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Synthesis

Consequences of porcine zona pellucida immunocontraception to feral horses

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Abstract: Porcine zona pellucida (PZP) immunocontraception was developed to provide a more humane, effective, and inexpensive method of population regulation for wildlife species. It has been used to regulate populations of several species including white tailed deer (*Odocoileus virginianus),* elk (*Cervus elaphus*), black bear (*Ursus americanus*), and the feral horse (*Equus ferus caballus*) with varying levels of success. Early studies on Assateague Island National Seashore, Maryland, USA, suggested PZP was as an ideal form of fertility control because it reduced the likelihood of conception to <10%, could be delivered remotely, was thought to be reversible, lacked debilitating physiological side effects, could not pass through the food chain, and showed minimal effects to social behaviors in a closed population of feral horses. However, research on Shackleford Banks, North Carolina, USA and on 3 western populations located in Little Brook Cliffs (Grand Junction, Colorado, USA), McCollough Peaks (east of Cody, Wyoming, USA), and the Pryor Mountains (Lovell, Wyoming, USA) has revealed behavioral and physiological side effects of long-term PZP use. When compared to untreated mares (those that have never received treatment), treated mares demonstrated decreased fidelity to the band stallion, increased and prolonged reproductive behavior, and an increased likelihood of extending reproductive cycling into the nonbreeding season. These effects were more pronounced in animals receiving more total and/or consecutive contraception treatments and can persist even after several years of treatment cessation. Finally, new data indicate that these changes to previously treated mares can affect the behavior and stress physiology of their band stallions, demonstrating the potential for the contraception of individuals to have population-level effects. These results are important to consider if we are to achieve both the effective management of feral horse populations in addition to the maintenance of their overall health and well-being.

Key words: behavior, *Equus ferus caballus*, feral horse, immunocontraception, physiology, porcine zona pellucida (PZP), reproductive, stress, treatment schedule

Feral horses (*Equus ferus caballus*) are a beloved icon of the American West. In 1971, the U.S. Congress, with the passage of the Wild Free-Roaming Horse and Burro Act (WFRHBA), designated feral horses as "wild" horses that are "living symbols of the historic and pioneer spirit of the West" (United States Congress 1971). The WFRHBA designation, the shift in public thinking that sparked it, and conflicting views on the status of feral horses as native or non-native wildlife (Weinstock et al. 2005, Mihlbachler et al. 2011), have likely contributed to the controversy surrounding feral horse management (Garrott et al. 1991). However, with few predators capable of taking feral horses (Eberhardt et al. 1982, Turner et al. 1992, Turner and Morrison 2001) and no legal hunting, unregulated feral horse populations can adversely impact both the local environment (Furbish 1990, Levin et al. 2002) and other wildlife (Ostermann-Kelm et al. 2008, Gooch et al. 2017, Hall et al. 2018) across the United States.

Feral horse population increases on Shackleford Banks, North Carolina, USA resulted in reduced vegetation, higher bird and crab diversity, and lower fish density and species richness in horse-grazed versus ungrazed marshes (Furbish and Albano 1994). In Nevada and Southern California, USA, feral horses outcompete bighorn sheep (*Ovis* spp.) and pronghorn (*Antilocapra americana*) for water; such competition can have serious implications in these desert habitats. Finally, conflict between humans and feral horse populations are particularly acute in the western United States, where the degradation of rangeland plants, soils, and water quality has been documented (Bureau of Land Management 2013).

Despite these impacts, the status of feral horses and their federal protection means that managers have few options to regulate population numbers. Gathers, in which animals are rounded up by helicopter and either re-released or removed from the range, divide social groups (commonly referred to as bands) and decrease band stability (Kirkpatrick et al. 1997). In addition, the use of hormonal contraception (in both male and female animals) proved problematic due to the dosage needed to achieve infertility, the issue of possible consumption by non-target species, and the subsequent changes to recipient behavior (Turner and Kirkpatrick 1991).

On the other hand, immunocontraception proved easier to administer, did not pass through the food chain, and because of its mechanism of action (in comparison to hormonal contraception in which treatment-induced changes to recipient hormone levels directly affect behavior [Turner and Kirkpatrick 1991]), immunocontraceptive treatment itself caused little to no change to recipient behavior (Kirkpatrick 1990, Turner and Kirkpatrick 1991, Kirkpatrick 1995, Kirkpatrick and Turner 2002). Immunocontraception induces the production of antibodies against structural or functional molecules necessary for reproduction. These antibodies obstruct the normal cell biology of reproduction, reducing the likelihood of pregnancy (Turner and Kirkpatrick 1991).

