Fertility control options for management of free-roaming horse populations

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Abstract: The management of free-roaming horses (*Equus ferus*) and burros (*E. asinus*) in the United States has been referred to as a "wicked problem" because, although there are population control options, societal values will ultimately determine what is acceptable and what is not. In the United States, free-roaming equids are managed by different types of organizations and agencies, and the landscapes that these animals inhabit vary widely in terms of access, size, topography, climate, natural resources, flora, and fauna. This landscape diversity, coupled with contemporary socioeconomic and political environments, means that adaptive management practices are needed to regulate these free-roaming populations. The Bureau of Land Management (BLM) currently manages free-roaming equids on 177 herd management areas in the United States by applying fertility control measures *in situ* and/or removing horses, which are either adopted by private individuals or sent to long-term holding facilities. The BLM off-range population currently includes >50,000 animals and costs approximately $50 million USD per year to maintain; on-range equid numbers were estimated in March 2022 to be approximately 82,384. On-range populations can grow at 15–20% annually, and current estimates far exceed the designated appropriate management level of 26,715. To reduce population recruitment, managers need better information about effective, long-lasting or permanent fertility control measures. Because mares breed only once a year, fertility control studies take years to complete. Some contraceptive approaches have been studied for decades, and results from various trials can collectively inform future research directions and actions. Employing 1 or more fertility control tools in concert with removals offers the best potential for success. Active, iterative, cooperative, and thoughtful management practices can protect free-roaming horses while simultaneously protecting the habitat. Herein, we review contraceptive vaccines, intrauterine devices, and surgical sterilization options for controlling fertility of free-roaming horses. This review provides managers with a "fertility control toolbox" and guides future research.

Key words: castration, *Equus asinus*, *Equus ferus*, feral burros, feral horses, fertility control, field implementation, immunocontraception, intrauterine devices, ovariectomy

Free-roaming horse (*Equus ferus*) and burro (*E. asinus*) populations that inhabit designated federal lands in the western United States are protected by the Wild Free-Roaming Horses and Burros Act of 1971 (Public Law 92-195). This legislation requires the Bureau of Land Management (BLM) and the U.S. Forest Service (USFS) to manage populations to achieve and maintain a thriving, natural ecological balance. The USFS oversees 34 Wild Horse and Burro Territories, while the BLM manages wild horses and burros on 177 Herd Management Areas (HMAs) across 10 western states. In an attempt to define an ecological balance, the BLM determined the Appropriate Management Level (AML) for each HMA (National Research Council [NRC]
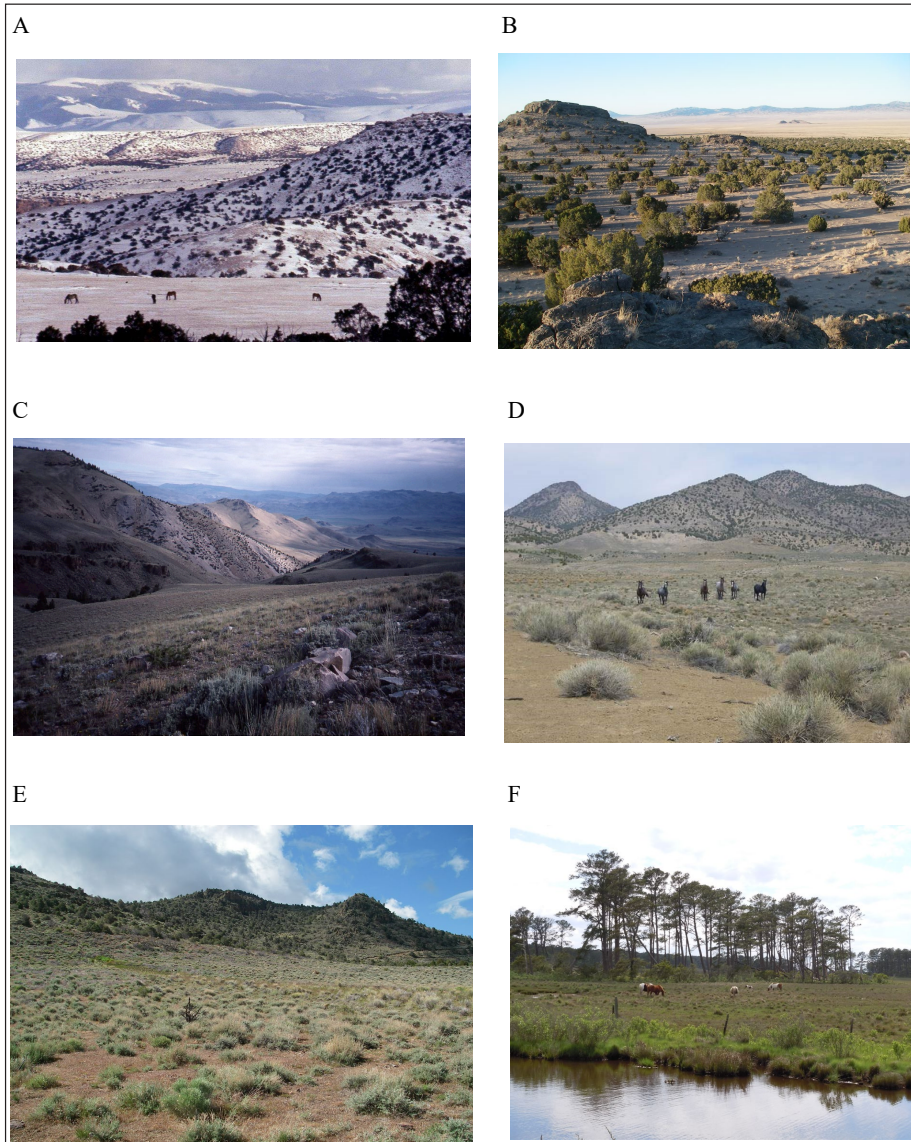
Figure 1. Different geographic landscapes supporting free-roaming horse (*Equus ferus*) populations in the United States. (A) The Pryor Mountains Herd Management Area (HMA) in southern Montana, USA, straddles the Crow Indian Reservation, Custer National Forest, and private land, covered in shrub-grass vegetation, Douglas fir (*Pseudotsuga menziesii*), lodgepole (*Pinus contorta*) and limber (*Pinus flexilis*) pine, and juniper (*Juniperus* spp.); temperatures range from -14–31°C. (B) The King Top Mountains HMA in west-central Utah, USA, has pinyon (*Pinus edulis*) and juniper communities on upper slopes and sagebrush (*Artemisia nova*), shadscale (*Atriplex confertifolia*), and ricegrass (*Oryzopsis hymenoides*) at lower elevations. (C) The Challis HMA in central Idaho, USA, ranges in elevation from 1,400 to nearly 2,450 m; high ridges have Douglas fir, sagebrush occupies wide basins, and riparian areas contain willows (*Salix wolfii*), birch (*Betula papyrifera*), and aspen (*Populus tremuloides*); temperatures range from -9–21°C. (D) The Sand Wash Basin HMA in north-western Colorado, USA, includes public, private, and state land, covered in sagebrush and bunchgrass (*Festuca viridula*), saltbush (*Atriplex confertifolia*) and pinon-juniper woodlands; temperatures range from -3–38°C. (E) The Pine Nut Mountains HMA in west-central Neva-da, USA, just east of Carson City, includes private and public lands with peak elevations of 2,700 m; vegetation consists of pinyon pine, juniper, sagebrush, and grasses; temperatures range from -12–38°C. (F) Chincoteague Island, northeastern Virginia, USA, just south of Assateague Island National Seashore, Maryland, USA, vegetation ranges from salt-tolerant plants (e.g., *Cakile edentula*) and grasses (e.g., *Ammophila breviligulata*) in dunes and salt marshes to loblolly pines (*Pinus taeda*) and shrubs (e.g., *Rubus argutus*) surrounding fresh-water wetlands; temperatures range from 17–38°C (*photos courtesy of J. Turner [A and C], D. Stoner [B and E], K. Grams [D], and U. Bechert [F]*).

2013). The current AML of HMAs has been set at 26,785 (BLM 2022); however, in March 2019, >80% of the HMAs exceeded their AML (BLM 2020). Garrott (2018) reported that given annual growth rates of 15–20%, populations are currently overwhelming management actions.

Because HMAs vary in size (from ~800 to >526,800 ha), topography, climate (Stoner et al. 2021), natural resources, and flora and fauna, they create different challenges for population management (Figure 1). Free-roaming burros may have positive effects on the landscape (e.g., shrub clearance, seed dispersal) as reported in 29% (10 of 34) of studies that examined environmental effects (Clancy et al. 2021). Lundgren et al. (2021) also reported free-roaming equids can expand water resources for other species by digging <2-m-deep wells in deserts. However, most studies report that overabundant equids can compact soils, increase erosion, and decrease plant cover (Ostermann-Kelm et al. 2009), compete with native fauna over resources, and be associated with loss of biodiversity (Davies and Boyd 2019, Coates et al. 2021). Native species like mule deer (*Odocoileus hemionus*) occur on 97% of BLM and USFS managed lands, and pronghorn (*Antilocapra americana*) are found on 82% (Stoner et al. 2021).

Climate change is altering habitats and ecosystem processes on some rangelands (McCollum et al. 2017) and will necessitate a review of AMLs. Factors such as drought or fire can have a more pronounced effect on vegetation and forage availability than locally overabundant free-roaming equid populations (Briske et al. 2005, Vetter 2005, Briske et al. 2015). Invasive species, like cheatgrass (*Bromus tectorum*), also affect rangelands and could be spread by horses (King et al. 2019). However, management goals should aim to preserve ecological processes rather than specific species (Perryman et al. 2021).

Free-roaming equids inhabit landscapes that are managed by different types of organizations and agencies in addition to the BLM and USFS, including First Nations, state and county governments, military organizations, the National Park Service (NPS), state and federal fish and wildlife agencies, private landowners, and other groups (Beever et al. 2018). Schoenecker et al. (2021) estimate that almost 300,000 feral equids now inhabit U.S. landscapes. Managing populations of free-roaming equids is thus

a significant challenge, involving animal welfare, environmental, sociological, public policy, and economic dimensions (Scasta et al. 2018). Because of changing socioeconomic and political variables as well as dynamic ecosystems, adaptive management of free-roaming equid populations is required. This is an iterative decision-making process that relies on consistent quantitative and qualitative monitoring, reliable models to develop predictions of future changes, and reviewing and adjusting management actions (Holling 1978, Briske et al. 2008).

Horses and burros, while both equids, have different survival and reproductive strategies. Burros are better adapted to hot, dry conditions and low-quality forage than are horses (Seegmiller and Ohmart 1981, Izraely et al. 1989, Burden and Thiemann 2015), and they can travel long distances (Gedir et al. 2021). Unlike the harem structure of horses, burros living in arid environments, like the southwestern United States, have a fission-fusion social system with no permanent bonds between adult individuals; however, in mesic environments, they can also form stable harem groups (Moehlman 1998).

Burro numbers were estimated to total 17,780 on BLM lands in March 2022, with the largest populations in Arizona, USA, followed by Nevada and California, USA (BLM 2022). Burros can become reproductively active as early as 10–12 months of age (Perryman and Muchlinski 1987); they are polyestrous and can mate throughout the year, although some populations have a peak breeding season in the late spring and summer (Grinder et al. 2006).

In a given population, up to 72% of the jennies may be pregnant (Wolfe et al. 1989), and poor physical condition does not appear to have a significant effect on fecundity (Perryman and Muchlinski 1987, Choquenot 1991). In 1 population, 60% of the lactating females were also pregnant, and males tended to live longer than females (Johnson et al. 1987). Burro populations can skew young with few animals living >10 years of age (Johnson et al. 1987). Immunocontraception requiring multiple doses of porcine zona pellucida (PZP) effectively contracepted small populations of jennies on geographically closed islands (Turner et al. 1996, French et al. 2020); however, open free-ranging populations could not be successfully managed this way (Gedir et al. 2021). Few studies

focused on fertility control of burros have been conducted (Clancy et al. 2021); however, it is possible that contraceptive options for horses could be adapted for burros in the future with consideration for how social system differences may affect male harassment of jennies in estrus.

In contrast, free-roaming horses consume primarily grasses (King and Schoenecker 2019), with forbs and shrubs comprising <8% of the diet (Hubbard and Hansen 1976). Horses usually belong to social groups or harems that occupy home ranges (McCort 1984). Mares become reproductively active up to 6 months later than jennies, and they are seasonal long-day breeders, mating only during the summer. The gestation period for the mare is 37–38 days shorter than the jenny (Fielding 1988), free-roaming mares usually live longer (up to 15 years or more), and the foal survival rate is comparatively greater (Johnson et al. 1987, Garrott et al. 1991).

