American Wild Horse Conservation®

What is the state of the science on the use of GonaCon™ in wild horses?

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Summary:

Gonadotropin Releasing Hormone (GnRH) immunocontraceptive, e.g., GonaCon™, is intended to be a reversible form of fertility control that is administered to female horses to manage their reproduction. Overall, it has not been as thoroughly researched in wild horses as PZP, and studies indicate that female horses may experience some unintended effects including permanent sterility and injection site reactions. Current studies on GonaCon™ generally show low efficacy upon initial treatment and improved efficacy upon boosting; however, efficacy between published studies has varied widely. More research is needed to determine the optimal booster schedule and the extent of potential side effects, and the possibility of darting this immunocontraceptive.

Information Considered:

How GonaCon Works

GnRH stimulates the pituitary gland to produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones in turn stimulate the growth of follicles and ovulation. GnRH vaccines like GonaCon™ interrupt this mechanism by causing the body to create anti-GnRH antibodies, effectively "removing" the GnRH from this hormone pathway. Without GnRH to stimulate FSH and LH, the ovary does not produce estrogen or progesterone and there is no ovulation. There are two formulations of GonaCon™: GnRH conjugated to (1) keyhole limpet hemocyanin protein (KLH), or (2) blue mollusk protein (B). Two other GnRH vaccines, Equity™ and Improvac®, are produced by Pfizer Animal Health, Australia. GonaCon™ is typically delivered by hand injection, though recent research has explored the use of darts to administer the vaccine.

GonaCon: Efficacy

Studies on the efficacy of GonaCon™ generally show low efficacy in the first year, and decreased efficacy over the remaining years in each study. Booster doses improve efficacy, but with varying results.

- Killian et al. (2008): efficacy of GonaCon-KLH (AdjuVac) over the 4 years of the study was **91, 52, 51, and 43%**, respectively, after a single dose.[1]

[1] Note that there is a discrepancy between the data reported in the abstract and the data reported in the body of the article—the latter data was used to calculate the efficacy. *See also* Killian et al., 2004; Killian et al., 2006.

- Gray et al. (2010): efficacy of GonaCon-B (AdjuVac) over the 3 years of the study was **35, 39, and 64%**, respectively, after a single dose.
- McCann et al. (2017) (unpublished): efficacy of GonaCon-Equine via Slo-inject™ remote delivery device was **45.8%** after a single dose.
  - Darting delivery success rate was 84.1%.
- Baker et al. (2018): efficacy of GonaCon-Equine (AdjuVac) over the first three years of the study was **37%, 28%, and 0%**, respectively, after a single dose; efficacy increased with a hand-injected booster dose (administered in year 4) to **100% and 81%** in the following two years.
- Baker et al. (2023): efficacy of GonaCon-Equine after hand-injected primer dose, followed by booster dose delivered via dart at—
  - 6 months: **62%** efficacy averaged over 3 years (78% 2018, 60% 2019, 48% 2020)
  - 1 year: **64%** efficacy averaged over 3 years (80% 2018, 53% 2019, 60% 2020)
  - 2 years: **61%** efficacy averaged over 4 years (63% 2017, 60% 2018, 53% 2019, 70% 2020)
  - 4 years: **88%** efficacy averaged over 6 years (100% 2015, 81% 2016 94% 2017, 88% 2018, 89% 2019, 77% 2020)

The BLM has been following a 30-day booster protocol that according to the agency has produced significantly better efficacy than a single dose (BLM indicated this was likely on the order of 80%+ efficacy), though efficacy with this protocol has not been published or peer-reviewed, and is not publically available. The EPA registration label does authorize boosting at 30 days. In previous studies with captive female elk, Powers et al. (2011) found maximum antibody concentrations peaked between three and eight months post-primary vaccination, which is why 6 months was considered by Baker to be the minimum interval for the 2023 re-immunization experiment.

Additionally, it was suggested by the National Academy of Sciences (NAS) that the discrepancy in efficacy between the Gray et al. (2010) study and the Killian et al. (2008) study was due either to the conservative method used to estimate efficacy in the former or a difference in body condition scores (BCS) of mares used in study. Mares at a lower nutritional status are expected to have a weaker immune response and therefore less likely to experience the contraceptive effects of the vaccine. Mares in the Killian et al. (2008) study were captive and thus likely in good condition, whereas the mares in the Gray et al. (2010) were free-ranging. Regardless, the relatively high efficacy after a single dose reported in Killian et al. (2008), has not been replicated.