In the case of the porcine zona pellucida (PZP) vaccine, antibodies bind sperm receptors on the egg's surface, preventing sperm attachment and fertilization over the course of 1 breeding season (Sacco 1977). Though hugely promising, initial work researching the potential effects of PZP on recipient animals did not consider the potential for behavioral effects of prolonged subfertility (Kirkpatrick and Turner 1991). Today, PZP is used with varying levels of success to control reproduction in several free-ranging ungulate species, including white-tailed deer (*Odocoileus virginianus;* McShea et al. 1997), elk (*Cervus elaphus;* Heilmann et al. 1998), and the feral horse (Kirkpatrick 1990, Turner et al. 2002).

Initially, most of the research examining the effects of PZP-induced subfertility on feral horses stemmed from 1 long-term study on Assateague Island National Seashore, Maryland, USA. Researchers reported no debilitating side effects to PZP recipients and only minor ovulation failure and depressed urinary oestrogen concentrations with repeated applications (Kirkpatrick et al. 1996). In addition, the contraceptive effects of PZP were shown to be reversible, safe for pregnant females, and did not adversely affect the survivorship or subsequent fertility of offspring born to treated individuals (Kirkpatrick and Turner 2002).

However, an important caveat was made; on average, mares receiving 4–5 consecutive treatments took 4.4 years to return to fertility (range = 1–8 years), and those receiving 7 consecutive treatments did not return to fertility during a 7-year, treatment-free interval (Kirkpatrick and Turner 2002). These results should have served as a warning to managers that more aggressive treatment could grossly affect future fertility in treated mares (potentially limiting future management options); however, such caution seems to have been taken only rarely (Ransom et al. 2013, Nuñez et al. 2017).

Early on, very little investigation was devoted to the potential for behavioral effects of PZP contraception. Powell (1999) found little to no effect; however, true controls (i.e., animals that had never been treated) were not available for comparison. More recent studies on feral horses living on Shackleford Banks and in the western United States demonstrate important differences in recipient behavior and physiology (Nuñez et al. 2009, Madosky et al. 2010, Nuñez et al. 2010, Ransom et al. 2010). For a gregarious species like the horse, such changes may have serious social and demographic consequences.

Herein, I provide a synthesis of the literature regarding the potential effects of PZP contraception on mares and band social structure. I compare the effects of PZP in different populations and use results from a 17-year dataset to offer management suggestions that may prevent or ameliorate these effects. The research reviewed here was conducted on horse populations in which the individual identity and treatment history of mares was known; therefore, these recommendations will likely not be applicable to all situations (Turner et al. 2001, Killian et al. 2008). However, the potential benefits to feral horse health and well-being and the assurance that future management options remain available (Nuñez et al. 2017) indicate that the suggestions proposed should be applied whenever possible.

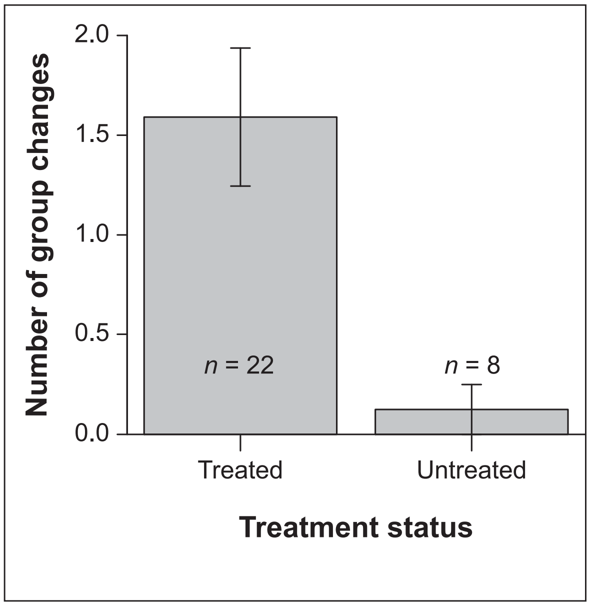

Figure 1. Number of group changes for treated and untreated mares. Even when controlling for the effect of age, treated mares changed groups more often than did their untreated counterparts. Adapted from Nuñez et al. (2009).

Effects of PZP-induced subfertility

PZP-induced subfertility can have important behavioral and physiological consequences for recipient animals (Nuñez et al. 2009, Madosky et al. 2010, Nuñez et al. 2010, Ransom et al. 2010, Ransom et al. 2013). This was especially the case with mares receiving ≥4 total and/or consecutive treatments (Nuñez 2005, unpublished data; Nuñez et al. 2010). Moreover, changes to mare behavior and physiology have the potential to become long-term (especially in mares that received ≥4 total treatments; Nuñez et al. 2017). Finally, such changes in recipient animals can significantly affect the behavior and physiology of their band stallions (Jones and Nuñez 2017, unpublished data). Although such aggressive treatment may be deemed necessary when rapid reductions in animal numbers are of paramount importance, the resultant changes to mare behavior and physiology can limit future management options (Nuñez et al. 2010, Ransom et al. 2014, Nuñez et al. 2017).