The BLM currently manages free-roaming horse populations by applying fertility control measures *in situ* and removing animals from the range. The BLM estimated the on-range horse population to be approximately 79,568 in March 2020 (BLM 2021) and 64,604 in March 2022 (BLM 2022), which is still significantly over AML. Horses removed from the range are either adopted by private individuals or sent to long-term holding facilities. Younger horses may be adopted by members of the public, but about half of the 84,741 horses removed between 2000 and 2009 ended up in long-term holding (Vincent 2010). Although the overall adoption rate has increased over the past 4 years (BLM 2020), the growing supply greatly exceeds the demand (Hendrickson 2018). The off-range population includes >45,000 horses and costs approximately $50 million USD per year to maintain (Garrott 2018), and in 2019, off-range holding costs consumed 67% of the BLM Wild Horse and Burro Program's budget (BLM 2019).

A suite of effective, long-lasting, or permanent fertility control solutions is needed to manage free-roaming horse populations. Research on a specific fertility control option typically starts with 1 or more laboratory-based or clinical studies to develop proof of concept before moving to field trials. However, not uncommonly, additional research with captive animals is needed to address challenges experienced during field trials, learn more about the mechanism(s) of action,

and/or fine-tune treatment strategies. Horses are seasonal long-day breeders that foal once a year, so trials to determine contraceptive efficacy (especially long-term efficacy) take years to complete. The choice of particular fertility control method(s) in each HMA will depend on management goals, the region's particular geographic, socioeconomic, and biological characteristics, as well as the demographic characteristics and approachability of the horses.

Our review will focus on horses because more fertility control research has been conducted with horses, they are physiologically different from burros (Burden and Thiemann 2015), and burro populations are comparatively smaller in size (Schoenecker et al. 2021). This review summarizes what is known about several horse contraceptive and sterilization options, as well as considerations for implementation, to provide managers with a "fertility control toolbox" and guide future research and management efforts.

Methods

The Free Roaming Equids and Ecosystems Sustainability Network (FREES; https://extension.usu.edu/freesnetwork/) Population Management Working Group developed a list of fertility control options that have been researched in horses, are currently available, or are in development. Members of the working group also gave presentations as part of a panel at the 2020 FREES Summit "Connecting the Dots" in Cody, Wyoming, USA, which included an overview of fertility control options, constraints of implementing fertility control and removals, and population modeling.

In this paper, we provide an in-depth review of horse fertility control options that the BLM has considered, including: immunocontraceptive vaccines, intrauterine devices (IUDs), and surgical options, with contributions from authors who have conducted research in these areas. Details of each fertility control option are reviewed based on previously published results, current projects are briefly described, and remaining questions and future research needs are identified. Considerations for implementation, which vary from 1 geographic location to another and include regulatory requirements, are also described.

Currently available tools (i.e., officially approved for use) as well as promising new fertil-
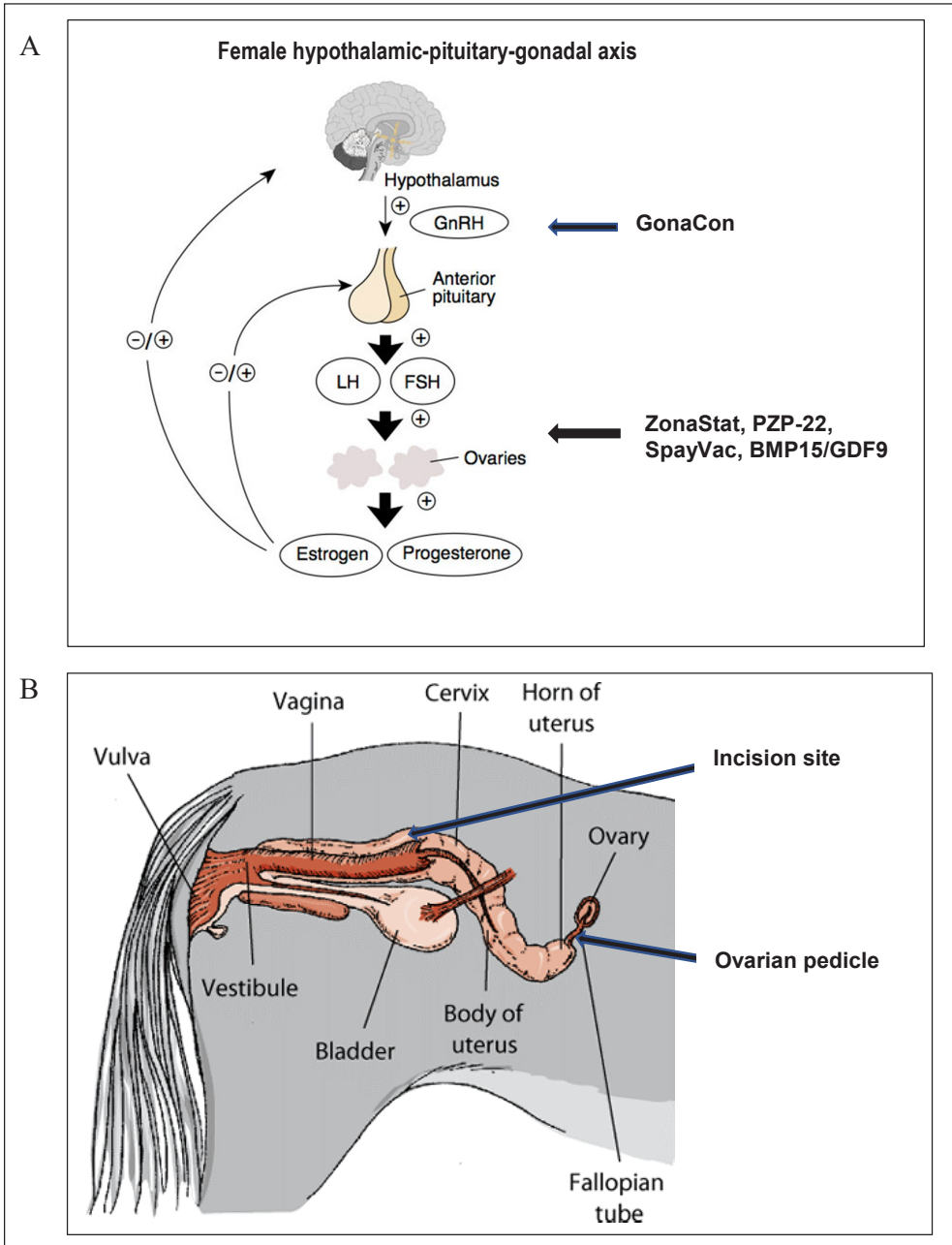
Figure 2. The reproductive system of the mare (*Equus ferus*) depicting (A) endocrine relationships (adapted from Hiller-Sturmhöfel and Bartke 1998) and immunocontraceptive targets; (B) side view of reproductive organs (adapted from Momont 2019) with ovariectomy incision and écraseur placement sites.

ity control options are included to help horse population managers develop a greater level of understanding and assess ability to implement 1 or more alternatives under different scenarios and management goals. Except for castration, all options focus on fertility control of mares because a single male can inseminate numer- ous females (Garrott and Siniff 1992). The use of contraception as a means of free-roaming horse fertility control was introduced in the 1970s with the successful field use of steroid hormones in males (Turner and Kirkpatrick 1982) and females (Eagle et al. 1992, Plotka et al. 1992). However, hormonal fertility control

options (e.g., estradiol-17β or progesterone implants) are not included in this review because these hormones could potentially enter the food chain and they are not considered to be an option by the BLM (Kane 2018).

Results and discussion
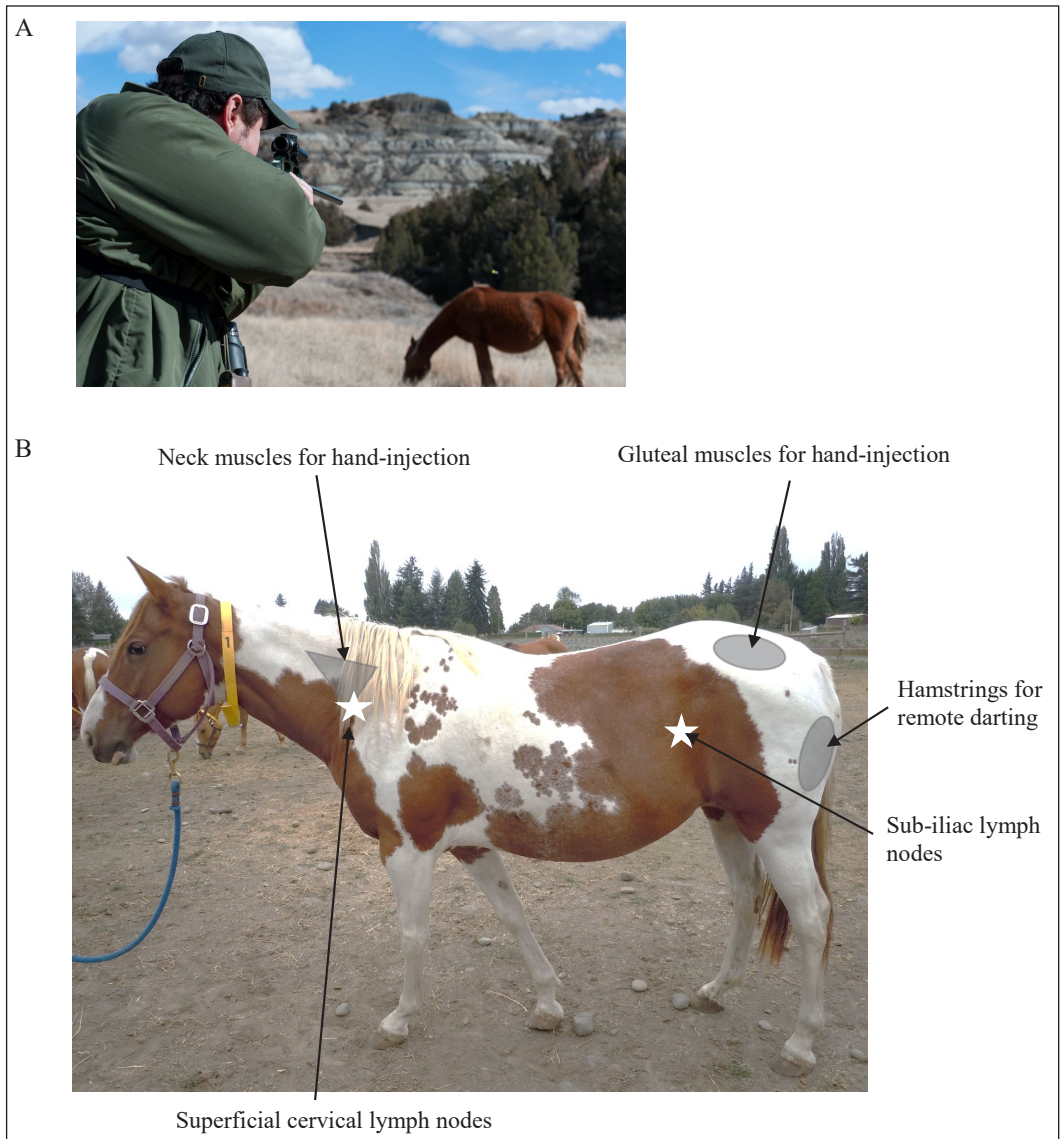### Immunocontraception

Contraceptive vaccines work by stimulating the immune system to produce antibodies that bind to specific molecules, usually proteins, affiliated with the reproductive system and impede fertility (Figure 2A). Vaccines are constructed by combining 3 primary components: (1) the antigen, (2) 1 or more adjuvants, and (3) the formulation or diluent that make up the final product and the way it is delivered. The antigen determines how the vaccine works. Importantly, the key to immunocontraception, which differs from traditional immunizations, is that the class of antigen is endogenous to the animal; thus, making the immune system responsive is often difficult. For example, PZP vaccines stimulate the immune system to produce antibodies to the zona pellucida (ZP), which surrounds the oocyte and facilitates sperm binding. When PZP antibodies occupy the mare's zona pellucida receptors, sperm are unable to bind to the oocyte, and fertilization cannot take place. The adjuvant, the second part of the immunocontraceptive, increases the immune response to an antigen in various ways (Awate et al. 2013, Brito et al. 2013). For example, Modified Freund's Adjuvant (MFA) is an oil-based adjuvant made with freeze-dried fractionated cell walls of *Mycobacterium butyricum* that stimulates immune cells to produce antibodies against the antigen component of the vaccine (Lyda et al. 2005). The formulation may also include 1 or more different classes of immune potentiators (Brito et al. 2013), including agonists of innate activation systems like Toll-like receptors (e.g., Poly(I:C) used in recombinant PZP vaccines; Nolan et al. 2018).

