Review of Fertility Control Research

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1. Introduction

A considerable amount of research and expertise has been directed toward fertility control in domestic and wildlife animals, including free-ranging horses, over the past 30 years. The purpose of this review is to assess current fertility control methods for free ranging feral horses. While methods for fertility control serve as an attractive alternative for reducing large-scale population growth rates for free-ranging or semi-captive feral horses throughout the United States, it also serves as an alternative means of maintaining limited equid population growth in smaller-scale scenarios. Because of the large number of published reports on contraception in free-ranging horses and its related issues, this review will attempt to focus on published reports salient to how we have arrived and where we are today with available fertility control agents. Any failure to cite other relevant reports is not intended. A more detailed and comprehensive review of the literature can be found in the National Research Council (NRC), 2013, "Using Science to Improve the BLM Wild Horse and Burro Program: A Way Forward."[1]

Despite accomplishments in the development of fertility control agents for horses, an optimum contraception strategy has yet to be achieved for wildlife suitable for management of large population considerations. A primary difficulty in the development of the optimal fertility control agent for large scale population reduction may be due to the failure of current methods to meet suitable management strategies on a large-scale basis and to meet the requirements set forth by regulatory agencies responsible for the horses and their stakeholders. Historically, the requirements set forth to limit populations of free-ranging horses suggest that agents used for fertility control must adhere to the following criteria: 1) be reversible and temporary, 2) the strategies used for contraception must be noninvasive procedures, 3) have no adverse effects (long-term or short-term) on targeted animals, 4) contraception agents capable of passing along the food-chain (predatory/prey) are not acceptable, 5) behavior must not be altered, 6) social structure and band integrity must not be interrupted, 7) a single injection achieving long-term contraception is essential for management considerations, and 8) it must be practical and economically suitable for ongoing management strategies of large-scale populations of free-ranging horses. These requirements justifiably were designed to ensure the overall management of reducing large populations of wildlife where necessary and ensure the humane treatment of the targeted animal while maintaining its natural state as much as possible. Recently, however, the overwhelming need to limit population growth rates has forced management agencies to rethink some of the limitations previously prescribed for fertility control agents. Longer lasting and permanent contraceptive agents are now of interest to managers of wild

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and feral animals. While protection of the food chain, the environment, and the welfare of the animals are still primary concerns, some agencies have come to accept that some side effects on social organization, behavior, and even the animals themselves (such as injection site reactions) may be unavoidable. As long as these effects do not have an adverse impact on animal welfare, they are now regarded by some as unfortunate but necessary for the application of effective fertility control on a large scale.

Several agents designed for use as fertility control agents in free-ranging horses are identical to those used for domestic horses to suppress estrus behavior. Therefore, it is worthy of clarifying that the formulations of the agents used to suppress estrous behavior and those used as fertility control agents are strikingly different, and its intended use and the desirable length of effectiveness varies considerably.

For horses, there are several chemical agents suitable and capable of inhibiting fertility. However, the use of these agents is dependent upon the reason(s) for their use and to which population of horses they are intended. While the majority of agents used in domestic horses are targeted primarily toward suppression of estrus behavior, the agents do disrupt cyclicity and, thus, could be considered a form of indirectly inhibiting fertility. For example, exogenous progesterone/progestins as well as gonadotropin-releasing hormone (GnRH) analogs are commonly used to suppress short-term estrus behavior in domestic mares. The agents have also been investigated for long-term contraception use, albeit in different formulations, in free-ranging horses and will be discussed later.

Agents used in domestic horses to suppress estrus behavior may also indirectly serve as agents for inhibiting fertility by prolonging the corpus luteum. Examples of agents used for extending the corpus luteum include administering 1500 to 3000 IU of hCG when a 30 mm follicle develops during the luteal phase of the estrous cycle, thus, inducing a luteal phase ovulation and extending the luteal phase of the estrous cycle.[2] The use of 60 units of oxytocin daily before day 10 after ovulation can also extend the luteal phase of the estrous cycle for at least 30 days.[3] Another method used to enhance prolonged endogenous secretion of progesterone is the insertion of a sterile 35 mm glass marble ball[4] or 20 mm water- filled propylene ball into the uterine lumen to mimic pregnancy in the mare.[5] In these two studies, the persistence of corpus luteum lasted for 60 to 90 days if the devices remained in the uterus of the mare (approximately 50% were spontaneously expelled) with no reported evidence of adverse effects on the uterus and mare.[4] Although these methods have primarily been investigated for use solely for estrus suppression in domestic horses and can also be considered an indirect form of inhibition of fertility, the present format would not be suitable for contraception use in large scale wild horse populations due to 1) the extensive monitoring required to ensure the correct timing of the estrous cycle when administering the agent, 2) the requirement for multiple injections or applications, and 3) the relatively short period of the effects.

