and effective dart configuration and injection system for remotely administering GonaCon vaccine to free-ranging horses by means of a syringe dart.

e) to develop a Bayesian model to forecast the consequences of different GonaCon vaccine treatments on feral horse population dynamics at THRO.

HYPOTHESIS:

H1: Female feral horses re-vaccinated with GonaCon will show significantly ($P \leq 0.05$) lower reproductive (yearly pregnancy and foaling) rates than non-treated control mares and contraceptive efficacy of re-vaccinated mares will be greater and longer lasting than that observed following the initial immunization.

Rationale: An immune response is a physiologic reaction to a foreign substance or antigen; especially one mediated by lymphocytes and involving recognition of antigens by specific antibodies or previously sensitized lymphocytes. Vaccines rely on the anamnestic response for optimal function. This response is a renewed rapid production of antibodies on the second (subsequent) encounter with the same antigen. This reaction is possible through memory cells that store information regarding the recognition of an antigen based upon previous exposure. Booster or repeat vaccinations generally result in a more rapid and stronger immune reaction to a second inoculation with the same antigen (Tizard 1982). However, the optimum re-vaccination schedule for GonaCon vaccine in feral horses or any other ungulate species has not yet been investigated or determined.

2. Technical Approach:

(Describe how the project will be conducted. The project design must contain enough detail to show the development of the project, including the relationship between the partners, milestones, and objectives. Clearly describe the techniques, procedures, and methodologies to be used; the data collection, analysis, and means of interpretation; the expected results and/or outcomes; and the procedures for evaluating project effectiveness, including appropriate performance measures and the probabilities of obtaining them.)

EXPERIMENTAL DESIGN AND METHODS

Study area and experimental horses

Theodore Roosevelt National Park (THRO) is located near the town of Medora in southwestern North Dakota (45º 55' N/103º 31'W) and consists of two units that are separated by approximately 115 km of federally and privately owned rangeland. The South Unit of the park, where this study will be conducted, comprises 19,000 ha and consists of eroded badlands with gullies and ravines separated by upland plateaus and small erosion-resistant buttes (Laird 1950). All feral horses used in these experiments are free-ranging and permanently reside in this unit of the park.

At present, there are approximately170 horses divided into roughly 10-15 individual bands and bachelor groups. Horses and bison are confined to the South Unit by a 1.8 to 2.4-m woven wire boundary fence. Feral horse history, distribution, habitat use, and population management at THRO have been previously described (Marlow et al. 1992). Individual horses are known by unique markings and band affiliations. Age and reproductive genealogy data for each animal has been retained in a database since 1993. The approximate date of birth (± 30 days) is known for each horse. Photographs have been taken of each mare from birth to adulthood to assist in the identification of individual horses.

Experimental treatments

In order to determine the optimum re-vaccination schedule for GonaCon vaccine in free-ranging

horses at THRO, we propose four post-primary vaccination treatment intervals of: a) four years, b) two years, c) one year, and d) six months (Table 1). The numbers of experimental treatments are limited by the availability of adult mares currently residing in the park. All experimental mares participating in these experiments have been assimilated into various bands such that each band contains one or more individuals from these treatment groups as well as untreated control mares.

Table 1. Summary of primary and secondary vaccination schedules and sample sizes for each experimental group of feral horses treated with GonaCon Immunological Vaccine or saline at THRO.

RE-VACCINATION TREATMENT	SAMPLE SIZE (N)	DATE OF PRIMARY VACCINATION	DATE OF SECONDARY VACCINATION
FOUR YEARS POST-PRIMARY	25	OCT - 2009	SEPT - 2013
TWO YEARS POST-PRIMARY	11	SEPT - 2013	SEPT - 2015
ONE YEAR POST-PRIMARY	16	SEPT - 2015	SEPT - 2016
SIX MONTH POST-PRIMARY	16	SEPT - 2015	MAR - 2016
SALINE CONTROL	25	OCT – 2009	SEPT - 2013

A description of each treatment group, the method of treatment application, and pertinent measurements and observations are presented below:

1) Four-year post-vaccination group. This experimental group was initially established and treated during the scheduled roundup at THRO in 2009. Ongoing measurements of foaling rates and biological side-effects following re-vaccination in 2013 are currently being conducted and will provide a four-year post-primary re-vaccination treatment group (n = 25) and control group (n = 25).

Experimental animals and treatment application: During a scheduled NPS gather and removal in September 2013, horses were herded by helicopter into permanent corrals and handling facilities. Fifty, adult mares (5-19 years of age) (25 GonaCon -treated: 25 saline-control) that had been previously vaccinated with a single inoculation of GonCon- or saline solution in October 2009 were identified and retained within the park for this experiment. Band stallions were also retained. All mares were identified individually using a photographic data base of pelage color and band association, as well as, previously implanted passive integrated transponder (PIT) tags. General health, pregnancy status, and body condition of each animal was assessed while horses were restrained in a hydraulic squeeze chute. Pregnancy status and approximate stage of gestation were determined using rectal palpation of the reproductive tract and transrectal ultrasound imaging (Bucca et al. 2005). Up to 50 mls of blood was collected and serum removed, frozen, and archived for future anti-GnRH antibody analyses (Powers et al. 2011). We collected hair samples from all horses to assess the genetic status of the population and fecal samples for pregnancy determination and prevalence of endoparasites. Body condition of mares was assessed and scored visually according to methods described by Henneke et al. (1983). Mares in the treatment group received an intramuscular booster inoculation, by hand-syringe, containing 2000μg (2 ml) of GonaCon (synthetic GnRH conjugate Blue Carrier protein and emulsified in

AdjuVac™ adjuvant (Miller et al. 2008) in the middle gluteus muscle on the opposite side from the primary vaccination. Mares in the control group were injected in the same way with an equal volume of saline solution. These treatments and procedures were identical to the ones used in 2009 except that injections were given on the right side of the body in 2013 rather than the left to allow differentiation from the previous injection site.

2) Two-year post-vaccination group. This vaccine treatment was applied at the 2013 scheduled roundup at THRO to investigate remote delivery of GonaCon vaccine. Re-vaccination of these mares in 2015 will provide a two-year post-vaccination treatment group.

Experimental animals and treatment application. Based on the promising results from the captive trial conducted in 2013, we will extend our evaluation of a remote dart delivery system of GonaCon from a controlled captive setting to a field test with these same mares that are now free-roaming in their respective bands at THRO. This field application will also provide an additional cohort of mares that have been re-vaccinated two years post-primary vaccination. During September 2015, the eleven mares that were previously administered a primary dose of GonaCon vaccine by means of syringe dart delivery, will be located in the park and re-immunized using the same dart configuration and delivery ballistics as that used for the captive trials in 2013. Each dart will be numbered and correspond to an individual mare. We will determine darting efficacy by measuring the precise dose of the vaccine delivered to each mare. This will be done by weighing each dart (± 0.01g) before and after injection. We will measure dart retention time in each animal and dart performance (i.e. failure rate, partial discharge, blow-out, bounce)**.** In the case of darts that fail to discharge or partially inject the vaccine, the animal will be re-darted until the full dose has been delivered. We will also record each animal's behavioral response to dart injection.

3) One year post-vaccination group and 4) six-month post-vaccination group. Including these two additional re-vaccination treatments will hopefully allow us to more clearly define the optimum re-immunization schedule for GonaCon vaccine in feral horses. However, we have no prior immunological evidence to support these time periods as being optimum or different from each other. These intervals were selected primarily on the basis of practical field application of the vaccine. It would generally be infeasible to locate and treat horses via remote dart delivery during the winter months (December-February) at THRO. Therefore, shorter time periods such as three months (which was the minimum time required for maximum antibody production in elk) (Powers et al. 2011) are not practical. Re-vaccination of mares at the 6 month interval will be conducted in March 2016 and for mares in the one-year interval group during September 2016.

Experimental animals and treatment application. Thirty-two free-ranging mares (1.5-3.5 years of age) will be selected for these treatment groups. A randomized complete block design consisting of either a one year or six-month GonaCon- re-vaccination group will be used in this analysis. Mares will be paired on the basis of age and pregnancy status such that animals within each block (n = 16 blocks of 2 mares each) will be as similar as possible. Within each pair, a mare will be randomly assigned to each experimental group. The general health, pregnancy status, and body condition of each mare will be determined in the field by trained biologist familiar with these animals. Pregnancy status will be determined by fecal estrogen assay (Baker et al. unpublished data). Body condition of all study mares will be evaluated visually and scored on a scale of 1 (very thin) to 9 (very fat) (Henneke et al. 1983). During September 2015, all 32 mares will receive a primary vaccination with GonaCon vaccine via remote dart delivery. Approximately 6 months (March 2016) following the initial vaccination, 16 mares will be re-vaccinated with GonaCon and 1 year later (September 2016) the remaining 16 mares will be similarly treated. All horses will receive the re-vaccination treatment using remote dart delivery.

Field Measurements:

Effects on reproduction. We will determine the effectiveness, duration of effects, and reversibility of a second immunization with GonaCon on reproduction during 2015-2020 (or beyond, if necessary) by comparing foaling and pregnancy rates of treated and control mares. Annual foaling rates will be estimated by observing all mares, at least weekly, during the breeding season (April – August) and documenting the presence of new foals and estimating approximate date of birth. We will continue to monitor reproductive rates in all experimental mares during 2015-2020 or until the magnitude of the difference in foaling rates between treatment and control mares is less than 50% or funding is no longer available. Supplementary to foaling rates, we will also collect fecal samples during approximately mid-gestation (October-February) and determine fecal estradiol concentrations to estimate pregnancy rates of all mares (Baker et al. unpublished data).

Biological side-effects. In conjunction with the above measurements, we will assess the safety and side effects of a second immunization with GonaCon. In both treatement and control groups of horses, we will evaluate the effects (if any) on general health, body condition, existing pregnancy, neonate survival and injection site reactions at weekly intervals during the breeding season and opportunistically throughout the year. In addition, we will observe all experimental mares for presence or absence of lameness (limping, gait alteration, reluctance to stand or bear weight, and evidence of swelling or discharge) at the site/side of vaccine injection. We will classify injection site reactions into four categories according to the scoring system of Roelle and Ransom (2009). Both the previous injection site in 2009 and the one in 2013 will be evaluated each year in conjunction with foaling observations.

Behavioral side-effects. We evaluated the effects of GonaCon vaccine on the daily activity patterns and social interactions of the four-year post vaccine group during March – August 2015. We used a restricted randomized design to balance observations as much as possible among all experimental animals while also trying to observe the behavior of each mare at least 6-8 times per month. We located bands containing selected mares by vehicle, foot, or horseback. Observations were balanced across time of day and conducted from distances of 50-100m with the aid of binoculars and spotting scopes. Each sampling period consisted of 20 min of continuous observation. We used a combination of instantaneous scan sampling procedure to record time budget data and all-occurrence sampling to record reproductive behaviors (Altmann 1974). We followed field and analytical methods described by Ransom and Cade (2009) to develop a herd-specific ethogram for selected behaviors at THRO. We will compare behavioral observations of GonaCon-treated mares and control mares the first breeding season following primary vaccination in 2010 and re-vaccination in 2013. Statistical analysis of data will follow those described by Ransom et al. (2014).

Statistical analysis

Our power analysis was originally developed for the four-year post-treatment group but offers an approximation of statistical power needed to detect a treatment effect for other treatments as well. We used a fixed sample size of available mares (n = 50, equally divided into 2 groups of 25 each), to estimate statistical power (1-β) for detecting a treatment effect (0.9 – 0.2) over time. We then used a 1-sided, two-sample t-test with a normal approximation together with software program SYSTAT 12.02.00 (SYSTAT Software, Inc.) to estimate the power for detecting effect sizes that vary from 0.20-0.90 (Kang and Kim 2004) (Table 2). Our current 2-year mean effect- size (difference between mean foaling rates in treatment [0.485] and control [0.759] groups) is 0.274. If repeat vaccination does not improve contraceptive efficacy, we will have little power to detect a difference between treatment groups and will conclude there is little

effect due to re-vaccination. However, if revaccination increases effect size to 0.6 or better we will have sufficient power to detect these effects.

We will determine the efficacy of re-vaccination treatments by comparing the proportion of fertile females in each treatment group with control females in the original four-year post-vaccination group combined across all foaling seasons. Females will be classified as being fertile, or infertile on the basis of the presence of a foal at heel, or fecal estrogen concentrations indicating pregnancy. We will use a linear mixed model analysis with restricted maximum likelihood estimation to determine treatment effects on fertility rates. A chi-squire test will be used to test for differences among fertility rates, foal survival, and seasonality of births. We define the foaling season to include March, April, May, June, and July. Results will be shown as means ± standard errors when appropriate.

We will also explore using Bayesian beta-bimodal (similar to the one used by Monello et al. 2014 to estimate elk survival) to examine the size of treatment effects. Power will be less of an issue in this approach because we will be able to show the probability distribution of differences attributable to treatment.

Table 2. Power calculations and corresponding contraceptive treatment effect size for the GonaCon field experiment with free-ranging mares at Theodore Roosevelt National Park.

Total Sample Size	Group Sample Size	THRO Foaling Rate	Effect Size	Alpha	Power (1-β)
50	25	0.759	0.9	0.1	0.977
50	25	0.759	0.8	0.1	0.949
50	25	0.759	0.7	0.1	0.898
50	25	0.759	0.6	0.1	0.817
50	25	0.759	0.5	0.1	0.706
50	25	0.759	0.4	0.1	0.570
50	25	0.759	0.3	0.1	0.425
50	25	0.759	0.2	0.1	0.290

Limitations in study design

One difficulty in this study is that, to our knowledge, there are no published data regarding the optimum re-vaccination schedule for GonaCon vaccine in horses or any other wild or domestic ungulate. Thus, while we may have adequate sample sizes to detect treatment differences between GonaCon-treated and control groups, our sample sizes may be inadequate to detect small differences among the four post-primary treatment groups. This limitation is due to the restricted availability of additional female horses at THRO for this experiment.

Moreover, the control group of mares used to compare treatment effects in this study was originally selected in 2009 to be as similar as possible to the four-year re-vaccination group. However, it is not necessarily representative of the re-vaccinated mares selected for the subsequent treatments. If this study was implemented in captivity, more appropriate control groups could have been established. Additionally, a more complex study design that incorporated different vaccination time-points and regimes could have more accurately determined the optimal time point for re-vaccination.

Our study was implemented to compliment practical management efforts at THRO that are determined by having reasonable access to study horses for treatment application. Regardless of efficacy outcome, this study will provide valuable information. If re-vaccination at these intervals is not

successful, our study will provide important information on the utility of this vaccine. If it is successful, the vaccine may have more wide-spread utility than previously observed.

Performance Measures and Reporting:

2015 - 2016

1. Collect and summarize four-year post-primary vaccination foaling rate estimates for GonaCon-treated mares and control mares for the 2015 and 2016 foaling seasons.

2. Collect and summarize data pertinent to foaling rates and side-effects of GonaCon-treated mares for the two-year post-primary vaccination group for the 2015 and 2016 foaling seasons.

3. Select and document successful re-vaccination of mares in the two-year post-primary vaccination group (11 mares) and primary vaccination of mares in the one-year (16 mares) and six month (16 mares) post-vaccination groups (September 2015).

4. Document successful re-vaccination of mares in the six month revaccination group during March 2016 and for the one-year group in September 2016.

5. Compare foaling rates on all vaccination schedules to their pregnancy rates estimated via fecal estrogen analysis.

6. Provide data analysis summarizing the effects of GonaCon vaccine on daily activity patterns and social interactions of feral horses at THRO during 2015-2016.

BUDGET

Table3. Yearly budget, by category, for proposed research at Theodore Roosevelt National Park 2015-2020.

Category	Year 1	Year 2	Year 3	Year 4	Year 5
Personnel	$40,898	$29,300	$29,878	$34,847	$67,473
Fringe benefits	$7,626	$5,866	$5,991	$7,033	$15,722
Travel	$3,003	$2,946	$1,964	$1,964	$1,964
Equipment	$ 0	$ 0	$ 0	$0	$ 0
Supplies	$4,550	$1,950	$1,950	$1,950	$1,950
Other	**$** 0	$ 0	$ 0	$1,000	$5,000
Direct costs	$56,077	$40,062	$39,783	$46,794	$92,109
Indirect costs	$9,813	$7,011	$6,962	$8,189	$16,119
Total costs	$65,890	$47,073	$46,745	$54,983	$108,228

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3. Qualifications, Experience, and Past Performance:
(Describe who will carry out the project activities. List all project personnel, including consultants, contractors, sub-recipients, etc., if known. Describe their responsibilities and the amount of time each will dedicate to the project. Briefly describe how their experience and qualifications are appropriate to successfully achieve the stated objectives.)

Dan L. Baker, Affiliate Faculty, Research Scientist, Colorado State University, Department of Biomedical Sciences/Animal Reproduction and Biotechnology Laboratory: will coordinate all project activities, study design, data collection and analysis, personnel management, reporting, interagency coordination. Dr. Baker has been the project leader in the evaluation of GonaCon in feral horses at Theodore Roosevelt National Park (THRO) since 2009. Prior to that (2006-2013) he was involved with similar research with this contraceptive vaccine in captive and free-ranging elk in Rocky Mountain National Park (ROMO) (50%).

Jenny G. Powers, Wildlife Veterinarian, National Park Service: attending veterinarian, assist with study design, and assessment of biological side-effects of GonaCon vaccine. Dr. Powers has been involved with the evaluation is this contraceptive agent at THRO since 2009 and was involved in similar research with captive and free-ranging elk in ROMO. Much of her previous research has been focused on the efficacy and physiological side-effects of various contraceptive agents. She will also facilitate animal care and use approval from NPS for this project.

Blake E. McCann, Wildlife Biologist, National Park Service, Theodore Roosevelt National Park: liaison and on-site project manager at THRO, study design, will lead efforts in dart delivery of GonaCon in free-ranging horses, will provide in-kind support for this research effort (i.e.

vehicles, office space, housing for field technicians) and coordinate research activities with ongoing NPS operations. Dr. McCann has been involved with the evaluation of GonaCon since 2013 has been instrumental in the design and evaluation of a GonaCon-specific dart configuration and ballistic system for feral horses.

N. Thompson Hobbs, Professor, Senior Research Scientist, Colorado State University (CSU), Department of ESS, Natural Resource Ecology Laboratory: will lead efforts to model effects of fertility control on feral horse population dynamics; provide statistical analysis of data, and coordinate administrative services and support for this project within NREL. Dr. Hobbs has been involved with several projects modeling the effects of fertility control on wild ungulates. He is currently working on a Bayesian state-space model of population dynamics of white-tailed deer to evaluate alternatives for population management including fertility control (5%).

Jason E. Bruemmer, Professor, Colorado State University, Department of Animal Science, Equine Reproduction Laboratory: provide technical expertise on reproductive physiology of feral horses, study design, interpretation of data, and manuscript preparation. Dr. Bruemmer has been involved with this investigation since 2009 and has provided pregnancy assessment of experimental mares at the 2009 and 2013 roundups. We have incorporated his mare pregnancy criteria and body condition scoring system into our field measurements.

Terry M. Nett, Professor, Colorado State University, Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory: provide laboratory services for fecal estrogen assay. Dr. Nett has been involved with this research project since 2009, as well as, similar research with this vaccine in captive and free-ranging elk and domestic horses. He is a leading authority on reproductive endocrinology and GnRH metabolism in mammals (1%).

Kathleen M. Eddy, Laboratory and field research technician, Colorado State University, Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory: Lead responsibility for developing and validating a fecal estrogen assay for pregnancy determination in horses; this assay will supplement foaling rate measurements to assess pregnancy status and treatment responses in experimental mares at THRO. In addition, she will assist with fecal collections and other field measurement (5%).

Douglas C. Eckery, Senior Scientist and Project Leader, USDA, APHIS, Wildlife Services, National Wildlife Research Center: will be primarily responsible for providing 100- 2ml doses of GonaCon-Equine vaccine packaged in 3ml plastic syringes for this study.

APPENDIX

Institutional Animal Care and Use Permits

G. HUMANE CARE AND USE OF ANIMALS

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort / Grant Application

Privileged Communication

Title of proposal: Evaluation of Re-Immunization with GonaCon-Equine™ on Reproduction and Side-Effects in Feral Horses

Investigators: Baker, Dan L.; Nett, Terry M.; Powers, Jenny G; Ransom, Jason I; Bruemmer, Jason E; Hobbs, N. Thompson; McCann, Blake E.

Pursuant to procedures established by the Bureau of Land Management, Wild Horse and Burro Research Program, I certify that the above described protocol follows guidelines set forth in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (#85-23) and the "Animal Welfare Act of 1966" (PL 89-544) as amended.

Signature _____ Date 4/30/14
Terry Engle, Ph.D., Chair, CSU Institutional Animal Care and Use Committee

Name of Institution: Colorado State University

NOTE: This completed form must be in receipt of the BLM WH&B Research Advisory Team before the initiation of funding or collaborative work can commence. Private individuals must seek local/regional institutional approval.

United States Department of the Interior
NATIONAL PARK SERVICE
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, Colorado 80525

National Park Service
Institutional Animal Care and Use Committee
Animal Research Protocol Approval

Principal Investigator(s): Dan Baker/ N. Thompson Hobbs
Telephone: 970.556.8518
Electronic Mail: danbaker@colostate.edu

Region: Midwest Region

Protocol Approval Number: MWR_THRO_Baker_Horse_2013.A3

Project Title: Remotely-delivered GnRH Vaccine (GonaCon-Equine) in Free-Ranging Horses: A Preliminary Investigation

Approval Date: 9/23/2013

Effective Date: 9/23/2013

Questionnaire Dates; Years 1 and 2 (if applicable): 9/23/2014, 9/23/2015

Expiration/Re-Submittal Date: 9/23/2016

Funding Agency(ies): None

Species: Horse (*Equus caballus*)

Number(s) of Animals: 10 horses/year, 30 total horses over three years

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This project study was reviewed by the National Park Service Institutional Animal Care and Use Committee. The following action(s) were taken:

Project Status: Approved

Midwest Region/ Intermountain Region/ NPS IACUC Chair: Dan Licht /s/, Mike Wrigley /s/, John Bryan /s/

Grant and Cooperative Agreement

CHOOSE ONE:
- [X] COOPERATIVE AGREEMENT
- [ ] GRANT

CHOOSE ONE: [ ] EDUCATION [ ] FACILITIES [ ] RESEARCH [ ] SDCR [ ] TRAINING

1. GRANT/COOPERATIVE AGREEMENT NUMBER	2. SUPPLEMENT NUMBER	3. EFFECTIVE DATE	4. COMPLETION DATE
L15AC00145		09/08/2015	09/07/2020

5. ISSUED TO
NAME/ADDRESS OF RECIPIENT (No., Street, City/County, State, Zip)
COLORADO STATE UNIVERSITY
Attn: TRACEY TRUJILLO, RSRCH ADMIN
601 S HOWES ST
FORT COLLINS CO 80521-2807

6. ISSUED BY BLM OR-ST OFC PROC MGMT BR(OR952)
Mailing Address: 1220 SW 3rd Avenue, 12th Floor
PORTLAND OR 97204

7. TAXPAYER IDENTIFICATION NO. (TIN)

8. COMMERCIAL & GOVERNMENT ENTITY (CAGE) NO.

9. PRINCIPAL INVESTIGATOR/ORGANIZATION'S PROJECT OR PROGRAM MGR. (*Name & Phone*)
Dan L. Baker, Senior Scientist
970-556-8518, danbaker@colostate.edu

10. RESEARCH, PROJECT OR PROGRAM TITLE
WILD HORSE AND BURRO CONTRACEPTIVE TECHNIQUES AND PROTOCOLS

11. PURPOSE
See Schedule.

12. PERIOD OF PERFORMANCE (*Approximately*)
09/08/2015 through 09/07/2020

13A.	AWARD HISTORY	13B.	FUNDING HISTORY
PREVIOUS	$0.00	PREVIOUS	$0.00
THIS ACTION	$159,708.00	THIS ACTION	$159,708.00
CASH SHARE	$0.00	**TOTAL**	$159,708.00
NON-CASH SHARE	$0.00		
RECIPIENT SHARE	$0.00		
TOTAL	$159,708.00		

14. ACCOUNTING AND APPROPRIATION DATA
01

PURCHASE REQUEST NO.	JOB ORDER NO.	AMOUNT	STATUS
0020082150			

15. POINTS OF CONTACT

	NAME	MAIL STOP	TELEPHONE	E-MAIL ADDRESS
TECHNICAL OFFICER	PO: Paul Griffin		970-226-9358	pgriffin@blm.gov
NEGOTIATOR				
ADMINISTRATOR	Walter Ullrey		503-808-6302	wullrey@blm.gov
PAYMENTS				

16. THIS AWARD IS MADE UNDER THE AUTHORITY OF :
The Wild Free-Roaming Horses and Burros Act of 1971, 16 USC 1336, PL 92-195, Section 6.

17. APPLICABLE STATEMENT(S), IF CHECKED:
- [ ] NO CHANGE IS MADE TO EXISTING PROVISIONS
- [ ] FDP TERMS AND CONDITIONS AND THE AGENCY-SPECIFIC REQUIREMENTS APPLY TO THIS GRANT

18. APPLICABLE ENCLOSURE(S), IF CHECKED:
- [ ] PROVISIONS
- [ ] SPECIAL CONDITIONS
- [ ] REQUIRED PUBLICATIONS AND REPORTS

UNITED STATES OF AMERICA		COOPERATIVE AGREEMENT RECIPIENT	
CONTRACTING/GRANT OFFICER	DATE	AUTHORIZED REPRESENTATIVE	DATE
Walter Ullrey	09/08/2015		

Grant and Cooperative Agreement

ITEM NO. (A)	ITEM OR SERVICE (Include Specifications and Special Instructions) (B)	QUANTITY (C)	UNIT (D)	ESTIMATED COST	
				UNIT PRICE (E)	AMOUNT (F)
	CFDA Number: 15.229 DUNS Number: 785979618 Funding Opportunity No. L14AS00048 WILD HORSE AND BURRO CONTRACEPTIVE TECHNIQUES AND PROTOCOLS Account Assignment: K G/L Account: 6100.411C0 Business Area: L000 Commitment Item: 411C00 Cost Center: LLWO260000 Functional Area: L10600000.PC0000 Fund: 15XL1109AF Fund Center: LLWO260000 Project/WBS: LX.SI.RSCH0000 PR Acct Assign Line: 01 Period of Performance: 09/08/2015 to 09/07/2020				
00010	Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance (Base Award, POP: 9/8/15-9/7/18) Obligated Amount: $159,708.00 ***IMPORTANT INFORMATION*** REPORTING FREQUENCY: Performance/Progress Reports: Semi-Annual Financial Status Reports (SF-425): Semi-Annual Semi-annual reporting periods end March 31st and September 30th of each year. Reports are due within 30 days after the end of the period. REPORT SUBMISSION: Submit reports via email to "blm_or_so_fa_reports@blm.gov" with a courtesy copy (cc) to the BLM Program Officer. Financial status reports should include documentation detailing from which budget categories funds were expended. Continued ...				159,708.00

Grant and Cooperative Agreement

ITEM NO. (A)	ITEM OR SERVICE (Include Specifications and Special Instructions) (B)	QUANTITY (C)	UNIT (D)	ESTIMATED COST UNIT PRICE (E)	ESTIMATED COST AMOUNT (F)
	BLM GRANTS MANAGEMENT OFFICER (GMO): Walter B. "Bert" Ullrey Bureau of Land Management, OR/WA State Office PO Box 2965, Portland, OR 97208 Telephone: 503-808-6302 Email: wullrey@blm.gov BLM PROGRAM OFFICER (PO): Paul Griffin, Ph.D., Research Coordinator BLM Wild Horse and Burro Program 2150 Centre Ave, Building C Fort Collins, CO 80526 Telephone: 970-226-9358 Email: pgriffin@blm.gov AWARD RECIPIENT: Tracey Trujillo, Research Administrator Colorado State University 601 South Howes Street Campus Delivery 2002 Fort Collins, CO 80523-2002 Telephone: 970-491-1560 Email: tracey.trujillo@colostate.edu RECIPIENT PROJECT MANAGER/PRINCIPAL INVESTIGATOR: Dan L. Baker, Senior Scientist Colorado State University Animal Reproduction and Biotechnology Laboratory 601 South Howes Street Fort Collins, CO 80523 Telephone: 970-556-8518 Email: danbaker@colostate.edu The total amount of award: $159,708.00. The obligation for this award is $159,708.00.				

I. STATEMENT OF JOINT OBJECTIVES

A. Purpose. This financial assistance agreement is made and entered into by the Department of the Interior, Bureau of Land Management, Oregon/Washington State Office (BLM), and Colorado State University, the recipient, for the purpose of transferring something of value to the recipient in order to carry out a public purpose of support or stimulation authorized by a law of the United States. This agreement is issued under the umbrella of the Great Plains Cooperative Ecosystem Studies Unit (CESU) Cooperative and Joint Venture Agreement, BLM No. KAA119001, the terms and conditions of which include a negotiated indirect cost rate not to exceed 17.5% of Modified Total Direct Costs (MTDC).

B. Objective. The objective of this cooperative agreement is to support both refinement of existing techniques and encourage development of new techniques and protocols in the contraception or permanent sterilization of either male or female wild horses and/or burros in the field. Projects conducted in a controlled environment will have the final goal of applying the sterilization or contraception techniques to free-roaming animals on the range.

C. Authority. **The Wild Free-Roaming Horses and Burros Act of 1971, 16 USC 1336, PL 92-195, Section 6. Section 1336.** The Secretary is authorized and directed to protect and manage wild free-roaming horses and burros as components of the public lands, and he may designate and maintain specific ranges on public lands as sanctuaries for their protection and preservation…Section 1336. The Secretary is authorized to enter into cooperative agreements with other landowners and with State and local governmental agencies and may issue such regulations as he deems necessary for the furtherance of the purpose of this chapter.

1. Public Benefit. The activity(ies) to be undertaken through this assistance provide the following public benefit(s): Overpopulation of wild horses and burros is damaging to rangelands and the health of the herds. Effective population growth suppression methods for wild horses and burros by is critical to effectively manage herd population growth rates and enable healthy herds to thrive on healthy rangelands. The public benefits from enhanced enjoyment of wild horse and burro hers and healthier, more productive rangelands. Population controls help to lessen the burden on already over-grazed range.

D. Performance Goals and Measures. The activity(ies) to be undertaken through this assistance agreement will be evaluated using the following BLM Performance Measures:

1. Goals & Estimated Timelines:
    a. Determine optimal timing of re-vaccination, to achieve reduced pregnancy rates.
    b. Determine dart efficiency in delivering vaccine.
    c. Assess body condition of animals in each treatment group.
    d. Assess effects of vaccine re-inoculation on mare and neonate health.
    e. Assess potential behavioral side-effects of vaccine re-inoculation.
    f. Estimate the foaling rate for re-vaccinated animals.
2. Measures:

a. A well-designed study of treatment groups with re-vaccination at 6 month, 1 year, 2 year, and 4-years after the initial vaccination, all comparable to control animals. The measurable outcomes for all treatment groups are pregnancy and foaling rates, as measured via fecal estrogen assay, and observations of mares and any foals.

b. Weigh pneumatic syringe darts before and after firing at subject animals. The difference is attributable to vaccine that was expelled. Other measures include dart retention rate and dart failure rate recording.

c. Body condition is scored according to the Henneke system, which has clearly defined scores from 1 to 9.

d. Observe mare and foal health from a distance, via weekly observations. Observations will include search for apparent lameness and injection site side-effects.

e. Analyze behavioral observations of re-vaccinated mares; thee observations were made in 2010 and 2013; analysis would be in year 4 of this study.

f. Analyses of foaling rate will make use of data from each treatment group, using linear mixed-models, and chi-square tests to assess statistical significance of differences between treatment groups.

II. PROJECT MANAGEMENT PLAN

A. The recipient and the BLM both agree the attached proposal entitled "Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance," and dated 08/21/15, is accepted.

B. Documents Incorporated by Reference. The following recipient documents are incorporated by reference: Standard Form (SF) 424, Application for Federal Assistance, SF-424A, Budget Information - Non-Construction Programs, SF-424B, Assurances - Non-Construction Programs, Budget Detail, and Certification Regarding Lobbying - Certification for Contracts, Grants, Loans and Cooperative Agreements.

III. TERM OF AGREEMENT

A. Term. This agreement shall become effective as of the date shown on the signed award cover page. It may remain in effect for a maximum of five (5) years. The BLM will consider continued support of the project upon; (a) the recipient showing progress satisfactory to the BLM toward program goals and the determination by the BLM that continuation of the program would be in the best interests of the Government, and/or (b) the availability of funds.

B. Modifications.

1. Recipients must request prior approvals from BLM's GMO for one or more of the following program or budget-related reasons: 1). Report deviations from budget or project scope or objective, 2). Any change in the project scope, key personnel, period of performance, budgeted costs, cost share or matching, administration or any other change to this agreement constitutes a modification of the agreement.

2. All requests for modification of the agreement shall be made in writing, provide a full description of the reason for the request, and be sent to the attention of the BLM Program Officer 30 calendar days before the expiration of the agreement and/or project/budget period. Requests involving additional support or funding will require new SF-424 Applications for Federal Assistance, including new project proposals and budgets. Any determination to modify, extend the period of performance, or provide follow-on funding for continuation of a project is solely at the discretion of the BLM.

3. All modifications to the agreement shall be in writing and signed by the GMO. No oral statements or any written statements made by any person other than the GMO, shall in any manner modify or otherwise affect the terms of the agreement. All modifications to the agreement may be signed unilaterally by the GMO, including actions to suspend or terminate the agreement in accordance with 2 CFR, Subpart D. Section 200.339, Termination.

C. Budget Revisions.

1. The budget submitted as part of the SF-424 Application for Federal Assistance and approved during the award process is the financial expression of the project scope, objective or program. Recipients are required to report deviations from the approved budget and program plans and request prior approval for revisions in accordance with 2 CFR Subpart C 200.308, Revision of budget and program plans.

2. The BLM may, at its option, restrict the transfer of funds among direct cost categories or programs, functions and activities for awards in which the federal share of the project exceeds $100,000 and the cumulative amount of such transfers exceeds, or is expected to exceed, ten percent (10%) of the total budget as last approved by the BLM. No revision or transfer of funds shall be used for purposes other than those consistent with the original intent of the award.

D. Termination. This agreement may be terminated in accordance with the provisions of 2 CFR, Subpart D. Section 200.339, Termination.

IV. FINANCIAL SUPPORT

A. Funding. This agreement may be funded each fiscal year (FY) based on the availability of BLM funding.

B. Per diem fees. Financial support is to be used for reimbursement of actual costs expended. If the recipient's estimated costs include a per diem (daily allowance) budgeted cost for animal care, the recipient is reminded that funding support awarded through financial assistance agreements is to be used for reimbursement of actual costs expended and shall not be calculated or justified using per diem rates.

C. Fiscal Year (FY) Carryover. As long as expenditures are within the approved Period of Performance, funds obligated but not expended by the recipient in a FY may be carried forward and expended in subsequent years.

D. Maximum Obligations. The total obligations, including modifications, represent the amount for which the BLM will be responsible under the terms of this agreement. The BLM shall not be responsible to pay for, nor shall the recipient be responsible to perform, any effort that will require the expenditure of Federal funds above the current obligated amount.

E. Cost Sharing or Matching.

1. Cost sharing or matching for this agreement shall be in accordance with 43 CFR, Subpart C, Section 12.64 and 2 CFR 200.306.

2. There is no cost share or match legislatively required for this award.

F. Program Income. Program income generated for this agreement shall be in accordance with 2 CFR, Subpart D, Section 200.307, Program income. Unless otherwise stated, program income shall be added to the funds committed to this agreement and be used for the purposes, and under the conditions of, the grant agreement.

G. Indirect Costs. Indirect costs are approved for reimbursement under this agreement at the Cooperative Ecosystem Studies Unit (CESU) Joint Venture Agreement participant rate of 17.5% (see Section I. A. Purpose). The indirect cost base shall be the same base identified in the recipient's Federal negotiated indirect cost rate agreement (NICRA).

V. PAYMENTS

A. Automated Standard Application for Payment (ASAP) System.

1. Payments will be made by the U.S. Department of the Treasury, Financial Management Service (FMS), ASAP system. The ASAP (https://www.asap.gov) system is an online recipient-initiated payment and information system for Financial Assistance Agreements. The recipient must register and request federal funds that are due directly from the Federal Reserve Bank on a reimbursable basis.

2. The ASAP Requestor ID, furnished by the Department of the Treasury, is used for account access and requesting reimbursement payments. The BLM will create an ASAP Account ID unique to this agreement. The first ten (10) characters will be the agreement number, and the remaining characters will identify BLM funding line items. Drawdown of funds must be taken from specific lines on the agreement.

B. Advance Payments. Payments are made by the Department of the Treasury through the ASAP system within three (3) days after request. Advance payments should not be required.

C. Drawdowns.

1. Treasury Circular 1075 (31 CFR 205) requires that drawdowns to a recipient organization shall be limited to the minimum amounts needed and shall be timed to be in accordance with the actual, immediate cash requirements of the recipient organization in carrying out the purposes of the approved program or project. The timing and amount of cash advances shall be as close as is administratively feasible to the actual disbursements by the recipient organization for direct program or project costs and the proportionate share of any allowable indirect costs.

VI. PROPERTY MANAGEMENT

A. Government-Furnished Property (GFP). Tools and equipment furnished by the BLM to the recipient shall be used for official purposes only and shall be subject to the terms of the agreement. Tools and equipment shall be returned in the same condition received except for normal wear and tear in project use.

B. Property Management Provisions. Any BLM property used or other property acquired under this agreement, including intangible property such as copyrights and patents shall be governed by the property management provisions of 2 CFR, Subpart D, Sections 200.310 to 200.316, Property Standards.

C. Defensive Driving. Recipient staff will be required to complete a BLM-approved Defensive Driving Course if driving a Government-owned vehicle (GOV).

D. All-Terrain Vehicles (ATV). Recipient staff will be required to complete a BLM-approved Four-wheel ATV safety and training program if using Government-furnished ATVs.

E. Power Equipment. Recipient staff will be required to complete a BLM-approved safety and training program if using Government-furnished power equipment, such as chainsaws, wood chippers, etc. The recipient will be responsible for meeting all protective equipment requirements if using Government-furnished equipment.

VII. LIABILITY, INSURANCE, AND INDEMNIFICATION

A. Liability. The BLM assumes no liability for any actions or activities conducted under this agreement except to the extent that recourse or remedies are provided by Congress under the Federal Tort Claims Act, 28 USC 2671.

B. Indemnification. The recipient hereby agrees:

1. To indemnify the federal government, Bureau of Land Management (BLM), from any act or omission of the recipient, its officers, employees, or (members, participants, agents, representatives, agents as appropriate) (1) against third party claims for damages arising from one or more activities carried out in connection with this financial assistance agreement and (2) for damage or loss to government property resulting from such an activity, to the extent the laws

of the State where the recipient is located permit. This obligation shall survive the termination of this agreement.

2. To pay the United States the full value for all damage to the lands or other property of the United States caused by the recipient, its officers, employees, or (members, participants, agents, representatives, agents as appropriate).

3. To provide workers' compensation protection to the recipient's officers, employees, and representatives.

4. To cooperate with the BLM in the investigation and defense of any claims that may be filed with the BLM arising out of the activities of the recipient, its agents, and employees.