Conversely, animals receiving fewer treatments exhibited behavior and reproductive physiology more typical of untreated animals. Moreover, animals receiving fewer treatments were more likely to return to fertility after the suspension of treatment (Nuñez et al. 2017), thereby maintaining flexibility should future management goals change. Although such considerations will not be practical with all populations (Turner et al. 2001, Killian et al. 2008), more careful consideration to feral horse behavior and physiology can help ensure more effective, flexible population management.

Behavior

On Shackleford Banks, PZP-treated mares were up to 10 times more likely to change bands than their untreated counterparts (Nuñez et al. 2009; Figure 1). Moreover, mares receiving 4–6 total treatments were more likely to engage in the behavior than those receiving 1–3 or 0 treatments (Nuñez 2005, unpublished data). This is an important shift in behavior, as band stability, which is affected by the degree of female loyalty to the band stallion (Kaseda et al. 1995), is important to overall animal health. Decreases in band stability have been associated with decreased physical condition and female fecundity, increased parasite load and offspring mortality, and less time spent in preferred behaviors (Linklater et al. 1999). Moreover, increased mare turnover can disturb resident females (Monard and Duncan 1996), causing an overall increase in their levels of aggression (Rutberg 1990, Monard and Duncan 1996). In addition, repeated changes to band composition may prevent the establishment of stable female dominance hierarchies, which are critical to maintaining social cohesion among mares and overall group stability (Berger 1977, Houpt and Wolski 1980, Heitor et al. 2006).

Evidence suggested that such decreases in mare fidelity to the band stallion were related to PZP-induced subfertility: mares that conceived more often over a 5-year period (i.e., mares that were left untreated or for which treatment had failed), were less likely to engage in group changing behavior (Nuñez et al. 2009; Figure 2). Though these results were confounded by PZP treatment (see Figure 2), given the vaccine's mechanism of action, and the demonstrated effects of treatment regime, it seems more likely that prolonged subfertility is the causal mechanism behind decreases in mare fidelity.

Similar to white-tailed deer does (McShea et al. 1997) and free-ranging elk cows (Heilmann et al. 1998), treated mares were also more likely to solicit and receive reproductive behaviors from males (Nuñez et al. 2009, Ransom et al. 2010).

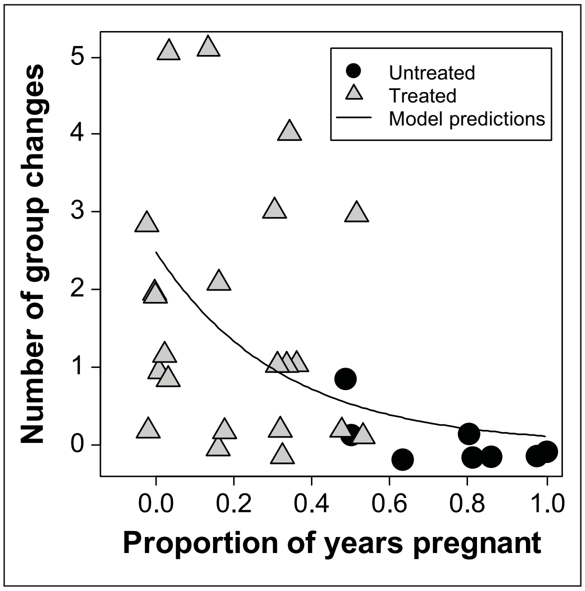

Figure 2. Number of group changes and the proportion of years pregnant from January 2000 to January 2005. Points have been jittered to allow clear visualization of every individual. The number of changes mares made decreased with the proportion of years they were pregnant. Adapted from Nuñez et al. (2009).

In addition, stallions in western populations were more likely to breed PZP-treated versus untreated mares regardless of their age or the probability of producing viable offspring, 2 factors that normally affect male mate choice (Ransom et al. 2010; Figure 3). In the absence of pregnancy, the increased frequency of reproductive cycling demonstrated by treated mares was undoubtedly a factor driving male behavior. In addition, there was the potential for a perceived increase in reproductive "failures" (by mares) to drive female reproductive behavior.

Stress physiology

Because stallions will vigorously attempt to retrieve and maintain resident mares, mares attempting to change groups are often subject to direct male harassment, including aggressive herds, chases, and increased reproductive activity by either the band or new stallion (Madosky 2011), and/or find themselves in close proximity to highly escalated male–male contests (Feist and McCullough 1976, Rubenstein 1981). In addition, if mares are successful at leaving their current band to join a new one, they are often subject to harassment by resident mares establishing their dominance (Rutberg 1990).