For longer-term contraception targeted for large scale population use, several chemical agents have been investigated in stallions and mares.

2. Fertility Control in the Male

In earlier studies, testosterone propionate was investigated for use in free-ranging horses. Several studies using various doses of testosterone propionate (200 μg to 1.3 g) reported successful attainment of oligospermia and reduction of foal crops in treated harems.[6] However, multiple doses were required in the majority of the studies, and the treatment was recognized as impractical for large-scale management purposes. GnRH vaccines have also been tested and reported in the stallion.[7–10] These reports demonstrate the effectiveness of GnRH vaccines to significantly reduce sperm quality while changes in behavior varied. However, multiple doses are also required during treatment, and in some studies a complete removal of sperm production was not evident.[9] Use of GnRH agonists in stallions has also been reported in earlier studies.[11–14] It appears that only transient effects were found following its use, and it has been postulated that perhaps larger doses may be required for a desired effect.

Vasectomy

Surgical vasectomy has been performed and reported in free-ranging horses.[15,16] Dominant stallions were used in these studies, and the results were successful in reducing foal production, particularly in bands where subordinate stallions were not present. However, foal production was eminent in bands where subordinate and bachelor stallions were present. While surgical vasectomy of stallions may not be a practical or economically feasible method for reducing large-scale populations of free-ranging horses, clearly, vasectomy of stallions, if performed in feral horse herds, should be inclusive of dominant, subordinate, and bachelor stallions, and a very large number of stallions being treated are a must in order to have any impact on foal production. Scientific scrutiny must provide evidence of its long-term effectiveness prior to its acceptance for large-scale use. Potentially, vasectomy, whether used chemically or surgically in a large number of stallions within select herd management areas, may have a deleterious effect on the genetic diversity of the herds. Surgical and chemical vasectomy has been investigated on the Sheldon Wildlife Refuge in the Western states. While the surgical procedures were thought to be successful, the vas deferens of the animals injected with a chemical vasectomy agent remained patent when examined some time later (Kane, personal commu-

nication). Chemical vasectomy is a minimally invasive procedure, and although it would more than likely be considered a sterilant if performed successfully, it may be an attractive alternative to limiting foal production in select areas where free-ranging horses are present and stallions thought to be a limiting factor. Development of chemical vasectomy, if proven successful and applied, will inevitably raise issues of stallion-targeted fertility control such as which and how many stallions will be required to have an effect on large-scale population dynamics and whether long-term effect on behavior or genetics will occur.

3. Stallion Versus Mares as Targets for Contraception

Controversy exists as to whether the male should be targeted rather than the female for large-scale population reduction. It is the author's opinion and speculation that the development of a chemical agent of fertility control in stallions is more than likely to have an effect on sexual behavior over time and is dependent upon the temperament and experience of the stallion. Any effect on sexual behavior in free-ranging dominant stallions could/would be an invitation for bachelor and subordinate stallions to meet the challenge and/or for mares of the affected stallion of a herd to wander during estrus. Additionally, a single fertile stallion (dominant, subordinate, or bachelor) may be capable of impregnating approximately 40 mares per year, although this scenario of access to mares would be unlikely in free-ranging horse herds. Regardless, in view of the number of bachelors available in free-ranging horse herds, it would require treating the majority of stallions (dominant, subordinate, and bachelors) in a given territory to have any effect on reducing foal crops for a given year. Would this have an effect on genetic diversity? What percentage of the stallions would be required to be treated to have an effect on population growth? Is this strategy feasible from a management perspective? These are a few of the many questions that require further investigation. Modeling studies may help answer questions of whether inhibition of fertility to favor males or females, or a combination of both, would be the best measurements of limiting foal production on a yearly basis. However, accurate data on population demographics and dynamics is required to inform and validate these models before they are truly useful.

