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Chapter 4

MANAGEMENT OF WILD HORSES WITH PORCINE ZONA PELLUCIDA: HISTORY, CONSEQUENCES, AND FUTURE STRATEGIES

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ABSTRACT

The advent of immunocontraception with porcine zona pellucida (PZP) has all but revolutionized wild horse management, providing a more humane method of population control than earlier strategies. Early studies on Assateague Island National Seashore have described it as an ideal form of fertility control in that it reduces the chance of conception to below 10%, can be delivered remotely, is reversible (after short-term use), lacks debilitating physiological side effects, cannot pass through the food chain, and shows minimal effects on social behaviors. However, recent research in other populations has revealed behavioral and physiological side effects of long-term PZP use. These results indicate that studies from one population may not necessarily be applicable to another, regardless of similarities in habitat and population structure. Careful study of the animals' demography, physiology, and behavior is necessary prior to and during treatment to ensure that a) the potential effects of PZP can be assessed accurately, and b) within managerial constraints, PZP effects are ameliorated as much as is possible. Here I explain the history of PZP use in wild horses, its side effects on the horses of Shackleford Banks, North Carolina in comparison to other populations, and offer management suggestions based upon wild horse biology and behavioral ecology, which may minimize or prevent these side effects in other populations.

INTRODUCTION

The modern horse (*Equus caballus*) thrived in North America for nearly five million years before its local extinction approximately 11,000 years ago [Weinstock et al. 2005]. In the late 15th century, horses were brought back to our continent by the Spanish and have since roamed free, forming feral herds in the United States and Canada [Weinstock et al. 2005]. Some argue that preservation of these animals is questionable given their recent history of reintroduction. Others maintain that they are an integral part of the United States' heritage and the natural history of our continent. Currently, the latter viewpoint has prevailed and wild horse preservation is assured by various acts in different parts of the country such as the Wild Free-Roaming Horse and Burro Act of 1971 and the Shackleford Banks Wild Horses Protection Act of 1997. The extirpation of their natural predators and modern pressures such as ranching and farming all but necessitate some form of population management throughout the country, however the "patchwork quilt" of policies regarding wild horse preservation means that populations under separate jurisdictions are treated differently.

Managers have few options to control population numbers. Culling is not readily accepted by the public, and "gathers," in which animals are rounded up by helicopter and removed, divide family groups, significantly decreasing herd stability [Kirkpatrick et al. 1997]. In addition, the use of hormonal contraception (in both male and female animals) has proven problematic due to the dosage needed to achieve infertility, the subsequent changes to behavior, and the issue of possible consumption by non-target species [Turner and Kirkpatrick 1991]. A more recent alternative, immunocontraception, induces the production of antibodies against structural or functional molecules important to reproduction. These antibodies impede the normal cell biology of reproduction, reducing the likelihood of conception [Turner and Kirkpatrick 1991]. In the case of porcine zona pellucida (PZP), antibodies bind sperm receptors on the egg's surface, thereby preventing sperm attachment and fertilization [Sacco 1977]. Today, PZP is used to control reproduction in several species of free-ranging ungulates including white-tailed deer (*Odocoileus virginianus*) [McShea et al. 1997], elk (*Cervus elaphus*) [Heilmann et al. 1998] and the wild horse [Kirkpatrick 1990, Turner et al. 2002].

The majority of studies examining the effects of PZP application to wild horses stem from a long-term study on Assateague Island National Seashore, Maryland. Most of these studies have focused on the physiological effects of the vaccine [Kirkpatrick et al. 1992, Kirkpatrick et al. 1997, Turner et al. 2002]. Researchers have 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 have been shown to be reversible, safe for pregnant females, and do not adversely affect the survivorship or subsequent fertility of offspring born to treated individuals [Kirkpatrick and Turner 2002].