On Shackleford Banks, this increased social instability affected mare stress physiology. During group changing behavior, mares exhibited increased fecal cortisol (Nuñez et al. 2014; Figure 4), a reliable measure of stress in several mammalian taxa (Wasser et al. 2000, Mostl and Palme 2002). This effect was not fleeting; mares changing groups more often continued to show elevated cortisol levels for up to 2 weeks post their group changing behavior (Nuñez et al. 2014; Figure 5).

These studies indicate that group transfer behavior can incur a significant cost to mares. Although stress in itself is not inherently detrimental to animal fitness or well-being (Moberg 2000), the stress response can become pathogenic when experienced chronically (Sapolsky 2005), resulting in adverse effects to cardiovascular and immune function, reproduction, and, in extreme cases, neurological functioning. On Shackleford Banks, mares changing bands more frequently can be subject to a negative feedback loop: their behavior leads to harassment, which may induce additional group changes, resulting in further harassment (Madosky et al. 2010; also see Linklater et al. 1999). It is unlikely that the mares observed in our study experienced chronic stress as defined by Sapolsky (2005). However, our data suggest that mares making more group changes experienced increased stress levels at more regular intervals than did mares making fewer group changes. There can be little doubt, therefore, that group changing behavior incurs a physiological cost to mares.

These results have important implications for feral horse management. On Shackleford, PZP-induced subfertility was associated with increased group changing behavior (see Behavior). Though the lack of any untreated mares at the time of our study precluded a direct comparison of the cortisol levels of treated versus untreated mares (Nuñez et al. 2014), the logic was clear: group changing behavior and the associated decreases to social stability induced a physiological stress response in mares. Mares that engage in this behavior more often may be at higher risk for the deleterious effects of the repeated stressor.

Contracepted mares consistently demonstrated improved physical condition and increased longevity when compared to uncontracepted mares (Kirkpatrick and Turner 2007, Nuñez et al. 2010). However, the potential effects of

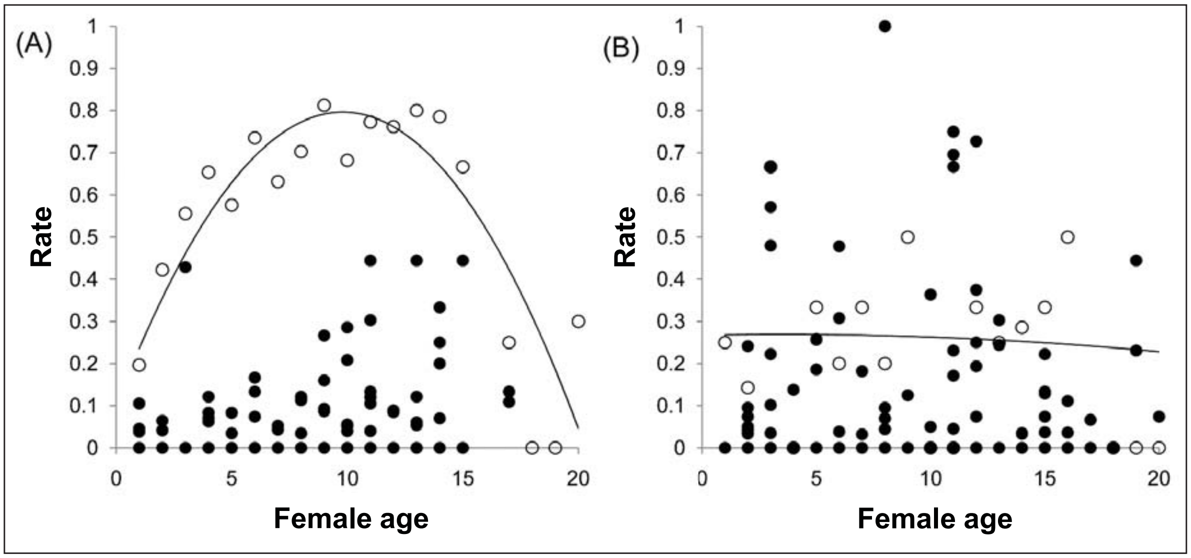

Figure 3. Rate of stallion reproductive behaviors exhibited per hour (●) as a function of mare age, and mean rate of viable foal production (○) as a function of mare age at conception, for repeated observations of 99 untreated mares (A) and 56 treated mares (B) at Little Book Cliffs Wild Horse Range, Grand Junction, Colorado, USA; McCullough Peaks Herd Management Area (HMA), east of Cody, Wyoming, USA; and Pryor Mountain Wild Horse Range, Wyoming and Montana, USA, 2003–2006. The rate of reproductive behavior expressed toward females (by males) generally followed the relationship between viable foal production and age of the female at the time of conception for untreated mares (A). This relationship deteriorated in treated mares (B) and the rate of reproductive behaviors expressed was both higher than control females and exhibited no clear pattern among age cohorts. Adapted from Ransom et al. (2010).