Studies that looked at ovarian suppression in relation to primer and booster doses:

- Botha et al. (2008), South Africa: mares treated with Improvac® twice (days 0 and 35)
  - At day = 35, 14.5% of mares had ovarian activity
  - At day = 70, 0% of mares had ovarian activity

- Elhay et al. (2007), Australia: mares treated with Equity™ twice (days 0 and 28)
  - By four weeks after the booster, the ovaries of the treated mares "resembled those of seasonally anovulatory mares." (NAS (2013), 114)

Botha et al. (2008) indicated that the 14.5% of mares with ovarian activity had their first vaccine during the luteal phase, concluding that the timing of the vaccination in relation to the mare's cycle may be important in maximizing efficacy.

GonaCon: Reversibility

The limited research available indicates that GonaCon™ can be reversible. However, records received through a Freedom of Information Act (FOIA) request reveal that GonaCon™ may result in prolonged efficacy, or effective sterilization, after just one booster dose (2 doses total). The researchers in the Baker et al. (2018) study initially treated mares with a single dose of GonaCon™ in 2009 and followed up with a single booster in 2013. The Baker et al. (2023) study included these mares and as of 2020, 17/24 mares had not regained fertility. It was concluded in Baker et al. 2023 that a definitive time frame of reversibility was not determined for any reimmunization treatment and it was not determined whether permanent infertility was a possible outcome.

The mares in this study were of an average age of 4 (ranging from 2–17) at the time of initial treatment. Thus, at the time of booster, the average age was 8 (6–21). The age of the mares who died during the study is unknown. However, assuming the age figures did not change significantly, the average age by 2020 would have been 15 (13–28). Given that mares generally cease to foal after 20–22 years of age, with declines in reproductive success beginning around 10–12, all members of the group would have passed peak reproductive age and some may not have returned to fertility regardless of any remaining impact resulting from GonaCon™ treatment.

- Baker et al. 2023: Booster doses HB. delivered at shorter intervals show marginally greater reversibility when compared to the 4 year booster:
  - 6 months: **46%** reversibility (6/13)
  - 1 year: **57%** reversibility (8/14)
  - 2 years: **36%** reversibility (4/11)
  - 4 years: **29%** reversibility (7/24)
- M.L. Schulman et al. (2013): Treated mares in three age groups with two doses of Improvac® 35 days apart:
  - Mean time to reverse = **417.8 days** (s.d. ± 23.9; range 232–488 days)
  - All mares showed suppression of cyclicity; **92.2% had reversed by day 720** (found it took longer in younger mares than older mares).

GonaCon: Darting

McCann et al. (2017) (unpublished) tested the delivery success rate of darting GonaCon-Equine via Slo-inject™ as well as the subsequent contraceptive efficacy (see above section). The study reported an 84.1% darting success rate upon the first attempt; mares that were unsuccessfully darted in the first round were successfully darted in the second for a 100% overall success in eventual dosing.

A novel dart-delivery protocol was tested in Theodore Roosevelt National Park and the full protocol was published in Baker et al. (2023) as supplementary material (available here). Although the recommended dose for GonaCon is 2 mL, only 1.8 mL of vaccine can be loaded into 2 cc darts. The dart must be loaded slowly and tapped periodically as due to the viscous nature of the fluid, air bubbles get trapped within the vaccine. GonaCon must use darts with a gel collar to ensure the entire dose is administered; the darts must remain in the muscle tissue for a minimum of 1 minute to achieve full injection per BLM protocols (Three Rivers Complex Burro Gather Plan Environmental Assessment, Appendix I (https://eplanning.blm.gov/public_projects/2025809/200562721/20084906/250091088/AppI_SOPFertCont.pdf). Baker et al. 2023 stated a minimum of 30 seconds for full administration. However, mean retention time of the dart in the marewas 10.33-13.14 minutes (with a standard error of 11.35-9.89 minutes) in Baker et al. 2023. Additionally, darts must be weighed when empty, when loaded with vaccine, and then be collected after discharge and weighed again, where each measurement must be accurate to the nearest hundredth gram. This is to ensure 90% (1.62 ml) of the vaccine has been injected. Animals receiving <50% of the dose should be darted with another full dose and those receiving >50% but <90% should receive a half dose. All darts need to be collected to ensure a proper dose has been administered. In the Three Rivers Complex SOP, they warned that failed delivery may be as high as 15%.