4. Fertility Control in the Female

A number of agents for use as contraceptives in female equids have been studied. The agents comprise of steroids, GnRH agonists, immunocontraceptives, and intrauterine devices.

Intrauterine Devices

Several forms of intrauterine devices have been investigated in the horse. Presumptively, the mechanism of action in horses is to incite a low grade endometritis, with the devices serving as a nidus, stimulating persistent inflammation within the uterine lumen as long as the device remains in the uterus. Persistent low grade endometritis represents a hostile environment within the uterus and is incompatible with survival of the embryo. Flexible, silastic O-ring devices were inserted into the uterus in six domestic mares.[17] The mares cycled normally when monitored by palpation and ultrasound. None of the mares conceived throughout one breeding season but all conceived after removal of the intrauterine devices. Another study examined the effectiveness of a copper T shaped intrauterine device in 15 feral mares over a three year period.[18] The mares were exposed to fertile stallions for 60 days and found 20 percent of the treated mares to have conceived compared with 75 percent of the controls in the first year of the study. However, 75% to 80% of the treated mares were found to be pregnant over the next two years with no evidence of the intrauterine device being present in the pregnant mares. The authors speculated that the intrauterine devices were spontaneously expelled from the uterus of treated mares after the first year. As mentioned previously, the insertion of glass marbles and water-filled propylene balls into the uterus of mares may also be considered as intrauterine devices, and they may also have an effect on fertility. However, the objective of these two investigations was aimed at estrous suppression and not long-term contraception. Subsequent and consistent losses over time of glass marbles inserted into mares when housed with stallions suggests that intrauterine devices may not serve effectively as a long-term contraceptive strategy for large-scale population growth reduction.

Estrogens and Progesterone

In several earlier studies, the use of native estrogen and progesterone as long-term contraception agents in horses proved not to be as successful as expected.[19,20] It is speculated that its failure to achieve a desired effectiveness was due to the rapid metabolism and enzymatic degradation of the native steroids and that significantly and impractical higher doses were required for a desired effect. These earlier studies led to the successful use of synthetic estrogens and progesterone or its combination thereof for contraception in mares. Formulations of the synthetic steroid ethinyl estradiol and natural progesterone into silastic implants for long acting effects have been reported.[21,22] The authors report that contraception effect from intraperitoneal-inserted implants in treated mares was 85% to 100% effective for 2 to 3 years. In another study by one of the investigators, ethinyl estradiol implants alone, when inserted into the peritoneal cavity of mares at concentrations of 1.5 g, 3.0 g, and 8.0 g, yielded a 75% to 100% contraception rate based on the rate of decline of plasma ethinyl estradiol.[23] Despite the successful outcome and relative inva-

siveness of the procedure, no data were collected on the behaviors of the treated mares, which may have potential adverse effects on band integrity and social organization. Thus, its acceptance for large-scale use in wild horse populations is questioned. Additionally, the use of steroids (synthetic or natural) as a contraceptive agent in feral horse populations may provide potential risks of passing along the food chain and may not be acceptable by regulatory agencies.

Progestagens

A significant number of studies have been reported on treatment of mares with progesterone or its synthetics. However, the majority of the studies were aimed at manipulation of the estrous cycle for short-term control and ovulation in domestic mares. Although synthetic progestagens formulated in implants are successfully used in zoo environments and in women, multiple injections and monitoring of the estrous cycle is required for beneficial effects and, thus, may not prove useful for fertility control in large-scale feral horse populations. One study using native progesterone (8 or 24 g) in silastic implants proved unsuccessful albeit estrous behavior was suppressed and ovulation and conception resulted from the treatment group.[22] At this time, altrenogest[a], a consistently effective progestagen for suppressing reproductive function, appears to be the only progestagen approved for use in domestic mares. The effect of altrenogest on reproductive function persists for approximately 30 days. Its potential as a contraceptive for fertility control in feral horse populations may warrant further investigation. However, side effects have been reported after long-term use in domestic mares such as clitoromegaly[24] and compromised foals at birth[25] and may require alternative formulations for use in large-scale feral horse population control.