However, recent studies on the horses living on Shackleford Banks (a barrier island off the North Carolina coast) and in the western United States, demonstrate differences in recipient behavior and physiology [Nunez et al. 2009, Nunez et al. in review, Ransom et al. 2010]. Shackleford mares receiving PZP change harem groups more often and treated horses in both areas both initiate and receive more reproductive behaviors than do untreated mares. For a gregarious species like the horse, such changes may have serious social and demographic consequences. Here I give an brief overview of wild horse biology, discuss the history of PZP use in wild horses, compare its effects in different populations, and offer management suggestions based upon the species' behavioral ecology that may prevent or ameliorate these effects.

WILD HORSE BIOLOGY AND BEHAVIORAL ECOLOGY

In wild horse societies, the harem is the core social group, consisting of usually one, but sometimes two or three harem male(s), one to several female(s), and their offspring [Feist and McCullough 1976, Rubenstein 1981; 1986, Linklater et al. 2000]. Harem groups are typically stable units, showing very few changes in composition over months or years [Klingel 1975], although this can vary among populations [Tyler 1972, Rutberg 1990]. Female loyalty to the harem male and the males' ability to retain females is paramount to maintaining this stability [Feist and McCullough 1976, Rubenstein 1981, Goodloe et al. 2000]. Decreases in harem stability have been shown to affect several aspects of mare well-being, resulting in lower overall reproductive success [Kaseda et al. 1995], less time for preferred behaviors, decreased body condition and fecundity, elevated parasite levels, and increased offspring mortality [Linklater et al. 1999].

Breeding normally occurs from March through August, with most births occurring in April and May [Crowell-Davis 2007]. Gestation lasts approximately 11-12 months [Asa 2002]. Temperate-zone equids are more strictly seasonal than are tropical-zone species. However, temperate individuals can show significant variability in reproductive timing and estrous behaviors during the fall and winter months which constitute the non-breeding season [Asa 1979, Asa et al. 1980]. Photoperiod is probably the most important cue for reproduction in temperate species [Ginther 1979; 1992], although birth peaks in the tropics suggest that nutritional factors are also important [Grubb 1981, Penzhorn 1988, Churcher 1993]. In all equids, estrus recurs until pregnancy or until the end of the breeding season [Asa 2002].

For the most part, wild horses are non-territorial, with several harems sharing both feeding and water resources [Feist and McCullough 1976, Rubenstein 1981, Cameron et al. 2003]. Given this ecology, changes in the behavior and/or physiology of individuals have the potential to affect interactions with neighboring harems and may, thereby, affect significant change at the population level. As such, understanding the potential effects of PZP on individual behavior and physiology is of broad importance to maintaining a functional population of feral horses.

PZP USE IN WILD HORSES

The management of wild horses across the U.S. is conducted for a variety of reasons. In the west, the Bureau of Land Management manages several populations in order to "establish appropriate management levels [based on] the ecological balance among wild horses and burros and the BLM's multiple use mission" [Bureau of Land Management 2010]. Multiple use includes ranching and, according to ranchers, wild horses outcompete their cattle for forage, and therefore adversely affect their herds. Thousands of horses are gathered, removed, and adopted off to the public annually [Bureau of Land Management 2010]. The fate of adopted animals is not tracked. On Assateague, management by the National Park Service (NPS) began primarily in response to an increase in the population from 21 horses in 1965 to 167 horses in 1975. Although the population remained relatively stable for the next nine years [Kirkpatrick 1995], concern over the animals' potential impact on the island's ecology prompted the NPS to quantify their effects on island vegetation. Horses seemed to have little impact on some plant species [Rodgers 1985], but serious impacts on others [Furbish 1980]. In 1986, research on the potential to manage the population with fertility control was initiated, and in 1994 the contraception program was officially begun. Such studies were not conducted on Shackleford Banks, but a more than doubling of the population over ten years from 100 horses in 1985 to ~220 in 1995 [Rubenstein and Dobson, unpublished report], prompted concern over the horses' potential impact on the island's vegetation, and resulted in their eventual management. The contraception program on Shackleford Banks began in the spring of 2000.