Contraceptive vaccines can target gamete (sperm or egg) production (e.g., gonadotropin releasing hormone; GnRH), gamete maturation (e.g., oocyte growth factors), gamete function (e.g., PZP), or gamete outcome (e.g., maternal recognition factors; Naz et al. 2005, Kaur and Prabha 2014). Other antigens, in addition to PZP, may target gamete function, but they

have not yet been tested in horse contraceptive vaccines. For example, a sperm surface protein (Izumo) facilitates binding to the egg receptor (Juno) and prevents polyspermy (Bianchi et al. 2014, Ohto et al. 2016). Vaccination against Izumo reduced fertility in female mice (*Mus musculus*) by inhibiting sperm penetration, and infertile women often have antibodies to Izumo (Naz 2014). Research has focused on other aspects of fertility including maternal recognition of pregnancy. Maternal recognition of pregnancy prevents lysis of the corpus luteum to maintain progesterone production and pregnancy; however, different agents serve as maternal recognition factors (MRF) in different species (Bazer 2015, Raheem 2017). For example, the MRF is human chorionic gonadotropin in humans, estrogen in pigs (*Sus domesticus*), interferon tau in ruminants, but it is not known what the agent is in mares (Raheem 2017). Equine chorionic gonadotropin plays a key role in the development of accessory corpora lutea in mares, but only after day 40 of pregnancy (Klein 2016).

In this section, we review 5 immunocontraceptive vaccines that vary by antigen, adjuvant, and formulation, as well as treatment protocols. Each vaccine's characteristics will make it more or less suitable to a particular scenario based on implementation constraints, contraceptive efficacy, safety, and management goals.

Porcine zona pellucida (PZP). Three different kinds of PZP-based vaccines (i.e., ZonaStat, PZP-22, and SpayVac®) have been used in horses, as well as experimental recombinant versions (Joonè et al. 2017*a*, Nolan et al. 2018). The ZP is comprised of 3 or 4 different glycoproteins. The mouse ZP has 3 glycoproteins (ZP1, ZP2, and ZP3); however, most mammalian species also have ZP4, including other rodents (Izquierdo-Rico et al. 2009), ungulates (Topper et al. 1997), rabbits (*Oryctolagus cuniculus*; Stetson et al. 2012), and humans (Lefievre et al. 2004). The glycoprotein ZP3 acts as the primary sperm receptor in mice; however, in humans and other species, ZP3 as well as ZP1 and ZP4 bind to sperm and induce the acrosome reaction, which allows for penetration of the ZP by sperm (Gupta et al. 2012). The primary ZP glycoproteins in the PZP-based vaccines described below include ZP2, ZP3, and ZP4 (D. Kroeger, SpayVac-for-Wildlife, Inc., personal communication). The PZP vaccines specifically

A

B

Neck muscles for hand-injection

Gluteal muscles for hand-injection

Hamstrings for remote darting

Sub-iliac lymph nodes

Superficial cervical lymph nodes

Figure 3. Vaccine delivery methods in horses (*Equus ferus*) include (A) remote darting targeting the hamstring muscles, and (B) hand-injections in either neck or gluteal muscles. The 2 major lymph nodes differ in distance to injection sites (*photos courtesy of M. Thompson [A] and U. Bechert [B]*).

target reproductive tissues, so they are safe to use (e.g., can be administered during pregnancy and lactation) and do not persist in the environment (Hobbs and Hinds 2018).

In 1985, based on successful fertility reduction (86%) using native PZP vaccine in 14 domestic mares (Liu et al. 1989), the team of Kirkpatrick, Turner, and Liu began a multi-study program testing PZP vaccine in free-roaming horses. Early in the research program, a list of "ideal" characteristics desired for a contraceptive vaccine was developed. It included: safety,

efficacy, reversibility, no passage through the food chain, continued basic social/sexual behavior, pregnancy and offspring unaffected, deliverable remotely, and cost effective (Kirkpatrick and Turner 1985).

The PZP antigens in ZonaStat-H (Science and Conservation Center, Billings, Montana, USA) are obtained by mincing pig ovaries (obtained from local abattoirs) using a ganged razorblades apparatus. Oocytes are counted and filtered to obtain the zonae pellucida in phosphate buffered saline (PBS). The solution

is then homogenized, solubilized at 70°C for 40–60 minutes, and stored frozen at -20°C. The vaccine is an emulsion of 100 µg aqueous PZP (0.5 mL) in PBS plus 0.5 mL MFA to create the primer dose, and the booster uses Freund's incomplete adjuvant, which lacks the mycobacterial component. When properly emulsified, the vaccine can be kept on ice for 24 hours prior to administration.

The first field trial of ZonaStat-H in free-roaming horses began in 1988 on Assateague Island (ASIS) National Seashore, Maryland, USA. Treatment consisted of an initial vaccination (primer) followed by a booster 3–4 weeks later all delivered via remote darting (similar to that depicted in Figure 3A). None of the 26 treated mares foaled the following year, compared to a 49% foaling rate for untreated mares (Kirkpatrick and Turner 1990). From 1989 to 1994, >50% (and up to 70%) of the ASIS mares received primer plus booster or annual booster treatments and averaged only a 3.5% fertility rate (Turner and Kirkpatrick 1991, Kirkpatrick 1995). A remote (fecal) early pregnancy determination method was also developed there (Kirkpatrick et al. 1991). In 1994, the (NPS) adopted ZonaStat-H as the sole population management tool for ASIS horses, achieving zero population growth by 2001 (Ballou et al. 2008). The NPS achieved a 32% decrease in population size by 2011 and has maintained their ZonaStat-H management goal since 2016 (NPS 2020; Figure 4). No ASIS horse has been removed for population control.

Importantly, the ASIS study established a clear profile of safety for PZP vaccines, showing reversibility and no effect on existing pregnancies (Kirkpatrick et al. 1992; Kirkpatrick and Turner 2002, 2003). Contraception was also associated with improved body condition and extended longevity (Kirkpatrick and Turner 2007, 2008). Behaviorally, basic social and sexual patterns were found to be similar among treated and untreated mares in other populations examined (Powell 1999). However, some studies have shown that PZP treatment can affect behavior after the usual breeding season, including reduced mare band fidelity and extension of estrous cycling with impacts on stallion behavior (Madosky et al. 2010, Ransom et al. 2010, Nuñez et al. 2017). Possible long-term consequences of such behavioral alterations or adaptation to them are not reported.

The use of ZonaStat-H in western free-roaming horses and elsewhere began in the early 1990s (Turner et al. 1996, 1997) with involvement of the BLM. Since 2005, >10,000 mares have been treated with ZonaStat-H (primarily by darting) in herds on BLM, NPS, USFS, state, sanctuary, and First Nations lands (K. Frank, Science and Conservation Center, and B. Wade, BLM Wild Horse and Burro Program, personal communications). In some smaller herds, in which 50–80% of mares have been treated annually, horse removals have been sharply reduced, and in some cases, zero population growth has been achieved (Kirkpatrick and Turner 2008, NPS 2020; K. Frank, Science and Conservation Center, personal communication). ZonaStat-H is registered by the U.S. Environmental Protection Agency (EPA, registration #86833-1) and is produced by the Science and Conservation Center in Billings, Montana.

Because the ASIS vaccination protocol of 2 initial injections with annual boosters is labor intensive and unsuitable for use on wary and widely dispersed herds common on western public lands, it was crucial to develop a single-injection PZP vaccine with multi-year effectiveness. Beginning in 1990, the BLM funded a series of studies testing PZP treatments that added PZP packaged in controlled-release lactide/glycolide polymers to the ZonaStat-H vaccine.

Large-scale field tests employing vaccine with controlled-release polymer microspheres were conducted on mares gathered by helicopter (as part of removal management actions) at the Antelope HMA (*n* = 127) in northeastern Nevada in 1992 and the Nevada Wild Horse Range in central Nevada (NWHR; *n* = 222) in 1996. In both studies, foaling rates of mares receiving a single injection of ZonaStat-H plus microsphere-encapsulated PZP were compared to mares receiving the 2-injection ASIS protocol. In the Antelope HMA study, the microsphere treatment reduced fertility by 73% but was less effective than the 2-injection protocol (Turner et al. 1997). However, addition of adjuvant (Carbopol 934) in microspheres at the NWHR study yielded contraceptive efficacy that matched the 2-injection protocol, with both preparations exceeding 80% fertility reductions over a year (Turner et al. 2000).

From 1996 to 2000, controlled-release preparations were modified and improved in the lab,
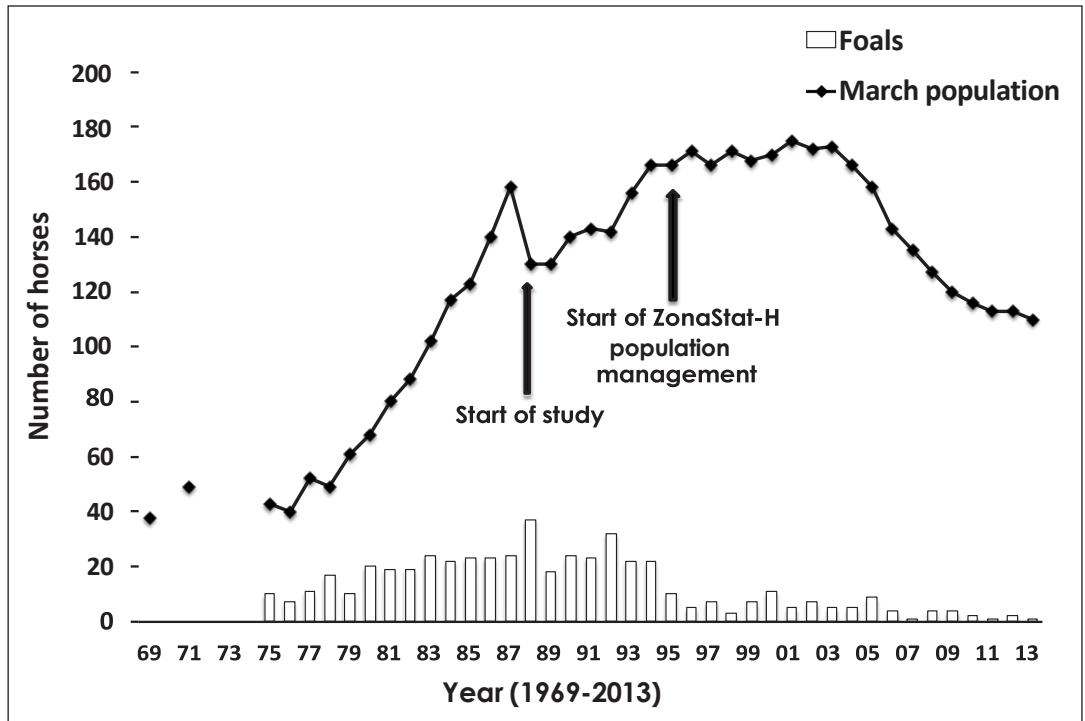
Figure 4. Effect of long-term ZonaStat-H vaccine treatment (primer + 1-month booster and subsequent annual boosters) delivered by dart to >50% of mares in the free-roaming horse (*Equus ferus*) population inhabiting Assateague Island National Seashore, Maryland, USA, from 1989 to 2013. Personnel performed horse census counts by individual identification annually in March starting in 1975.

and then tested in captive mares and in the field (Turner et al. 2002, 2007). Studies of PZP antibody titers across 2 years in captive mares yielded a preparation (eventually named PZP-22; Science and Conservation Center, Billings, Montana) that maintained contraceptive-level titers for 22 months post-immunization (Liu et al. 2005). PZP-22 consists of a single injection containing the ZonaStat-H primer dose plus 3 different polymer mini-pellets matrixed with PZP and a saponin adjuvant (QA-21) to mimic the initial and first annual ZonaStat-H boosters (Turner et al. 2008).

Offering the prospect of a single injection, 2-year PZP vaccine, PZP-22 was used in its first field trial on 100 mares gathered, treated, and released at Clan Alpine HMA, Nevada, beginning in 2000. In the 3 years following PZP-22 treatment, fertility was reduced by 89%, 74%, and 41% relative to untreated mares, returning to full fertility in the fourth year (Turner et al. 2007). This study demonstrated PZP-22 multi-year efficacy and reversibility.

With the support of the Annenberg Foundation, the Humane Society of the United States,

and the BLM, 3 subsequent field trials of PZP-22 were carried out between 2008 and 2014 at Cedar Mountain HMA, Utah, Sand Wash Basin HMA, Colorado, and Jarita Mesa Wild Horse Territory, New Mexico, USA. While designed to examine population-level and longer-term behavioral effects, these studies also provided data about the impacts of seasonal timing and method of PZP-22 delivery on contraceptive efficacy. Mares in New Mexico were treated with PZP-22 via remote dart and had significantly lower foaling rates the following year (Carey et al. 2019). Cedar Mountain and Sand Wash Basin mares had the highest rates of contraceptive efficacy when hand-injected PZP-22 was administered in February, moderate rates in December, and the lowest rates of effectiveness when administered in October (Rutberg et al. 2017), demonstrating the importance of season in timing treatments. For mares at Cedar Mountain and Sand Wash Basin that were given a ZonaStat-H or PZP-22 booster 2–3 years after initial vaccination with PZP-22 ($n = 107$): (1) mares experienced reduced fertility by 66–78% over 3–4 years ($P < 0.01$ for all years); (2) booster

treatments were effective when delivered by hand-injection or remote dart; and (3) 2 cycles of gathers and treatments sharply reduced population growth (Rutberg et al. 2017). Because these 3 studies required regular observations (e.g., physical condition, basic behaviors) of >200 individual horses during April through October for 4–7 years, strong confirmation of PZP-22 field use viability was established.