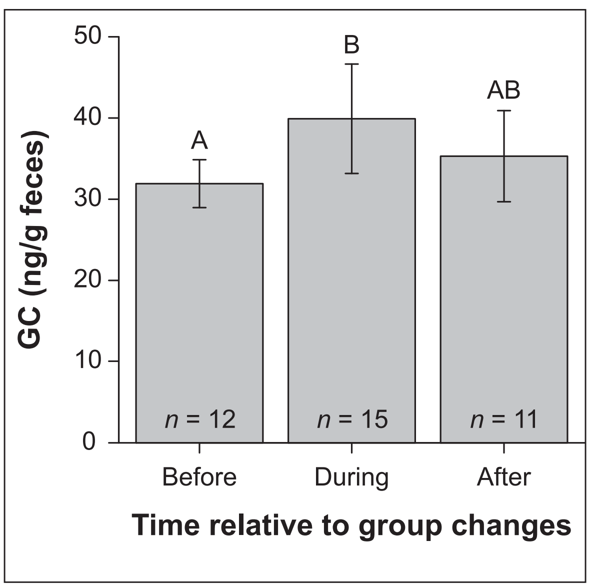

Figure 4. Mare cortisol level and the timing relative to group change behavior. Mares showed increases in cortisol during group changes. Adapted from Nuñez (2014).

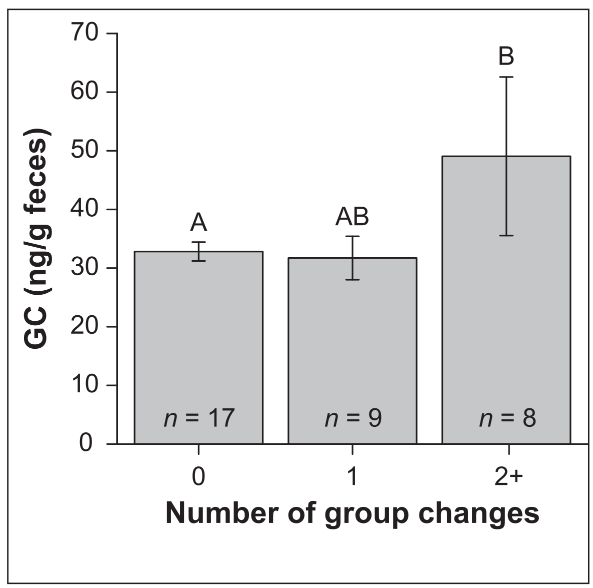

Figure 5. Mare cortisol level 2 weeks post group changing behavior. Mares making more group changes exhibited higher cortisol levels 2 weeks post behavior than did mares making fewer group changes. Adapted from Nuñez et al. (2014).

repeated stressors to treated mares is not trivial. For instance, repeated stress can result in more frequently dysregulated glucocorticoid secretion, elevated food consumption, insulin resistance, and increased fat deposition, which, in some species, can contribute to high condition scores (Leibowitz and Hoebel 1997, McEwen and Wingfield 2003, Sapolsky 2005). Moreover, recent research in other taxa suggests that accepted condition measures do not reliably predict animal survival (crimson finches [*Neochmia phaeton*]) or other constituents of body condition, including