Management organizations have limited options to control wild horse populations. Lethal methods are not accepted by the general public and are currently illegal in several states. Gather and removal, also criticized by the public, is commonly conducted by helicopter, all terrain vehicle, "Judas" horse, and/or some combination of these. Horses are herded, often from great distances, into corrals. In some cases, efforts are made to keep harem groups together, in others the groups are split, male from female, foal from dam. In all cases, horses are confined to a relatively small area (often for several days before they are removed and either sold or adopted off) and are put in direct contact with individuals they would not normally encounter in the wild. For a socially complex species like the horse, the experience is highly stressful. Moreover, the removal of the animals permanently affects the genetic makeup of the remaining herd and increases the reproductive efficiency of the horses that remain [Keiper and Houpt 1984, Kirkpatrick and Turner 1991, Cameron et al. 2003].

In the mid-late 80s, research investigating the use of immunoantifertility achieved contraception by raising antibodies against 1) gonadotropin releasing hormone (GnRH) in both males and females, 2) spermatozoa, and 3) ovarian zona pellucida [Turner and Kirkpatrick 1991]. The latter, which did not affect hormone levels in the target animal, was investigated more intensively and the porcine zona pellucida (PZP) vaccine was developed. Females of several species including mice [Jilek and Pavlok 1975], hamsters [Oikawa and Yanagimachi 1975], and rabbits [Sacco 1977] immunized with PZP showed reduced fertility. Studies with wild horses on Assateague Island National Seashore proved particularly promising. PZP was highly effective, reversible, could be remotely delivered, and had only low-level side effects [Kirkpatrick 1990]. These results were especially well received, given the alternatives to wild horse population management.

EFFECTS OF PZP IN WILD HORSES

Behavior

We know very little about the potential for PZP to affect recipient behavior. Powell's study on Assateague Island National Seashore [1999] represents one of the few studies to systematically address this question. He found that recipient and non-recipient mares did not differ in their activity budgets or their spatial relationships relative to the harem stallion. This is in contrast to what has been found with Shackleford Banks horses. Finer-scale measures of activity budgets show an interaction with age such that younger mares (aged 2-3 years) receiving PZP spent less time grazing and more time standing than did non-recipient mares [Rogers 2001]. Although treated mares are often in better physical condition than untreated mares [Kirkpatrick et al. 1996], decreases in the performance of important maintenance behaviors may present problems for the animals' long-term health [Sadleir 1969, Ransom et al. 2010].

In addition, during the non-breeding season, recipient mares on Shackleford exhibit differences in their spatial relationships within the harem. They spend a greater percentage of time in the center of the group and exhibit closer distances to the harem male, on average, than do non-recipient mares (Generalized Linear Model: mare placement, $P = 0.03$; distance to the harem male, $P = 0.007$, see Figure 1, A and B). This effect is significant even when controlling for mare and harem male age, group size, and the presence/absence of recipient and/or non-recipient mares. Moreover, in harems containing both contracepted and uncontracepted females, males are more likely to have contracepted females as their near neighbors (Generalized Linear Model: $P = 0.05$, see Figure 1, C). Perhaps more striking, is the fact that during both the breeding and non-breeding season, Shackleford mares receiving PZP change harem groups more often and visit more groups than do non-recipients [Nunez et al. 2009, Smith 2010]. Such changes in behavior can adversely affect harem group stability [Feist and McCullough 1976, Rubenstein 1981, Goodloe et al. 2000] and therefore, have the potential to affect the overall health and well-being of all harem group members [Kaseda et al. 1995, Linklater et al. 1999]. The addition of new females (especially strangers) often disturbs resident females [Monard and Duncan 1996], resulting in an overall increase in their aggression [Rutberg 1990, Monard and Duncan 1996]. Additionally, repeated changes to harem composition are likely to preclude the establishment of stable female dominance hierarchies, which are necessary for the maintenance of social cohesion among mares and overall group stability [Berger 1977, Houpt and Wolski 1980, Heitor et al. 2006]. The instability caused by switching females may reduce group cohesion even further by adversely affecting the resident females' relationship with the harem male. Finally, because of their tendency to interact with several different groups, the effects of contracepted mares on harem stability level are potentially widespread throughout the population.