5. In the event of damage to or destruction of the buildings and facilities assigned for the use of the recipient in whole or in part by any cause whatsoever, nothing herein contained shall be deemed to require the BLM to replace or repair the buildings or facilities. If the BLM determines in writing, after consultation with the recipient that damage to the buildings or portions thereof renders such buildings unsuitable for continued use by the recipient, the BLM shall assume sole control over such buildings or portions thereof. If the buildings or facilities rendered unsuitable for use are essential for conducting operations authorized under this agreement, then failure to substitute and assign other facilities acceptable to the recipient will constitute termination of this agreement by the BLM.

C. Flow-down. For the purposes of this clause, "recipient" includes such subrecipients, contractors, or subcontractors as, in the judgment of the recipient and subject to the Government's determination of sufficiency, have sufficient resources and/or maintain adequate and appropriate insurance to achieve the purposes of this clause.

D. Identified Activities. All activities carried out in connection with this financial assistance agreement.

VIII. REPORTING REQUIREMENTS

A. Periodic Reporting. Submission of periodic financial, performance/progress, and (if applicable) youth employment reports is required whether or not any work has been attempted or completed and/or whether or not any funds have been drawn down or expended.

B. Federal Financial Reports.

1. Recipients of federal financial assistance are required to submit periodic financial reports which document the financial status of their awards. The Federal Financial Report form (FFR), also known as Standard Form (SF) 425, is the standard form used to report financial status. Award expenditures and/or income may be reported either on a cash or accrual basis, whichever method is normally used by the recipient. Financial reports are reviewed to identify questionable patterns of expenditures, such as accelerated or delayed drawdowns, and to assess whether performance or financial management problems exist.

2. In addition to the SF-425, the recipient must include detailed information on the costs for which the funds were used, listed by budget category as approved on their SF-424A Budget Information form. This additional information must be cumulative and may include a brief narrative describing the grant activities supported by the funds.

3. Recipients must sign the SF-425 in Box 13b certifying that the information being reported is complete, accurate, consistent with the recipient's accounting system, and that all expenditures and obligations are for the purposes set forth in the agreement. The SF-425 represents a claim to the Federal government. Filing a false claim may result in civil or criminal penalties.

4. Blank SF-425 forms and instructions are available on the Office of Management & Budget's (OMB) web site, address: http://www.whitehouse.gov/omb/grants_forms.

5. Standard Form (SF) 425 financial reports for this cooperative agreement shall be submitted: **SEMI-ANNUALLY.**

a. The first financial report shall cover from the date of award through: **March 31, 2016.**

b. Semi-annual financial reports are due 30 calendar days past the reporting period end date and each reporting period end date thereafter for the term of the agreement. The table below shows the reporting periods and their corresponding report submission due dates:

Semi-Annual Reporting Period	Submit Reports By:
October 1 to March 31	April 30th
April 1 to September 30	October 30th

c. Email Financial Reports to: BLM_OR_SO_FA_Reports@blm.gov. In addition, send courtesy copies (cc) to the BLM Program Officer(s) and Technical Advisor(s) as listed on the award cover pages.

6. Final SF-425 financial reports shall be submitted no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement.

C. Performance/Progress Status Reports

1. The recipient shall submit periodic performance reports to the GMO. The performance report must be prepared in accordance with 2 CFR, Subpart D, Section 200.328, Monitoring and reporting program performance.

2. The performance report shall include a narrative summary both of completed activities and activities in progress, a calculation of percent of completed work based on work

identified in the Recipient's submitted proposal, Project Management Plan, the reason for slippage if objectives or milestones are not met, a prediction of future activities and how they will be accomplished, and a discussion of issues and problems which may impact the ability to complete the work on time. Recommendations to overcome problems shall also be provided. In addition, the performance report should reflect the BLM Performance Measures as listed in Section III, Paragraph B. A Performance Report Template is available at our Public Web Site, address: http://www.blm.gov/or/procurement/agreements.php.

2. Performance/progress reports for this cooperative agreement shall be submitted: **SEMI-ANNUALLY.**

a. The first performance/progress report covers from the date of award through: **March 31, 2016.**

b. Annual performance/progress reports are due 30 calendar days past the reporting period end date and each reporting period end date thereafter for the term of the agreement. The table below shows the reporting periods and their corresponding report submission due dates:

Semi-Annual Reporting Period	Submit Reports By:
October 1 to March 31	April 30th
April 1 to September 30	October 30th

c. Email Performance/Progress Reports to address: BLM_OR_SO_FA_Reports@blm.gov. In addition, send courtesy copies (cc) to the BLM Program Officer(s) and Technical Advisor(s) as listed on the award cover pages.

3. Final performance/progress reports shall be submitted no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement.

E. Non-compliance: Failure to comply with the reporting requirements contained in this agreement may be considered a material non-compliance with the terms and conditions of the award. Non-compliance may result in withholding of future payments, suspension or termination of the agreement, recovery of funds paid under the agreement, and withholding of future awards.

F. Agency Review: If a recipient has a history of poor performance, financial instability, has a management system not meeting standards prescribed by the Uniform Administrative Requirements, has not conformed to the terms and conditions of the award, and/or is not otherwise responsible in safeguarding federal funds, they may be placed on Agency Review. Agency Review limits a recipient's access to funds by requiring that all drawdowns must be requested, reviewed, and approved prior to their being released. Recipients on agency review must submit a completed Standard Form (SF) 270 Request for Advance Payment or Reimbursement for each payment requested along with a detailed explanation of how the costs correspond to the approved budget categories as listed on their Application for Federal

Assistance SF-424A Budget Information and their Detailed Budget Breakdown or Challenge Cost Share Program Commitment Document, whichever is applicable. This process does not relieve the recipient of their required SF-425 financial report or performance report submission requirements.

IX. MONITORING

A. General. The recipient is responsible for oversight of the operations of the federal award supported activities to assure compliance with applicable Federal requirements and performance expectations. The BLM conducts pre-award and post-award, programmatic and financial monitoring. Depending upon the program, monitoring activities may include desk reviews (review of the award file including discussion(s) with the recipient regarding reporting, award activities, and project status), monitoring reviews (analysis of performance/progress and financial reports), and onsite or virtual site visits (discussion(s) of specific issues related to project implementation, observation of project activity, and review of planned versus actual progress).

1. Programmatic Monitoring. Program monitoring addresses the content and substance of the program. It is a qualitative review to determine performance, innovation, and contributions to the field. The BLM may make site visits as warranted by program needs. In addition, the BLM has the right of timely and unrestricted access to any books, documents, papers, or other records of the recipient's that are pertinent to the award, in order to make audits, examinations, excerpts, transcripts and copies of such document. This right also includes timely and reasonable access to recipient personnel for the purpose of interviews and discussions related to such documents.

2. Financial Monitoring. Financial monitoring ensures compliance with financial guidelines and general accounting practices. Onsite or internal financial reviews are conducted to determine if: (1) award recipients are properly accounting for the receipt and expenditures of federal funds; (2) expenditures are in compliance with federal requirements and award special conditions; and (3) proper documentation on financial monitoring activities is prepared, maintained, and distributed as appropriate.

B. Inspection. The BLM has the right to inspect and evaluate the work performed or being performed under this agreement, and the premises where the work is being performed, at all reasonable times and in a manner that will not unduly delay the work. If BLM performs inspection or evaluation on the premises of the recipient or a sub-recipient, the recipient shall furnish and shall require sub-recipients to furnish all reasonable facilities and assistance for the safe and convenient performance of these duties.

C. Audit Requirements.

1. Non-Federal entities that expend $750,000 or more in federal funds during a year shall have a single or program-specific audit conducted for that year in accordance with the Single Audit Act Amendments of 1996 (31 U.S.C. 7501-7507) and revised OMB Circular A-133, which is available at http://www.whitehouse.gov/omb/grants/grants_circulars.html .

Federal awards are defined as federal financial assistance and federal cost-reimbursement contracts that non-federal entities receive directly from federal awarding agencies or indirectly from pass-through entities. They do not include procurement contracts under grants or contracts used to buy goods or services from vendors. Non-federal entities that expend less than $750,000 for a fiscal year in federal awards are exempt from federal audit requirements for that year, except as noted in A-133, §_215(a), but records must be available for review or audit by appropriate officials of the federal agency, pass-through entity, and General Accounting Office (GAO).

2. Audits shall be made by an independent auditor in accordance with generally accepted government auditing standards covering financial audits. Additional audit requirements applicable to this agreement are found at 2 CFR, Subpart F, Section 200.501, Audits.

3. This and any other federal financial assistance award should be reported under its appropriate Catalog of Federal Domestic Assistance (CFDA) number.

4. For more information on the Single Audit process, go to the Federal Audit Clearinghouse Web Site at https://harvester.census.gov/facweb/Default.aspx.

X. KEY OFFICIALS

The key officials on this agreement are listed on the award cover page(s) and are considered to be essential to ensure maximum coordination and communication between the parties and the work being performed. Upon written notice, either party may designate an alternate to act in the place of their designated key official.

XI. STANDARD AWARD TERMS AND CONDITIONS

A. Due to changes to Federal grant regulations on December 26, 2013, the Office of Management and Budget (OMB) issued final rules for implementation of the new Uniform Guidance for grants. The Uniform Guidance consolidates guidance previously contained in the OMB circulars governing grants administration: A-21 (2 CFR Part 220), A-87 (2 CFR Part 225), A-110 (2 CFR Part 215), A-122 (2 CFR part 230), A-89, A-102, A-133, and the guidance in Circular A-50 on Single Audit Act follow-up). The new Uniform Guidance provides a streamlined format to improve clarity and consistency and may affect how you receive, manage, expend and report Federal grant funds. Currently, guidance is posted in Title 2 of the Code of Federal Regulations (CFR).

B. The U.S. Department of the Interior agencies, including the Bureau of Land Management implemented the new regulations on December 26, 2014 in the 2 CFR, Part 200—UNIFORM ADMINISTRATIVE REQUIREMENTS, COST PRINCIPLES, AND AUDIT REQUIREMENTS FOR FEDERAL AWARDS

1. Administrative and National Policy Requirements.

a. Office of Management and Budget Circulars. By accepting Federal funding under the current Federal assistance, your organization agrees to abide by the applicable OMB Circulars in the expenditure of Federal funds and performance under this program. OMB circulars are available at the following web site: http://www.whitehouse.gov/omb/circulars/

2. Administrative Requirements.

a. 2 CFR Part 200 Subparts A through D - Uniform Administrative Requirements and Cost Principles.

b. 2 CFR, Subpart B, 200.112 - Conflict of Interest

The Recipient must establish safeguards to prohibit its employees and Subrecipients from using their positions for purposes that constitute or present the appearance of a personal or organizational conflict of interest. The Recipient is responsible for notifying the Grants Officer in writing of any actual or potential conflicts of interest that may arise during the life of this award. Conflicts of interest include any relationship or matter which might place the Recipient or its employees in a position of conflict, real or apparent, between their responsibilities under the agreement and any other outside interests. Conflicts of interest may also include, but are not limited to, direct or indirect financial interests, close personal relationships, positions of trust in outside organizations, consideration of future employment arrangements with a different organization, or decision-making affecting the award that would cause a reasonable person with knowledge of the relevant facts to question the impartiality of the Recipient and/or Recipient's employees and Sub-recipients in the matter.

The Grants Officer and the servicing Ethics Counselor will determine if a conflict of interest exists. If a conflict of interest exists, the Grants Officer will determine whether a mitigation plan is feasible. Mitigation plans must be approved by the Grants Officer in writing. Failure to resolve conflicts of interest in a manner that satisfies the government may be cause for termination of the award.

Failure to make required disclosures may result in any of the remedies described in 2 CFR § 200.338, Remedies for Noncompliance, including suspension or debarment (see also 2 CFR Part 180).

Definitions: This section incorporates by reference 2 CFR Part 200, Subpart A, Acronyms and Definitions including, but not limited to the following additional terms:

(1) Conflict of Interest is defined as any relationship or matter which might place the Recipient, its employees, and/or its Subrecipients in a position of conflict, real or apparent, between their responsibilities under the agreement and any other outside interests. Conflicts of interest may also include, but are not limited to, direct or indirect financial interests, close personal relationships, positions of trust in outside organizations, consideration of future employment arrangements with a different organization, or decision-making affecting the award that would cause a reasonable person with knowledge of the relevant facts to question the impartiality of the Recipient and/or Recipient's employees and Subrecipients in the matter.

(2) Close Personal Relationship means a Federal award program

employee's childhood or other friend, sibling, or other family relations that may compromise or impair the fairness and impartiality of the Proposal Evaluator and Advisor and Grants Officer in the review, selection, award, and management of a financial assistance award.

(3) Discretionary Federal Financial Assistance means Federal awards including grants and agreements that are awarded at the discretion of the agency.

(4) Employment means:

(a) In any capacity, even if otherwise permissible, by any applicant or potential applicant for a Federal financial assistance award;

(b) Employment within the last 12 months with a different organization applying for some portion of the award's approved project activities and funding to complete them OR expected to apply for and to receive some portion of the award; and/or

(c) Employment with a different organization of any member of the organization employee's household or a relative with whom the organization's employee has a close personal relationship who is applying for some portion of the award's approved project activities and funding to complete them OR expected to apply for and to receive some portion of the award.

(5) Non-Federal entity means a State, local government, Indian tribe, institution of higher education, or nonprofit organization that carries out a Federal award as a Recipient or Subrecipient.

(6) Recipient means a non-Federal entity that receives a Federal award directly from a Federal awarding agency to carry out an activity under a Federal program. The term Recipient does not include Subrecipients.

(7) Subrecipient means a non-Federal entity that receives a subaward from a pass-through entity to carry out part of a Federal program, but does not include an individual who is a beneficiary of such program. A Subrecipient may also be a recipient of other Federal awards directly from a Federal awarding agency.

c. 2 CFR Part 200 Subpart F - Audit Requirements. Non-Federal entities that expend $750,000.00, or more, in federal awards in a single year shall have a single or program-specific audit conducted for that year in accordance with the Single Audit Act Amendments of 1996 (31 U.S.C. 7501-7507) and revised OMB Circular A-133, available at: http://www.whitehouse.gov/omb/circulars_default.

d. Indirect Facilities and Administration (F&A) Costs.

(1) 2 CFR Part 200.414 - Indirect (F&A) Costs

(2) 2 CFR, Appendix III to Part 200 - Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs)

(3) Appendix IV to Part 200 - Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Nonprofit Organizations

(4) Appendix V to Part 200 - State/Local Government-wide Central Service Cost Allocation Plans

(a) The provisions of 2 CFR 200.414(c) require Federal agencies to accept federally negotiated indirect cost rates. The BLM has applied the following policies, procedures and general decision-making criteria for deviations from negotiated Indirect Cost Rates for financial assistance programs and agreements.

(b) Distribution Basis. For all deviations to the Federal negotiated indirect cost rate, including statutory, regulatory, programmatic, and voluntary, the basis of direct costs against which the indirect cost rate is applied must be:

i The same base identified in the recipient's negotiated indirect cost rate agreement, if the recipient has a federally negotiated indirect cost rate agreement; or

ii The Modified Total Direct Cost (MTDC) base in cases where the recipient does not have a federally negotiated indirect cost rate agreement or, with prior approval of the Awarding Agency, when the recipient's federally negotiated indirect cost rate agreement base is only a subset of the MTDC (such as salaries and wages) and the use of the MTDC still results in an overall reduction in the total indirect cost recovered. MTDC is the base defined by 2 CFR 200.68, "Modified Total Direct Cost (MTDC)."

iii In cases where the recipient does not have a federally negotiated indirect cost rate agreement, under no circumstances will the Department use a modified rate based upon Total Direct Cost or other base not identified in the federally negotiated indirect cost rate agreement or defined within 2 CFR 200.68. The purpose of this restriction is to ensure that the reduced rate is applied against a base that does not include any potentially distorting items (such as pass-through funds, subcontracts in excess of $25,000, and participant support costs) and is based on the requirements outlined in 2 CFR 200.68; 2 CFR 200.414(f); 2 CFR 200 Appendix III, Section C.2.; 2 CFR 200 Appendix IV, Section B.3.f.; and Appendix VII, Section C.2.c.

(c) Indirect Cost Rate Reductions Used as Cost-Share. Instances where the recipient elects to use a rate lower than the federally negotiated indirect cost rate, and uses the balance of the unrecovered indirect costs to meet a cost-share or matching requirement required by the program and/or statute, are not considered a deviation from 2 CFR 200.414(c) as the federally negotiated indirect cost rate is being applied under the agreement in order to meet the terms and conditions of the award.

3. Program Legislation and/or Regulations. N/A

4. Standard Award Terms and Conditions.

a. Code of Federal Regulations/Regulatory Requirements, as applicable (contact your program officer with any questions regarding the applicability of the following):

(1) 2 CFR Part 25, *Universal Identifier and System of Award Management*

(2) 2 CFR Part 170, *Reporting Subawards and Executive Compensation*

(3) 2 CFR Part 175, *Award Term for Trafficking in Persons*

(4) 2 CFR Part 1400, *Government-wide Debarment and Suspension (Non-procurement)*

(5) 2 CFR Part 1401, *Requirements for Drug-Free Workplace (Financial Assistance)*

(6) 43 CFR 18, *New Restrictions on Lobbying*: Submission of an application also represents the applicant's certification of the statements in 43 CFR Part 18, Appendix A, *Certification Regarding Lobbying*.

(7) 41 USC §4712, *Pilot Program for Enhancement of Recipient and Sub-recipient Employee Whistleblower Protection*: This requirement applies to all awards issued after July 1, 2013 and shall be in effect until January 1, 2017.

(8) 41 USC §6306, *Prohibition on Members of Congress Making Contracts with Federal Government*: No member of or delegate to the United States Congress or Resident Commissioner shall be admitted to any share or part of this award, or to any benefit that may arise therefrom; this provision shall not be construed to extend to an award made to a corporation for the public's general benefit.

(9) Executive Order 13513, *Federal Leadership on Reducing Text Messaging while Driving*: Recipients are encouraged to adopt and enforce policies that ban text messaging while driving, including conducting initiatives of the type described in section 3(a) of the order.

(10) Executive Order 13658, *Minimum Wage for Contractors*, seeks to increase the efficiency and cost savings in the work performed by parties who contract with the Federal Government by increasing the hourly minimum wage paid by those contractors (see 79 CFR 9851). The Executive Order requires agencies to include a clause in applicable contracts and Contract like instruments that specifies, as a condition of payment, that the Executive Order Minimum wage be paid to workers in the performance of the contract and any subcontracts.

(11) Executive Order 13043, *Increase Seat Belt Use in the United States* Recipients of grants/cooperative agreements and/or sub-awards are encouraged to adopt and enforce on-the-job seat belt use policies and programs for their employees when operating company-owned, rented, or personally owned vehicles. These measures include, but are not limited to, conducting education, awareness, and other appropriate programs for their employees about the importance of wearing seat belts and the consequences of not wearing them.

(12) Opposition to Any Legislation. In accordance with the Department of the Interior, Environment, and Related Agencies Act, 2006, Title IV, Section 402, no part of any appropriation contained in this Act shall be available for any activity or the publication or distribution of literature that in any way tends to promote public support or opposition to any legislative proposal on which Congressional action is not complete other than to communicate to Members of Congress as described in 18 U.S.C. 1913.

(13) Metric Conversion. All performance and final reports, other reports, or publications, produced under this agreement, shall employ the metric system of measurements to the maximum extent practicable. Both metric and inch-pound units (dual units) may be used if necessary during and transition period(s). However, the recipient may use non-metric measurements to the extent the recipient has supporting documentation that the use of metric measurements is impracticable or is likely to cause significant inefficiencies or loss of markets to the recipient, such as when foreign competitors are producing competing products in non-metric units.

(14) Reimbursable Costs and Limitations. The recipient shall not incur costs or obligate funds for any purpose pertaining to operation of the program or activities beyond the expiration date stated in the agreement. The only costs which are authorized for a period of up to 90 days following the award expiration date are those strictly associated with closeout activities for preparation of the final report.

(15) The BLM's financial participation is limited. The BLM will only fund up to its share of those amounts requested in the project proposal and as are subsequently approved and funded in the agreement. The recipient shall not be obligated to continue performance under the agreement or to incur costs in excess of the costs set forth in the proposal and subsequent agreement. However, if the Recipient chooses to expend funds in excess of the approved project budget, the Recipient will be responsible to fund the excess without funding participation by the Bureau.

(16) 2 CFR, Part 200 Procurement Standards.

(17) 200-317 Procurement by States. When procuring property and services under a Federal award, a state must follow the same policies and procedures it uses for procurements from its non-Federal funds. The state will comply with §200.322 Procurement of recovered materials and ensure that every purchase order or other contract includes any clauses required by section §200.326 Contract provisions.

(18) All other non-Federal entities, including subrecipients of a state, will follow §§200.318 General procurement standards through 200.326 Contract provisions.

(19) §200.318 General procurement standards.

(20) §200.319 Competition.

(a) All procurement transactions must be conducted in a manner providing full and open competition consistent with the standards of this section. In order to ensure

objective contractor performance and eliminate unfair competitive advantage, contractors that develop or draft specifications, requirements, statements of work, or invitations for bids or requests for proposals must be excluded from competing for such procurements.

(21) §200.320 Methods of procurement to be followed.

(a) The non-Federal entity must use one of the following methods of procurement:

i Procurement by micro-purchases.

ii Procurement by small purchase procedures.

iii Procurement by sealed bids (formal advertising).

(22) Compliance with Buy American Act. NOTICE: Pursuant to Section 307 of the Omnibus Consolidated Appropriations Act of 1997, Public Law 104-208, 110 Stat. 3009, please be advised of the following: In the case of any equipment or product that may be authorized to be purchased with financial assistance provided using funds made available in this act, it is the sense of the Congress that entities receiving the assistance should, in expending the assistance, purchase only American-made equipment and products.

(23) Endorsements.

(a) Recipient shall not publicize or otherwise circulate, promotional material (such as advertisements, sales brochures, press releases, speeches, still and motion pictures, articles, manuscripts or other publications) which states or implies governmental, Departmental, bureau, or government employee endorsement of a product, service, or position which the recipient represents. No release of information relating to this award may state or imply that the Government approves of the recipient's work products, or considers the recipient's work product to be superior to other products or services.

(b) All information submitted for publication or other public releases of information regarding this project shall carry the following disclaimer:

> *The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Government. Mention of trade names or commercial products does not constitute their endorsement by the U.S. Government.*

(c) Recipient must obtain prior Government approval for any public information releases concerning this award which refer to the Department of the Interior or any bureau or employee (by name or title). The specific text, layout photographs, etc. of the proposed release must be submitted with the request for approval.

(d) A recipient further agrees to include this provision in a subaward to and subrecipient, except for a subaward to a State government, a local government, or to a federally recognized Indian tribal government.

(e) See also XII. SPECIAL TERMS AND CONDITIONS, B. Data Management, Paragraph 6, below.

(24) Intangible Property and Rights to Data.

a. Recipients are subject to the administrative standards set forth in 2 CFR, Subpart D, Sections 200.310 to 200.316, Property Standards.

b. See also XII. SPECIAL TERMS AND CONDITIONS, B. Data Management, below.

(25) Retention and Access Requirements for Records.

a. All recipient financial and programmatic records, supporting documents, statistical records, and other grants-related records shall be maintained and available for access in accordance with 2 CFR, Subpart D, Sections 200.333 through 200.337, Record Retention and Access.

b. Inspector General's (IG's) Office Access to Records - Recipients shall provide additional access for the IG's office to examine recipient's records and to interview officers/employees of recipient.

(25) Order of Precedence. Any inconsistency in this agreement shall be resolved by giving precedence in the following order: (a) Any national policy requirements and administrative management standards; (b) 43 CFR Part 12; (c) requirements of the applicable OMB Circulars and Treasury regulations; (d) special terms and conditions; (e) all agreement sections, documents, exhibits, and attachments; and (f) the recipient's project proposal.

XII. SPECIAL TERMS AND CONDITIONS

A. Scientific Integrity. Scientific integrity is vital to Department of the Interior (DOI) activities under which scientific research, data, summaries, syntheses, interpretations, presentations, and/or publications are developed and used. Failure to uphold the highest degree of scientific integrity will result not only in potentially flawed scientific results, interpretations, and applications but will damage DOI's reputation and ability to uphold the public's trust. All work performed must comply with the DOI Scientific Integrity Policy posted to http://www.doi.gov, or its equivalent as provided by their organization or State law.

B. Data Management.

1. Recipients should follow practices and guidelines for data management that are commensurate with those required by the National Institutes of Health (NIH), and by their own

university. The following guidelines for the sharing of research results are based on NIH standards:

2. The results and accomplishments of activities funded by the BLM should be made available to the public. Principal Investigators (PI) and awardee organizations are expected to make the results and accomplishments of their activities available to the research community and to the public at large. If the outcomes of the research result in inventions, the provisions of the Bayh-Dole Act of 1980, as implemented in 37 CFR 401, apply. As long as awardees abide by the provisions of the Bayh-Dole Act, as amended by the Technology Transfer Commercialization Act of 2000 (P.L. 106-404), and 37 CFR 401, they have the right to retain title to any invention conceived or first actually reduced to practice using BLM financial assistance funds.

3. In general, awardees own the rights in data resulting from a project supported by a BLM financial assistance agreement (grant or cooperative agreement). Special terms and conditions of the award may indicate alternative rights, e.g., under a cooperative agreement or based on specific programmatic considerations as stated in the applicable Request for Applications (RFA). Except as otherwise provided in the terms and conditions of the award, any publications, data, or other copyrightable works developed under a BLM cooperative agreement may be copyrighted without BLM approval. For this purpose, "data" means recorded information, regardless of the form or media on which it may be recorded, and includes writings, films, sound recordings, pictorial reproductions, drawings, designs, or other graphic representations, procedural manuals, forms, diagrams, work flow charts, equipment descriptions, data files, data processing or computer programs (software), statistical records, and other research data.

4. Rights in data also extend to students, fellows, or trainees under awards whose primary purpose is educational, with the authors free to copyright works without BLM approval. In all cases, BLM must be given a royalty-free, nonexclusive, and irrevocable license for the Federal government to reproduce, publish, or otherwise use the material and to authorize others to do so for Federal purposes. Data developed by a consortium participant also is subject to this policy.

5. As a means of sharing knowledge, BLM encourages awardees to arrange for publication of BLM-supported original research in primary scientific journals. Awardees also should assert copyright in scientific and technical articles based on data produced under the award where necessary to effect journal publication or inclusion in proceedings associated with professional activities.

6. All awardees must acknowledge Federal funding when issuing statements, press releases, requests for proposals, bid invitations, and other documents describing projects or programs funded in whole or in part with Federal money. Each publication, press release, or other document about research supported by a BLM award must include:

1. An acknowledgment of BLM support such as:

"Research reported in this [publication, release] was supported by the Bureau of Land Management under award number [cite the proper agreement number here]."

2. A disclaimer that says:
"The content is solely the responsibility of the authors and does not necessarily represent the official views of the Bureau of Land Management."

7. If the awardee plans to issue a press release about research supported by a BLM Wild Horse and Burro (WHB) program award, it should notify the BLM WHB program in advance to allow for coordination.

C. Publications.

1. Publications resulting from work performed under a BLM financial assistance-supported project must be included as part of the semi-annual or final Performance/Progress report submitted to the BLM. When publications are available electronically, the URL or the PMCID number must be provided. If not available electronically, one copy of the publication may be provided along with the progress report.

2. In addition to any requirements listed in the Project Management Plan, two (2) copies of each applicable publication produced under this agreement shall be sent to the Natural Resources Library with a transmittal that identifies the sender and the publication, and states that the publication is intended for deposit in the Natural Resources Library. Publications shall be sent to the following address:

U.S. Department of the Interior
Natural Resources Library
Interior Service Center
Gifts and Exchanges Section
1849 C Street, N.W.
Washington, D.C. 20240

D. Recipient/Subrecipient Personnel Security and Suitability Requirements

1. As implemented by Homeland Security Presidential Directive-12 (HSPD-12), if performance of this agreement requires recipient/subrecipient personnel to have a Federal government-issued Personal Identity Verification (PIV) credential before being allowed unsupervised access to a DOI facility and/or information system, the Program Officer will be the sponsoring official and will make the arrangements through a DOI Access Card Sponsor for personal identity verification and DOI Access Card issuance.

2. At least two weeks before start of agreement performance, the recipient must identify all recipient and subrecipient personnel who will require physical and/or logical access for performance of work under this agreement. Physical Access means routine, unescorted or unmonitored access to non-public areas of a Federally-controlled facility. Logical Access means routine, unsupervised access to a Federally-controlled information system. The recipient and

subrecipient must make their personnel available at the place and time specified by the Program Officer in order to initiate screening and background investigations. The following forms, or their equivalent, may be used to initiate the credentialing process:

a. OPM Standard Form 85 or 85P

b. OF 306

c. National Criminal History Check (NCHC) (local procedures may require the fingerprinting to be done at a police station; in this case, any charges are to be borne by the recipient or subrecipient, as applicable)

d. Release to Obtain Credit Information

e. PIV card application (web-based)

3. Before starting work under this agreement, a National Criminal History Check (NCHC) will be initiated to verify the identity of the individual applying for clearance and to determine the individual's suitability for the position. If the NCHC adjudication is favorable, a DOI Access Card will be issued for that individual. If the adjudication is unfavorable, the credentials will not be issued and the recipient or subrecipient must make other arrangements for performance of the work. In the event of a disagreement between the recipient/subrecipient and the Government concerning the suitability of an individual to perform work under this agreement, DOI shall have the right of final determination.

4. Recipient and subrecipient employees must give, and authorize others to give, full, frank, and truthful answers to relevant and material questions needed to reach a suitability determination. Refusal or failure to furnish or authorize provision of information may constitute grounds for denial or revocation of credentials. Government personnel may contact the recipient or subrecipient personnel being screened or investigated in person, by telephone or in writing, and the recipient or subrecipient must ensure they are available for such contact.

5. Alternatively, if an individual has already been credentialed by another agency through the Office of Personnel Management (OPM), and that credential has not yet expired, further clearance may not be necessary. In that case, the recipient/subrecipient must provide the sponsoring office with documentation that supports the individual's credentialed status.

6. Recipient and subrecipient employees who have been successfully adjudicated will be issued DOI Access Cards, which must be activated at a USAccess Credentialing Center. Those Recipient or subrecipient employees not located within a reasonable travel time of a USAccess Credentialing Center will be screened and issued alternate credentials, such as temporary access badges.

7. During performance of this agreement, the recipient must keep the Program Officer apprised of changes in personnel to ensure that performance is not delayed by compliance with credentialing processes. Cards that have been lost, damaged, or stolen must be reported to the

Program Officer, Grants Management Officer, and Issuing Office within 24 hours. If reissuance of expired credentials is needed, it will be coordinated through the Program Officer.

8. At the end of this agreement's performance, or when a recipient/subrecipient employee is no longer working under this agreement, the recipient will ensure that all identification cards are returned to the Program Officer.

E. Federal Information Systems Security Awareness Training. Before the recipient, or any of its employees or subrecipients, are granted access to the BLM Federal computer system, they must first successfully complete the U.S. Department of the Interior's (DOI) Federal Information Systems Security Awareness Online Course. This course was designed specifically for users of Federal computer systems. The course is a Web-based training product that explains the importance of Information Systems Security and takes approximately one hour to complete. This course is mandatory for all DOI employees, contractors, recipients, and all other users of DOI computer resources. Topics covered in the course include: threats and vulnerabilities, malicious code, user responsibilities, and new developments affecting Information Systems Security.

END OF AGREEMENT

BUREAU OF LAND MANAGEMENT
OR-WA State Office
Grants & Cooperative Agreements

PROJECT PROPOSAL

Instructions: A Project Proposal must be submitted with the Standard Form (SF) 424 Application for Financial Assistance for all Financial Assistance Agreements. A new proposal must be included with any request for modification which involves a revision to funding, project scope, period of performance, or key personnel.

Agreement or Funding Opportunity No.: L14AS00048 Date: 08/21/15

Organization Name: Colorado State University

Project Title: Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance

Current Funding Estimated Period of Performance (PoP): **08/24/15** to 8/23/20

Name & Title of Person Submitting Proposal: Dan L. Baker, Senior Scientist
Terry M. Nett, Senior Scientist

* If a specific start work date is needed, contact your BLM Program Officer. Do not start work without prior approval from the Grants Management Officer.

1. Purpose, Objectives, and Relevance:

(Describe why the project is needed, the applicant's objectives, how the applicant's objectives support their mission, and how this project benefits the general public.)

BACKGROUND

1. Re-immunization

In many areas of the western United States, overabundant and rapidly expanding populations of feral horses (*Equus caballus*) pose a significant dilemma for natural resource managers. The Wild Free-Roaming Horses and Burros Act of 1971(P.L.92-195) provided protection for feral horses and burros (*Equus asinus*) on most federal lands and established guidance for their management as a wildland species (Wagner 1983). There is, however, wide-spread concern among state, federal, and private land management agencies that unregulated feral horse populations are severely altering native plant communities and limiting the abundance and diversity of habitat resources allocated for native wildlife and other domestic livestock species.

Current population control methods such as utilizing periodic roundups and adoption or sale of excess animals, or maintaining excess feral horses in long-term holding facilities are expensive, resource intensive, and unsustainable. Clearly, more efficient, cost effective, and humane approaches to reducing feral horse densities on public lands are needed. Controlling the fertility of female horses offers a potential non-lethal alternative to conventional methods (National Research Council 2013).

A promising immunological approach to contraception in feral horses involves

immunization against the neuropeptide gonadotropin releasing hormone (GnRH). Scientists at the National Wildlife Research Center (NWRC) have conjugated synthetic GnRH peptides to a highly immunogenic carrier protein that, when combined with a potent adjuvant, stimulates the host's immune system to produce antibodies that bind to endogenous GnRH. This, in turn, prevents synthesis and secretion of important downstream reproductive hormones necessary for reproduction. Animals generally return to fertility as antibodies concentrations decline (Powers et al. 2011).

Multiple years of infertility have been achieved in captive and free-ranging wild ungulates with a single inoculation with the GnRH-based vaccine, known as GonaCon. This vaccines has been experimentally tested and found to provide multiple years of infertility after a single application in white-tailed deer (*Odocoileus virginianus)(* Miller et al. 2008, Gionfriddo et al. 2011*a*), bison (*Bison bison)(* Miller et al. 2004), elk (*Cervus elaphus)(* Killian et al. 2009, Powers et al. 2011, 2014), wild pig (*Sus scrofa)(* Massei et al. 2012), and feral horses (Killian et al. 2008, Gray et al. 2010, Baker et al. 2013). However, multiple years of infertility are only experienced in a fraction of vaccinated animals. In free-ranging elk, there was approximately a 90% treatment effect the first year after vaccination but that dropped to 50% by the second year and by the third year of the study, there was no measureable response (Powers et al. 2014). Similarly, during the first 3 years of our current investigation in feral horses at THRO, we observed a 25-35% decrease in foaling in treated versus control mares for the first and second years of the study but no effect by year three (Baker et al. 2013).

Repeat vaccinations generally result in a more profound and longer-lasting antibody production due to the anamnestic response (Tizard 1982). Therefore, we expect longer- lasting contraceptive effects in re-vaccinated mares. The single-injection GonaCon vaccine is unique in that the formulation initiates high antibody titers that remain elevated in some applications; however, to our knowledge, no research has been conducted to evaluate booster doses of this vaccine in any mammalian species.

Booster immunizations using a variety of GnRH vaccines in domestic horses have been shown to improve contraceptive efficacy and to suppress behavioral and physiological estrus (Garza et al.1986, Elhay et al. 2007, Botha et al 2008). However, these GnRH vaccines differ from GonaCon in that they incorporate different protein carrier molecules and adjuvants, and are formulated for short duration (< 1 yr.) contraceptive effectiveness that is generally achieved by using a primary immunization followed 35 days later by a booster inoculation.

While a single vaccination is often preferred from a management perspective, GonaCon vaccine may prove to be more effective if repeat vaccinations are delivered on a periodic basis. Efficacy data collected from 25 mares treated with single application of GonaCon in 2009, at Theodore Roosevelt National Park (THRO) revealed a moderate 2-year decline of approximately 30% in foaling rates, with all mares regaining fertility by three years post-primary vaccination treatment (Baker et al. 2013). Surprisingly, re-vaccination of these same mares in the fall 2013 (four years post-primary vaccination) has resulted to date, in complete infertility during the 2015 foaling season (the first season to expect a re-vaccination effect on fertility). Clearly, these results are both statistically and biologically significant, as well as encouraging from a fertility control perspective.

If these results persist over time and these mares remain infertile, it would lend support to our hypothesis that re-vaccination with GonaCon, even four years post-primary vaccination produces a strong anamnestic response in horses that stimulates anti-GnRH antibodies and suppresses fertility. At present, however, it is premature to predict how many of these re-vaccinated mares failed to conceive during the 2014 breeding season and will not foal or regain fertility during 2015 and beyond. It is possible that the booster vaccination simply delayed the estrous cycle in these mares, which could result in foals being born later in the foaling season.

While these findings are tentative and inconclusive, they suggest that repeat vaccinations are likely needed to achieve high efficacy of GonaCon vaccine in free-ranging horses and these

effects have not been investigated or determined. Thus, our proposed research offers a unique opportunity to address this question at THRO and will have relevance, not only to feral horses, but also to other wild ungulates that have been treated with a single treatment of GonaCon vaccine. Our proposed research will begin to define the vaccination schedule needed to maintain infertility in free-ranging horses and whether or not long-term or permanent sterility is a possible outcome. We will investigate the safety and efficacy of a repeat vaccination under the hypothesis that this vaccine will be more efficacious and longer-lasting than the original primary immunization.

2. Remote Dart Delivery

Fundamental to practical field application of GonaCon vaccine in free-ranging horses is a safe, reliable, and effective method of administering a single dose of the vaccine to free-ranging horses by means of a syringe dart. Many contraceptive agents have been successfully applied via syringe dart or biodegradable implant to an assortment of wild ungulate species including white-tailed deer (Turner et al. 1992, Jacobsen et al. 1995, DeNicola et al. 1997), elk (Shideler et al. 2002, Baker et al. 2005), feral horses (Kirkpatrick et al.1990, Roelle and Ransom 2009), and elephants (*Loxodonta Africana*) (Delsink et al. 2002). However, to our knowledge, evaluation of remotely-delivered GonaCon vaccine is limited to one field investigation with white-tailed deer (DeNicola unpublished data). Although dart performance in this study was less than expected, it provided important basic information regarding optimum dart configuration and delivery ballistics. Using this preliminary data, technicians at Pneu-Dart, Inc. developed a prototype dart configuration for delivering this highly viscous vaccine formulation to free-ranging horses.

We tested this GonaCon-specific dart delivery system with captive feral horses at the 2013 scheduled roundup at THRO. Eleven adult mares (2-4 years of age), that had not been previously vaccinated, were held in small paddocks and remotely darted in the biceps femoris muscle with 2 ml (2000 µg) of GonaCon vaccine. All darts were weighed (± 0.01g) before and after injection to determine the precise dose delivered. Darting distance varied from 10-15 m. Nine out of 11 darts delivered, on average, 95% of the GonaCon vaccine formulation. Two darts failed to discharge possibly due to low muzzle velocity. All darts appeared to dispense the vaccine deep into the muscle mass and none of the darts were observed to bounce without penetration, partially discharge, blow-out, or show evidence of subcutaneous delivery of the vaccine. The two horses in which the darts failed to discharge were subsequently re-treated and the second darts successfully delivered a full dose. With 85% of the 2015 foaling season complete, 7/11 (63%) of these mares have not foaled. In contrast, only 16% of the untreated mares have not foaled to date. A dependable dart delivery system for administering GonCon remotely to free-ranging horses is critical to the determination of an optimum re-vaccination schedule in our proposed study. If successful, this technology will potentially provide resource managers with an alternative strategy for managing this feral horse population.

