

Appendix B

Scientific Literature Review

This appendix includes scientific literature reviews addressing five topics: effects of gathers, effects of wild horses and burros on rangeland ecosystems, effects of fertility control vaccines and sex ratio manipulations, effects of sterilization, and effects of intrauterine devices (IUDs). This scientific literature review was compiled by the BLM's Wild Horse and Burro Program Research Coordinator (Paul Griffin, Ph.D.) and is considered current as of October 2020.

Effects of Gathers on Wild Horses and Burros

Gathering any wild animals into pens has the potential to cause impacts to individual animals. There is also the potential for impacts to individual horses and burros during transportation, short-term holding, long-term holding that take place after a gather. However, BLM follows guidelines to minimize those impacts and ensure humane animal care and high standards of welfare. The following literature review summarizes the limited number of scientific papers and government reports that have examined the effects of gathers and holding on wild horses and burros.

Two early papers, by Hansen and Mosley (2000) and Ashley and Holcomb (2001) examined limited effects of gathers, including behavioral effects and effects on foaling rates. Hansen and Mosley (2000) observed BLM gathers in Idaho and Wyoming. They monitored wild horse behaviors before and after a gather event and compared the behavioral and reproductive outcomes for animals that were gathered by helicopter against those outcomes for animals that were not. This comparison led to the conclusion that gather activities used at that time had no effect on observed wild horse foraging or social behaviors, in terms of time spent resting, feeding, vigilant, traveling, or engaged in agonistic encounters (Hansen and Mosley 2000). Similarly, the authors did not find any statistically significant difference in foaling rates in the year after the gather in comparisons between horses that were captured, those that were chased by a helicopter but evaded capture, or those that were not chased by a helicopter. The authors concluded that the gathers had no deleterious effects on behavior or reproduction. Ashley and Holcomb (2001) conducted observations of reproductive rates at Garfield Flat HMA in Nevada, where horses were gathered in 1993 and 1997, and compared those observations at Granite Range HMA in Nevada, where there was no gather. The authors found that the two gathers had a short-term effect on foaling rates; pregnant mares that were gathered had lower foaling rates than pregnant mares that were not gathered. The authors suggested that BLM make changes to the gather methods used at that time, to minimize the length of time that pregnant mares are held prior to their release back to the range. Since the publications by Hansen and Mosley (2000) and by Ashley and Holcomb (2001), BLM did make changes to reduce the stress that gathered animals, including pregnant females, may experience as a result of gather and removal activities; these measures have been formalized as policy in the comprehensive animal welfare program (BLM IM 2015-151).

A thorough review of gather practices and their effects on wild horses and burros can be found in a 2008 report from the Government Accounting Office. The report found that the BLM had controls in place to help ensure the humane treatment of wild horses and burros (GAO 2008). The controls included SOPs for gather operations, inspections, and data collection to monitor animal welfare. These procedures led to humane treatment during gathers, and in short-term and long-term holding facilities. The report found that cumulative effects associated with the capture and removal of excess wild horses include gather-related

mortality averaged only about 0.5% and approximately 0.7% of the captured animals, on average, are humanely euthanized due to pre-existing conditions (such as lameness or club feet) in accordance with BLM policy. Scasta (2020) found the same overall mortality rate (1.2%) for BLM WH&B gathers in 2010-2019, with a mortality rate of 0.25% caused directly by the gather, and a mortality rate of 0.94% attributable to euthanasia of animals with pre-existing conditions such as blindness or club-footedness. Scasta (2020) summarized mortality rates from 70 BLM WH&B gathers across nine states, from 2010-2019. Records for 28,821 horses and 2,005 burros came from helicopter and bait/water trapping. For wild burro bait / water trapping, mortality rates were 0.05% due to acute injury caused by the gather process, and death for burros with pre-existing conditions was 0.2% (Scasta 2020). For wild horse bait / water trapping, mortality rates were 0.3% due to acute injury, and the mortality rate due to pre-existing conditions was 1.4% (Scasta 2020). For wild horses gathered with the help of helicopters, mortality rates were only slightly lower than for bait / water trapping, with 0.3% due to acute causes, and 0.8% due to pre-existing conditions (Scasta 2020). Scasta (2020) noted that for other wildlife species capture operations, mortality rates above 2% are considered unacceptable and that, by that measure, BLM WH&B "...welfare is being optimized to a level acceptable across other animal handling disciplines."

The GAO report (2008) noted the precautions that BLM takes before gather operations, including screening potential gather sites for environmental and safety concerns, approving facility plans to ensure that there are no hazards to the animals there, and limiting the speeds that animals travel to trap sites. BLM used SOPs for short-term holding facilities (e.g., corrals) that included procedures to minimize excitement of the animals to prevent injury, separating horses by age, sex, and size, regular observation of the animals, and recording information about the animals in a BLM database. The GAO reported that BLM had regular inspections of short-term holding facilities and the animals held there, ensuring that the corral equipment is up to code and that animals are treated with appropriate veterinary care (including that hooves are trimmed adequately to prevent injury). Mortality was found to be about 5% per year associated with transportation, short term holding, and adoption or sale with limitations. The GAO noted that BLM also had controls in place to ensure humane care at long-term holding facilities (i.e., pastures). BLM staff monitor the number of animals, the pasture conditions, winter feeding, and animal health. Veterinarians from the USDA Animal and Plant Health Inspection Service inspect long-term facilities annually, including a full count of animals, with written reports. Contract veterinarians provide animal care at long-term facilities, when needed. Weekly counts provide an incentive for contractors that operate long-term holding facilities to maintain animal health (GAO 2008). Mortality at long-term holding was found to be about 8% per year, on average (GAO 2008). The mortality rates at short-term and long-term holding facilities are comparable to the natural annual mortality rate on the range of about 16% per year for foals (animals under age 1), about 5-10% per year for horses ages 1-10 years, and about 10-25% for animals aged 10-20 years (Ransom et al. 2016).

In 2010, the American Association of Equine Practitioners (AAEP 2011) was invited by the BLM to visit the BLM operations and facilities, spend time on WH&B gathers and evaluate the management of the wild equids. The AAEP Task Force evaluated horses in the BLM Wild Horse and Burro Program through several visits to wild horse gathers, and short- and long-term holding facilities. The task force was specifically asked to "review animal care and handling within the Wild Horse and Burro Program, and make whatever recommendations, if any, the Association feels may be indicated, and if possible, issue a public statement regarding the care and welfare of animals under BLM management." In their report (AAEP 2011), the task force concluded "that the care, handling and management practices utilized by the agency are appropriate for this population of horses and generally support the safety, health status and welfare of the animals."

In June 2010 BLM invited independent observers organized by American Horse Protection Association (AHPA) to observe BLM gathers and document their findings. AHPA engaged four independent

credentialed professionals who are academia-based equine veterinarians or equine specialists. Each observer served on a team of two and was tasked specifically to observe the care and handling of the animals for a 3-4-day period during the gather process and submit their findings to AHPA. An Evaluation Checklist was provided to each of the observers that included four sections: Gather Activities; Horse Handling During Gather; Horse Description; and Temporary Holding Facility. The independent group visited three separate gather operations and found that “BLM and contractors are responsible and concerned about the welfare of the horses before, during and after the gather process” and that “gentle and knowledgeable, used acceptable methods for moving horses... demonstrated the ability to review, assess and adapt procedures to ensure the care and well-being of the animals” (Greene et al. 2013).

BLM commissioned the Natural Resources Council of the National Academies of Sciences (NAS) to conduct an independent, technical evaluation of the science, methodology, and technical decision-making approaches of the BLM Wild Horse and Burro Management Program. Among the conclusions of their 2013 report, NAS (2013) concluded that wild horse populations grow at 15-20 percent a year, and that predation will not typically control population growth rates of free-ranging horses. The report (NAS 2013) also noted that, because there are human-created barriers to dispersal and movement (such as fences and highways) and no substantial predator pressure, maintaining a herd within an AML requires removing animals in roundups, also known as gathers, and may require management actions that limit population growth rates. The report (NAS 2013) examined a number of population growth suppression techniques, including the use of sterilization, fertility control vaccines, and sex ratio manipulation.

The effects of gathers as part of feral horse management have also been documented on National Park Service Lands. Since the 1980s, managers at Theodore Roosevelt National Park have used periodic gathers, removals, and auctions to maintain the feral horse herd size at a carrying capacity level of 50 to 90 horses (Amberg et al. 2014). In practical terms, this carrying capacity is equivalent to an AML. Horse herd sizes at those levels were determined to allow for maintenance of certain sensitive forage plant species. Gathers every 3-5 years did not prevent the herd from self-sustaining. The herd continues to grow, to the point that the NPS now uses gathers and removals along with temporary fertility control methods in its feral horse management (Amberg et al. 2014).

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Effects of Wild Horses and Burros on Rangeland Ecosystems

The presence of wild horses and wild burros can have substantial effects on rangeland ecosystems, and on the capacity for habitat restoration efforts to achieve landscape conservation and restoration goals.

In the biological sense, all free-roaming horses and burros in North America are feral, meaning that they are descendants of domesticated animals brought to the Americas by European colonists. Horses went extinct in the Americas by the end of the Pleistocene, about 10,000 years ago (Webb 1984; MacFadden 2005). Burros evolved in Eurasia (Geigl et al. 2016). The published literature refers to free-roaming horses and burros as either feral or wild. In the ecological context the terms are interchangeable, but the terms 'wild horse' and 'wild burro' are associated with a specific legal status. The following literature review on the effects of wild horses and burros on rangeland ecosystems draws on scientific studies of feral horses and burros, some of which also have wild horse or wild burro legal status. The following literature review draws on Parts 1 and 2 of the 'Science framework for conservation and restoration of the sagebrush biome' interagency report (Chambers et al. 2017, Crist et al. 2019).

Because of the known damage that overpopulated wild horse and burro herds can cause in rangeland ecosystems, the presence of wild horses and burros is considered a threat to Greater sage-grouse habitat quality, particularly in the bird species' western range (Beever and Aldridge 2011, USFWS 2013). Wild horse population sizes on federal lands have more than doubled in the five years since the USFWS report (2013) was published (BLM 2018). On lands administered by the BLM, there were an estimated 81,951 BLM-administered wild horses and burros as of March 1, 2018, which does not include foals born in 2018. Lands with wild horses and burros are managed for multiple uses, so it can be difficult to parse out their ecological effects. Despite this, scientific studies designed to separate out those effects, which are summarized below, point to conclusions that landscapes with greater wild horse and burro abundance will tend to have lower resilience to disturbance and lower resistance to invasive plants than similar landscapes with herds at or below target AML levels.

In contrast to managed livestock grazing, neither the seasonal timing nor the intensity of wild horse and burro grazing can be managed, except through efforts to manage their numbers and distribution. Wild horses live on the range year round, they roam freely, and wild horse populations have the potential to

grow 15-20% per year (Wolfe 1980; Eberhardt et al. 1982; Garrott et al 1991; Dawson 2005; Roelle et al. 2010; Scorolli et al. 2010). Although this annual growth rate may be lower in some areas where mountain lions can take foals (Turner and Morrison 2001, Turner 2015), horses tend to favor use of more open habitats (Schoenecker 2016) that are dominated by grasses and shrubs and where ambush is less likely. Horses can compete with managed livestock in forage selected (Scasta et al. 2016). For the majority of wild horse herds, there is little overall evidence that population growth is significantly affected by predation. As a result of the potential for wild horse populations to grow rapidly, impacts from wild horses on water, soil, vegetation, and native wildlife resources (Davies and Boyd 2019) can increase exponentially unless there is active management to limit their population sizes.

The USFWS (2008), Beever and Aldridge (2011), and Chambers et al (2017) summarize much of the literature that quantifies direct ecosystem effects of wild horse presence. Beever and Aldridge (2011) present a conceptual model that illustrates the effects of wild horses on sagebrush ecosystems. In the Great Basin, areas without wild horses had greater shrub cover, plant cover, species richness, native plant cover, and overall plant biomass, and less cover percentage of grazing-tolerant, unpalatable, and invasive plant species, including cheatgrass, compared to areas with horses (Smith 1986; Beever et al. 2008; Davies et al. 2014; Zeigenfuss et al. 2014; Boyd et al. 2017). There were also measurable increases in soil penetration resistance and erosion, decreases in ant mound and granivorous small mammal densities, and changes in reptile communities (Beever et al. 2003; Beever and Brussard 2004; Beever and Herrick 2006; Ostermann-Kelm et al. 2009). Intensive grazing by horses and other ungulates can damage biological crusts (Belnap et al. 2001). In contrast to domestic livestock grazing, where post-fire grazing rest and deferment can foster recovery, wild horse grazing occurs year-round. These effects imply that horse presence can have broad effects on ecosystem function that could influence conservation and restoration actions.

Many studies corroborate the general conclusion that wild horses can lead to biologically significant changes in rangeland ecosystems, particularly when their populations are overabundant relative to water and forage resources, and other wildlife living on the landscape (Eldridge et al. 2020). Horses are primarily grazers (Hanley and Hanley 1982), but shrubs – including sagebrush – can represent a large part of a horse’s diet, at least in summer in the Great Basin (Nordquist 2011). Grazing by wild horses can have severe impacts on water source quality, aquatic ecosystems and riparian communities as well (Beever and Brussard 2000; Barnett 2002; Nordquist 2011; USFWS 2008; Earnst et al. 2012; USFWS 2012, Kaweck et al. 2018), sometimes excluding native ungulates from water sources (Ostermann-Kelm et al. 2008; USFWS 2008; Perry et al. 2015; Hall et al. 2016; Gooch et al. 2017; Hall et al. 2018). Impacts to riparian vegetation per individual wild horse can exceed impacts per individual domestic cow (Kaweck et al. 2018). Bird nest survival may be lower in areas with wild horses (Zalba and Cozzani 2004), and bird populations have recovered substantially after livestock and / or wild horses have been removed (Earnst et al. 2005; Earnst et al. 2012; Batchelor et al. 2015). Wild horses can spread non-native plant species, including cheatgrass, and may limit the effectiveness of habitat restoration projects (Beever et al. 2003; Couvreur et al. 2004; Jessop and Anderson 2007; Loydi and Zalba 2009). Riparian and wildlife habitat improvement projects intended to increase the availability of grasses, forbs, riparian habitats, and water will likely attract and be subject to heavy grazing and trampling by wild horses that live in the vicinity of the project. Even after domestic livestock are removed, continued wild horse grazing can cause ongoing detrimental ecosystem effects (USFWS 2008; Davies et al. 2014) which may require several decades for recovery (e.g., Anderson and Inouye 2001).

Most analyses of wild horse effects have contrasted areas with wild horses to areas without, which is a study design that should control for effects of other grazers, but historical or ongoing effects of livestock grazing may be difficult to separate from horse effects in some cases (Davies et al. 2014). Analyses have generally not included horse density as a continuous covariate; therefore, ecosystem effects have not been

quantified as a linear function of increasing wild horse density. One exception is an analysis of satellite imagery confirming that varied levels of feral horse biomass were negatively correlated with average plant biomass growth (Ziegenfuss et al. 2014).

Horses require access to large amounts of water; an individual can drink an average of 7.4 gallons of water per day (Groenendyk et al. 1988). Despite a general preference for habitats near water (e.g., Crane et al. 1997), wild horses will routinely commute long distances (e.g., 10+ miles per day) between water sources and palatable vegetation (Hampson et al. 2010).

Wild burros can also substantially affect riparian habitats (e.g., Tiller 1997), native wildlife (e.g., Seegmiller and Ohmart 1981), and have grazing and trampling impacts that are similar to wild horses (Carothers et al. 1976; Hanley and Brady 1977; Douglas and Hurst 1983). Where wild burros and Greater sage-grouse co-occur, burros' year-round use of low-elevation habitats may lead to a high degree of overlap between burros and Greater sage-grouse (Beever and Aldridge 2011).

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Effects of Fertility Control Vaccines and Sex Ratio Manipulations

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Although fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

An extensive body of peer-reviewed scientific literature details the impacts of fertility control methods on wild horses and burros. No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros, but NEPA analysis has been required. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of fertility control vaccine use in mares, then of sex ratio manipulation. This review does not examine effects of spaying and neutering. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not yet addressed in horses or burros specifically. While most studies reviewed here refer to horses, burros are extremely similar in terms of physiology, such that expected effects are comparable, except where differences between the species are noted.

On the whole, the identified impacts are generally transient and affect primarily the individuals treated. Fertility control that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single herd management areas (HMAs), in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of “metapopulations” – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception alone does not remove excess horses from an HMA’s population, so one or more gathers

are usually needed in order to bring the herd down to a level close to AML. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), fertility control methods such as immunocontraceptive vaccines and sex ratio manipulation are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even more modest fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers. Bartholow (2007) concluded that the application of 2-year or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs. Because applying contraception to horses requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000).