Gonadotropin-Releasing Hormone Analogs

Gonadotropin-releasing hormone, produced by the hypothalamus, is the master control of reproductive function in the male and female. Upon production and secretion, GnRH stimulates the pituitary gland to produce follicle-stimulating hormone and luteinizing hormone, which in turn is responsible for follicular development and ovulation. Inhibition of GnRH with synthetic or native analogs prevents reproductive function through the absence of follicular growth, oocyte maturation, and subsequent ovulation. (Fig. 1B)

Several formulations of GnRH have been investigated for use as potential contraception agents in the horse. Many other formulations, however, have been tested for use in the manipulation of the estrous cycle and ovulation in domestic mares and may not be relevant in this review. In one of the earlier studies aimed at contraception, immunization of domestic mares against GnRH conjugated with human serum albumin resulting in high variability of response and antibody titers.[27] However, three of the five inoculated mares ceased ovulation for a period of 4 months. Another study using GnRH conjugated to keyhole limpet hemocyanin and triple adjuvant in 29 feral mares showed no difference in foaling rates compared with controls following treatment.[28] More recent work with GnRH[b] as contraception agents in feral horses demonstrated that 93%, 64%, 57%, and 43%, after the first, second, third, and fourth year respectively, were not pregnant when the mares were inoculated with GnRH conjugated to keyhole limpet hemocyanin.[29] Another formulation of GnRH[b], was investigated in 24 free-ranging feral horses. In this study, 61%, 58%, and 69% in years 1, 2, and 3, respectively, were found to be not pregnant over a three year period.[30] Both investigations used the GnRH vaccine[b], and a M. avium adjuvant[c].

Studies using other GnRH conjugated formulations[d,e] also inhibit cyclicity in mares, and there are considerable variations as to the length of estrus and follicular suppression among these studies.[31–34] Inhibition of estrus suppression, cyclicity, and follicular development varied from 4 to 100 weeks. Formulations of GnRH vaccine for use in contraception, although limited in published reports in horses, appear at this time to be a potential GnRH formulation for the application and management suitability of fertility control for the large-scale reduction of feral horse populations.

GnRH Agonists

Actions of GnRH agonists on reproduction function in the mare are similar to those of native GnRH. The most widely used GnRH agonist is deslorelin acetate and is formulated as implants for subcutaneous insertion. Although the agonist implant[f] is designed specifically to induce ovulation in the cycling mare, another injectable[g] was developed for contraception use in other animal species. The injectable form[g] is commonly used as a contraception agent in captive wildlife and is designed to be effective for 12 to 18 months. However, the implants have not been tested in horses specifically for contraception to the author's knowledge. Despite the requirement for multiple injections to achieve a long-term desired effect, the injectable form[g] may serve as an attractive potential for long-term contraception in horses if reformulated as a single injection agent.

Porcine Zona Pellucida-Based Vaccines

Porcine zona pellucida (pZP) is the acellular membrane that surrounds the oocyte. This membrane is responsible for sperm binding through receptors, penetration and prevention of polyspermy, and is a prerequisite for fertilization in the horse and many other mammalian species. Inoculation of porcine zona pellucida, a similar but heterogenic set of proteins, into mares elicits an antibody response, which prevents the binding of sperm to sperm receptor