3. Biological Side-Effects

Evaluation of the biological side-effects of GonaCon vaccine treatments have been reported for numerous wild ungulate species including white-tailed deer (Curtis et al. 2008, Gionfriddo et al. 2011*b*), elk (Powers et al. 2011, 2012, 2014), bison (Miller et al. 2004) and feral horses (Baker et al. 2013). Results from these investigations generally conclude that GonaCon does not cause serious adverse effects on general health, body condition, existing pregnancy, neonatal health, major organ systems, or fertility of male and female offspring of females treated during pregnancy.

Granulomatous intramuscular injection-site lesions, that occasionally break and drain as abscesses, are the only adverse effect of vaccination consistently reported in these studies. The formation of these injection site lesions may be necessary for stimulation of a strong immune response and infertility. GonaCon vaccine contains AdjuVac; a water-in-oil based adjuvant

developed from a USDA approved Johnes disease vaccine called Myocopar™ (Fort Dodge Animal Health). AdjuVac contains killed *Mycobacterium avium,* which is needed to induce a rapid, strong, and sustained contraceptive response (Miller et al. 2008*a*, Perry et al. 2008). This combination of water - in- oil emulsion and killed mycobacteria results in a highly potent adjuvant that stimulates both humoral and cellular immunity (Warren et al. 1986).

Vaccines, like GonaCon, that contain mycobacteria may induce strong immune responses because of the formation of a repository or depot at the injection site (Fukanoki et al. 2000). In response to the presence of the depot, a granuloma forms as the immune system attempts to isolate the foreign material. The continued existence of this depot, which initiates a chronic inflammatory response, likely provides a long-term source of antigen stimulation and persistent antibody production. We speculate that this is the mechanism by which a single vaccination can provide multiple years of infertility in a portion of the population in many species that have been studied.

However, even with this prolonged antigenic stimulation, the immune response from a single vaccination does not consistently provide multiple years of infertility in all or even a high proportion of animals (Powers et al. 2014, Baker et al. 2013). In all studies, where post-mortem examinations were performed, prevalence of injection-site inflammation and granulomas were present but in some species, such as white-tailed deer and elk, they were not apparent antemortem (Curtis et al. 2008, Powers et al. 2011, Gionfriddo et al. 2011*b*).

In contrast to these species, injection site reactions in feral horses, following GonaCon vaccination at THRO, are readily observable as subcutaneous swellings. In past studies at THRO (2009-2013), all injection site reactions appeared to be confined to the general gluteus muscle where the vaccine was first hand-injected. Reactions to the vaccine were first observed 30 days post-treatment in 17.2% (5/29) of mares and by the second breeding season, 79.3% (23/29) of treated females showed some evidence of inflammation or swelling at the injection site. Saline control mares displayed no evidence of injection site reactions. Swellings of various sizes (marble to baseball size) were most common, followed by nodules, and rarely a draining abscess. Most of these reactions were observable for three years post-treatment, then began to resolve and become less visible by year 4 (many that could not be visually observed were still manually palpable at the 2013 roundup).

However, similar to other studies where injection site reactions have been evaluated, we did not observe any clinical evidence of lameness, impaired mobility, depression, or decreased health or fitness in any animal that was associated with GonaCon vaccine treatment. While results from the above investigations are generally consistent relative to the effects of GonaCon-induced injection site reactions, they are also limited to the consequences of a single vaccination usually delivered by hand-injection.

At the 2013 THRO round-up, GonaCon –treated mares were re-vaccinated, four years post-primary vaccination, with a booster dose on the opposite side in the biceps femoris muscle. This investigation is in progress but thus far, injection site reactions appear to be less apparent than those observed following the 2009 vaccination (Baker et al. unpublished). At this time, the cumulative effects of re-vaccination are unknown and the potential for more intense immune reactions with additional doses of this vaccine delivered by syringe dart is a consideration (Broderson 1989, Roelle and Ransom 2009).

4. Behavioral Side-Effects

Behavioral side-effects of GonaCon vaccination in wild ungulates have not been extensively investigated (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014). Given the physiological mechanism of action, GonaCon vaccine has the potential to suppress fertility and diminish the reproductive behaviors typically associated with estrus. However, in GonaCon-vaccinated female elk (Powers et al. 2011) and free-ranging horses (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014) such behaviors were maintained throughout the first breeding season

after immunization and were not different from untreated females.

In a previous study at THRO during 2009-2010, daily activity patterns, social interactions, and reproductive behaviors were similar for GonaCon treated and control mares (Baker et al. 2012, Ransom et al. 2014). But, since GonaCon only prevented conception in 50% of treated mares (n = 28), behavioral observations were limited to only 14 infertile females. Thus, inferences to free-ranging feral horse populations are not definitive and deserve further investigation prior to use in management applications.

In an attempt to further our understanding of the behavioral side-effects GonaCon vaccine, we conducted behavioral observations during the first breeding season following re-vaccination of these same mares at THRO in 2013. We measured the effects of this vaccine on sociosexual behavior, harem dynamics, and activity budgets of treated (n = 25) and control (n =25) horses. To date (July 20 2015), none of the re-vaccinated mares have foaled, whereas 84% (21/25) of the control mares have done so. As a result of higher vaccine efficacy in treated mares, our sample size increased by 44% and offered a more rigorous quantitative investigation into potential effects of GonaCon treatment on feral horse behaviors.

5. Population Modeling

We will integrate contraceptive efficacy and population monitoring data at THRO to estimate parameters and unobserved states in a Bayesian hierarchal model (Dulberger et al. 2010, Monello et al. 2014, Hobbs and Hooten 2015, Hobbs et al. 2015, Rahio et al. in review).We will use the model to evaluate the population-level effects of GonaCon on the free-ranging horse population at THRO. We will forecast the consequences of alternative contraceptive strategies on population performance with rigorous evaluation of uncertainty. There is an urgent need to extend studies of efficacy of individuals to populations (Ransom et at. 2014). A key extension of our experimental research is to determine the effects of different GonaCon delivery regimes on the growth rate of the THRO population.

OBJECTIVES:

The primary objectives of this research are:

a) to begin to determine the optimum and most effective re-vaccination schedule with GonaCon vaccine for suppressing reproductive rates in free-ranging horses, the duration of effectiveness, and the return to fertility following treatment.

b) to determine the safety and physiological side-effects (if any) in feral horses following re-vaccination with GonaCon including visual assessment of general health, body condition, injection site reactions, effects on current pregnancy, and neonatal health and survival.

c) to determine the effects of GonaCon vaccination on the behavioral side-effects (if any) in free-ranging horses including quantitative assessment of the effects on daily activity patterns and social interactions.

d) to develop and test a safe and effective dart configuration and injection system for remotely administering GonaCon vaccine to free-ranging horses by means of a syringe dart.

e) to develop a Bayesian model to forecast the consequences of different GonaCon vaccine treatments on feral horse population dynamics at THRO.

HYPOTHESIS:

H1: Female feral horses re-vaccinated with GonaCon will show significantly ($P \leq 0.05$) lower reproductive (yearly pregnancy and foaling) rates than non-treated control mares and contraceptive efficacy of re-vaccinated mares will be greater and longer lasting than that observed following the initial immunization.

Rationale: An immune response is a physiologic reaction to a foreign substance or antigen; especially one mediated by lymphocytes and involving recognition of antigens by specific antibodies or previously sensitized lymphocytes. Vaccines rely on the anamnestic response for optimal function. This response is a renewed rapid production of antibodies on the second (subsequent) encounter with the same antigen. This reaction is possible through memory cells that store information regarding the recognition of an antigen based upon previous exposure. Booster or repeat vaccinations generally result in a more rapid and stronger immune reaction to a second inoculation with the same antigen (Tizard 1982). However, the optimum re-vaccination schedule for GonaCon vaccine in feral horses or any other ungulate species has not yet been investigated or determined.

2. Technical Approach:

(Describe how the project will be conducted. The project design must contain enough detail to show the development of the project, including the relationship between the partners, milestones, and objectives. Clearly describe the techniques, procedures, and methodologies to be used; the data collection, analysis, and means of interpretation; the expected results and/or outcomes; and the procedures for evaluating project effectiveness, including appropriate performance measures and the probabilities of obtaining them.)

EXPERIMENTAL DESIGN AND METHODS

Study area and experimental horses

Theodore Roosevelt National Park (THRO) is located near the town of Medora in southwestern North Dakota (45º 55' N/103º 31'W) and consists of two units that are separated by approximately 115 km of federally and privately owned rangeland. The South Unit of the park, where this study will be conducted, comprises 19,000 ha and consists of eroded badlands with gullies and ravines separated by upland plateaus and small erosion-resistant buttes (Laird 1950). All feral horses used in these experiments are free-ranging and permanently reside in this unit of the park.

At present, there are approximately170 horses divided into roughly 10-15 individual bands and bachelor groups. Horses and bison are confined to the South Unit by a 1.8 to 2.4-m woven wire boundary fence. Feral horse history, distribution, habitat use, and population management at THRO have been previously described (Marlow et al. 1992). Individual horses are known by unique markings and band affiliations. Age and reproductive genealogy data for each animal has been retained in a database since 1993. The approximate date of birth (± 30 days) is known for each horse. Photographs have been taken of each mare from birth to adulthood to assist in the identification of individual horses.

Experimental treatments

In order to determine the optimum re-vaccination schedule for GonaCon vaccine in free-ranging

horses at THRO, we propose four post-primary vaccination treatment intervals of: a) four years, b) two years, c) one year, and d) six months (Table 1). The numbers of experimental treatments are limited by the availability of adult mares currently residing in the park. All experimental mares participating in these experiments have been assimilated into various bands such that each band contains one or more individuals from these treatment groups as well as untreated control mares.

Table 1. Summary of primary and secondary vaccination schedules and sample sizes for each experimental group of feral horses treated with GonaCon Immunological Vaccine or saline at THRO.

RE-VACCINATION TREATMENT	SAMPLE SIZE (N)	DATE OF PRIMARY VACCINATION	DATE OF SECONDARY VACCINATION
FOUR YEARS POST-PRIMARY	25	OCT - 2009	SEPT - 2013
TWO YEARS POST-PRIMARY	11	SEPT - 2013	SEPT - 2015
ONE YEAR POST-PRIMARY	16	SEPT - 2015	SEPT - 2016
SIX MONTH POST-PRIMARY	16	SEPT - 2015	MAR - 2016
SALINE CONTROL	25	OCT – 2009	SEPT - 2013

A description of each treatment group, the method of treatment application, and pertinent measurements and observations are presented below:

1) Four-year post-vaccination group. This experimental group was initially established and treated during the scheduled roundup at THRO in 2009. Ongoing measurements of foaling rates and biological side-effects following re-vaccination in 2013 are currently being conducted and will provide a four-year post-primary re-vaccination treatment group (n = 25) and control group (n = 25).

Experimental animals and treatment application: During a scheduled NPS gather and removal in September 2013, horses were herded by helicopter into permanent corrals and handling facilities. Fifty, adult mares (5-19 years of age) (25 GonaCon -treated: 25 saline-control) that had been previously vaccinated with a single inoculation of GonCon- or saline solution in October 2009 were identified and retained within the park for this experiment. Band stallions were also retained. All mares were identified individually using a photographic data base of pelage color and band association, as well as, previously implanted passive integrated transponder (PIT) tags. General health, pregnancy status, and body condition of each animal was assessed while horses were restrained in a hydraulic squeeze chute. Pregnancy status and approximate stage of gestation were determined using rectal palpation of the reproductive tract and transrectal ultrasound imaging (Bucca et al. 2005). Up to 50 mls of blood was collected and serum removed, frozen, and archived for future anti-GnRH antibody analyses (Powers et al. 2011). We collected hair samples from all horses to assess the genetic status of the population and fecal samples for pregnancy determination and prevalence of endoparasites. Body condition of mares was assessed and scored visually according to methods described by Henneke et al. (1983). Mares in the treatment group received an intramuscular booster inoculation, by hand-syringe, containing 2000μg (2 ml) of GonaCon (synthetic GnRH conjugate Blue Carrier protein and emulsified in

AdjuVac™ adjuvant (Miller et al. 2008) in the middle gluteus muscle on the opposite side from the primary vaccination. Mares in the control group were injected in the same way with an equal volume of saline solution. These treatments and procedures were identical to the ones used in 2009 except that injections were given on the right side of the body in 2013 rather than the left to allow differentiation from the previous injection site.

2) Two-year post-vaccination group. This vaccine treatment was applied at the 2013 scheduled roundup at THRO to investigate remote delivery of GonaCon vaccine. Re-vaccination of these mares in 2015 will provide a two-year post-vaccination treatment group.

Experimental animals and treatment application. Based on the promising results from the captive trial conducted in 2013, we will extend our evaluation of a remote dart delivery system of GonaCon from a controlled captive setting to a field test with these same mares that are now free-roaming in their respective bands at THRO. This field application will also provide an additional cohort of mares that have been re-vaccinated two years post-primary vaccination. During September 2015, the eleven mares that were previously administered a primary dose of GonaCon vaccine by means of syringe dart delivery, will be located in the park and re-immunized using the same dart configuration and delivery ballistics as that used for the captive trials in 2013. Each dart will be numbered and correspond to an individual mare. We will determine darting efficacy by measuring the precise dose of the vaccine delivered to each mare. This will be done by weighing each dart (± 0.01g) before and after injection. We will measure dart retention time in each animal and dart performance (i.e. failure rate, partial discharge, blow-out, bounce)**.** In the case of darts that fail to discharge or partially inject the vaccine, the animal will be re-darted until the full dose has been delivered. We will also record each animal's behavioral response to dart injection.

3) One year post-vaccination group and 4) six-month post-vaccination group. Including these two additional re-vaccination treatments will hopefully allow us to more clearly define the optimum re-immunization schedule for GonaCon vaccine in feral horses. However, we have no prior immunological evidence to support these time periods as being optimum or different from each other. These intervals were selected primarily on the basis of practical field application of the vaccine. It would generally be infeasible to locate and treat horses via remote dart delivery during the winter months (December-February) at THRO. Therefore, shorter time periods such as three months (which was the minimum time required for maximum antibody production in elk) (Powers et al. 2011) are not practical. Re-vaccination of mares at the 6 month interval will be conducted in March 2016 and for mares in the one-year interval group during September 2016.

Experimental animals and treatment application. Thirty-two free-ranging mares (1.5-3.5 years of age) will be selected for these treatment groups. A randomized complete block design consisting of either a one year or six-month GonaCon- re-vaccination group will be used in this analysis. Mares will be paired on the basis of age and pregnancy status such that animals within each block (n = 16 blocks of 2 mares each) will be as similar as possible. Within each pair, a mare will be randomly assigned to each experimental group. The general health, pregnancy status, and body condition of each mare will be determined in the field by trained biologist familiar with these animals. Pregnancy status will be determined by fecal estrogen assay (Baker et al. unpublished data). Body condition of all study mares will be evaluated visually and scored on a scale of 1 (very thin) to 9 (very fat) (Henneke et al. 1983). During September 2015, all 32 mares will receive a primary vaccination with GonaCon vaccine via remote dart delivery. Approximately 6 months (March 2016) following the initial vaccination, 16 mares will be re-vaccinated with GonaCon and 1 year later (September 2016) the remaining 16 mares will be similarly treated. All horses will receive the re-vaccination treatment using remote dart delivery.

Field Measurements:

Effects on reproduction. We will determine the effectiveness, duration of effects, and reversibility of a second immunization with GonaCon on reproduction during 2015-2020 (or beyond, if necessary) by comparing foaling and pregnancy rates of treated and control mares. Annual foaling rates will be estimated by observing all mares, at least weekly, during the breeding season (April – August) and documenting the presence of new foals and estimating approximate date of birth. We will continue to monitor reproductive rates in all experimental mares during 2015-2020 or until the magnitude of the difference in foaling rates between treatment and control mares is less than 50% or funding is no longer available. Supplementary to foaling rates, we will also collect fecal samples during approximately mid-gestation (October-February) and determine fecal estradiol concentrations to estimate pregnancy rates of all mares (Baker et al. unpublished data).

Biological side-effects. In conjunction with the above measurements, we will assess the safety and side effects of a second immunization with GonaCon. In both treatement and control groups of horses, we will evaluate the effects (if any) on general health, body condition, existing pregnancy, neonate survival and injection site reactions at weekly intervals during the breeding season and opportunistically throughout the year. In addition, we will observe all experimental mares for presence or absence of lameness (limping, gait alteration, reluctance to stand or bear weight, and evidence of swelling or discharge) at the site/side of vaccine injection. We will classify injection site reactions into four categories according to the scoring system of Roelle and Ransom (2009). Both the previous injection site in 2009 and the one in 2013 will be evaluated each year in conjunction with foaling observations.

Behavioral side-effects. We evaluated the effects of GonaCon vaccine on the daily activity patterns and social interactions of the four-year post vaccine group during March – August 2015. We used a restricted randomized design to balance observations as much as possible among all experimental animals while also trying to observe the behavior of each mare at least 6-8 times per month. We located bands containing selected mares by vehicle, foot, or horseback. Observations were balanced across time of day and conducted from distances of 50-100m with the aid of binoculars and spotting scopes. Each sampling period consisted of 20 min of continuous observation. We used a combination of instantaneous scan sampling procedure to record time budget data and all-occurrence sampling to record reproductive behaviors (Altmann 1974). We followed field and analytical methods described by Ransom and Cade (2009) to develop a herd-specific ethogram for selected behaviors at THRO. We will compare behavioral observations of GonaCon-treated mares and control mares the first breeding season following primary vaccination in 2010 and re-vaccination in 2013. Statistical analysis of data will follow those described by Ransom et al. (2014).

Statistical analysis

Our power analysis was originally developed for the four-year post-treatment group but offers an approximation of statistical power needed to detect a treatment effect for other treatments as well. We used a fixed sample size of available mares (n = 50, equally divided into 2 groups of 25 each), to estimate statistical power (1-β) for detecting a treatment effect (0.9 – 0.2) over time. We then used a 1-sided, two-sample t-test with a normal approximation together with software program SYSTAT 12.02.00 (SYSTAT Software, Inc.) to estimate the power for detecting effect sizes that vary from 0.20-0.90 (Kang and Kim 2004) (Table 2). Our current 2-year mean effect- size (difference between mean foaling rates in treatment [0.485] and control [0.759] groups) is 0.274. If repeat vaccination does not improve contraceptive efficacy, we will have little power to detect a difference between treatment groups and will conclude there is little

effect due to re-vaccination. However, if revaccination increases effect size to 0.6 or better we will have sufficient power to detect these effects.

We will determine the efficacy of re-vaccination treatments by comparing the proportion of fertile females in each treatment group with control females in the original four-year post-vaccination group combined across all foaling seasons. Females will be classified as being fertile, or infertile on the basis of the presence of a foal at heel, or fecal estrogen concentrations indicating pregnancy. We will use a linear mixed model analysis with restricted maximum likelihood estimation to determine treatment effects on fertility rates. A chi-squire test will be used to test for differences among fertility rates, foal survival, and seasonality of births. We define the foaling season to include March, April, May, June, and July. Results will be shown as means ± standard errors when appropriate.

We will also explore using Bayesian beta-bimodal (similar to the one used by Monello et al. 2014 to estimate elk survival) to examine the size of treatment effects. Power will be less of an issue in this approach because we will be able to show the probability distribution of differences attributable to treatment.

Table 2. Power calculations and corresponding contraceptive treatment effect size for the GonaCon field experiment with free-ranging mares at Theodore Roosevelt National Park.

Total Sample Size	Group Sample Size	THRO Foaling Rate	Effect Size	Alpha	Power (1-β)
50	25	0.759	0.9	0.1	0.977
50	25	0.759	0.8	0.1	0.949
50	25	0.759	0.7	0.1	0.898
50	25	0.759	0.6	0.1	0.817
50	25	0.759	0.5	0.1	0.706
50	25	0.759	0.4	0.1	0.570
50	25	0.759	0.3	0.1	0.425
50	25	0.759	0.2	0.1	0.290

Limitations in study design

One difficulty in this study is that, to our knowledge, there are no published data regarding the optimum re-vaccination schedule for GonaCon vaccine in horses or any other wild or domestic ungulate. Thus, while we may have adequate sample sizes to detect treatment differences between GonaCon-treated and control groups, our sample sizes may be inadequate to detect small differences among the four post-primary treatment groups. This limitation is due to the restricted availability of additional female horses at THRO for this experiment.

Moreover, the control group of mares used to compare treatment effects in this study was originally selected in 2009 to be as similar as possible to the four-year re-vaccination group. However, it is not necessarily representative of the re-vaccinated mares selected for the subsequent treatments. If this study was implemented in captivity, more appropriate control groups could have been established. Additionally, a more complex study design that incorporated different vaccination time-points and regimes could have more accurately determined the optimal time point for re-vaccination.

Our study was implemented to compliment practical management efforts at THRO that are determined by having reasonable access to study horses for treatment application. Regardless of efficacy outcome, this study will provide valuable information. If re-vaccination at these intervals is not

successful, our study will provide important information on the utility of this vaccine. If it is successful, the vaccine may have more wide-spread utility than previously observed.

Performance Measures and Reporting:

2015 - 2016

1. Collect and summarize four-year post-primary vaccination foaling rate estimates for GonaCon-treated mares and control mares for the 2015 and 2016 foaling seasons.

2. Collect and summarize data pertinent to foaling rates and side-effects of GonaCon-treated mares for the two-year post-primary vaccination group for the 2015 and 2016 foaling seasons.

3. Select and document successful re-vaccination of mares in the two-year post-primary vaccination group (11 mares) and primary vaccination of mares in the one-year (16 mares) and six month (16 mares) post-vaccination groups (September 2015).

4. Document successful re-vaccination of mares in the six month revaccination group during March 2016 and for the one-year group in September 2016.

5. Compare foaling rates on all vaccination schedules to their pregnancy rates estimated via fecal estrogen analysis.

6. Provide data analysis summarizing the effects of GonaCon vaccine on daily activity patterns and social interactions of feral horses at THRO during 2015-2016.

BUDGET

Table3. Yearly budget, by category, for proposed research at Theodore Roosevelt National Park 2015-2020.

Category	Year 1	Year 2	Year 3	Year 4	Year 5
Personnel	$40,898	$29,300	$29,878	$34,847	$67,473
Fringe benefits	$7,626	$5,866	$5,991	$7,033	$15,722
Travel	$3,003	$2,946	$1,964	$1,964	$1,964
Equipment	$ 0	$ 0	$ 0	$0	$ 0
Supplies	$4,550	$1,950	$1,950	$1,950	$1,950
Other	**$** 0	$ 0	$ 0	$1,000	$5,000
Direct costs	$56,077	$40,062	$39,783	$46,794	$92,109
Indirect costs	$9,813	$7,011	$6,962	$8,189	$16,119
Total costs	$65,890	$47,073	$46,745	$54,983	$108,228

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Powers, J.G., D.L. Baker, M.G. Ackerman, J.E. Bruemmer, T.R. Spraker, M.M. Conner, and T.M. Nett. 2012. Passive transfer of maternal GnRH antibodies does not affect reproductive

development in elk (*Cervus elaphus nelsoni)* calves. Theriogenology 78:830-841.
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Ransom, J. I., J. G. Powers, H. M. Garbe, M. W. Oehler, T. M. Nett, and D. L. Baker. 2014. Behavior of feral horses in response to culling and GnRH immunocontraception. Applied Animal Behavior Science 157:81-92.
Ransom, J. I., J. G. Powers, N. Thompson Hobbs, and D. L. Baker. 2014. REVIEW: Ecological feedbacks can reduce population-level efficacy of wildlife fertility control. Journal of Applied Ecology **51**:259-269.
Roelle, J.E. and J.I. Ransom. 2009. Injection-site reactions in wild horses (*Equus caballus)* receiving an immunocontraceptive vaccine. U.S. Geological Survey Scientific Investigation Report 2009-5038, 15p.
Shideler, S. E., M. A. Stoops, N. A. Gee, J. A. Howell, and B. L. Lasley. 2002. Use of porcine zona pellucida (PZP) vaccine as a contraceptive agent in free-ranging tule elk (*Cervus elaphus nannodes).* Reproduction 60(Suppl):169-176.
Tizard, I.R. 1982. An introduction to veterinary immunology. Second edition. W.B. Saunders Company, Philadelphia, Pennsylvania, USA.
Turner, J.W., Jr., I.K.M. Liu, and J. F. Kirkpatrick. 1992. Remotely delivered immunocontraception in white-tailed deer. Journal of Wildlife Management 56:154-157.
Wagner, F. N. 1983. Status of wild horse and burro management on public rangelands. North American and Natural Resources Conference 48:116-133.
Warren, H.S., F.R Vogel, and L.A. Chedid. 1986. Current status of immunological adjuvants. Annual Review of Immunology 4:369-388.

3. Qualifications, Experience, and Past Performance:
(Describe who will carry out the project activities. List all project personnel, including consultants, contractors, sub-recipients, etc., if known. Describe their responsibilities and the amount of time each will dedicate to the project. Briefly describe how their experience and qualifications are appropriate to successfully achieve the stated objectives.)

Dan L. Baker, Affiliate Faculty, Research Scientist, Colorado State University, Department of Biomedical Sciences/Animal Reproduction and Biotechnology Laboratory: will coordinate all project activities, study design, data collection and analysis, personnel management, reporting, interagency coordination. Dr. Baker has been the project leader in the evaluation of GonaCon in feral horses at Theodore Roosevelt National Park (THRO) since 2009. Prior to that (2006-2013) he was involved with similar research with this contraceptive vaccine in captive and free-ranging elk in Rocky Mountain National Park (ROMO) (50%).

Jenny G. Powers, Wildlife Veterinarian, National Park Service: attending veterinarian, assist with study design, and assessment of biological side-effects of GonaCon vaccine. Dr. Powers has been involved with the evaluation is this contraceptive agent at THRO since 2009 and was involved in similar research with captive and free-ranging elk in ROMO. Much of her previous research has been focused on the efficacy and physiological side-effects of various contraceptive agents. She will also facilitate animal care and use approval from NPS for this project.

Blake E. McCann, Wildlife Biologist, National Park Service, Theodore Roosevelt National Park: liaison and on-site project manager at THRO, study design, will lead efforts in dart delivery of GonaCon in free-ranging horses, will provide in-kind support for this research effort (i.e.

vehicles, office space, housing for field technicians) and coordinate research activities with ongoing NPS operations. Dr. McCann has been involved with the evaluation of GonaCon since 2013 has been instrumental in the design and evaluation of a GonaCon-specific dart configuration and ballistic system for feral horses.

N. Thompson Hobbs, Professor, Senior Research Scientist, Colorado State University (CSU), Department of ESS, Natural Resource Ecology Laboratory: will lead efforts to model effects of fertility control on feral horse population dynamics; provide statistical analysis of data, and coordinate administrative services and support for this project within NREL. Dr. Hobbs has been involved with several projects modeling the effects of fertility control on wild ungulates. He is currently working on a Bayesian state-space model of population dynamics of white-tailed deer to evaluate alternatives for population management including fertility control (5%).

Jason E. Bruemmer, Professor, Colorado State University, Department of Animal Science, Equine Reproduction Laboratory: provide technical expertise on reproductive physiology of feral horses, study design, interpretation of data, and manuscript preparation. Dr. Bruemmer has been involved with this investigation since 2009 and has provided pregnancy assessment of experimental mares at the 2009 and 2013 roundups. We have incorporated his mare pregnancy criteria and body condition scoring system into our field measurements.

Terry M. Nett, Professor, Colorado State University, Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory: provide laboratory services for fecal estrogen assay. Dr. Nett has been involved with this research project since 2009, as well as, similar research with this vaccine in captive and free-ranging elk and domestic horses. He is a leading authority on reproductive endocrinology and GnRH metabolism in mammals (1%).

Kathleen M. Eddy, Laboratory and field research technician, Colorado State University, Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory: Lead responsibility for developing and validating a fecal estrogen assay for pregnancy determination in horses; this assay will supplement foaling rate measurements to assess pregnancy status and treatment responses in experimental mares at THRO. In addition, she will assist with fecal collections and other field measurement (5%).

Douglas C. Eckery, Senior Scientist and Project Leader, USDA, APHIS, Wildlife Services, National Wildlife Research Center: will be primarily responsible for providing 100- 2ml doses of GonaCon-Equine vaccine packaged in 3ml plastic syringes for this study.

APPENDIX

Institutional Animal Care and Use Permits

G. HUMANE CARE AND USE OF ANIMALS

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort / Grant Application

Privileged Communication

Title of proposal: Evaluation of Re-Immunization with GonaCon-Equine™ on Reproduction and Side-Effects in Feral Horses

Investigators: Baker, Dan L.; Nett, Terry M.; Powers, Jenny G; Ransom, Jason I; Bruemmer, Jason E; Hobbs, N. Thompson; McCann, Blake E.

Pursuant to procedures established by the Bureau of Land Management, Wild Horse and Burro Research Program, I certify that the above described protocol follows guidelines set forth in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (#85-23) and the "Animal Welfare Act of 1966" (PL 89-544) as amended.

Signature _____ Date 4/30/14
Terry Engle, Ph.D., Chair, CSU Institutional Animal Care and Use Committee

Name of Institution: Colorado State University

NOTE: This completed form must be in receipt of the BLM WH&B Research Advisory Team before the initiation of funding or collaborative work can commence. Private individuals must seek local/regional institutional approval.

United States Department of the Interior
NATIONAL PARK SERVICE
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, Colorado 80525

National Park Service
Institutional Animal Care and Use Committee
Animal Research Protocol Approval

Principal Investigator(s): Dan Baker/ N. Thompson Hobbs
Telephone: 970.556.8518
Electronic Mail: danbaker@colostate.edu

Region: Midwest Region

Protocol Approval Number: MWR_THRO_Baker_Horse_2013.A3

Project Title: Remotely-delivered GnRH Vaccine (GonaCon-Equine) in Free-Ranging Horses: A Preliminary Investigation

Approval Date: 9/23/2013

Effective Date: 9/23/2013

Questionnaire Dates; Years 1 and 2 (if applicable): 9/23/2014, 9/23/2015

Expiration/Re-Submittal Date: 9/23/2016

Funding Agency(ies): None

Species: Horse (*Equus caballus*)

Number(s) of Animals: 10 horses/year, 30 total horses over three years

---

This project study was reviewed by the National Park Service Institutional Animal Care and Use Committee. The following action(s) were taken:

Project Status: Approved

Midwest Region/ Intermountain Region/ NPS IACUC Chair: Dan Licht /s/, Mike Wrigley /s/, John Bryan /s/

UNITED STATES DEPARTMENT OF THE INTERIOR

INTER/INTRA-AGENCY AGREEMENT (IAA)

1. Period of Performance

START	END
2/10/2020	2/10/2022

Buyer has work performed for them by the Seller named in item 6b. Seller to perform work as described herein for the agency named in item 6a.

SEE INSTRUCTIONS ON PAGE 2

2. Common Document Number (Agreement Number)
L20PG00022 Page 1 of 7

3. Check appropriate box
☒ Original ☐ Modification No.

4. Under the authority of (Cite authorities):
☐ 43 U.S.C. 1701 et seq., (FLPMA)
☒ Service First, Title IV, Section 428
☐ 31 U.S.C. 1535 (the Economy Act)
☐ Working Capital Fund (WCF)
☐ Southern Nevada Public Land Management Act

5. Description of Work.: Ongoing feral horse fertility control research. PROJECT TITLE: BLM-THRO(NPS) Wild Horse Research IAA

Buyer	Seller
6a. Agency: Bureau of Land Management	6b. Agency: National Park Service, Theodore Roosevelt National Park
Address: 1340 Financial Blvd. Reno, NV 89502	Address: 315 Second Ave, P.O. Box 7 Medora, ND 58645
Finance POC: David Appold, Contracting Officer	Finance POC: Kevin Melzo, Budget Officer
Email: dappold@blm.gov Phone: (775) 861-6417 Fax: (775) 861-6634	Email: kevin_Melzo@nps.gov Phone: (701) 623-4730 Fax: (701) 623-4840
Technical Point of Contact: Bruce Rittenhouse	Technical Point of Contact: Blake McCann, Ph.D.
Email: britten@blm.gov Phone:(202) 912-7648 Fax: (202) 912-7182	Email: blake_mccann@nps.gov Phone: (701) 623-4730 , ext. 1433 Fax:

ACCOUNT DATA	BUYER	SELLER
7. Agency Location Code	7a. 14-11-0008	7b. 14-10-0099
8. BPN Number (DUNS #) FSN	8a. 084359236	8b. 927589424
9. Treasury Account Symbol (TAFS)	9a. **14X1109**	9b. 14201036
10. Standard General Ledger	10a.	10b.
11. Cost Structure/Account	11a. LLWO260000 L106000000.PC00000 20XL11009AF LX.SI.RSCH00000 $19,000.00	**11b.**
12. Business Event Type Code	12a. DISB	12b.COLL
13. Requisition Number for Buyer/Project Account for Seller	13a. 40473695	13b.
14. Contract Line Number for Buyer/ Proposal Number or other data for Seller	14a. 0010	14b.
15. Buyer provide Expiration of Funding Source (Date or indefinite)	15a. 02/10/2022	15b. NOTE: Seller, ensure project completion by this date (Seller must not incur additional costs) **See Block 15a**

a. Initial or current obligation:	$ 19,000.00
b. Modification Amount (check one) ☐ Increase ☐ Decrease	
c. Total obligation:	$ 19,000.00

17. Bill To (Name and Address, including zip code of Finance **Office**):
Name: OC622-Payments Section, Bureau of Land Management
Address: Denver Federal Center, Bldg. 50, POB 25047
Denver, CO 80225

18. Billing for Federal Agencies and DOD will be processed via IPAC. (billing will be done ☐ bi-weekly ☐ monthly ☒ quarterly ☐ in advance)

Upon Approval, this agreement constitutes an obligation against Buyer requesting the work; or authority to proceed with work by Seller for the herein named agency in anticipation of reimbursement.

19. Approved for Buyer: _____ (Contracting Officer or other Authorized Signature) *other only for WCF	20. Approved by Seller: _____ (Seller's Authorizing Signature)
19a. Name (Type): David Appold	20a. Name (Type): Blake McCann

19b. Title: Contracting Officer	19c. Date:	20b. Title: Chief of Resource Management	20c. Date: 2/3/2020

INSTRUCTIONS FOR INTER/INTRA-AGENCY AGREEMENT (IAA)

NOTE: Information highlighted is to be completed by, or obtained from, the Seller Agency

IAA – BUYER TO HAVE WORK PERFORMED BY A PARTICIPATING (SELLER) AGENCY

***for a single funding line – complete continuation page for multiple lines of funding**

The Buyer executes this form, completes and obligates information under Buyer data elements.

1. Enter the start and end date (period of performance) in which work will be completed.
2. Enter the Common Document Number (Inter/intra Agency Agreement number).
3. Check "Original" if first submission, "Modification" and enter modification number if modification.
4. Check 31 U.S.C. 1535" unless another specific legislative authority exists, in which case that authority is shown under "other". If 31 U.S.C. 1535 is checked, an Economy Act Determination **must** be prepared by the project manager and approved by a warranted Contracting Officer with delegated authority.
5. Provide a Project Title and description of the work to be performed in accordance with Acquisition, Section 1510-17.5.
6. Enter the Buyer Agency office name, city, state, zip code, Buyer technical and administrative contact names and phone nos. with area code also include fax and Email address.

6a. Enter the Seller Agency office name, city, State, Buyer technical and administrative contact names and phone nos. with area code, also include fax and Email address. These fields can be completed by the Seller if unknown to the Buyer.

This data will be referenced on your Treasury IPAC bill

7a. Provide your 8 digit Agency Location Code (ALC) assigned by Treasury.
8a. Type your Business Partner Network DUNS Number as registered in Fed-Reg this is also referred to as the FSN for Dept. of Defense.
9a. Provide the Treasury Account Symbol (TAS) for this funding line.
10a. Determine the Treasury Standard General Ledger accounts (SGL) for this funding request.
11a. Enter the account cost structure for your Agency. This may include an office identifier, program and budget object class.
12a. Provide the Business Event Type Code (BETC) for this action.
13a. Type the Requisition Number referenced to support this Agreement.
14a. Contract Line Number for this funding.
15a. provide the Fund Expiration date, or type 'Indefinite' (for no year funds).

***Items 9a – 14a are specific for each line of funding on the obligation document. See * above.**

7b-14b. Participating Seller Agency completes these items. This data will be used to cross-reference the IA with the Participating Agency's reimbursable account.

16. For an original IA; enter the amount to complete items a, c, and d. For modification; complete items a, b, c, and d.
16a. Enter the Initial or current obligation amount
16b. Enter the Modification Amount
16c. Check appropriate box to indicate if the funding is being increased or decreased by this action.
17. Enter the Buyer Agency, Bill To - Finance Office address; include office name, city, state, and zip code.

Forward a copy of this draft Agreement for completion of the Participating Seller Agency account data. Obtain a signed, accepted copy of this Agreement from the Buyer Agency. Ensure that the data elements in 7b-14b have been completed.

18. Check the preferred billing schedule for the Buyer Agency and ensure that the term is acceptable for both Buyer and Seller.
19. IA must be signed by a warranted Contracting Officer with delegated authority. IA is not signed by the Buyer until approved in block 20 by the participating agency.
20. Signature of approving official for the participating agency.

Send a fully executed copy of this Agreement to the Seller Agency after obligation is recorded in the IDEAS/PRISM system.

PARTICIPATING SELLER AGENCY TO SUPPORT THE BUYER AGENCY

The Draft IAA is received for completion by the Seller Agency.

This data will be used to cross-reference the IAA with the Participating seller Agency's reimbursable account in FFS or Prism.

6b. Enter the Participating Seller Agency office name, city, State, Buyer technical and administrative contact names and phone nos. with area code, also include fax and Email address. These fields can be completed by the Buyer
7b. Provide your 8 digit Agency Location Code (ALC) assigned by Treasury.
8b. Type your Business Partner Network DUNS Number as registered in Federal Register this is also referred to as the FSN for Dept. of Defense.
9b. Provide the Treasury Account Symbol (TAS) for this funding line.
10b. Determine the Treasury Standard General Ledger accounts (SGL) for your reimbursable account.
11b. Enter the cost structure / account classification for your Agency's reimbursable. This may include an office identifier, program and budget object class. (Note: This cost structure **must** be charged with time or expenditures for billing to occur.)
12b. Provide the Business Event Type Code (BETC) for this action.
13b. Type the Project or Job Number assigned to track expenses for completing the work requested in Agreement.
14b. Enter any additional Seller account reference data. (Project code assigned, Proposal number, sub-agreement contract reference)
15b. Ensure completion by this date.

16 To be completed by Buyer

17. Ensure that the billing term is acceptable by Seller Agency.
20. Ensure that the approval signature is an agent authorized to accept or behalf of the Seller Agency.

Return the IAA copy to the Contracting Officer for execution and obligation.

After Receipt of the fully executed copy of this Agreement, create a reimbursable account in FFS, PCAS or SAP, SD to track expenses that will be IPAC billed against this Obligation.