In the context of BLM wild horse and burro management, fertility control vaccines and sex ratio manipulation rely on reducing the number of reproducing females. Taking into consideration available literature on the subject, the National Academies of Sciences concluded in their 2013 report that forms of fertility control vaccines were two of the three ‘most promising’ available methods for contraception in wild horses and burros (NAS 2013). That report also noted that sex ratio manipulations where herds have approximately 60% males and 40% females can expect lower annual growth rates, simply as a result of having a lower number of reproducing females.

Fertility Control Vaccines

Fertility control vaccines (also known as immunocontraceptives) meet BLM requirements for safety to mares and the environment (EPA 2009a, 2012). Because they work by causing an immune response in treated animals, there is no risk of hormones or toxins being taken into the food chain when a treated mare dies. The BLM and other land managers have mainly used three fertility control vaccine formulations for fertility control of wild horse mares on the range: ZonaStat-H, PZP-22, and GonaCon-Equine. As other formulations become available, they may be applied in the future.

In any vaccine, the antigen is the stimulant to which the body responds by making antigen-specific antibodies. Those antibodies then signal to the body that a foreign molecule is present, initiating an immune response that removes the molecule or cell. Adjuvants are additional substances that are included in vaccines to elevate the level of immune response. Adjuvants help to incite recruitment of lymphocytes and other immune cells which foster a long-lasting immune response that is specific to the antigen.

Liquid emulsion vaccines can be injected by hand or remotely administered in the field using a pneumatic dart (Roelle and Ransom 2009, Rutberg et al. 2017, McCann et al. 2017) in cases where mares are relatively approachable. Use of remotely delivered (dart-delivered) vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached within 50 m (BLM 2010). Booster doses can be safely administered by hand or by dart. Even with repeated booster treatments of the vaccines, it is expected that most mares would eventually return to fertility, though some individual mares treated repeatedly may remain infertile. Once the herd size in a project area is at AML and population growth seems to be stabilized, BLM can make adaptive determinations as to the required frequency of new and booster treatments.

BLM has followed SOPs for fertility control vaccine application (BLM IM 2009-090). Herds selected for fertility control vaccine use should have annual growth rates over 5%, have a herd size over 50 animals, and have a target rate of treatment of between 50% and 90% of female wild horses or burros. The IM

requires that treated mares be identifiable via a visible freeze brand or individual color markings, so that their vaccination history can be known. The IM calls for follow-up population surveys to determine the realized annual growth rate in herds treated with fertility control vaccines.

Vaccine Formulations: Porcine Zona Pellucida (PZP)

PZP vaccines have been used on dozens of horse herds by the National Park Service, US Forest Service, Bureau of Land Management, and Native American tribes and PZP vaccine use is approved for free-ranging wild and feral horse herds in the United States (EPA 2012). PZP use can reduce or eliminate the need for gathers and removals, if very high fractions of mares are treated over a very long time period (Turner et al. 1997). PZP vaccines have been used extensively in wild horses (NAS 2013), and in feral burros on Caribbean islands (Turner et al. 1996, French et al. 2017). PZP vaccine formulations are produced as ZonaStat-H, an EPA-registered commercial product (EPA 2012, SCC 2015), as PZP-22, which is a formulation of PZP in polymer pellets that can lead to a longer immune response (Turner et al. 2002, Rutberg et al. 2017), and as Spayvac, where the PZP protein is enveloped in liposomes (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018). ‘Native’ PZP proteins can be purified from pig ovaries (Liu et al. 1989). Recombinant ZP proteins may be produced with molecular techniques (Gupta and Minhas 2017, Joonè et al. 2017a, Nolan et al. 2018a).

When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2012). In keeping with the EPA registration for ZonaStat-H (EPA 2012; reg. no. 86833-1), certification through the Science and Conservation Center in Billings Montana is required to apply that vaccine to equids.

For maximum effectiveness, PZP is administered within the December to February timeframe. When applying ZonaStat-H, first the primer with modified Freund’s Complete adjuvant is given and then the booster with Freund’s Incomplete adjuvant is given 2-6 weeks later. Preferably, the timing of the booster dose is at least 1-2 weeks prior to the onset of breeding activity. Following the initial 2 inoculations, only annual boosters are required. For the PZP-22 formulation, each released mare would receive a single dose of the two-year PZP contraceptive vaccine at the same time as a dose of the liquid PZP vaccine with modified Freund’s Complete adjuvant. The pellets are applied to the mare with a large gauge needle and jab-stick into the hip. Although PZP-22 pellets have been delivered via darting in trial studies (Rutberg et al 2017, Carey et al. 2019), BLM does not plan to use darting for PZP-22 delivery until there is more demonstration that PZP-22 can be reliably delivered via dart.

Vaccine Formulations: Gonadotropin Releasing Hormone (GnRH)

GonaCon (which is produced under the trade name GonaCon-Equine for use in feral horses and burros) is approved for use by authorized federal, state, tribal, public and private personnel, for application to free-ranging wild horse and burro herds in the United States (EPA 2013, 2015). GonaCon has been used on feral horses in Theodore Roosevelt National Park and on wild horses administered by BLM (BLM 2015). GonaCon has been produced by USDA-APHIS (Fort Collins, Colorado) in several different formulations, the history of which is reviewed by Miller et al. (2013). GonaCon vaccines present the recipient with hundreds of copies of GnRH as peptides on the surface of a linked protein that is naturally antigenic because it comes from invertebrate hemocyanin (Miller et al 2013). Early GonaCon formulations linked many copies of GnRH to a protein from the keyhole limpet (GonaCon-KHL), but more recently produced formulations where the GnRH antigen is linked to a protein from the blue mussel (GonaCon-B) proved less expensive and more effective (Miller et al. 2008). GonaCon-Equine is in the category of GonaCon-B vaccines.

As with other contraceptives applied to wild horses, the long-term goal of GonaCon-Equine use is to reduce or eliminate the need for gathers and removals (NAS 2013). GonaCon-Equine contraceptive

vaccine is an EPA-approved pesticide (EPA, 2009a) that is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and is produced in a USDA-APHIS laboratory. GonaCon is a pharmaceutical-grade vaccine, including aseptic manufacturing technique to deliver a sterile vaccine product (Miller et al. 2013). If stored at 4° C, the shelf life is 6 months (Miller et al 2013).

Miller et al. (2013) reviewed the vaccine environmental safety and toxicity. When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2009b). EPA waived a number of tests prior to registering the vaccine, because GonaCon was deemed to pose low risks to the environment, so long as the product label is followed (Wang-Cahill et al., *in press*).

GonaCon-Equine can safely be reapplied as necessary to control the population growth rate; booster dose effects may lead to increased effectiveness of contraception, which is generally the intent. Even after booster treatment of GonaCon-Equine, it is expected that most, if not all, mares would return to fertility at some point. Although the exact timing for the return to fertility in mares boosted more than once with GonaCon-Equine has not been quantified, a prolonged return to fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

The adjuvant used in GonaCon, Adjuvac, generally leads to a milder reaction than Freund's Complete Adjuvant (Powers et al. 2011). Adjuvac contains a small number of killed *Mycobacterium avium* cells (Miller et al. 2008, Miller et al. 2013). The antigen and adjuvant are emulsified in mineral oil, such that they are not all presented to the immune system right after injection. It is thought that the mineral oil emulsion leads to a 'depot effect' that is associated with slow or sustained release of the antigen, and a resulting longer-lasting immune response (Miller et al. 2013). Miller et al. (2008, 2013) have speculated that, in cases where memory-B leukocytes are protected in immune complexes in the lymphatic system, it can lead to years of immune response. Increased doses of vaccine may lead to stronger immune reactions, but only to a certain point; when Yoder and Miller (2010) tested varying doses of GonaCon in prairie dogs, antibody responses to the 200µg and 400µg doses were equal to each other but were both higher than in response to a 100µg dose.

Direct Effects: PZP Vaccines

The historically accepted hypothesis explaining PZP vaccine effectiveness posits that when injected as an antigen in vaccines, PZP causes the mare's immune system to produce antibodies that are specific to zona pellucida proteins on the surface of that mare's eggs. The antibodies bind to the mare's eggs surface proteins (Liu et al. 1989), and effectively block sperm binding and fertilization (Zoo Montana, 2000). Because treated mares do not become pregnant but other ovarian functions remain generally unchanged, PZP can cause a mare to continue having regular estrus cycles throughout the breeding season. More recent observations support a complementary hypothesis, which posits that PZP vaccination causes reductions in ovary size and function (Mask et al. 2015, Joonè et al. 2017b, Joonè et al. 2017c, Nolan et al. 2018b, 2018c). PZP vaccines do not appear to interact with other organ systems, as antibodies specific to PZP protein do not cross-react with tissues outside of the reproductive system (Barber and Fayrer-Hosken 2000).

Research has demonstrated that contraceptive efficacy of an injected liquid PZP vaccine, such as ZonaStat-H, is approximately 90% or more for mares treated twice in the first year (Turner and Kirkpatrick 2002, Turner et al. 2008). The highest success for fertility control has been reported when the vaccine has been applied November through February. High contraceptive rates of 90% or more can be maintained in horses that are given a booster dose annually (Kirkpatrick et al. 1992). Approximately 60% to 85% of mares are successfully contracepted for one year when treated simultaneously with a liquid primer and PZP-22 pellets (Rutberg et al. 2017, Carey et al. 2019). Application of PZP for fertility control

would reduce fertility in a large percentage of mares for at least one year (Ransom et al. 2011). The contraceptive result for a single application of the liquid PZP vaccine primer dose along with PZP vaccine pellets (PZP-22), based on winter applications, can be expected to fall in the approximate efficacy ranges as follows (based on figure 2 in Rutberg et al. 2017). Below, the approximate efficacy is measured as the relative decrease in foaling rate for treated mares, compared to control mares:

Year 1	Year 2	Year 3
0 (developing fetuses come to term)	~30-75%	~20-50%

If mares that have been treated with PZP-22 vaccine pellets subsequently receive a booster dose of either the liquid PZP vaccine or the PZP-22 vaccine pellets, the subsequent contraceptive effect is apparently more pronounced and long-lasting. The approximate efficacy following a booster dose can be expected to be in the following ranges (based on figure 3 in Rutberg et al. 2017).

Year 1	Year 2	Year 3	Year 4
0 (developing fetuses come to term)	~50-90%	~55-75%	~40-75%

The fraction of mares treated in a herd can have a large effect on the realized change in growth rate due to PZP contraception, with an extremely high portion of mares required over many years to be treated to totally prevent population-level growth (e.g., Turner and Kirkpatrick 2002). Gather efficiency does not usually exceed 85% via helicopter, and may be less with bait and water trapping, so there will almost always be a portion of the female population uncaptured that is not treated in any given year. Additionally, some mares may not respond to the fertility control vaccine, but instead will continue to foal normally.

Direct Effects: GnRH Vaccines

GonaCon-Equine is one of several vaccines that have been engineered to create an immune response to the gonadotropin releasing hormone peptide (GnRH). GnRH is a small peptide that plays an important role in signaling the production of other hormones involved in reproduction in both sexes. When combined with an adjuvant, a GnRH vaccine stimulates a persistent immune response resulting in prolonged antibody production against GnRH, the carrier protein, and the adjuvant (Miller et al., 2008). The most direct result of successful GnRH vaccination is that it has the effect of decreasing the level of GnRH signaling in the body, as evidenced by a drop in luteinizing hormone levels, and a cessation of ovulation.

GnRH is highly conserved across mammalian taxa, so some inferences about the mechanism and effects of GonaCon-Equine in horses can be made from studies that used different anti-GnRH vaccines, in horses and other taxa. Other commercially available anti-GnRH vaccines include: Improvac (Imboden et al. 2006, Botha et al. 2008, Janett et al. 2009a, Janett et al. 2009b, Schulman et al. 2013, Dalmau et al. 2015,

Nolan et al. 2018c), made in South Africa; Equity (Elhay et al. 2007), made in Australia; Improvest, for use in swine (Bohrer et al. 2014); Repro-BLOC (Boedeker et al. 2011); and Bopriva, for use in cows (Balet et al. 2014). Of these, GonaCon-Equine, Improvac, and Equity are specifically intended for horses. Other anti-GnRH vaccine formulations have also been tested, but did not become trademarked products (e.g., Goodloe 1991, Dalin et al 2002, Stout et al. 2003, Donovan et al. 2013, Schaut et al. 2018, Yao et al. 2018). The effectiveness and side-effects of these various anti-GnRH vaccines may not be the same as would be expected from GonaCon-Equine use in horses. Results could differ as a result of differences in the preparation of the GnRH antigen, and the choice of adjuvant used to stimulate the immune response. For some formulations of anti-GnRH vaccines, a booster dose is required to elicit a contraceptive response, though GonaCon can cause short-term contraception in a fraction of treated animals from one dose (Powers et al. 2011, Gionfriddo et al. 2011a, Baker et al. 2013, Miller et al 2013).

GonaCon can provide multiple years of infertility in several wild ungulate species, including horses (Killian et al., 2008; Gray et al., 2010). The lack of estrus cycling that results from successful GonaCon vaccination has been compared to typical winter period of anoestrus in open mares. As anti-GnRH antibodies decline over time, concentrations of available endogenous GnRH increase and treated animals usually regain fertility (Power et al., 2011).

Females that are successfully contracepted by GnRH vaccination enter a state similar to anestrus, have a lack of or incomplete follicle maturation, and no ovarian cycling (Botha et al. 2008, Nolan et al. 2018c). A leading hypothesis is that anti-GnRH antibodies bind GnRH in the hypothalamus – pituitary ‘portal vessels,’ preventing GnRH from binding to GnRH-specific binding sites on gonadotroph cells in the pituitary, thereby limiting the production of gonadotropin hormones, particularly luteinizing hormone (LH) and, to a lesser degree, follicle-stimulating hormone (FSH) (Powers et al. 2011, NAS 2013). This reduction in LH (and FSH), and a corresponding lack of ovulation, has been measured in response to treatment with anti-GnRH vaccines (Boedeker et al. 2011, Garza et al. 1986).

Females successfully treated with anti-GnRH vaccines have reduced progesterone levels (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay 2007, Botha et al. 2008, Killian et al. 2008, Miller et al. 2008, Janett et al. 2009, Schulman et al. 2013, Balet et al 2014, Dalmau et al. 2015) and β -17 estradiol levels (Elhay et al. 2007), but no great decrease in estrogen levels (Balet et al. 2014). Reductions in progesterone do not occur immediately after the primer dose, but can take several weeks or months to develop (Elhay et al. 2007, Botha et al. 2008, Schulman et al. 2013, Dalmau et al. 2015). This indicates that ovulation is not occurring and corpora lutea, formed from post-ovulation follicular tissue, are not being established.

Antibody titer measurements are proximate measures of the antibody concentration in the blood specific to a given antigen. Anti-GnRH titers generally correlate with a suppressed reproduction system (Gionfriddo et al. 2011a, Powers et al. 2011). Various studies have attempted to identify a relationship between anti-GnRH titer levels and infertility, but that relationship has not been universally predictable or consistent. The time length that titer levels stay high appears to correlate with the length of suppressed reproduction (Dalin et al. 2002, Levy et al. 2011, Donovan et al. 2013, Powers et al. 2011). For example, Goodloe (1991) noted that mares did produce elevated titers and had suppressed follicular development for 11-13 weeks after treatment, but that all treated mares ovulated after the titer levels declined. Similarly, Elhay (2007) found that high initial titers correlated with longer-lasting ovarian and behavioral anoestrus. However, Powers et al. (2011) did not identify a threshold level of titer that was consistently indicative of suppressed reproduction despite seeing a strong correlation between antibody concentration and infertility, nor did Schulman et al. (2013) find a clear relationship between titer levels and mare acyclicity.

In many cases, young animals appear to have higher immune responses, and stronger contraceptive effects of anti-GnRH vaccines than older animals (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). Vaccinating with GonaCon at too young an age, though, may prevent effectiveness; Gionfriddo et al. (2011a) observed weak effects in 3-4-month-old fawns. It has not been possible to predict which individuals of a given age class will have long-lasting immune responses to the GonaCon vaccine. Gray (2010) noted that mares in poor body condition tended to have lower contraceptive efficacy in response to GonaCon-B. Miller et al. (2013) suggested that higher parasite loads might have explained a lower immune response in free-roaming horses than had been observed in a captive trial. At this time, it is unclear what the most important factors affecting efficacy are.