GonaCon is being dart-delivered in the Piceance HMA (CO), Hog Creek HMA (OR), Cold Springs HMA (OR), Sand Springs HMA (OR), Coyote Lake HMA (OR), Onaqui Mountain HMA (UT), and Range Creek HMA (UT); these programs have not reported on any results.

GonaCon: Side Effects: Physical and Physiological

The presence and extent of injection-site reactions from GnRH vaccines have varied considerably. Gray et al. (2010) noted no evidence of injection-site reactions in free-ranging mares treated with GonaCon-B. Botha et al. (2008) noted transient injection-site reactions—gone by day 6—in mares treated with Improvac in the hip. Improvac administered in the neck, Imboden et al. (2006), resulted in more severe reactions to the injections (swelling, pain, stiffness, pyrexia, and apathy), though these signs disappeared within 5 days. This suggested that the location of injection sites could be a contributing factor in reactivity.

However, Baker et al. (2018) found significant injection-site reactivity from GonaCon™ administered in the hip: "approximately 72% of treated mares (21/29) displayed a visible reaction at the site of injection after a single vaccination with GonaCon-Equine. 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." The authors did note, however, that there was no measurable effect on soundness, locomotion, or body condition.

As reported in Baker et al. (2018), Baker et al. (2023) also found significant injection-site reactivity. While only four of the saline control injections showed evidence of reaction (4/51), 89% (115/129) of GonaCon-Equine injections by any delivery method developed visible reactions. Swelling at the injection site accounted for 68% (88/129), while draining abscess accounted for 21% (27/129) and regardless of vaccine delivery, a higher proportion of draining abscesses were recorded after revaccination compared to primary vaccination. No cases of lameness were recorded in the study.

Other side effects include reduced ovarian volume, follicle size, and progesterone concentrations, and the absence of corpora lutea (NAS (2013), 115). GnRH receptors have additionally been identified in the heart, central nervous system, bladder, ovary, testes and placenta which raises concerns that anti-GnRH vaccines could lead to adverse effects outside the reproductive system, as discussed by Kirkpatrick et al. (2011). However, no published data has addressed these concerns directly.

<u>*GonaCon: Side Effects: Pregnancy, Birth Seasonality, and Survival*</u>

The National Academy of Sciences cautioned that "GonaCon should not be administered during early pregnancy because abortion could occur." (NAS (2013), Table 4-1). The risk of abortion results from the inhibition of LH production necessary for progesterone production, which supports the pregnancy during the first 2–3 months (NAS (2013), 98–99). Without LH, there is insufficient progesterone and the pregnancy aborts. Baker et al. (2018) concluded that inoculation during the second trimester did not affect the pregnancy or the neonatal health or survival. One mare in the Gray et al. (2010) study lost her fetus for unknown reasons. This study treated two groups of mares—one in May and one in June—that were combined in the analysis. It is not clear which group the mare came from or her pregnancy status at the time of treatment. Baker et al. 2023 concluded that reimmunization is safe for pregnant females and does not affect neonatal or post-neonatal health or survival when applied mid-gestation.

In addition, treatment during early pregnancy is not advised as titer levels will have decreased by breeding season, leading to a decreased contraceptive effect. In Powers et al. (2011), antibody titers in Elk were measured from time of injection and found that titers were at their highest 3–8 months post-injection; as a result, treatments should be administered 3-8 months prior to breeding to maximize efficacy (Baker et al. 2023).

Neither Gray et al. (2010) nor Baker et al. (2018) found any effects on the birth seasonality, which can shift if there is an extended breeding season, or on foal survival or gender. However, in Baker et al. 2023 the range of parturition dates was more widespread in treated mares (range

349 days) than untreated mares (range 277 days), with a foaling peak 34 days later in treated mares. Additionally, Baker et al. (2023) found that 3% foals born to untreated mares were born out-of-season, compared to 30% for treated mares. However, the birth season did not significantly affect foal survival. Interestingly "68% (11/16) of the treated mares that foaled out-of-season either failed to produce a foal the next year or, if they did foal, the foal was born in-season".