FOR BLM USE ONLY, DATE RECEIVED _____

FOR BLM USE ONLY, PROPOSAL # _____
(MM-YY-####)

WH&B FINAL REPORT

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort

Study Project Name:

Institution:

Title of Project:

Principal Investigator:

Co-Investigators:

Starting Date:

Completion Date:

Executive Summary: (limit to 250 words)

Summary of Objectives: (Please restate, in writing, each objective and then summarize progress, were the objectives accomplished? Explain why or why not; limit to no more than three pages per objective)

Explain Major Findings and Limitations:

Information of Practical Value to the WH&B Program (e.g., how could this information be used for program improvements):

Publications and time frame for publications:

BUREAU OF LAND MANAGEMENT
Grants & Cooperative Agreements

PERFORMANCE/PROGRESS REPORT

Agreement Number: _____

Project Title: _____

Reporting Period Begin & End Dates: _____ **to** _____ **Final Report? ☐YES ☐NO**

Recipient Organization:

Name:
Address:
Telephone:
Email:

Reporting Frequency:

☐ Quarterly
☐ Semi-annual
☐ Annual

Report On: (Project accomplishments, developments, problems, etc., for this period and how they relate to the agreement Objectives and Performance Measures.)

Certification: I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

_____

Printed Name & Title of Person Completing Report

_____ _____

Signature *Date Submitted*

From: Griffin, Paul C
To: McCann, Blake E
Subject: Questions from my CO
Date: Friday, January 31, 2020 10:04:18 AM

Hi Blake,

My CO is asking:

- What's the Theodore Roosevelt Nat'l Park Service
    1. Agency Location code (ALC)
    2. Duns #
    3. Treasury Account Symbol (TAS)_
- Allowing monthly or quarterly billing?

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Annual Report 2019
Date: Monday, November 11, 2019 9:26:04 AM

Hi Dan,
Thank you for looking into the financial report side of things. Yes, I'd be happy to get together some time soon. Would you be available on Tuesday the 19th? My schedule is pretty flexible that day, but could I suggest maybe meeting at 9? I haven't been to the place yet, but let me suggest "Cuppy's Coffee" at 353 W Drake Rd #120. I'm suggesting it just as a local coffeehouse with a name that doesn't start with 'Starbuck's.' But if that time or place is not good for you, I have no real attachment to either, and could meet somewhere else at a time that works.
Looking forward to talking soon,
Paul

On Fri, Nov 8, 2019 at 3:37 PM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> Sorry about the financial report. It totally slipped my mind. I'll get it to you as soon as they send it to me.
>
> Our accountant and I are working on the interagency agreement budget now and should be able to get that to you as a draft next week.
>
> I'm flexible next week for coffee, as well. Pick a day and time and I'm sure I can make it work. I think the Wild Boar went out of business so we'll have to pick another place for coffee. Your choice.
>
> Dan
>
> On 11/8/2019 12:53 PM, Griffin, Paul wrote:
>
> > Hi Dan,
> > Thank you very much for that report. I'll share that with the research advisory team.
> > I forgot to also remind you to, please, ask your office of sponsored programs for a semi-annual financial report (form SF-425) for this project. Maybe next week, after the long weekend.
> > I'd be glad to see you any time. My schedule is pretty flexible in the coming weeks. It is definitely still on our radar to try to form an interagency agreement with NPS, in support of the remaining 2020 fieldwork funding needs for the project at THRO.
> > Thank you,
> > Paul
> >
> > On Thu, Nov 7, 2019 at 8:41 AM Dan Baker <danbaker@colostate.edu> wrote:
> >
> > > Hi Paul,
> > >
> > > Thanks for allowing me additional time to include the updated graph of

the foaling summary. I think that it makes the report more timely. Let me know if you have questions or would like to have more specific information included in the report.

I have some ideas on future research at THRO that would address the question of permanent infertility in these GonaCon-treated mares. Maybe we could go for coffee sometime soon.

Regards,

Dan

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

--
Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

--
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970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: Dan Baker
To: Griffin, Paul C
Subject: Re: [EXTERNAL] Annual Report 2019
Date: Monday, November 11, 2019 2:16:09 PM
Importance: High

Hi Paul,

That sounds good. See you then.

Dan

On 11/11/2019 9:26 AM, Griffin, Paul wrote:

> Hi Dan,
> Thank you for looking into the financial report side of things. Yes, I'd be happy to get together some time soon. Would you be available on Tuesday the 19th? My schedule is pretty flexible that day, but could I suggest maybe meeting at 9? I haven't been to the place yet, but let me suggest "Cuppy's Coffee" at 353 W Drake Rd #120. I'm suggesting it just as a local coffeehouse with a name that doesn't start with 'Starbuck's.' But if that time or place is not good for you, I have no real attachment to either, and could meet somewhere else at a time that works.
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>
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> question of permanent infertility in these GonaCon-treated mares. Maybe
> we could go for coffee sometime soon.
>
> Regards,
>
> Dan
>
> --
> Dan L. Baker, PhD
> Affiliate Faculty
> Department of Biomedical Sciences
> Animal Reproduction and Biotechnology Laboratory
> Colorado State University
> Fort Collins, Colorado 80535 USA
> Phone: 970-556-8518
> Email: danbaker@colostate.edu

--
Paul Griffin, Ph.D.
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--
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--

Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] annual report
Date: Monday, November 4, 2019 9:09:07 AM

Hi Dan,
Sorry for the delay in responding. Sure, no problem. Some time this week for that report would be fine.
Thank you,
Pual

On Tue, Oct 29, 2019 at 10:52 AM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> I've completed my annual report except for an updated graph showing a summary of foaling rates for control and 4 yr booster from 2009-2019. Our statistician has better statistical graphing software than I do but he is out of town until Monday. Would it be possible to get the report to you after I get the updated graph? I should get it by Monday or Tuesday of next week. If not, I can reformat the data to a table and send it to you by Thursday. I also plan on sending this graph plus explanatory test to PLos ONE. At some point, I would like to visit with you about a future experiment with the TR horses that would address the question of permanent sterility and differences in hand versus dart injection of GonaCon.
>
> Hope all is well with you.
>
> Best,
>
> Dan
>
> --
> Dan L. Baker, PhD
> Affiliate Faculty
> Department of Biomedical Sciences
> Animal Reproduction and Biotechnology Laboratory
> Colorado State University
> Fort Collins, Colorado 80535 USA
> Phone: 970-556-8518
> Email: danbaker@colostate.edu

--
Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: Griffin, Paul C
To: Dan Baker; Powers, Jenny; Galloway, Nathan L
Subject: Re: [EXTERNAL] BLM Final Report
Date: Friday, December 4, 2020 4:20:34 PM

Hi Dan,
Thank you for your message today. My family and I are fine, and I hope the same is true of you, Jenny, Dr. Nett, and Kathleen.
I don't have the authority to grant the request for a delay on the final report, but I have forwarded it to the grants management officers (currently Sherry Healey or Leona Parker, because Brandon Riley left the BLM), along with reasons I think it is a reasonable request. Hopefully, they will agree, and let us know early next week.
Really good to see those three papers that you are working on. Please be sure to include funding from BLM agreement L15AC00145 in the acknowledgements. As with the papers, when Kathleen's thesis is approved, please do send me a pdf copy for our records.
Most important of all, stay safe out there. Common sense says: don't hurry anything along if it elevates risk of exposure to coronavirus.
Looking forward to communicating more next week.
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 631-4808 (mobile / pandemic)
(970) 226-9358 (office)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, December 4, 2020 10:11 AM
To: Griffin, Paul C <pgriffin@blm.gov>; Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
Subject: [EXTERNAL] BLM Final Report

Hi Paul,
I hope all is well with you and your family during these most challenging times.
I'm writing to give you an update on the status of our final report that is due on December 6. Unfortunately, we will not be able to provide a complete and meaningful final report by this

date and are requesting an extension of this deadline. We apologize for this inconvenience and are diligently working to provide this information as soon as possible. We are planning to submit three manuscripts for potential publication in peer-reviewed journals. The information from these papers will provide the basis for the final report and we should be able to provide a meaningful document in the next few weeks that should be acceptable to the BLM. These manuscripts are in different stages of preparation and include the following:

Manuscript 1. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine in free-ranging horses *(Equus caballus):* Limitations and side effects - Update 2018-2020

This paper will update our previous 2018 publication in PLoS ONE and will include data from the 2018, 2019, and 2020 foaling seasons. Our thoughts now are to again submit this manuscript to PLoS ONE for publication. This paper will not be nearly as long as the previous one since we will be able to reference the Methods from the previous paper. To date, I've written a partial draft for this paper and only need final statistical analysis of foaling proportions and effectiveness to update previous results. With this information, we should be able to provide reliable summary of these results for the BLM report and well-before submitting the manuscript to PLoS ONE for the review process.

Manuscript 2. Optimum reimmunization interval for delivery of GnRH immunocontraceptive vaccine (GonaCon-Equine) to feral horses (*Equus caballus)* using prototype syringe darts.

This manuscript will combine data on remote dart delivery of GonaCon and an assessment of the optimum reimmunization interval . We have a partial draft of this paper and are currently conducting a comprehensive statistical analysis comparing foaling proportions and effectiveness for the four treatment intervals across 2017, 2018, 2019, and 2020. Similar to Manuscript 1, once we have a preliminary analysis of these data, I can include this information in the final report. We have not yet decided on the ultimate outlet for publication of the results from study, possibly Wildlife Society Bulletin. Any suggestions?

Manuscript 3. Pregnancy diagnosis in captive and free-ranging horses (*Equus caballus)* using serum and fecal estradiol analysis.

I would like to include this information in the final report because, as you know, it was funded in large part by the BLM. Except for re-running a few samples, the laboratory phase of this study has been completed the results are reported in Kathleen Eddy's MS thesis, which she successfully defended last month. Dr. Terry Nett and I are assisting her in converting her thesis into a publishable manuscript. However, she also has teaching responsibilities until the end of this semester and at present has limited time to devote to this effort. We will make a concerted effort to provide, at least, a preliminary summary of this research as soon as possible. We are considering Animal Reproduction Science and Wildlife Society Bulletin as possible journals for this publication.

Again, I apologize for having to request an extension to the current deadline but as you can see, the funding from the BLM for these studies as resulted in plethora of novel and exciting information that should be invaluable to the management of free-ranging horses. We just need a little more time to complete our data analysis, interpret results, and discuss the significance of these results in a well-written final report or publication. Thank you for your consideration of this request. Please give me a call if you would like to discuss this matter.

Kind regards,

Dan

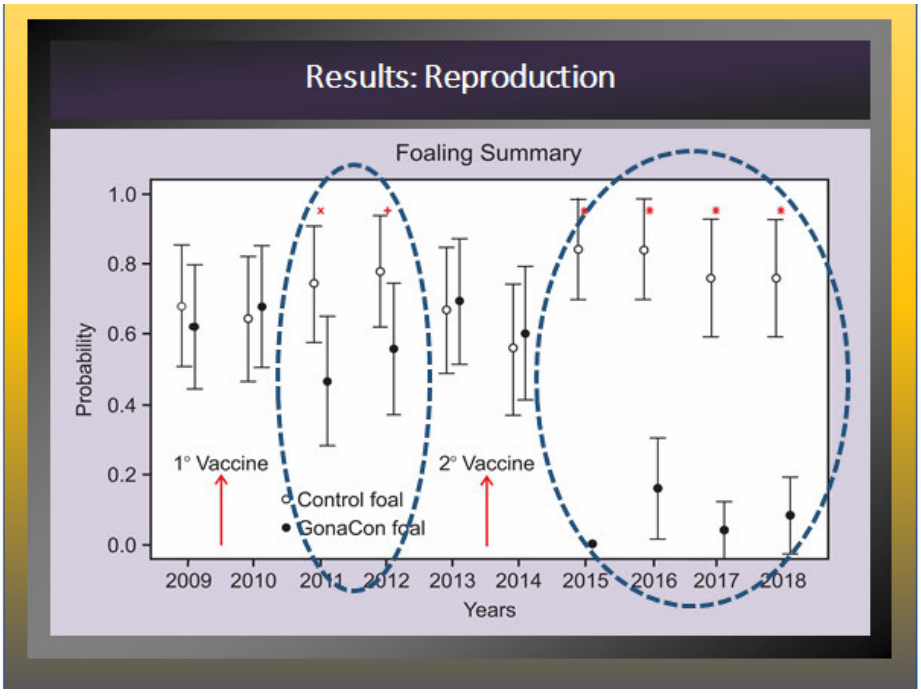
--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory

Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin_Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Federal Grant No. L15AC00145: Project review and request for additional funds for FY 2020
Date: Tuesday, March 5, 2019 12:25:03 PM
Attachments: onabfhbihlmnhhbh.png
aegoohgajjaobpfo.png

Dan,
Thank you very much. This looks just right in terms of information to share with the research team.
Paul

On Tue, Mar 5, 2019 at 12:19 PM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> The purpose of this email is to provide you and the BLM WHB research team with a brief summary of the salient results of our research efforts in evaluating GonaCon-Equine in free-ranging horses at T. Roosevelt N. P., the current status of this research, and to request additional funds to continue this results through FY 2020. In general, I think that we have made considerable progress over the last four years of the project and arguably have provided a one of the most significant breakthroughs in the field of fertility control in free-ranging horses and other wild ungulates. This has come about due to many factors including an excellent team of committed scientist, collaboration with resource managers and agencies, excellent field and lab research technicians, and the willingness and foresightedness of the BLM to fund this novel research effort.
>
> Summary of Progress (2015-2019):
>
> 1. **Effects of Reimmunization**
>
> Our results, to date, have exceeded most expectations. We have provided strong evidence that reimmunization with GonaCon-Equine vaccine can provide a highly effective and longer lasting suppression of fertility in feral horses than any other wildlife fertility control agent currently reported in the literature and without significant side-effects. We have published these results in the online, open access, international scientific journal *PLoS-One (*see attachment, Fig. 1). These results extend through 2017 and reveal that this vaccine is significantly effective in suppressing fertility in feral mares for three breeding seasons. We continued to see effective suppression of fertility in these mares during the 2018 foaling season and these results were presented at the Free-Ranging Horse and Burro Fertility Control Workshop during November 8-10 in Albuquerque, NM (see below).
>
> 
>
>
> Fig 1. Comparative probability of foaling and pregnancy for treatment (solid circles) and control (open circles) groups of feral mares during 2009-2018. Red arrows represent primary and reimmunization time points. Bars represent 95% CI and symbols represent p-values.
>
> 2. **Optimum Reimmunization Intervals**

To our knowledge, prior to this study, the optimum reimmunization schedule for GonaCon Equine had not been reported for any wild or domestic species and, equally important, it is unknown if decreasing the interval between the primary and secondary immunization will result in decreased persistence and effectiveness of this vaccine. For resource managers, knowledge of the minimal effective interval for reimmunization is essential for recommending a vaccination schedule for treating feral mares in a natural environment. Thus, in 2013, we requested an additional 40 mares from THRO to conduct a companion experiment to evaluate potential differences in long-term reproductive responses resulting from variation in immunization intervals of booster treatments. We established booster intervals of 6mo, 1yr, 2yr, and used the current 4yr interval to represent the reference point for defining the maximum reimmunization interval.

We accomplished treatment applications in these experimental mares developing and testing a dependable (91% success rate) remote dart delivery system for GonaCon-Equine and applying treatment and booster vaccinations during 2015-2016. Results of development of dart delivery for this vaccine and first and second year effects on foaling proportions are currently being analyzed and will be submitted to a peer-reviewed scientific journal in October 2019. Initial foaling results for year one are were presented at the above workshop in New Mexico (see below).

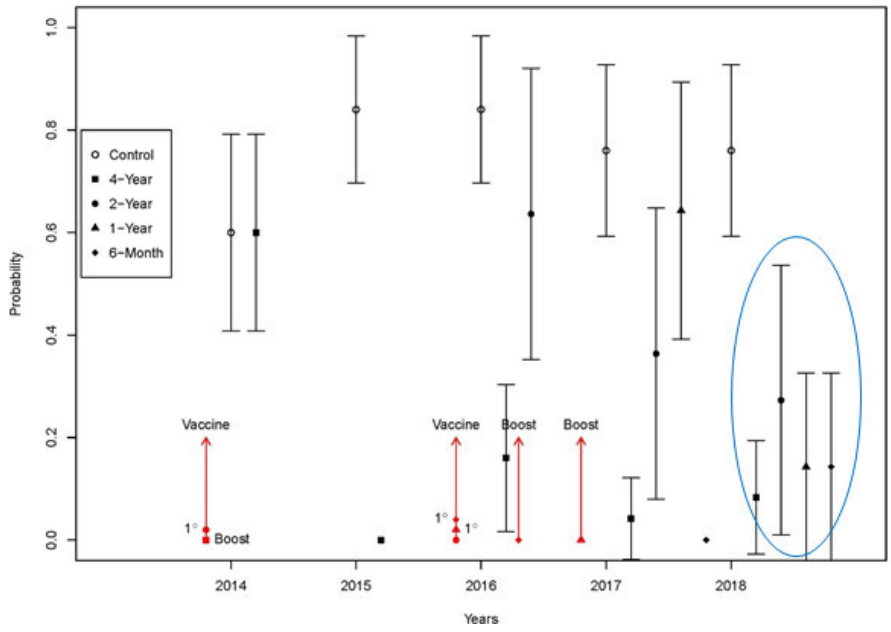


Fig. 2. Comparative probability of foaling and pregnancy for treatment (solid circles) and control (open circles) groups of feral mares during 2009-2018. Red arrows represent primary and reimmunization time points. Bars represent 95% CI and symbols represent p-values. The first year that the vaccine could have an effect on foaling proportions was 2018. Preliminary results suggest that foaling proportions were significantly different between control and all booster treatments but that booster treatments were not different from each other.

Research Status

Both of these experiments are currently ongoing. We have not yet determined the duration of effective contraception for any of the reimmunization intervals or if permanent infertility is a possible outcome. Furthermore, if reversible contraception is observed then we need to assess the possible effects on altered birth phenology.

3. **Budget Status**

While our current budget is sufficient to cover project cost for FY 2019, we will not have sufficient funds to complete field work in 2020. This deficiency has come about primarily because, in our original budget proposal, we underestimated the amount of field time necessary to apply primary and booster treatments in FY 2015-2016 to forty additional mares and the amount of field technician time necessary to monitor annual foaling events in these experimental animals. As a result, each year we exceeded our budget such that we currently need approximately $15,000-$17,000 to conduct field work in FY 2020. This amount would include primarily field technician time, and publication costs. If no additional funds are possible, I will start actively seeking support from private institutions such as Bostiber Institute, Morris Animal Foundation, etc. We have invested too many resources into this research and our results are encouraging. It would be a travesty not to complete the objectives of this project.

I can provide a more detailed itemized budget proposal for FY 2020, if necessary.

Let me know if this is too much or too little information.

Dan

--
Dan L Baker, PhD
Research Biologist
Faculty Affiliate
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, CO 80535
(970) 556-8518
danbaker@colostate.edu

--
Paul Griffin, Ph D
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Final paperwork
Date: Friday, September 18, 2020 2:36:25 PM

Hi Dan,
No problem.
"The funding agreement (L15AC00145) ended recently (9/7/20). Is there any information you need from me about closing out that agreement? I believe what I'll need from you is just:
-a final financial report (SF-425)...no more spending is allowed for costs after the last date of the agreement.
-a final report on the research and results.
Both of these are due to BLM "no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement." So...that'd be December 6th."

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
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(970) 631-4808 (mobile)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, September 18, 2020 2:02 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Final paperwork

Hi Paul,

I apologize for this inconvenience but could you please resend your last email regarding the list of items that the BLM needs for completion of our project. I misfiled it and wasn't able to recover it. Sorry about that. Thanks.

Dan

--
Dan L. Baker, PhD
Affiliate Faculty

Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] final report
Date: Tuesday, December 8, 2020 2:21:43 PM

Hi Dan,
Sure, I'd be glad to talk. Could we try each other at 3?

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
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(970) 226-9358 (office)

From: Dan Baker <danbaker@colostate.edu>
Sent: Tuesday, December 8, 2020 9:17 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] final report

Hi Paul,

I have a couple of questions regarding the final report. Could I give you a call at your convenience? Thanks.

Dan

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] final report
Date: Tuesday, December 8, 2020 3:21:31 PM
Attachments: Performance Final Report Template_Dec2016.docx
Performance Progress Report Template_Nov2016.docx

Final and progress report templates attached

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
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(970) 631-4808 (mobile / pandemic)
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From: Dan Baker <danbaker@colostate.edu>
Sent: Tuesday, December 8, 2020 9:17 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] final report

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Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Powers, Jenny
To: Dan Baker; Griffin, Paul C; McCann, Blake E; Jason Bruemmer; Terry Nett; Doug Eckery; Galloway, Nathan L
Subject: Re: [EXTERNAL] Foaling data - 2020
Date: Friday, September 18, 2020 10:52:23 AM

Thanks for sending Dan. Great info to have.

Let me know when you want to dig back into the manuscript. I should have a little time this fall/winter.

Jenny

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, September 18, 2020 10:34 AM
To: Griffin, Paul C <pgriffin@blm.gov>; Powers, Jenny <Jenny_Powers@nps.gov>; McCann, Blake E <blake_mccann@nps.gov>; Jason Bruemmer <jason.bruemmer@colostate.edu>; Terry Nett <terry.nett@colostate.edu>; Doug Eckery <douglas.c.eckery@aphis.usda.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
Subject: [EXTERNAL] Foaling data - 2020

All,

Attached are "quick and dirty" preliminary results from the 2020 foaling season and a brief comparative summary of data for foaling proportions and vaccine effectiveness from previous years. Clearly, a more sophisticated statistical analysis will be forthcoming as we move into the data analysis and writing phases of this research. Your input will be most appreciated. Let me know if you have questions or comments. Thanks.

Dan

Dan L. Baker, PhD
Affiliate Faculty Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory
Colorado State University Fort Collins, Colorado 80535
USA Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Powers, Jenny
To: Dan Baker; Griffin, Paul C; McCann, Blake E; Jason Bruemmer; Terry Nett; Doug Eckery; Galloway, Nathan L
Subject: Re: [EXTERNAL] Foaling data - 2020
Date: Friday, September 18, 2020 10:52:23 AM

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Jenny

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, September 18, 2020 10:34 AM
To: Griffin, Paul C <pgriffin@blm.gov>; Powers, Jenny <Jenny_Powers@nps.gov>; McCann, Blake E <blake_mccann@nps.gov>; Jason Bruemmer <jason.bruemmer@colostate.edu>; Terry Nett <terry.nett@colostate.edu>; Doug Eckery <douglas.c.eckery@aphis.usda.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
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Dan

Dan L. Baker, PhD
Affiliate Faculty Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory
Colorado State University Fort Collins, Colorado 80535
USA Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker; Jason Bruemmer; Terry Nett; Doug Eckery
Subject: Re: [EXTERNAL] Follow-up research at TR
Date: Wednesday, September 16, 2020 10:56:31 AM

Hi Dan,
Thank you for that update. I think that's good to look on the positive side, at all the great information that has come from this project. I hope it's satisfying for you to know that BLM is increasing its use of boostered Gonacon substantially.

I'll look forward to any information you can share about this year's foaling.

A a separate, procedural note, the funding agreement (L15AC00145) ended recently (9/7/20). Is there any information you need from me about closing out that agreement? I believe what I'll need from you is just:
-a final financial report (SF-425)...no more spending is allowed for costs after the last date of the agreement.
-a final report on the research and results.
Both of these are due to BLM "no later than 90 calendar days after the expiration, termination, and/or project completion of this agreement." So...that'd be December 6th.

Paul

Paul Griffin, Ph.D.
Research Coordinator
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(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Dan Baker <danbaker@colostate.edu>
Sent: Wednesday, September 16, 2020 9:48 AM
To: Jason Bruemmer <jason.bruemmer@colostate.edu>; Terry Nett <terry.nett@colostate.edu>; Doug Eckery <douglas.c.eckery@aphis.usda.gov>; Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Follow-up research at TR

All,

Sorry for the delay in getting back to you regarding my efforts to convince the park to continue evaluating GonaCon in treated mares. I have spent several hours of discussion with Blake and park staff trying to explain to them the importance of continuing to monitor the 19/24 mares that have not regained fertility since being boosted in 2013.

Based upon several of your suggestions, I have provided alternatives for continued monitoring of this group of horses using volunteers and the fecal estrogen assay that is being validated by Kathleen and Terry. I have also proposed capturing a subset of this group of mares and transporting them to CSU for further investigations into whether they are permanently infertile or not and, if so, why. I know that Jenny and Paul Griffin have lobbied for continued evaluation with potential funding from the BLM and/or other sources.

The park has rejected all of our alternatives and I am convinced that efforts to pursue any collaboration will fail. It's depressing to me and a great loss of opportunity but in retrospect, I feel that, with the past cooperation from the park, our research at TR has been quite successful and we have be able to advance the understanding and application of this contraceptive vaccine in feral horses.

I'll be sending out a brief summary of our foaling results from 2020 and potential alternatives for future publications of our research findings.

Thanks for your support. Give me a call if you have questions.

Dan

Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences Animal Reproduction
and Biotechnology Laboratory
Colorado State University Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker; Eckery, Douglas C - APHIS
Subject: Re: [EXTERNAL] Fwd: GonaCon Bulletin for Review
Date: Monday, April 27, 2020 10:11:29 AM
Attachments: WHB_StandardLogo_Color_transparent.png
blm_logo_transparent.png

Thank you Dan. That looks like a good drafft. Yes, that would be great and appropriate to please ask that BLM be included in the acknowledgements. Here, I'm attaching a logo for the WHB program. Or, if they prefer just the BLM logo, that is attached as well.

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
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(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Dan Baker <danbaker@colostate.edu>
Sent: Monday, April 27, 2020 9:46 AM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>; Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Fwd: GonaCon Bulletin for Review

Hi Doug and Paul,

I just received this daft from TR and wanted to get your comments. I've offered some comments on content but I also I think that it would be appropriate to include acknowledgements to NWRC, BLM, and Morris Animal Foundation.

Thanks,

Dan

-------- Forwarded Message --------
Subject: GonaCon Bulletin for Review
Date: Mon, 27 Apr 2020 06:47:53 -0800
From: Klosterman, Megan E <megan_klosterman@nps.gov>
To: McCann, Blake E <blake_mccann@nps.gov>, McCann, Amy J <Amy_McCann@nps.gov>, Baker,Danny <Dan2.Baker@ColoState.EDU>
CC: Sedlacek, Katherine M <Katherine_Sedlacek@nps.gov>, Lincoln Eddy <eddylinc@gmail.com>, Eddy, Lincoln R <Lincoln_Eddy@nps.gov>

Hi everyone,

We have put together a draft bulletin for your review. We would like to hear all your thoughts,

comments, and concerns pertaining to the content and the layout. If you have any references that you think would be good to make available to the public (we already have the two that Dan shared with us previously), please let us know about that as well. We plan to include one link on the bulletin that will bring people to our website where we will have a list of further references. We were not sure if NWRC would want to be mentioned on this bulletin, so if anyone has any insight on that, please let us know.

Thank you Kate and Lincoln for all your hard work on this!!

Megan E. Klosterman | Resource Management Specialist
NPS · Theodore Roosevelt National Park
☎ (701) 623-4730 ext.1407 | ✉ Megan_Klosterman@nps.gov (she/her)
*☎ (during telework) (937) 974-1245

From: Griffin, Paul C
To: McCann, Blake E; Dan Baker
Cc: Shepherd, Alan B
Subject: Re: [EXTERNAL] interagency transfer
Date: Monday, December 16, 2019 4:15:30 PM

Hi Blake and Dan,
Thanks for initiating the process that we would need for IAA formation. We in the BLM WHB program had a chance to share with BLM and DOI leadership last week how important NPS leadership has been in moving GonaCon research forward.
Yes, I'd be available to talk this week, except tomorrow morning and Friday. If he is also free, we should also try to include Alan Shepherd (ashepher@blm.gov, cc'd here). Blake, can you 'see' my google calendar (pgriffin@blm.gov) to check out when I'll be free?
Keeping in mind Alan's, Blake's and my schedule from what I can see, can I suggest **Wednesday 1 pm Mountain time (noon Pacific)**? If that works for you, we could all call in to: b(5) [redacted]
Paul

On Mon, Dec 16, 2019 at 3:17 PM McCann, Blake <blake_mccann@nps.gov> wrote:

> Hello Dan and Paul;
>
> I will initiate the process in anticipation of an IAA.
>
> Blake
>
> On Mon, Dec 16, 2019 at 3:12 PM Dan Baker <danbaker@colostate.edu> wrote:
>
> > Hi Paul,
> >
> > I know that you are probably in "catch up" mode due to the holiday season but I just wanted to make sure that you received my email regarding the interagency transfer of funds from BLM to Blake to CSU for technician time in FY2020. I just visited with Blake and he emphasized the urgency of starting this process as soon as possible so that funds would be available for field technicians starting March 1. You mentioned previously that we might want to set-up a conference call with Blake this week to discuss this process. Is that still a possibility for you? I think that Blake would be available if that's still an option. Please let me know if I can do anything to facilitate this effort from my end.
> >
> > Best,
> >
> > Dan
> >
> > --
> > Dan L. Baker, PhD
> > Affiliate Faculty
> > Department of Biomedical Sciences
> > Animal Reproduction and Biotechnology Laboratory
> > Colorado State University
> > Fort Collins, Colorado 80535 USA
> > Phone: 970-556-8518

Email: danbaker@colostate.edu

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

--
Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: Dan Baker
To: Griffin, Paul C
Subject: Re: [EXTERNAL] Re: 1-month extension for BLM Final Report, L15AC00145
Date: Monday, December 7, 2020 9:40:41 AM

Hi Paul,
Thanks for your comments.

I wanted to ask you if there was a required format for the final report. The format that I wanted to use was more aligned with a journal format and a presentation of results and discussion related to the three experiments that we conducted at THRO and CSU. Thanks. Dan

On 12/7/2020 7:28 AM, Griffin, Paul C wrote:

> Hi Dan,
> Thank you for your message. I'm happy to know that the GMO gave you the extension. It's no problem at all for me to help with those communications. Good luck with manuscript preparations. Totally understood that those won't all be submitted before the report to BLM is complete.
> Paul
> Paul Griffin, Ph.D.
> Research Coordinator
> BLM Wild Horse and Burro Program
> 2150 Centre Ave. Building C
> Fort Collins, CO 80526 USA
> (970) 631-4808 (mobile / pandemic)
> (970) 226-9358 (office)
>
> ---
>
> **From:** Baker,Danny <Dan2.Baker@ColoState.EDU>
> **Sent:** Friday, December 4, 2020 5:23 PM
> **To:** Griffin, Paul C <pgriffin@blm.gov>
> **Cc:** Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
> **Subject:** [EXTERNAL] Re: 1-month extension for BLM Final Report, L15AC00145
>
> **This email has been received from outside of DOI - Use caution before clicking on links, opening attachments, or responding.**
>
> Hi Paul,
> Thanks for your support in getting us an extension on the deadline for the final report. I hope that I didn't put you on the "spot" with this request. Your support throughout this project is much appreciated by all of us.
>
> While these manuscripts will probably still be in the review process in January, this should be sufficient time to provide the BLM a high quality final report.

Thanks for the reminder on the BLM funding acknowledgement and no problem getting you a copy of Kathleen's thesis.

Best to you. Take care.

Dan

Sent from my iPhone

> On Dec 4, 2020, at 4:47 PM, Griffin, Paul C <pgriffin@blm.gov> wrote:
>
> Hi Dan,
> Despite it being Friday afternoon, GMO Leona Parker let me know that she has approved a 1-month extension for CSU to return the final performance report to BLM (i.e., January 7). We already received the final financial report (form SF-425) from your university. So, good luck with your final analyses. If you would still like to talk next week, I will be available.
> Paul
>
> Paul Griffin, Ph.D.
> Research Coordinator
> BLM Wild Horse and Burro Program
> 2150 Centre Ave. Building C
> Fort Collins, CO 80526 USA
> (970) 631-4808 (mobile / pandemic)
> (970) 226-9358 (office)
>
> ---
>
> **From:** Griffin, Paul C <pgriffin@blm.gov>
> **Sent:** Friday, December 4, 2020 4:20 PM
> **To:** Dan Baker <danbaker@colostate.edu>; Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
> **Subject:** Re: [EXTERNAL] BLM Final Report
> Hi Dan,
> Thank you for your message today. My family and I are fine, and I hope the same is true of you, Jenny, Dr. Nett, and Kathleen.
> I don't have the authority to grant the request for a delay on the final report, but I have forwarded it to the grants management officers

(currently Sherry Healey or Leona Parker, because Brandon Riley left the BLM), along with reasons I think it is a reasonable request. Hopefully, they will agree, and let us know early next week.
Really good to see those three papers that you are working on. Please be sure to include funding from BLM agreement L15AC00145 in the acknowledgements. As with the papers, when Kathleen's thesis is approved, please do send me a pdf copy for our records.
Most important of all, stay safe out there. Common sense says: don't hurry anything along if it elevates risk of exposure to coronavirus.
Looking forward to communicating more next week.
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 631-4808 (mobile / pandemic)
(970) 226-9358 (office)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, December 4, 2020 10:11 AM
To: Griffin, Paul C <pgriffin@blm.gov>; Powers, Jenny <Jenny_Powers@nps.gov>; Galloway, Nathan L <Nathan_Galloway@nps.gov>
Subject: [EXTERNAL] BLM Final Report

Hi Paul,
I hope all is well with you and your family during these most challenging times.
I'm writing to give you an update on the status of our final report that is due on December 6. Unfortunately, we will not be able to provide a complete and meaningful final report by this date and are requesting an extension of this deadline. We apologize for this inconvenience and are diligently working to provide this information as soon as possible. We are planning to submit three manuscripts for potential publication in peer-reviewed journals. The information from these papers will provide the basis for the final report and we should be able to provide a meaningful document in the next few weeks that should be acceptable to the BLM. These manuscripts are in different

stages of preparation and include the following:

Manuscript 1. Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine in free-ranging horses *(Equus caballus):* Limitations and side effects - Update 2018-2020

This paper will update our previous 2018 publication in PLoS ONE and will include data from the 2018, 2019, and 2020 foaling seasons. Our thoughts now are to again submit this manuscript to PLoS ONE for publication. This paper will not be nearly as long as the previous one since we will be able to reference the Methods from the previous paper. To date, I've written a partial draft for this paper and only need final statistical analysis of foaling proportions and effectiveness to update previous results. With this information, we should be able to provide reliable summary of these results for the BLM report and well-before submitting the manuscript to PLoS ONE for the review process.

Manuscript 2. Optimum reimmunization interval for delivery of GnRH immunocontraceptive vaccine (GonaCon-Equine) to feral horses (*Equus caballus)* using prototype syringe darts.

This manuscript will combine data on remote dart delivery of GonaCon and an assessment of the optimum reimmunization interval . We have a partial draft of this paper and are currently conducting a comprehensive statistical analysis comparing foaling proportions and effectiveness for the four treatment intervals across 2017, 2018, 2019, and 2020. Similar to Manuscript 1, once we have a preliminary analysis of these data, I can include this information in the final report. We have not yet decided on the ultimate outlet for publication of the results from study, possibly Wildlife Society Bulletin. Any suggestions?

Manuscript 3. Pregnancy diagnosis in captive and free-ranging horses (*Equus caballus)* using serum and fecal estradiol analysis.

I would like to include this information in the final report because, as you know, it was funded in large part by the BLM. Except for re-running a few samples, the laboratory phase of this study has been completed the results are reported in Kathleen Eddy's MS thesis, which she successfully defended last month. Dr. Terry Nett and I are assisting her in converting her thesis into a publishable manuscript. However, she also has teaching responsibilities until the end of this semester and at present has limited time to devote to this effort. We will make a concerted effort to provide, at least, a preliminary summary of this research as soon as possible. We are considering Animal Reproduction Science and Wildlife Society Bulletin as possible journals for this publication.

Again, I apologize for having to request an extension to the current deadline but as you can see, the funding from the BLM for these studies as resulted in plethora of novel and exciting information that should be invaluable to the management of free-ranging horses. We just need a little more time to complete our data analysis, interpret results, and discuss the significance of these results in a well-written final report or publication. Thank you for your consideration of this

request. Please give me a call if you would like to discuss this matter.
Kind regards,
Dan

--
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Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: Any update on the noninvasive pregnancy assay paper?
Date: Friday, July 8, 2022 9:20:27 AM

Tanks Dan. I may be able to call you today at 10:30 am. Could that work, too? Are you still at: 970-556-8518?

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, July 8, 2022 9:08 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: Re: [EXTERNAL] Re: Any update on the noninvasive pregnancy assay paper?

Hey Paul. I'm available just about anytime on M, Tu, or Wed. Thanks. Dan

On 7/8/2022 9:05 AM, Griffin, Paul C wrote:

> **** Caution: EXTERNAL Sender ****
> Hi Dan -- yes, sure I'd be glad to talk. I'm wrapped up in administrative tasks today. What could be good days and times for you next week?
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> Paul Griffin, Ph.D.
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> ---
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> **From:** Dan Baker <danbaker@colostate.edu>
> **Sent:** Friday, July 8, 2022 8:49 AM
> **To:** Griffin, Paul C <pgriffin@blm.gov>

Subject: [EXTERNAL] Re: Any update on the noninvasive pregnancy assay paper?

This email has been received from outside of DOI - Use caution before clicking on links, opening attachments, or responding.

Hi Paul,
Good to hear from you. Do you have time for a brief phone call today or early next week.
Thanks,
Dan

On 7/7/2022 12:42 PM, Griffin, Paul C wrote:

> **** Caution: EXTERNAL Sender ****
> Hi Dan!
> I hope this message finds you doing well.
> Just checking in to see if there is any update on publication of the pregnancy assay paper.
> Thank you,
> Paul
>
> Paul Griffin, Ph.D.
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From: Dan Baker
To: Griffin, Paul C
Subject: Re: [EXTERNAL] Re: Any update on the noninvasive pregnancy assay paper?
Date: Friday, July 8, 2022 9:22:37 AM

That works for me. Yes, same phone number.

On 7/8/2022 9:20 AM, Griffin, Paul C wrote:

> **** Caution: EXTERNAL Sender ****
> Tanks Dan. I may be able to call you today at 10:30 am. Could that work, too? Are you still at: 970-556-8518?
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Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: BLM Wild Horse and Burro research funding opportunity
Date: Thursday, November 18, 2021 10:06:52 AM

Thank you very much Dan,
Hey, let's talk some time soon; probably best right after the Thanksgiving break, as I'll be out most of next week. A) Yes, I certainly encourage you to apply if you have ideas you'd want to pursue. and B) we intend to compile what data we have on GonaCon applications that the BLM has done, with variable lengths of time between primer and booster, and I'd value you as a reviewer or bouncer-off-of-ideas; for many of those herds, we wouldn't expect to see effects / reduced foaling rates until 2022, so this is just preparatory...
Paul

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(970) 226-9358 (office)
Pronouns: he, him, his

From: Dan Baker <danbaker@colostate.edu>
Sent: Thursday, November 18, 2021 9:53 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Re: BLM Wild Horse and Burro research funding opportunity

Hi Paul,

Thanks for the notification. I'll visit with those involved in our research project and see if there is interest in submitting a proposal. I would like to pursue a population-level evaluation of GonaCon-Equine but as you mentioned previously there may not be a suitable experimental population that can provide adequate demographic data for a study like this.