Several studies have monitored animal health after immunization against GnRH. GonaCon treated mares did not have any measurable difference in uterine edema (Killian 2006, 2008). Powers et al. (2011, 2013) noted no differences in blood chemistry except a mildly elevated fibrinogen level in some GonaCon treated elk. In that study, one sham-treated elk and one GonaCon treated elk each developed leukocytosis, suggesting that there may have been a causal link between the adjuvant and the effect. Curtis et al. (2008) found persistent granulomas at GonaCon-KHL injection sites three years after injection, and reduced ovary weights in treated females. Yoder and Miller (2010) found no difference in blood chemistry between GonaCon treated and control prairie dogs. One of 15 GonaCon treated cats died without explanation, and with no determination about cause of death possible based on necropsy or histology (Levy et al. 2011). Other anti-GnRH vaccine formulations have led to no detectable adverse effects (in elephants; Boedeker et al. 2011), though Imboden et al. (2006) speculated that young treated animals might conceivably have impaired hypothalamic or pituitary function.

Kirkpatrick et al. (2011) raised concerns that anti-GnRH vaccines could lead to adverse effects in other organ systems outside the reproductive system. GnRH receptors have been identified in tissues outside of the pituitary system, including in the testes and placenta (Khodr and Siler-Khodr 1980), ovary (Hsueh and Erickson 1979), bladder (Coit et al. 2009), heart (Dong et al. 2011), and central nervous system, so it is plausible that reductions in circulating GnRH levels could inhibit physiological processes in those organ systems. Kirkpatrick et al. (2011) noted elevated cardiological risks to human patients taking GnRH agonists (such as leuprolide), but the National Academy of Sciences (2013) concluded that the mechanism and results of GnRH agonists would be expected to be different from that of anti-GnRH antibodies; the former flood GnRH receptors, while the latter deprive receptors of GnRH.

Reversibility and Effects on Ovaries: PZP Vaccines

In most cases, PZP contraception appears to be temporary and reversible, with most treated mares returning to fertility over time (Kirkpatrick and Turner 2002). The ZonaStat-H formulation of the vaccine tends to confer only one year of efficacy per dose. Some studies have found that a PZP vaccine in long-lasting pellets (PZP-22) can confer multiple years of contraception (Turner et al. 2007), particularly when boosted with subsequent PZP vaccination (Rutberg et al. 2017). Other trial data, though, indicate that the pelleted vaccine may only be effective for one year (J. Turner, University of Toledo, Personal Communication to BLM).

The purpose of applying PZP vaccine treatment is to prevent mares from conceiving foals, but BLM acknowledges that long-term infertility, or permanent sterility, could be a result for some number of individual wild horses receiving PZP vaccinations. The rate of long-term or permanent sterility following vaccinations with PZP is hard to predict for individual horses, but that outcome appears to increase in likelihood as the number of doses increases (Kirkpatrick and Turner 2002). Permanent sterility for mares treated consecutively in each of 5-7 years was observed by Nuñez et al. (2010, 2017). In a graduate thesis, Knight (2014) suggested that repeated treatment with as few as three to four years of PZP treatment may lead to longer-term sterility, and that sterility may result from PZP treatment before puberty. Repeated

treatment with PZP led long-term infertility in Przewalski's horses receiving as few as one PZP booster dose (Feh 2012). However, even if some number of mares become sterile as a result of PZP treatment, that potential result would be consistent with the contraceptive purpose that motivates BLM's potential use of the vaccine.

In some number of individual mares, PZP vaccination may cause direct effects on ovaries (Gray and Cameron 2010, Joonè et al. 2017b, Joonè et al. 2017c, Joonè et al. 2017d, Nolan et al. 2018b). Joonè et al. (2017a) noted reversible effects on ovaries in mares treated with one primer dose and booster dose. Joonè et al. (2017c) and Nolan et al. (2018b) documented decreased anti-Mullerian hormone (AMH) levels in mares treated with native or recombinant PZP vaccines; AMH levels are thought to be an indicator of ovarian function. Bechert et al. (2013) found that ovarian function was affected by the SpayVac PZP vaccination, but that there were no effects on other organ systems. Mask et al. (2015) demonstrated that equine antibodies that resulted from SpayVac immunization could bind to oocytes, ZP proteins, follicular tissues, and ovarian tissues. It is possible that result is specific to the immune response to SpayVac, which may have lower PZP purity than ZonaStat or PZP-22 (Hall et al. 2016). However, in studies with native ZP proteins and recombinant ZP proteins, Joonè et al. (2017a) found transient effects on ovaries after PZP vaccination in some treated mares; normal estrus cycling had resumed 10 months after the last treatment. SpayVac is a patented formulation of PZP in liposomes that led to multiple years of infertility in some breeding trials (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018), but unacceptably poor efficacy in a subsequent trial (Kane 2018). Kirkpatrick et al. (1992) noted effects on horse ovaries after three years of treatment with PZP. Observations at Assateague Island National Seashore indicated that the more times a mare is consecutively treated, the longer the time lag before fertility returns, but that even mares treated seven consecutive years did eventually return to ovulation (Kirkpatrick and Turner 2002). Other studies have reported that continued PZP vaccine applications may result in decreased estrogen levels (Kirkpatrick et al. 1992) but that decrease was not biologically significant, as ovulation remained similar between treated and untreated mares (Powell and Monfort 2001). Bagavant et al. (2003) demonstrated T-cell clusters on ovaries, but no loss of ovarian function after ZP protein immunization in macaques.

Reversibility and Effects on Ovaries: GnRH Vaccines

The NAS (2013) review pointed out that single doses of GonaCon-Equine do not lead to high rates of initial effectiveness, or long duration. Initial effectiveness of one dose of GonaCon-Equine vaccine appears to be lower than for a combined primer plus booster dose of the PZP vaccine Zonastat-H (Kirkpatrick et al. 2011), and the initial effect of a single GonaCon dose can be limited to as little as one breeding season. However, preliminary results on the effects of boosted doses of GonaCon-Equine indicate that it can have high efficacy and longer-lasting effects in free-roaming horses (Baker et al. 2017, 2018) than the one-year effect that is generally expected from a single booster of Zonastat-H.

Too few studies have reported on the various formulations of anti-GnRH vaccines to make generalizations about differences between products, but GonaCon formulations were consistently good at causing loss of fertility in a statistically significant fraction of treated mares for at least one year (Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2017, 2018). With few exceptions (e.g., Goodloe 1991), anti-GnRH treated mares gave birth to fewer foals in the first season when there would be an expected contraceptive effect (Botha et al. 2008, Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2018). Goodloe (1991) used an anti-GnRH-KHL vaccine with a triple adjuvant, in some cases attempting to deliver the vaccine to horses with a hollow-tipped 'biobullet,' but concluded that the vaccine was not an effective immunocontraceptive in that study.

Not all mares should be expected to respond to the GonaCon-equine vaccine; some number should be expected to continue to become pregnant and give birth to foals. In studies where mares were exposed to

stallions, the fraction of treated mares that are effectively contracepted in the year after anti-GnRH vaccination varied from study to study, ranging from ~50% (Baker et al. 2017), to 61% (Gray et al. 2010), to ~90% (Killian et al. 2006, 2008, 2009). Miller et al. (2013) noted lower effectiveness in free-ranging mares (Gray et al. 2010) than captive mares (Killian et al. 2009). Some of these rates are lower than the high rate of effectiveness typically reported for the first year after PZP vaccine treatment (Kirkpatrick et al. 2011). In the one study that tested for a difference, darts and hand-injected GonaCon doses were equally effective in terms of fertility outcome (McCann et al. 2017).

In studies where mares were not exposed to stallions, the duration of effectiveness also varied. A primer and booster dose of Equity led to anoestrus for at least 3 months (Elhay et al. 2007). A primer and booster dose of Improvac also led to loss of ovarian cycling for all mares in the short term (Imboden et al. 2006, Nolan et al. 2018c). It is worth repeating that those vaccines do not have the same formulation as GonaCon.

Results from horses (Baker et al. 2017, 2018) and other species (Curtis et al. 2001) suggest that providing a booster dose of GonaCon-Equine will increase the fraction of temporarily infertile animals to higher levels than would a single vaccine dose alone.

Longer-term infertility has been observed in some mares treated with anti-GnRH vaccines, including GonaCon-Equine. In a single-dose mare captive trial with an initial year effectiveness of 94%, Killian et al. (2008) noted infertility rates of 64%, 57%, and 43% in treated mares during the following three years, while control mares in those years had infertility rates of 25%, 12%, and 0% in those years. GonaCon effectiveness in free-roaming populations was lower, with infertility rates consistently near 60% for three years after a single dose in one study (Gray et al. 2010) and annual infertility rates decreasing over time from 55% to 30% to 0% in another study with one dose (Baker et al. 2017, 2018). Similarly, gradually increasing fertility rates were observed after single dose treatment with GonaCon in elk (Powers et al. 2011) and deer (Gionfriddo et al. 2011a).

Baker et al. (2017, 2018) observed a return to fertility over 4 years in mares treated once with GonaCon, but then noted extremely low fertility rates of 0% and 16% in the two years after the same mares were given a booster dose four years after the primer dose. Four of nine mares treated with primer and booster doses of Improvac did not return to ovulation within 2 years of the primer dose (Imboden et al. 2006), though one should probably not make conclusions about the long-term effects of GonaCon-Equine based on results from Improvac.

It is difficult to predict which females will exhibit strong or long-term immune responses to anti-GnRH vaccines (Killian et al. 2006, Miller et al. 2008, Levy et al. 2011). A number of factors may influence responses to vaccination, including age, body condition, nutrition, prior immune responses, and genetics (Cooper and Herbert 2001, Curtis et al. 2001, Powers et al. 2011). One apparent trend is that animals that are treated at a younger age, especially before puberty, may have stronger and longer-lasting responses (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). It is plausible that giving GonaCon-Equine to prepubertal mares will lead to long-lasting infertility, but that has not yet been tested.

To date, short term evaluation of anti-GnRH vaccines, show contraception appears to be temporary and reversible. Killian et al. noted long-term effects of GonaCon in some captive mares (2009). However, Baker et al. (2017) observed horses treated with GonaCon-B return to fertility after they were treated with a single primer dose; after four years, the fertility rate was indistinguishable between treated and control mares. It appears that a single dose of GonaCon results in reversible infertility. If long-term treatment

resulted in permanent infertility for some treated mares, such permanent infertility fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

Other anti-GnRH vaccines also have had reversible effects in mares. Elhay (2007) noted a return to ovary functioning over the course of 34 weeks for 10 of 16 mares treated with Equity. That study ended at 34 weeks, so it is not clear when the other six mares would have returned to fertility. Donovan et al. (2013) found that half of mares treated with an anti-GnRH vaccine intended for dogs had returned to fertility after 40 weeks, at which point the study ended. In a study of mares treated with a primer and booster dose of Improvac, 47 of 51 treated mares had returned to ovarian cyclicity within 2 years; younger mares appeared to have longer-lasting effects than older mares (Schulman et al. 2013). Joonè et al. (2017) analyzed samples from the Schulman et al. (2013) study and found no significant decrease in anti-Mullerian hormone (AMH) levels in mares treated with GnRH vaccine. AMH levels are thought to be an indicator of ovarian function, so results from Joonè et al. (2017) support the general view that the anoestrus resulting from GnRH vaccination is physiologically similar to typical winter anoestrus. In a small study with a non-commercial anti-GnRH vaccine (Stout et al. 2003), three of seven treated mares had returned to cyclicity within 8 weeks after delivery of the primer dose, while four others were still suppressed for 12 or more weeks. In elk, Powers et al. (2011) noted that contraception after one dose of GonaCon was reversible. In white-tailed deer, single doses of GonaCon appeared to confer two years of contraception (Miller et al. 2000). Ten of 30 domestic cows treated became pregnant within 30 weeks after the first dose of Bopriva (Balet et al. 2014).

Permanent sterility as a result of single-dose or boosted GonaCon-Equine vaccine, or other anti-GnRH vaccines, has not been recorded, but that may be because no long-term studies have tested for that effect. It is conceivable that some fraction of mares could become sterile after receiving one or more booster doses of GonaCon-Equine. If some fraction of mares treated with GonaCon-Equine were to become sterile, though, that result would be consistent with text of the WFRHBA of 1971, as amended, which allows for sterilization to achieve population goals.

In summary, based on the above results related to fertility effects of GonaCon and other anti-GnRH vaccines, application of a single dose of GonaCon-Equine to gathered or remotely-darted wild horses could be expected to prevent pregnancy in perhaps 30%-60% of mares for one year. Some smaller number of wild mares should be expected to have persistent contraception for a second year, and less still for a third year. Applying one booster dose of GonaCon to previously treated mares may lead to four or more years with relatively high rates (80+%) of additional infertility expected (Baker et al. 2018). There is no data to support speculation regarding efficacy of multiple boosters of GonaCon-Equine; however, given it is formulated as a highly immunogenic long-lasting vaccine, it is reasonable to hypothesize that additional boosters would increase the effectiveness and duration of the vaccine.

GonaCon-Equine only affects the fertility of treated animals; untreated animals will still be expected to give birth. Even under favorable circumstances for population growth suppression, gather efficiency might not exceed 85% via helicopter, and may be less with bait and water trapping. Similarly, not all animals may be approachable for darting. The uncaptured or undarted portion of the female population would still be expected to have normally high fertility rates in any given year, though those rates could go up slightly if contraception in other mares increases forage and water availability.

Changes in hormones associated with anti-GnRH vaccination lead to measurable changes in ovarian structure and function. The volume of ovaries reduced in response to treatment (Garza et al. 1986, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Botha et al. 2008, Gionfriddo 2011a, Dalmau et al. 2015). Treatment with an anti-GnRH vaccine changes follicle development (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay et al. 2007, Donovan et al. 2013, Powers et al. 2011, Balet et al. 2014), with the result that ovulation does not occur. A related result is that the ovaries can exhibit less activity

and cycle with less regularity or not at all in anti-GnRH vaccine treated females (Goodloe 1991, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Janett et al. 2009a, Powers et al. 2011, Donovan et al. 2013). In studies where the vaccine required a booster, hormonal and associated results were generally observed within several weeks after delivery of the booster dose.

Effects on Existing Pregnancies, Foals, and Birth Phenology: PZP Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

If a mare is already pregnant, the PZP vaccine has not been shown to affect normal development of the fetus or foal, or the hormonal health of the mare with relation to pregnancy (Kirkpatrick and Turner 2003). Studies on Assateague Island (Kirkpatrick and Turner 2002) showed that once female offspring born to mares treated with PZP during pregnancy eventually breed, they produce healthy, viable foals. It is possible that there may be transitory effects on foals born to mares or jennies treated with PZP. For example, in mice, Sacco et al. (1981) found that antibodies specific to PZP can pass from mother mouse to pup via the placenta or colostrum, but that did not apparently cause any innate immune response in the offspring: the level of those antibodies were undetectable by 116 days after birth. There was no indication in that study that the fertility or ovarian function of those mouse pups was compromised, nor is BLM aware of any such results in horses or burros. Unsubstantiated, speculative connections between PZP treatment and ‘foal stealing’ has not been published in a peer-reviewed study and thus cannot be verified. ‘Foal stealing,’ where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including spayed mares in a wild horse herd. McDonnell (2012) noted that “foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling,” such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season. Similarly, although Nettles (1997) noted reported stillbirths after PZP treatments in cynomolgus monkeys, those results have not been observed in equids despite extensive use in horses and burros.

On-range observations from 20 years of application to wild horses indicate that PZP application in wild mares does not generally cause mares to give birth to foals out of season or late in the year (Kirkpatrick and Turner 2003). Nuñez’s (2010) research showed that a small number of mares that had previously been treated with PZP foaled later than untreated mares and expressed the concern that this late foaling “may” impact foal survivorship and decrease band stability, or that higher levels of attention from stallions on PZP-treated mares might harm those mares. However, that paper provided no evidence that such impacts on foal survival or mare well-being actually occurred. Rubenstein (1981) called attention to a number of unique ecological features of horse herds on Atlantic barrier islands, such as where Nuñez made observations, which calls into question whether inferences drawn from island herds can be applied to western wild horse herds. Ransom et al. (2013), though, did identify a potential shift in reproductive timing as a possible drawback to prolonged treatment with PZP, stating that treated mares foaled on average 31 days later than non-treated mares. Results from Ransom et al. (2013), however, showed that over 81% of the documented births in that study were between March 1 and June 21, i.e., within the normal, peak, spring foaling season. Ransom et al. (2013) pointedly advised that managers should consider carefully before using fertility control vaccines in small refugia or rare species. Wild horses and burros managed by BLM do not generally occur in isolated refugia, nor are they at all rare species. The US Fish and Wildlife Service denied a petition to list wild horses as endangered (USFWS 2015). Moreover, any effect of shifting birth phenology was not observed uniformly: in two of three PZP-treated wild horse populations studied by Ransom et al. (2013), foaling season of treated mares extended three

weeks and 3.5 months, respectively, beyond that of untreated mares. In the other population, the treated mares foaled within the same time period as the untreated mares. Furthermore, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. If there are shifts in birth phenology, though, it is reasonable to assume that some negative effects on foal survival for a small number of foals might result from particularly severe weather events (Nuñez et al. 2018).