GonaCon: Side Effects: Behavior and Social Dynamics

Gray et al. (2010) found that "there were no treatment effects on activity budget, rates of sexual behavior, proximity between stallions and mares, attempts to initiate proximity, aggression given or received, or band-changing by mares." (NAS (2013), 116) At the time of writing, the NAS report indicated that further studies of the behavioral effects were needed. Published the following year, Ransom et al. (2014) similarly found that while vaccinated mares used their time differently (e.g., time spent feeding), there was no noted difference in social behavior.

Ransom et al. (2014) demonstrated that the vaccine inhibits estrous behavior in a way similar to pregnant mares. Some estrous behavior may remain despite the lack of ovulation, but this phenomenon is also found in some pregnant mares and is believed to be a bonding mechanism. Baker et al. (2018) speculated that vaccinating with GonaCon™ may avoid extending the length and intensity of the breeding season 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."

Analysis:

Side effects and Injection Site Reactions:

The presence and extent of injection-site reactions from GnRH vaccines have varied considerably. Most studies showed reactions resolving within one week of injection. However, both Baker et al. (2018) and Baker et al. (2023) reported high instances of injection side reactions, albeit with no evidence of lameness, altered gait or abnormal range of motion.

Both Baker et al. (2018) and Baker et al. (2023) used saline as the control, as opposed to the adjuvant and foreign protein carrier molecule without the antigen; it is possible that similar injection site reactions would have occurred in the control group had the adjuvant been delivered on its own. Based on this theory, research on alternative adjuvants is currently underway to reduce—and hopefully eliminate—injection site reactions while maintaining efficacy.

Baker et al. 2023 found extended birth phenology in all GonaCon-Equine treatment groups, where contraceptive mares gave birth over a wider time period than untreated mares. However, as stated before this is a single study in a small, isolated population, so it is unclear if these results would be replicated with other, larger populations.

Impact on Reproductive and Social Behaviors:

As social animals, interactions among individuals play a large role in the welfare of wild horses. Though fertility control itself (regardless of the type) may cause changes to reproductive or other social behaviors there is an interest in fertility control methods that minimize such changes.

Though further study is warranted, researchers speculate that GonaCon may offer relief from some negative impacts on reproductive behavior since it mimics a state of pregnancy, much more similar to a mare's natural state during 11 months of the year.

Impact on Fertility—Efficacy and Reversibility:

With one non-reproducible exception, initial doses of GonaCon have failed to provide the high level of efficacy needed for effective management. Once boosted, GonaCon shows significant improvement. With the exception of the 4-year booster protocol, no other boosting protocol achieved higher than 80% effectiveness (Baker et al. 2023). However, all these boosting protocols showed prolonged effectiveness of 48% or greater over the following years.

It is not yet understood the extent to which sterilization is a risk factor for GonaCon—age is a factor that should be accounted for when interpreting the Baker study results. The Baker study involved mares of an average age of 4 (ranging from 2–17) when they were initially treated in 2009. By the time they received their booster 4 years later, the average age would have been 8 (6–21) years old. According to several sources, mares stop giving birth at around 20–22 years old on average, with declines in reproductive success beginning around ages 10–12.[2] In other words, some of these study mares may have aged out of their reproductive eras by the time they received the booster in 2013. By 2020, the mares were an average 15 (13–28) years old—all should have been past their prime and several would have been past the accepted reproductive age entirely.

The raw data has not been released so the age distribution for this group of mares is unknown, and it is unknown if prolonged infertility corresponded with age. We are unaware of any follow up studies that addressed the concern of permanent sterility. Despite this suggestion, the age of the mares should be considered in analyzing the sterility noted in the Baker study.

Both Baker studies (2018 and 2023) had high instances of injection site reactions. These included both swelling and draining abscesses. As the control groups were injected with saline instead of the adjuvant and protein carrier molecule, it is unclear if this is due to these adjuvants or GonaCon-equine itself.

[2] E.g., Jason I. Ransom et al., *Wild and Feral Equid Population Dynamics*, at 69, in Wild Equids: Ecology, Management, and Conservation (2016, eds. Jason I. Ransom & Petra Kaczensky); Cassandra M. V. Nuñez, *Consequences of Porcine Zona Pellucida Immunocontraception to Feral Horses*, 12 HUMAN-WILDLIFE INTERACTIONS 135 (2018).