By the way, we should have two manuscripts relating to the T. Roosevelt feral horse study ready to submit for peer review by the end of the year. One will relate to remote dart delivery of GonaCon-Equine and the other on long-term vaccine effectiveness.

I hope that you are doing well and I'm sure staying busy. Have a wonderful and relaxing

Thanksgiving Holiday.

Kind regards,

Dan

On 11/16/2021 1:39 PM, Griffin, Paul C wrote:

> I am writing you today because the US Department of the Interior's Bureau of Land Management is announcing a funding opportunity for new wild horse and burro related research partnerships with universities, other federal, state, or local agencies, tribal, or non-governmental organizations. Please share this message with colleagues in and outside of your institution.
>
> The BLM is seeking research proposals from scientists who can develop new research projects that support the goals outlined in its 2021 Strategic Research Plan, and described in a related blog post. The process for proposal submission is different for non-federal, as opposed to federal researchers. Federal researchers should respond to the Request for Proposals for Federal Agencies: Wild Horse and Burro Research, and non-federal researchers should respond to the parallel Notice of Funding Opportunity Announcement (NOFO): L22AS00069; Wild Horse and Burro Research for non-federal researchers. All qualifying proposals will be evaluated by scientific review panels, composed of external peer reviewers. Deadline for submissions is January 18, 2022.
>
> Because of the need for more effective population growth suppression, the BLM's top research priority remains the development of safe, practical, effective and long-lasting fertility control methods for wild horses and burros. The BLM's secondary research priority is to improve understanding of the relationship between wild horses and burros and their environment, including how climate change will impact management and protection.
>
> The BLM is not funding other, lower priority, wild horse and burro research topics at this time. Those include estimating herd size and demographic modeling; population genetics; animal health, handling and welfare; private care placement; and the human dimension (socio-economic) of wild horse and burro management. However, the BLM accepts unsolicited research proposals at any time. Unsolicited proposals addressing those or any other topics are typically evaluated by a technical team, which solicits external peer review as needed; unsolicited projects are approved based on merit, responsiveness to agency management needs, and available funding.
>
> Thank you for considering this opportunity, and sharing with any colleagues, as appropriate.
>
> Sincerely,
>
> Paul Griffin

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Research Coordinator

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From: Dan Baker
To: Griffin, Paul C
Subject: Re: [EXTERNAL] Re: BLM Wild Horse and Burro research funding opportunity
Date: Thursday, November 18, 2021 5:59:54 PM

Hi Paul,

Sounds good. I will give you a call during the week after Thanksgiving. Dan

On 11/18/2021 10:06 AM, Griffin, Paul C wrote:

> Thank you very much Dan,
> Hey, let's talk some time soon; probably best right after the Thanksgiving break, as I'll be out most of next week. A) Yes, I certainly encourage you to apply if you have ideas you'd want to pursue. and B) we intend to compile what data we have on GonaCon applications that the BLM has done, with variable lengths of time between primer and booster, and I'd value you as a reviewer or bouncer-off-of-ideas; for many of those herds, we wouldn't expect to see effects / reduced foaling rates until 2022, so this is just preparatory...
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> **From:** Dan Baker <danbaker@colostate.edu>
> **Sent:** Thursday, November 18, 2021 9:53 AM
> **To:** Griffin, Paul C <pgriffin@blm.gov>
> **Subject:** [EXTERNAL] Re: BLM Wild Horse and Burro research funding opportunity
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Sincerely,

Paul Griffin

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From: Griffin, Paul C
To: Powers, Jenny
Cc: Dan Baker
Subject: Re: [EXTERNAL] Re: Can we call to catch up?...and might you be free briefly March 13-14?
Date: Monday, February 11, 2019 1:55:15 PM

Jenny -- no problem, and sounds good. Dan -- looking forward to tomorrow. Thank you, Paul

On Mon, Feb 11, 2019 at 1:48 PM Powers, Jenny <jenny_powers@nps.gov> wrote:

> Hi guys,
>
> I can't make it tomorrow. Go for it without me. Dan knows the research better than I do of course!
>
> I am teaching an anesthesia class March 12-15 so won't be able to make your advisory board meeting. Sorry about that.
>
> Jenny
>
> On Mon, Feb 11, 2019 at 1:45 PM Dan Baker <danbaker@colostate.edu> wrote:
>
> > Paul,
> >
> > You're probably right. See you there.
> >
> > Dan
> >
> > On 2/11/2019 1:37 PM, Griffin, Paul wrote:
> >
> > > Hi Dan,
> > > That time & place works for me. I'm still not sure whether Jenny can make it. If we don't hear from her, though, let's assume that she is swamped with pre-possible-shutdown-2 business, and just you and I can meet Tuesday at 3 at that cafe.
> > > Thank you,
> > > Paul
> > >
> > > On Mon, Feb 11, 2019 at 1:03 PM Dan Baker <danbaker@colostate.edu> wrote:
> > >
> > > > Hi Paul,
> > > >
> > > > Are we still meeting this week on Tuesday at 3pm at the Wild Boar?
> > > > Thanks.
> > > >
> > > > Dan
> > > >
> > > > On 2/8/2019 4:40 PM, Griffin, Paul wrote:
> > > >
> > > > > Thanks, Dan -- Sorry about that Jenny. I hit 'reply' instead of

'reply all.' If that cafe wher ewe met in December is better for you, or somewhere else, we can locate to there, or stick to a phone call.
Hoping to see you soon.
Paul

On Fri, Feb 8, 2019 at 4:13 PM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> The 12th at 3pm at the Wild Boar works for me. Let's hear from Jenny before we confirm.
>
> Thanks.
>
> Dan
>
> On 2/8/2019 3:04 PM, Griffin, Paul wrote:
>
>> Hi Dan,
>> Thanks for your flexibility. Could we talk Tuesday the 12th at 3 pm? If that time works for you, I'd be very glad to meet you somewhere for a hot drink.
>> I don't remember where you are on campus...would somewhere like the 'wild boar cafe' (1510 College Ave) be convenient for you? Or, maybe somewhere else you'd prefer? If that timing is tight for you, we can also just talk by phone.
>> Paul
>>
>> On Fri, Feb 8, 2019 at 2:30 PM Dan Baker <danbaker@colostate.edu> wrote:
>>
>>> Hi Paul,
>>>
>>> Good to hear from you. I'm available next week for a visit. Do you want to do a conference call or get together for coffee. I'm available on the 11, 12, and 13 just about any time. I'm also available on March 13 or 14 for a meeting with the research advisory team. I'll try to keep those dates open and just let me know when you have a definite time.
>>>
>>> Dan
>>>
>>> On 2/7/2019 3:59 PM, Griffin, Paul wrote:

Hi Dan and Jenny,
It was very nice to see you before the shutdown last year. With a long delay due to the shutdown, I'm hoping that we might be able to talk soon, preferably next week (February 11-15) about recent developments and coming expectations for your BLM-funded research. I like to be up to date on how your research has been going, and I feel that I have not kept up lately.
Please let me know what days and times you might be available to talk. I'm aiming for next week because there is a chance that the Department of the Interior will again be shutdown after February 15, though I certainly hope that does not happen.
When we speak I also will be asking whether you might be able to set aside time to speak briefly with the BLM wild horse and burro (WHB) research advisory team that I chair, some time on March 13 or the morning of March 14. Our team will be holding our annual in-person meeting at that time (in Fort Collins). Originally, we on BLM's WHB research team had been planning to have a meeting on February 20-21, but because of uncertainty about the potential for another shutdown after February 15, we thought it would be prudent to push it back to March 13-14.
Thank you,
Paul

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Paul Griffin, Ph.D.

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From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: Checking in on coronavirus-caused changes to the Wild Horse project
Date: Friday, March 27, 2020 4:27:09 PM

Hi Dan,
Tuesday at 10 sounds great. Looking forward to talking with you then.
Paul

Paul Griffin, Ph.D.
Research Coordinator
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From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, March 27, 2020 4:17 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: Re: [EXTERNAL] Re: Checking in on coronavirus-caused changes to the Wild Horse project

Hi Paul,

How about Tuesday morning at 10am? Other times will also work for me. Thanks.
Dan

On 3/27/2020 3:42 PM, Griffin, Paul C wrote:

> Hi Dan,
> Great to hear from you. Can we aim to talk some time on Monday afternoon, or Tuesday (any time except 11:30-12)? Please pick a time that's good for you, and we'll aim for that. I can be reached via cell phone, 970-631-4808
> Thank you very much,
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> Paul Griffin, Ph.D.
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> 2150 Centre Ave. Building C
> Fort Collins, CO 80526 USA
> (970) 226-9358 (office)
> (970) 631-4808 (mobile)
>
> ---
>
> **From:** Baker,Danny <Dan2.Baker@ColoState.EDU>

Sent: Wednesday, March 25, 2020 4:11 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Re: Checking in on coronavirus-caused changes to the Wild Horse project

Hey Paul,
Good to hear from you. Doing fine here. Hope you are, too. Glad to visit anytime next week. We do have a few novel circumstances at TR that we are dealing with to talk about. Let me know a good time for you.

Regards,
Dan

Sent from my iPhone

> On Mar 25, 2020, at 12:44 PM, Griffin, Paul C <pgriffin@blm.gov> wrote:
>
> Hi Dan,
> I hope this message finds you well (presumably at home).
> Might there be a time in the next week or so when we could check in by phone to talk about the status of the project, and any limitations that the coronavirus is causing for your research?
> Thank you,
> Paul
>
> Paul Griffin, Ph.D.
> Research Coordinator
> BLM Wild Horse and Burro Program
> 2150 Centre Ave. Building C
> Fort Collins, CO 80526 USA
> (970) 226-9358 (office)
> (970) 631-4808 (mobile)

--
Dan L. Baker, PhD
Affiliate Faculty
Department of Biomedical Sciences
Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: GonaCon darting SOPs
Date: Monday, July 11, 2022 8:44:04 AM

Hi Dan,
Thanks again for talking with me last week. Here is contact information for Shaney, who may be unavailable on and off in the next few weeks.
Shaney Rockefeller, WHB Specialist
BLM Oregon Vale district office
(208) 859-2501 (mobile)

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, July 8, 2022 4:26 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Re: GonaCon darting SOPs

Hi Paul,

Very enjoyable conversation with you. Thanks for your time. I didn't realize we had so much to visit about. I always value your counsel. Also, thanks for reaching out to Shaney on my behalf. I will try to contact her on Monday. Can you send me her contact information? Have a wonderful weekend.

Dan

On 7/8/2022 11:39 AM, Griffin, Paul C wrote:

Hi Dan and Shaney,

Good talking with you today Dan. If you and your coauthors are comfortable with it, I think you'll find Shaney is the best BLM resource for providing any review of the draft SOPs for GonaCon delivery that you are preparing as part of your publication. I'm happy to provide feedback, too, but my experience is very limited.

Shaney, if Dan ends up sending you draft SOPs please treat then with the same level of confidentiality that we approach all proposals and unpublished manuscripts with. Hopefully they will publish soon and we'd be able to share and reference the document then.

Thank you,

Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
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(he / him)

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Dan L. Baker, PhD
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Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: Please send any materials for WHB research team by tomorrow
Date: Tuesday, March 5, 2019 10:16:06 AM

Noon would leave me plenty of time. Thanks, Dan.

On Tue, Mar 5, 2019 at 9:55 AM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> I should be able to get you this information to by noon today. Is that OK? Or, do you need it sooner?
>
> Dan
>
> On 3/4/2019 4:37 PM, Griffin, Paul wrote:
>
> > Hi Dan,
> > Yes, a brief itemization would be good, along with a brief description of why the previously-funded amount is not going to be enough to get you through the full 2020 field season. I.e., was it a cost overrun? you had to work a longer field season due to the later pregnancies? assay costs came in higher than budgeted? etc... That'd help people understand the rationale there.
> > Yes, I do also think a table of the results you presented at Albuquerque would be good. Alan Shepherd and I were there, but it'd be good for the others to be reminded of those successes.
> > Thank you,
> > Paul
> >
> > On Mon, Mar 4, 2019 at 4:30 PM Dan Baker <danbaker@colostate.edu> wrote:
> >
> > > Hi Paul,
> > >
> > > Thanks for the heads-up. I think that I would be requesting $15,000-$17,000 for 2020. This would be primarily to cover ongoing field technician costs and publication expenses for follow-up data reporting. Would you like for me to itemize these request? I could also provide a summary of data from the New Mexico conference.
> > >
> > > Dan
> > >
> > > On 3/4/2019 3:50 PM, Griffin, Paul wrote:
> > >
> > > > Hi Dan,
> > > > Just a reminder that if you have any materials you'd like me to share with the BLM WHB research team, then tomorrow (Tuesday)is the day by which you should send me those materials. I'm thinking of a justification for your extra funding request, for example.
> > > > Thank you,
> > > > Paul

--
Paul Griffin, Ph.D.
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pgriffin@blm.gov

From: Griffin, Paul C
To: Dan Baker
Subject: Re: [EXTERNAL] Re: THRO is closed for visitors; for researchers, too?
Date: Sunday, April 19, 2020 3:33:14 PM

Hi Dan,
That is all great to hear, a testament to the good relations you've worked to maintain, and a sign of how much priority the park clearly places on your research.
Paul

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(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Dan Baker <danbaker@colostate.edu>
Sent: Friday, April 17, 2020 5:41 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] Re: THRO is closed for visitors; for researchers, too?

Hi Paul,

Thanks for your concerns. It's been keeping me awake at night lately just wondering when we might be shown the door at THRO. However, we are still dodging bullets and the project is still full on. As you are aware, right now the park is closed to all visitors and recreation activities but not to permitted research projects like ours.

Luckily, two of my veteran techs from previous years made it up there before they banned any techs from Colorado from working in the park this year due to COVID-19. With the help from a couple of local volunteers, my techs are still contacting all mares each week for evidence of foaling. Thanks in large part to Blake, I think that our project will survive and our data will not be compromised. I'll keep you posted.

Regards,
Dan

On 4/17/2020 3:29 PM, Griffin, Paul C wrote:

> Hi Dan,
> I just saw the news that THRO will be closed to visitors. Just checking: does that mean, as you suspected, that it'll be closed to your technicians also?
> Thank you,

Paul

Paul Griffin, Ph.D.
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Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
Email: danbaker@colostate.edu

From: Eckery, Douglas C - APHIS
To: Griffin, Paul C
Subject: RE: [EXTERNAL] RE: What does 2mL of GonaCon weigh?
Date: Monday, July 27, 2020 12:47:40 PM

Hi Paul,
Yes, I was copied in an email thread between Cary Mundell and Mike Tweddell on the failed shipment. You are correct, the vaccine should not be used and Cary is going to ship new vaccine tomorrow.
Regards,
Doug

Douglas C. Eckery, PhD
Assistant Director
National Wildlife Research Center
USDA APHIS Wildlife Services
4101 LaPorte Avenue
Fort Collins, CO 80521
Office: 970-266-6164
Mobile: 970-692-7387

From: Griffin, Paul C [mailto:pgriffin@blm.gov]
Sent: Monday, July 27, 2020 12:25 PM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Subject: Re: [EXTERNAL] RE: What does 2mL of GonaCon weigh?

Thank you Doug.
That information on mass per mL will be useful as I tailor BLM's SOPs for GonaCon use. The SOPs I got from Dan & Blake specified that darts be weighed, and specified the desirable volume of vaccine for dart delivery, but did not say what the target change in dart weight should be.
FYI, unfortunately, I heard today that a shipment of 60 doses, sent to Fillmore, UT, took 5 days to get there, and arrived warm. The specialist there will be in touch with Cary to see what to do about it. But, in a nutshell, those doses are not useful any more if they say warm for a long time, right?
Paul

Paul Griffin, Ph.D.
Research Coordinator
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(970) 631-4808 (mobile)

From: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Sent: Monday, July 27, 2020 9:26 AM
To: Dan Baker <danbaker@colostate.edu>
Cc: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] RE: What does 2mL of GonaCon weigh?

I'll have Cary weigh some vaccine from the next batch he makes.
Regards,
Doug

Douglas C. Eckery, PhD
Assistant Director
National Wildlife Research Center
USDA APHIS Wildlife Services
4101 LaPorte Avenue
Fort Collins, CO 80521
Office: 970-266-6164
Mobile: 970-692-7387

From: Dan Baker [mailto:danbaker@colostate.edu]
Sent: Saturday, July 25, 2020 11:41 AM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Cc: Paul Griffin <pgriffin@blm.gov>
Subject: Fwd: What does 2mL of GonaCon weigh?

Hi Doug,
I think that you would be the most qualified to answer Paul's question. Thanks.
Dan

-------- Forwarded Message --------
Subject: What does 2mL of GonaCon weigh?
Date: Fri, 24 Jul 2020 22:03:00 +0000
From: Griffin, Paul C <pgriffin@blm.gov>
To: Baker,Danny <Dan2.Baker@ColoState.EDU>
CC: Dan Baker <danbaker@colostate.edu>

Hi Dan,
I hope this email finds you dong all right. Quick question, to follow up on your helpful GonaCon SOPs. How much does GonaCon weigh, in grams per mL? I ask because you say folks should weigh before and after, to be sure about how many mL get delivered via dart.
Thank you,
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: Eckery, Douglas C - APHIS
To: Griffin, Paul C
Subject: RE: [EXTERNAL] RE: What does 2mL of GonaCon weigh?
Date: Tuesday, July 28, 2020 6:28:13 PM

Hi Paul,
Cary took the average weight of 10 x 2 ml doses which came to 1.9g. So, 0.95 g/ml.

Regards,
Doug

Douglas C. Eckery, PhD
Assistant Director
National Wildlife Research Center
USDA APHIS Wildlife Services

4101 LaPorte Avenue
Fort Collins, CO 80521

Office: 970-266-6164
Mobile: 970-692-7387

From: Griffin, Paul C [mailto:pgriffin@blm.gov]
Sent: Monday, July 27, 2020 12:25 PM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Subject: Re: [EXTERNAL] RE: What does 2mL of GonaCon weigh?

Thank you Doug.
That information on mass per mL will be useful as I tailor BLM's SOPs for GonaCon use. The SOPs I got from Dan & Blake specified that darts be weighed, and specified the desirable volume of vaccine for dart delivery, but did not say what the target change in dart weight should be.
FYI, unfortunately, I heard today that a shipment of 60 doses, sent to Fillmore, UT, took 5 days to get there, and arrived warm. The specialist there will be in touch with Cary to see what to do about it. But, in a nutshell, those doses are not useful any more if they say warm for a long time, right?
Paul

Paul Griffin, Ph.D.
Research Coordinator

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From: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Sent: Monday, July 27, 2020 9:26 AM
To: Dan Baker <danbaker@colostate.edu>
Cc: Griffin, Paul C <pgriffin@blm.gov>
Subject: [EXTERNAL] RE: What does 2mL of GonaCon weigh?

I'll have Cary weigh some vaccine from the next batch he makes.

Regards,
Doug

Douglas C. Eckery, PhD
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4101 LaPorte Avenue
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Office: 970-266-6164
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From: Dan Baker [mailto:danbaker@colostate.edu]
Sent: Saturday, July 25, 2020 11:41 AM
To: Eckery, Douglas C - APHIS <douglas.c.eckery@usda.gov>
Cc: Paul Griffin <pgriffin@blm.gov>
Subject: Fwd: What does 2mL of GonaCon weigh?

Hi Doug,

I think that you would be the most qualified to answer Paul's question. Thanks.

Dan

-------- Forwarded Message --------
Subject: What does 2mL of GonaCon weigh?
Date: Fri, 24 Jul 2020 22:03:00 +0000
From: Griffin, Paul C <pgriffin@blm.gov>
To: Baker,Danny <Dan2.Baker@ColoState.EDU>
CC: Dan Baker <danbaker@colostate.edu>

Hi Dan,
I hope this email finds you dong all right. Quick question, to follow up on your helpful GonaCon SOPs. How much does GonaCon weigh, in grams per mL? I ask because you say folks should weigh before and after, to be sure about how many mL get delivered via dart.
Thank you,
Paul

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Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From:	Griffin, Paul C
To:	Dan Baker
Cc:	McCann, Blake E; Rittenhouse, Bruce H; Shepherd, Alan B; Reiland, Michael J
Subject:	Re: [EXTERNAL] Supplemental budget request 2020
Date:	Monday, December 16, 2019 4:10:51 PM
Attachments:	Budget Justification FY 2020a.docx

Dan,
For administrative reasons, I do not expect it would be possible for BLM to provide the $19K of extra funds to CSU under your existing financial assistance agreement (grant) number L15AC00145. However, with this email I am sharing your request for the funding needed to finish the fieldwork in 2020 with BLM WHB program leadership (cc'd here).
Instead, what I suggest is that BLM create an interagency agreement directly with the Park Service to accomplish the needed goals that your project has identified, keeping in mind what funding would be needed for NPS to accomplish the outstanding field work.
BLM values and recognizes the importance of the work that the NPS and CSU have already completed. Having the final year of foaling rate data from 2020 would be extremely valuable for evaluating the duration of GonaCon booster dose effectiveness, especially for animals that were boostered 6 months, 1 year, or 2 years after the first dose.
Thank you,
Paul

On Fri, Dec 13, 2019 at 10:48 AM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> SUBJ: BLM Agreement Number: L15AC00145.
>
> Study Project Name: Reimmunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance.
>
> Regrettably, the purpose of this letter is to inform you that, after several meetings with our accountant, Maura Link here at CSU, we came to the conclusion that we are unlikely to have sufficient funds to complete our research project objectives for FY2020. This situation began in 2015, with our original BLM budget request that underestimated the amount of personnel time needed to conduct the intensive field measurements to evaluate the effects of GonaCon-Equine in 90 experimental horses at Theodore Roosevelt N. P. Even though we have previously requested and gratefully received supplemental funding from the BLM, it apparently was not sufficient to offset the deficit that gradually accumulated each year over the period of our contract (2015-2019).
>
> We feel that our collaborative research efforts with the BLM, USDA/APHIS, NPS, and CSU have been a huge success and have resulted in a more effective approach in the application of GonaCon-Equine in free-ranging horses. The culmination of this effort has been reported in a scientific publication (Baker et al. 2018) and with another publication on the development and testing of dart delivery of this vaccine on the way (McCann et al. in press). However, as presented in

these papers and in our original BLM study proposal, this research is not complete. Two primary objectives remain unanswered and need to be addressed: 1) to determine the most effective reimmunization schedule for GonaCon-Equine for suppressing foaling rates in free-ranging horses, and 2) to determine the duration of effective contraception in all post-primary revaccination treatment groups. In order to complete, or at least provide insight into these objectives, additional funding will be needed for FY2020. Without this funding, a complete and thorough evaluation of this vaccine will be significantly compromised.

I have worked closely with our accountant to estimate the amount of additional funds necessary to accomplish our remaining objectives in FY2020. Since the backbone of our research project is well-trained field research technicians, our funding request is for support for these positions. Thus, additional funds requested for personnel in the final year of this project is $16,175 direct funds and $2,825 indirect (17.5%) for a total of $19,000.

Thank you for consideration of this request. Please give me a call if you have questions.

Dan Baker

--
Dan L. Baker, PhD
Affiliate Faculty
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Animal Reproduction and Biotechnology Laboratory
Colorado State University
Fort Collins, Colorado 80535 USA
Phone: 970-556-8518
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pgriffin@blm.gov

From: Griffin, Paul C
To: McCann, Blake E
Subject: Re: [EXTERNAL] Supplemental funding
Date: Monday, January 13, 2020 1:07:35 PM

On Mon, Jan 13, 2020 at 1:03 PM McCann, Blake <blake_mccann@nps.gov> wrote:

> Let's both try to email - I will try not to forget, but it happens to me sometimes.
>
> On Mon, Jan 13, 2020 at 11:42 AM Griffin, Paul <pgriffin@blm.gov> wrote:
>
> > Sounds great. Thanks, Blake. Looking forward to it. I'll look for your email Wednesday.
> > Paul
> >
> > On Mon, Jan 13, 2020 at 11:30 AM McCann, Blake <blake_mccann@nps.gov> wrote:
> >
> > > Yes, at Oakridge.
> > >
> > > Let's try Thursday. Can we check in by email Wednesday to set up the time?
> > >
> > > Blake
> > >
> > > On Mon, Jan 13, 2020 at 11:22 AM Griffin, Paul <pgriffin@blm.gov> wrote:
> > >
> > > > Hi Blake,
> > > > I'd be happy to meet during one of your lunch breaks. Maybe on the Wednesday or the Thursday? Are you going to be in the NPS building on Oakridge Drive?
> > > > Paul
> > > >
> > > > On Mon, Jan 13, 2020 at 10:49 AM McCann, Blake <blake_mccann@nps.gov> wrote:
> > > >
> > > > > Thanks Paul;
> > > > >
> > > > > Yes, it would be good to meet. I will have lunch breaks, and my evenings should be free. Not sure about availability during regular business hours otherwise.
> > > > >
> > > > > I will be leaving Friday morning and could possibly visit for a bit before I leave town on that day.
> > > > >
> > > > > Blake
> > > > >
> > > > > On Mon, Jan 13, 2020 at 10:02 AM Griffin, Paul <pgriffin@blm.gov> wrote:
> > > > >
> > > > > > I'll try to start the BLM side of the IAA with NPS asap. I don't think you are part of the BLM-NPS process, Dan, but thanks for asking.
> > > > > > Blake, if you have any breaks in your schedule in Fort Collins, I'd be very happy to come meet you in person wherever you are. Please let me know; you can also text me via my gov't cell phone (970-631-4808).
> > > > > > Thank you,
> > > > > > Paul
> > > > > >
> > > > > > On Mon, Jan 13, 2020 at 8:07 AM McCann, Blake <blake_mccann@nps.gov> wrote:
> > > > > >
> > > > > > > Hello Dan and Paul;
> > > > > > >
> > > > > > > I have reviewed Pauls SOW, and pertinent information is all there. We can move forward with the

IAA at any time. It has to originate from BLM and should come to Kevin Melzo (referenced in prior email) and myself. I need to get moving on the agreement side of things to facilitate the CESU CA with CSU. I have started that process, but I have not yet submitted forms.

I am here today and will be traveling to Ft. Collins tomorrow - weather permitting. FYI

Blake

On Sun, Jan 12, 2020 at 9:17 AM Dan Baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> It's been a while since we lasted visited with Blake regarding our request for supplemental funding for 2020. I checked my notes from that conversation and just wanted to make sure that you were not needing additional documentation from me in this process.
>
> Thanks.
>
> Dan
>
> --
> Dan L. Baker, PhD
> Affiliate Faculty
> Department of Biomedical Sciences
> Animal Reproduction and Biotechnology Laboratory
> Colorado State University
> Fort Collins, Colorado 80535 USA
> Phone: 970-556-8518
> Email: danbaker@colostate.edu

--
Blake McCann, Ph.D.
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Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

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pgriffin@blm.gov

From: Griffin, Paul C
To: Dan baker
Subject: Re: [EXTERNAL] T. Roosevelt Wild Horse Project
Date: Monday, August 19, 2019 4:30:41 PM

Hi Dan,
That sounds great. Looking forward to talking Tuesday at 10.
Paul

On Mon, Aug 19, 2019 at 3:47 PM Dan baker <danbaker@colostate.edu> wrote:

> Hi Paul,
>
> How about tomorrow morning (8/20) at 10 am? Thanks.
>
> Dan
>
> On 8/19/2019 10:27 AM, Griffin, Paul wrote:
>
> > Hi Dan,
> > Thank you for reaching out. Yes, I'd be very glad to talk. I'm mostly available for a call all week after 9 am, except for the following times:
> > Monday 11:15 - 2
> > Tuesday 11:15 - 1:30
> > So...name a day and time. The sooner the better.
> > Looking forward to talking with you,
> > Paul
> >
> > On Mon, Aug 19, 2019 at 9:31 AM Dan baker <danbaker@colostate.edu> wrote:
> >
> > > Hi Paul,
> > >
> > > I would like to give you a brief update on our study results for this season. When would be a good time to give you a call?
> > >
> > > Thanks,
> > >
> > > Dan
> > >
> > > --
> > > Dan L Baker, PhD
> > > Research Biologist
> > > Faculty Affiliate
> > > Department of Biomedical Sciences
> > > Animal Reproduction and Biotechnology Laboratory
> > > Colorado State University
> > > Fort Collins, CO 80535
> > > (970) 556-8518
> > > danbaker@colostate.edu

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Research Coordinator, BLM Wild Horse and Burro Program
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970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: McCann, Blake E
To: Griffin, Paul C
Cc: Shepherd, Alan B; Melzo, Kevin A; Appold, David W
Subject: Re: draft 1, SOW for BLM-NPS IAA
Date: Thursday, January 9, 2020 3:26:22 PM
Importance: High

Hello Paul;

Sorry for the delayed response. We do need to move relatively quickly - it appears that things will not be as urgent as previously thought, but our Agreements deadlines are compressed this year, regardless. The SOW looks to be comprehensive. It seems more like a Cooperative Agreement than what I have used for IAAs in the past. Content is appropriate, however. I suggest we move forward.

Yes, Kevin is the appropriate budget contact. Kevin, can you advise regarding payment?

Thank you.

Blake

On Thu, Dec 26, 2019 at 1:18 PM Griffin, Paul <pgriffin@blm.gov> wrote:

> Blake,
> Attached is a draft statement of work / articles for an interagency agreement in support of NPS finishing the GonaCon field work at Theodore Roosevelt National Park in 2020. Please let me know your thoughts and suggested edits. I appreciate what you said in our phone conversation about the need to move quickly on this, to allow time for finishing all aspects of agreement formation in time for hiring fieldwork staff.
> One question: Is Kevin Melzo the right person to list as both a budget contact and a billing / payment contact?
> Note: I've written this in a way that would have a $19K obligation, but with a maximum of up to $24K, in case some unforeseen need arises. However, if you are aware of any $20K threshold for any particular level of approvals needed, we can also back it down to just the $19K, for simplicity and speed of approvals.
> I am cc-ing Contracting Officer Dave Appold, who may be involved with agreement formation in the new year, as well as BLM WHB program on-range branch chief Alan Shepherd, BLM WHB program budget advisory Michael Reiland, and BLM WHB program division chief Bruce Rittenhouse.
> Thank you,
> Paul
>
> --
> Paul Griffin, Ph.D.
> Research Coordinator, BLM Wild Horse and Burro Program
> 2150 Centre Ave, Building C, Fort Collins, CO 80526
> 970-226-9358 office, 970-631-4808 mobile
> pgriffin@blm.gov

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park

315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From: McCann, Blake
To: Griffin, Paul C
Subject: Re: draft 1, SOW for BLM-NPS IAA
Date: Tuesday, January 21, 2020 10:31:44 AM

Hello Paul;

It looks like our best option will be to hire the positions as either GS-03 or 04 Bio-techs. We would also only have them on NPS time from May - July - I need to confirm with Dan that he has enough funds on the original project to get his staff through the beginning of May.

Regardless, are you able to provide language in the Budget section to support GS-03 and GS-04, full time for five or six pay periods ($12-15k), and perhaps som GS-06 coverage (a pay period or two, the remainder) for our lead tech to assist with the field work to complete the project this summer?

If so, then I think we can move forward.

Blake

On Thu, Dec 26, 2019 at 1:18 PM Griffin, Paul <pgriffin@blm.gov> wrote:

> Blake,
> Attached is a draft statement of work / articles for an interagency agreement in support of NPS finishing the GonaCon field work at Theodore Roosevelt National Park in 2020. Please let me know your thoughts and suggested edits. I appreciate what you said in our phone conversation about the need to move quickly on this, to allow time for finishing all aspects of agreement formation in time for hiring fieldwork staff.
> One question: Is Kevin Melzo the right person to list as both a budget contact and a billing / payment contact?
> Note: I've written this in a way that would have a $19K obligation, but with a maximum of up to $24K, in case some unforeseen need arises. However, if you are aware of any $20K threshold for any particular level of approvals needed, we can also back it down to just the $19K, for simplicity and speed of approvals.
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Blake McCann, Ph.D.
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Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7

Medora, ND 58645
701-623-4730 ext. 1433

From: McCann, Blake
To: Griffin, Paul C
Cc: Melzo, Kevin A
Subject: Re: Expect IAA articles for your signature early next week
Date: Friday, January 24, 2020 10:56:22 AM

Hello Paul;

Yes, the text appropriately describes the scope of the work. Sorry that I did not get back to you. The GS levels indicated will work for our purposes.

Thank you.

Blake

On Fri, Jan 24, 2020 at 10:50 AM Griffin, Paul C <pgriffin@blm.gov> wrote:

> Hi Blake,
> Contracting Officer Dave Appold told me today that you can expect to see the interagency agreement articles for NPS signature early next week. Those will include the changes you suggested, about GS level employees. Specifically, the text on that last page will say:
> "Description of 2020 Field Work Staff Positions
>
> Crew Leader The Crew Leader (i.e., GS-6) will be the daily, onsite contact person responsible for planning, training and scheduling daily work assignments for two field research technicians, as well as daily oversight and quality control of data collection. Any Crew Leader that will be supported by NPS THRO would be well-versed and familiar with the objectives, methods of data collection, and logistics of this research project. In addition, the Crew Leader will have knowledge of the park landmarks and terrain, identification of individual experimental horses and location identification of individual experimental horses, and their band associations. The Crew Leader will play a role in the training of two new technicians.
>
> Project Field Research Technicians Two field research technicians (i.e., GS-3 or GS-4). The role of these technicians will be daily observations and measurements on ~85 experimental free-ranging mares at NPS THRO during 1 March-1August, 2020. Measurements will include identification of individual mares and determination of presence/absence of a foal, body condition of each mare/foal, evaluation of injection site reactions from GonaCon vaccinations, and band composition. Additional roles will be daily communication and data transfer to crew leader, interactions with NPS THRO staff, and park visitors."
>
> Does that sound OK? Thank you very much,
>
> Paul

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park

315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From: Griffin, Paul C
To: Powers, Jenny
Cc: Dan Baker; McCann, Blake E; Shepherd, Alan B
Subject: Re: funding mechanism
Date: Tuesday, February 19, 2019 11:38:44 AM

Hi Jenny,
Thank you for writing. Yes, what I had mentioned to Dan was the difficulty in adding money to his existing grant; because the total amount of his grant is over $50,000, approval from the Secretary of the Interior's office is required to add any more money to that agreement.
Your idea about funding NPS to assist with the missing field staffer for the 2020 season has potential.
The BLM WHB research team will be meeting in March, at which time we'll be discussing any and all outstanding requests for supplemental funding for research projects, and if and how to cover them. So, I can't give you any definite plan at this time.
On the bright side, I'm very glad that Dan's funding is already obligated and covers all expected 2019 expenses, and all but about $20K of the 2020 expenses.
Paul

On Tue, Feb 19, 2019 at 11:23 AM Powers, Jenny <jenny_powers@nps.gov> wrote:

> Hi Paul,
>
> I was catching up with Dan yesterday and he mentioned that there doesn't seem to be a good funding mechanism to extend THRO funding for next year. I'm wondering if you could use an Interagency Agreement (IAA) to either THRO or to us to get the work done? We can then spend the money (Dan was mentioned $20k) on a seasonal employee to complete the foaling monitoring in FY 20.
>
> Let me know if that might be a possibility and Blake and I can put the planning in place from our side.
>
> Thanks for all the support on this project!
> Jenny
> --
>
> _____
>
> Jenny Powers, DVM, PhD
> National Park Service
> Biological Resources Division, Wildlife Health Branch
> 1201 Oakridge Dr. #200
> Fort Collins, CO 80525
>
> (970) 267-2122 (office)
> (970) 214-2933 (cell)
> (970) 225-3585 (fax)
> jenny_powers@nps.gov

--
Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: Powers, Jenny
To: Griffin, Paul C
Cc: Dan Baker; McCann, Blake E; Shepherd, Alan B
Subject: Re: funding mechanism
Date: Tuesday, February 19, 2019 11:48:15 AM
Importance: High

Thanks for the follow up Paul. I completely understand budget uncertainties! Just keep us posted. From a hiring perspective we would need to get started in August to make spring happen so knowing by then if we need to go the fed route would be helpful.

On Tue, Feb 19, 2019 at 11:38 AM Griffin, Paul <pgriffin@blm.gov> wrote:

> Hi Jenny,
> Thank you for writing. Yes, what I had mentioned to Dan was the difficulty in adding money to his existing grant; because the total amount of his grant is over $50,000, approval from the Secretary of the Interior's office is required to add any more money to that agreement.
> Your idea about funding NPS to assist with the missing field staffer for the 2020 season has potential.
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> Paul
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> On Tue, Feb 19, 2019 at 11:23 AM Powers, Jenny <jenny_powers@nps.gov> wrote:
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> > Hi Paul,
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> > Thanks for all the support on this project!
> > Jenny
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(970) 225-3585 (fax)
jenny_powers@nps.gov

From: Rockefeller, Shaney L
To: Griffin, Paul C; Dan Baker
Subject: Re: GonaCon darting SOPs
Date: Thursday, July 21, 2022 10:21:11 AM

Paul,
No problem with reviewing and coordinating with Dan.

Dan,
I am out in the field a lot this summer without any kind of cell/data service so if you email me, I will get back to you when I get back into town.

Shaney Rockefeller, Wild Horse Specialist
Vale District BLM, 100 Oregon St., Vale, OR 97918
desk 541-473-6221 cell 208-859-2501

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, July 8, 2022 11:39 AM
To: Dan Baker <danbaker@colostate.edu>; Rockefeller, Shaney L <srockefe@blm.gov>
Subject: GonaCon darting SOPs

Hi Dan and Shaney,
Good talking with you today Dan. If you and your coauthors are comfortable with it, I think you'll find Shaney is the best BLM resource for providing any review of the draft SOPs for GonaCon delivery that you are preparing as part of your publication. I'm happy to provide feedback, too, but my experience is very limited.
Shaney, if Dan ends up sending you draft SOPs please treat then with the same level of confidentiality that we approach all proposals and unpublished manuscripts with. Hopefully they will publish soon and we'd be able to share and reference the document then.
Thank you,
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From: McCann, Blake E
To: Griffin, Paul C
Subject: RE: January 31 is new deadline for BLM wild horse and burro research proposals
Date: Friday, January 14, 2022 1:39:37 PM

Thanks Paul.

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, January 14, 2022 1:38 PM
To: McCann, Blake E <blake_mccann@nps.gov>
Subject: Re: January 31 is new deadline for BLM wild horse and burro research proposals

Hi Blake,
No problem; I just wanted to be sure you were kept in the loop. I'm cautiously optimistic that we will get some really good, practical proposals. Awards won't be probably until May or June, I figure. We'll probably have a big press announcement when they are out.
Good luck out there --
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From: McCann, Blake E <blake_mccann@nps.gov>
Sent: Friday, January 14, 2022 1:33 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: RE: January 31 is new deadline for BLM wild horse and burro research proposals

Thanks Paul;

It does not appear that anyone in my radius will be submitting a proposal. I discussed with Kate Schoenecker and made some connections for her with a Tribe where she was considering a project, but I have not heard any further feedback. Not sure if that was intended for this call.

FYI

Blake

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Wednesday, January 12, 2022 1:24 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Cc: Glass, Patricia A <pglass@blm.gov>; Beckstead, Melanie J <mbeckstead@blm.gov>
Subject: January 31 is new deadline for BLM wild horse and burro research proposals

The BLM has extended the submission deadline for research proposals related to the BLM's Wild Horse and Burro Program. The new deadline is January 31, 2022.

