

Porcine Zona Pellucida (PZP) Literature Review (as of May 2018)

BLM has identified fertility control as a method that could be used to protect rangeland ecosystem health and to reduce the frequency of wild horse and wild burro gathers and removals. Expanding the use of population growth suppression to slow population growth rates and reduce the number of animals removed from the range and sent to off-range pastures (ORPs) is a BLM priority. The WFRHBA of 1971 specifically provides for contraception and sterilization (section 3.b.1). No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros. The following literature review is intended to summarize what is known and what is not known about potential effects of treating mares with porcine zona pellucida (PZP) vaccine. As noted below, some negative consequences of vaccination are possible. PZP vaccines are administered only to females.

Contraception has been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used with other techniques, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). All fertility control methods in wild animals are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception by itself does not remove excess horses from an HMA's population, so if a wild horse population is in excess of AML, then contraception alone would result in some continuing environmental effects of horse overpopulation. Successful contraception reduces future reproduction. Limiting future population increases of horses could limit increases in environmental damage from higher densities of horses than currently exist. Horses are long-lived, potentially reaching 20 years of age or more in the wild and, if the population is above AML, treated horses returned to the HMA may continue exerting negative environmental effects, throughout their life span. In contrast, if horses above AML are removed when horses are gathered, that can lead to an immediate decrease in the severity of ongoing detrimental environmental effects to rangeland water, soils and vegetation.

Successful contraception would be expected to reduce the frequency of horse gather activities, as well as wild horse management costs to taxpayers. Bartholow (2007) concluded that the application of 2 or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs. He also concluded that contraceptive treatment would likely reduce the number of horses that must be removed in total, with associated cost reductions in the number of adoptions and total holding costs. If applying contraception to horses requires capturing and handling horses, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs. Population suppression becomes less

expensive if fertility control is long-lasting (Hobbs et al. 2000). Although contraceptive treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, detailed below, those concerns do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013). Whether to use or not use this method to reduce population growth rates in wild horses is a decision that must be made considering those effects as well as the potential negative consequences of inaction, such as continued overpopulation and rangeland health degradation.

Porcine Zona Pellucida (PZP) Vaccine Formulations

PZP vaccines have been used on dozens of horse herds by the National Park Service, US Forest Service, Bureau of Land Management, and Native American tribes and its use is approved for free-ranging wild horse herds. Taking into consideration available literature on the subject, the National Research Council concluded in their 2013 report that PZP was one of the preferable available methods for contraception in wild horses and burros (NRC 2013). PZP use can reduce or eliminate the need for gathers and removals (Turner et al. 1997). PZP vaccines meet most of the criteria that the National Research Council (2013) used to identify promising fertility control methods, in terms of delivery method, availability, efficacy, and side effects. It has been used extensively in wild horses (NRC 2013), and in feral burros on Caribbean islands (Turner et al. 1996, French et al. 2017). PZP is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and is produced as ZonaStat-H, an EPA-registered commercial product (EPA 2012, SCC 2015), or as PZP-22, which is a formulation of PZP in polymer pellets that can lead to a longer immune response (Turner et al. 2002, Rutberg et al. 2017). ‘Native’ PZP proteins can be purified from pig ovaries (Liu et al. 1989). Recombinant ZP proteins may be produced with molecular techniques (Gupta and Minhas 2017, Joonè et al. 2017a). It can easily be remotely administered in the field in cases where mares are relatively approachable and winter access is available. Use of remotely delivered (dart-delivered) vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached within 50 m (BLM 2010).

Both forms of PZP can safely be reapplied as necessary to control the population growth rate. Even with repeated booster treatments of PZP, it is expected that most mares would return to fertility, though some mares treated repeatedly may not (see PZP Direct Effects, below).

The BLM currently uses two PZP formulations for fertility control of wild horse mares, ZonaStat-H (PZP Native) and PZP-22. PZP can be applied via hand injections when animals are gathered into corrals and chutes. In keeping with the EPA registration for ZonaStat-H (EPA 2012; reg. no. 86833-1), certification through the Science and Conservation Center in Billings Montana is required to apply that vaccine to equids.

When applying native PZP (i.e., ZonaStat-H), first the primer with modified Freund's Complete adjuvant is given and then the booster with Freund's Incomplete adjuvant is given 2-6 weeks later. Preferably, the timing of the booster dose is at least 1-2 weeks prior to the onset of breeding activity. Following the initial 2 inoculations, only annual boosters are required. For maximum effectiveness, PZP should be administered within the December to February timeframe. The procedures to be followed for application of PZP are detailed in *Instruction Memorandum No. 2009-909 Attachment 1: Standard Operating Procedures for Population-level Porcine Zona Pellucida Fertility Control Treatments*.