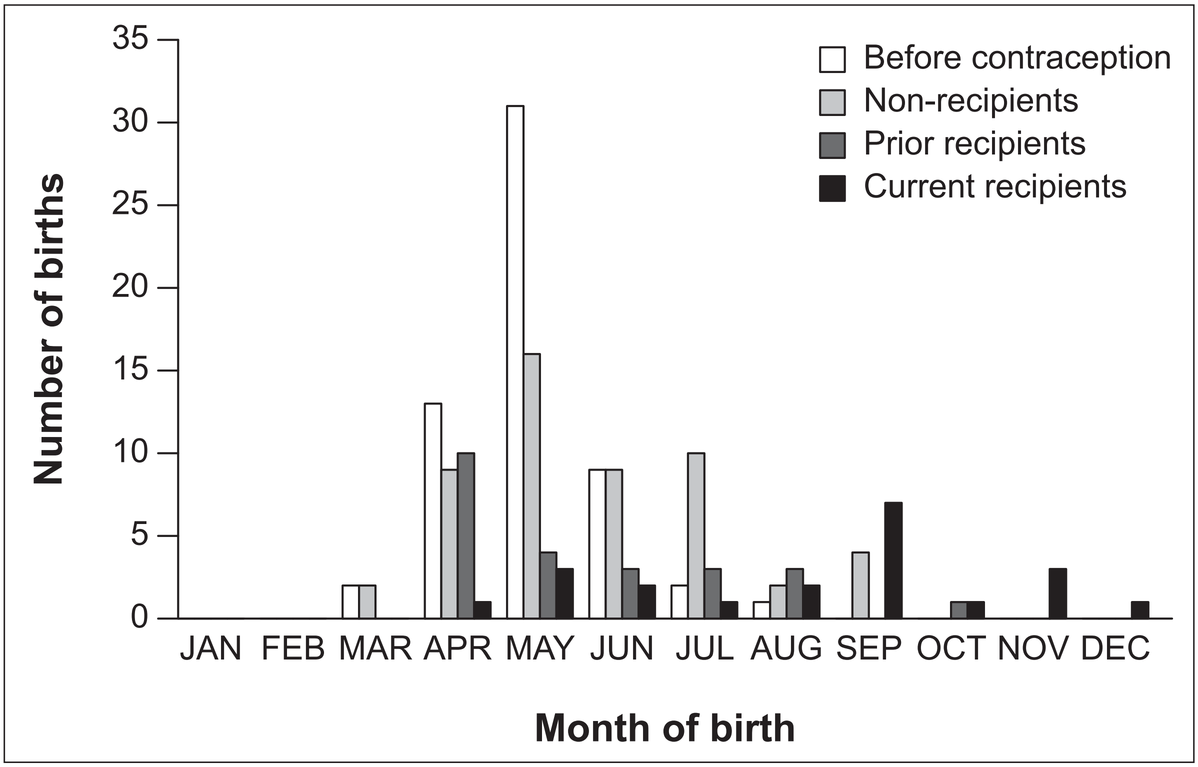

Figure 6. The distribution of births for mares on Shackleford Banks, North Carolina, USA, pre-contraception and post-contraception management. Mares gave birth over a wider range of months and later, on average, after the onset of contraception; this effect was more pronounced in PZP recipients than non-recipients. Adapted from Nuñez (2010).

fat and lean mass (lizards [*Anolis sagrei*]), suggesting that such scoring methods, regardless of what they may indicate, may not be sufficient for the assessment of integrative animal health (Milenkaya et al. 2015, Warner et al. 2016).

Reproductive physiology

In both the Shackleford and western populations studied, mares treated with PZP were more likely to extend reproductive cycling into what is typically considered the non-breeding season (fall and winter) than were untreated mares. This resulted in an increased number of fall and winter births to treated, compared to untreated, mares (Nuñez et al. 2010, Ransom et al. 2013; Figures 6 and 7). On Shackleford, this effect was especially pronounced in mares receiving a greater number of consecutive applications. Each additional consecutive treatment resulted in a 25-day delay in foaling (Nuñez et al. 2010).

These changes constituted an important shift in reproductive physiology, as mares living in temperate climates are typically anovulatory during the non-breeding season (Asa 2002). Gestation lasts approximately 11–13 months in feral horses (Asa 2002). Consequently, mares give birth to foals in seasonal conditions similar to those in which they conceive (i.e., they give birth during the same season the following year). A cessation of ovulatory cycling during the non-breeding season is adaptive in that it precludes conception and subsequent birth during a time of year when resources are at their lowest (Stevens 1990).

Continued cycling into the non-breeding season and the associated fall and winter births could contribute to higher foal mortality; however, management interventions (such as the removal of "at risk" animals) has made the accurate analysis of this possibility infeasible on Shackleford Banks. In areas like the western United States, harsher climatic conditions will likely exacerbate the potential effects of extended cycling to offspring and mare health (Ransom et al. 2013).

Potential for long-term effects of PZP treatment

The potential for long-term effects of PZP-induced subfertility is highly dependent upon the number of treatments females receive during