The 'Notice of Funding Opportunity' for non-federal applicants is still available at grants.gov, as amended announcement L22AS00069. That NOFO is also attached here as a pdf file. Applicants with questions about required documents for grants.gov submission are encouraged to ask Patricia Glass: pglass@blm.gov

The 'Request for Proposals' for federal applicants has not changed, except that the new deadline for application submission is January 31. That RFP is also attached here as a word file.

Thank you for your interest in supporting the BLM's wild horse and burro program, through scientific research.

Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From:	McCann, Blake
To:	Appold, David W
Cc:	Melzo, Kevin A; Rittenhouse, Bruce H; Griffin, Paul C; Shepherd, Alan B; Reiland, Michael J
Subject:	Re: New IAA L20PG00022 Cover and Articles
Date:	Monday, February 3, 2020 2:25:20 PM
Attachments:	L20PG0022_COVER.pdf

Hello All;

Attached please find the document with my electronic signature in box 20. I discussed with Paul an issue with the Articles document: we wish to have Part A, No. 4 removed, as the publication of this work is not contingent upon the IAA, nor would NPS be able to agree to fulfilment of that objective, given the larger collaborative framework. It is really the field data collection (described under Part A, No. 5) that is key.

If BLM is agreeable to this minor change, then please accept the signed document to move this process forward. Otherwise, please advise regarding next steps to resolve.

Thank you.

Blake

On Fri, Jan 31, 2020 at 4:26 PM Appold, David W <dappold@blm.gov> wrote:

> Blake/Kevin,
>
> Please see the attached cover and articles for your review and signature.
>
> Thanks,.
>
> Dave
>
> David W. Appold
>
> Supervisory Procurement Analyst/Contracting Officer
>
> BLM Nevada State Office
>
> (O) 775-861-6417
>
> (F) 775-861-6634
>
> dappold@blm.gov

--
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From: Griffin, Paul C
To: McCann, Blake E; Appold, David W
Cc: Melzo, Kevin A; Rittenhouse, Bruce H; Shepherd, Alan B; Reiland, Michael J
Subject: Re: New IAA L20PG00022 Cover and Articles
Date: Monday, February 3, 2020 2:40:51 PM

Dave,
I discussed the minor change to the articles that Park Service has suggested, and I recommend that you please approve of that request. Removing item A.4. from the articles will still lead to BLM getting what is needed out of the interagency agreement.
BLM was not really looking for the Park Service to write a peer-reviewed paper based on the field data collection. BLM is just looking for Park Service to collect those data, and share them with their appropriate coauthors.
Please contact me if you have any questions, but if you are OK with the very-slightly revised articles, please move ahead with your final approval of the IAA.

Thank you very much,
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
2150 Centre Ave. Building C
Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: McCann, Blake <blake_mccann@nps.gov>
Sent: Monday, February 3, 2020 2:24 PM
To: Appold, David W <dappold@blm.gov>
Cc: Melzo, Kevin A <kevin_melzo@nps.gov>; Rittenhouse, Bruce H <brittenh@blm.gov>; Griffin, Paul C <pgriffin@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>; Reiland, Michael J <mreiland@blm.gov>
Subject: Re: New IAA L20PG00022 Cover and Articles

Hello All;

Attached please find the document with my electronic signature in box 20. I discussed with Paul an issue with the Articles document: we wish to have Part A, No. 4 removed, as the publication of this work is not contingent upon the IAA, nor would NPS be able to agree to fulfilment of that objective, given the larger collaborative framework. It is really the field data collection (described under Part A, No. 5) that is key.

If BLM is agreeable to this minor change, then please accept the signed document to move this process forward. Otherwise, please advise regarding next steps to resolve.

Thank you.

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315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From: Griffin, Paul C
To: McCann, Blake E
Subject: Re: Phone call re: BLM funds
Date: Thursday, April 23, 2020 12:21:31 PM

Hi Blake,

Thank you for your message. I'm supportive of the park using the funds. Of the options you suggested, the ones tha tmake the most sense to me, in light of the text of the IAA, are 1) continue to pay for field work beyond that date, and 3) task a GS-05 Bio-Tech (primary role weed control) with collateral duty horse research.
BLM obligated $19,000 toward this IAA.
However, I am not the BLM technical contact or the budget contact on the IAA. Even though modifications of the IAA need to be in writing, this doesn't strike me as a modification of the IAA, because if the Park uses the money in support of the horse research project, that is fundamentally in keeping with the purpose.
To be transparent, though, we should get the OK in writing from the designated budget contact. This isn't a major budget change, so I expect he (Michael Reiland) will be supportive. I'll cc you on an email to Michael later today.
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
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Fort Collins, CO 80526 USA
(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: McCann, Blake E <blake_mccann@nps.gov>
Sent: Thursday, April 23, 2020 8:33 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: Phone call re: BLM funds

Hey Paul;
With our pandemic response, we have had to scale back on seasonal staff, and this has affected how I have hired for horse research. Luckily, I have two staff that have worked on the project who are currently in ND. I am bringing both on as GS-06 Bio-Techs and feel that their salary fits the IAA description reasonably well. It looks, however, like we may have around $4000 remaining from the IAA by August 15. Options are to 1) continue to pay for field work beyond that date, 2) cover housing for CSU techs currently in the park, 3) task a GS-05 Bio-Tech (primary role weed control) with collateral duty horse research, 4) send the remainder back, or 5) some other solution that I am not envisioning here?
Regardless, the good news is that we will be able to staff field work for the research project this summer. I thought we could talk by phone to identify the most responsible method to manage funds. Are you available for a call?
Thank you.
Blake
Blake McCann, Ph.D.
Chief of Resource Management

Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: Griffin, Paul C
To: McCann, Blake E
Cc: Reiland, Michael J; Shepherd, Alan B; Rittenhouse, Bruce H
Subject: Re: Phone call re: BLM funds
Date: Friday, April 24, 2020 8:21:30 AM

Good morning Blake,
BLM is glad to hear that the research into GonaCon's effects is continuing this summer. Thank you for your request yesterday to spend what you projected to be a relative surplus of about $4,000, to be used on further costs in support of the research outlined in agreement L20PG00022.
The initial obligation from BLM for the agreement was $19,000. Let this email serve as a written approval for NPS to spend up to that full amount for related expenses such as those you suggested. Here, I am cc-ing the BLM budget contact on the agreement (Michael Reiland), who has let me know this approval can be given to NPS, and the technical contact on the agreement (Bruce Rittenhouse).
Of the costs that you suggested, BLM would be most supportive of items 1 or 3 (continuing to pay for more field work through the period of performance of the agreement, which extend through February 2022), or tasking a GS-05 Bio-Tech with collateral duty horse research.
Please call or write me any time if you have further questions.
Stay well,
Paul

Paul Griffin, Ph.D.
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Sent: Thursday, April 23, 2020 8:33 AM
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Regardless, the good news is that we will be able to staff field work for the research project this summer. I thought we could talk by phone to identify the most responsible method to manage funds. Are you available for a call?

Thank you.

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Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From:	McCann, Blake E
To:	Griffin, Paul C
Cc:	Reiland, Michael J; Shepherd, Alan B; Rittenhouse, Bruce H; Melzo, Kevin A; Klosterman, Megan E
Subject:	RE: Phone call re: BLM funds
Date:	Friday, April 24, 2020 8:45:05 AM

Thank you Paul;
We will expend funds as requested for extension of field season and/or collateral duty GS-05 staff time to complete research operations.
Blake

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, April 24, 2020 8:21 AM
To: McCann, Blake E <blake_mccann@nps.gov>
Cc: Reiland, Michael J <mreiland@blm.gov>; Shepherd, Alan B <ashepher@blm.gov>; Rittenhouse, Bruce H <brittenh@blm.gov>
Subject: Re: Phone call re: BLM funds

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Please call or write me any time if you have further questions.

Stay well,
Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
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Fort Collins, CO 80526 USA
(970) 226-9358 (office)
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Sent: Thursday, April 23, 2020 8:33 AM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: Phone call re: BLM funds

Hey Paul;
With our pandemic response, we have had to scale back on seasonal staff, and this has affected how I have hired for horse research. Luckily, I have two staff that have worked on the project who are currently in ND. I am bringing both on as GS-06 Bio-Techs and feel that their salary fits the IAA description reasonably well. It looks, however, like we may have around $4000 remaining from the IAA by August 15. Options are to 1) continue to pay for field work beyond that date, 2) cover housing for CSU techs currently in the park, 3) task a GS-05 Bio-Tech (primary role weed control) with collateral duty horse research, 4) send the remainder back, or 5) some other solution that I am not envisioning here?
Regardless, the good news is that we will be able to staff field work for the research project this summer. I thought we could talk by phone to identify the most responsible method to manage funds. Are you available for a call?
Thank you.
Blake

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: McCann, Blake
To: Melzo, Kevin A
Cc: Griffin, Paul C
Subject: Re: Questions from my CO
Date: Monday, February 3, 2020 8:32:47 AM

Thanks Kevin;

Paul, let me know if you need anything else.

Blake

On Fri, Jan 31, 2020 at 2:56 PM Melzo, Kevin <kevin_melzo@nps.gov> wrote:

> Hello Paul,
>
> (b) (4) [redacted]
>
> Let's go with monthly.
>
> Let me know if you need anything else.
>
> Kevin A. Melzo
> Administrative Officer
> Theodore Roosevelt National Park
>
> Phone (701) 623-4730 ext 1403
> Fax (701) 623-4840
>
> On Fri, Jan 31, 2020 at 10:15 AM McCann, Blake <blake_mccann@nps.gov> wrote:
>
> > Can you reply to Paul with this information?
> >
> > ---------- Forwarded message ---------
> > From: **Griffin, Paul C** <pgriffin@blm.gov>
> > Date: Fri, Jan 31, 2020 at 10:04 AM
> > Subject: Questions from my CO
> > To: McCann, Blake E <blake_mccann@nps.gov>
> >
> > Hi Blake,
> > My CO is asking:
> > - What's the Theodore Roosevelt Nat'l Park Service
> >   1. Agency Location code (ALC)
> >   2. Duns #
> >   3. Treasury Account Symbol (TAS)_
> > - Allowing monthly or quarterly billing?

Paul Griffin, Ph.D.
Research Coordinator
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(970) 226-9358 (office)
(970) 631-4808 (mobile)

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Theodore Roosevelt National Park
315 Second Avenue; P.O. Box 7
Medora, ND 58645
701-623-4730 ext. 1433

From: Griffin, Paul C
To: McCann, Blake E
Subject: Re: Thoughts on IAA
Date: Wednesday, January 15, 2020 2:33:57 PM

Thanks Blake. See you Thursday at noon, out front. My work cell phone # is 970-631-4808.
Paul

On Wed, Jan 15, 2020 at 2:29 PM McCann, Blake <blake_mccann@nps.gov> wrote:

> OK, sounds good. My lunch break will be from 12:00 - 1:00, and I am at 1201 Oakview. I can meet you out front shortly after noon and we can just walk across the street for lunch, if you like.
>
> My cell is 701-430-9281
>
> Blake
>
> On Wed, Jan 15, 2020 at 12:28 PM Griffin, Paul <pgriffin@blm.gov> wrote:
>
> > Hi Blake,
> > Thanks for asking. We can talk more in person tomorrow around lunch if the timing still work for you. Reminder to let me know where & when to come down and meet. Or, I can come down during a coffee break if you'll be tied up during lunch.
> > But no, there would be no issues at all from BLM's perspective for THRO to handle getting the field work done however you see fit. BLM would not want to put any limitations on NPS in doing the field work however you feel is appropriate. BLM has no explicit or implicit requirement that NPS to enter into a 'subcontracting' type agreement. Whatever way you see best to get it done works for us.
> > Paul
> >
> > On Wed, Jan 15, 2020 at 12:12 PM McCann, Blake <blake_mccann@nps.gov> wrote:
> >
> > > Hey Paul;
> > >
> > > It sounds like our FA folks think we may have issues going forward with approvals for CESU (we asked this question months ago, and they saw no problem - must have been recently advised). Is there a limitation on how the funds are used from your standpoint? For instance, could I simply hire a Bio-tech to complete the work? I have a unique opportunity where the current field tech for CSU has rehire abilities for a GS-06 for the park, and I could also likely contribute other staff time to the research, if I had some level of funding support. Another advantage is that the funds would go directly to personnel services (field work) rather than overhead with a University and/or PI salary line items (if included).
> > >
> > > Your thoughts?
> > >
> > > FYI, I am still working on the agreements option - just looking at alternatives as a backup.
> > >
> > > Blake
> > >
> > > --
> > > Blake McCann, Ph.D.
> > > Chief of Resource Management
> > > Theodore Roosevelt National Park
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> > > Medora, ND 58645
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pgriffin@blm.gov

From: McCann, Blake E
To: Griffin, Paul C
Subject: RE: THRO horses in the news
Date: Wednesday, March 23, 2022 2:22:25 PM

Thanks Paul.

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Wednesday, March 23, 2022 2:01 PM
To: McCann, Blake E <blake_mccann@nps.gov>
Subject: THRO horses in the news

Hi Blake
I saw that THRO is in the news:
https://www.inforum.com/news/north-dakota/plans-for-wild-horse-herd-at-theodore-roosevelt-national-park-range-from-no-change-to-no-horses
I hope your planning process goes well -- good luck!

Paul

Paul Griffin, Ph.D.
Research Coordinator
BLM Wild Horse and Burro Program
https://www.blm.gov/programs/wild-horse-and-burro/herd-management/science-and-research
(970) 631-4808 (mobile / pandemic)
(he / him)

From: Griffin, Paul C
To: McCann, Blake E
Subject: Re: What does 2mL of GonaCon weigh?
Date: Friday, July 24, 2020 4:02:10 PM

Thanks, Blake. I'll check with Dan. Have a great weekend.

Paul Griffin, Ph.D.
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(970) 631-4808 (mobile)

From: McCann, Blake E <blake_mccann@nps.gov>
Sent: Friday, July 24, 2020 3:59 PM
To: Griffin, Paul C <pgriffin@blm.gov>
Subject: RE: What does 2mL of GonaCon weigh?

I just got off the phone with him, so he should be available.

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, July 24, 2020 3:56 PM
To: McCann, Blake E <blake_mccann@nps.gov>
Subject: What does 2mL of GonaCon weigh?

Hi Blake,
I hope this email finds you dong all right. Quick question, to follow up on your helpful GonaCon SOPs. How much does GonaCon weigh, in grams per mL? I ask because you say folks should weigh before and after, to be sure about how amny mL get delivered via dart.
Thank you,
Paul

Paul Griffin, Ph.D.
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Fort Collins, CO 80526 USA

(970) 226-9358 (office)
(970) 631-4808 (mobile)

From: McCann, Blake E
To: Griffin, Paul C
Subject: RE: What does 2mL of GonaCon weigh?
Date: Friday, July 24, 2020 3:59:34 PM

I just got off the phone with him, so he should be available.
Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

From: Griffin, Paul C <pgriffin@blm.gov>
Sent: Friday, July 24, 2020 3:56 PM
To: McCann, Blake E <blake_mccann@nps.gov>
Subject: What does 2mL of GonaCon weigh?

Hi Blake,

I hope this email finds you dong all right. Quick question, to follow up on your helpful GonaCon SOPs. How much does GonaCon weigh, in grams per mL? I ask because you say folks should weigh before and after, to be sure about how amny mL get delivered via dart.

Thank you,

Paul

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PLOS ONE

RESEARCH ARTICLE

Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine (GonaCon-Equine) in free-ranging horses (*Equus caballus*): Limitations and side effects

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* danbaker@colostate.edu

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Abstract

Wildlife and humans are increasingly competing for resources worldwide, and a diverse, innovative, and effective set of management tools is needed. Controlling abundance of wildlife species that are simultaneously protected, abundant, competitive for resources, and in conflict with some stakeholders but beloved by others, is a daunting challenge. Free-ranging horses (*Equus caballus*) present such a conundrum and managers struggle for effective tools for regulating their abundance. Controlling reproduction of female horses presents a potential alternative. During 2009–2017, we determined the long-term effectiveness of GnRH vaccine (GonaCon-Equine) both as a single immunization and subsequent reimmunization on reproduction and side effects in free-ranging horses. At a scheduled management roundup in 2009, we randomly assigned 57 adult mares to either a GonaCon-Equine treatment group ($n = 29$) or a saline control group ($n = 28$). In a second roundup in 2013, we administered a booster vaccination to these same mares. We used annual ground observations to estimate foaling proportions, social behaviors, body condition, and injection site reactions. We found this vaccine to be safe for pregnant females and neonates, with no overt deleterious behavioral side effects during the breeding season. The proportion of treated mares that foaled following a single vaccination was lower than that for control mares for the second ($P = 0.03$) and third ($P = 0.08$) post-treatment foaling seasons but was similar ($P = 0.67$) to untreated mares for the fourth season, demonstrating reversibility of the primary vaccine treatment. After two vaccinations, however, the proportion of females giving birth was lower ($P < 0.001$) than that for control mares for three consecutive years and ranged from 0.0–0.16. The only detectable adverse side effect of vaccination was intramuscular

L14AS00048) to DLB. Additionally, NPS provided funding (2008–2010), as well as significant in-kind support each year of the investigation.

Competing interests: The authors have declared that no competing interests exist.

swelling at the vaccination site. Regardless of vaccine treatment (primary/secondary), approximately 62% (34/55) of immunized mares revealed a visible reaction at the vaccine injection site. However, none of these mares displayed any evidence of lameness, altered gait or abnormal range of movement throughout the 8 years they were observed in this study. Our research suggests that practical application of this vaccine in feral horses will require an initial inoculation that may provide only modest suppression of fertility followed by reimmunization that together could result in greater reduction in population growth rates over time.

Introduction

Anthropogenic disturbance of landscapes and natural resources is pervasive across much of the earth, resulting in increased conflict between humans and wildlife and a need for effective resource management [1]. Humans indeed have tried to control animal abundance in some capacity for over 13,000 years [2]. Regulating abundance of wild animals using fertility control or contraception is a relatively new development, emerging only 50 years ago [3]. Such tools are appealing to wildlife managers and stakeholders because they present a non-lethal solution for regulating abundance when species pose a risk to human interests and safety, and when wildlife densities are high enough to disrupt ecosystem function [4,5].

Feral horses (*Equus caballus*) present perhaps one of the most unique wildlife management problems worldwide. Humans have spent centuries propagating and dispersing domestic horses to every continent except Antarctica over the last several centuries, only to have inadvertently created expansive feral populations that now compete with humans, wildlife, and domestic animals for resources [6]. The unique relationship between humans and horses has resulted in a precarious dichotomy, with the struggle for relief from conflict and resource competition challenged by a mutualistic societal view where feral horses are perceived as part of our social environment. This struggle is elevated in the United States, where federal law (*P*. L. 92–195, as amended) provides protection for feral horses and burros (*Equus asinus*) on large expanses of public land, and establishes guidance for their management as a wildland species [7].

Current methods of population control for free-ranging horses in the U.S. involve periodic removals and adoption or sale of surplus animals, or maintaining excess animals in long-term holding facilities which are expensive, resource intensive, and unsustainable [8]. Clearly, more efficient, cost effective, and humane approaches to reducing feral horse densities on public lands are needed. Controlling the fertility of female horses offers a potential complementary or alternative strategy for limiting the growth of some populations [9].

A promising immunological approach to contraception in feral horses and other wild ungulate species involves immunization against gonadotropin- releasing hormone (GnRH), a small neuropeptide that performs an obligatory role in mammalian reproduction [10]. When conjugated to a highly immunogenic carrier protein and combined with a potent adjuvant, GnRH vaccination actively stimulates a persistent immune response resulting in prolonged antibody production against endogenous GnRH. These antibodies induce transient infertility by binding to GnRH, thus preventing attachment to receptors on pituitary gonadotropes, suppression of gonadotropin release, and ultimately ovulation in females [11, 12]. As anti-GnRH antibodies decline over time, the availability of endogenous GnRH increases and treated animals generally regain normal fertility [13–17].

The GnRH-based contraceptive agent known as GonaCon-Equine (National Wildlife Research Center, Fort Collins, CO, USA; [18] is registered by the United States Environmental Protection Agency as a restricted-use pesticide for contraception of adult female feral horses and burros. A single immunization with this or earlier versions of this vaccine (more generally referred to as GonaCon) have been shown to induce extended infertility ($\geq$ 2 yr) in numerous wild ungulate species including captive and free-ranging elk (*Cervus elaphus*) [15–17] white-tailed deer (*Odocoileus virginianus*) [18–20], bison (*Bison bison* [21]), and feral horses [22– 24]. However, multiple years of infertility are only experienced in a fraction of vaccinated animals. In free-ranging elk, for example, there was approximately a 90% treatment effect the first year after vaccination but this declined to 50% by the second year; with no measurable effect by year three [16]. Similar declines in effectiveness have been reported for captive feral horses treated with the same vaccine [22].

Booster vaccinations generally result in a more profound and longer-lasting antibody production due to the anamnestic (cell memory) response [25]. Traditional veterinary vaccinology suggests that non-replicating vaccines most often require two initial doses 2–6 weeks apart followed by booster vaccinations every 1–3 years [26]. Repeat immunizations using a variety of GnRH vaccines in domestic horses improves contraceptive efficacy and suppress behavioral and physiological estrus [27–29]) However, these GnRH vaccines differ from GonaCon-Equine in that they incorporate different protein carrier molecules and adjuvants, and are formulated for short duration ($<$ 1 yr.) effectiveness. They are also administered on a more traditional vaccination schedule with a primary set of immunizations followed by periodic boosters.

Other forms of wildlife fertility control vaccines have adopted comparable initial and booster recommendations [30–32]. However, this intensive vaccination schedule places significant logistical barriers on application in free-ranging animals. GonaCon vaccine is formulated with highly immunostimulating mycobacteria as a component of the adjuvant. This may prolong the initial and subsequent booster vaccination windows for optimum efficacy as initial antibody concentrations are maximal 2–12 months post-primary vaccination [15]. GonaCon vaccine is one of the rare exceptions among animal vaccines in that the formulation initiates high antibody titers that remain elevated in some individuals after a single-injection ; however, little research has been conducted to evaluate booster doses of this vaccine in any free-ranging wild ungulate [17, 24] or domestic species [33]. While a single immunization against GnRH may be preferred from a practical perspective, there may be a more optimal vaccination schedule that balances the need for minimizing animal handling or contact while maximizing vaccine effectiveness. Thus, it's imperative to investigate the safety and long-term effectiveness of repeat vaccination and to evaluate its potential to limit fertility in this long-lived and perennially pregnant species.

In female wild ungulates, adverse side effects following a single immunization against GnRH appear to be minimal. Evaluation of biological side effects has been reported for numerous wild ungulate species including white-tailed deer [13, 34], elk [15, 16, 35], feral pigs [36], bison [21], and free-ranging horses [17, 24]. A summary of results from these investigations indicate that GonaCon is reversible, safe for use in pregnant females, does not significantly change social behaviors [37] or negatively affect neonatal development, survival, or maturation [15, 35]. No adverse effects of vaccination have been shown to be related to general health, body condition, blood chemistry parameters, or hematology of treated animals. The most apparent pathological side effect has been the development and persistence of non-debilitating granulomatous and often purulent inflammation at the site of injection. In all studies, where post-mortem examinations have been conducted, injection-site lesions were pervasive, but in some species, such as white-tailed deer and elk, they were not apparent antemortem. Likewise,

in cases where injection-site reactions have been documented, no clinical evidence of lameness, impaired mobility, or depression, have been reported [13, 15–17, 21, 24, 34, 35].

While documentation of contraceptive efficacy and side effects of GonaCon have been described for a variety of wild ungulates, similar evidence for feral horses is limited. To our knowledge, only two long-term ($\geq$ 3 years) empirical investigations have been conducted using GonaCon-Equine. These include a clinical trial with captive feral mares [22] and the other with free-ranging mares in a natural environment [23]. In the study with free-ranging horses, vaccination significantly reduced foaling rates of treated females, however, effectiveness was inconsistent over time and was substantially lower than that reported for captive feral mares treated with the same vaccine [22]. Furthermore, neither of these studies integrated revaccination as a strategy to increase vaccine efficacy. Lastly, these inquiries provide little quantitative evidence of the reversibility of the effects of this vaccine, the presence or absence of adverse side effects related to inoculation of pregnant mares, and neither examined the potential for increased side effects with reimmunization.

Knowledge of the effects of GonaCon-Equine on equid fetal health, neonatal survival, and body condition is largely anecdotal, whereas injections site reactions to booster immunization and the efficacy of revaccination are limited to two investigations [24, 33]. Clearly, additional research is needed to further define the long-term therapeutic effectiveness and contraindications of this potential technology before resource managers can make informed decisions regarding its practical application for stabilizing the growth rate of free-ranging feral horse populations.

Consequently, the fundamental objectives of this investigation were: 1) to determine the duration, effectiveness, and reversibility of both a single immunization and subsequent reimmunization against GnRH in suppressing reproductive rates of free-ranging mares in a natural environment, 2) to determine the safety and adverse side effects (if any) in free-ranging mares including assessment of general health, body condition, effects on current pregnancy, injection site reactions, and neonatal health and survival and, 3) to compare the effects of a single vaccination against GnRH on time budgets and social behaviors [37] to similar behaviors following reimmunization. Based on evidence from prior studies with feral horses and other wildlife species, we predicted ($H_{1:}$) that a single vaccination against GnRH would suppress fertility for multiple years with decreasing effectiveness over time but would not result in permanent infertility. Furthermore, we surmised ($H_{2:}$) that the anamnestic immune response to revaccination would be more effective and longer lasting in suppressing fertility than the initial immunization alone. Moreover, we reasoned ($H_{3:}$) that except for localized inflammatory reactions at the injection site, we would not observe other adverse side effects (i.e. lameness, detrimental effects on existing pregnancy, neonatal health and survival, body condition, behavioral changes). Apart from determination of return to normal fertility of treated mares, these objectives and hypotheses were addressed and accomplished in this investigation.

Materials and methods

Study area

We conducted this research in the South Unit of Theodore Roosevelt National Park (THRO), USA) (45˚ 55'N/103˚ 31'W). This unit is located near the town of Medora in southwestern North Dakota and encompasses approximately 19,000 ha of native vegetation. The landscape is topographically diverse and consists of eroded badlands with gullies and ravines separated by relatively large upland plateaus and small erosion-resistant buttes capped by scoria. Elevation ranges from 683 m to 870 m. Its continental climate is characterized by short, arid summers (mean temperature 21^0 C) and long, cold winters (mean temperature -12^0 C) [38].

Precipitation is irregular in amount and distribution with a long-term annual mean of 38 cm with most of this falling as rain showers from April to June [39].

Vegetation is primarily mixed-grass prairie dominated by needle-and -thread grass (*Hesperostipa comata)*, western wheatgrass (*Pascopyrum smithii)*, threadleaf sedge (*Carex filifolia)*, blue gramma (*Boutelous gracilis)*, and little blue-stem (*Schizachyrium scoparium)*. Cottonwood (*Populus deltoides)* gallery forests occur along perennial water courses while hardwood stands of green ash (*Fraxinus pennsylvanica)* and chokecherry (*Prunus virginiana)* dominate the upland drainages. Dense stands of Rocky Mountain juniper (*Juniper scopulorum*) are common on steep north-facing slopes [40].

Besides feral horses, sympatric wild ungulate species include bison, elk, mule deer (*Odocoileus hemionus)*, white-tailed deer, and pronghorn (*Antilocapra americana)*. Horses and bison are confined to the South Unit of the Park by a 1.8–2.4 m woven-wire boundary fence. Currently, horse numbers are controlled through periodic live capture and removal of select individuals. Free-ranging horses at THRO are classified by the National Park Service (NPS) as "feral livestock" and managed as a "historical demonstration herd". The most recent estimate of population size (2017) is 150–175 horses and the Park has set a management goal for this herd at approximately 50–90 animals.

The social structure of this population consists of 14–16 social groups (bands) that include a single dominant stallion, subdominant stallions, and 1–5 adult mares, yearlings, and foals of both sexes. Males greater than 1 year of age that have not acquired a band are usually found in ephemeral bachelor groups of 3–6 individuals. These bands are non-territorial and are spatially distributed across the South Unit primarily east of the Little Missouri River. All horses are known by unique coloration and markings and have been previously identified and assigned individual identifiers by managers. Photographs of each animal from birth to adulthood assist in the identification of individuals. Age, reproductive history, and genealogy data for each animal has been maintained since 1993.

In spring/summer 2009, we collected pre-treatment data on all mares and bands within THRO. The purpose of this effort was: 1) to determine the sample size and sampling intensity required to achieve acceptable statistical power ($\geq$ 80%) to detect fixed differences ($\geq$ 50%) in foaling proportions of experimental groups, 2) to assess unknown logistical limitations of locating and identifying specific study mares within bands of horses, and 3) to train field technicians to observation protocols, and collect pre-treatment time budget and social behavioral data.

Experimental animals and treatments

Primary vaccination (2009–2013). During a scheduled management roundup at THRO (18–23 October 2009), 160 horses were guided by helicopter into permanent corrals and handling facilities. An attempt was made to capture the entire population to maximize sample sizes for this research project and to remove excess horses to meet desired herd management objectives. A total of 57 adult mares (2–17 years of age) and associated foals, and band stallions, were captured, identified, treated, and retained in the Park for this experiment. Using a randomized complete block design, we established two experimental groups consisting of a GonaCon-Equine treatment group (n = 29) and a saline control group (n = 28). Mares were paired (blocked) based on age and pregnancy status such that animals within a block were as similar as possible. Within each block, individual mares were then randomly assigned to either a control or treatment group.

Equine veterinarians and a reproductive specialist, blinded to treatment status, assessed the general health, body condition, pregnancy status, and approximate gestational stage of each

mare. We determined pregnancy status and gestational age by transrectal palpation and ultrasonography of the reproductive tract [41]. We collected whole blood (up to 50 mL) via jugular venipuncture (BD Vacutainer SST; Becton Dickinson and Co., Franklin Lakes, NJ) then centrifuged these samples at the capture site, and temporarily stored serum in cryovials at -20^0 C. We later transferred frozen serum on dry ice to Fort Collins, Colorado, where it was stored at -80^0 C. We also assessed serum for exposure to common pathogens known to cause abortions in horses (e.g. equine herpesvirus-1, equine infectious anemia, equine viral arteritis and contagious equine metritis) that could confound the interpretation of treatment-induced infertility [42].

We applied treatments while mares were restrained in a squeeze chute. Females in the treatment group received an intramuscular injection in the lower left gluteal musculature, by hand-held syringe (18-gauge, 3.8 cm needle) containing GonaCon-Equine (2.0 mg GnRH conjugate + adjuvant; 2.0 mL). The vaccine contained multiple synthetic copies of GnRH coupled to a large immunogenic carrier protein (Blue Carrier; Biosonda, Santiago, Chile) that was combined with a water-in-oil adjuvant containing killed *Mycobacterium avium* ssp. *avium* (Adju-Vac, National Wildlife Research Center) [18]. Mares in the control group were injected in a similar manner, with an equal volume of physiologic saline solution (0.9% NaCl; 2.0 mL). We chose to inject the vaccine into the gluteus muscle (~ 15 cm distal to the point of the hip) rather than the neck because of greater safety for hand-injection, enhanced detection of potential injection site reactions under field conditions, and the preferred location for potential remote dart delivery of the vaccine.

Secondary vaccination (2013–2017). Four years later, during 23–25 September 2013, we similarly rounded up the entire THRO horse population and moved and handled them through existing corrals and chute systems to remove excess animals from the Park. Given this unique opportunity and endorsement from the Park, we retained all available mares previously immunized and control mares, retreated them, assessed pregnancy status, and determined body condition using techniques identical to those applied at the 2009 roundup. Two mares in the control group and 4 mares in the treatment group died between 2009–2013 and therefore, were not available for this experiment. We attributed these mortalities to malnutrition, dystocia, broken appendage, and unknown causes not related to treatments. The one exception in our 2013 protocol was that we injected the booster vaccination into the opposite (right) hip from where the primary (left hip) vaccination was previously administered. This provided the opportunity to simultaneously evaluate injection site reactions related to both immunizations. Treatment mares again received 2.0 mL GonaCon-Equine and control mares 2.0 mL saline.

Field measurements

Using 2–3 trained technicians and occasional equally trained volunteers, we conducted field measurements and observations consistently from year to year. Prior to field observations, technicians were provided with photographic images of individual horses and required to recognize them by band association, natural markings, and pelage coloration. They were also trained or had previous experience in identifying prepartum characteristics of pregnancy (e.g., enlarged abdomen, mammary gland development, waxing teats, behavior, etc.), as well as, body condition scoring, and the appearance and classification of injection site reactions to the vaccine. We collected all data from ground surveys (foot, vehicle, horseback) using binoculars and spotting scopes. Although technicians were unaware of treatment assignments of individual mares, the presence of injection site reactions in several GonaCon-treated mares could have revealed their treatment designation.

Reproduction. We predicted that pregnant females inoculated with GnRH at the fall gathers of 2009 and 2013 would give birth to a healthy foal the following spring (2010 or 2014) and presumably be infertile during subsequent breeding seasons. Thus, the effects of the primary or booster vaccinations on reproduction (foaling proportions) would not be observed until the 2011 and 2015 foaling seasons, respectively. These are the first breeding seasons that a treatment or retreatment effect on mare fertility could be detected when using foaling observations to assess successful contraception by the vaccine.

We determined the effectiveness, duration of effects, and reversibility of the primary and booster vaccinations on reproduction by comparing foaling proportions of treated and control mares during 1 March to 31 December 2009–2017. We chose to use the term vaccine "effectiveness" rather than "efficacy" because it more realistically represents how GonaCon-Equine affects fertility under more natural field conditions compared to a controlled clinical trial [43, 44]. We defined vaccine effectiveness (VE) as the proportional reduction in annual foaling (F = number of mares with a foal/ total number of mares in a treatment group) between control and treated mares. Vaccine effectiveness is equivalent to relative risk reduction (RRR) in medical statistics and was calculated from the risk ratio $\left(\mathrm{RR} = \frac{\mathrm{F}_{Trt}}{\mathrm{F}_{Con}}\right)$ where F_{Con} = foaling proportion of the control mares, and F_{Trt} = the foaling proportion of the treated mares. Risk ratio was calculated using the *fmsb* package in program R [45–47] and we then solved for VE as follows:

$$\mathrm{VE} = \frac{\mathrm{F}_{Con} - \mathrm{F}_{Trt}}{\mathrm{F}_{Con}} = 1 - \frac{\mathrm{F}_{Trt}}{\mathrm{F}_{Con}} = 1 - \mathrm{RR}$$

Each year of the study, we estimated annual foaling proportions by locating all bands to identify individual mares and determine the presence or absence of foals. During the intensive sampling period (1 March–1 August), we attempted to observe 95% or greater of all experimental mares and foals (when present) at least weekly and 100% of them every two weeks, then opportunistically until 31 December. We did not attempt to assess contraceptive effect based on visual characteristics of pregnancy but did use these criteria to prioritize weekly observations of individual mares. Instead, we defined foaling as a parturition event or neonatal foal by side, as detected by direct observation. We matched foals with dams through observations of nursing and repeated close association during feeding, bedding, and traveling [48, 49]. We collected neonatal data at first sighting of a foal and estimated date of birth by observing the foal's level of activity, presence of an umbilicus, and elapsed time since the dam was last observed pregnant [50]. We photographed and estimated the age of each new foal when first observed, recorded its sex, general health (vigorous, average, poor), markings, and band association, and gave it a unique identifier; then entered these observations into a herd database. Finally, we assessed the utility of using foaling proportions as a proxy for pregnancy proportions by comparing pregnancy proportions determined at the time of each gather in 2009 and 2013 to foaling proportions observed in 2010 and 2014.

Side effects

Behavioral. We repeated thee behavioral measurements with the same treatment groups of mares that were previously conducted during an earlier phase of this project [37]. We proposed that, if greater contraceptive effectiveness after reimmunization against GnRH was achieved, it would potentially provide a larger and more statistically powerful sample size of contracepted animals in which to detect behavioral changes related to this vaccine (if they occurred).

We completed an intensive behavioral study during an earlier phase of this larger GnRH study during 2009–2010 [37]. To make these analyses directly comparable to that prior study, we followed the same behavioral sampling design as that described previously [50]. Briefly, this included blocking observations into three daylight time periods (08:00–12:00 h, 12:01–16:00 h, and 16:01–20:00 h) with observations conducted during the primary breeding season, 1 March– 1 August 2014. Each observation session included collection of a 20-min instantaneous scan sample of time budgets at 1 min intervals for each adult band member (≥1 year old), and all-occurrence data collection for social interactions [37].

Primary behavior categories included feeding, resting, locomotion, maintenance, and social behaviors [51]. Social behavior data included herding, reproduction, agonism, harem-tending, and harem-social behavior, and were collected at all occurrences throughout the observation sessions. Harem-social behavior was not collected through all-occurrence sampling in our previous study; however, it was collected during the scan sample in the previous study and was worthy of further consideration here. We defined this category as interactions between two individuals that did not meet the definition of the other all-occurrence behaviors (e.g. allogrooming and non-reproductive olfactory investigation).

We observed all horses from the nearest distance that did not elicit attention to the presence of the observer, typically 50–200 m. All observations were conducted using a 15–45 × 600 mm spotting scope or 10 × 42 mm binoculars when the distance between horses and observers was too far to allow unassisted detailed observation. We observed each band of horses weekly or bi-weekly in conjunction with other field assessments.

Physiological. Concurrently with foaling and behavior observations, we evaluated and compared potential adverse side-effects of treatment on injection-site reactions, body condition, success of existing pregnancy, and neonatal survival in treated and control mares. We made assessments of these potential side-effects monthly during the primary foaling season and opportunistically for the remainder of the year. We observed each mare for the presence or absence of visible lesions, swellings, or discharge at the injection site. In addition, we documented evidence of lameness (e.g. limping, gait alteration, reluctance to stand or bear weight on a limb), as well as behavioral depression, muscle tremors, or other systemic reactions that could be related to the vaccine treatment. We classified injection-site reactions according to the following criteria: 1) **abscess**–an open sore usually with fluid drainage or discharge, 2) **swelling**–a raised area of tissue of variable size and shape with no visible fluid drainage, 3) **lameness**–any abnormal range of movement or stiffness in the leg where the vaccine injection was delivered, 4) **none**–no observable reaction [52]. These categories were not mutually exclusive with respect to a single observation and both sides of the animal were observed, when possible. For these observations, we approached as near as possible to individual horses (≤ 50 m) and assessed and photographed each injection-site reaction for later evaluation. At the same time, we visually evaluated body condition of each mare and scored condition as previously described [53]. We evaluated the success of the existing pregnancy by comparing foaling proportions between treated and control groups in 2010 and 2014. We measured neonatal survival as the proportion of foals surviving to 14 days of age and post-natal survival to 200 days.

Statistical analysis

Reproduction. Yearly foaling data are reported as the proportion of mares observed with a foal in each group. We used asymptotic approximation to the binomial distribution to compute 95% confidence intervals for these proportions using package *binom* in program R [45, 47]. We used a risk ratio analysis ($\alpha = 0.05$) to compare all observed annual proportions between treatment groups. We used the same method to evaluate the success of the existing

pregnancy between groups during the first foaling season post-vaccination (2010 and 2014). All comparisons between treatment groups were made within a single year and without multiple testing corrections.