Practicality of Darting:

Baker et al. 2023 reported of the 88 shots taken at 40 individual mares, 67 successfully delivered full doses at first attempt. Additionally, in eight cases delivery of <90% (86.7-89.4%) were deemed successful. Of the 88 shots, only 3 darts were not recovered. However, as noted in the study, these horses were conditioned to human presence and behavioral aversion to darting was not evident. However, the complexity of delivering GonaCon via dart is a major concern. These darts must be weighed to the nearest hundredth of a gram, before loading, after loading, and then after delivery. This is further complicated by the gel collar on the dart which requires the dart to stay in the mare longer. Baker et al. 2023 had retention time of the darts in the mare average ranged 10.33-13.14 minutes with a large standard error (11.35-9.89) meaning these darts can be expected to stay embedded in the mare anywhere from immediate ejection to upwards of 23 minutes. Although the vaccine may be stored refrigerated, loaded darts must be stored well ventilated while refrigerated, elsewise the gel collar will be compromised, and the dart will be unusable.

Still Unknown:

It is still unknown whether there are long-term physiological effects or risk of effective sterilization. Given that GnRH receptors occur throughout the body, the long-term impact of blocking GnRH production on the heart, central nervous system, and other organs warrants investigation. (Kirkpatrick, Lyda & Frank 2011).

As the most robust studies (Baker et al. 2018 & 2023) were performed on the same confined herd, it is unknown how individual genetics affect these results. Additionally, no large population scale studies have been performed, so it is unclear if the Baker results can be replicated. Changes in parturition seasonality were only reported on in one study (Baker et al. 2023) so it is unclear if those findings would be replicated elsewhere. Additionally, as the Baker et al. (2018) study measured foaling - as opposed to contraception - research is needed to determine whether the efficacy results can be explained by a reduction in pregnancy rate or whether some mares became pregnant and lost the foals due to uterine issues.

Summary:

Based on the current published literature available:

1. Gonacon has unpredictable efficacy and unpredictable reversibility with efficacy often reported at levels lower than with PZP.
    a. Efficacy with a single dose ranged from 35-64% when excluding the Killian study that was not on free-roaming horses and has never been replicated.
    b. The published studies on efficacy after a booster dose are Baker et al. (2018) and Baker et al. (2023), which were performed on the same herd. Efficacy was:

i. 6 months: 62% efficacy averaged over 3 years (Max: 78%)
ii. 1 year: 64% efficacy averaged over 3 years (Max: 80%)
iii. 2 years: 61% efficacy averaged over 4 years (Max: 70%)
iv. 4 years: 88% efficacy averaged over 6 years (Max: 100%)

c. There have been **0** studies released or published on the efficacy of Gonacon with a 30 day booster so its efficacy is completely unknown.
d. It is not clear what population coverage would be required to show population decline with these lower efficacy results, assumably it would require >80%.
e. When all boosting treatments were combined, Baker et al. 2023 showed an overall return to fertility of only 40% (25/62).
   i. This indicates there could be a potential for sterilization after only 2 doses; however, multiple factors including age and sample size need to be taken into account when interpreting these results.

2. Significant research on Gonacon is still necessary to answer questions on population level effects, efficacy and reversibility.
   a. Although there are multiple HMAs receiving GonaCon treatment, there is no data available on the success of these programs, and it is unclear if data is being collected on these herds. If/when there are multiple studies from multiple populations, data on efficacy and reversibility will be significantly more reliable.
   b. Baker et. al. 2023 even concludes that population-level experiments are still needed to prove GonaCon can produce meaningful reductions.
3. Gonacon darting protocol is complex, introduces a large chance for error, and requires significantly more effort than darting PZP.
   a. Darts must be weighed to the nearest hundredth of a gram, before loading, after loading, and then after delivery.
   b. This is further complicated by the darts staying in the mare on the order of 10-15 minutes, meaning the darter would have to follow each and every mare for this length of time and then find the dart in order to confirm appropriate dosage was delivered.
   c. As Gonacon cannot fit the required 2cc dose into darts, a very small amount of undelivered vaccine would mean the mare did not receive a full dose and would need to be darted again.

Based on this review, it is concluded that there is insufficient data on the vaccine's efficacy, reversibility, potential side effects, and darting feasibility to justify its use as a management tool for federally-protected herds at this time.

<u>Sources</u>:

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