For the first administration of the PZP-22 vaccine pellet formulation given to any mare, she would receive a single dose of the two-year PZP contraceptive vaccine at the same time as a dose of the liquid PZP vaccine with modified Freund's Complete adjuvant. The pellets are applied to the mare with a large gauge needle and jab-stick into the hip. Subsequent 'booster' doses given to mares that have received the PZP-22 vaccine pellets may be either of ZonaStat-H, or of PZP-22 vaccine pellets (Rutberg et al. 2017). Although PZP-22 pellets have been delivered via darting in trial studies (Rutberg et al 2017), BLM does not plan to use darting for PZP-22 delivery until there is more demonstration that PZP-22 can be reliably delivered via dart. Therefore, WH&Bs must be gathered for each application of this formulation.

PZP Direct Effects

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

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

al. 2017). Application of PZP for fertility control would reduce fertility in a large percentage of mares for at least one year (Ransom et al. 2011).

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

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

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

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

The efficacies noted above, which are based on results in Rutberg et al. (2017), call into question population and economic models that assume PZP-22 can have an 85% efficacy in years 2 and 3 after immunization, such as Fonner and Bohara (2017).

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

Reversibility of PZP vaccine and Effects on Ovaries

In most cases, PZP contraception appears to be temporary and reversible, with most treated mares returning to fertility over time (Kirkpatrick and Turner 2002). The NRC (2013) criterion by which PZP is not optimal for wild horse contraception was duration. The ZonaStat-H formulation of the vaccine tends to confer only one year of efficacy per dose. Some studies have found that a PZP

vaccine in long-lasting pellets (PZP-22) can confer multiple years of contraception (Turner et al. 2007), particularly when boosted with subsequent PZP vaccination (Rutberg et al. 2017). Other trial data, though, indicate that the pelleted vaccine may only be effective for one year (J. Turner, University of Toledo, Personal Communication).

The purposes of applying PZP treatment is to prevent mares from conceiving foals, but BLM acknowledges that long-term infertility, or permanent sterility, could be a result for some number of wild horses receiving PZP vaccinations. The rate of long-term or permanent sterility following vaccinations with PZP is hard to predict for individual horses, but that outcome appears to increase in likelihood as the number of doses increases (Kirkpatrick and Turner 2002). Permanent sterility for mares treated consecutively 5-7 years was observed by Nuñez et al. (2010, 2017). In a graduate thesis, Knight (2014) suggested that repeated treatment with as few as three to four years of PZP treatment may lead to longer-term sterility, and that sterility may result from PZP treatment before puberty. Repeated treatment with PZP led to long-term infertility in Przewalski's horses receiving as few as one PZP booster dose (Feh 2012). However, even if some number of mares become sterile as a result of PZP treatment, that potential result would be consistent with the contraceptive purpose that motivates BLM's potential use of the vaccine.

In some mares, PZP vaccination may cause direct effects on ovaries (Gray and Cameron 2010, Joonè et al. 2017b, Joonè et al. 2017c, Joonè et al. 2017d). Joonè et al. (2017a) noted reversible effects on ovaries in mares treated with one primer dose and booster dose. Joonè et al. (2017c) documented decreased anti-Mullerian hormone (AMH) levels in mares treated with native or recombinant PZP vaccines; AMH levels are thought to be an indicator of ovarian function. Bechert et al. (2013) found that ovarian function was affected by the SpayVac PZP vaccination, but that there were no effects on other organ systems. Mask et al. (2015) demonstrated that equine antibodies that resulted from SpayVac immunization could bind to oocytes, ZP proteins, follicular tissues, and ovarian tissues. It is possible that result is specific to the immune response to SpayVac, which may have lower PZP purity than ZonaStat or PZP-22 (Hall et al. 2016b). However, in studies with native ZP proteins and recombinant ZP proteins, Joonè et al. (2017a) found transient effects on ovaries after PZP vaccination in some treated mares; normal estrus cycling had resumed 10 months after the last treatment. SpayVac is a patented formulation of PZP in liposomes that led to multiple years of infertility in some breeding trials (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018), but unacceptably poor efficacy in a subsequent trial (Kane 2018). Kirkpatrick et al. (1992) noted effects on horse ovaries after three years of treatment with PZP. Observations at Assateague Island National Seashore indicate that the more times a mare is consecutively treated, the longer the time lag before fertility returns, but that even mares treated 7 consecutive years did eventually return to ovulation (Kirkpatrick and Turner 2002). Other studies have reported that continued applications of PZP may result