Effects on Existing Pregnancies, Foals, and Birth Phenology: GnRH Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

GonaCon and other anti-GnRH vaccines can be injected while a female is pregnant (Miller et al. 2000, Powers et al. 2011, Baker et al. 2013) – in such a case, a successfully contracepted mare will be expected to give birth during the following foaling season, but to be infertile during the same year’s breeding season. Thus, a mare injected in November of 2018 would not show the contraceptive effect (i.e., no new foal) until spring of 2020.

GonaCon had no apparent effect on pregnancies in progress, foaling success, or the health of offspring, in horses that were immunized in October (Baker et al. 2013), elk immunized 80-100 days into gestation (Powers et al. 2011, 2013), or deer immunized in February (Miller et al. 2000). Kirkpatrick et al. (2011) noted that anti-GnRH immunization is not expected to cause hormonal changes that would lead to abortion in the horse, but this may not be true for the first 6 weeks of pregnancy (NAS 2013). Curtis et al. (2011) noted that GonaCon-KHL treated white tailed deer had lower twinning rates than controls, but speculated that the difference could be due to poorer sperm quality late in the breeding season, when the treated does did become pregnant. Goodloe (1991) found no difference in foal production between treated and control animals.

Offspring of anti-GnRH vaccine treated mothers could exhibit an immune response to GnRH (Khodr and Siler-Khodr 1980), as antibodies from the mother could pass to the offspring through the placenta or colostrum. In the most extensive study of long-term effects of GonaCon immunization on offspring, Powers et al. (2012) monitored 15 elk fawns born to GonaCon treated cows. Of those, 5 had low titers at birth and 10 had high titer levels at birth. All 15 were of normal weight at birth, and developed normal endocrine profiles, hypothalamic GnRH content, pituitary gonadotropin content, gonad structure, and gametogenesis. All the females became pregnant in their second reproductive season, as is typical. All males showed normal development of secondary sexual characteristics. Powers et al. (2012) concluded that suppressing GnRH in the neonatal period did not alter long-term reproductive function in either male or female offspring. Miller et al. (2013) report elevated anti-GnRH antibody titers in fawns born to treated white tailed deer, but those dropped to normal levels in 11 of 12 of those fawns, which came into breeding condition; the remaining fawn was infertile for three years.

Direct effects on foal survival are equivocal in the literature. Goodloe (1991), reported lower foal survival for a small sample of foals born to anti-GnRH treated mares, but she did not assess other possible explanatory factors such as mare social status, age, body condition, or habitat in her analysis (NAS 2013). Gray et al. (2010) found no difference in foal survival in foals born to free-roaming mares treated with GonaCon.

There is little empirical information available to evaluate the effects of GnRH vaccination on foaling phenology, but those effects are likely to be similar to those for PZP vaccine treated mares in which the effects of the vaccine wear off. It is possible that immunocontracepted mares returning to fertility late in the breeding season could give birth to foals at a time that is out of the normal range (Nuñez et al. 2010,

Ransom et al 2013). Curtis et al. (2001) did observe a slightly later fawning date for GonaCon treated deer in the second year after treatment, when some does regained fertility late in the breeding season. In anti-GnRH vaccine trials in free-roaming horses, there were no published differences in mean date of foal production (Goodloe 1991, Gray et al. 2010). Unpublished results from an ongoing study of GonaCon treated free-roaming mares indicate that some degree of seasonal foaling is possible (D. Baker, Colorado State University, personal communication to Paul Griffin, BLM WH&B Research Coordinator). Because of the concern that contraception could lead to shifts in the timing of parturitions for some treated animals, Ransom et al. (2013) advised that managers should consider carefully before using PZP immunocontraception in small refugia or rare species; the same considerations could be advised for use of GonaCon, but wild horses and burros in most areas do not generally occur in isolated refugia, they are not a rare species at the regional, national, or international level, and genetically they represent descendants of domestic livestock with most populations containing few if any unique alleles (NAS 2013). Moreover, in PZP-treated horses that did have some degree of parturition date shift, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season; however, this may be more related to stochastic, inclement weather events than extended foaling seasons. If there were to be a shift in foaling date for some treated mares, the effect on foal survival may depend on severity of weather and local conditions; for example, Ransom et al. (2013) did not find consistent effects across study sites.

Effects of Marking and Injection

Standard practices require that immunocontraceptive-treated animals be readily identifiable, either via brand marks or unique coloration (BLM 2010). Some level of transient stress is likely to result in newly captured mares that do not have markings associated with previous fertility control treatments. It is difficult to compare that level of temporary stress with the long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013). Handling may include freeze-marking, for the purpose of identifying that mare and identifying her vaccine treatment history. Under past management practices, captured mares experienced increased stress levels from handling (Ashley and Holcombe 2001), but BLM has instituted guidelines to reduce the sources of handling stress in captured animals (BLM 2015).

Most mares recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long term effects from the fertility control injections, other than the direct consequence of becoming temporarily infertile. Injection site reactions associated with fertility control treatments are possible in treated mares (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017, Baker et al. 2018), but swelling or local reactions at the injection site are expected to be minor in nature. Roelle and Ransom (2009) found that the most time-efficient method for applying PZP is by hand-delivered injection of 2-year pellets when horses are gathered. They observed only two instances of swelling from that technique. Whether injection is by hand or via darting, GonaCon-Equine is associated with some degree of inflammation, swelling, and the potential for abscesses at the injection site (Baker et al. 2013). Swelling or local reactions at the injection site are generally expected to be minor in nature, but some may develop into draining abscesses. Use of remotely delivered vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached. The dart-delivered PZP formulation produced injection-site reactions of varying intensity, though none of the observed reactions appeared debilitating to the animals (Roelle and Ransom 2009) but that was not observed with dart-delivered GonaCon (McCann et al. 2017). Joonè et al. (2017a) found that injection site reactions had healed in most mares within 3 months after the booster dose, and that they did not affect movement or cause fever.

Long-lasting nodules observed did not appear to change any animal's range of movement or locomotor patterns and in most cases did not appear to differ in magnitude from naturally occurring injuries or scars. Mares treated with one formulation of GnRH-KHL vaccine developed pyogenic abscesses (Goodloe

1991). Miller et al. (2008) noted that the water and oil emulsion in GonaCon will often cause cysts, granulomas, or sterile abscesses at injection sites; in some cases, a sterile abscess may develop into a draining abscess. In elk treated with GonaCon, Powers et al. (2011) noted up to 35% of treated elk had an abscess form, despite the injection sites first being clipped and swabbed with alcohol. Even in studies where swelling and visible abscesses followed GonaCon immunization, the longer-term nodules observed did not appear to change any animal's range of movement or locomotor patterns (Powers et al. 2013, Baker et al. 2017, 2018). The result that other formulations of anti-GnRH vaccine may be associated with less notable injection site reactions in horses may indicate that the adjuvant formulation in GonaCon leads a single dose to cause a stronger immune reaction than the adjuvants used in other anti-GnRH vaccines. Despite that, a booster dose of GonaCon-Equine appears to be more effective than a primer dose alone (Baker et al. 2017). Horses injected in the hip with Improvac showed only transient reactions that disappeared within 6 days in one study (Botha et al. 2008), but stiffness and swelling that lasted 5 days were noted in another study where horses received Improvac in the neck (Imboden et al. 2006). Equity led to transient reactions that resolved within a week in some treated animals (Elhay et al. 2007). Donovan et al. noted no reactions to the canine anti-GnRH vaccine (2013). In cows treated with Bopriva there was a mildly elevated body temperature and mild swelling at injection sites that subsided within 2 weeks (Balet et al. 2014).

Indirect Effects: PZP Vaccines

One expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health (Turner and Kirkpatrick 2002). Many treated mares would not experience the biological stress of reproduction, foaling and lactation as frequently as untreated mares. The observable measure of improved health is higher body condition scores (Nuñez et al. 2010). After a treated mare returns to fertility, her future foals would be expected to be healthier overall and would benefit from improved nutritional quality in the mare's milk. This is particularly to be expected if there is an improvement in rangeland forage quality at the same time, due to reduced wild horse population size. Past application of fertility control has shown that mares' overall health and body condition remains improved even after fertility resumes. PZP treatment may increase mare survival rates, leading to longer potential lifespan (Turner and Kirkpatrick 2002, Ransom et al. 2014a) that may be as much as 5-10 years (NPS 2008). To the extent that this happens, changes in lifespan and decreased foaling rates could combine to cause changes in overall age structure in a treated herd (i.e., Turner and Kirkpatrick 2002, Roelle et al. 2010), with a greater prevalence of older mares in the herd (Gross 2000, NPS 2008). Observations of mares treated in past gathers showed that many of the treated mares were larger than, maintained higher body condition than, and had larger healthy foals than untreated mares (BLM, anecdotal observations).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and may reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991).

Because successful fertility control in a given herd reduces foaling rates and population growth rates, another indirect effect should be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception may change a herd's age structure, with a relative increase in the fraction of older animals in the herd (NPS 2008). Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily

adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding.

A principle motivation for use of contraceptive vaccines or sex ratio manipulation is to reduce population growth rates and maintain herd sizes at AML. Where successful, this should allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse and burro habitat quality, and well-being of animals living on the range. As the population nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses and burros, there should also be less trailing and concentrated use of water sources. Lower population density should lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Among mares in the herd that remain fertile, a higher level of physical health and future reproductive success would be expected in areas where lower horse and burro population sizes lead to increases in water and forage resources. While it is conceivable that widespread and continued treatment with fertility control vaccines could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated in almost every year.

Indirect Effects: GnRH Vaccines

As noted above to PZP vaccines, an expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health. Body condition of anti-GnRH-treated females was equal to or better than that of control females in published studies. Ransom et al. (2014b) observed no difference in mean body condition between GonaCon-B treated mares and controls. Goodloe (1991) found that GnRH-KHL treated mares had higher survival rates than untreated controls. In other species, treated deer had better body condition than controls (Gionfriddo et al. 2011b), treated cats gained more weight than controls (Levy et al. 2011), as did treated young female pigs (Bohrer et al. 2014).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called by some a ‘rebound effect.’ Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and could negate the compensatory reproduction that can follow removals (Kirkpatrick and Turner 1991).

Because successful fertility control would reduce foaling rates and population growth rates, another indirect effect would be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception would be expected to lead to a relative increase in the fraction of older animals in the herd. Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding. Among mares in the herd that remain fertile, a high level of physical health and future reproductive success would be expected because reduced population sizes should lead to more availability of water and forage resources per capita.

Reduced population growth rates and smaller population sizes could also allow for continued and increased environmental improvements to range conditions within the project area, which would have

long-term benefits to wild horse habitat quality. As the local horse abundance nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available to wild horses and wildlife throughout the area. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses across the range, there should also be less trailing and concentrated use of water sources. Lower population density would be expected to lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Should GonaCon-Equine treatment, including booster doses, continue into the future, with treatments given on a schedule to maintain a lowered level of fertility in the herd, the chronic cycle of overpopulation and large gathers and removals might no longer occur, but instead a consistent abundance of wild horses could be maintained, resulting in continued improvement of overall habitat conditions and animal health. While it is conceivable that widespread and continued treatment with GonaCon-Equine could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated with primer and booster doses, and perhaps repeated booster doses.

Behavioral Effects: PZP Vaccines

Behavioral difference, compared to mares that are fertile, should be considered as potential results of successful contraception. The NAS report (2013) noted that all forms of fertility suppression have effects on mare behavior, mostly because of the lack of pregnancy and foaling, and concluded that fertility control vaccines were among the most promising fertility control methods for wild horses and burros. The resulting impacts may be seen as neutral in the sense that a wide range of natural behaviors is already observable in untreated wild horses, or mildly adverse in the sense that effects are expected to be transient and to not affect all treated animals.

PZP vaccine-treated mares may continue estrus cycles throughout the breeding season. Ransom and Cade (2009) delineated wild horse behaviors. Ransom et al. (2010) found no differences in how PZP-treated and untreated mares allocated their time between feeding, resting, travel, maintenance, and most social behaviors in three populations of wild horses, which is consistent with Powell's (1999) findings in another population. Likewise, body condition of PZP-treated and control mares did not differ between treatment groups in Ransom et al.'s (2010) study. Nuñez (2010) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation. Knight (2014) found that PZP-treated mares had better body condition, lived longer and switched harems more frequently, while mares that foaled spent more time concentrating on grazing and lactation and had lower overall body condition.

In two studies involving a total of four wild horse populations, both Nuñez et al. (2009) and Ransom et al. (2010) found that PZP vaccine treated mares were involved in reproductive interactions with stallions more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Killian 1997, Heilmann et al. 1998, Curtis et al. 2001, Duncan et al. 2017). There was no evidence, though, that mare welfare was affected by the increased level of herding by stallions noted in Ransom et al. (2010). Nuñez's later analysis (2017) noted no difference in mare reproductive behavior as a function of contraception history.

Ransom et al. (2010) found that control mares were herded by stallions more frequently than PZP-treated mares, and Nuñez et al. (2009, 2014, 2017, 2018) found that PZP-treated mares exhibited higher infidelity to their band stallion during the non-breeding season than control mares. Madosky et al. (2010) and

Knight (2014) found this infidelity was also evident during the breeding season in the same population that Nuñez et al. (2009, 2010, 2014, 2017, 2018) studied. Nuñez et al. (2014, 2017, 2018) concluded that PZP-treated mares changing bands more frequently than control mares could lead to band instability. Nuñez et al. (2009), though, cautioned against generalizing from that island population to other herds. Also, despite any potential changes in band infidelity due to PZP vaccination, horses continued to live in social groups with dominant stallions and one or more mares. Nuñez et al. (2014) found elevated levels of fecal cortisol, a marker of physiological stress, in mares that changed bands. The research is inconclusive as to whether all the mares' movements between bands were related to the PZP treatments themselves or the fact that the mares were not nursing a foal, and did not demonstrate any long-term negative consequence of the transiently elevated cortisol levels. Nuñez et al. 2014 wrote that these effects "...may be of limited concern when population reduction is an urgent priority." Nuñez (2018) and Jones et al. (2019, 2020) noted that band stallions of mares that have received PZP treatment can exhibit changes in behavior and physiology. Nuñez (2018) cautioned that PZP use may limit the ability of mares to return to fertility, but also noted that, "such aggressive treatments may be necessary when rapid reductions in animal numbers are of paramount importance...If the primary management goal is to reduce population size, it is unlikely (and perhaps less important) that managers achieve a balance between population control and the maintenance of more typical feral horse behavior and physiology."

In contrast to transient stresses, Creel et al. (2013) highlight that variation in population density is one of the most well-established causal factors of chronic activation of the hypothalamic-pituitary-adrenal axis, which mediates stress hormones; high population densities and competition for resources can cause chronic stress. Creel et al. (2013) also state that "...there is little consistent evidence for a negative association between elevated baseline glucocorticoids and fitness." Band fidelity is not an aspect of wild horse biology that is specifically protected by the WFRHBA of 1971. It is also notable that Ransom et al. (2014b) found higher group fidelity after a herd had been gathered and treated with a contraceptive vaccine; in that case, the researchers postulated that higher fidelity may have been facilitated by the decreased competition for forage after excess horses were removed. At the population level, available research does not provide evidence of the loss of harem structure among any herds treated with PZP. No biologically significant negative impacts on the overall animals or populations overall, long-term welfare or well-being have been established in these studies.

The National Research Council (2013) found that harem changing was not likely to result in serious adverse effects for treated mares:

"The studies on Shackleford Banks (Nuñez et al., 2009; Madosky et al., 2010) suggest that there is an interaction between pregnancy and social cohesion. The importance of harem stability to mare well-being is not clear, but considering the relatively large number of free-ranging mares that have been treated with liquid PZP in a variety of ecological settings, the likelihood of serious adverse effects seem low."

Nuñez (2010) stated that not all populations will respond similarly to PZP treatment. Differences in habitat, resource availability, and demography among conspecific populations will undoubtedly affect their physiological and behavioral responses to PZP contraception and need to be considered. Kirkpatrick et al. (2010) concluded that: "the larger question is, even if subtle alterations in behavior may occur, this is still far better than the alternative," and that the "...other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that will only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not."

The NAS report (2013) provides a comprehensive review of the literature on the behavioral effects of contraception that puts research up to that date by Nuñez et al. (2009, 2010) into the broader context of all of the available scientific literature, and cautions, based on its extensive review of the literature that:

“ . . . in no case can the committee conclude from the published research that the behavior differences observed are due to a particular compound rather than to the fact that treated animals had no offspring during the study. That must be borne in mind particularly in interpreting long-term impacts of contraception (e.g., repeated years of reproductive “failure” due to contraception).”