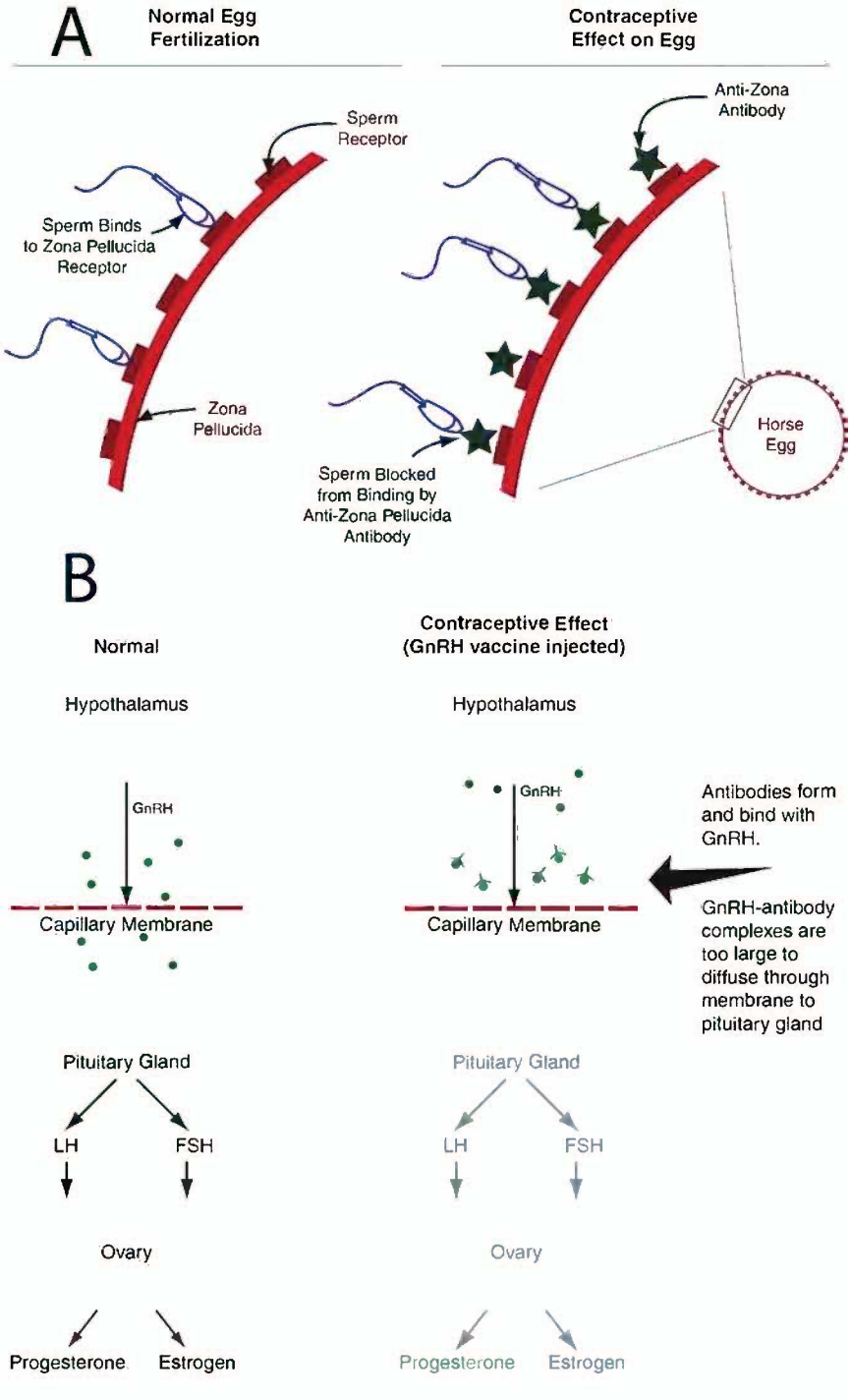

Fig. 1. A, Mechanism of the action of porcine zona pellucida in mares. Normal and inhibition of sperm binding by anti-zona pellucida antibody.[1] B, Mechanism of action of gonadotropin-releasing hormone. Normal and inhibition of GnRH function by anti-GnRH antibody.[1] Adapted from Asa et al. (1996).[26]

sites on the zona pellucida of horse oocytes. When binding of sperm is inhibited, fertilization is prevented (Fig. 1A).[35]

Porcine zona pellucida-based vaccines have been the most extensively studied contraception agent in free-ranging horse populations. Initial studies on the successful use of pZP in wild horses was reported in the late 1980s, and progress in its development for optimal use in the management of free-ranging horses has been slow. There are currently three formulations of pZP-based vaccines used under experimental conditions in an effort to find a suitable