Accumulated field reports of injection site reactions to ZonaStat-H and PZP-22 vaccines were <25% across studies, with reactions primarily mild and disappearing without consequence in several weeks (K. Grams, Science and Conservation Center, personal communication); an observational study of 306 injection site reactions in 85 mares found that none were debilitating (Roelle and Ransom 2009). From a safety viewpoint, PZP-22 differs from EPA-registered ZonaStat-H only in the addition of controlled-release pellet components, which have already been approved by the U.S. Food and Drug Administration for other uses (e.g., grafts, sutures, implants, food). Across the years of use, both ZonaStat-H and PZP-22 injections were delivered in the upper rump.

The PZP-22 costs more per treatment than ZonaStat-H but requires less frequent administration, which offsets the cost difference across years, especially when access to horses is challenging. Recent models using published data (Turner et al. 2007, Rutberg et al. 2017) for PZP-22 with a ZonaStat-H booster in a 50% treatment and 50% removal pattern across 10 years yielded a 39% lower cost than removals alone (J. Turner, University of Toledo, unpublished data). Technical improvements on PZP-22 to further reduce costs, increase efficacy, eliminate injection site reactions, and simplify handling are in progress. These include innovations like no-mix primers, increased precision in the timing of vaccine release from pellets, novel adjuvants, and using recombinant-ZP as a substitute for PZP in ZonaStat-H. As a reversible contraceptive, PZP-22 can be integrated with limited removals and other tools, enabling free-roaming horses to have quality lives and a role in determining their own genetic future.

SpayVac. This contraceptive vaccine was developed in the early 1990s to control grey seal (*Halichoerus grypus*) populations along the Atlantic coast of Canada (Bechert and Fraker 2018).

Liposomes were used to encapsulate PZP antigens in an effort to provide sustained release over an extended period of time. Liposomes are multi-lamellar, concentric spheres made up of phospholipid bilayers separated by aqueous compartments and may themselves be considered immunological adjuvants (Allison and Gregoriadis 1974, Shek and Sabiston 1982). Fertility rates for 101 female seals treated with a single injection of SpayVac dropped from 70–75% to 9–15% for years 2–5 (Brown et al. 1997*a*) and 10 years post-vaccination (Fraker and Brown 2011). These remarkable results served as the foundation to launch IMV, Inc. (Halifax, Nova Scotia, Canada), a biopharmaceutical company that now develops human cancer immunotherapies and infectious disease vaccines using this patented liposome technology (IMV, Inc. 2021). SpayVac-for-Wildlife, Inc. (Sidney, British Columbia, Canada) has an exclusive, global license from IMV to use this technology in contraceptive vaccines for native wildlife and feral mammals.

The PZP in SpayVac (SpayVac-for-Wildlife, Inc.) is isolated by grinding trimmed ovaries and then passing the ground tissue through a series of nylon screens as described by Brown et al. (1997*b*). Either 200 or 400 µg PZP doses have been used in SpayVac horse vaccines. Two types of adjuvants have been used in SpayVac formulations: MFA or AdjuVac, which uses *Mycobacterium avium* in mineral oil (National Wildlife Research Center [NWRC], Fort Collins, Colorado). Both MFA and AdjuVac produce less severe inflammatory responses compared to Freund's Complete Adjuvant (Lyda et al. 2005, Powers et al. 2007). IMV, Inc. has developed 2 patented liposome-based formulations: (1) VacciMax, a water-in-oil emulsion (MacDonald et al. 2010), and (2) a freeze-dried version (https://www.imv-inc.com/the-dpx-platform), which undergoes a lyophilization step with the components resuspended in oil just prior to injection (Brewer et al. 2018). VacciMax formulations need to remain frozen until shortly prior to use, whereas freeze-dried vaccines can be stored under less stringent conditions and reconstituted when needed, making them easier to use in the field. Most SpayVac field trials in wildlife have used the original VacciMax formulation.

Three SpayVac trials in horses have been conducted. The first trial used SpayVac VacciMax formulated with 400 µg PZP and AdjuVac in-

jected intramuscularly (IM) in the neck (Killian et al. 2008). Pregnancy rates dropped to 0, 17, 17, and 17%, respectively, 1–4 years after treatment of mares with a single SpayVac injection ($n = 12$), compared to 75, 75, 88, and 100% for untreated mares ($n = 8$).

The second study used 200 μg PZP with MFA and both types of liposome formulations in 2 treatment groups (Bechert et al. 2013). Three to 4 months post-vaccination IM in the neck with SpayVac VacciMax ($n = 7$) or the freeze-dried version ($n = 7$), 93% of treated mares ceased cycling as evidenced by lower serum concentrations of progesterone ($P < 0.025$) and smaller ovaries with fewer follicles ($P < 0.001$) compared to controls ($n = 7$), while other organ systems were unaffected. The direct effects of vaccination on the ovaries of treated horses may represent a second contraceptive mechanism of action for PZP, in addition to interference with sperm binding. A subsequent study in ponies (Joonè et al. 2017a), using a similar experimental design and different PZP vaccines administered in the gluteal muscles (100 μg PZP in MFA followed by a booster with another 100 μg PZP in Freund's Incomplete Adjuvant), also found suppression of ovarian function in 86% of treated mares ($n = 7$). Earlier endocrine data based on longitudinal fecal samples collected from mares treated with ZonaStat-H on Assateague Island had suggested that ovulatory failure occurred frequently (Kirkpatrick et al. 1995, Powell and Monfort 2001). A variety of mechanisms of action for ovulatory failure has been suggested, including contamination of the antigen with non-ZP ovarian proteins and effects of cytotoxic T-cells (Bechert et al. 2013, Joonè et al. 2017b).

The third SpayVac trial in horses also used 200 μg PZP with MFA and both types of liposome formulations in 2 treatment groups; however, single injections were administered into gluteal muscles instead of the neck (Roelle et al. 2017). Fertility for the VacciMax group ($n = 30$) was lower ($P < 0.001$) at 13, 47, and 43% for years 1–3 post-vaccination, compared to 100, 98, and 100% of controls ($n = 30$). Although fertility in mares treated with the freeze-dried formulation ($n = 30$) was 17% in the first year, this increased to 76% in year 2, and this group was removed from the study. The immune response and contraceptive efficacy clearly differed from the first 2 SpayVac studies in horses.

In an effort to understand the difference, frozen serum samples from the VacciMax treated group in the Roelle et al. (2017) study were used to determine potential differences in specific immunoglobulin G (IgG) isotype responses (Bechert et al. 2018). The predominant antibody class in equine serum and the major component of humoral immunity is IgG, and horses have 7 different IgG isotypes (Wagner et al. 2004). Previous studies have demonstrated a positive correlation between levels of PZP antibodies produced and contraceptive effect (Liu et al. 1989); however, individual mares that were consistently infertile did not necessarily have the highest antibody titers (Roelle et al. 2017). Instead of using ELISA (enzyme-linked immunosorbent assay) to determine PZP antibody titers, a more sensitive fluorescent bead-based assay was used to distinguish IgG isotype responses against PZP. Serum samples collected 1, 2, and 4 years post-vaccination from mares that were continuously infertile ($n = 14$) were compared with samples from those that had foaled at least once during the 4-year period ($n = 14$). Samples from the continuously infertile mares were also collected and analyzed 5 years post-vaccination. While IgG1 antibodies were generally higher in infertile compared to fertile mares, only IgG4/7 antibody levels were significantly higher in infertile mares during years 1 and 2 post-vaccination ($P < 0.05$; Bechert et al. 2018). Surprisingly, IgG4/7 isotype levels were significantly higher during year 5 compared to year 4 in the continuously infertile mares ($P < 0.02$). SpayVac's ability to preferentially stimulate IgG4/7 antibodies may contribute to its long-term immunocontraceptive efficacy, and measuring IgG4/7 isotypes may help differentiate effectively contracepted mares from those that may need additional vaccination. However, an understanding of why some of these mares mounted a more robust immune response compared to others was still lacking.

Horses are typically given IM injections in either the neck or the rump, which are generally viewed as equivalent. However, in other species, injection site is known to influence vaccine efficacy. For example, hepatitis B vaccinations are successful when given into deltoid, but not gluteal, muscles in humans (Caldwell et al. 1985), and the reason is proximity to regional lymph nodes (Eggert et al. 1999, Mould

et al. 2017). Horses have 2 pairs of primary lymph nodes: the superficial cervical lymph nodes, located very close to the neck injection site, and the sub-iliac lymph nodes, which are located further from hindquarter injection sites (Gaedke et al. 2013, Blenntoft 2020; Figure 3B). Dendritic cells are located in tissues like the skin where their receptors trap antigens; then they migrate to lymph nodes where they interact with T and B cells to stimulate an immune response (Eggert et al. 1999, Randolph et al. 2005). After successful antigen stimulation or vaccination, long-lived plasma cells produce large amounts of high-affinity IgG antibodies. Vaccine efficacy can be improved by enhancing dendritic cell maturation and migration to the lymph nodes (Mould et al. 2017) and considering IgG isotypes in vaccine development (Goodman et al. 2012). We suspect that the closer proximity of the neck injection site to the superficial cervical lymph node facilitates dendritic cell uptake of PZP antigens, resulting in a more robust immune response compared to rump injection sites, which are further from the sub-iliac lymph nodes.

Future and ongoing research with SpayVac for horses include studies to: (1) confirm how injection site may affect immune response and contraceptive efficacy, (2) explore a potential increase in contraceptive efficacy when a booster dose is administered several years after the initial SpayVac injection, (3) develop a freeze-dried formulation that would make SpayVac even easier to administer in the field, and (4) test SpayVac in burros. An EPA application to register SpayVac-Equid, formulated with 400 µg PZP and MFA, is expected to be submitted within the next year.

Gonadotropin releasing hormone (GnRH). GonaCon-Equine is registered by the EPA for application in adult female horses and burros (NWRC, Fort Collins, Colorado). It takes advantage of the role played by GnRH in regulating mammalian reproduction (Figure 1A). The GnRH stimulates release of gonadotropins (i.e., follicle stimulating hormone and luteinizing hormone) from the anterior pituitary gland, which in turn regulate both endocrine function and gamete maturation in the ovaries and testes (Garza et al. 1986). The GnRH is a small neuropeptide that is a weak antigen, but when conjugated to a highly immunogenic carrier protein (Blue Carrier; Biosonda,

Santiago, Chile) and combined with AdjuVac, vaccination stimulates an immune response resulting in antibody production against endogenous GnRH. These antibodies subsequently induce transient infertility by binding to GnRH, thus preventing attachment to receptors on pituitary gonadotrophs, inhibiting gonadotropin release, and ultimately suppressing follicular development, estrus, and ovulation in females (Hazum and Conn 1988). As anti-GnRH antibodies decline over time, the availability of endogenous GnRH increases, and treated animals generally regain normal fertility (Gionfriddo et al. 2011*b*, Powers et al. 2014, Baker et al. 2018).

A single immunization with GonaCon induced infertility for up to 2+ years in numerous wild ungulate species, including captive and free-ranging white-tailed deer (*Odocoileus virginianus*; Miller et al. 2008, Gionfriddo et al. 2011*a*), bison (*Bison bison*; Miller et al. 2004), elk (*Cervus elaphus*; Killian et al. 2009; Powers et al. 2011, 2012, 2014), feral cattle (*Bos taurus*; Massei et al. 2015, 2018), and horses (Killian et al. 2008, Gray et al. 2010, Baker et al. 2018). Multiple years of infertility, however, are experienced in only a fraction of vaccinated animals, and this limitation has been shown to be particularly true for free-roaming horses (Killian et al. 2008, Gray et al. 2010, Baker et al. 2018).

Adverse side effects following a single or booster immunization appear to be minimal in a majority of wild ungulates studied, including white-tailed deer (Curtis et al. 2008, Gionfriddo et al. 2011*b*), elk (Powers et al. 2011, 2012, 2014), bison (Miller et al. 2004), and free-roaming horses (Baker et al. 2018). GonaCon-Equine has been demonstrated to be safe for pregnant females and neonates and does not result in adverse behavioral side effects (Baker et al. 2018). Several studies have shown that vaccinated and control mares display no differences in frequency or intensity of social behaviors that maintain herd structure (e.g., herding, tending, and defending) and social hierarchies (Ransom et al. 2014, Baker et al. 2018). The most apparent pathology is the development of persistent, non-debilitating granulomatous and purulent inflammation at the site of injection; however, this does not result in lameness, impaired mobility, or depression (Baker et al. 2018).