Behavioral Effects: GnRH Vaccines

The result that GonaCon treated mares may have suppressed estrous cycles throughout the breeding season can lead treated mares to behave in ways that are functionally similar to pregnant mares. Where it is successful in mares, GonaCon and other anti-GnRH vaccines are expected to induce fewer estrous cycles when compared to non-pregnant control mares. This has been observed in many studies (Garza et al. 1986, Curtis et al. 2001, Dalin et al. 2002, Killian et al. 2006, Dalmau et al. 2015). Females treated with GonaCon had fewer estrous cycles than control or PZP-treated mares (Killian et al. 2006) or deer (Curtis et al. 2001). Thus, any concerns about PZP treated mares receiving more courting and breeding behaviors from stallions (Nuñez et al. 2009, Ransom et al. 2010) are not generally expected to be a concern for mares treated with anti-GnRH vaccines (Botha et al. 2008).

Ransom et al. (2014b) and Baker et al. (2018) found that GonaCon treated mares had similar rates of reproductive behaviors that were similar to those of pregnant mares. Among other potential causes, the reduction in progesterone levels in treated females may lead to a reduction in behaviors associated with reproduction. Despite this, some females treated with GonaCon or other anti-GnRH vaccines did continue to exhibit reproductive behaviors, albeit at irregular intervals and durations (Dalin et al. 2002, Stout et al. 2003, Imboden et al. 2006), which is a result that is similar to spayed (ovariectomized) mares (Asa et al. 1980). Gray et al. (2009a) and Baker et al. (2018) found no difference in sexual behaviors in mares treated with GonaCon and untreated mares. When progesterone levels are low, small changes in estradiol concentration can foster reproductive estrous behaviors (Imboden et al. 2006). Owners of anti-GnRH vaccine treated mares reported a reduced number of estrous-related behaviors under saddle (Donovan et al. 2013). Treated mares may refrain from reproductive behavior even after ovaries return to cyclicity (Elhay et al. 2007). Studies in elk found that GonaCon treated cows had equal levels of precopulatory behaviors as controls (Powers et al. 2011), though bull elk paid more attention to treated cows late in the breeding season, after control cows were already pregnant (Powers et al. 2011).

Stallion herding of mares, and harem switching by mares are two behaviors related to reproduction that might change as a result of contraception. Ransom et al. (2014b) observed a 50% decrease in herding behavior by stallions after the free-roaming horse population at Theodore Roosevelt National Park was reduced via a gather, and mares there were treated with GonaCon-B. The increased harem tending behaviors by stallions were directed to both treated and control mares. It is difficult to separate any effect of GonaCon in this study from changes in horse density and forage following horse removals.

With respect to treatment with GonaCon or other anti-GnRH vaccines, it is probably less likely that treated mares will switch harems at higher rates than untreated animals, because treated mares are similar to pregnant mares in their behaviors (Ransom et al. 2014b). Indeed, Gray et al. (2009a) found no difference in band fidelity in a free-roaming population of horses with GonaCon treated mares, despite differences in foal production between treated and untreated mares. Ransom et al. (2014b) actually found increased levels of band fidelity after treatment, though this may have been partially a result of changes in overall horse density and forage availability.

Gray et al. (2009) and Ransom et al. (2014b) monitored non-reproductive behaviors in GonaCon treated populations of free-roaming horses. Gray et al. (2009a) found no difference between treated and untreated mares in terms of activity budget, sexual behavior, proximity of mares to stallions, or aggression. Ransom et al. (2014b) found only minimal differences between treated and untreated mare time budgets, but those differences were consistent with differences in the metabolic demands of pregnancy and lactation in untreated mares, as opposed to non-pregnant treated mares.

Genetic Effects of Fertility Control Vaccines

In HMAs where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, contraception is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviate potential inbreeding concerns (BLM 2010).

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM, such that most alleles that are present in any given mare are likely to already be well represented in her siblings, cousins, and more distant relatives. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Even if it is the case that repeated treatment with a fertility control vaccine may lead to prolonged infertility, or even sterility in some mares, most HMAs have only a low risk of loss of genetic diversity if logistically realistic rates of contraception are applied to mares. Wild horses in most herd management areas are descendants of a diverse range of ancestors coming from many breeds of domestic horses. As such, the existing genetic diversity in the majority of HMAs does not contain unique or historically unusual genetic markers. Past interchange between HMAs, either through natural dispersal or through assisted migration (i.e., human movement of horses) means that many HMAs are effectively indistinguishable and interchangeable in terms of their genetic composition (i.e., see the table of F_{st} values in NAS 2013). Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Their results show that the risk of the loss of genetic heterozygosity is extremely low except in case where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the female population are permanently sterilized.

It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before she is treated with contraceptives.

One concern that has been raised with regards to genetic diversity is that treatment with immunocontraceptives could possibly lead to an evolutionary increase in the frequency of individuals whose genetic composition fosters weak immune responses (Cooper and Larson 2006, Ransom et al. 2014a). Many factors influence the strength of a vaccinated individual's immune response, potentially including genetics, but also nutrition, body condition, and prior immune responses to pathogens or other antigens (Powers et al. 2013). This premise is based on an assumption that lack of response to any given fertility control vaccine is a heritable trait, and that the frequency of that trait will increase over time in a population of vaccine-treated animals. Cooper and Herbert (2001) reviewed the topic, in the context of concerns about the long-term effectiveness of immunocontraceptives as a control agent for exotic species in Australia. They argue that immunocontraception could be a strong selective pressure, and that selecting for reproduction in individuals with poor immune response could lead to a general decline in immune function in populations where such evolution takes place. Other authors have also speculated that differences in antibody titer responses could be partially due to genetic differences between animals (Curtis et al. 2001, Herbert and Trigg 2005). However, Magiafolou et al. (2013) clarify that if the variation in immune response is due to environmental factors (i.e., body condition, social rank) and not due to genetic factors, then there will be no expected effect of the immune phenotype on future generations. It is possible that general health, as measured by body condition, can have a causal role in determining immune response, with animals in poor condition demonstrating poor immune reactions (NAS 2013).

Correlations between physical factors and immune response would not preclude, though, that there could also be a heritable response to immunocontraception. In studies not directly related to immunocontraception, immune response has been shown to be heritable (Kean et al. 1994, Sarker et al. 1999). Unfortunately, predictions about the long-term, population-level evolutionary response to immunocontraceptive treatments are speculative at this point, with results likely to depend on several factors, including: the strength of the genetic predisposition to not respond to the fertility control vaccine; the heritability of that gene or genes; the initial prevalence of that gene or genes; the number of mares treated with a primer dose of the vaccine (which generally has a short-acting effect); the number of mares treated with one or more booster doses of the vaccine; and the actual size of the genetically-interacting metapopulation of horses within which the vaccine treatment takes place.

BLM is not aware of any studies that have quantified the heritability of a lack of response to immunocontraception such as PZP vaccine or GonaCon-Equine in horses or burros. At this point, there are no studies available from which one could make conclusions about the long-term effects of sustained and widespread immunocontraception treatments on population-wide immune function. Although a few, generally isolated, feral horse populations have been treated with high fractions of mares receiving PZP immunocontraception for long-term population control (e.g., Assateague Island National Park, and Pryor Mountains Herd Management Area), no studies have tested for changes in immune competence in those areas. Relative to the large number of free-roaming feral horses in the western United States, immunocontraception has not been, and is not expected to be used in the type of widespread or prolonged manner that might be required to cause a detectable evolutionary response.

Sex Ratio Manipulation

Skewing the sex ratio of a herd so that there are more males than females is an established BLM management technique for reducing population growth rates. As part of a wild horse and burro gather process, the number of animals returned to the range may include more males, the number removed from the range may include more females, or both. By reducing the proportion of breeding females in a population (as a fraction of the total number of animals present), the technique leads to fewer foals being born, relative to the total herd size.

Sex ratio is typically adjusted in such a way that 60 percent of the horses are male. In the absence of other fertility control treatments, this 60:40 sex ratio can temporarily reduce population growth rates from approximately 20% to approximately 15% (Bartholow 2004). While such a decrease in growth rate may not appear to be large or long-lasting, the net result can be that fewer foals being born, at least for a few years – this can extend the time between gathers, and reduce impacts on-range, and costs off-range. Any impacts of sex ratio manipulation are expected to be temporary because the sex ratio of wild horse and burro foals at birth is approximately equal between males and females (NAS 2013), and it is common for female foals to reproduce by their second year (NAS 2013). Thus, within a few years after a gather and selective removal that leads to more males than females, the sex ratio of reproducing wild horses and burros will be returning toward a 50:50 ratio.

Having a larger number of males than females is expected to lead to several demographic and behavioral changes as noted in the NAS report (2013), including the following. Having more fertile males than females should not alter the fecundity of fertile females. Wild mares may be distributed in a larger number of smaller harems. Competition and aggression between males may cause a decline in male body condition. Female foraging may be somewhat disrupted by elevated male-male aggression. With a greater number of males available to choose from, females may have opportunities to select more genetically fit sires. There would also be an increase in the genetic effective population size because more stallions would be breeding and existing females would be distributed among many more small harems. This last beneficial impact is one reason that skewing the sex ratio to favor males is listed in the BLM wild horse and burro handbook (BLM 2010) as a method to consider in herds where there may be concern about the loss of genetic diversity; having more males fosters a greater retention of genetic diversity.

Infanticide is a natural behavior that has been observed in wild equids (Feh and Munktuya 2008, Gray 2009), but there are no published accounts of infanticide rates increasing as a result of having a skewed sex ratio in wild horse or wild burro herds. Any comment that implies such an impact would be speculative.

The BLM wild horse and burro management handbook (BLM 2010) discusses this method. The handbook acknowledges that there may be some behavioral impacts of having more males than females. The handbook includes guidelines for when the method should be applied, specifying that this method should be considered where the low end of the AML is 150 animals or greater, and with the result that males comprise 60-70 percent of the herd. Having more than 70 percent males may result in unacceptable impacts in terms of elevated male-male aggression. In NEPA analyses, BLM has chosen to follow these guidelines in some cases, for example:

In the 2015 Cold Springs HMA Population Management Plan EA (DOI-BLM-V040-2015-022), the low end of AML was 75. Under the preferred alternative, 37 mares and 38 stallions would remain on the HMA. This is well below the 150 head threshold noted above.

In the 2017 Hog Creek HMA Population Management Plan EA (DOI-BLM-ORWA-V000-2017-0026-EA), BLM clearly identified that maintaining a 50:50 sex ratio was appropriate because the herd size at the low end of AML was only 30 animals.

It is relatively straightforward to speed the return of skewed sex ratios back to a 50:50 ratio. The BLM wild horse and burro handbook (BLM 2010) specifies that, if post-treatment monitoring reveals negative impacts to breeding harems due to sex ratio manipulation, then mitigation measures could include removing males, not introducing additional males, or releasing a larger proportion of females during the next gather.

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Effects of Sterilization, Including Spaying and Neutering

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000), such as with sterilization methods that may include spaying and neutering. Sterilizing a female horse (mare) or burro (jenny) can be accomplished by several methods, some of which are surgical and others of which are non-surgical. In this review, ‘spaying’ is defined to be surgical sterilization, usually accomplished by removal of the ovaries, but other surgical methods such as tubal ligation that lead to sterility may also be considered a form of spaying. Unlike in dog and cat spaying, spaying a horse or burro does not entail removal of the uterus. Here, ‘neutering’ is defined to be the sterilization of a male horse (stallion) or burro (jack), either by removal of the testicles (castration, also known as gelding) or by vasectomy, where the testicles are retained but no sperm leave the body by severing or blocking the vas deferens or epididymis.

In the context of BLM wild horse and burro management, sterilization is expected to be successful to the extent that it reduces the number of reproducing females. By definition, sterilizing a given female is 100% effective as a fertility control method for that female. Neutering males may be effective in one of two ways. First, neutered males may continue to guard fertile females, preventing the females from breeding with fertile males. Second, if neutered males are included in a herd that has a high male-to-female sex ratio, then the neutered males may comprise some of the animals within the appropriate management level (AML) of that herd, which would effectively reduce the number of females in the herd. Although these and other fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

Peer-reviewed scientific literature details the expected impacts of sterilization methods on wild horses and burros. No finding of excess animals is required for BLM to pursue sterilization in wild horses or wild burros, but NEPA analysis has been required. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of female sterilization, then neuter use in males. This review does not examine effects of reversible fertility control vaccines. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not yet addressed in horses or burros specifically.

While most studies reviewed here refer to horses, burros are extremely similar in terms of physiology, such that expected effects are comparable, except where differences between the species are noted.

On the whole, the identified impacts at the herd level are generally transient. The principle impact to individuals treated is sterility, which is the intended outcome. Sterilization that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single HMAs, in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of “metapopulations” – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity.

Discussions about herds that are ‘non-reproducing’ in whole or in part are in the context of this ‘metapopulation’ structure, where self-sustaining herds are not necessarily at the scale of single HMAs. So long as the definition of what constitutes a self-sustaining herd includes the larger set of HMAs that have past or ongoing demographic and genetic connections – as is recommended by the NAS 2013 report – it is clear that single HMAs can be managed as non-reproducing in whole or in part while still allowing for a self-sustaining population of wild horses or burros at the broader spatial scale. Wild horses are not an endangered species (USFWS 2015), nor are they rare. Nearly 67,000 adult wild horses and nearly 15,000 adult wild burros roam BLM lands as of March 1, 2018, and those numbers do not include at least 10,000 WH&B on US Forest Service lands, and at least 50,000 feral horses on tribal lands in the Western United States.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception methods alone do not remove excess horses from an HMA’s population, so one or more gathers are usually needed to bring the herd down to a level close to AML. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), spaying and neutering are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even modest levels of fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000), such as with sterilization. Because sterilizing animals requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs.

Effects of handling and marking

Surgical sterilization techniques, while not reversible, may control horse reproduction without the kind of additional handling or darting that can be needed to administer contraceptive vaccines. In this sense, sterilization surgeries can be used to achieve herd management objectives with a relative minimum level of animal handling and management over the long term. The WFRHBA (as amended) indicates that management should be at the minimum level necessary to achieve management objectives (CFR 4710.4), and if gelding some fraction of a managed population can reduce population growth rates by replacing breeding mares, it then follows that spaying or neutering some individuals can lead to a reduced number of handling occasions and removals of excess horses from the range, which is consistent with legal guidelines. Other fertility control options that may be temporarily effective on male horses, such as the

injection of GonaCon-Equine immunocontraceptive vaccine, apparently require multiple handling occasions to achieve longer-term male infertility. Similarly, some formulations of PZP immunocontraception that is currently available for use in female wild horses and burros require handling or darting every year (though longer-term effects may result after 4 or more treatments; Nuñez et al. 2017). By some measures, any management activities that require multiple capture operations to treat a given individual would be more intrusive for wild horses and potentially less sustainable than an activity that requires only one handling occasion.

It is prudent for sterilized animals to be readily identifiable, either via freeze brand marks or unique coloration, so that their treatment history is easily recognized (e.g., BLM 2010). Markings may also be useful into the future to determine the approximate fraction of geldings in a herd and could provide additional insight regarding gather efficiency. BLM has instituted capture and animal welfare program guidelines to reduce the sources of handling stress in captured animals (BLM 2015). Handling may include freeze-marking, for the purpose of identifying an individual. Some level of transient stress is likely to result in newly captured horses that are not previously marked. Under past management practices, captured horses experienced increased, transient stress levels from handling (Ashley and Holcombe 2001). It is difficult to compare that level of temporary stress with long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013), which could occur in the absence of herd management.

Most horses recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long-term effects from gelding, other than the direct consequence of becoming infertile.

Observations of the long-term outcomes of sterilization may be recorded during routine resource monitoring work. Such observations could include but not be limited to band size, social interactions with other geldings and harem bands, distribution within their habitat, forage utilization and activities around key water sources. Periodic population inventories and future gather statistics could provide additional anecdotal information.

Neutering Males

Castration (the surgical removal of the testicles, also called gelding or neutering) is a surgical procedure for the horse sterilization that has been used for millennia. Vasectomy involves severing or blocking the vas deferens or epididymis, to prevent sperm from being ejaculated. The procedures are fairly straight forward and has a relatively low complication rate. As noted in the review of scientific literature that follows, the expected effects of gelding and vasectomy are well understood overall, even though there is some degree of uncertainty about the exact quantitative outcomes for any given individual (as is true for any natural system).

Including a portion of neutered males in a herd can lead to a reduced population-level per-capita growth rate if they cause a marginal decrease in female fertility or if the neutered males take some of the places that would otherwise be occupied by fertile females. By having a skewed sex ratio with fewer females than males (fertile stallions plus neutered males), the result will be that there will be a lower number of breeding females in the population. Including neutered males in herd management is not new for BLM and federal land management. Geldings have been released on BLM lands as a part of herd management in the Barren Valley complex in Oregon (BLM 2011), the Challis HMA in Idaho (BLM 2012), and the Conger HMA in Utah (BLM 2016). Vasectomized males and geldings were also included in US Fish and Wildlife Service management plans for the Sheldon National Wildlife Refuge that relied on sterilization and removals (Collins and Kasbohm 2016). Taking into consideration the literature available at the time,

the National Academies of Sciences concluded in their 2013 report that a form of vasectomy was one of the three most promising methods for WH&B fertility control (NAS 2013).