Recipient mares on Shackleford also solicit and receive more instances of reproductive behavior than do non-recipients during the non-breeding season [Nunez et al. 2009]. Similar results regarding reproductive behavior have been found in three discrete populations of wild horses in the western United States, with recipient females receiving 54.5% more reproductive behaviors than non-recipients [Ransom et al. 2010]. Moreover, the factors determining the rate of reproductive behavior expressed toward non-recipients (by the harem male) do not affect the rates expressed toward recipient mares [Ransom et al. 2010]. With non-recipient mares, harem males show an increase in the rate of reproductive behavior toward females aged 9-14 years that are more likely to produce a viable foal. This relationship breaks down with recipient females: the harem males' rate of reproductive behavior is both higher and shows no clear pattern among age cohorts or the likelihood of viable offspring production. Such changes in harem male behavior in response to female treatment indicate the potential pervasiveness of PZP's effects in social species.

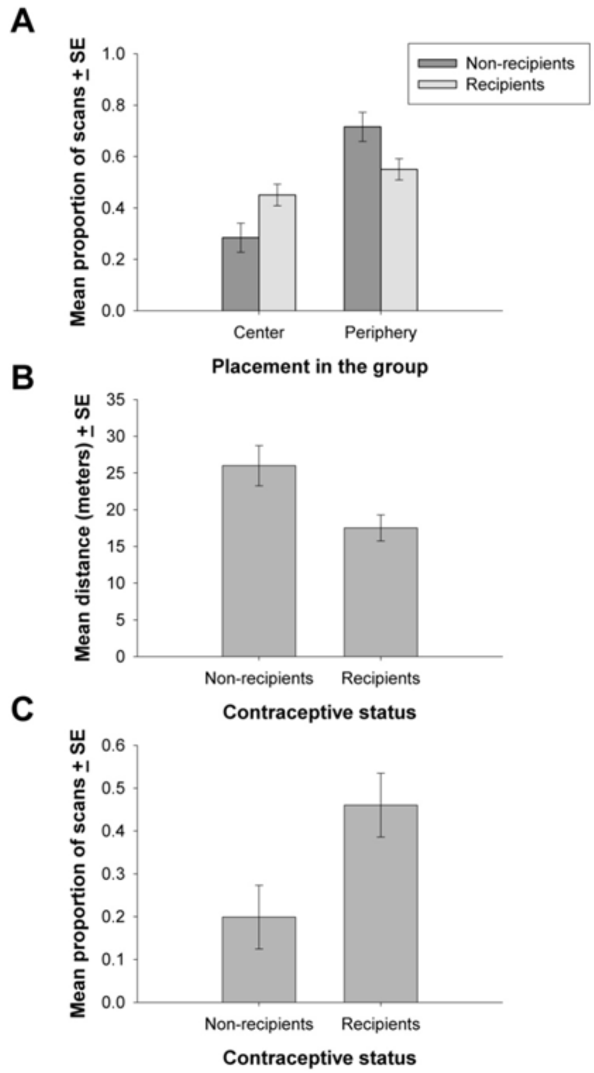

Figure 1. Mares receiving PZP exhibit different spatial relationships with the harem male than do non-recipients. (A) the mean proportion of scans in which mares were located in the center or on the periphery of the group, *P*=0.03; (B) the mean distance between mares and the harem male, *P*=0.007; (C) the mean proportion of scans in which mares were the harem male's nearest neighbor, *P*=0.05 Data were analyzed by Generalized Linear Model. Initial variables in the model included mare and harem male age, group size, and the presence/absence of recipient and/or non-recipient mares. Nonsignificant variables were removed by backwards elimination [see Nunez et al. 2009]

The differences in the results of these studies may be due to a variety of factors including the individual populations' demography and ecology, and the time of study (breeding vs. non-breeding season). Moreover, it should be noted that 18 of the 19 "non-recipients" in the Assateague population had received PZP for the three seasons previous to the study [Powell 1999]. It is not likely that these animals constitute true controls as their behavior may have been affected by the previous contraceptive treatment [Nunez et al. 2009]. The non-recipients on Shackleford Banks and in the three western populations had never received PZP during their lifetime. It is therefore likely that data on these animals more accurately represents the natural behavior of uncontracepted individuals.

