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Immunocontraception decreases group fidelity in a feral horse population during the non-breeding season

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ABSTRACT

The behavioral effects of the immunocontraceptive agent porcine zona pellucida (PZP) have not been adequately studied. Important managerial decisions for several species, including the wild horse (*Equus caballus*), have been based on this limited research. We studied 30 horses on Shackleford Banks, North Carolina, USA to determine the effects of PZP contraception on female fidelity to the harem male. We examined two classes of females: contracepts, recipients of the PZP vaccine ($n = 22$); and controls, females that have never received PZP ($n = 8$). We conducted the study during the non-breeding season from December 2005 to February 2006, totaling 102.2 h of observation. Contracepted mares changed groups more often than control mares ($P = 0.04$). Contracepts also visited more harem groups than did control mares ($P = 0.02$) and exhibited more reproductive interest ($P = 0.05$). For both contracepted and control females, the number of group changes ($P = 0.01$) and number of groups visited ($P = 0.003$) decreased with the proportion of years mares were pregnant. Our study shows that the application of PZP has significant consequences for the social behavior of Shackleford Banks horses. In gregarious species such as the horse, PZP application may disrupt social ties among individuals and inhibit normal social functioning at the population level.

1. Introduction

Due to the extirpation of their natural predators, ungulate populations in North America have expanded, necessitating their regulation through culling or contraception management (Eberhardt et al., 1982). Immunocontraceptives are widely used to control reproduction in free-ranging ungulates (Kirkpatrick et al., 1990; Turner et al., 1992). In females, the most common form of immunocontraception, porcine zona pellucida (PZP), stimulates the production of antibodies that bind sperm receptors on the egg's surface, thereby preventing sperm attachment and fertilization (Sacco, 1977). While PZP effectively inhibits conception in several different mammalian species (Kirkpatrick et al., 1996), little is known about its potential effects on recipient behavior. Studies in free-ranging elk (*Cervus elaphus*) and white-tailed deer (*Odocoileus virginianus*) indicate that females receiving PZP extend reproductive behaviors into the post-breeding season (McShea et al., 1997; Heilmann et al., 1998). Authors suggest that in response to repeated unsuccessful mating attempts, females continue cycling in an attempt to gain additional reproductive opportunities. Such changes in behavior can have serious consequences for social species, particularly for those that are polygynous with males defending and retaining several females.

Several studies have examined the effects of PZP application to wild horses (*Equus caballus*). These studies have focused primarily on the physiological effects (both

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reproductive and otherwise) of the vaccine (Kirkpatrick et al., 1992, 1997; Turner and Kirkpatrick, 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).

Researchers claim that the contraceptive has no effect on recipient behavior (Kirkpatrick et al., 1996, 1997; Powell and Monfort, 2001), but present no quantitative data to support their conclusions. In fact, no systematic study has specifically addressed this issue with true controls, animals that have never received PZP during their lifetime (Powell, 1999). Additionally, no study on wild horses has focused on the post-breeding period, when the effects of PZP appear most pronounced in other ungulates (McShea et al., 1997; Heilmann et al., 1998).

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). Female loyalty to the harem male and the male's 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).

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, decreases in the stability of individual harems have the potential to affect the interactions and social relationships of neighboring harems and thereby, may affect significant change at the population level. As such, understanding the potential effects of PZP on individual behavior is of broad importance to maintaining a functional population of feral horses.

In this study we investigate the behavioral effects of PZP on the horses of Shackleford Banks, North Carolina, USA during the non-breeding season. Specifically, we examine female propensity to switch harems, the number of harem groups visited, and the occurrence of reproductive behavior. Since the first application of the contraceptive in January 2000, a reduction in the fidelity of resident mares to their harem males has been noted, albeit anecdotally (C. Mason, personal observation). Based on this information, we hypothesized that contracepted females would change groups more often, would visit more groups, and would exhibit reproductive behaviors more often than would control mares (those never having received the vaccine).

2. Materials and methods

2.1. Study area

Shackleford Banks is a barrier island approximately 3 km off the coast of North Carolina, USA located at 34°40′04.94″N and 76°35′39.39″W. The island stretches 15 km in length, and varies between 0.5 and 3 km in width. The specific study area extended approximately 7 km and was located in the center of the island. This site contained all study animals.