Nelson (1980) and Garrott and Siniff (1992) modeled potential efficacy of male-oriented contraception as a population management tool, and both studies agreed that while slowing growth, sterilizing only dominant males (i.e., harem-holding stallions) would result in only marginal reduction in female fertility rates. Eagle et al. (1993) and Asa (1999) tested this hypothesis on HMAs where dominant males were vasectomized. Their findings agreed with modeling results from previous studies, and they also concluded that sterilizing only dominant males would not provide the desired reduction in female fertility and overall population growth rate, assuming that the numbers of fertile females is not changed. While bands with vasectomized harem stallions tended to have fewer foals, breeding by bachelors and subordinate stallions meant that population growth still occurred – female fertility was not dramatically reduced. Collins and Kasbohm (2016) demonstrated that there was a reduced fertility rate in a feral horse herd with both spayed and vasectomized horses – some geldings were also present in that herd. Garrott and Siniff (1992) concluded from their modeling that male sterilization would effectively cause there to be zero population growth (the point where births roughly equal deaths) only if a large proportion of males (i.e., >85%) could be sterilized. In cases where the goal of harem stallion sterilization is to reduce population growth rates, success appears to be dependent on a stable group structure, as strong bonds between a stallion and mares reduce the probability of a mare mating an extra-group stallion (Nelson 1980, Garrott and Siniff 1992, Eagle et al. 1993, Asa 1999).

Despite these studies, neutered males can be used to reduce overall growth rates in a management strategy that does not rely on any expectation that geldings will retain harems or lead to a reduction in per-female fertility rates. The primary goal of including neutered males in a herd need not necessarily be to reduce female fertility (although that may be one result). Rather, by including some neutered males in a herd that also has fertile mares and stallions, the neutered males would take some of the spaces toward AML that would otherwise be taken by fertile females. If the total number of horses is constant but neutered males are included in the herd, this can reduce the number of fertile mares, therefore reducing the absolute number of foals produced. Put another way, if neutered males occupy spaces toward AML that would otherwise be filled by fertile mares, that will reduce growth rates merely by the fact of causing there to be a lower starting number of fertile mares.

Direct Effects of Neutering

No animals which appear to be distressed, injured, or in poor health or condition would be selected for gelding. Stallions would not typically be neutered within 72 hours of capture. The surgery would be performed by a veterinarian using general anesthesia and appropriate surgical techniques. The final determination of which specific animals would be gelded would be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer (i.e., See the SOPs for neutering in the Antelope / Triple B gather EA, DOI-BLM-NV-E030-2017-010-EA).

Though neutering males is a common surgical procedure, especially gelding, some level of minor complications after surgery may be expected (Getman 2009), and it is not always possible to predict when postoperative complications would occur. Fortunately, the most common complications are almost always self-limiting, resolving with time and exercise. Individual impacts to the stallions during and following the gelding process should be minimal and would mostly involve localized swelling and bleeding. Complications may include, but are not limited to: minor bleeding, swelling, inflammation, edema, infection, peritonitis, hydrocele, penile damage, excessive hemorrhage, and eventration (Schumacher 1996, Searle et al. 1999, Getman 2009). A small amount of bleeding is normal and generally subsides quickly, within 2-4 hours following the procedure. Some degree of swelling is normal, including swelling of the prepuce and scrotum, usually peaking between 3-6 days after surgery (Searle et al. 1999). Swelling

should be minimized through the daily movements (exercise) of the horse during travel to and from foraging and watering areas. Most cases of minor swelling should be back to normal within 5-7 days, more serious cases of moderate to severe swelling are also self-limiting and are expected to resolve with exercise after one to 2 weeks. Older horses are reported to be at greater risk of post-operative edema, but daily exercise can prevent premature closure of the incision and prevent fluid buildup (Getman 2009). In some cases, a hydrocele (accumulation of sterile fluid) may develop over months or years (Searle et al. 1999). Serious complications (eventration, anesthetic reaction, injuries during handling, etc.) that result in euthanasia or mortality during and following surgery are rare (e.g., eventration rate of 0.2% to 2.6% noted in Getman 2009, but eventration rate of 4.8% noted in Shoemaker et al. 2004) and vary according to the population of horses being treated (Getman 2009). Normally one would expect serious complications in less than 5% of horses operated under general anesthesia, but in some populations these rates have been as high as 12% (Shoemaker 2004). Serious complications are generally noted within 3 or 4 hours of surgery but may occur any time within the first week following surgery (Searle et al. 1999). If they occur, they would be treated with surgical intervention when possible, or with euthanasia when there is a poor prognosis for recovery. Vasectomized stallions may remain fertile for up to 6 weeks after surgery, so it is optimal if that treatment occurs well in advance of the season of mare fertility starting in the spring (NAS 2013). The NAS report (2013) suggested that chemical vasectomy, which has been developed for dogs and cats, may be appropriate for wild horses and burros.

For intact stallions, testosterone levels appear to vary as a function of age, season, and harem size (Khalil et al 1998). It is expected that testosterone levels will decline over time after castration. Testosterone levels should not change due to vasectomy. Vasectomized stallions should retain their previous levels of libido. Domestic geldings had a significant prolactin response to sexual stimulation but lacked the cortisol response present in stallions (Colborn et al. 1991). Although libido and the ability to ejaculate tends to be gradually lost after castration (Thompson et al. 1980), some geldings continue to mount mares and intromit (Rios and Houpt 1995, Schumacher 2006).

Indirect Effects of Neutering

Other than the short-term outcomes of surgery, neutering is not expected to reduce males' survival rates. Castration is actually thought to increase survival as males are released from the cost of reproduction (Jewell 1997). In Soay sheep castrates survived longer than rams in the same cohort (Jewell 1997), and Misaki horse geldings lived longer than intact males (Kaseda et al. 1997, Khalil and Murakami 1999). Moreover, it is unlikely that a reduced testosterone level will compromise gelding survival in the wild, considering that wild mares survive with low levels of testosterone. Consistent with geldings not expending as much energy toward in attempts to obtain or defend a harem, it is expected that wild geldings may have a better body condition than wild, fertile stallions. In contrast, vasectomized males may continue to defend or compete for harems in the way that fertile males do, so they are not expected to experience an increase in health or body condition due to surgery.

Depending on whether an HMA is non-reproducing in whole or in part, reproductive stallions may or may not still be a component of the population's age and sex structure. The question of whether a given neutered male would or would not attempt to maintain a harem is not germane to population-level management. It is worth noting, though, that the BLM is not required to manage populations of wild horses in a manner that ensures that any given individual maintains its social standing within any given harem or band. Neutering a subset of stallions would not prevent other fertile stallions and mares from continuing with the typical range of social behaviors for sexually active adults. For fertility control strategies where gelding is intended to reduce growth rates by virtue of sterile males defending harems, the NAS (2013) suggested that the effectiveness of gelding on overall reproductive rates may depend on the pre-castration social roles of those animals. Having a post-gather herd with some neutered males and a lower fraction of fertile mares necessarily reduces the absolute number of foals born per year, compared

to a herd that includes more fertile mares. An additional benefit is that geldings that would otherwise be permanently removed from the range (for adoption, sale or other disposition) may be released back onto the range where they can engage in free-roaming behaviors.

Behavioral Effects of Neutering

Feral horses typically form bands composed of an adult male with 1 to 3 adult females and their immature offspring (Feist and McCullough 1976, Berger 1986, Roelle et al. 2010). In many populations subordinate ‘satellite’ stallions have been observed associating with the band, although the function of these males continues to be debated (see Feh 1999, and Linklater and Cameron 2000). Juvenile offspring of both sexes leave the band at sexual maturity (normally around two or three years of age (Berger 1986), but adult females may remain with the same band over a span of years. Group stability and cohesion is maintained through positive social interactions and agonistic behaviors among all members, and herding and reproductive behaviors from the stallion (Ransom and Cade 2009). Group movements and consortship of a stallion with mares is advertised to other males through the group stallion marking dung piles as they are encountered, and over-marking mare eliminations as they occur (King and Gurnell 2006).

In horses, males play a variety of roles during their lives (Deniston 1979): after dispersal from their natal band they generally live as bachelors with other young males, before associating with mares and developing their own breeding group as a harem stallion or satellite stallion. In any population of horses not all males will achieve harem stallion status, so all males do not have an equal chance of breeding (Asa 1999). Stallion behavior is thought to be related to androgen levels, with breeding stallions having higher androgen concentrations than bachelors (Angle et al. 1979, Chaudhuri and Ginsberg 1990, Khalil et al. 1998). A bachelor with low libido had lower levels of androgens, and two-year-old bachelors had higher testosterone levels than two year olds with undescended testicles who remained with their natal band (Angle et al. 1979).

Vasectomized males continue to attempt to defend or gain breeding access to females. It is generally expected that vasectomized WH&B will continue to behave like fertile males, given that the only physiological change in their condition is a lack of sperm in their ejaculate. If a vasectomized stallion retains a harem, the females in the harem will continue to cycle until they are fertilized by another stallion, or until the end of the breeding season. As a result, the vasectomized stallion may be involved in more aggressive behaviors to other males through the entire breeding season (Asa 1999), which may divert time from foraging and cause him to be in poorer body condition going into winter. Ultimately, this may lead to the stallion losing control of a given harem. A feral horse herd with high numbers of vasectomized stallions retained typical harem social structure (Collins and Kasbohm 2016). Again, it is worth noting that the BLM is not required to manage populations of wild horses in a manner that ensures that any given individual maintains its social standing within any given harem or band.

Neutering males by gelding adult male horses is expected to result in reduced testosterone production, which is expected to directly influence reproductive behaviors (NAS 2013). However, testosterone levels alone are not a predictor of masculine behavior (Line et al. 1985, Schumacher 2006). In domestic geldings, 20-30% continued to show stallion-like behavior, whether castrated pre- or post-puberty (Line et al. 1985). Gelding of domestic horses most commonly takes place before or shortly after sexual maturity, and age-at-gelding can affect the degree to which stallion-like behavior is expressed later in life. In intact stallions, testosterone levels peak increase up to an age of ~4-6 years, and can be higher in harem stallions than bachelors (Khalil et al 1998). It is assumed that free roaming wild horse geldings would generally exhibit reduced aggression toward other horses and reduced reproductive behaviors (NAS 2013). The behavior of wild horse geldings in the presence of intact stallions has not been well

documented, but the literature review below can be used to make reasonable inferences about their likely behaviors.

Despite livestock being managed by neutering males for millennia, there is relatively little published research on castrates' behaviors (Hart and Jones 1975). Stallion behaviors in wild or pasture settings are better documented than gelding behaviors, but it inferences about how the behaviors of geldings will change, how quickly any change will occur after surgery, or what effect gelding an adult stallion and releasing him back in to a wild horse population will have on his behavior and that of the wider population must be surmised from the existing literature. There is an ongoing BLM study in Utah focused on the individual and population-level effects of including some geldings in a free-roaming horse population (BLM 2016) but results from that study are not yet available. However, inferences about likely behavioral outcomes of gelding can be made based on available literature.

The effect of castration on aggression in horses has not often been quantified. One report has noted that high levels of aggression continued to be observed in domestic horse geldings who also exhibited sexual behaviors (Rios and Houpt 1995). Stallion-like behavior in domestic horse geldings is relatively common (Smith 1974, Schumacher 1996), being shown in 20-33% of cases whether the horse was castrated pre- or post-puberty (Line et al. 1985, Rios and Houpt 1995, Schumacher 2006). While some of these cases may be due to cryptorchidism or incomplete surgery, it appears that horses are less dependent on hormones than other mechanisms for the maintenance of sexual behaviors (Smith 1974). Domestic geldings exhibiting masculine behavior had no difference in testosterone concentrations than other geldings (Line et al. 1985, Schumacher 2006), and in some instances the behavior appeared context dependent (Borsberry 1980, Pearce 1980).

Dogs and cats are commonly neutered, and it is also common for them to continue to exhibit reproductive behaviors several years after castration (Dunbar 1975). Dogs, ferrets, hamsters, and marmosets continued to show sexually motivated behaviors after castration, regardless of whether they had previous experience or not, although in beagles and ferrets there was a reduction in motivation post-operatively (Hart 1968, Dunbar 1975, Dixson 1993, Costantini et al. 2007, Vinke et al. 2008). Ungulates continued to show reproductive behaviors after castration, with goats and llamas continuing to respond to females even a year later in the case of goats, although mating time and the ejaculatory response was reduced (Hart and Jones 1975, Nickolmann et al. 2008).

The likely effects of castration on geldings' social interactions and group membership can be inferred from available literature. In a pasture study of domestic horses, Van Dierendonk et al. (1995) found that social rank among geldings was directly correlated to the age at which the horse was castrated, suggesting that social experiences prior to sterilization may influence behavior afterward. Of the two geldings present in a study of semi-feral horses in England, one was dominant over the mares whereas a younger gelding was subordinate to older mares; stallions were only present in this population during a short breeding season (Tyler 1972). A study of domestic geldings in Iceland held in a large pasture with mares and sub-adults of both sexes, but no mature stallions, found that geldings and sub-adults formed associations amongst each other that included interactions such as allo-grooming and play, and were defined by close proximity (Sigurjónsdóttir et al. 2003). These geldings and sub-adults tended to remain in a separate group from mares with foals, similar to castrated Soay sheep rams (*Ovis aries*) behaving like bachelors and grouping together or remaining in their mother's group (Jewell 1997). In Japan, Kaseda et al. (1997) reported that young males dispersing from their natal harem and geldings moved to a different area than stallions and mares during the non-breeding season. Although the situation in Japan may be the equivalent of a bachelor group in natural populations, in Iceland this division between mares and the rest of the horses in the herd contradicts the dynamics typically observed in a population containing mature stallions. Sigurjónsdóttir et al. (2003) also noted that in the absence of a stallion, allo-grooming between adult females increased drastically. Other findings included increased social interaction among yearlings,

display of stallion-like behaviors such as mounting by the adult females, and decreased association between females and their yearling offspring (Sigurjónsdóttir et al. 2003). In the same population in Iceland Van Dierendonck et al. (2004) concluded that the presence of geldings did not appear to affect the social behavior of mares or negatively influence parturition, mare-foal bonding, or subsequent maternal activities. Additionally, the welfare of broodmares and their foals was not affected by the presence of geldings in the herd (Van Dierendonck et al. 2004). These findings are important because treated geldings will be returned to the range in the presence of pregnant mares and mares with foals of the year.

The likely effects of castration on geldings' home range and habitat use can also be surmised from available literature. Bands of horses tend to have distinct home ranges, varying in size depending on the habitat and varying by season, but always including a water source, forage, and places where horses can shelter from inclement weather or insects (King and Gurnell 2005). By comparison, bachelor groups tend to be more transient, and can potentially use areas of good forage further from water sources, as they are not constrained by the needs of lactating mares in a group. The number of observations of gelded wild stallion behavior are still too few to make general predictions about whether a particular gelded stallion individual will behave like a harem stallion, a bachelor, or form a group with geldings that may forage and water differently from fertile wild horses.

Sterilizing wild horses does not change their status as wild horses under the WFRHBA (as amended). In terms of whether geldings will continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that geldings would continue to roam unhindered once they are returned to the range. Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a gelded animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior. Despite marginal uncertainty about subtle aspects of potential changes in habitat preference, there is no expectation that gelding wild horses will cause them to lose their free-roaming nature. It is worth noting that individual choices in wild horse group membership, home range, and habitat use are not protected under the WFRHBA. BLM acknowledges that geldings may exhibit some behavioral differences after surgery, compared to intact stallions, but those differences are not expected to remove the geldings' rebellious and feisty nature, or their defiance of man. While it may be that a gelded horse could have a different set of behavioral priorities than an intact stallion, the expectation is that geldings will choose to act upon their behavioral priorities in an unhindered way, just as is the case for an intact stallion. In this sense, a gelded male would be just as much 'wild' as defined by the WFRHBA as any intact stallion, even if his patterns of movement differ from those of an intact stallion. Congress specified that sterilization is an acceptable management action (16 USC § 1333.b.1). Sterilization is not one of the clearly defined events that cause an animal to lose its status as a wild free-roaming horse (16 USC § 1333.2.C.d). Several academics have offered their opinions about whether gelding a given stallion would lead to that individual effectively losing its status as a wild horse (Rutberg 2011, Kirkpatrick 2012, Nock 2017). Those opinions are based on a semantic and subjective definition of 'wild,' while BLM must adhere to the legal definition of what constitutes a wild horse, based on the WFRHBA (as amended). Those individuals have not conducted any studies that would test the speculative opinion that gelding wild stallions will cause them to become docile. BLM is not obliged to base management decisions on such opinions, which do not meet the BLM's principle and practice to "Use the best available scientific knowledge relevant to the problem or decision being addressed, relying on peer reviewed literature when it exists" (Kitchell et al. 2015).