Although not previously reported for GonaCon-Equine, additional studies are warranted

to evaluate the potential long-term side effects related to the presence of GnRH receptors found in multiple organs throughout the body (Kakar and Jennes 1995), including the pancreas (Wang et al. 2001) and bladder (Coit et al. 2009). The role GnRH plays throughout the body is still being discovered, but it has been shown to regulate cardiac contractile function (Dong et al. 2011) and can affect the antler cycle in deer vis à vis effects on testosterone (Durrant et al. 1996). However, most of the receptors identified in these tissues have very low binding affinity to GnRH, so effects of GonaCon vaccination on major organ function would likely be negligible (T. Nett, Colorado State University, personal communication).

Comprehensive reproductive studies were conducted from 2009 to 2013 with individually identified free-roaming horses at Theodore Roosevelt National Park (TRNP), North Dakota, USA. GonaCon-Equine vaccines were administered by hand injection into gluteal muscles, with the majority of mares pregnant at the time of treatment. Foaling rates were 46% in treated mares (13/28) compared to 73% for control mares (19/26) for the second year ($P = 0.04$), and 55% (15/27) compared to 78% (21/27) in treated and control mares, respectively, during the third year ($P = 0.08$). Despite that, contraceptive effectiveness was low to modest and estimated to be only 37% (95% CI = 0.01–0.60) and 28% (95% CI = -0.06–0.51) for the second and third seasons, respectively (Baker et al. 2018). Similar results were reported for captive (Killian et al. 2008) and free-roaming (Gray et al. 2010) horses in Nevada, suggesting that while a single vaccination against GnRH may be preferred from a practical perspective, multiple years of infertility may be experienced in only a fraction of immunized animals.

More recent research, however, has demonstrated that GonaCon-Equine can provide more effective and long-term contraception in free-ranging ungulates when a single vaccination is followed by reimmunization (Massei et al. 2015, Baker et al. 2018). Unlike results from the single-dose trial, a booster administered 4 years after the primary vaccination resulted in foaling rates of 0% (0/25), 16% (4/25), and 4% (1/24) in treated mares ($n = 25$) compared to 84% for control mares ($n = 25$), and these differences remained significant for 3 years ($P < 0.001$; Baker

et al. 2018). During this 3-year period, average vaccine effectiveness was estimated to be 92% (95% CI = 0.81–1.00).

These results were not surprising because it is well-documented that booster vaccinations generally result in a more potent and enduring antibody production due to the anamnestic (cell memory) response (Tizard 1982, Sallusto et al. 2010). However, variation in the length of time between primary and booster immunizations may lead to different levels and qualities of immune response (Dwivedi and Yadav 2012). Multiple studies have demonstrated that when primary and booster immunizations are applied too close together in time, memory cells may not be able to mount an adequate immune response, and the long-term effectiveness of the booster can be diminished (Wistrom et al. 1999, Castiglione et al. 2012, Ledgerwood et al. 2013). These studies recommend that the number of immunizations and intervals between vaccinations be carefully considered to optimize vaccine efficacy and reduce potential side effects. From a practical standpoint, there is a need to determine an optimal vaccination schedule for free-roaming horses that balances the need for minimizing animal handling while maximizing vaccine effectiveness.

In a collaborative research effort, scientists at Colorado State University, the BLM, NPS, and the NWRC attempted to address this question in a corollary experiment to Baker et al. (2018) at TRNP during 2015–2020. The experimental design consisted of 4 booster intervals of decreasing length (i.e., 4, 2, 1, and 0.5 years) to test the hypothesis that decreasing the interval between primary and booster immunizations would reduce the contraceptive efficacy of GonaCon-Equine in free-roaming horses. Although results from this investigation are incomplete, preliminary results are promising. GonaCon-Equine reimmunizations reduced ($P \leq 0.001$) the probability of foaling between treated and control mares for all treatment groups for all years of the experiment (D. Baker, Colorado State University, unpublished data). Average effectiveness (defined as the proportional reduction in annual foaling [number of mares with foals/total number of mares in group] between treated and control mares) was greatest for the 4-year treatment group and was estimated to average 88% over a 6-year period, followed by 60% for the

Table 1. Timing of primary and booster vaccinations with GonaCon-Equine, including sample size, mode of vaccine delivery, contraceptive efficacy, and age at time of treatment for female free-roaming horses (*Equus ferus*) at Theodore Roosevelt National Park, North Dakota, USA.

Booster interval (years)	Sample size	Delivery method	Date of primary	Date of booster	Effective years*	Mean age ± SE (years)		Median age (range in years)	
						Primary	Booster	Primary	Booster
4	25	Hand	10/2009	9/2013	2015–2020	5.4 ± 0.6	9.4 ± 0.7	4 (2–17)	8 (6–21)
2	11	Dart	9/2013	9/2015	2017–2020	3.0 ± 0.2	4.9 ± 0.2	3 (2–4)	5 (4–6)
1	15	Dart	9/2015	9/2016	2018–2020	1.6 ± 0.3	2.6 ± 0.3	1 (1–5)	2 (2–6)
0.5	14	Dart	9/2015	3/2016	2018–2020	2.3 ± 0.8	3.3 ± 0.8	1 (1–14)	2 (2–15)
Control**	25	Hand	10/2009	10/2013	N/A	5.2 ± 0.6	9.2 ± 0.7	3 (2–11)	7 (6–15)

*Years of contraception significantly different ($P < 0.001$) from controls.
**Control females were injected with saline solution.

2-year interval (over 4 years), and 64% and 62% (over 3 years) for the 1- and 0.5-year intervals, respectively (Table 1). Reversibility was 24% (6/25), 45% (5/11), 50% (7/14), and 54% (7/13) for the 4-, 2-, 1- and 0.5-year booster interval treatment groups, respectively (D. Baker, Colorado State University, unpublished data). Collectively, these preliminary data support the hypothesis that increasing the interval between the primary and booster immunizations will increase the level of effectiveness of GonaCon-Equine in free-roaming horses.

In summary, initial vaccination with GonaCon-Equine may provide only modest (≤2 years) suppression of reproduction, but when followed by reimmunization from 6 months up to 4 years, will result in a greater and longer-lasting reduction in fertility in free-roaming horses. Future research will further refine primary and booster vaccination intervals, test remote delivery of vaccines, and evaluate potential long-term side effects of multi-year contraception using GonaCon-Equine.

Oocyte-specific growth factors. Mammalian reproductive success depends on an adequate number of healthy oocytes being present within the ovaries. The total number of oocytes contained in the ovaries is established either before or shortly after birth, depending on the species (Eckery et al. 1996, Peters et al. 1976, Sawyer et al. 2001, Tingen et al. 2009). Each primordial follicle consists of an oocyte surrounded by a few flattened somatic (i.e., granulosa) cells. Primordial follicle growth is initiated by means that are not yet fully understood. This growth is described by both an increase in oocyte size and proliferation of granulosa cells due to interactions between these cell types. Initiation of growth is a committed step, and the once quiescent follicles cannot return to a non-growing state. Consequently, nearly all follicles become atretic at some stage. It is estimated that <0.1% of the oocytes populating the ovary are ever ovulated; many follicles are lost during the process of selecting a dominant follicle for ovulation (Holesh et al. 2021). Entry of follicles into the growth phase is tightly controlled to ensure that an adequate number of follicles is available throughout the reproductive life of the animal (Reddy et al. 2010, Monniaux et al. 2014).

The physiological mechanisms controlling initiation of follicular growth, follicular development, and thus the number of oocytes released at ovulation involve a complex signaling cascade among various organs, glands, and the ovaries (endocrine) and a local exchange of molecules directly between the oocyte and its surrounding somatic cells (paracrine) within the ovaries (Peters et al. 1976, McNatty et al. 2007b, Binelli and Murphy 2010). Communication between the oocyte and granulosa cells is crucial for the survival and growth of the follicles (Matzuk et al. 2002, Gilchrist et al. 2004). Significant discoveries have shown that the oocyte itself produces 2 key regulatory growth factors, namely growth and differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), that are essential for regulating follicular growth and the ovulation rate (Dong et al. 1996, Galloway et al. 2000). These discoveries also led to a paradigm shift in recognizing that oocytes act as the control center of follicles and play a major role in their own

growth and ovulation.

Both of these oocyte-specific growth factors (OGFs: GDF9 and BMP15) have been shown to be critical for early follicular growth in several mammalian species (Shimasaki et al. 2004), including a marsupial (*Trichosurus vulpecula*; Eckery et al. 2002), sheep (*Ovis aries*; Juengel et al. 2002), and humans (Juengel and McNatty 2005). These OGFs cooperate in a species-specific way to regulate maturation of the oocyte and communicate with the granulosa cells (Lin et al. 2012). Ewes that have double copy mutations in the genes encoding either GDF9 or BMP15 are sterile, but otherwise healthy. In these animals, follicles do not progress beyond the primary stage of growth. Interestingly, in animals with only a single copy mutation in either of these genes, and thus presumably producing only half the amount of protein, ovulation rate is increased (Galloway et al. 2000, McNatty et al. 2005). In laboratory mice, GDF9 knockout females (i.e., females in which genes responsible for GDF9 have been made inoperative) are sterile, but BMP15 knockouts only demonstrate decreased litter size (Moore and Shimasaki 2005). Mutations in GDF9 or BMP15 have also been found in women, and are believed to be a cause of premature ovarian failure (Di Pasquale et al. 2004, Pouresmaeili and Fazeli 2014).

McNatty et al. (2007*a*) demonstrated that ewes could be sterilized by immunization against either GDF9 or BMP15. Moreover, specific regions of each growth factor were identified that were important for the biological activity of the respective proteins. This enabled the production of effective peptide vaccines that were specific to those regions. The homologous regions were identified in cows and sheep and found to have 100% amino acid sequence identity. In cows, vaccination against these peptide regions resulted in suppressed reproductive cycles in some animals, while others experienced an increase in ovulation rate (Juengel et al. 2009). In white-tailed deer, BMP15 vaccinated animals became more fecund (Eckery et al. 2014). This may have been due to the biological activity of BMP15 being only partially blocked (similar to the effect of a single copy mutation in sheep) and causing an increase in ovulation rate. Deer vaccinated against GDF9 were also more fecund during the first year but were infertile in years 2 and 3. The effects observed during the first year could have

been because of the timing of vaccination in relation to onset of breeding (i.e., does may not have had an opportunity to mount a sufficient immune response prior to breeding). Regardless, it appears that GDF9 was only partially inhibited during the first breeding season. Results from all of these studies demonstrate that vaccination against GDF9 and BMP15 has the potential to control fertility in a range of species.

Genes for both GDF9 and BMP15 have been identified in the mare (Wade et al. 2009) and shown to be expressed in equine oocytes (Campos-Chillon et al. 2015). Therefore, it is likely that these growth factors have similar functions in horses. We recently tested the hypothesis that vaccination against either GDF9 or BMP15 would alter reproductive function in the mare. Domestic mares (*n* = 10) received an initial dose and 3 boosters administered in neck muscles at 6-week intervals beginning in February 2016. The vaccines used keyhole limpet hemocyanin as a carrier protein and Seppic Montanide™ Pet Gel A as a mild adjuvant. While BMP15-immunized mares experienced a 50% decrease in ovulations, those vaccinated against GDF9 did not differ from controls, yet both groups demonstrated altered estrous activity and a significant decrease in follicle size prior to ovulation (Davis et al. 2018).

With this information, a study was initiated for an additional 2-year experiment in which free-roaming mares in a holding facility (*n* = 10) were immunized against both GDF9 and BMP15 simultaneously. The vaccine was administered in the first year only, as described above. Preliminary results are encouraging, as a marked decrease in ovulations (>88%) has been observed over the 2-year period (D. Eckery and J. Bruemmer, NWRC, unpublished data). Additional studies focused on identifying an ideal adjuvant, length of efficacy of a single injection, and safety in pregnant mares are either ongoing or planned for the near future.