Daylight hours, measured from sunrise to sunset times, ranged from 9 h and 53 min at the beginning of the study on 10 December 2005 to 10 h and 35 min at the conclusion of the study on 3 February 2006 (U.S. Naval Observatory Data Services, 2008). In Beaufort, NC, 7.8 km from the study site, average daily temperatures $\pm$ 1 S.E. for the past 20 years for December, January, and February were 7.93 $\pm$ 0.40 °C, 7.08 $\pm$ 0.28 °C, and 7.95 $\pm$ 0.26 °C, respectively. During the present study average daily temperatures $\pm$ 1 S.E. in Beaufort, NC for December, January, and February were 6.86 $\pm$ 1.65 °C, 7.19 $\pm$ 0.48 °C, and 7.26 $\pm$ 0.30 °C, respectively (National Climate Data Center, 2008).

2.2. Study subjects

The reproductive units of Shackleford horses are typical of feral equids, consisting of coherent harem groups of one or, sometimes two or three stallion(s) with one to several mare(s) and their offspring (Rubenstein, 1981). While multi-male harems are more common in some populations (Linklater and Cameron, 2000), they occur less frequently on Shackleford Banks, accounting for only 19% of all harems on the island at the time of this study. For the most part, these social units are not territorial, and the animals move freely within their overlapping home ranges.

Normally, harem groups are long lasting with most changes involving the dispersal of immature individuals (both male and female). Harem males will sometimes fight to acquire mares from other groups, but stallions almost always retain their mares (Feist and McCullough, 1976; Rubenstein, 1981).

The application of PZP for the purposes of immunocontraception was begun by the National Park Service in January 2000. At that time, eight control mares were identified; one from each of the distinct genetic lineages on the island. These mares would not receive the vaccine at any point during their lifetime. Females younger than 2 years of age were not considered for control status. These procedures determined the current age distribution of control and contracepted animals on Shackleford Banks. The authors of this study were not involved in establishing the number of control and/or contracepted animals.

We observed 30 females that organized themselves into 13 harem groups. Twenty-two mares were treated with PZP at least once between January 2000 and January 2005; the remaining control animals had never been treated. Six of the harem groups investigated contained contracepted females only; two groups contained control females only; the remaining five groups contained both contracepted and control females (see Table 1). All harems considered in this study contained only one harem male. At the time of the study, five of the control mares were pregnant; three of which were nursing foals. An additional control mare nursed a foal, but was not pregnant. Three contracepted mares were pregnant. Two of these females had not received PZP treatment the previous spring; the remaining mare's pregnancy suggests a failure of the treatment. Two other contracepted mares nursed foals; these mares had not received treatment the previous spring. The inoculation, pregnancy, and foaling records for all study animals are shown in Tables 2 and 3.

2.3. PZP contraception

The National Park Service administers PZP from late February through April each year. Mares are first treated at 2 years of age. Each injection contains 100 μg of PZP plus an adjuvant (mixed at the darting site). Initial doses contain Freund's Complete Adjuvant, Modified, *Mycobacterium butyricum* (Calbiochem, Gibbstown, NJ, USA, #344289). All subsequent doses contain Freund's Incomplete Adjuvant (Sigma, St. Louis, MO, USA, #F5506).

For the animals in this study, PZP deterred pregnancy in 97% of cases when administered during the same year. This efficacy dropped to 76% in

Table 1
Initial harem configurations of focal animals.

Harem male	Group type	Control females	Contracepted females	Focal females
Adam	Control	1	0	1
Edge	Control	1	0	1
Axl*	Contracept	0	2	1
Duchovny	Contracept	0	1	1
Pacino	Contracept	0	6	6
Phinius*	Contracept	0	4	1
Teddy*	Contracept	0	5	1
Toro*	Contracept	0	4	4
Clint	Mixed	1	1	2
Dionysis	Mixed	1	1	2
Satellite	Mixed	1	2	3
Stobbs	Mixed	1	2	3
Winston	Mixed	2	2	4

Four of the harems listed reside outside of the study area and were not observed as focal groups (*). However, at least one female from each of these harems moved into focal groups during our study. The focal females column reflects only the number of individuals from each harem that were observed systematically during the study. Other columns show the initial group composition of each harem at the study's outset.

the second year after PZP inoculation. These values are similar to those published for Assateague horses, 94% and 86%, in the first and second years, respectively (Turner et al., 2007).

While we were unable to obtain blood samples for mares during this study, anti-PZP antibody titers in domestic mares remain above control levels for up to 40 weeks post-injection when using similar doses and adjuvant mixtures (Willis, 1994). The National Park Service routinely inoculates mares from February through April. Therefore, in animals inoculated in 2005, anti-PZP antibody levels would have been high during the breeding season, but were likely approaching control levels at the time of this study.