formulation to meet the needs of large scale reduction of free-ranging horse populations. Although all three formulations are pZP-based, two formulations, a liquid pZP emulsion[h] and pZP-22 (a pelleted form of pZP) use pZP harvested from pig ovaries similarly whereas a third pZP-based vaccine[i], is produced slightly different. Additionally, pZP incorporated in liposomes[i] incorporates a diffusion-release mechanism (lipid bilayer liposomes). PZP-22 uses liquid pZP[h] as a primary inoculation and pellets containing pZP incorporated in d-lactide-glycolide copolymers as a controlled, time-release mechanism and QA-21 as the adjuvant for long-acting, booster effects when administered as a paired injection. Liquid pZP[h] is not formulated with controlled release mechanisms and must be administered on a yearly basis to maintain long-term contraceptive effect. The differences in methods of production of pZP from pig ovaries as well as the formulations for long-term effects may be related to the differences in results obtained when used as contraception agents. In particular, the differences between long-term and adverse effects are evident when pZP incorporated in liposomes[i] is administered in comparison with liquid pZP and pZP-22. In general, the major differences are related to effects on follicular development, uterine pathology, and reversibility post-inoculation. Reductions in the number and sizes of follicular development and persistent uterine edema have been reported after the administration of alternate formulations of pZP incorporated in liposomes[i].[29,36] In addition, evidence of reversibility of the contraceptive effect after the administration of pZP incorporated in liposomes[i] have not been consistently observed or reported. Although oocyte evaluation was not performed, the use of liquid pZP[h] also reported a decline in ovarian estrogen production and ovulation rates in treated mares after 3 to 7 years of consecutive yearly injections of the agent. The rate of decline for ovulations reached 10% after seven consecutive years of treatment. In this study, the absence of ovulation was determined by an assay for nonspecific progesterone metabolite iPdG assay from fecal samples.[37] No effects on cyclicity in the mare have been reported as yet after the use of pZP-22. However, further investigations are required to evaluate its effect on cyclicity in the mare post-injection. It is speculated that disruption of cyclicity of mares post pZP treatment may be 1) due to the presence of other ovarian proteins present in the inoculate, in view of the manner in which pZP is currently being processed and used and 2) additional mechanisms of infertility induced by pZP other than blocking of attachment of the zona pellucida by steric hindrance.

Injection site abscesses have also been reported following the use of liquid pZP[h] and pZP-22.[38] In this study, significantly large numbers of abscesses, nodules, and transient swellings were found in mares when pZP was remotely delivered with a rifle. Hand injection of the agent, in contrast, produced minimal numbers of presumptively granulomatous nodules at the injection site.

The contraceptive effectiveness of pZP-based vaccines vary considerably throughout the literature and may lead to much confusion among the reading audience. In general, however, pZP-based vaccines are considered to be 85% to 100% effective for the first year. With long-acting, single injection formulations, the effectiveness begins to decrease after the second and third years at a range of 83% to 85% and 68% to 85%, respectively.[29,39] Unfortunately, recent unpublished data using pZP (pZP-22 and pZP incorporated in liposomes[i]) designed for long-acting contraceptive effects as well as a GnRH vaccine[b], have provided evidence of suboptimal and inconsistent results compared with those previously reported (Kane A, personal communication). Variations in results using similar or modified formulations of pZP-based agents may be attributable to a host of factors, such as delivery method (hand injection, jab-stick injection, remote delivery), alteration of formulations for long-term effects, adjuvant modifications, methods for monitoring, personnel administering of the vaccine, health and nutritional status of the mares, accurate identity of the vaccinated mares, and other uncontrollable factors in free-ranging horse management that may influence or limit the outcome of obtaining optimal and consistent results.

Many studies have emerged and have been reported in the literature on the successful use of liquid pZP,[h] as a fertility control agent. However, a major disadvantage of this pZP formulation is that it requires repeated injections on a yearly basis to maintain contraceptive effect in the mare. From a free-ranging, feral horse management perspective, the necessity to inoculate any mare on a yearly basis to achieve effective contraception on a long-term basis would likely be considered impractical. Liquid pZP[h] and pZP-22 have demonstrated safety for use in mares with no apparent short term, adverse effects with the exception of limited nodule formation at the injection site post-treatment.[38,39] Use of liquid pZP[h] after several consecutive years has also been reported to reduce ovarian estrogen production and ovulation in treated mares as mentioned previously. Despite these disadvantages, liquid pZP[h] and pZP-22 is safe for use in pregnant mares,[40] evidence for reversibility after inoculation has been reported,[41] pZP in inoculated animals will not pass along the food chain,[42] and there are no detrimental behavior changes in inoculated mares.[43,44] Additionally, it has been shown that mares inoculated with liquid pZP[h] on a yearly basis improve the overall body condition and longevity of the mares.[45]

Concerns that pZP inoculated mares may cause an extension of the breeding season have been raised. The treated mares' failure to conceive but continue to cycle normally, thereby causing excessive out-of-season activity of stallions and the production of out-of-season foals past the normal

physiological breeding and foaling season are controversial and not clearly defined. Photoperiod via the retinohypothalamic tract is a major regulator of the physiological breeding season in mares, as well as in stallions. Cyclicity and follicular development in approximately 70% to 80% of domestic mares and receptivity to the stallion decreases significantly as day length (northern hemisphere, 38–43° N) shortens toward the end of the physiological breeding season.[46] It is the author's opinion that it is unlikely that there are any physiological differences in photoperiod receptivity in feral, free-ranging mares, and thus, out-of-season breeding activity may not be a consistent finding among pZP-inoculated mares despite treatment. Other factors also play a role in influencing seasonality in mares such as temperature, age, nutrition, lactation, and stress. These modifiers of seasonality would seem to be more prominent and critical among free-ranging horse mares than domestic mares and may have a stronger influence or larger impact on seasonality of the estrous cycle in the majority of wild horse mares.