In addition, it is likely that the scheduling of PZP administration has contributed strongly to the differences in recipient response. In 1995, after nine years of research, an environmental assessment was prepared for management-level application of PZP to the Assateague herd [Kirkpatrick 1995]. The priorities for treatment followed a hierarchical approach that was based on prior breeding success of the population to ensure that all mares were given an opportunity to reproduce. Females for which there was a high priority for treatment included those that had either a) produced two or more generations of surviving offspring, b) produced more than one surviving offspring, or c) produced one surviving offspring. Low priority females included those that were aged less than four years. Females greater than four years old that had not produced surviving offspring did not receive treatment. In addition, the plan stipulated that only mares that had produced at least three surviving offspring or two generations of offspring would receive more than three consecutive years of treatment. Foals were not to be removed due to the subsequent effects on mare fecundity [Keiper and Houpt 1984, Kirkpatrick and Turner 1991, Cameron et al. 2003]. Finally, it was recognized that this plan was subject to change as the population numbers decreased [Kirkpatrick 1995]. As of 2006, recipient mares were being contracepted upon reaching maturity and were contracepted for three consecutive years. Mares were then allowed to breed normally until one foal was produced, after which time they were contracepted for the rest of their reproductive lives [Zimmerman et al. 2006].

Upon the inception of contraception management on Shackleford Banks in 2000, eight mares were designated as controls based on their age, prior breeding success, and genetic representation in the population. In a given year, ~60% of the foals birthed by these mares were removed [Nunez et al. 2009]. Control mares were not meant to receive the vaccine during their lifetime, but were administered the vaccine in 2009, primarily because of their increased productivity [C. Mason, personal communication], likely due to their offspring's removal [Keiper and Houpt 1984, Kirkpatrick and Turner 1991]. Regardless of their productivity, mares designated as recipients receive contraception upon reaching two years of age and are administered an average of 3.39 $\pm$ 0.17 consecutive years of contraception, after which, any foals born are likely to be removed. This difference in PZP administration may play a key role in the responses of Shackleford mares, as the ability to conceive after dispersal is likely critical, not only to establishing lasting fidelity to a new harem male, but also to normal, physiological functioning [Turner and Kirkpatrick 1991].

Physiology

Prior research has shown little to no effect of PZP on wild horse physiology [Kirkpatrick et al. 1996, Kirkpatrick et al. 1997, Powell and Monfort 2001]. For example, on Assateague, PZP has had no effect on the duration of individual estrous cycles [Powell and Monfort 2001], and as many as seven consecutive years of treatment resulted in no debilitating side effects. In addition, the contraceptive effects of PZP proved reversible after up to four consecutive years of treatment, and even long-term treatment (5-7 years) caused only minor ovulation failure and depressed urinary oestrogen concentrations [Kirkpatrick et al. 1996].

Finally, PZP proved 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, behavioral research on Shackleford Banks horses [Nunez et al. 2009], white-tailed deer [McShea et al. 1997], and free-ranging elk [Heilmann et al. 1998] suggests that PZP may affect the reproductive physiology of recipient animals. In each of these studies, females treated with PZP extended reproductive behaviors into what is typically the non-breeding season. These results are indicative of an extension of ovulatory cycling into the post-breeding season, when most females are normally anovulatory.