2.4. Pregnancy testing

Fecal samples are collected by the National Park Service in January of each year. All pregnancy testing is completed by enzymeimmunoassay of fecal material at the Science and Conservation Center at ZooMontana in Billings, MT, USA. Using the methods of Kirkpatrick et al. (1991), water extracts of fecal samples are assayed for estrone conjugates and nonspecific progesterone metabolites. Foaling records from the summers following testing were used to supplement assay results.

2.5. Behavioral and demographic sampling

The study was conducted by one observer (C.M.V. Nuñez) during the non-breeding season from December 2005 to February 2006, totaling 102.2 h of observation. Horses were identified individually by color, sex, age, physical condition, and other distinguishing markings including freeze brands. Ages are known from long-term records for the identified horses of Shackleford Banks (Nuñez, 2000).

We located each harem and noted its composition an average of four times each week. We recorded its GPS location and composition, paying particular attention to the presence or absence of females. These data allowed us to assess female willingness (or ability) to remain with their harems. The following measures were analyzed:

- Number of changes that females made, i.e. how many times females switched groups during the study.
- Number of different groups that females visited, i.e. the total number of groups in whom a female was seen during the study.
- The age of the harem male with whom a female was most often associated.
- The size of the group in which the female was most often found.

All incidences of reproductive interest (including copulation, mounting, genital sniffing, and rump rubbing) directed to and initiated by mares were recorded *ad libitum* during scan sampling (Altmann, 1974). Behaviors of reproductive interest were defined as follows:

- Mounting—male places forelimbs around a female's flank; does not include insertion of the penis.
- Copulation—male mounts female; insertion of penis achieved.
- Genital sniffing—animal (male or female) actively places the snout to the genitals of another animal of the opposite sex.
- Rump rubbing—the initiator (male or female) places the chin and/or neck on the rump of a recipient of the opposite sex; initiator rubs its neck back and forth horizontally over recipient's rump.

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2.6. Statistical analyses

We analyzed the effect of contraception on the number of group changes, the number of different groups females visited, and the occurrence of reproductive interest (either received or initiated by mares) using generalized linear models in R (version 2.7.1). All variables were poisson distributed and were analyzed using models with a quasipoisson error distribution and a log link function. All models were weighted by the number of times a mare was observed.

Many factors in addition to PZP treatment may affect the number of group changes, the number of groups visited, and the occurrence of reproductive interest. Such factors include mare and harem male age, group size, pregnancy status, the presence of a foal, and the percentage of females contracepted in each group (Feist and McCullough, 1976; Rutberg and Greenberg, 1990; Linklater et al., 2000). We included mare age, PZP treatment, and their interaction in the initial, maximal generalized linear models discussed above. As PZP treatment was correlated with harem male age, group size, pregnancy status, the presence of a foal, and the percentage of contracepted mares in a group, these latter terms were not included in our models to avoid multicolinearity. Non-significant terms were removed from the

Table 2
Pregnancy and foaling histories for control mares.

Mare	2000	2001		2002		2003		2004		2005	
	Pr	F	Pr	F	Pr	F	Pr	F	Pr	F	Pr
Biff	+	**	+	+	+	**	+	+	+	+	+
Carrot	+	+	—	—	+	+	+	+	+	+	+
Damigo	+	+	+	+	—	—	+	**	+	+	+
Hercules	+	**	+	+	—	—	+	+	—	—	—
Julie	+	**	+	**	+	+	+	**	—	—	+
Kelty	+	+	—	—	+	+	—	—	+	+	—
Laurie	+	+	—	—	+	**	+	**	+	**	—
Wallace	—	—	+	**	+	+	—	—	+	**	+

Column headings: Pr, pregnant during post-breeding season (fall) of the listed year; F, foal present (was conceived in prior year). "+" indicates the presence of a foal or that the animal was pregnant; "—" indicates the absence of a foal or that the animal was not pregnant; "**" indicates that an animal foaled, but the offspring died before reaching 1 year of age.

Table 3
Inoculation, pregnancy, and foaling histories for contracepted mares.