Other considerations for immunocontraception. In addition to vaccine design considerations like antigen target, adjuvant and formulation, delivery method, and vaccination sites and schedules, a variety of intrinsic factors can affect an animal's ability to mount an immune response. Abolins et al. (2011) demonstrated that wild mice have more variable immune responses than do laboratory strains. Environmental variables may affect an animal's health and nutritional status, reducing
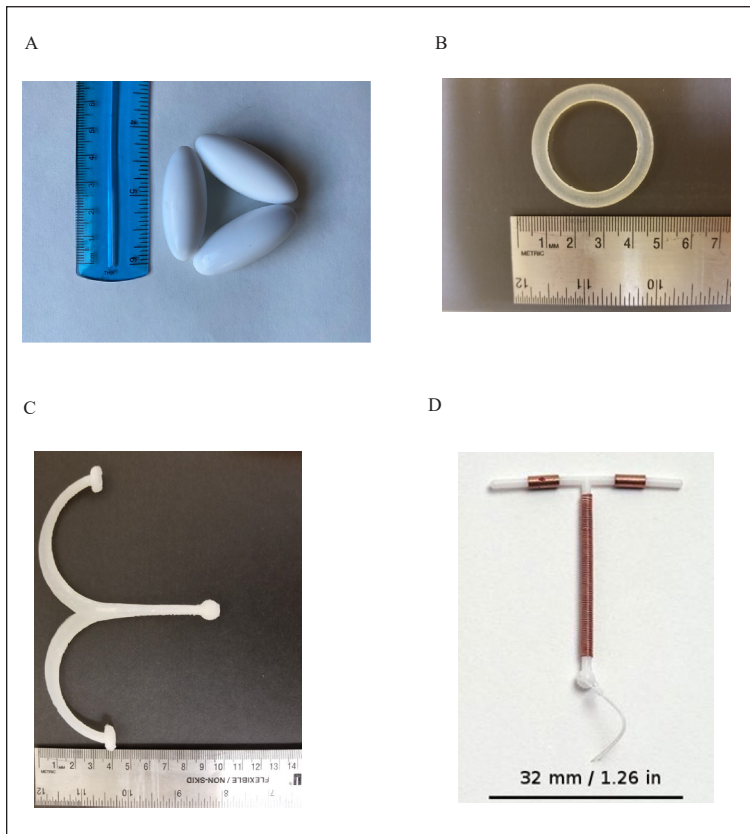
Figure 5. Some commonly used intrauterine devices (IUDs) in mares (*Equus ferus*) include (A) magnetic iUPOD®, (B) silastic O-ring, (C) silastic Y-design, and (D) T-shaped copper IUDs (*photos courtesy of C. Gradil [A] and G. Holyoak [B, C, and D]*).

its ability to mount an effective immune response after vaccination (Gross and Newberne 1980, Ponton et al. 2011), and closed versus open populations will affect field trial results (Dawson and Hone 2012). The range of variables that may affect response to vaccination can make it challenging to draw firm conclusions from initial trials. In the future, new antigen targets may be identified, and different research tools can be used to evaluate vaccine efficacy. For example, IgG4/7 detection may more precisely gauge immune response (Bechert et al. 2018), and anti-Müllerian hormone may further characterize reproductive effects of vaccination (Jooné et al. 2018).

Intrauterine devices

One method of contraception receiving renewed interest is the IUD (Holyoak et al. 2021, Joonè et al. 2021). Insertion of an IUD into the uterus of a free-roaming horse unaccustomed to handling may require mild sedation. Use of an IUD in pregnant mares would not be advised because cervical manipulation may result in a high incidence of abortion while also expelling the IUD. While the actual mechanism of action for contraception resulting from the placement of IUDs is uncertain, multiple theories exist, such as mechanical interference with sperm transport, copper IUDs possessing sperm cytotoxic properties, IUD interference with embryonic migration through the uterus in the early days of pregnancy (Ortiz et al. 1996), or chronic inflammation surrounding the IUD, which interferes with embryonic development (Killian et al. 2008, Joonè et al. 2021). Some veterinary practitioners also use IUDs in an attempt to modify estrus behavior in mares; however, reports of success in prevention of estrus behavior are anecdotal. Successful IUD placement (i.e., high retention and decreased pregnancy rates) appears to be affected by size, shape, weight, and material composition of the IUD (Figure 5).

Retention and contraception. All results for various IUDs presented below are from captive or pasture trials with domestic horses.

Daels and Hughes (1995) performed a 10-month trial using silastic O-ring IUDs; all treated mares (*n* = 6) retained the IUD for the study duration and failed to become pregnant in the presence of a fertile stallion. However, the silastic O-ring did not provide the same rate of success for Baldrighi et al. (2017), where differing sizes and durometers (hardness) of the silastic O-rings resulted in a loss rate of 60% in 24 mares housed with 2 breeding stallions. Mares pasture bred within a week after IUD insertion were at highest risk for losing their IUD (Baldrighi et al. 2017). Holyoak et al. (2021) redesigned the silastic O-ring IUD and performed a 20-month pasture breeding study with a silastic Y-design, "fundus-seeking" IUD (*n* = 20). The Y-design IUD had a 75% retention rate and prevented all pregnancies when IUDs were retained, after pairing insertion with a single injection of bio-release progesterone, which prevented breeding behavior in the days immediately following insertion.

Copper IUDs, used for contraception in women, have been tested in mares with some success. When compared with a copper linear shaped IUD, and a silastic-coated copper ring IUD, the 380 Copper T IUDs were the most promising. Copper T IUDs were placed in 7 pony mares and were effective for 3 years (*n* = 1), 4 years (*n* = 2) and 5 years (*n* = 1; Killian et al. 2008).

Nie et al. (2003) placed glass ball IUDs into the uteri of 24 mares. Within 11 days, 6 of 12 mares with a 25-mm glass ball expelled their IUD; however, all 12 mares given 35-mm glass balls retained theirs for at least 4 estrous cycles. The retention rate of IUDs in the presence of breeding activity was not tested. Argo and Turnbull (2010) inserted 35-mm borosilicate glass balls into the uteri of 4 Welsh pony mares, and they all retained the IUD until manual removal after 2–5 estrous cycles. Even though the larger glass balls had higher retention rates, Rivera del Alamo et al. (2008) and Klein et al. (2016) lost only 1 IUD when using 20-mm water-filled plastic balls over 4 months (*n* = 30 for the 2 studies).

Magnetic IUDs (comprised of 3 separate, shatter-proof, 26-mm oblong-shaped arms) were retained in the uteri of 29 mares for 2–9 months until manual removal (Gradil et al. 2019). Joonè et al. (2021) inserted magnetic IUDs into the uteri of 7 mares following artificial insemination; mares failed to become pregnant and retained the IUD over 3 months. Retention of the magnetic IUDs in the presence of breeding behavior with a stallion has yet to be reported.

Uterine health. Some risks and potential negative long-lasting impacts of IUDs have been described, such as endometritis or pathology, as well as severe complications induced from glass balls shattering (Klabnik-Bradford et al. 2013, Turner et al. 2015, Vanderwall 2015). Although IUDs cause chronic inflammation, they generally do not induce irreversible pathologies (Daels and Hughes 1995, Nie et al. 2003, Gradil et al. 2019, Holyoak et al. 2021, Joonè et al. 2021). Daels and Hughes (1995) reported that all 6 mares treated with a silastic O-ring IUD experienced a worsening of endometrial biopsy score as a result of inflammation; however, neutrophilic uterine cytologies (i.e., bacterial infections) resolved within 1 week following IUD removal, and all mares subsequently became pregnant. In contrast, Rivera del Alamo et al. (2008) reported that only 3 of 9 mares had a neutrophilic uterine cytology. The Y-design IUD, made of the same silastic material as O-ring IUDs, also differed in that 11 of 20 mares had either no change or showed an improvement in endometrial biopsy score; 12 of the 20 mares became pregnant after IUD removal (Holyoak et al. 2021). Nie et al. (2003) reported that of 24 treated mares, only 1 mare experienced a decline in endometrial biopsy score, and 17 of 23 mares conceived following glass ball removal. In the Gradil et al. (2019) study, all 8 mares became pregnant via artificial insemination in the breeding season following IUD removal. Uterine fluid in the presence of an IUD does not appear to impact future pregnancy rates once the IUD is removed (Nie et al. 2003, Holyoak et al. 2021). Endometrial biopsy score differences could be due to varying durations of project length, differences in IUD material, or subjective interpretation by those scoring the biopsies.

Ovarian cyclicity and estrus behavior. Multiple publications describe uninterrupted ovarian cyclicity in the presence of an IUD (Daels and Hughes 1995, Argo and Turnbull 2010, Holyoak et al. 2021). Reported mean inter-ovulatory intervals were 19 ± 1.4 days (Daels and Hughes 1995) and 24 ± 0.57 days (Argo and Turnbull

Table 2. Comparison of administration requirements and animal welfare impacts of different fertility control and sterilization options for free-roaming horses (*Equus ferus*). Modeled after Hampton et al. (2015).

Treatment	Administration*				Animal welfare impact***
	Capture	Surgery	Multiple doses	Frequency of treatment**	
Immunocontraception					
ZonaStat-H (primer + booster at 4 weeks)	-	-	+	1 year	+
PZP-22	±	-	+	2 years	+
SpayVac	±	-	-	4 years	+
GonaCon-Equine****	±	-	+	2 years	+
BMP15-GDF9	±	-	?	?	+
Intrauterine devices	+	-	-	?	++
Sterilization					
Bilateral ovariectomy	+	+	-	Permanent	+++
Castration	+	+	-	Permanent	+++

*Administration: - not required; ± may be required; + required; ? unknown
**Frequency of treatment: based on best results from a single treatment representing a significant difference from controls in published studies (note: ZonaStat-H requires boosters as part of the single treatment)
***Impact of treatment: + minimal animal welfare impact; ++ mild animal welfare impact (some restraint; procedure is minimally invasive); +++ highest animal welfare impact (restraint and sedation required; procedure is invasive)
****GonaCon-Equine boosters given 4 years after the primary vaccination provide 6 years of contraceptive efficacy (see Table 1; D. Baker, Colorado State University, unpublished data).

2010), compared to 21 ± 2.4 days for controls (Ginther and Pierson 1989). Similarly, Holyoak et al. (2021) reported continued evidence of ovulation in mares possessing the silastic Y-design IUD. On the other hand, Nie et al. (2001) reported extended luteal function in 42% of mares treated with glass balls; a follow-up study revealed a 29% rate of extended luteal function (7 of 24) in treated mares compared to 13% for control mares (Nie et al. 2003). Gradil et al. (2019) reported that all mares (*n* = 14) experienced a prolonged diestrus (51 ± 22 days) when magnetic IUDs were inserted at any stage of the estrous cycle, and this increased to 73 ± 36 days when the IUD was inserted during estrus.

Mares with an IUD have failed to demonstrate any change in social or sexual behavior (Argo and Turnbull 2010). Mares teased to a stallion demonstrated behavior consistent with the concentration of circulating progesterone, rather than presence of the IUD (Nie et al. 2003). Gradil et al. (2019) are the only researchers to report that mares with magnetic IUDs exhibited "subdued estrus-related behaviors" with "improved

rideability" as related to a "general demeanor shift." These findings are subjective, and blinded studies are necessary for confirmation.

Despite hypotheses of prostaglandin secretion being affected by the presence of IUDs (i.e., interference with maternal recognition of pregnancy), how IUDs may prolong the life span of corpora lutea has yet to be elucidated (Rivera del Alamo et al. 2008, Klein et al. 2016, Rivera del Alamo et al. 2018). Larger scale, long-term contraceptive studies in free-roaming horses are currently underway to assess how long IUDs can be maintained in the uteri of mares being bred by a harem stallion.

Surgical options

The BLM has been supporting research to explore 3 methods of surgical sterilization in free-ranging mares (BLM 2016): tubal ligation (McCue et al. 2000), hysteroscopically guided laser ablation of oviduct papilla (Edwards et al. 2021), and ovariectomy via colpotomy (Collins and Kasbohm 2017). Various methods of vasectomizing and castrating stallions have also been explored (Scully et al. 2015). Surgical options result

A

One hand secures the ovary in the abdominal cavity and places the chain écraseur around the pedicle, while the other hand steadies the écraseur. An assistant tightens the écraseur.

One hand secures the ovary in the abdominal cavity and places the chain écraseur around the pedicle, while the other hand tightens the écraseur.

B

Figure 6. Different physical environments and available resources for ovariectomies conducted on mares (*Equus ferus*) in (A) an indoor surgical suite, and (B) a horse stall in the field. Note several veterinarians in (A) are fully gowned and gloved for surgery, the room is climate-controlled, and the hospital provides access to a complete suite of medical drugs, supplies, and equipment. Ovariectomies conducted in the field are less able to control environmental variables (e.g., wind, temperature) and, depending on location, have comparatively fewer medical resources to draw upon (*photos courtesy of T. Prado [A] and U. Bechert [B]*).

in permanent sterility; however, these methods are comparatively more invasive, time consuming, and expensive over the short-term than previously described fertility control options (Table 2).

Additionally, procedures associated with few complications when performed with domestic horses are likely to have higher complication rates when performed with free-roaming horses because horse demeanor is different, and the same precautions, sterile environments, and access to medical resources are difficult to duplicate in the field (Figure 6).