in decreased estrogen levels (Kirkpatrick et al. 1992) but that decrease was not biologically significant, as ovulation remained similar between treated and untreated mares (Powell and Monfort 2001). Permanent sterility for mares treated consecutively 5-7 years was observed by Nuñez et al. (2010, 2017). Bagavant et al. (2003) demonstrated T-cell clusters on ovaries, but no loss of ovarian function after ZP protein immunization in macaques. Skinner et al. (1984) raised concerns about PZP effects on ovaries, based on their study in laboratory rabbits, as did Kaur and Prabha (2014), though neither paper was a study of PZP effects in equids.

Effects of PZP on Existing Pregnancies, Foals, and Birth Phenology

If a mare is already pregnant, the PZP vaccine has not been shown to affect normal development of the fetus or foal, or the hormonal health of the mare with relation to pregnancy (Kirkpatrick and Turner 2003). It is possible that there may be transitory effects on foals born to mares or jennies treated with PZP. In mice, Sacco et al. (1981) found that antibodies specific to PZP can pass from mother mouse to pup via the placenta or colostrum, but that did not apparently cause any innate immune response in the offspring: the level of those antibodies were undetectable by 116 days after birth. There was no indication in that study that the fertility or ovarian function of those mouse pups was compromised, nor is BLM aware of any such results in horses or burros. Unsubstantiated speculative connections between PZP treatment and foal stealing has not been published in a peer-reviewed study and thus cannot be verified. Similarly, although Nettles (1997) noted reported stillbirths after PZP treatments in cynomolgus monkeys, those results have not been observed in equids despite extensive use.

On-range observations from 20 years of application to wild horses indicate that PZP application in wild mares does not generally cause mares to give birth to foals out of season or late in the year (Kirkpatrick and Turner 2003). Nuñez's (2010) research showed that a small number of mares that had previously been treated with PZP foaled later than untreated mares and expressed the concern that this late foaling "may" impact foal survivorship and decrease band stability, or that higher levels of attention from stallions on PZP-treated mares might harm those mares. However, that paper provided no evidence that such impacts on foal survival or mare well-being actually occurred. Rubenstein (1981) called attention to a number of unique ecological features of horse herds on Atlantic barrier islands, which calls into question whether inferences drawn from island herds can be applied to western wild horse herds.

Parturition phenology (birthing season) for North American feral horses has been shown to peak during May (Berger 1986, Garrott and Siniff 1992, Nunez et al. 2010) and photoperiod and temperature are powerful inputs driving the biological rhythms of conception and birth in horses. With an 11-month gestation period, this timing maximizes the likelihood that foals will be born and spend their first few months of life at a time when the weather is warm and food is plentiful (Crowell-Davis 2007). Ransom et al. (2013) identified a potential shift in

reproductive timing as a possible drawback to prolonged treatment with PZP, stating that treated mares foaled on average 31 days later than non-treated mares. Results from Ransom et al. (2013), however, showed that over 81% of the documented births in this study were between March 1 and June 21, i.e., within the normal, peak, spring foaling season. Ransom et al. (2013) pointedly advised that managers should consider carefully before using PZP in small refugia or rare species. Wild horses and burros managed by BLM do not generally occur in isolated refugia, nor are they rare species. Moreover, an effect of shifting birth phenology was not observed uniformly: in two of three PZP-treated wild horse populations studied by Ransom et al. (2013), foaling season of treated mares extended three weeks and 3.5 months, respectively, beyond that of untreated mares. In the other population, the treated mares foaled within the same time period as the untreated mares. Furthermore, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. If there are shifts in birth phenology, though, it is reasonable to assume that some negative effects on foal survival might result from particularly severe weather events (Nuñez et al. 2018).