	2000		2001			2002			2003			2004			2005-6		
Mare	**PZP**	**Pr**	**F**	**PZP**	**Pr**	**F**	**PZP**	**Pr**	**F**	**PZP**	**Pr**	**F**	**PZP**	**Pr**	**F**	**PZP**	**Pr**
Alexa	+	—	—	—	—	—	—	—	—	+	—	—	+	—	—	+	—
Bo	—	+	+	—	+	+	—	—	—	+	—	—	—	—	—	+	—
C'susha	n/a	n/a	n/a	n/a	n/a	n/a	n/a	—	—	+	—	—	+	—	—	+	—
Darcy	—	+	+	—	+	+	+	—	—	+	—	—	+	—	—	+	—
Doobie	+	—	—	—	+	+	+	—	—	+	—	—	+	—	—	+	—
Dotu	—	—	—	+	—	—	+	—	—	+	—	—	—	—	—	+	—
Dumé	—	—	—	—	+	+	—	+	+	+	—	—	+	—	—	—	—
Dusty	—	—	—	+	—	—	+	—	—	+	—	—	—	+	+	+	—
Hardee	+	—	—	—	—	—	+	—	—	+	+	**	—	+	+	—	—
Helena	+	—	—	—	—	—	—	—	—	+	—	—	+	—	—	+	—
Jaqui	—	—	—	+	—	—	—	—	—	+	—	—	+	—	—	+	—
Juniper	+	—	—	—	—	—	+	—	—	+	—	—	+	—	—	—	—
Larissa	n/a	n/a	n/a	n/a	n/a	n/a	n/a	—	—	+	—	—	+	—	—	+	—
Noir	—	—	—	—	—	—	—	+	+	—	—	—	—	+	**	+	—
Paula	—	+	+	—	+	+	—	+	+	+	—	—	+	—	—	+	—
Serenac	—	—	—	+	—	—	—	+	+	+	—	—	+	—	—	+	—
Shag	+	—	—	—	—	—	+	—	—	+	—	—	—	—	—	—	+
Slug	—	+	+	—	—	—	+	—	—	+	—	—	+	—	—	+	+
Sydney	—	+	+	—	—	—	+	—	—	+	—	—	—	—	—	—	+
Tatya	n/a	n/a	n/a	n/a	n/a	n/a	n/a	—	—	+	—	—	+	—	—	+	—
Wire	—	+	**	—	+	+	—	+	+	+	—	—	+	—	—	+	—
Zelda	—	+	**	—	+	+	—	+	+	+	—	—	+	—	—	—	—

Column headings: F, foal present (was conceived in prior year); PZP, contraception during the pre-breeding season (spring) of the listed year; Pr, pregnant during post-breeding season (fall) of the listed year. "+" indicates the presence of a foal, that the animal was pregnant, and/or that the animal was inoculated with PZP; "—" indicates the absence of a foal, that the animal was not pregnant, and/or that an animal was not inoculated with PZP; "**" indicates that an animal foaled, but that the offspring died before reaching 1 year of age; "n/a" indicates that an animal was 0–2 years old and not eligible for contraception. PZP administration began in January 2000; foals present that year are not indicative of PZP efficacy and are not included. The status for the animals during the study period is highlighted.

models by backwards elimination. As sample sizes were limited, terms were retained if their P-value was less than 0.10.

To address whether harem male age, group size, pregnancy status, the presence of a foal, and the percentage of contracepted mares in a group had a significant influence on mare behavior, we analyzed them separately for control and PZP groups using Spearman rank correlations against the following variables: number of group changes, groups visited, and occurrences of reproductive interest (see Table 4).

Table 4
Spearman's rank correlations between response variables and predictor variables that correlated with contraceptive treatment.

Predictor variable	Response variable		
	Total changes among groups	Number of groups visited	Instances of reproductive behavior
Male age	*Controls*: $\rho = -0.58$, $P = 0.13$ *Contracepts*: $\rho = -0.16$, $P = 0.47$	*Controls*: $\rho = -0.58$, $P = 0.13$ *Contracepts*: $\rho = -0.18$, $P = 0.43$	*Controls*: $\rho = 0.08$, $P = 0.85$ *Contracepts*: $\rho = -0.03$, $P = 0.88$
Group size	*Controls*: $\rho = -0.44$, $P = 0.28$ *Contracepts*: $\rho = -0.28$, $P = 0.22$	*Controls*: $\rho = -0.44$, $P = 0.28$ *Contracepts*: $\rho = -0.22$, $P = 0.35$	*Controls*: $\rho = 0.01.$, $P = 0.99$ *Contracepts*: $\rho = 0.02$, $P = 0.95$
Percentage of group members contracepted	*Controls*: $\rho = 0.18$, $P = 0.68$ *Contracepts*: $\rho = 0.11$, $P = 0.67$	*Controls*: $\rho = 0.18$, $P = 0.68$ *Contracepts*: $\rho = 0.09$, $P = 0.71$	*Controls*: $\rho = 0.62$, $P = 0.10$ *Contracepts*: $\rho = 0.13$, $P = 0.59$
Pregnant or with foal during study	*Controls*: $\rho = -0.66$, $P = 0.08$ *Contracepts*: $\rho = -0.41$, $P = 0.06$	*Controls*: $\rho = -0.66$, $P = 0.08$ *Contracepts*: $\rho = -0.41$, $P = 0.06$	*Controls*: $\rho = -0.66$, $P = 0.08$ *Contracepts*: $\rho = -0.12$, $P = 0.58$

Each correlation was performed separately for control ($n = 8$) and contracepted ($n = 22$) groups.