Behavior. We used the same statistical approach for the analyses in 2014 as that used in 2010 [37]. We modeled the frequency of each behavior using mixed-effects linear regression, where individual female identity and sampling time (time of day) were included as random effects on the intercept term of each model. This accounted for variation that may have been present among individuals who were sampled repeatedly, though not always equally over time, and for temporal variation in behavior when samples were not equally collected across all times of the day. Time budget behaviors sampled at 1-min intervals were aggregated into proportion of time spent per behavior to calculate an independent measure of behavior per observation session. We used the *lme4* package of R version 3.1.2 (The R Foundation for Statistical Computing 2014) and SYSTAT 12.02.00 (SYSTAT Software, Inc. 2007) to calculate descriptive statistics and obtain mixed-effects model estimates using restricted maximum likelihood [54]. Separate models were fitted for each time budget behavior with the fixed effects of treatment group (treated or control), foal presence (dependent foal $<$ 1 year of age present with the female, or no foal present with the female), female age, and band size. In the previous study, we considered band fidelity (number of times a female moved bands within a year), but data were too homogeneous to consider that factor in 2014: only 8 horses moved bands at all (4 treated/4 saline) and five of those moved collectively to a different stallion.

Physiological. We used descriptive statistics (arithmetic means with ± 95% CI) to compare, occurrence of lesions at the injection site and 1-tailed Fisher's exact test ($\alpha = 0.05$, 1df) to compare foal survival proportions of treated females to that of controls. We used normal binomial distributions to compute confidence limits for the differences between proportions using Jeffrey's interval for small sample sizes [55]. Effects on body condition scores were examined using generalized linear models in the *lmer* package in program R [56]. We employed random effects for year and individuals and then compared this nested model to full models which added the effect of either treatment or foaling using an ANOVA.

This research was approved by the Institutional Animal Care and Use Committees of the National Park Service (NPS) (Permit Numbers: MWR THRO Baker Horse 2013.A3, MWR THRO Baker Horse 2015.A3) and Colorado State University (IACUC Protocol No. 17-7651A). This study was conducted in accordance with good laboratory practices (GLP) and oversight from United States Department of Agriculture/National Wildlife Research Center (No.QA1647). All data collections were conducted after obtaining a scientific collection permit issued by Theodore Roosevelt National Park (THRO-2010-SCI-0010). All work, other than animal handling and vaccination at the two feral horse roundups, was observational. Every effort was made to prevent and minimize disruption of natural band dynamics and individual horse behavior and well-being during handling and treatment application.

Results

The statistical process used to select experimental mares for this investigation resulted in two treatment groups that were relatively homogeneous in age, body condition, body mass, and pregnancy status [S1 Table]. Results of pregnancy assessment indicated that most mares were pregnant at the 2009 (0.86 (49/57), 95% CI = 0.74–0.93) and 2013 (0.90 (46/51), 95% CI = 0.79–0.96) roundups, thus providing sufficient opportunity to evaluate and compare the safety and potential side effects of vaccine treatment on pregnancy and neonatal survival.

Transrectal ultrasonography revealed that the fetuses of most pregnant females were approximately 120+ days old at the roundup and that most had descended over the pelvic rim

preventing a more accurate assessment of gestational age at treatment application [57]. To provide a more precise estimate, we used an estimated gestation period for horses of 342 days [58] and the approximate foaling date (± 5 days) of each mare in 2010 and 2014 and then back-calculated to the date of treatment application at the 2009 (18–23 October) and 2013 (23–25 September) roundups. Using these calculations, we estimated mean gestational age at vaccine inoculation in 2009 to be 162 days (95% CI = 150–175) for treated mares and 154 days (95% CI = 138–170 days) for control mares. For 2013, we used the same calculation and projected that, on average, females were reimmunized against GnRH at approximately 129 days (95% CI = 105–151 days) of gestation and saline-treated control mares at 132 days (95% CI = 119–144 days).

Following the 2009 and 2013 roundups and release, experimental mares distributed themselves among 16–19 individual bands. At least one treated or control mare was present in all bands during 2010–2017. Likewise, the composition of adult mares in each band, as well as the band stallions, remained relatively stable during this period. By the end of the 2017 foaling season, 14% (4/29) of treated mares and 11% (3/28) of control mares had died of various causes (e.g., malnutrition, broken appendage, dystocia, unknown causes). Except for these mares and one vaccinated mare that was not re-captured at the 2013 gather, all others were observed for foaling and other field measurements for all eight years of this investigation.

We met our sampling objective by observing more than 95% of all mares weekly (and sometimes more often) from 1 March to 1 August each year of the study. It is possible that some foals were born and died without being detected but given the intensity of the sampling observations, we feel that this was highly unlikely. Observations during the remainder of the year and following winter were less intense and more opportunistic depending upon available personnel, weather, and road conditions. During this time, mortality of foals was more likely to have gone undetected.

Vaccine effectiveness

Primary vaccination (2009–2013). Mean foaling proportions of treated (0.62 (18/29) 95% CI = 0.44–0.79) and control (0.68 (19/28) 95% CI = 0.50–0.85) mares during the 2009 pre-treatment foaling season were not different ($P = 0.65$) indicating that prior to contraception, treatment groups exhibited equal fertility [S1 Table]. Further evidence was provided by individual mares at the 2009 gather and primary vaccine inoculation. The proportion of treated (0.86 (25/29), 95% CI = 0.71–0.95 and control (0.85 (24/28), 95% CI = 0.70–0.95) mares determined to be pregnant, via transrectal ultrasonography, were not different ($P = 0.63$) [S1 Table, Fig 1]. This provided an opportunity to compare the effects of GonaCon-Equine vaccination on the existing pregnancy of treated mares and neonatal health and survival to that of untreated control mares. Foaling proportions of treated (0.68 (19/28) 95% CI = 0.50–0.85) and control (0.64 (18/28), 95% CI = 0.46–0.82) mares during 2010 were not different ($P = 0.78$) (Fig 1). Births occurred from early March to early September with 97% (35/36) observed during the first four months of the foaling season (1 March to 1 June). Average foaling dates in 2010 for treated and control mares were 5 May (95% CI = 22 April–18 May) and 10 May (95% CI = 25 April–25 May), respectively. No foal was detected for 12 mares (6 treated: 6 control) that were determined to be pregnant at the 2009 gather. None of these mares showed evidence of pregnancy during the intensive foaling period or for the remainder of the year. We surmised that most of these foals were either aborted or died as neonates between the periods from 20 October 2009 (gather) to 1 March 2010 (beginning of foaling observations). Regardless of timing or cause of death, the proportion of mares that foaled in

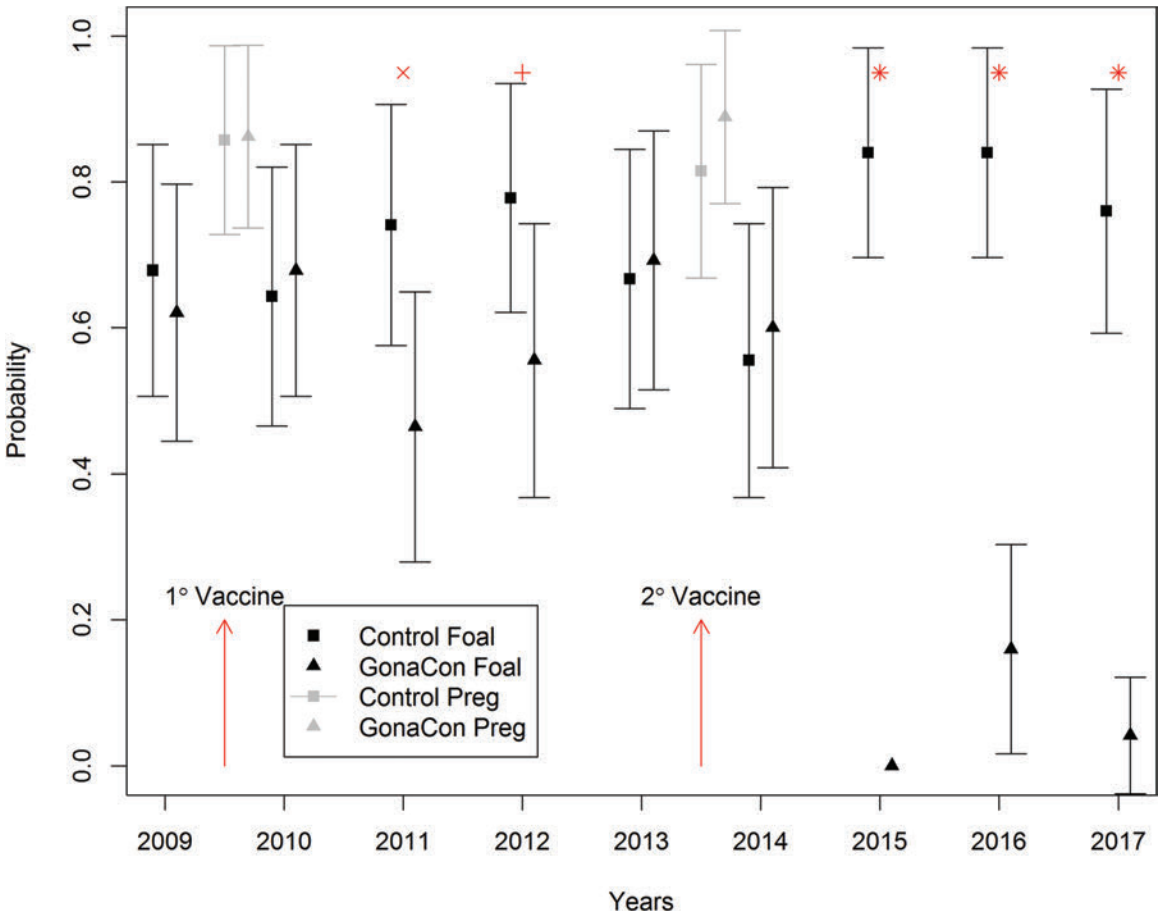

Fig 1. Comparative probability of foaling and pregnancy for treatment and control groups of free ranging feral horses (*Equus caballus)* mares selected for this experiment. Mares were treated with a primary vaccination of GonaCon Equine in October 2009 and then reimmunized with the same vaccine in September 2013 at scheduled gathers at Theodore Roosevelt National Park, North Dakota, USA. GonaCon vaccinations occurred at the time points represented by the red arrows. Symbols correspond to observed p values for relative risk comparisons between treatment groups within years (p value between 0.05 and 0.1 = +, for < 0.05 = x, and for $< 1x10^{05}$ = *).

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2010 underestimated the proportion of mares that were determined to be pregnant at the 2009 gather by 24% for treated mares and 21% for control mares.

Estimated age of all foals at first observation was 2.4 days (95% CI = 1.7–3.1days). Most neonates (97%), from both experimental groups, were classified as vigorous and in good to excellent condition when first observed. Neonatal survival rate from parturition to 14 days of age was estimated to be 0.95 (18/19, 95% CI = 0.75–0.99) for foals born to GonaCon-treated females and 0.88 (16/18, 95% CI = 0.64–0.98) for foals born to control mares ($P = 0.54$). After 14 days of age, post-neonatal survival rates (14–200 da) averaged 0.97 (30/31, 95% CI = 0.84–0.99) and were similar for both experimental groups ($P = 0.57$). These results support our prediction (H_3) that inoculation with GonaCon-Equine vaccine, during approximately the second trimester of pregnancy, does not affect the existing pregnancy of treated females or neonatal health and survival.

The proportion of treated mares that foaled (13/28) following a single vaccination was lower than that for control mares (19/26) for the second (2011) ($P = 0.04$) and third (15/27 vs 21/27) (2012) ($P = 0.08$) post-treatment foaling seasons but was similar (18/26 vs (18/27)

($P = 0.67$) to control mares for the fourth (2013) season, demonstrating reversibility of the primary vaccine treatment (Fig 1). Even though we observed a significant reduction in foaling proportions between treated and control mares during 2011 and a declining effect in 2012, therapeutic effectiveness and relative risk reduction estimates were low to modest and estimated to be 0.37 (95% CI = 0.01–0.60) and 0.28 (95% CI = -0.06–0.51), respectively (Table 1). These findings lend support to our hypotheses *(H_1)* that a single vaccination with GonaCon-Equine is reversible and suppresses fertility for multiple years post-treatment in a portion of treated animals but with diminished effectiveness over time.

Secondary vaccination (2013–2017). At the scheduled gather in October 2013, we extended our evaluation of GonaCon-Equine by assessing the effects of revaccination on fertility and safety in these same experimental mares treated four years after the primary vaccination. Evidence of similar fertility for individual mares was demonstrated at the 2013 gather, where pregnancy proportions of treated (0.92 (23/25), 95% CI = 0.75–0.98) and control (0.88 (23/26), 95% CI = 0.71–0.96) mares were similar ($P = 0.86$) [S1 Table]. Except for one treated and one control mare, all others had conceived and given birth to at least one foal during 2009–2013. For the 2013 foaling season, foaling proportions of treated (0.69 (18/26), 95% CI = 0.51–0.87) and control (0.66 (18/27) 95% CI = 0.49–0.84) mares were not different ($P = 0.84$) providing additional evidence that treatments groups were of equal fertility prior to reimmunization (Fig 1).

Like 2010, mean foaling proportions during the first post-treatment foaling season (2014) were not different ($P = 0.74$) between treated (0.60 (15/25), 95% CI = 0.41–0.79) and control (0.56 (15/27), 95% CI = 0.37–0.74) mares (Fig 1) supporting similar observations in 2010 that revaccination could be applied to pregnant mares, during mid-gestation, without risk to the existing pregnancy. Foaling date distribution was comparable to that observed in 2010 following the primary vaccination. Average foaling date for treated mares was estimated to be 27 April (95% CI = 5 April– 20 May) and 19 April (95% CI = 6 April– 2 May) for controls. No foal was observed for 15 mares (8 treated: 7 control) that were determined to be pregnant at the 2013 gather. Like 2010 estimates, foaling proportions underestimated pregnancy proportions determined at the 2013 gather for both treated and control mares by approximately 30% and 34%, respectively (Fig 1). These data, together with similar observations in 2010, support the inference that foaling proportions are not an accurate proxy for pregnancy proportions but provide a limited but practicable field measurement for determining contraceptive

Table 1. Comparative relative risk reduction (RRR), 95% confidence intervals, and p values associated with differences in foaling proportions between GonaCon treated and control mares during 2009 2017.

Year	Relative Risk Reduction	95% Confidence Interval		p value
	(RRR)	Lower	Upper	
2009	0.0852	0.3757	0.3402	0.6500
2010	0.0555	0.2750	0.5301	0.7797
2011	**0.3732**	**0.6028**	**0.0109**	**0.0381***
2012	0.2857	0.5178	0.0581	0.0861
2013	0.0384	0.2826	0.5032	0.8430
2014	0.08	0.3216	0.7194	0.7482
2015	**1**	**1**	**NA**	**2.57E 09***
2016	0.8095	0.9236	0.5247	1.94E 06*
2017	0.9451	0.9920	0.6217	4.15E 07*

*Significant p values (<0.05).

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effectiveness. Average foal age at first observation across both treatment and control groups was 2.6 (95% CI = 1.5–3.3) days. Nearly all foals born to revaccinated and control mares were classified as vigorous and found to be in good to excellent condition when first observed.

Neonatal survival rate to 14 days of age for foals born to revaccinated mares was 0.87 (13/15), 95% CI = 0.62–0.96 and 0.93 (14/15), 95% CI = 0.70–0.98) for foals born to control mares ($P = 0.49$). After 14 days of age, post-neonatal survival rates were 0.80 (12/15), 95% CI = 0.55–0.92) for revaccinated mares and 0.73 (11/15), 95% CI = 0.48–0.89) for control mares ($P = 0.55$). These results reflect similar findings following a primary vaccination with GonaCon-Equine and reinforces the deduction (H_3) that reimmunization is safe for treatment of pregnant females and does not affect neonatal or post-neonatal health or survival when applied at approximately mid-gestation.

Unlike results from the single vaccination trial, we observed, not only highly significant reduction in foaling proportions between treated and control mares following reimmunization but also a remarkably effective contraceptive response. Except for the first foaling season following treatment application, (2014) in which the vaccine was not expected to have an effect ($P = 0.75$), foaling proportions in reimmunized mares were lower ($P < 0.001$) than that for control mares for all subsequent years (2015–2017) (Fig 1). This was particularly evident for the second post-treatment foaling season (2015) when none 0.00 (0/25), 95% CI = 0.0) of the reimmunized mares produced a foal while the proportion of control mares foaling was estimated to be 0.84 (21/25, 95% CI = 0.69–0.98). During the third post-treatment foaling season (2016), four treated mares produced a foal resulting in a foaling proportion of 0.16 (4/25), 95% CI = 0.01–0.30) while the proportion of control mares foaling was identical to that observed in 2015 (Fig 1). These foals were determined to be vigorous and in good to excellent condition at birth, however, two of these foals, born in September, were not observed the following spring and were categorized as post-natal mortalities and presumed to have died during winter (2016/2017).

In 2017, no additional treated mares produced a foal or showed evidence of pregnancy. However, one of the treated mares that had foaled in 2016 died of apparent natural causes (age-related malnutrition) during 2017 and two other revaccinated mares that had foaled in 2016 failed to produce a foal that year resulting in a foaling proportion of 0.041 (1/24), 95% CI = 0.03–0.12) (Fig 1). The foaling proportion for mares in the control group (2017) was 0.84 (21/25, 95% CI = 0.69–0.98) and higher ($P < 0.001$) than that for GonaCon-treated mares (Fig 1). It should be noted that the apparent decrease in foaling proportions in GonaCon-treated mares from 2016–2017 and resulting increase in vaccine effectiveness (Table 1) is likely due to the inherent error associated with the small sample size ($n = 4$) of mares in this treatment group that regained fertility. Overall, there was both a substantial decrease in foaling proportions (Fig 1) and an exceedingly high level of effectiveness (Table 1) for treated mares compared to controls for 3 years post-revaccination (2015–2017) ($P < 0.001$). Thus, fertility measurements during 2015–2017 support our prediction (H_2) that revaccination with GonaCon-Equine would be more effective in suppressing foaling proportions in treated females compared to controls than a single immunization (Fig 1, Table 1).

Side effects

Behavioral. We collected behavioral data on 73 feral horses (22 males, 25 treated females, 26 saline females) for 218.3 h in 2014. The median age of observed stallions was 12 years (range = 9–19 years), median age of observed control females was 8 years (range = 7–20), median age of observed treated females was 9 years (range = 7–22), and median band size was 8 horses (range = 2–14). There were no differences detected between treatment groups in any

time budget behavior category (Table 2). As band size increased, feeding decreased 1.24% (95% CI = 0.48–2.00) per additional horse in the band. Likewise, locomotion increased 0.20% (95% CI = 0.07–0.33) and maintenance decreased 0.10% (95% CI = 0.01–0.19) per additional horse in the band.

Foal presence influenced locomotion, with barren females moving 1.24% (95% C I = 0.40–2.08) more than females with dependents. Foal presence also influenced the social behavior component of time budgets, with barren females interacting with others 2.74% (95% CI = 0.59–4.90) more than females with dependents.

Variance among individuals had little influence on any of the behaviors modeled (Table 2). Variance was also minimal between time periods of observation; however, there were some significant differences in amount of activity by time of day. An estimated 6.88% (95% C I = -0.73–14.5) more feeding occurred in the 1601–2000 h time-period than did earlier in the day, and this was reciprocated by an estimated 3.33% (95% CI = 1.09–7.79) less resting, 0.34% (95% CI = 0.15–0.82) less maintenance, and 1.30% (95% CI = 0.51–3.11) less social behavior during the same period.

There were no differences detected between treatment groups in herding, reproduction, or agonism, but treatment group did influence harem-social behavior. Observed instances of harem-tending behavior provided too few data to model. Because these social behaviors were not as dependent on other broad categories as is the case with compositional time budgets [51], we re-estimated the social behavior models with only treatment and supported effects to allow for clearer interpretation of the results.

Stallions initiated harem-social behavior 13.9% (95% CI = 3.25–24.68) less toward control females than toward treated females. Though all harem-social records were analyzed as a group, it should be noted that 55.8% of the 308 harem-social events were sub-categorized as allogrooming. While the significant difference between treatment groups was detected, the variance among individuals for this behavior was near zero (Table 2).

Physiological. No study mares exhibited antibody titers to any of the infectious diseases that were surveyed for (i.e., equine herpesvirus-1, equine infectious anemia, equine viral arteritis and contagious equine metritis) thus eliminating this factor as a potential cause of infertility in GonaCon-treated females.

No control mares, treated with saline, showed any evidence of injection site reactions. Swelling and discharge were never observed in this group. Likewise, these mares showed no evidence of lameness or gait abnormalities in either hind limb. Consistent with our hypothesis (H_3), approximately 72% of treated mares (21/29) displayed a visible reaction at the site of injection after a single vaccination with GonaCon-Equine (S1 Photo). A single mare developed a draining abscess after the initial vaccination. These lesions were persistent over multiple years. At the time of the 2013 roundup and revaccination, 81% (21/26) of vaccinated mares continued to have palpable swelling at the original site of vaccine injection.

Like initial vaccination reactions, during the first-year post-revaccination, approximately 50% (13/26) of mares continued to show swelling on the left hip at the site of the 2009 injection and 50% developed a reaction on the right hip at the site of revaccination in 2013. Two of these new reactions were draining abscesses. Yet again, injection site reactions were persistent with approximately half of the mares with swellings at one or both injection sites, 3 years after revaccination. None of the GonaCon-treated mares displayed any evidence of lameness, altered gait or abnormal range of movement throughout the 8 years they were observed.

While body condition varied between individuals and study years, it did not vary between treatment groups ($P = 0.14$) over the course of the study. Likewise, there was no effect of presence of a foal on body condition ($P = 0.16$). Average body condition ranged from 3.7–4.9

Table 2. Treatment and supported effects in a mixed effects linear regression of feral horse (*Equus caballus*) time budget behaviors (e.g. feeding, resting, locomotion, maintenance, social) and all occurrence social behaviors (e.g. herding, reproduction, agonism, harem social) at Theodore Roosevelt National Park, USA. Variance for the random effects of time of day (j) and individual horse identity (k) are shown as σ_j^2 and σ_k^2.

Behavior	Effect	*t*	*P*	Difference	95% confidence limit		σ_j^2	σ_k^2
					Lower	Upper		
Feeding	Treatment	0.125	0.900				0.004	0.003
	Band Size	3.193	0.001	0.012	0.020	0.005		
Resting	Treatment	0.590	0.555				0.001	0.001
Locomotion	Treatment	0.143	0.886				<0.001	<0.001
	Band Size	3.047	0.002	0.002	0.001	0.003		
	Foal Presence	2.900	0.004	0.012	0.004	0.021		
Maintenance	Treatment	1.193	0.233				<0.001	<0.001
	Band Size	2.238	0.025	0.001	0.002	0.001		
Social	Treatment	0.037	0.970				<0.001	0.001
	Foal Presence	2.499	0.013	0.027	0.006	0.049		
Herding	Treatment	0.909	0.368				0.009	<0.001
Reproduction	Treatment	1.555	0.159				<0.001	<0.001
Agonism	Treatment	0.669	0.528				<0.001	0.048
Harem social	Treatment	2.620	0.012	0.140	0.033	0.247	0.007	<0.001

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(moderately thin to moderate body condition) for all study animals over the 8 years that mares were observed. Individual body condition scores ranged from 1–7.

Discussion

Reproduction

This study demonstrated that a single vaccination against GnRH, using GonaCon-Equine, administered during mid-gestation, was safe, initiated short duration (2 yrs.) infertility in some mares, and was reversible, but was minimally effective in reducing fertility of treated females compared to controls. For two foaling seasons following vaccine treatment, we observed statistically significant reductions (28–38%) in foaling proportions of treated versus control mares but no effect by the third-year post-treatment, thus confirming the reversibility of the vaccine.

These results parallel similar findings from other experimental evaluations of GonaCon-Equine reported for captive and free-ranging mares. In a comparable study in Nevada with feral horses in a natural environment, GonaCon-Equine reduced foaling proportions by an average of 33% over a 3-year period but, like our study, contraception was only modestly effective over this period [23]. In contrast, contraceptive effectiveness of captive mares treated with GonaCon was greater and longer lasting ($\geq$ 4yrs) than either of these studies [22]. The disparity between captive and free-ranging animals in contraceptive response to GonaCon vaccine is not limited to feral horses but has also been observed between captive and free-ranging white-tailed deer [14, 18, 20] and elk [15, 16, 19]. Although these investigations did not suggest a definitive causation for these differences, they all pointed to suppressed and less persistent GnRH antibody concentrations in free-ranging ungulates compared to their captive counterparts suggesting a relatively compromised or weakened immune response to the vaccine that resulted in reduced contraceptive effectiveness.

It is widely acknowledged that differences in vaccine effectiveness can be attributed to increased environmental stressors (i.e., nutritional status, injuries, parasite load, pathogen exposure, and social dynamics) that can inhibit a more vigorous immune response in free-

ranging animals in a natural environment [59, 60]. It follows that while efficacy trials with captive animals can provide an important first approximation of vaccine safety and performance under controlled conditions; they may offer only limited inference to free-ranging animals that are not buffered against natural stressors that may decrease immune response and vaccine effectiveness. Regardless of the factor(s) contributing to the limited effectiveness of GonaCon in free-ranging animals, it appears that the immune response from a single vaccination does not consistently provide multiple years of infertility in all or even a high proportion of these animals.

In comparison to a single inoculation with GonaCon-Equine, the effect of reimmunization on foaling proportions was highly significant which allowed clear differentiation between treated and control mares for multiple breeding seasons. Compared to a single vaccination, reimmunization of mares in this study resulted in a much higher (58%) average vaccine effectiveness (range = 0.80–0.94) than the single vaccination for a 3-year period (2015–2017). Likewise, this level of effectiveness following reimmunization was on average higher than that previously reported for free-ranging mares treated with a single application with GonaCon-Equine [23] and 32% above what was reported for captive mares treated with the same vaccine formulation [22]. These results support the conclusion that a booster immunization with GonaCon-Equine can provide a highly effective, multi-year suppression of fertility in free-ranging horses and these results may be consistent in other animal species, as well.

It is fundamental knowledge that a secondary response to a vaccine generally results in a more rapid production of antibodies that are produced in greater amounts and over a longer time compared to the primary vaccination [25]. Repeat immunizations using a variety of GnRH vaccines in domestic horses have been shown to improve contraceptive efficacy. However, unlike commercially available short duration vaccines ($<$ 1 yr.) developed for domestic horses [29, 61], GonaCon-Equine is formulated by combining a non-biodegradable oil in water-based emulsion and an optimum concentration of immunostimulatory killed mycobacteria to form a depot usually deep in muscle tissue. This depot injection is thought to allow for a slow release and prolonged stimulation so that the formulation can act for much longer periods of time (years) than is possible with standard injections (months). This effect is thought to be responsible for the extended antibody response of 3–4 years in vaccinated deer [14, 18, 20, 62], elk [15, 16], and horses [22].

While this response was not unexpected, the magnitude and duration of effectiveness of GonaCon-Equine following revaccination, even 4 years after the initial vaccination, is salient and relevant to the management of fertility in free-ranging horses. First, it demonstrates that a booster vaccination can stimulate a highly effective immune response that can result in multiple years ($\geq$ 3 yrs.) of contraception. Second, it provides an initial reference point for defining the optimum revaccination schedule required for long-term reproductive management of female horses in a natural environment. And finally, it supports the consideration that while a single application may be preferred from a practical management perspective, GonaCon-Equine is more effective, in free-ranging horses, if repeat vaccinations are delivered on a periodic basis. While initial results are encouraging, additional research is needed to complete the objectives of this study including: 1) to define the duration of effective contraception post-revaccination, 2) to determine if long-term or permanent infertility is a possible outcome, and 3) to assess if return to fertility (if it occurs) results in altered birth phenology of treated mares. We will investigate these questions over the next three years of this study. Additionally, there may be a more optimal revaccination schedule which allows for altered duration of effectiveness or is more conducive to management schedules.

Side effects

After revaccinated in October 2013, time budget and social behaviors of mares in spring/summer of 2014 were comparable to those observed during the same period in 2010, following the initial treatment in October 2009. We found no evidence of differences in frequency or intensity of social behaviors including estrous behavior associated with treatment. Both treatment and control groups displayed few estrus behaviors in either 2010 [37] or during 2014. Behaviors associated with estrus were observed only 17 times in treated and 57 times in control mares out of 1148 observed social behavior events. This supports our earlier findings that pregnant mares rarely show overt estrous-related behaviors and similarly GonaCon-Equine treated mares only occasionally display these behaviors, although each for different reasons. Once a mare is pregnant, progesterone likely subverts much of the estrous type behavior that would generally be displayed with high estrogen levels, and only occasionally do domestic horses display and stand for mounting when pregnant [63]. Relatively small amounts of estrogen are secreted as follicles develop and then regress. In the absence of progesterone, relatively small amounts of estrogen are likely sufficient to induce erratic estrous behavior as was observed in these mares. However, the small amounts of estrogen were likely insufficient to induce an LH surge and subsequent ovulation.

Regardless of the underlying endocrinology associated with these behaviors, vaccinated and control mares both displayed social interactions that maintained herd structure; herding, tending, and defending behaviors from the stallion; and social hierarchies. The only meaningful factor that influenced the amount of time spent in social behaviors (e.g. allo-grooming, herding and tending) was the presence or absence of a foal. Mares with foals spent more time alone with the foal than those without off-spring, which is to be expected given their social and nutrient requirements during the neonatal and post-natal periods [50]. It is possible that long-term absence of foals could influence social behavior on a longitudinal scale, but additional studies are needed to investigate such phenomena on an appropriate time scale.

Other techniques for reducing the fertility of free-ranging species, such as vaccination with the native porcine zona pellucida vaccine (PZP) and tubal ligation, maintain the competency of the endocrine aspects of fertility. This can lead to unintended consequences with repeated estrous cycling in polyestrous species. In fact, in a population of white-tailed deer, where most reproductive females had received tubal ligations, fawning was negligible; however, there was more than a 700% increase in the number mature males attracted to the area occupied by a high number of estrous cycling females [64]. Similarly, PZP vaccination has extended the length and intensity of breeding seasons in horses [49, 65–68], deer [69, 70], and elk [71]. GonaCon-Equine may avoid these inadvertent consequences by functionally inducing mimicry of pregnancy in females which continues to be an important part of the social structure of the group but does not invite intense adverse breeding behaviors.

Researchers have generally hypothesized that by alleviating the energetic demands of gestation and lactation, contracepted females will attain improved body condition over pregnant females that require additional food resources to produce and rear an offspring. However, for free-ranging large ungulates, empirical evidence supporting [72] or refuting [73–75] this prediction is limited and equivocal. In this investigation, contracepted mares that experienced no gestation and lactation did not exhibit improved body condition over mares that successfully reproduced. Individual mares in each experimental group, attained an average BCS of 5.0 (moderate) or better, which has been reported to be the minimally optimal level of stored fat necessary to achieve maximum reproductive efficiency during pregnancy and lactation [53, 76]. These levels of body condition were reflected in the high proportion of pregnant mares (0.85–0.92) observed in each treatment group at the management roundups in 2009 and 2013.

We acknowledge that our sampling intensity and/or sensitivity of our ocular index to body condition may not have enabled us to detect fine-scale differences between experimental groups. However, we conducted these evaluations during time periods when differences in body condition between pregnant and non-pregnant (GonaCon-treated) females should have been the greatest. Namely, during early spring (March) when fats deposits are depleted over winter and during April–August when the energetic demands of late gestation and lactation are increasing.

The body condition of an animal is dependent on a balance between energy intake and expenditure. When intake is not sufficient to meet energy requirements for various activities (i.e. maintenance, growth, activity, gestation, lactation, etc.), fat reserves and eventually lean body tissue will be lost. The fact that pregnant and lactating mares in this study were in similar body condition to that of contracepted ones suggest that food is unlikely a limiting factor for free-ranging horses at THRO. This is primarily due to the conservative management of multiple species of ungulates and their food resources [77–79]. The consequence of this approach is that only under the most extreme climatic conditions, such as prolonged drought, will forage be limiting to herbivores at THRO, regardless of reproductive status.

The only detectable adverse side effect of vaccination was intramuscular swelling at the vaccination site. Mares treated with GonaCon-Equine consistently showed evidence of inflammatory reactions at the injection site. While we never observed lameness associated with this reaction, several mares revealed draining abscesses within one-year post-vaccination. This is consistent with results for other wild ungulates treated with the same or similar GonaCon vaccines [13, 15, 34]. Given the designed highly inflammatory nature of both the adjuvant, which contains killed mycobacteria and non-biodegradable oil, as well as, the foreign protein carrier molecule, these types of reactions are predictable. In fact, they are likely necessary for optimum vaccine efficacy [80]. It is impossible to assess the total impact of these lesions on animal welfare; however, in this investigation, these did not have a measurable effect on body condition, locomotion, or social behaviors. Therefore, until additional research suggests otherwise, we conclude that the presence of injection site lesions following GonCon vaccination do not pose a serious contraindication associated with the application of this vaccine, and there appear to be minimal long-term effects on individual animal welfare.

Conclusions

Controlling abundance of wildlife species that are simultaneously protected, abundant, competitive for resources, and in conflict with some stakeholders is a formidable challenge for resource managers. We demonstrated that the GnRH vaccine, GonaCon-Equine, could be an effective immunocontraceptive for free-ranging feral horses, particularly when the primary vaccination is followed by reimmunization four years later. This vaccine was shown to be safe for pregnant females and neonates and did not result in deleterious behavioral side effects during the foaling/breeding season. The only adverse reactions to vaccination were non-debilitating inflammatory responses at injection sites. One noteworthy implication has emerged regarding long-term management of free-ranging horse populations using GonaCon-Equine vaccine: effective management and development of population models will need to incorporate repeat immunizations of this vaccine to optimize management strategies aimed at stabilizing the growth rate of feral horse populations. Our research suggests that practical application of this vaccine in feral horses will require an initial inoculation that may provide only modest suppression of fertility followed by reimmunization over time that together could result in greater reduction in population growth rates. Future research will begin to define the most effective revaccination schedule with GonaCon-Equine for suppressing reproductive rates in

free-ranging horses, the duration of effectiveness, and the return to fertility following treatment. Moreover, applying GonaCon-Equine to control the growth of feral horse populations will require that resource managers choose specific tactics for treating animals. Choices must be made on the number and age to treat and the frequency of treatment needed to maintain the desired population age structure and genetic diversity. Decisions on the most beneficial tactics will depend on overarching management goals and long-term objectives for the population.

Supporting information

S1 Table. Comparative metrics and pregnancy proportions, by age class, for treatment and control groups of free-ranging mares selected for this experiment.
(PDF)

S1 Photo. An Intramuscular swelling (raised area of tissue) observed in a Theodore Roosevelt National Park horse, 9 months after hand-injection with GonaCon-Equine vaccine.
(TIF)

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From: Griffin, Paul C
To: Dan Baker; Bernadette.Basaraba@colostate.edu
Subject: Requesting Financial report (SF-425) for L15AC00145 (late)
Date: Friday, November 22, 2019 11:36:30 AM

Hi Bernadette and Dan,
Please prepare a financial report (SF-425) for federal agreement number L15AC00145, for activities through September 30, 2019. This report was due at the end of October. .
Please send a copy to me and to the following 2 email addresses:
briley@blm.gov
BLM_WO_FAreports@blm.gov
Thank you very much,
Paul

--

Paul Griffin, Ph.D.
Research Coordinator, BLM Wild Horse and Burro Program
2150 Centre Ave, Building C, Fort Collins, CO 80526
970-226-9358 office, 970-631-4808 mobile
pgriffin@blm.gov

From: McCann, Blake E
To: Griffin, Paul C
Subject: Research on horse spatial ecology
Date: Monday, July 27, 2020 9:22:13 AM

Hello Paul;
Any idea who among academic researchers is interested in horse spatial ecology studies?
Thinking of possibilities to get a graduate research project going.
Thank you.
Blake

Blake McCann, Ph.D.
Chief of Resource Management
Theodore Roosevelt National Park
P.O. Box 7, Medora, ND 58645
701-623-4730 x1433

BUREAU OF LAND MANAGEMENT
OR-WA State Office
Grants & Cooperative Agreements

PROJECT PROPOSAL

Instructions: A Project Proposal must be submitted with the Standard Form (SF) 424 Application for Financial Assistance for all Financial Assistance Agreements. A new proposal must be included with any request for modification which involves a revision to funding, project scope, period of performance, or key personnel.

Agreement or Funding Opportunity No.: L14AS00048 Date: 08/21/15

Organization Name: Colorado State University

Project Title: Re-immunization of Free-Ranging Horses with GonaCon Immunological Vaccine: Effects on Reproduction, Side-Effects, and Population Performance

Current Funding Estimated Period of Performance (PoP): **08/24/15** to 8/23/20

Name & Title of Person Submitting Proposal: Dan L. Baker, Senior Scientist
Terry M. Nett, Senior Scientist

* If a specific start work date is needed, contact your BLM Program Officer. Do not start work without prior approval from the Grants Management Officer.

1. Purpose, Objectives, and Relevance:

(Describe why the project is needed, the applicant's objectives, how the applicant's objectives support their mission, and how this project benefits the general public.)

BACKGROUND

1. Re-immunization

In many areas of the western United States, overabundant and rapidly expanding populations of feral horses (*Equus caballus*) pose a significant dilemma for natural resource managers. The Wild Free-Roaming Horses and Burros Act of 1971(P.L.92-195) provided protection for feral horses and burros (*Equus asinus*) on most federal lands and established guidance for their management as a wildland species (Wagner 1983). There is, however, wide-spread concern among state, federal, and private land management agencies that unregulated feral horse populations are severely altering native plant communities and limiting the abundance and diversity of habitat resources allocated for native wildlife and other domestic livestock species.

Current population control methods such as utilizing periodic roundups and adoption or sale of excess animals, or maintaining excess feral horses in long-term holding facilities are expensive, resource intensive, and unsustainable. Clearly, more efficient, cost effective, and humane approaches to reducing feral horse densities on public lands are needed. Controlling the fertility of female horses offers a potential non-lethal alternative to conventional methods (National Research Council 2013).

A promising immunological approach to contraception in feral horses involves

immunization against the neuropeptide gonadotropin releasing hormone (GnRH). Scientists at the National Wildlife Research Center (NWRC) have conjugated synthetic GnRH peptides to a highly immunogenic carrier protein that, when combined with a potent adjuvant, stimulates the host's immune system to produce antibodies that bind to endogenous GnRH. This, in turn, prevents synthesis and secretion of important downstream reproductive hormones necessary for reproduction. Animals generally return to fertility as antibodies concentrations decline (Powers et al. 2011).

Multiple years of infertility have been achieved in captive and free-ranging wild ungulates with a single inoculation with the GnRH-based vaccine, known as GonaCon. This vaccines has been experimentally tested and found to provide multiple years of infertility after a single application in white-tailed deer (*Odocoileus virginianus)(* Miller et al. 2008, Gionfriddo et al. 2011*a*), bison (*Bison bison)(* Miller et al. 2004), elk (*Cervus elaphus)(* Killian et al. 2009, Powers et al. 2011, 2014), wild pig (*Sus scrofa)(* Massei et al. 2012), and feral horses (Killian et al. 2008, Gray et al. 2010, Baker et al. 2013). However, multiple years of infertility are only experienced in a fraction of vaccinated animals. In free-ranging elk, there was approximately a 90% treatment effect the first year after vaccination but that dropped to 50% by the second year and by the third year of the study, there was no measureable response (Powers et al. 2014). Similarly, during the first 3 years of our current investigation in feral horses at THRO, we observed a 25-35% decrease in foaling in treated versus control mares for the first and second years of the study but no effect by year three (Baker et al. 2013).