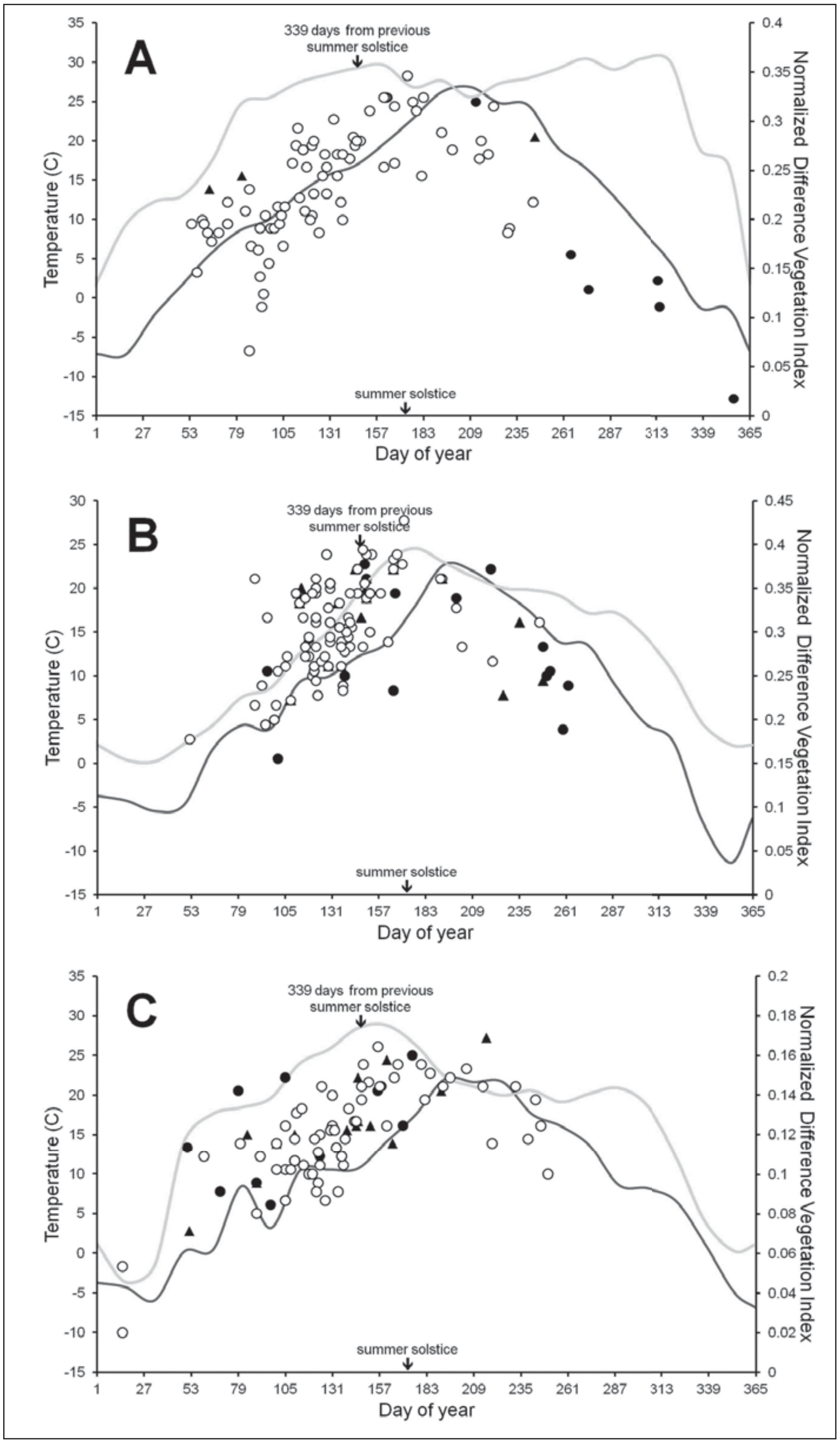

Figure 7. Birth phenology of untreated (○) and post-treated mares (● = first post-treatment birth, ▲ = birth subsequent to ●). Births are shown as a function of temperature at approximate conception date for populations at (A) Little Book Cliffs Wild Horse Range, Grand Junction, Colorado, USA, 2005–2011; (B) Pryor Mountain Wild Horse Range, Wyoming and Montana, USA, 2005–2011; and (C) McCullough Peaks Herd Management Area (HMA), east of Cody, Wyoming, USA, 2007–2011. Post-treated females were previously inoculated with the immunocontraceptive porcine zona pellucida (PZP). Mean Normalized Difference Vegetation Index (shown in lighter gray) represents temporal availability of forage. Mean daily surface temperature is also included (shown in darker gray). Adapted from Ransom et al. (2013).

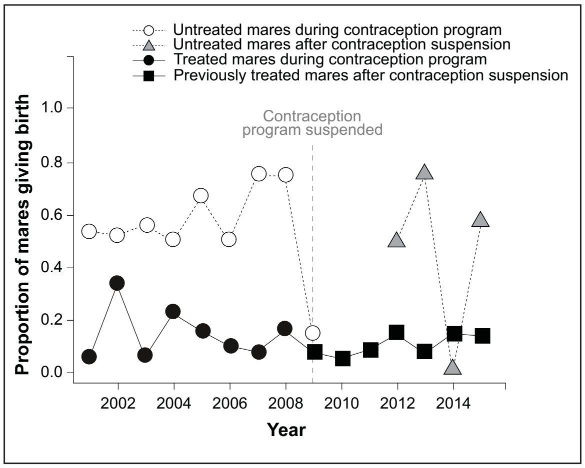

Figure 8. Proportion of mares giving birth in the years during and after contraception management. During the contraception program, treated mares showed markedly lower birth rates than did untreated mares. In 2009, all untreated mares received PZP, leaving no untreated animals of reproductive age on the island in 2010 and 2011. Even after the suspension of treatment, previously treated mares did not return to a level of fertility comparable to untreated mares before 2009 or to younger, untreated animals that became reproductive between 2012 and 2015. Adapted from Nuñez et al. (2017).

their lifetime (Nuñez et al. 2017). In 2009, after 9 years of intensive contraception management on Shackleford Banks, the program was largely but temporarily suspended to allow for controlled population growth (S. Stuska, National Park Service, personal communication). Even when controlling for the effects of mare age on fertility, previously treated mares were less likely to foal than were untreated mares during 5 years of contraception suspension (Figure 8). However, for mares that previously received fewer total treatments (1–3 applications), foaling probability increased with time since their last treatment before declining at approximately 6 years post-treatment. This was not the case for mares that previously received more intensive treatment (≥4 [range = 4–9] total applications); for these mares, there was no increase in foaling probability after contraception suspension. Again, these effects were significant even when controlling for the effects of mare age on fertility.