Mare Sterilization

Surgical sterilization (spaying mares by removing a mare's ovaries), via colpotomy, has been an established veterinary technique since 1903 (Loesch and Rodgers 2003, NAS 2013). Spaying via colpotomy has the advantage of not leaving any external wound that could become infected. For this

reason, it has been identified as a good choice for sterilization of feral or wild mares (Rowland et al. 2018). The procedure has a relatively low complication rate, although post-surgical mortality and morbidity are possible, as with any surgery. Herd-level birth rate is expected to decline in direct proportion to the fraction of spayed mares in the herd because spayed mares cannot become pregnant. Spaying mares has already been shown to be an effective part of feral horse management that reduced herd growth rates on federal lands (Collins and Kasbohm 2016).

Current Methods of Sterilization

This literature review of mare sterilization impacts focuses on 4 methods: spaying via flank laparoscopy, spaying via colpotomy, non-surgical physical sterilization, and pharmacological or immunocontraceptive sterilization. The anticipated effects are both physical and behavioral. Physical effects of surgical methods would be due to post-treatment healing and the possibility for complications.

Colpotomy is a surgical technique in which there is no external incision, reducing susceptibility to infection. For this reason, ovariectomy via colpotomy has been identified as a good choice for feral or wild horses (Rowland et al. 2018). Ovariectomy via colpotomy is a relatively short surgery, with a relatively quick expected recovery time. In 1903, Williams first described a vaginal approach, or colpotomy, using an ecraseur to ovariectomize mares (Loesch and Rodgerson 2003). The ovariectomy via colpotomy procedure has been conducted for over 100 years, normally on open (non-pregnant), domestic mares. It is expected that the surgeon should be able to access ovaries with ease in mares that are in the early- or mid-stage of pregnancy. The anticipated risks associated with the pregnancy are described below. When wild horses are gathered or trapped for fertility control treatment there would likely be mares in various stages of gestation. Removal of the ovaries is permanent and 100 percent effective, however the procedure is not without risk.

Flank laparoscopy (Lee and Hendrickson 2008, Devick et al. 2018, Easley et al. 2018) is commonly used in domestic horses for application in mares due to its minimal invasiveness and full observation of the operative field. Ovariectomy via flank laparoscopy was seen as the lowest risk method considered by a panel of expert reviewers convened by USGS (Bowen 2015). In a review of unilateral and bilateral laparoscopic ovariectomy on 157 mares, Röcken et al. (2011) found that 10.8% of mares had minor post-surgical complications and recorded no mortality. Mortality due to this type of surgery, or post-surgical complications, is not expected, but is a possibility. In two studies, ovariectomy by laparoscopy or endoscope-assisted colpotomy did not cause mares to lose weight, and there was no need for rescue analgesia following surgery (Pader et al. 2011, Bertin et al. 2013). This surgical approach entails three small incisions on the animal's flank, through which three cannulae (tubes) allow entry of narrow devices to enter the body cavity: these are the insufflator, endoscope, and surgical instrument. The surgical procedure involves the use of narrow instruments introduced into the abdomen via cannulas for the purpose of transecting or sealing (Easley 2018) the ovarian pedicle, but the insufflation should allow the veterinarian to navigate inside the abdomen without damaging other internal organs. The insufflator blows air into the cavity to increase the operating space between organs, and the endoscope provides a video feed to visualize the operation of the surgical instrument. This procedure can require a relatively long duration of surgery but tends to lead to the lowest post-operative rates of complications. Flank laparoscopy may leave three small (<5 cm) visible scars on one side of the horse's flank, but even in performance horses these scars are considered minimal. It is expected that the tissues and musculature under the skin at the site of the incisions in the flank will heal quickly, leaving no long-lasting effects on horse health. Monitoring for up to two weeks at the facility where surgeries take place will allow for veterinary inspection of wound healing. The ovaries may be dropped into the abdomen, but this is not expected to cause any health problem; it is usually done in ovariectomies in cattle (e.g., the Willis Dropped Ovary Technique) and Shoemaker et al. (2014) found no problems with revascularization or necrosis in a study of young horses using this method.

Non-surgical, physical sterilization would include any physical form of sterilization that does not involve surgery. This could include any form of physical procedure that leads a mare to be unable to become pregnant, or to maintain a pregnancy. For example, one form of physical, non-surgical sterilization causes a long-term blockage of the oviduct, so that fertile eggs cannot go from the ovaries to the uterus. One form of this procedure infuses medical cyanoacrylate glue into the oviduct to cause long-term blockage (Bigolin et al. 2009). Treated mares would need to be screened to ensure they are not pregnant. The procedure is transcervical, so the treated mare cannot have a fetus in the uterus at the time of treatment. The mare would be sterile, although she would continue to have estrus cycles.

Pharmacological or immunocontraceptive sterilization methods would use an as-yet undetermined drug or vaccine to cause sterilization. At this time, BLM has not yet identified a pharmacological or immunocontraceptive method to sterilize mares that has been proven to reliably and humanely sterilize wild horse mares. However, there is the possibility that future development and testing of new methods could make an injectable sterilant available for wild horse mares. Analyses of the effects of having sterile mares as a part of a wild horse herd, such as due to surgical sterilization, would likely be applicable to non-surgical methods as well.

Effects of Spaying on Pregnancy and Foal

The average mare gestation period ranges from 335 to 340 days (Evans et al. 1977, p. 373). There are few peer reviewed studies documenting the effects of ovariectomy on the success of pregnancy in a mare. A National Research Council of the National Academies of Sciences (NAS) committee that reviewed research proposals in 2015 explained, “The mare’s ovaries and their production of progesterone are required during the first 70 days of pregnancy to maintain the pregnancy” (NAS 2015). In female mammals, less progesterone is produced when ovaries are removed, but production does not cease (Webley and Johnson 1982). In 1977, Evans et al. stated that by 200 days, the secretion of progesterone by the corpora lutea is insignificant because removal of the ovaries does not result in abortion (p. 376). “If this procedure were performed in the first 120 days of pregnancy, the fetus would be resorbed or aborted by the mother. If performed after 120 days, the pregnancy should be maintained. The effect of ovary removal on a pregnancy at 90–120 days of gestation is unpredictable because it is during this stage of gestation that the transition from corpus luteum to placental support typically occurs” (NAS 2015). In 1979, Holtan et al. evaluated the effects of bilateral ovariectomy at selected times between 25 and 210 days of gestation on 50 mature pony mares. Their results show that abortion (resorption) of the conceptus (fetus) occurred in all 14 mares ovariectomized before day 50 of gestation, that pregnancy was maintained in 11 of 20 mares after ovariectomy between days 50 and 70, and that pregnancy was not interrupted in any of 12 mares ovariectomized on days 140 to 210. Those results are similar to the suggestions of the NAS committee (2015).

For those pregnancies that are maintained following the procedure, likely those past approximately 120 days, the development of the foal is not expected to be affected. However, because this procedure is not commonly conducted on pregnant mares the rate of complications to the fetus has not yet been quantified. There is the possibility that entry to the abdominal cavity could cause premature births related to inflammation. However, after five months the placenta should hormonally support the pregnancy regardless of the presence or absence of ovaries. Gestation length was similar between ovariectomized and control mares (Holtan et al. 1979).

Direct Effects of Spaying

Between 2009 and 2011, the Sheldon NWR in Nevada conducted ovariectomy via colpotomy surgeries (August through October) on 114 feral mares and released them back to the range with a mixture of sterilized stallions and untreated mares and stallions (Collins and Kasbohm 2016). Gestational stage was not recorded, but a majority of the mares were pregnant (Gail Collins, US Fish and Wildlife Service

(USFWS), pers. comm.). Only a small number of mares were very close to full term. Those mares with late term pregnancies did not receive surgery as the veterinarian could not get good access to the ovaries due to the position of the foal (Gail Collins, USFWS, pers. comm.). After holding the mares for an average of 8 days after surgery for observation, they were returned to the range with other treated and untreated mares and stallions (Collins and Kasbohm 2016). During holding the only complications were observed within 2 days of surgery. The observed mortality rate for ovariectomized mares following the procedure was less than 2 percent (Collins and Kasbohm 2016, Pielstick pers. comm.).

During the Sheldon NWR ovariectomy study, mares generally walked out of the chute and started to eat; some would raise their tail and act as if they were defecating; however, in most mares one could not notice signs of discomfort (Bowen 2015). In their discussion of ovariectomy via colpotomy, McKinnon and Vasey (2007) considered the procedure safe and efficacious in many instances, able to be performed expediently by personnel experienced with examination of the female reproductive tract, and associated with a complication rate that is similar to or less than male castration. Nevertheless, all surgery is associated with some risk. Loesch et al. (2003) lists that following potential risks with colpotomy: pain and discomfort; injuries to the cervix, bladder, or a segment of bowel; delayed vaginal healing; eventration of the bowel; incisional site hematoma; intraabdominal adhesions to the vagina; and chronic lumbar or bilateral hind limb pain. Most horses, however, tolerate ovariectomy via colpotomy with very few complications, including feral horses (Collins and Kasbohm 2016). Evisceration is also a possibility, but these complications are considered rare (Prado and Schumacher, 2017). Mortality due to surgery or post-surgical complications is not anticipated, but it is a possibility and therefore every effort would be made to mitigate risks.

In September 2015, the BLM solicited the USGS to convene a panel of veterinary experts to assess the relative merits and drawbacks of several surgical ovariectomy techniques that are commonly used in domestic horses for potential application in wild horses. A table summarizing the various methods was sent to the BLM (Bowen 2015) and provides a concise comparison of several methods. Of these, ovariectomy via colpotomy was found to be relatively safe when practiced by an experienced surgeon and was associated with the shortest duration of potential complications after the operation. The panel discussed the potential for evisceration through the vaginal incision with this procedure. In marked contrast to a suggestion by the NAS report (2013), this panel of veterinarians identified evisceration as not being a probable risk associated with ovariectomy via colpotomy and “none of the panel participants had had this occur nor had heard of it actually occurring” (Bowen 2015).

Most spay surgeries on mares have low morbidity and with the help of medications, pain and discomfort can be mitigated. Pain management is an important aspect of any ovariectomy (Rowland et al. 2018); according to surgical protocols that would be used, a long-lasting direct anesthetic would be applied to the ovarian pedicle, and systemic analgesics in the form of butorphanol and flunixin meglumine would be administered, as is compatible with accepted animal husbandry practices. In a study of the effects of bilateral ovariectomy via colpotomy on 23 mares, Hooper and others (1993) reported that postoperative problems were minimal (1 in 23, or 4%). Hooper et al. (1993) noted that four other mares were reported by owners as having some problems after surgery, but that evidence as to the role the surgery played in those subsequent problems was inconclusive. In contrast Röcken et al. (2011) noted a morbidity of 10.8% for mares that were ovariectomized via a flank laparoscopy. “Although 5 mares in our study had problems (repeated colic in 2 mares, signs of lumbar pain in 1 mare, signs of bilateral hind limb pain in 1 mare, and clinical signs of peritonitis in 1 mare) after surgery, evidence is inconclusive in each as to the role played by surgery” (Hooper et al. 1993). A recent study showed a 2.5% complication rate where one mare of 39 showed signs of moderate colic after laparoscopic ovariectomy (Devick 2018 personal communication).

Behavioral Effects of Mare Sterilization

No fertility control method exists that does not affect physiology or behavior of a mare (NAS 2013). Any action taken to alter the reproductive capacity of an individual has the potential to affect hormone production and therefore behavioral interactions and ultimately population dynamics in unforeseen ways (Ransom et al. 2014). The health and behavioral effects of spaying wild horse mares that live with other fertile and infertile wild horses has not been well documented, but the literature review below can be used to make reasonable inferences about their likely behaviors.

Horses are anovulatory (do not ovulate/express estrous behavior) during the short days of late fall and early winter, beginning to ovulate as days lengthen and then cycling roughly every 21 days during the warmer months, with about 5 days of estrus (Asa et al. 1979, Crowell-Davis 2007). Estrus in mares is shown by increased frequency of proceptive behaviors: approaching and following the stallion, urinating, presenting the rear end, clitoral winking, and raising the tail towards the stallion (Asa et al. 1979, Crowell-Davis 2007). In most mammal species other than primates, estrus behavior is not shown during the anovulatory period, and reproductive behavior is considered extinguished following spaying (Hart and Eckstein 1997). However, mares may continue to demonstrate estrus behavior during the anovulatory period (Asa et al. 1980). Similarly, ovariectomized mares may also continue to exhibit estrous behavior (Scott and Kunze 1977, Kamm and Hendrickson 2007, Crabtree 2016), with one study finding that 30% of mares showed estrus signs at least once after surgery (Roessner et al 2015) and only 60 percent of ovariectomized mares cease estrous behavior following surgery (Loesch and Rodgeron 2003). Mares continue to show reproductive behavior following ovariectomy due to non-endocrine support of estrus behavior, specifically steroids from the adrenal cortex. Continuation of this behavior during the non-breeding season has the function of maintaining social cohesion within a horse group (Asa et al. 1980, Asa et al. 1984, NAS 2013). This may be a unique response of the horse (Bertin et al. 2013), as spaying usually greatly reduces female sexual behavior in companion animals (Hart and Eckstein 1997). In six ponies, mean monthly plasma luteinizing hormone levels in ovariectomized mares were similar to intact mares during the anestrous season, and during the breeding season were similar to levels in intact mares at mid-estrus (Garcia and Ginther 1976).

The likely effects of spaying on mares' social interactions and group membership can be inferred from available literature, even though wild horses have rarely been spayed and released back into the wild, resulting in few studies that have investigated their behavior in free-roaming populations. Wild horses and burros are instinctually herd-bound and this behavior is expected to continue. Overall, the BLM anticipates that some spayed mares may continue to exhibit estrus behavior which could foster band cohesion. If free-ranging ovariectomized mares show estrous behavior and occasionally allow copulation, interest of the stallion may be maintained, which could foster band cohesion (NAS 2013). This last statement could be validated by the observations of group associations on the Sheldon NWR where feral mares were ovariectomized via colpotomy and released back on to the range with untreated horses of both sexes (Collins and Kasbohm 2016). No data were collected on inter- or intra-band behavior (e.g. estrous display, increased tending by stallions, etc.), during multiple aerial surveys in years following treatment, all treated individuals appeared to maintain group associations, and there were no groups consisting only of treated males or only of treated females (Collins and Kasbohm 2016). In addition, of solitary animals documented during surveys, there were no observations of solitary treated females (Collins and Kasbohm 2016). These data help support the expectation that ovariectomized mares would not lose interest in or be cast out of the social dynamics of a wild horse herd. As noted by the NAS (2013), the ideal fertility control method would not eliminate sexual behavior or change social structure substantially.

A study conducted for 15 days in January 1978 (Asa et al. 1980), compared the sexual behavior in ovariectomized and seasonally anovulatory (intact) pony mares and found that there were no statistical differences between the two conditions for any measure of proceptivity or copulatory behavior, or days in

estrous. This may explain why treated mares at Sheldon NWR continued to be accepted into harem bands; they may have been acting the same as a non-pregnant mare. Five to ten percent of pregnant mares exhibit estrous behavior (Crowell-Davis 2007). Although the physiological cause of this phenomenon is not fully understood (Crowell-Davis 2007), it is thought to be a bonding mechanism that assists in the maintenance of stable social groups of horses year round (Ransom et al. 2014b). The complexity of social behaviors among free-roaming horses is not entirely centered on reproductive receptivity, and fertility control treatments that suppress the reproductive system and reproductive behaviors should contribute to minimal changes to social behavior (Ransom et al. 2014b, Collins and Kasbohm 2016).

BLM expects that wild horse harem structures would continue to exist under the proposed action because fertile mares, stallions, and their foals would continue to be a component of the herd. It is not expected that spaying a subset of mares would significantly change the social structure or herd demographics (age and sex ratios) of fertile wild horses.

'Foal stealing,' where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including spayed mares in a wild horse herd. McDonnell (2012) noted that "foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling," such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season.

Indirect Effects of Mare sterilization

The free-roaming behavior of wild horses is not anticipated to be affected by mare sterilization, as the definition of free-roaming is the ability to move without restriction by fences or other barriers within a HMA (BLM H-4700-1, 2010) and there are no permanent physical barriers being proposed.