Effects of Marking and Injection

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

Most mares recover from the stress of capture and handling quickly once released back to the HMA, and none are expected to suffer serious long-term effects from the fertility control injections, other than the direct consequence of becoming temporarily infertile. Injection site reactions associated with fertility control treatments are possible in treated mares (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017), but swelling or local reactions at the injection site are expected to be minor in nature. Roelle and Ransom (2009) found that the most time-efficient method for applying PZP is by hand-delivered injection of 2-year pellets when horses are gathered. They observed only two instances of swelling from that technique. Use of remotely delivered, 1-year PZP is generally limited to populations where individual animals can be accurately identified and repeatedly approached. The dart-delivered formulation produced injection-site reactions of

varying intensity, though none of the observed reactions appeared debilitating to the animals (Roelle and Ransom 2009). Joonè et al. (2017a) found that injection site reactions had healed in most mares within 3 months after the booster dose and that they did not affect movement or cause fever. The longer-term nodules observed did not appear to change any animal's range of movement or locomotor patterns and in most cases did not appear to differ in magnitude from naturally occurring injuries or scars.

Indirect Effects of PZP vaccination

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

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

Because successful fertility control would reduce foaling rates and population growth rates, another indirect effect should be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception would be expected to lead to a relative increase in the fraction of older animals in the herd. Reducing the numbers of wild horses that

would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding. Among mares in the herd that remain fertile, a high level of physical health and future reproductive success would be expected because reduced population sizes should lead to more availability of water and forage resources per capita.

Reduced population growth rates and smaller population sizes could also allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse habitat quality. As the population nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available to wild horses and wildlife throughout HMAs. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses across the HMA, there should also be less trailing and concentrated use of water sources. Lower population density would be expected to lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Should PZP booster treatment continue into the future, the chronic cycle of overpopulation and large gathers and removals would no longer occur, but instead a consistent cycle of balance and stability would ensue, resulting in continued improvement of overall habitat conditions and animal health. While it is conceivable that widespread and continued treatment with PZP could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated in almost every year.

Behavioral Effects of PZP vaccine

The NRC report (2013) noted that all fertility suppression has effects on mare behavior, mostly as a result of the lack of pregnancy and foaling, and concluded that PZP was a good choice for use in the program. The result that PZP-treated mares may continue estrus cycles throughout the breeding season can lead to behavioral differences (as discussed below), when compared to mares that are fertile. Such behavioral differences should be considered as potential consequences of successful contraception.

Ransom and Cade (2009) delineate behaviors that can be used to test for quantitative differences due to treatments. Ransom et al. (2010) found no differences in how PZP-treated and untreated mares allocated their time between feeding, resting, travel, maintenance, and most social behaviors in three populations of wild horses, which is consistent with Powell's (1999) findings in another population. Likewise, body condition of PZP-treated and control mares

did not differ between treatment groups in Ransom et al.'s (2010) study. Nuñez (2010) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation. Knight (2014) found that PZP-treated mares had better body condition, lived longer and switched harems more frequently, while mares that foaled spent more time concentrating on grazing and lactation and had lower overall body condition. Studies on Assateague Island (Kirkpatrick and Turner 2002) showed that once fillies (female foals) that were born to mares treated with PZP during pregnancy eventually breed, they produce healthy, viable foals.

In two studies involving a total of four wild horse populations, both Nuñez et al. (2009) and Ransom et al. (2010) found that PZP-treated mares were involved in reproductive interactions with stallions more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Killian 1997, Heilmann et al. 1998, Powell 1999, Curtis et al. 2001, Duncan et al. 2017). There was no evidence, though, that mare welfare was affected by the increased level of herding by stallions noted in Ransom et al. (2010). Nuñez's later analysis (2017) noted no difference in mare reproductive behavior as a function of contraception history.

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

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

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

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

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

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

". . . in no case can the committee conclude from the published research that the behavior differences observed are due to a particular compound rather than to the fact that treated animals had no offspring during the study. That must be borne in

mind particularly in interpreting long-term impacts of contraception (e.g., repeated years of reproductive “failure” due to contraception).”

Genetic Effects Specific to PZP Vaccination

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

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

BLM is not aware of any studies that have quantified the heritability of a lack of response to immunocontraception such as PZP vaccine or GonaCon-Equine in horses. At this point, there are no studies available from which one could make conclusions about the long-term effects of sustained and widespread

immunocontraception treatments on population-wide immune function. Although a few, generally isolated, feral horse populations have been treated with high fractions of mares receiving PZP immunocontraception for long-term population control (e.g., Assateague Island and Pryor Mountains), no studies have tested for changes in immune competence in those areas. Relative to the large number of free-roaming feral horses in the western United States, immunocontraception has not been used in the type of widespread or prolonged manner that might be required to cause a detectable evolutionary response.

Although this topic may merit further study, lack of clarity should not preclude the use of immunocontraceptives to help stabilize extremely rapidly growing herds.

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