Repeat vaccinations generally result in a more profound and longer-lasting antibody production due to the anamnestic response (Tizard 1982). Therefore, we expect longer- lasting contraceptive effects in re-vaccinated mares. The single-injection GonaCon vaccine is unique in that the formulation initiates high antibody titers that remain elevated in some applications; however, to our knowledge, no research has been conducted to evaluate booster doses of this vaccine in any mammalian species.

Booster immunizations using a variety of GnRH vaccines in domestic horses have been shown to improve contraceptive efficacy and to suppress behavioral and physiological estrus (Garza et al.1986, Elhay et al. 2007, Botha et al 2008). However, these GnRH vaccines differ from GonaCon in that they incorporate different protein carrier molecules and adjuvants, and are formulated for short duration (< 1 yr.) contraceptive effectiveness that is generally achieved by using a primary immunization followed 35 days later by a booster inoculation.

While a single vaccination is often preferred from a management perspective, GonaCon vaccine may prove to be more effective if repeat vaccinations are delivered on a periodic basis. Efficacy data collected from 25 mares treated with single application of GonaCon in 2009, at Theodore Roosevelt National Park (THRO) revealed a moderate 2-year decline of approximately 30% in foaling rates, with all mares regaining fertility by three years post-primary vaccination treatment (Baker et al. 2013). Surprisingly, re-vaccination of these same mares in the fall 2013 (four years post-primary vaccination) has resulted to date, in complete infertility during the 2015 foaling season (the first season to expect a re-vaccination effect on fertility). Clearly, these results are both statistically and biologically significant, as well as encouraging from a fertility control perspective.

If these results persist over time and these mares remain infertile, it would lend support to our hypothesis that re-vaccination with GonaCon, even four years post-primary vaccination produces a strong anamnestic response in horses that stimulates anti-GnRH antibodies and suppresses fertility. At present, however, it is premature to predict how many of these re-vaccinated mares failed to conceive during the 2014 breeding season and will not foal or regain fertility during 2015 and beyond. It is possible that the booster vaccination simply delayed the estrous cycle in these mares, which could result in foals being born later in the foaling season.

While these findings are tentative and inconclusive, they suggest that repeat vaccinations are likely needed to achieve high efficacy of GonaCon vaccine in free-ranging horses and these

effects have not been investigated or determined. Thus, our proposed research offers a unique opportunity to address this question at THRO and will have relevance, not only to feral horses, but also to other wild ungulates that have been treated with a single treatment of GonaCon vaccine. Our proposed research will begin to define the vaccination schedule needed to maintain infertility in free-ranging horses and whether or not long-term or permanent sterility is a possible outcome. We will investigate the safety and efficacy of a repeat vaccination under the hypothesis that this vaccine will be more efficacious and longer-lasting than the original primary immunization.

2. Remote Dart Delivery

Fundamental to practical field application of GonaCon vaccine in free-ranging horses is a safe, reliable, and effective method of administering a single dose of the vaccine to free-ranging horses by means of a syringe dart. Many contraceptive agents have been successfully applied via syringe dart or biodegradable implant to an assortment of wild ungulate species including white-tailed deer (Turner et al. 1992, Jacobsen et al. 1995, DeNicola et al. 1997), elk (Shideler et al. 2002, Baker et al. 2005), feral horses (Kirkpatrick et al.1990, Roelle and Ransom 2009), and elephants (*Loxodonta Africana*) (Delsink et al. 2002). However, to our knowledge, evaluation of remotely-delivered GonaCon vaccine is limited to one field investigation with white-tailed deer (DeNicola unpublished data). Although dart performance in this study was less than expected, it provided important basic information regarding optimum dart configuration and delivery ballistics. Using this preliminary data, technicians at Pneu-Dart, Inc. developed a prototype dart configuration for delivering this highly viscous vaccine formulation to free-ranging horses.

We tested this GonaCon-specific dart delivery system with captive feral horses at the 2013 scheduled roundup at THRO. Eleven adult mares (2-4 years of age), that had not been previously vaccinated, were held in small paddocks and remotely darted in the biceps femoris muscle with 2 ml (2000 µg) of GonaCon vaccine. All darts were weighed (± 0.01g) before and after injection to determine the precise dose delivered. Darting distance varied from 10-15 m. Nine out of 11 darts delivered, on average, 95% of the GonaCon vaccine formulation. Two darts failed to discharge possibly due to low muzzle velocity. All darts appeared to dispense the vaccine deep into the muscle mass and none of the darts were observed to bounce without penetration, partially discharge, blow-out, or show evidence of subcutaneous delivery of the vaccine. The two horses in which the darts failed to discharge were subsequently re-treated and the second darts successfully delivered a full dose. With 85% of the 2015 foaling season complete, 7/11 (63%) of these mares have not foaled. In contrast, only 16% of the untreated mares have not foaled to date. A dependable dart delivery system for administering GonCon remotely to free-ranging horses is critical to the determination of an optimum re-vaccination schedule in our proposed study. If successful, this technology will potentially provide resource managers with an alternative strategy for managing this feral horse population.

3. Biological Side-Effects

Evaluation of the biological side-effects of GonaCon vaccine treatments have been reported for numerous wild ungulate species including white-tailed deer (Curtis et al. 2008, Gionfriddo et al. 2011*b*), elk (Powers et al. 2011, 2012, 2014), bison (Miller et al. 2004) and feral horses (Baker et al. 2013). Results from these investigations generally conclude that GonaCon does not cause serious adverse effects on general health, body condition, existing pregnancy, neonatal health, major organ systems, or fertility of male and female offspring of females treated during pregnancy.

Granulomatous intramuscular injection-site lesions, that occasionally break and drain as abscesses, are the only adverse effect of vaccination consistently reported in these studies. The formation of these injection site lesions may be necessary for stimulation of a strong immune response and infertility. GonaCon vaccine contains AdjuVac; a water-in-oil based adjuvant

developed from a USDA approved Johnes disease vaccine called Myocopar™ (Fort Dodge Animal Health). AdjuVac contains killed *Mycobacterium avium,* which is needed to induce a rapid, strong, and sustained contraceptive response (Miller et al. 2008*a,* Perry et al. 2008). This combination of water - in- oil emulsion and killed mycobacteria results in a highly potent adjuvant that stimulates both humoral and cellular immunity (Warren et al. 1986).

Vaccines, like GonaCon, that contain mycobacteria may induce strong immune responses because of the formation of a repository or depot at the injection site (Fukanoki et al. 2000). In response to the presence of the depot, a granuloma forms as the immune system attempts to isolate the foreign material. The continued existence of this depot, which initiates a chronic inflammatory response, likely provides a long-term source of antigen stimulation and persistent antibody production. We speculate that this is the mechanism by which a single vaccination can provide multiple years of infertility in a portion of the population in many species that have been studied.

However, even with this prolonged antigenic stimulation, the immune response from a single vaccination does not consistently provide multiple years of infertility in all or even a high proportion of animals (Powers et al. 2014, Baker et al. 2013). In all studies, where post-mortem examinations were performed, prevalence of injection-site inflammation and granulomas were present but in some species, such as white-tailed deer and elk, they were not apparent antemortem (Curtis et al. 2008, Powers et al. 2011, Gionfriddo et al. 2011*b*).

In contrast to these species, injection site reactions in feral horses, following GonaCon vaccination at THRO, are readily observable as subcutaneous swellings. In past studies at THRO (2009-2013), all injection site reactions appeared to be confined to the general gluteus muscle where the vaccine was first hand-injected. Reactions to the vaccine were first observed 30 days post-treatment in 17.2% (5/29) of mares and by the second breeding season, 79.3% (23/29) of treated females showed some evidence of inflammation or swelling at the injection site. Saline control mares displayed no evidence of injection site reactions. Swellings of various sizes (marble to baseball size) were most common, followed by nodules, and rarely a draining abscess. Most of these reactions were observable for three years post-treatment, then began to resolve and become less visible by year 4 (many that could not be visually observed were still manually palpable at the 2013 roundup).

However, similar to other studies where injection site reactions have been evaluated, we did not observe any clinical evidence of lameness, impaired mobility, depression, or decreased health or fitness in any animal that was associated with GonaCon vaccine treatment. While results from the above investigations are generally consistent relative to the effects of GonaCon-induced injection site reactions, they are also limited to the consequences of a single vaccination usually delivered by hand-injection.

At the 2013 THRO round-up, GonaCon –treated mares were re-vaccinated, four years post-primary vaccination, with a booster dose on the opposite side in the biceps femoris muscle. This investigation is in progress but thus far, injection site reactions appear to be less apparent than those observed following the 2009 vaccination (Baker et al. unpublished). At this time, the cumulative effects of re-vaccination are unknown and the potential for more intense immune reactions with additional doses of this vaccine delivered by syringe dart is a consideration (Broderson 1989, Roelle and Ransom 2009).

4. Behavioral Side-Effects

Behavioral side-effects of GonaCon vaccination in wild ungulates have not been extensively investigated (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014). Given the physiological mechanism of action, GonaCon vaccine has the potential to suppress fertility and diminish the reproductive behaviors typically associated with estrus. However, in GonaCon-vaccinated female elk (Powers et al. 2011) and free-ranging horses (Gray et al. 2010, Baker et al. 2012, Ransom et al. 2014) such behaviors were maintained throughout the

first breeding season after immunization and were not different from untreated females.

In a previous study at THRO during 2009-2010, daily activity patterns, social interactions, and reproductive behaviors were similar for GonaCon treated and control mares (Baker et al. 2012, Ransom et al. 2014). But, since GonaCon only prevented conception in 50% of treated mares (n = 28), behavioral observations were limited to only 14 infertile females. Thus, rigorous quantitative investigation into the potential effects of GonaCon treatment on feral horse behavior is missing from the assessment of this immunocontraceptive as a potential management tool. Inferences to free-ranging feral horse populations are, therefore not definitive and deserve further investigation.

In an attempt to further our understanding of the behavioral side-effects GonaCon vaccine, we conducted behavioral observations during the first breeding season (2014) following re-vaccination of these same mares at THRO in 2013. We measured the effects of this vaccine on sociosexual behavior, harem dynamics, and activity budgets of treated (n = 25) and control (n =25) horses. To date (July 20 2015), none of the re-vaccinated mares have foaled, whereas 84% (21/25) of the control mares have done so. As a result of higher vaccine efficacy in treated mares, our sample size increased by 44% and offered a more thorough investigation into potential effects of GonaCon treatment on feral horse behaviors. We postulated that based on the assumed mechanism of action of GonaCon that re-vaccination would suppress reproductive behaviors in treated females compared to controls.

5. Population Modeling

We will integrate contraceptive efficacy and population monitoring data at THRO to estimate parameters and unobserved states in a Bayesian hierarchal model (Dulberger et al. 2010, Monello et al. 2014, Hobbs and Hooten 2015, Hobbs et al. 2015, Rahio et al. in review).We will use the model to evaluate the population-level effects of GonaCon on the free-ranging horse population at THRO. We will forecast the consequences of alternative contraceptive strategies on population performance with rigorous evaluation of uncertainty. There is an urgent need to extend studies of efficacy of individuals to populations (Ransom et at. 2014). A key extension of our experimental research is to determine the effects of different GonaCon delivery regimes on the growth rate of the THRO population.

OBJECTIVES:

The primary objectives of this research are:

a) To begin to determine the optimum and most effective re-vaccination schedule with GonaCon vaccine for suppressing reproductive rates in free-ranging horses, the duration of effectiveness, and the return to fertility following treatment.

b) To determine the safety and physiological side-effects (if any) in feral horses following re-vaccination with GonaCon including visual assessment of general health, body condition, injection site reactions, effects on current pregnancy, and neonatal health and survival.

c) To determine the effects of GonaCon vaccination on the behavioral side-effects (if any) in free-ranging horses including quantitative assessment of the effects on daily activity patterns and social interactions.

d) To develop and test a safe and effective dart configuration and injection system for remotely administering GonaCon vaccine to free-ranging horses by means of a syringe dart.

e) To develop a Bayesian model to forecast the consequences of different GonaCon vaccine treatments on feral horse population dynamics at THRO.

HYPOTHESIS:

H1: Female feral horses re-vaccinated with GonaCon will show significantly ($P \leq 0.05$) lower reproductive (yearly pregnancy and foaling) rates than non-treated control mares and contraceptive efficacy of re-vaccinated mares will be greater and longer lasting than that observed following the initial immunization.

Rationale: An immune response is a physiologic reaction to a foreign substance or antigen; especially one mediated by lymphocytes and involving recognition of antigens by specific antibodies or previously sensitized lymphocytes. Vaccines rely on the anamnestic response for optimal function. This response is a renewed rapid production of antibodies on the second (subsequent) encounter with the same antigen. This reaction is possible through memory cells that store information regarding the recognition of an antigen based upon previous exposure. Booster or repeat vaccinations generally result in a more rapid and stronger immune reaction to a second inoculation with the same antigen (Tizard 1982). However, the optimum re-vaccination schedule for GonaCon vaccine in feral horses or any other ungulate species has not yet been investigated or determined.

2. Technical Approach:

(Describe how the project will be conducted. The project design must contain enough detail to show the development of the project, including the relationship between the partners, milestones, and objectives. Clearly describe the techniques, procedures, and methodologies to be used; the data collection, analysis, and means of interpretation; the expected results and/or outcomes; and the procedures for evaluating project effectiveness, including appropriate performance measures and the probabilities of obtaining them.)

EXPERIMENTAL DESIGN AND METHODS

Study area and experimental horses

Theodore Roosevelt National Park (THRO) is located near the town of Medora in southwestern North Dakota (45º 55’ N/103º 31’W) and consists of two units that are separated by approximately 115 km of federally and privately owned rangeland. The South Unit of the park, where this study will be conducted, comprises 19,000 ha and consists of eroded badlands with gullies and ravines separated by upland plateaus and small erosion-resistant buttes (Laird 1950). All feral horses used in these experiments are free-ranging and permanently reside in this unit of the park.

At present, there are approximately170 horses divided into roughly 10-15 individual bands and bachelor groups. Horses and bison are confined to the South Unit by a 1.8 to 2.4-m woven wire boundary fence. Feral horse history, distribution, habitat use, and population management at THRO have been previously described (Marlow et al. 1992). Individual horses are known by unique markings and band affiliations. Age and reproductive genealogy data for each animal has been retained in a database since 1993. The approximate date of birth (± 30 days) is known for each horse. Photographs have been taken of each mare from birth to adulthood to assist in the identification of individual horses.

Experimental treatments

In order to determine the optimum re-vaccination schedule for GonaCon vaccine in free-ranging horses at THRO, we propose four post-primary vaccination treatment intervals of: a) four years, b) two years, c) one year, and d) six months (Table 1). The numbers of experimental treatments are limited by the availability of adult mares currently residing in the park. All experimental mares participating in these experiments have been assimilated into various bands such that each band contains one or more individuals from these treatment groups as well as untreated control mares.

Table 1. Summary of primary and secondary vaccination schedules and sample sizes for each experimental group of feral horses treated with GonaCon Immunological Vaccine or saline at THRO.

RE-VACCINATION TREATMENT	SAMPLE SIZE (N)	DATE OF PRIMARY VACCINATION	DATE OF SECONDARY VACCINATION
FOUR YEARS POST-PRIMARY	25	OCT - 2009	SEPT - 2013
TWO YEARS POST-PRIMARY	11	SEPT - 2013	SEPT - 2015
ONE YEAR POST-PRIMARY	16	SEPT - 2015	SEPT - 2016
SIX MONTH POST-PRIMARY	16	SEPT - 2015	MAR - 2016
SALINE CONTROL	25	OCT – 2009	SEPT - 2013

A description of each treatment group, the method of treatment application, and pertinent measurements and observations are presented below:

1) Four-year post-vaccination group. This experimental group was initially established and treated during the scheduled roundup at THRO in 2009. Ongoing measurements of foaling rates and biological side-effects following re-vaccination in 2013 are currently being conducted and will provide a four-year post-primary re-vaccination treatment group (n = 25) and control group (n = 25).

Experimental animals and treatment application: During a scheduled NPS gather and removal in September 2013, horses were herded by helicopter into permanent corrals and handling facilities. Fifty, adult mares (5-19 years of age) (25 GonaCon -treated: 25 saline-control) that had been previously vaccinated with a single inoculation of GonCon- or saline solution in October 2009 were identified and retained within the park for this experiment. Band stallions were also retained. All mares were identified individually using a photographic data base of pelage color and band association, as well as, previously implanted passive integrated transponder (PIT) tags. General health, pregnancy status, and body condition of each animal was assessed while horses were restrained in a hydraulic squeeze chute. Pregnancy status and approximate stage of gestation were determined using rectal palpation of the reproductive tract and transrectal ultrasound imaging (Bucca et al. 2005). Up to 50 mls of blood was collected and serum removed, frozen, and archived for future anti-GnRH antibody analyses (Powers et al. 2011). We collected hair samples from all horses to assess the genetic status of the population and fecal samples for

pregnancy determination and prevalence of endoparasites. Body condition of mares was assessed and scored visually according to methods described by Henneke et al. (1983). Mares in the treatment group received an intramuscular booster inoculation, by hand-syringe, containing 2000µg (2 ml) of GonaCon (synthetic GnRH conjugate Blue Carrier protein and emulsified in AdjuVac^{TM} adjuvant (Miller et al. 2008) in the middle gluteus muscle on the opposite side from the primary vaccination. Mares in the control group were injected in the same way with an equal volume of saline solution. These treatments and procedures were identical to the ones used in 2009 except that injections were given on the right side of the body in 2013 rather than the left to allow differentiation from the previous injection site.

2) Two-year post-vaccination group. This vaccine treatment was applied at the 2013 scheduled roundup at THRO to investigate remote delivery of GonaCon vaccine. Re-vaccination of these mares in 2015 will provide a two-year post-vaccination treatment group.

Experimental animals and treatment application. Based on the promising results from the captive trial conducted in 2013, we will extend our evaluation of a remote dart delivery system of GonaCon from a controlled captive setting to a field test with these same mares that are now free-roaming in their respective bands at THRO. This field application will also provide an additional cohort of mares that have been re-vaccinated two years post-primary vaccination. During September 2015, the eleven mares that were previously administered a primary dose of GonaCon vaccine by means of syringe dart delivery, will be located in the park and re-immunized using the same dart configuration and delivery ballistics as that used for the captive trials in 2013. Each dart will be numbered and correspond to an individual mare. We will determine darting efficacy by measuring the precise dose of the vaccine delivered to each mare. This will be done by weighing each dart (± 0.01g) before and after injection. We will measure dart retention time in each animal and dart performance (i.e. failure rate, partial discharge, blow-out, bounce)**.** In the case of darts that fail to discharge or partially inject the vaccine, the animal will be re-darted until the full dose has been delivered. We will also record each animal's behavioral response to dart injection.

3) One year post-vaccination group and 4) six-month post-vaccination group. Including these two additional re-vaccination treatments will hopefully allow us to more clearly define the optimum re-immunization schedule for GonaCon vaccine in feral horses. However, we have no prior immunological evidence to support these time periods as being optimum or different from each other. These intervals were selected primarily on the basis of practical field application of the vaccine. It would generally be infeasible to locate and treat horses via remote dart delivery during the winter months (December-February) at THRO. Therefore, shorter time periods such as three months (which was the minimum time required for maximum antibody production in elk) (Powers et al. 2011) are not practical. Re-vaccination of mares at the 6 month interval will be conducted in March 2016 and for mares in the one-year interval group during September 2016.

Experimental animals and treatment application. Thirty-two free-ranging mares (1.5-3.5 years of age) will be selected for these treatment groups. A randomized complete block design consisting of either a one year or six-month GonaCon- re-vaccination group will be used in this analysis. Mares will be paired on the basis of age and pregnancy status such that animals within each block (n = 16 blocks of 2 mares each) will be as similar as possible. Within each pair, a mare will be randomly assigned to each experimental group. The general health, pregnancy status, and body condition of each mare will be determined in the field by trained biologist familiar with these animals. Pregnancy status will be determined by fecal estrogen assay (Baker et al. unpublished data). Body condition of all study mares will be evaluated visually and scored on a scale of 1 (very thin) to 9 (very fat) (Henneke et al. 1983). During September 2015, all 32 mares will receive a primary vaccination with GonaCon vaccine via remote dart delivery. Approximately 6 months (March

2016) following the initial vaccination, 16 mares will be re-vaccinated with GonaCon and 1 year later (September 2016) the remaining 16 mares will be similarly treated. All horses will receive the re-vaccination treatment using remote dart delivery.

Field Measurements:

Effects on reproduction. We will determine the effectiveness, duration of effects, and reversibility of a second immunization with GonaCon on reproduction during 2015-2020 (or beyond, if necessary) by comparing foaling and pregnancy rates of treated and control mares. Annual foaling rates will be estimated by observing all mares, at least weekly, during the breeding season (April – August) and documenting the presence of new foals and estimating approximate date of birth. We will continue to monitor reproductive rates in all experimental mares during 2015-2020 or until the magnitude of the difference in foaling rates between treatment and control mares is less than 50% or funding is no longer available. Supplementary to foaling rates, we will also collect fecal samples during approximately mid-gestation (October-February) and determine fecal estradiol concentrations to estimate pregnancy rates of all mares (Baker et al. unpublished data).

Biological side-effects. In conjunction with the above measurements, we will assess the safety and side effects of a second immunization with GonaCon. In both treatement and control groups of horses, we will evaluate the effects (if any) on general health, body condition, existing pregnancy, neonate survival and injection site reactions at weekly intervals during the breeding season and opportunistically throughout the year. In addition, we will observe all experimental mares for presence or absence of lameness (limping, gait alteration, reluctance to stand or bear weight, and evidence of swelling or discharge) at the site/side of vaccine injection. We will classify injection site reactions into four categories according to the scoring system of Roelle and Ransom (2009). Both the previous injection site in 2009 and the one in 2013 will be evaluated each year in conjunction with foaling observations.

Behavioral side-effects. **We evaluated the effects of GonaCon vaccine on the daily activity patterns and social interactions of the four-year post vaccine group during March – August 2014. We used a restricted randomized design to balance observations as much as possible among all experimental animals while also trying to observe the behavior of each mare at least 6-8 times per month. We located bands containing selected mares by vehicle, foot, or horseback. Observations were balanced across time of day and conducted from distances of 50-100m with the aid of binoculars and spotting scopes. Each sampling period consisted of 20 min of continuous observation. We used a combination of instantaneous scan sampling procedure to record time budget data and all-occurrence sampling to record reproductive behaviors (Altmann 1974). We followed field and analytical methods described by Ransom and Cade (2009) to develop a herd-specific ethogram for selected behaviors at THRO. We will compare behavioral observations of GonaCon-treated mares and control mares the first breeding season following primary vaccination in 2010 and following re-vaccination in 2013. Statistical analysis of data followed those described by Ransom et al. (2014). Analysis of these behavioral data will be completed during spring of 2016 and a draft manuscript will be submitted to a peer-reviewed journal for publication (Ransom et al., in preparation).**

Statistical analysis

Our power analysis was originally developed for the four-year post-treatment group but offers an approximation of statistical power needed to detect a treatment effect for other treatments as well. We used a fixed sample size of available mares (n = 50, equally divided into 2

groups of 25 each), to estimate statistical power (1-β) for detecting a treatment effect (0.9 – 0.2) over time. We then used a 1-sided, two-sample t-test with a normal approximation together with software program SYSTAT 12.02.00 (SYSTAT Software, Inc.) to estimate the power for detecting effect sizes that vary from 0.20-0.90 (Kang and Kim 2004) (Table 2). Our current 2-year mean effect- size (difference between mean foaling rates in treatment [0.485] and control [0.759] groups) is 0.274. If repeat vaccination does not improve contraceptive efficacy, we will have little power to detect a difference between treatment groups and will conclude there is little effect due to re-vaccination. However, if revaccination increases effect size to 0.6 or better we will have sufficient power to detect these effects.

We will determine the efficacy of re-vaccination treatments by comparing the proportion of fertile females in each treatment group with control females in the original four-year post-vaccination group combined across all foaling seasons. Females will be classified as being fertile, or infertile on the basis of the presence of a foal at heel, or fecal estrogen concentrations indicating pregnancy. We will use a linear mixed model analysis with restricted maximum likelihood estimation to determine treatment effects on fertility rates. A chi-squire test will be used to test for differences among fertility rates, foal survival, and seasonality of births. We define the foaling season to include March, April, May, June, and July. Results will be shown as means ± standard errors when appropriate.

We will also explore using Bayesian beta-bimodal (similar to the one used by Monello et al. 2014 to estimate elk survival) to examine the size of treatment effects. Power will be less of an issue in this approach because we will be able to show the probability distribution of differences attributable to treatment.

Table 2. Power calculations and corresponding contraceptive treatment effect size for the GonaCon field experiment with free-ranging mares at Theodore Roosevelt National Park.

Total Sample Size	Group Sample Size	THRO Foaling Rate	Effect Size	Alpha	Power (1-β)
50	25	0.759	0.9	0.1	0.977
50	25	0.759	0.8	0.1	0.949
50	25	0.759	0.7	0.1	0.898
50	25	0.759	0.6	0.1	0.817
50	25	0.759	0.5	0.1	0.706
50	25	0.759	0.4	0.1	0.570
50	25	0.759	0.3	0.1	0.425
50	25	0.759	0.2	0.1	0.290

Limitations in study design

One difficulty in this study is that, to our knowledge, there are no published data regarding the optimum re-vaccination schedule for GonaCon vaccine in horses or any other wild or domestic ungulate. Thus, while we may have adequate sample sizes to detect treatment differences between GonaCon-treated and control groups, our sample sizes may be inadequate to detect small differences among the four post-primary treatment groups. This limitation is due to the restricted availability of additional female horses at THRO for this experiment.

Moreover, the control group of mares used to compare treatment effects in this study was originally selected in 2009 to be as similar as possible to the four-year re-vaccination group. However, it is not necessarily representative of the re-vaccinated mares selected for the

subsequent treatments. If this study was implemented in captivity, more appropriate control groups could have been established. Additionally, a more complex study design that incorporated different vaccination time-points and regimes could have more accurately determined the optimal time point for re-vaccination.

Our study was implemented to compliment practical management efforts at THRO that are determined by having reasonable access to study horses for treatment application. Regardless of efficacy outcome, this study will provide valuable information. If re-vaccination at these intervals is not successful, our study will provide important information on the utility of this vaccine. If it is successful, the vaccine may have more wide-spread utility than previously observed.

Performance Measures and Reporting:

2015 - 2016

1. Collect and summarize four-year post-primary vaccination foaling rate estimates for GonaCon-treated mares and control mares for the 2015 and 2016 foaling seasons.

2. Collect and summarize data pertinent to foaling rates and side-effects of GonaCon-treated mares for the two-year post-primary vaccination group for the 2015 and 2016 foaling seasons.

3. Select and document successful re-vaccination of mares in the two-year post-primary vaccination group (11 mares) and primary vaccination of mares in the one-year (16 mares) and six month (16 mares) post-vaccination groups (September 2015).

4. Document successful re-vaccination of mares in the six month revaccination group during March 2016 and for the one-year group in September 2016.

5. Compare foaling rates on all vaccination schedules to their pregnancy rates estimated via fecal estrogen analysis.

6. Provide data analysis summarizing the effects of GonaCon vaccine on daily activity patterns and social interactions of feral horses at THRO during 2015-2016.

BUDGET

Table3. Yearly budget, by category, for proposed research at Theodore Roosevelt National Park 2015-2020.

Category	Year 1	Year 2	Year 3	Year 4	Year 5
Personnel	$40,898	$29,300	$29,878	$34,847	$67,473
Fringe benefits	$7,626	$5,866	$5,991	$7,033	$15,722
Travel	$3,003	$2,946	$1,964	$1,964	$1,964
Equipment	$ 0	$ 0	$ 0	$0	$ 0
Supplies	$4,550	$1,950	$1,950	$1,950	$1,950
Other	$4,500	$ 0	$ 0	$ 0	$ 0
Direct costs	$60,577	$40,062	$39,783	$45,794	$87,109
Indirect costs	$10,601	$7,011	$6,962	$8,014	$15,244
Total costs	$71,178	$47,073	$46,745	$53,808	$102,353

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3. Qualifications, Experience, and Past Performance:
(Describe who will carry out the project activities. List all project personnel, including consultants, contractors, sub-recipients, etc., if known. Describe their responsibilities and the amount of time each will dedicate to the project. Briefly describe how their experience and qualifications are appropriate to successfully achieve the stated objectives.)

Dan L. Baker, Affiliate Faculty, Research Scientist, Colorado State University, Department of Biomedical Sciences/Animal Reproduction and Biotechnology Laboratory: will coordinate all project activities, study design, data collection and analysis, personnel management, reporting, interagency coordination. Dr. Baker has been the project leader in the evaluation of GonaCon in feral horses at Theodore Roosevelt National Park (THRO) since 2009. Prior to that (2006-2013) he was involved with similar research with this contraceptive vaccine in captive and free-ranging elk in Rocky Mountain National Park (ROMO) (50%).

Jenny G. Powers, Wildlife Veterinarian, National Park Service: attending veterinarian, assist with study design, and assessment of biological side-effects of GonaCon vaccine. Dr. Powers has been involved with the evaluation is this contraceptive agent at THRO since 2009 and was involved in similar research with captive and free-ranging elk in ROMO. Much of her

previous research has been focused on the efficacy and physiological side-effects of various contraceptive agents. She will also facilitate animal care and use approval from NPS for this project.

Blake E. McCann, Wildlife Biologist, National Park Service, Theodore Roosevelt National Park: liaison and on-site project manager at THRO, study design, will lead efforts in dart delivery of GonaCon in free-ranging horses, will provide in-kind support for this research effort (i.e. vehicles, office space, housing for field technicians) and coordinate research activities with ongoing NPS operations. Dr. McCann has been involved with the evaluation of GonaCon since 2013 has been instrumental in the design and evaluation of a GonaCon-specific dart configuration and ballistic system for feral horses.

N. Thompson Hobbs, Professor, Senior Research Scientist, Colorado State University (CSU), Department of ESS, Natural Resource Ecology Laboratory: will lead efforts to model effects of fertility control on feral horse population dynamics; provide statistical analysis of data, and coordinate administrative services and support for this project within NREL. Dr. Hobbs has been involved with several projects modeling the effects of fertility control on wild ungulates. He is currently working on a Bayesian state-space model of population dynamics of white-tailed deer to evaluate alternatives for population management including fertility control (5%).

Jason E. Bruemmer, Professor, Colorado State University, Department of Animal Science, Equine Reproduction Laboratory: provide technical expertise on reproductive physiology of feral horses, study design, interpretation of data, and manuscript preparation. Dr. Bruemmer has been involved with this investigation since 2009 and has provided pregnancy assessment of experimental mares at the 2009 and 2013 roundups. We have incorporated his mare pregnancy criteria and body condition scoring system into our field measurements.

Terry M. Nett, Professor, Colorado State University, Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory: provide laboratory services for fecal estrogen assay. Dr. Nett has been involved with this research project since 2009, as well as, similar research with this vaccine in captive and free-ranging elk and domestic horses. He is a leading authority on reproductive endocrinology and GnRH metabolism in mammals (1%).

Kathleen M. Eddy, Laboratory and field research technician, Colorado State University, Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory: Lead responsibility for developing and validating a fecal estrogen assay for pregnancy determination in horses; this assay will supplement foaling rate measurements to assess pregnancy status and treatment responses in experimental mares at THRO. In addition, she will assist with fecal collections and other field measurement (5%).

Douglas C. Eckery, Senior Scientist and Project Leader, USDA, APHIS, Wildlife Services, National Wildlife Research Center: will be primarily responsible for providing 100- 2ml doses of GonaCon-Equine vaccine packaged in 3ml plastic syringes for this study.

APPENDIX

Institutional Animal Care and Use Permits

G. HUMANE CARE AND USE OF ANIMALS

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort / Grant Application

Privileged Communication

Title of proposal: Evaluation of Re-Immunization with GonaCon-Equine™ on Reproduction and Side-Effects in Feral Horses

Investigators: Baker, Dan L.; Nett, Terry M.; Powers, Jenny G; Ransom, Jason I; Bruemmer, Jason E; Hobbs, N. Thompson; McCann, Blake E.

Pursuant to procedures established by the Bureau of Land Management, Wild Horse and Burro Research Program, I certify that the above described protocol follows guidelines set forth in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (#85-23) and the "Animal Welfare Act of 1966" (PL 89-544) as amended.

Signature: _____ Date 4/30/14
Terry Engle, Ph.D., Chair, CSU Institutional Animal Care and Use Committee

Name of Institution: Colorado State University

NOTE: This completed form must be in receipt of the BLM WH&B Research Advisory Team before the initiation of funding or collaborative work can commence. Private individuals must seek local/regional institutional approval.

United States Department of the Interior
NATIONAL PARK SERVICE
Biological Resource Management Division
1201 Oakridge Drive, Suite 200
Fort Collins, Colorado 80525

National Park Service
Institutional Animal Care and Use Committee
Animal Research Protocol Approval

Principal Investigator(s): Dan Baker/ N. Thompson Hobbs
Telephone: 970.556.8518
Electronic Mail: danbaker@colostate.edu

Region: Midwest Region

Protocol Approval Number: MWR_THRO_Baker_Horse_2013.A3

Project Title: Remotely-delivered GnRH Vaccine (GonaCon-Equine) in Free-Ranging Horses: A Preliminary Investigation

Approval Date: 9/23/2013

Effective Date: 9/23/2013

Questionnaire Dates; Years 1 and 2 (if applicable): 9/23/2014, 9/23/2015

Expiration/Re-Submittal Date: 9/23/2016

Funding Agency(ies): None

Species: Horse (*Equus caballus*)

Number(s) of Animals: 10 horses/year, 30 total horses over three years

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This project study was reviewed by the National Park Service Institutional Animal Care and Use Committee. The following action(s) were taken:

Project Status: Approved

Midwest Region/ Intermountain Region/ NPS IACUC Chair: Dan Licht /s/, Mike Wrigley /s/, John Bryan /s/

Standard Operating Procedures for Remote Delivery of GonaCon-Equine in Free-Ranging Horses

Orders for GonaCon – Equine should be placed with the Unites States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS), Pocatello Supply Depot (PSD). The PSD requires all orders to be placed in writing. Orders can be emailed to ws.psd@usda.gov and should include the name of the product being ordered, the quantity being ordered, a physical shipping address for UPS shipping and contact information for the person that should receive the billing invoice. Once the PSD receives the order and determines the shipping charges, an invoice and payment instructions will be emailed to the designated person. Payment can be made via credit card on the pay.gov webpage. Orders for GonaCon-Equine will be shipped once payment confirmation has been received at the PSD. Any questions regarding the ordering process can be sent to ws.psd@usda.gov or call 208-236-6920.

Preparation of Darts for Remote Delivery:

1. The vaccine is distributed as preloaded doses (2 mL) in labeled syringes. Upon receipt, the vaccine should be kept refrigerated (4° C) until use. Do not freeze. The vaccine has a 6-month shelf-life from the time of production and the expiration date will be noted on each syringe that is provided. Important: label instructions must be followed for this product.
2. Although infrequent, dart injections can result in partial injections of the vaccine, and shots are missed. As a precaution, it is recommended that extra doses of the vaccine be ordered to accommodate failed delivery (~15 %). To determine the amount of vaccine delivered, the dart must be weighed before loading, and before and after delivery in the field.
3. For best results, darts with a gel barb should be used. (i.e. 2 cc Pneu-Dart brand darts configured with Slow-inject technology, 3.81 cm long 14 ga.tri-port needles, and gel collars positioned 1.27 cm ahead of the ferrule)
4. Wearing latex gloves, darts are numbered and filled with vaccine by attaching a loading needle (7.62 cm; provided by dart manufacturer) to the syringe containing vaccine and placing the needle into the cannula of the dart to the fullest depth possible. Slowly depress the syringe plunger and begin filling the dart. Periodically, tap the dart on a hard surface to dislodge air bubbles trapped within the vaccine. Due to the viscous nature of the fluid, air entrapment typically results in a maximum of approximately 1.8 ml of vaccine being loaded in the dart. The dart is filled to max once a small amount of the vaccine can be seen at the tri-ports.
5. Important! Do not load and refrigerate darts the night before application. When exposed to moisture and condensation, the edges of gel barbs soften, begin to dissolve, and will not hold the dart in the muscle tissue long enough for full injection of the vaccine. The dart needs to remain in the muscle tissue for a minimum of 1 minute to achieve dependable full injection. Sharp gel barbs are critical. If necessary to load darts the night before application, store loaded darts in an open container within the refrigeration unit.
6. Darts (configured specifically as described above) can be loaded in the field and stored in a cooler prior to application. Darts loaded, but not used can be maintained in a cooler at about 4° C and used the next day, but do not store in a refrigerator or any other container likely to cause condensation.

Standard Operating Procedures for Remote Delivery of GonaCon-Equine in Free-Ranging Horses

Administering the Vaccine:

1. For initial and booster treatments, mares would ideally receive 2.0 ml of GonaCon-Equine. However, experience has demonstrated that only 1.8 ml of vaccine can typically be loaded into 2 cc darts, and this dose has proven successful. Calculations below reflect a 1.8 ml dose.
2. With each injection, the vaccine should be injected into the left or right hind quarters of the mare, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).
3. Darts should be weighed to the nearest hundredth gram by electronic scale when empty, when loaded with vaccine, and after discharge, to ensure that 90% (1.62 ml) of the vaccine has been injected. Animals receiving <50% should be darted with another full dose; those receiving >50% but <90% should receive a half dose (1 ml). All darts should be weighed to verify a combination of ≥1.62 ml has been administered. Therefore, every effort should be made to recover darts after they have fallen from animals.
4. A booster vaccine may be administered 90 or more days after the first injection to improve efficacy of the product over subsequent years.
5. Free ranging animals may be photographed using a telephoto lens and high quality digital receiver as a record of treated individuals, and the injection site can be recorded on data sheets to facilitate identification by animal markings and potential injection scars.

Standard Operating Procedures for Remote Delivery of GonaCon-Equine in Free-Ranging Horses

1. A tracking system would be maintained by NPO detailing the lot number(s) of the vaccine, quantity of vaccine issued, the quantity used, the date of vaccination, disposition of any unused vaccine, the date disposed, the number of treated mares by HMA, field office, and State along with the freeze-mark(s) applied by HMA and date.

FOALING DATA X TREATMENT GROUP FOR 2020 FOALING SEASON

Treatment	Proportion	Effectiveness
Control	0.81 (17/21)	
4yr	0.21 (5/24)	0.74
2yr	0.27 (3/11)	0.66
1yr	0.36 (5/14)	0.55
0.5yr	0.46 (6/13)	0.43

SUMMARY OF FOALING DATA X TREATMENT GROUP ACROSS YEARS

Treatment	Years	Foaling Proportions Range	Effectiveness Range	Total Mares Reversed %
Control	6 (2015-2020	0.76-0.84	n/a	n/a
4yr (hand)	6 (2015-2020	0.00-0.21	0.74-1.00	0.20 (5/25)
2yr (dart)	4 (2017-2020	0.27-0.45	0.42-0.66	0.45 (5/11)
1yr (dart)	3 (2018-2020	0.14-0.36	0.53-0.82	0.57 (8/14)
.5yr (dart)	3 (2018-2020	0.15-0.46	0.43-0.81	0.46 (6/13)