Gestation in wild horses lasts approximately 11-12 months [Asa 2002, see above]. It is therefore possible to reliably estimate conception date from foaling date. Since the contraception program began on Shackleford Banks, foaling has occurred later and over a significantly broader range than it had before the program [Nunez et al. in review]. This effect is still significant even when controlling for mare age and physical condition, temperature, rainfall, and the presence/absence of other management practices [Nunez et al. in review, see Figure 2, A]. Although the effect is more prevalent in recipient mares (particularly those receiving several consecutive applications), this change is also exhibited by non-recipients. A similar study conducted on Assateague did not find such differences [Kirkpatrick and Turner 2003, see Figure 2, B]. Management regimes at the two sites differ and may contribute to these divergent patterns (see above). Additionally, the Assateague study did not discriminate between foaling patterns pre- and post- the contraception program, which may have masked some important effects.

By preventing pregnancy without altering hormonal cycling, effective PZP treatment, by default, increases the average number of ovulatory cycles a mare undergoes each breeding season [Powell 1999]. Typically a mare will conceive during the breeding season after which time she ceases to cycle. This is not the case for a successfully contracepted mare; she will continue to cycle throughout the breeding season [Asa 2002]. Therefore, PZP treatment likely extends the reproductive behavior of both treated mares and their harem males throughout the whole of the breeding season [Powell 1999]. Changes in foaling date indicate a more dramatic change in the schedule of ovulatory cycling, whereby recipient mares extend reproductive physiology into what is typically the non-breeding season.

Such changes have the potential to disrupt behaviors important to sufficient nutrient acquisition by all group members and may, therefore, have both social and demographic consequences. In temperate environments, food availability is lowest during the fall and winter months [Sadleir 1969]. During these seasons, free-ranging horses increase the time spent foraging (at the expense of other activities) which likely increases consumption and lowers their overall energetic costs [Duncan 1985, Morel et al. 2006]. Prior to contraception management, Shackleford horses increased inter-individual spacing during this time of year which likely reduced competition [personal observation].

The extension of cycling into the post-breeding season by mares increases male attentiveness [Nunez et al. 2009] which may limit female movement, decrease group spread, and reduce foraging efficiency [Tyler 1972, Feist and McCullough 1976]. In addition, this more active defense of females during the fall/winter months likely requires higher energy expenditure by males during harsh environmental conditions [Stevens 1990] and could lead to a reduction in their body condition [Ransom et al. 2010]. Offspring conceived (and born) during what is normally the post-breeding season may also be subject to decreased resources since low quality forage likely affects mares' ability to produce sufficient milk [Sadleir 1969], but see Kirkpatrick and Turner [2003].