Moreover, previously treated mares receiving any level of treatment continued to conceive later into the year than did untreated mares, demonstrating prolonged reproductive cycling, and mares previously receiving ≥4 treatments continued to change groups more often than did untreated mares (even after 4–6 years without treatment), demonstrating a prolonged decrease in loyalty to their band stallions (Nuñez et al. 2017).

These results demonstrate the potential for long-term behavioral and physiological effects of PZP-induced subfertility (even after the cessation of treatment). They also show that such effects can be ameliorated with less intense application schedules. When at all possible, careful consideration to the frequency of PZP treatment is important to the maintenance of more typical feral horse behavior and physiology. Moreover, and perhaps just as important, the likelihood that mares will return to fertility (and thus, the ability to maintain future management options) may be compromised if females are kept subfertile for extended periods of time.

Finally, it is critical to note that these results are not anomalous. Similar effects have been demonstrated in western populations of feral horses (Ransom et al. 2013), in other free-ranging species including bison (*Bison bison*; Duncan et al. 2017) and white-tailed deer (Miller et al. 2001), and in several captive species including Asian elephants (*Elephas maximus*), African elephants (*Loxodonta africana*), Seba's bats (*Carollia perspicillata*), big cats (*Felidae*), wildebeest (*Connochaetes*), white rhinoceros (*Ceratotherium simum*), and stingrays (*Myliobatoidei*; Songer 1996).

Effects to untreated associates

In social species, the effects of prolonged PZP-induced subfertility are likely to affect the behavior and physiology of their untreated associates. For instance, increased female group changing behavior could result in increased male–male and male–female aggression (Madosky 2011). Such changes to behavior could subsequently impact the endocrinology of untreated associates, including increases in cortisol and/or testosterone levels. Such impacts have been demonstrated in several taxa including primates, birds, and fish (Wikelski et al. 1999, Cavigelli and Pereira 2000, Hirschenhauser and Oliveira 2006).

Preliminary work on Shackleford Banks strongly suggested such patterns (Jones and Nuñez 2017, unpublished data) indicating that when managing social species, the changes we effect in target animals are likely to affect the behavior and physiology of their non-target associates. When at all possible, we should attempt to minimize such changes to target animals.

The studies reviewed here show that mares receiving fewer (1–3) total and/or consecutive treatments demonstrate more typical behavior and physiology than do mares receiving more (≥4) treatments. 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. However, in areas or situations in which the social and physiological functioning of animals is of concern and mare identity and treatment history can be tracked, the application of fewer treatments, including "breaks" during which mares are allowed to reproduce, is recommended. Such treatment will better ensure the health and overall well-being of the population and, depending on managerial goals, more effective, flexible management.

Management implications

Feral horse management via direct population regulation will continue to be a difficult task. With every decision, managers and policymakers must consider the efficacy, feasibility, costs, and benefits of different strategies, the potential effects to feral horse health and welfare, and the impacts of public opinion on management implementation. In the absence of natural predators to regulate feral horse populations, any method we choose will likely necessitate ongoing human intervention. Obtaining balance between the costs and benefits to both feral horse and human populations and the ecology of western rangelands remains challenging. However, results from the studies reviewed here show that with fewer total and/or consecutive treatments, we can maintain more natural behavior and physiology in feral horses.

Mares receiving fewer treatments are less likely to engage in group changing behavior and give birth at times of the year that more closely approximate those of untreated mares. Moreover, these mares are more likely to return to fertility after treatment suspension, maintaining future management options, more natural behavior and physiology, and more typical band stallion behaviors. Finally, given the fact that group changing behavior induces a physiological stress response in mares, the data reviewed here strongly suggest that mares receiving fewer total and/or consecutive treatments will be at lower risk for repeated stressors. Such refinements to contraception scheduling are likely to apply regardless of the methods or vaccines used, as long as it is mare subfertility and not the treatment itself that causes changes to mare behavior and physiology. Lastly, though such methods may not be possible or practical in all areas, in smaller, closed populations, where the identification of individuals and the careful tracking of treatment history is plausible, care should be taken to maintain more typical animal behavior and physiology.

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