In domestic animals, spaying is often associated with weight gain and associated increase in body fat (Fettman et al 1997, Becket et al 2002, Jeusette et al. 2006, Belsito et al 2009, Reichler 2009, Camara et al. 2014). Spayed cats had a decrease in fasting metabolic rate, and spayed dogs had a decreased daily energy requirement, but both had increased appetite (O'Farrell & Peachey 1990, Hart and Eckstein 1997, Fettman et al. 1997, Jeusette et al. 2004). In wild horses, contracepted mares tend to be in better body condition than mares that are pregnant or that are nursing foals (Nuñez et al. 2010); the same improvement in body condition is likely to take place in spayed mares. In horses spaying has the potential to increase risk of equine metabolic syndrome (leading to obesity and laminitis), but both blood glucose and insulin levels were similar in mares before and after ovariectomy over the short-term (Bertin et al. 2013). In wild horses the quality and quantity of forage is unlikely to be sufficient to promote over-eating and obesity.

Coit et al. (2009) demonstrated that spayed dogs have elevated levels of LH-receptor and GnRH-receptor mRNA in the bladder tissue, and lower contractile strength of muscles. They noted that urinary incontinence occurs at elevated levels in spayed dogs and in post-menopausal women. Thus, it is reasonable to suppose that some ovariectomized mares could also suffer from elevated levels of urinary incontinence.

Sterilization had no effect on movements and space use of feral cats or brushtail possums (Ramsey 2007, Guttilla & Stapp 2010), or greyhound racing performance (Payne 2013). Rice field rats (*Rattus argentiventer*) tend to have a smaller home range in the breeding season, as they remain close to their litters to protect and nurse them. When surgically sterilized, rice field rats had larger home ranges and moved further from their burrows than hormonally sterilized or fertile rats (Jacob et al. 2004). Spayed possums and foxes (*Vulpes vulpes*) had a similar core range area after spay surgery compared to before and were no more likely to shift their range than intact females (Saunders et al. 2002, Ramsey 2007).

The likely effects of spaying on mares' home range and habitat use can also be surmised from available literature. Bands of horses tend to have distinct home ranges, varying in size depending on the habitat and varying by season, but always including a water source, forage, and places where horses can shelter from inclement weather or insects (King and Gurnell 2005). It is unlikely that spayed mares will change their spatial ecology but being emancipated from constraints of lactation may mean they can spend more time away from water sources and increase their home range size. Lactating mares need to drink every day, but during the winter when snow can fulfill water needs or when not lactating, horses can traverse a wider area (Feist & McCullough 1976, Salter 1979). During multiple aerial surveys in years following the mare ovariectomy study at the Sheldon NWR, it was documented that all treated individuals appeared to maintain group associations, no groups consisted only of treated females, and none of the solitary animals observed were treated females (Collins and Kasbohm 2016). Because treated females maintained group associations, this indicates that their movement patterns and distances may be unchanged.

Spaying wild horses does not change their status as wild horses under the WFRHBA (as amended). In terms of whether spayed mares would continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that spayed mares would continue to roam unhindered. Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a spayed animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior. Despite marginal uncertainty about subtle aspects of potential changes in habitat preference, there is no expectation that spaying wild horses will cause them to lose their free-roaming nature.

In this sense, a spayed wild mare would be just as much 'wild' as defined by the WFRHBA as any fertile wild mare, even if her patterns of movement differ slightly. Congress specified that sterilization is an acceptable management action (16 USC §1333.b.1). Sterilization is not one of the clearly defined events that cause an animal to lose its status as a wild free-roaming horse (16 USC §1333.2.C.d). Any opinions based on a semantic and subjective definition of what constitutes a 'wild' horse are not legally binding for BLM, which must adhere to the legal definition of what constitutes a wild free-roaming horse, based on the WFRHBA (as amended). BLM is not obliged to base management decisions on personal opinions, which do not meet the BLM's principle and practice to "Use the best available scientific knowledge relevant to the problem or decision being addressed, relying on peer reviewed literature when it exists" (Kitchell et al. 2015).

Spaying is not expected to reduce mare survival rates on public rangelands. Individuals receiving fertility control often have reduced mortality and increased longevity due to being released from the costs of reproduction (Kirkpatrick and Turner 2008). Similar to contraception studies, in other wildlife species a common trend has been higher survival of sterilized females (Twigg et al. 2000, Saunders et al. 2002, Ramsey 2005, Jacob et al. 2008, Seidler and Gese 2012). Observations from the Sheldon NWR provide some insight into long-term effects of ovariectomy on feral horse survival rates. The Sheldon NWR ovariectomized mares were returned to the range along with untreated mares. Between 2007 and 2014, mares were captured, a portion treated, and then recaptured. There was a minimum of 1 year between treatment and recapture; some mares were recaptured a year later, and some were recaptured several years later. The long-term survival rate of treated wild mares appears to be the same as that of untreated mares (Collins and Kasbohm 2016). Recapture rates for released mares were similar for treated mares and untreated mares.

Effects of Spaying on Bone Histology

The BLM knows of no scientific, peer-reviewed literature that documents bone density loss in mares following ovariectomy. A concern has been raised in an opinion article (Nock 2013) that ovary removal in mares could lead to bone density loss. That paper was not peer reviewed nor was it based on research

in wild or domestic horses, so it does not meet the BLM's standard for "best available science" on which to base decisions (Kitchell et al. 2015). Hypotheses that are forwarded in Nock (2013) appear to be based on analogies from modern humans leading sedentary lives. Post-menopausal women have a greater chance of osteoporosis (Scholz-Ahrens et al. 1996), but BLM is not aware of any research examining bone loss in horses following ovariectomy. Bone loss in humans has been linked to reduced circulating estrogen. There have been conflicting results when researchers have attempted to test for an effect of reduced estrogen on animal bone loss rates in animal models; all experiments have been on laboratory animals, rather than free-ranging wild animals. While some studies found changes in bone cell activity after ovariectomy leading to decreased bone strength (Jerome et al. 1997, Baldock et al. 1998, Huang et al. 2002, Sigrist et al. 2007), others found that changes were moderate and transient or minimal (Scholz-Ahrens et al. 1996, Lundon et al. 1994, Zhang et al. 2007), and even returned to normal after 4 months (Sigrist et al. 2007).

Consistent and strenuous use of bones, for instance using jaw bones by eating hard feed, or using leg bones by travelling large distances, may limit the negative effects of estrogen deficiency on micro-architecture (Mavropoulos et al. 2014). The effect of exercise on bone strength in animals has been known for many years and has been shown experimentally (Rubin et al. 2001). Dr. Simon Turner, Professor Emeritus of the Small Ruminant Comparative Orthopaedic Laboratory at Colorado State University, conducted extensive bone density studies on ovariectomized sheep, as a model for human osteoporosis. During these studies, he did observe bone density loss on ovariectomized sheep, but those sheep were confined in captive conditions, fed twice a day, had shelter from inclement weather, and had very little distance to travel to get food and water (Simon Turner, Colorado State University Emeritus, written comm., 2015). Dr. Turner indicated that an estrogen deficiency (no ovaries) could potentially affect a horse's bone metabolism, just as it does in sheep and human females when they lead a sedentary lifestyle, but indicated that the constant weight bearing exercise, coupled with high exposure to sunlight ensuring high vitamin D levels, are expected to prevent bone density loss (Simon Turner, Colorado State University Emeritus, written comm., 2015).

Home range size of horses in the wild has been described as 4.2 to 30.2 square miles (Green and Green 1977) and 28.1 to 117 square miles (Miller 1983). A study of distances travelled by feral horses in "outback" Australia shows horses travelling between 5 and 17.5 miles per 24-hour period (Hampson et al. 2010a), travelling about 11 miles a day even in a very large paddock (Hampson et al. 2010b). Thus, extensive movement patterns of wild horses are expected to help prevent bone loss. The expected daily movement distance would be far greater in the context of larger pastures typical of BLM long-term holding facilities in off-range pastures. A horse would have to stay on stall rest for years after removal of the ovaries in order to develop osteoporosis (Simon Turner, Colorado State University Emeritus, written comm., 2015) and that condition does not apply to any wild horses turned back to the range or any wild horses that go into off-range pastures.

Genetic Effects of Spaying and Neutering

It is true that spayed females and neutered males are unable to contribute to the genetic diversity of the herd. BLM is not obligated to ensure that any given individual in a herd has the chance to sire a foal and pass on genetic material. Management practices in the BLM Wild Horse and Burro Handbook (2010) include measures to increase population genetic diversity in reproducing herds where monitoring reveals a cause for concern about low levels of observed heterozygosity. These measures include increasing the sex ratio to a greater percentage of fertile males than fertile females (and thereby increasing the number of males siring foals) and bringing new animals into a herd from elsewhere.

In herds that are managed to be non-reproducing, it is not a concern to maintain genetic diversity because the management goal would be that animals in such a herd would not breed.

In reproducing herds where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, spaying and neutering is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before she is treated with contraceptives. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviate potential inbreeding concerns (BLM 2010). The NAS report (2013) recommended that managed herds of wild horses would be better viewed as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements.

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM. As a result, most alleles that are present in any given mare are likely to already be well represented in her siblings, cousins, and more distant relatives on the HMA. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Although those results are specific to mares, some inferences about potential effects of stallion sterilization may also be made from their results. Roelle and Oyler-McCance (2015) showed that the risk of the loss of genetic heterozygosity is extremely low except in cases where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the population are permanently sterilized. Given that 94 of 102 wild horse herds sampled for genetic diversity did not meet a threshold for concern (NAS 2013), the starting level of genetic diversity in most wild-horse herds is relatively high.

In a breeding herd where more than 85% of males in a population are sterile, there could be genetic consequences of reduced heterozygosity and increased inbreeding coefficients, as it would potentially allow a very small group of males to dominate the breeding (e.g., Saltz et al. 2000). Such genetic

consequences could be mitigated by natural movements or human-facilitated translocations (BLM 2010). Garrott and Siniff's (1992) model predicts that gelding 50-80% of mature males in the population would result in reduced, but not halted, mare fertility rates. However, neutering males tends to have short-lived effects, because within a few years after any male sterilization treatment, a number of fertile male colts would become sexually mature stallions who could contribute genetically to the herd.

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Intrauterine Devices (IUDs)

Up through the present time (September 2020), BLM has not used intrauterine devices (IUDs) to control fertility as a wild horse and burro fertility control method on the range. The BLM has supported and continues to support research into the development and testing of effective and safe IUDs for use in wild horse mares (Baldrighi et al. 2017, Holyoak et al. unpublished data). However, existing literature on the use of IUDs in horses allows for inferences about expected effects of any management alternatives that might include use of IUDs, and support the apparent safety and efficacy of some types of IUDs for use in horses. Overall, as with other methods of population growth suppression, use of IUDs and other fertility control measures are expected to help reduce population growth rates, extend the time interval between gathers, and reduce the total number of excess animals that will need to be removed from the range.

The 2013 National Academies of Sciences (NAS) report considered IUDs and suggested that research should test whether IUDs cause uterine inflammation, and should also test how well IUDs stay in mares that live and breed with fertile stallions. Since that report, a recent study by Holyoak et al. (unpublished data) indicate that a flexible, inert, y-shaped, medical-grade silicone IUD design prevented pregnancies in all the domestic mares that retained the device, even when exposed to fertile stallions. Domestic mares in that study lived in large pastures, mating with fertile stallions. Biweekly ultrasound examinations showed that IUDs stayed in 75% of treated mares over the course of two breeding seasons. The IUDs were then removed so the researchers could monitor the mares' return to fertility. Uterine health, as measured in terms of inflammation, was not seriously affected by the IUDs, and most mares became pregnant within months after IUD removal. The overall results are consistent with results from an earlier study (Daels and Hughes 1995), which used O-shaped silicone IUDs.

IUDs are considered a temporary fertility control method that does not generally cause future sterility (Daels and Hughes 1995). Use of IUDs is an effective fertility control method in women, and IUDs have historically been used in livestock management, including in domestic horses. Insertion of an IUD can be a very rapid procedure, but it does require the mare to be temporarily restrained, such as in a squeeze chute. IUDs in mares may cause physiological effects including discomfort, infection, perforation of the uterus if the IUD is hard and angular, endometritis, uterine edema (Killian et al. 2008), and pyometra (Klabnik-Bradford et al. 2013). In women, deaths attributable to IUD use may be as low as 1.06 per million (Daels and Hughes 1995).

The exact mechanism by which IUDs prevent pregnancy is uncertain (Daels and Hughes 1995), but the presence of an IUD in the uterus may, like a pregnancy, prevent the mare from coming back into estrus (Turner et al. 2015). However, some domestic mares did exhibit repeated estrus cycles during the time when they had IUDs (Killian et al. 2008, Gradil et al. 2019). The main cause for an IUD to not be effective at contraception is its failure to stay in the uterus (Daels and Hughes 1995). As a result, one of

the major challenges to using IUDs to control fertility in mares on the range is preventing the IUD from being dislodged or otherwise ejected over the course of daily activities, which could include, at times, frequent breeding. No potentially harmful consequences of a mare being treated with both an IUD and an immunocontraceptive vaccine have been identified, as those two forms of fertility control are thought to have different mechanisms of action. An IUD-treated mare may be temporarily infertile longer than she would have been, if only given a dose of immunocontraceptive vaccine. The overall duration of infertility for individuals treated with both methods would be expected to depend on whether the specific mare's immune response to the vaccine ceases first, or whether the IUD is dislodged from the uterus first.

At this time, it is thought that any IUD inserted into a pregnant mare may cause the pregnancy to terminate, which may also cause the IUD to be expelled. For that reason, it is expected that IUDs would only be inserted in non-pregnant (open) mares. Wild mares receiving IUDs would be checked for pregnancy prior to insertion of an IUD. This can be accomplished by transrectal palpation and/or ultrasound performed by a veterinarian. Pregnant mares would not receive an IUD. The IUD is inserted into the uterus using a thin, tubular applicator similar to a shielded culture tube, and would be inserted in a manner similar to that routinely used to obtain uterine cultures in domestic mares. If a mare has a zygote or very small, early phase embryo, it is possible that it will fail to be detected in screening, and may develop further, but without causing the expulsion of the IUD. Wild mares with IUDs would be individually marked and identified, so that they can be monitored occasionally and examined, if necessary, in the future, consistent with other BLM management activities.

Using metallic or glass marbles as IUDs may prevent pregnancy in horses (Nie et al. 2003) but can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015). Marbles may break into shards (Turner et al. 2015), and uterine irritation that results from marble IUDs may cause chronic, intermittent colic (Freeman and Lyle 2015). Metallic IUDs may cause severe infection (Klabnik-Bradford et al. 2013).

In domestic ponies, Killian et al. (2008) explored the use of three different IUD configurations, including a silastic polymer O-ring with copper clamps, and the "380 Copper T" and "GyneFix" IUDs designed for women. The longest retention time for the three IUD models was seen in the "T" device, which stayed in the uterus of several mares for 3-5 years. Reported contraception rates for IUD-treated mares were 80%, 29%, 14%, and 0% in years 1-4, respectively. They surmised that pregnancy resulted after IUD fell out of the uterus. Killian et al. (2008) reported high levels of progesterone in non-pregnant, IUD-treated ponies.

Soft IUDs may cause relatively less discomfort than hard IUDs (Daels and Hughes 1995). Daels and Hughes (1995) tested the use of a flexible O-ring IUD, made of silastic, surgical-grade polymer, measuring 40 mm in diameter; in five of six breeding domestic mares tested, the IUD was reported to have stayed in the mare for at least 10 months. In mares with IUDs, Daels and Hughes (1995) reported some level of uterine irritation but surmised that the level of irritation was not enough to interfere with a return to fertility after IUD removal.

More recently, several types of IUDs have been tested for use in breeding mares. When researchers attempted to replicate the O-ring study (Daels and Hughes 1995) in an USGS / Oklahoma State University (OSU) study with breeding domestic mares, using various configurations of silicone O-ring IUDs, the IUDs fell out at unacceptably high rates over time scales of less than 2 months (Baldrighi et al. 2017). Subsequently, the USGS / OSU researchers tested a Y-shaped IUD to determine retention rates and assess effects on uterine health; retention rates were greater than 75% for an 18-month period, and mares returned to good uterine health and reproductive capacity after removal of the IUDs (Holyoak et al., unpublished results). These Y-shaped silicone IUDs are considered a pesticide device by the EPA, in that they work by physical means (EPA 2020). The University of Massachusetts has developed a magnetic IUD that has been effective at preventing estrus in non-breeding domestic mares (Gradil et al.

2019, Joonè et al. 2021). After insertion in the uterus, the three subunits of the device are held together by magnetic forces as a flexible triangle. A metal detector can be used to determine whether the device is still present in the mare. In an early trial, two sizes of those magnetic IUDs fell out of breeding domestic mares at high rates (Holyoak et al., unpublished results). In 2019, the magnetic IUD was used in two trials where mares were exposed to stallions, and in one where mares were artificially inseminated; in all cases, the IUDs were reported to stay in the mares without any pregnancy (Gradil 2019, Joonè et al. 2021).

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