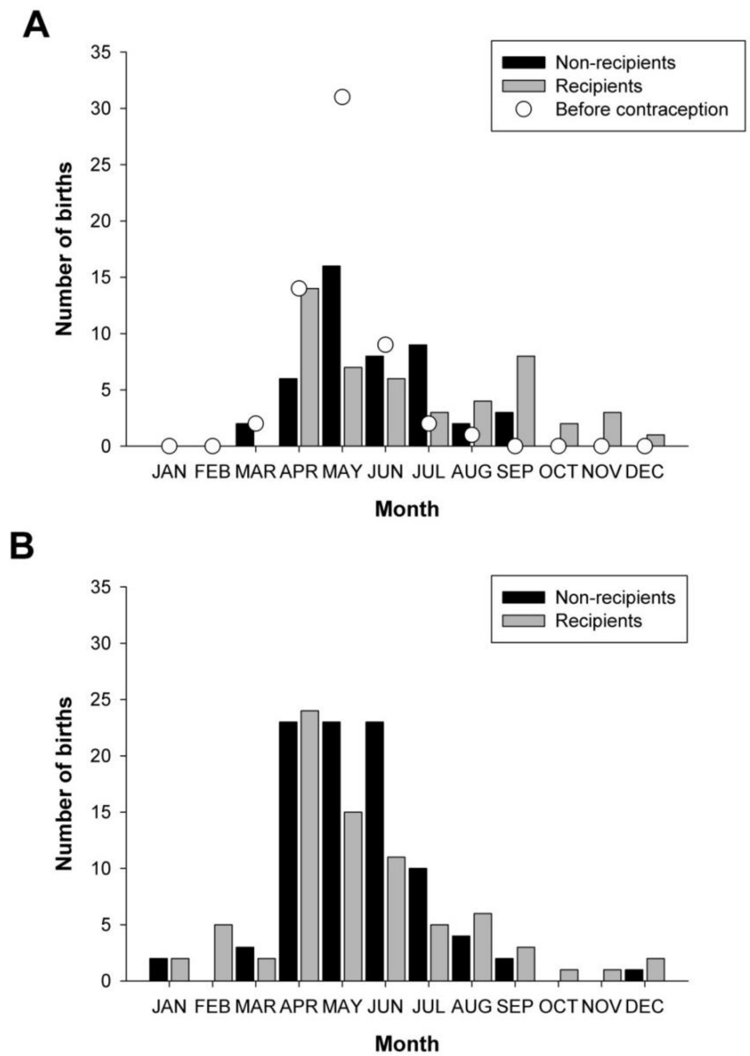

Figure 2. Distribution of foaling dates for recipient and non-recipient mares on (A) Shackleford Banks, NC and (B) Assateague Island National Seashore, MD. Assateague data redrawn from Kirkpatrick and Turner, 2003

The increased reproductive behavior between recipient mares and their stallions may have the additional effect of entraining non-recipients to continue reproductive behaviors and cycling into the non-breeding season. Such entrainment would help explain why even non-recipients have shown shifts in reproductive cycling after PZP management on Shackleford Banks [Nunez et al. in review]. Reproductively active conspecifics are commonly used to induce receptivity in several domestic species including horses [Davies Morel 2003], pigs [Morris 2001], and cows [Hornbuckle et al. 1995]. In the wild, courtship signals from conspecifics advance gonadal cycles or maturation in several taxa, including mammals [Marsden and Bronson 1964, Whitten et al. 1968, McComb 1987], birds [Ball and Balthazart

2002], amphibians [Lea et al. 2001], and reptiles [Lindzey and Crews 1988]. The potential for PZP treatment to thusly affect the reproductive physiology of multiple individuals within a population, regardless of treatment status, is an important consideration.

CONCLUSION

When the alternatives are considered, PZP is currently managers' most humane and effective option for population control. PZP has been used to control the Assategue Island population for 16 years, and recipient mares show no behavioral and only minor physiological responses [Kirkpatrick et al. 1996, Kirkpatrick et al. 1997, Powell 1999, Powell and Monfort 2001, Kirkpatrick and Turner 2002, Turner et al. 2002]. However, horses on Shackleford Banks [Nunez et al. 2009] and in the western United States [Ransom et al. 2010] alter social and reproductive behaviors in response to PZP. These differences indicate 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. Careful study of the animals' physiology, behavior, and demography is necessary prior to and during treatment to ensure that potential effects of PZP can be assessed accurately and that, within managerial constraints, PZP effects are ameliorated as much as is possible.

A thorough understanding of a species' biology and behavioral ecology is imperative in the planning stages of contraception scheduling. Seemingly small differences in the management regime could lead to very different and potentially detrimental outcomes. If feral horse herds are to be maintained in as natural a state as possible, we recommend that sub-adult, dispersing females be allowed to have (and keep) at least one foal before receiving contraception [Kirkpatrick 1995], and that mares should not receive PZP for several consecutive seasons. Such management regimes would of course necessitate a higher minimum population level and may involve additional costs. However, given the expense of removing foals and the efforts required to find safe homes for them, allowing mares to keep their offspring may offset these costs. Moreover, given the potential benefit to the health and well-being of both target and non-target animals, these strategies are worth serious consideration. For a social species like the horse, they could bridge the gap between managing population size and maintaining behaviorally functional populations.

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