Ovariectomy. Spaying (i.e., ovariohysterectomy) is widely practiced in companion animals and involves removal of the ovaries, oviducts, and uterus. Domestic horses are not usually

spayed; instead, ovariectomies (i.e., removal of the ovaries) are sometimes performed to remove tumors, create a teaser mare, produce a recipient mare for embryo transfer, or diminish behavioral signs of estrus (Hooper et al. 1993). However, bilateral ovariectomy commonly results in continued display of sexual receptiveness, with loss of normal cyclic activity, so it is unlikely to resolve unwanted sexual behavior (Hedberg et al. 2007). This is because progesterone is the hormone responsible for inhibiting behavioral signs of estrus (Watson and Hinrichs 1989), and progesterone is produced by corpora lutea, which form after ovulation and would not be present in the absence of the ovaries.

Progesterone is also necessary to maintain pregnancy in mammals, but ovaries become less necessary for the maintenance of pregnancy in horses between days 50 and 70 of gestation. In 1 study, all mares aborted when ovariectomized prior to 45 days of gestation, about half of the mares carried their foal to term when they were ovariectomized at 50 to 70 days of gestation, and all mares carried their pregnancy to term when ovariectomized after 140 days of gestation (Holtan et al. 1979). However, the stage of gestation will affect the difficulty of performing an ovariectomy, with advanced pregnancy increasing the risk of complications.

There are different surgical approaches for removing the ovaries of a mare (Hooper et al. 1993, Pader et al. 2011); however, bilateral ovariectomy performed through a colpotomy (incision via the vagina) is comparatively quick and less expensive than other approaches, which require general anesthesia. A colpotomy is performed with the mare standing; it can be completed in 15–20 minutes by an experienced veterinarian; a surgical facility is not required; and the only specialized instrument required is a chain écraseur (Prado and Schumacher 2019). Briefly, after surgical preparation, an incision 1–3 cm long is made in the anterior-dorsal-lateral aspect of the vagina, which is enlarged by blunt dissection to allow the surgeon's hand to enter the abdomen (Figures 2B and 6). The ovary is identified and isolated by direct palpation, and a local anesthetic agent is injected into the ovarian pedicle, which contains the ovarian artery and vein. An écraseur is placed around the pedicle and tightened using a rachet until the pedicle is severed. The ovary is then removed manually, and the same procedure is performed on the remaining ovary (Prado and Schumacher 2019).

The vaginal incision is generally left unsutured to heal by second intention because suturing is difficult and may induce vaginitis, causing the mare to strain. Within about 3 days, the incision contracts to a diameter that accommodates only 1 finger, and the hole is usually sealed from the abdomen within 3 weeks (Prado and Schumacher 2019). Because the vaginal incision is left unsutured, ovariectomy through a colpotomy should not be performed on mares that suffer from vesicovaginal reflux (i.e., pooled urine). Mares having poor perineal conformation should receive a Caslick's vulvoplasty, or perhaps even a vestibuloplasty, to prevent pneumovagina and subsequent contamination of the vestibule, vagina, and abdomen, which can result in septic peritonitis (Prado and Schumacher 2019).

Complications of bilateral ovariectomy performed through a colpotomy are rare when a domestic mare is correctly prepared for surgery and precautions are taken during and after the procedure (Prado and Schumacher 2019). Prior to surgery, colonic fecal balls should be eliminated because they can be mistaken for ovaries, food should be withheld for 24 hours, a laxative (e.g., mineral oil) should be given by nasogastric intubation, and the mare's hematocrit and serum total protein levels should be determined. Sedatives should be given just prior to and during the surgery as needed, and later, antimicrobials and anti-inflammatory drugs should be administered. Movement of mares after surgery should be limited, and individuals should be held and observed for 5–7 days to detect any post-surgical problems.

Complications after a bilateral ovariectomy via colpotomy can include infections and peritonitis, hernias, vaginal adhesions, post-operative pain, but most importantly hemorrhage and evisceration (Embertson 2009). The most serious potential complication of performing ovariectomies through a colpotomy is fatal hemorrhage because, with this approach, hemostasis of the ovarian arteries and veins relies on crushing them with an écraseur, and intra-abdominal hemorrhage is difficult to detect because the procedure is performed blindly. Inadvertent perforation of the vaginal branch of the uter-

ine artery would cause fatal hemorrhage, with death occurring within minutes; however, perforation can be avoided if the artery is first identified by palpation and by incising the fornix of the vagina at the 10:30 or 1:30 o'clock position (Embertson 2009, Prado and Schumacher 2019). Clinical signs associated with substantial blood loss (e.g., fast heart and respiratory rates, weak pulse, pale mucous membranes, prolonged capillary refill) may be difficult to observe if the mare cannot be safely handled (Mudge 2014). If possible, the mare's hematocrit and serum total protein levels should be determined periodically after surgery; however, a decrease in total serum protein is often not evident for at least 6 hours, and a decrease in hematocrit may not be evident for 12–24 hours, making hemorrhaging difficult to identify (Getman 2009).

To decrease the likelihood of evisceration, some surgeons recommend leaving the mare tied for 4–5 days after colpotomy to prevent recumbency (Nickels 1988). However, in 1 study, none of 51 mares that were bilaterally ovariectomized via colpotomy eviscerated, even though they were left untied in stalls for 7 days (Colbern and Reagan 1987). Generally, ovariectomized mares can be allowed unrestricted exercise after about 5 days (Colbern 1993, Moll and Slone 1998, Embertson 2009, Pader et al. 2011).

Bilateral ovariectomy was used as a means to control fertility of free-roaming horses at the Sheldon National Wildlife Refuge in northern Nevada. Mares (n = 114) were ovariectomized by colpotomy, held for 3–8 days after surgery, and then returned to the range along with 36 untreated mares (Collins and Kasbohm 2017). Losses due to complications with ovariectomies were not reported; however, 2% of ovariectomized mares and vasectomized stallions (n = 8 of 385 total) were lost pre-release (Collins and Kasbohm 2017). Post-surgical observations were made aerially, and the recapture rate a year later was 85.3% for the 150 released mares; survival rates for treated mares (85.1%) did not differ significantly from untreated mares (86.1%).

Protests from horse advocacy groups have halted additional ovariectomy field studies, and a report by the NRC (2013) states, "The possibility that ovariectomy may be followed by prolonged bleeding or peritoneal infection makes it inadvisable for field application" (130). Veterinary surgeons agree with this because of the serious complications that can accompany the procedure and the challenge of addressing these in the field (J. Schumacher, P. Mulon, and P. Jones, University of Tennessee, personal communication). Future research could explore safer, alternative forms of permanent sterilization for free-roaming mares, with consideration given to the unique challenges encountered in the field.

Castration. Castration is familiar to most people as a tool to control fertility and behavior in males. In pets and livestock, castration is used to curb undesirable reproductive behaviors and aggression (McGreevy et al. 2018). In the United States, the majority of male pet dogs (*Canis familiaris*) and cats (*Felis catus*) are castrated (Houlihan 2017), and castration is among the most common surgeries performed on stallions (Schumacher 1996; Figure 7). Despite this, there is surprisingly little longitudinal research on the effects of castration, although the literature is increasing for dogs (e.g., Hart et al. 2020). Castration affects male behaviors by removing production of androgens in the testicles, with concentrations of testosterone decreasing rapidly (within 6 hours) after surgery in the horse (Thompson et al. 1980). Removal of the testicles and their production of hormones also affects other endocrine glands (Taravat et al. 2017), thus compounding effects on behavior. The literature on a wide range of species shows mixed effects of castration on behavior, partly because behavior can be influenced by so many factors. Castration has been found to reduce aggression in rodents (Barfield et al. 1972, Christie and Barfield 1979, Hume and Wynne-Edwards 2005, Lofgren et al. 2012, Gale et al. 2019), Japanese macaques (*Macaca fuscata*; Takeshita et al. 2017), and domestic dogs (Maarschalkerweerd et al. 1997), although no changes were reported after castration in free-ranging dogs (Garde et al. 2016), prairie voles (*Microtus ochrogaster*; Demas et al. 1999), and other non-human primates (Wilson and Vessey 1968, Epple 1978). In horses, effects of castration on aggression have rarely been examined, with some authors reporting that castration controlled aggressive behavior (Smith 1974), while others report that aggression can continue to be problematic (Line et al. 1985, i Rios and Houpt 1995, Schumacher 2006). To put this in context, 1 study found a similar frequency of aggression in a group of geldings as a group of mares (Vervaecke et al. 2007).

Figure 7. Recovering free-roaming geldings (*Equus ferus*) a few hours after castration at the Bureau of Land Management Delta Wild Horse and Burro Corral, Utah, USA. Shaved patches are related to freeze-marking for individual identification and not related to surgery (*photo courtesy of S. King*).

Although castration is most commonly thought to extinguish reproductive behaviors, in the few studies that have explored this, most have found that expression of sexual behavior remained but may diminish over time—for example, in non-human primates (Wilson and Vessey 1968, Phoenix 1974, Zumpe et al. 1992), goats (*Capra aegagarus hircus*; Hart and Jones 1975), and free-ranging dogs (Garde et al. 2016). In domestic horses, persistence of reproductive behavior following castration was reported as problematic by 69% of respondents to a survey (i Rios and Houpt 1995) and was shown in 20–30% of geldings whether castrated pre- or post-pubertally (Smith 1974, Schumacher 2006). Reproductive behavior shown by domestic geldings included herding, penile erection, mounting, and copulation (Pearce 1980, Cox 1986, i Rios and Houpt 1995, Schumacher 2006). These behaviors were not associated with prior sexual experience (Cox 1986); in some horses, these behaviors were exhibited from the time of castration, with others showing it only later in life (i Rios and Houpt 1995). Exhibition of sexual behaviors may be situational in geldings: 1 author reported that it was shown by geldings only when moved to a new environment (Pearce 1980), indicating that these behaviors could be less dependent on hormones than on cerebral or social mechanisms (Smith 1974, Fritz et al. 2019).

For free-roaming horses, castration as a male fertility control method that has no impact on their aggressive or reproductive behavior sounds promising, as this implies that it could reduce foaling rates but will have little im-

pact on their social structure. However, until a recent study (King et al. 2022), there was no research on how geldings behave in a reproductive herd. In King et al.'s study (2022), behavioral data were collected on free-roaming stallions both before and after castration and in comparison to intact males at Conger HMA in western Utah. There was no effect of castration on expression of agonistic behaviors. Reproductive behaviors continued to be shown by geldings throughout the study, with little difference between them and intact males. Although some geldings continued to associate with mares, the number of mares in a gelding's harem decreased over the course of the study in comparison to intact controls (King et al. 2022). Research on Icelandic ponies in a large pasture, where mares, foals, and juveniles were kept with geldings but no intact stallions, found that while mares displayed threat behavior to geldings more often than to other mares, there was no effect of geldings on the welfare of mares or foals (Van Dierendonck et al. 2004, Jørgensen et al. 2009). In a population in Japan where geldings were in a reproductive herd (Kaseda 1981, Kaseda et al. 1997), and in the studies in Iceland where geldings were with mares and foals (Sigurjónsdóttir et al. 2003, Van Dierendonck et al. 2004), geldings tended to group together; however, this was not seen at Conger HMA (King et al. 2022). Testosterone levels in intact males vary by season, and harem stallions have higher levels of testosterone than bachelors (Berndtson et al. 1974, Angle et al. 1979, Turner and Kirkpatrick 1982, McDonnell and Murray 1995, Sterregaard et al. 2002). As geldings have no production of testosterone, this would be expected to affect their dominance rank, yet geldings were found to be dominant over mares in some studies (Montgomery 1957, Tyler 1972, Houpt and Keiper 1982), although the social structure in Icelandic ponies was dominated by mares (Sigurjónsdóttir et al. 2003).

Success of a fertility control method is ultimately assessed by its effect on foaling rate. Preliminary data indicate that castrating stallions in a free-roaming herd reduces foaling rate over the short-term, but the impact is reduced over subsequent years as intact males, which were young at the time of castration, mature (King et al. 2022). Two other studies where male fertility was controlled in a free-roaming horse herd,

by microencapsulated testosterone propionate (Kirkpatrick et al. 1982, Turner and Kirkpatrick 1982) or vasectomy (Eagle et al. 1993), found similar results: during the first breeding season after treatment, there were fewer foals in groups where the harem stallion had been treated. Foals born in these harems were due to pregnant mares changing groups (Eagle et al. 1993). Mares exhibit mate choice (Pickerel et al. 1993), meaning that they select the stallions with which to copulate. In populations with few group changes, most foals are sired by the harem stallion (Bowling and Touchberry 1990, Feh 1990, Kaseda and Khalil 1996, Gray et al. 2011). Thus, in stable populations, male fertility control will likely show some success at reducing foaling rate initially. However, in unstable populations with high rates of mares changing groups (i.e., low mare fidelity to a stallion), there would be reduced efficacy of male fertility control unless most males are castrated (Nelson 1980, Miller 1981). Stability of horse groups can be affected by gathers (Gray et al. 2011, Nuñez et al. 2015). As current free-roaming horse management relies on gathers and removals in addition to fertility treatments, castrating stallions as a form of population growth control cannot depend on group stability for success and is likely to have a substantial and longer-term effect on foaling rates only when the majority of males (>80%) in a population are castrated (Garrott and Siniff 1992).