At this writing, while short-term contraceptive agents are available, there is no ideal contraceptive agent that meets the needs of controlling overpopulation of free-ranging horses. Although presumptively promising, limited information is available on long-term fertility control agents such as pZP-22, pZP incorporated into liposomes[i], GnRH and chemical vasectomies. Ongoing preliminary studies have indicated inconsistent findings among all fertility control agents tested to date. Critical to science is consistency in the repeatability of results, and as such, current usage of long-term contraceptive agents in large-scale population reduction of wild horses is not feasible. In view of the absence of an ideal contraception agent, free-ranging horse populations continue to increase at alarming rates—rates concerning enough to entertain the possibility of incorporating sterilization modalities in select groups within a population. If sterilization of select groups of mares and stallions is, in part, an end goal of reducing feral horse populations, formulations in the development of current contraception vaccines may require significant modifications, or other alternative methods will be required to achieve that end goal. From the author's perspective, a perspective that may not be shared by all, sterilization of a select group (older mares) of overpopulated wildlife may have several advantages. Firstly and although there are many anecdotal reports of exceptions, the fertility life of a domestic mare is generally accepted to decline at approximately 15 to 16 years of age. More than likely, the decline in fertility is at a younger age in free-ranging horses in view of the relative hostile environment a free-ranging mare is subjected to when compared with domestic mares. Pregnancy (at least in horses) takes a significant toll on a mare's well-being due to energy demands required to maintain pregnancy and lactation. Additionally, the incidence of uterine pathology in the mare increases with multiple births and age. Body condition scores and longevity increases in mares that have been contracepted for multiple years using yearly injections of a contraceptive agent.[45] However, body condition scores, whether contracepted or not, may be dependent upon available nutrition and the social environment of the area in which the mare resides. From a veterinary medical perspective, sterilization of mares eliminates or decreases the incidences of common, pregnancy-associated conditions such as chronic uterine infections, pyometras, cervical and uterine pathology, and most importantly, the life of a mare during dystocias. All together, the advantages of remaining "open" in older mares far outweigh the physiological disadvantages of being pregnant. Perhaps the combination of sterilization and contraception methods applied to select groups of mares and in select herd areas of management may potentially accelerate the reduction of population growth where there is critical need. Regardless of which method(s) are used, modeling studies are critical to accurately estimate the number of horses required to be treated in order to have any impact on population growth.

In summary, there are several potential fertility agents for the reduction of feral horse populations. However, based on current data available in the literature, long-acting fertility control agents such as pZP-based vaccines (pZP-22, pZP incorporated in liposomes[i]), GnRH-based vaccines[b], and chemical vasectomy require further development and investigation with consistent repeatable results in order to meet the needs and requirements of large-scale application for fertility control in feral horse populations. Essential criteria for a best-fit fertility control method when integrated with large-scale management of feral horse populations are based on a single injection protocol, long-term effectiveness, high percent effectiveness, and positive effect on health and behavior of the targeted animal and its environment.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Regumate, Intervet, Inc., Millsboro, DE 19966.

[b]Gonacon-K and Gonacon-B, National Wildlife Research Center, USDA/APHIS, Fort Collins, CO 80521.

[c]Adjuvac, National Wildlife Research Center, USDA/APHIS, Fort Collins, CO 80521.
[d]Improvac, Zoetis, Florham Parks, NJ 07932.
[e]Equity, Zoetis, Auckland, NZ.
[f]Ovuplant, Thorn Bioscience LLC, Louisville, KY 40204.
[g]Suprelorin, Peptech Animal Health/Virbac, Australia.
[h]Zonastat-H, The Science and Conservation Center, Billings, MT 59106.
[i]SpayVac, Terramar Environmental Research LLC, Ashland, OR 97520-1830.