Considerations for implementation

Operational hurdles, including regulatory, financial, and logistical issues, must be negotiated before fertility control measures for free-roaming horse populations can be effectively implemented. Such constraints can be placed into 5 categories: (1) comprehensive plan development, (2) regulatory requirements, (3) animal welfare, (4) environmental conditions, and (5) logistics and key personnel.

Comprehensive plan development. A comprehensive plan for managing free-roaming horses is essential for defining a course of action, securing funding, and demonstrating buy-in from stakeholders. Although there is no regulatory requirement for a comprehensive plan, the cost and complexity involved in management actions makes this necessary. In addition to Resource Management Plans (RMPs) and National Environmental Policy Act (NEPA) documents, a comprehensive plan develops and justifies budgets and helps build consensus among stakeholders. In addition to FREES, a consortium of conservation, animal welfare, and agricultural groups all support the use of contraception to keep free-roaming horse populations within AML (American Society for the Prevention of Cruelty to Animals [ASPCA] et al. 2020); however, some animal rights advocates, such as Friends of Animals, oppose any intervention into the autonomy of free-roaming animals, including the use of contraceptives. A well thought out management plan and supporting implementation documents are more likely to survive inevitable court challenges, which can seriously disrupt operations by diverting resources and interfering with plan implementation.

Regulatory requirements. Part of the planning process includes complying with NEPA requirements, which come into play if federal funds or approvals are required in an action that could significantly affect the environment. Actions taken by the BLM, USFS, tribal agencies, and the NPS all fit into this category; however, actions taken by states, tribes, and private landowners are exempt from NEPA, if federal funds and approvals are not involved. The BLM develops RMPs for multiple planning unit areas, and plans are revised every 10–20 years. For each RMP, NEPA requires a comprehensive Environmental Impact Statement (EIS) "if a proposed major federal action is determined to significantly affect the quality of the human environment" (EPA 2020). This process is not intended to guarantee that no adverse environmental impact will occur, only that potential impacts are identified and given due consideration (and impacts may be positive). An Environmental Assessment (EA) considers the potential effects of proposed actions within the scope of an existing EIS. These documents also consider the consequences of alternatives, such as other forms of fertility control, as well as taking no action.

With respect to managing free-roaming horse populations, all types of potential actions must be considered in the RMP. These would include removals as well as the use of 1 or more fertility control options. An amendment to the RMP is required if an action that was not contemplated initially is now being considered or if significant new scientific information about potential

effects has become available. Major plan revisions or new plans trigger the need for a new EIS, while an amendment could require preparation of either an EA or EIS, depending on the scope of the change. Court challenges are inevitable and can be costly because they may derail the planning and preparations that have taken place. The best way to ensure that actions planned by federal management agencies can survive court challenges is to employ seasoned personnel who are skilled in preparing defensible NEPA documents; the BLM often contracts with vendors to achieve this (BLM 2020).

Contraceptive agents must be registered with the appropriate government agency unless they are used under an Experimental Use Permit or similar approval. For fertility control agents like immunocontraceptive vaccines, the appropriate legislation is the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and EPA is the responsible agency. However, because of the data requirements and costs associated with registering such products, many organizations have stopped pursuing registration if product sales would be limited (Fagerstone et al. 1990). The costs of registration under FIFRA, which can total several hundred thousand U.S. dollars, have to have a reasonable prospect of being recovered through sales. However, many companies may be reluctant to invest substantial amounts of money where the apparent returns appear quite limited.

Animal welfare. The well-being of horses as well as staff is paramount during capture, handling, and treatment. One aim is to bring free-roaming horse populations into balance with their environment to ensure that they do not have to face starvation or dehydration, which is 1 of the 5 freedoms of animal welfare (ASPCA 2021). Gathers are governed by a comprehensive animal welfare policy (BLM et al. 2015), which ensures that contractors follow animal welfare protocols (e.g., use of handling aids such as signaling flags, hand signals, and voice commands). Helicopters are used so that animals are encouraged to move in the desired direction but are not panicked. Visual barriers (e.g., burlap or plywood) on corrals and drift wings must meet standards that minimize stress to the animals. For example, when foals become separated from their dams, efforts must be made to reunite them. A veterinarian must be present at each gather to ensure that any ani-

mal in distress is properly attended. Should the veterinarian believe that an animal needs to be removed to receive off-range attention or be euthanized, the recommendation is made to the BLM officer in charge who makes the final decision (BLM, unpublished memorandum). Animals to be taken off-range must be separated and transported, according to sex and age, and with specified minimum space requirements.

A functional management plan for free-roaming horses must address both short- and long-term aspects, and employing 1 or more fertility control tools in concert with needed removals may offer the best opportunity for success (NRC 2013, Garrott 2018). For many populations, it is necessary to capture and remove excess animals from the range to bring the herd size down to AML quickly. Gathers also provide opportunities to administer contraceptive agents, which should be done when ambient temperatures are not extreme. Historically, BLM has performed removals at an HMA approximately every 4 years, with exceptions for emergency circumstances resulting from range fires, extreme drought, and animals that pose public safety hazards along highways. Capture methods for free-roaming horses used by BLM usually have good animal welfare outcomes in comparison to wildlife capture operations (Scasta 2020).

Environmental conditions. The productivity of field operations is influenced by weather, forage and water availability, and accessibility within an HMA. The ability to work in an HMA during some seasons can be restricted by environmental conditions, including mud, terrain ruggedness, elevation change, tree cover, and extreme temperatures (Turner 2015). Snow and rain can significantly interfere with access to some areas during particular seasons. The HMAs encompass a wide range of climatic, edaphic, and vegetation conditions (Figure 1), which can affect horse herd growth rates, abundance, and animal body condition. Thus, these influence management options, such as the prioritization of areas in greatest need of action.

Logistics. For gathers at large and remote HMAs, the BLM relies on contractors with portable corral systems, temporary fencing, transport trucks, helicopters, field holding facilities, and other essential items (e.g., water troughs, high quality hay) that are needed to capture, handle, and accommodate animals to be removed from

the range. Additionally, the BLM has worked with local volunteers to dart free-roaming horses with contraceptives, but this is possible only when the population is small and approachable, individual horses are known, and volunteers are willing and able to be trained.

Management implications

Cooperation among multiple stakeholders is needed to orchestrate permit requirements, accurately characterize HMAs, adjust AMLs, select 1 or more fertility control options to implement in conjunction with removals for herd management, and monitor a myriad of factors to continually refine population management strategies. However, additional consideration should be given to how population control methods, even when deemed successful, may affect long-term management outcomes. For example, successful management of the closed population of Assateague Island horses resulted in increased longevity of individual mares—an unforeseen consequence of fertility control. Methods used to determine AMLs in order to maintain "a thriving natural ecological balance" have been questioned, especially when policy decisions determine forage allocations among multiple users. The sociopolitical framework within which the BLM and other organizations must operate adds additional layers of complexity to effective management of free-roaming horses, and educating politicians and the general public may facilitate implementation of science-based management strategies.

Acknowledgments

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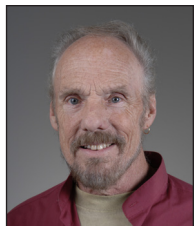

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URSULA S. BECHERT, DVM, PhD, earned her B.S. degree from Utah State University in 1985 and her DVM from Washington State University in 1991. After working in private practice and a free-roaming wildlife park in Oregon for several years, she earned a Ph.D. degree in animal sciences from Oregon State University. She has been conducting research for >25 years with interests in the reproductive physiology of endangered species, development of novel population management tools, as well as nutritional and pharmacokinetic studies with wildlife. She is currently serving as the research advisor for the Bureau of Land Management Wild Horse and Burro Advisory Board. At the University of Pennsylvania, she helps shape the strategic goals of graduate-level programming for the College of Liberal and Professional Studies in the School of Arts and Sciences.
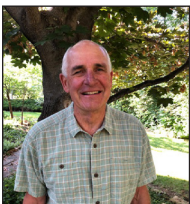
JOHN W. TURNER, JR. is an endocrinologist (Ph.D., Cornell University; postdoctoral training, University of California, Los Angeles) with extensive expertise in reproductive biology and stress physiology. He is a professor of physiology engaged in teaching and research at the University of Toledo College of Medicine, and his research endeavors have included pioneering research in the field of wildlife contraception, effects of chronically deteriorating habitats on stress levels of wildlife and fish, and the psychophysiology of human stress-related disorders and their treatment. He has 45 years of direct field experience with >10 different wild horse populations and has been heavily involved in the research, development, and testing of native PZP and PZP-22 controlled-release contraceptive vaccines and their applications in multiple species. His research efforts have involved collaboration with investigators at 12 U.S. and 4 foreign universities and have yielded 120 peer-reviewed journal papers and reviews, with 71 of those in wildlife contraception and PZP-related research. He has authored multiple book chapters and edited 6 books. He has served as a wildlife-contraception management consultant for the U.S. Forest Service and for >20 parks and preserves worldwide. His awards include the U.S. Forest Service Chief Award (2005) and 2 University Career Research Awards (2006, 2012).

DAN L. BAKER is an affiliate faculty member at the Animal Reproduction and Biotechnology Laboratory in the Department of Biomedical Sciences at Colorado State University. He previously worked as a wildlife scientist for the Colorado Division of Wildlife for 25 years, where his primary research interests were nutritional ecology and reproduction in wild ungulates. He has been associated with Colorado State University as an affiliate faculty member since 2006, where his research interest included the development and testing of alternative fertility control technologies in free-ranging wild ungulates. His professional activities involve supervising graduate students, submitting proposals for research grants, preparing manuscripts for publication, and presenting research findings at professional meetings. His current research project relates to the evaluation of GnRH (GonaCon-Equine) immunocontraceptive vaccine in feral horses using prototype syringe darts at Theodore Roosevelt National Park, North Dakota, USA.

DOUGLAS C. ECKERY is an assistant director at the U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center. His research focuses on the development of methods of fertility control that can be integrated into wildlife management strategies. He is an affiliate faculty in the Department of Biomedical Sciences and Animal Reproduction and Biotechnology Laboratory at Colorado State University.
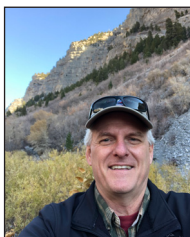
JASON E. BRUEMMER is a supervisory research wildlife biologist at the U.S. Department of Agriculture's National Wildlife Research Center (NWRC) in Fort Collins, Colorado. As the leader of NWRC's Fertility Control Project, his research interests include development of novel contraceptives for a multitude of species. Prior to taking his position at NWRC, he served for >2 decades as a professor in the Department of Animal Sciences at Colorado State University focusing of reproductive physiology, particularly in horses.

CANDACE C. LYMAN, DVM, DACT, completed her veterinary training at Kansas State University College of Veterinary Medicine in 2007. Following completion of a residency in Large Animal Reproduction at the University of Pennsylvania School of Veterinary Medicine (New Bolton Center), she became a diplomate in the American College of Theriogenologists in 2011. She has spent time during her career working in both equine private practice and academia. Most recently, she was on faculty at Oklahoma State University College of Veterinary Medicine until joining the faculty at Auburn University College of Veterinary Medicine. Her areas of research include ovarian follicular synchronization and recruitment for the purposes of transvaginal aspiration of oocytes for in-vitro embryo production in the horse, development and modification of contraceptive devices for population control in free-roaming mares, and evaluation of the reproductive microbiome in the canine and equine.

TULIO M. PRADO was born in, Venezuela, South America. He received his Diploma of Medico Veterinario (DVM) at the University of Zulia, Venezuela. In the United States, he completed a Theriogenology Residency and an M.S. degree in reproductive physiology at Oklahoma State University. He worked as a visiting assistant professor at Oklahoma State University, and now he is an associate professor at the University of Tennessee as a theriogenologist working with all species. He is first author and coauthor of several publications related to reproduction. He serves on the editorial board as topic editor of the journal *Animals*, as a scientific reviewer of the *Journal of Animal Reproduction*, and PI and CO-PI of scientific and clinical research.

SARAH R. B. KING is a research scientist at Colorado State University. After attaining a B.S. and Ph.D. degree from Queen Mary, University of London, her work has focused on how understanding behavioral ecology of endangered and abundant species can inform their conservation and management. Most of her research has been applied to equids, including Przewalski's horses in Mongolia and free-roaming horses and burros in the United States. She serves as co-chair of the IUCN/SSC Equid Specialist Group.

MARK A. FRAKER, CWB®, RPBio, has been a practicing wildlife biologist for 5 decades, working in both the United States and Canada. In 1998, he began researching the efficacy of SpayVac®, a long-lasting, single-dose contraceptive vaccine, first in deer, and later in horses and African elephants. In 2017, he co-founded SpayVac-for-Wildlife, Inc. to advance Spay-Vac to provide fertility control in native and feral mammals.