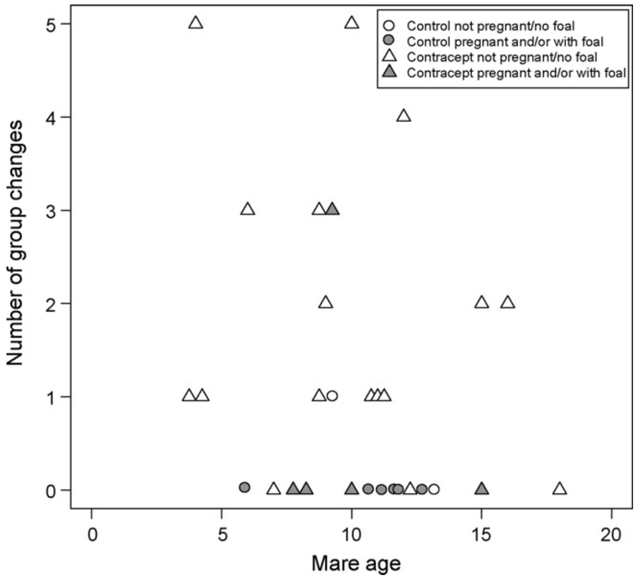

Fig. 1. Number of group changes during the study period by mare age for control ($n = 8$) and contracepted mares ($n = 22$). Even when controlling for the effect of age, contracepted mares change groups more often than do controls. Filled symbols represent mares that were either pregnant or nursing a foal at the time of the study. In the events of ties, mare age has been jittered by 0.2 years to allow clear visualization of every individual.

3. Results

3.1. Number of group changes

A generalized linear model shows that PZP treated mares changed groups significantly more often than did controls, even when accounting for mare age (analysis of deviance, overall GLM: $F_{2,27} = 6.73$, $P = 0.004$; PZP treatment: estimate = 1.99, $t = 2.11$, $P = 0.04$; mare age: estimate = −0.13, $t = -1.92$, $P = 0.07$, see Fig. 1). Pregnancy and/or the presence of a foal seemed to have a marginal effect (see Section 3.3). Spearman rank correlations within treatment groups show that harem male age, group size, and the percentage of contracepted mares in the group had no effect on the number of group changes (see Table 4), suggesting that their influence was not substantial.

3.2. Number of groups visited

A separate generalized linear model shows that contracepted females visited significantly more groups than did control mares, again controlling for mare age (analysis of deviance, overall model: $F_{2,27} = 6.83$, $P = 0.004$; PZP treatment: estimate = 0.49, $t = 2.42$, $P = 0.02$; mare age: estimate = −0.06, $t = -2.39$, $P = 0.02$, see Fig. 2). As above, pregnancy and/or the presence of a foal seemed to have a marginal effect (see below). Spearman rank correlations within treatment groups show that harem male age, group size, and the percentage of contracepted mares in the group had no effect on the number of males consorted with (see Table 4).

3.3. Pregnancy and foal presence

Both control and contracepted mares that were pregnant and/or had foals tended to change groups less often and visit fewer groups than did other mares (Spearman rank correlation: controls, $\rho = -0.66$, $P = 0.08$; contracepts, $\rho = -0.41$, $P = 0.06$; also see Table 4). Given this trend, we investigated whether a mare's history of pregnancy or foaling (over multiple years) affected behavior. For each female, we calculated the proportion of years pregnant and the proportion of years with a foal from January 2000 to January 2005, considering only those years in which the mare was sexually mature. A generalized linear model shows that mares pregnant for a greater proportion of years changed groups less often (overall model: $F_{1,28} = 10.75$, $P = 0.003$; % years pregnant: estimate = −3.11, $t = -2.79$, $P = 0.01$, see Fig. 3A) and visited fewer groups (overall model: $F_{1,28} = 11.77$, $P = 0.002$; % years pregnant: estimate = −1.03, $t = -3.31$, $P = 0.003$, see Fig. 3B). Mare age did not contribute significant explanatory power to these models and was thus removed. The proportion of years that mares had foals from 2000 to 2005 did not affect mare behavior (Group changes, overall model: $F_{2,27} = 2.64$, $P = 0.09$; % years with foal: estimate = −1.96, $t = -1.16$, $P = 0.25$. Groups visited, overall model: $F_{2,27} = 4.63$, $P = 0.04$; % years with foal: estimate = −1.39, $t = -1.44$, $P = 0.16$).

3.4. Reproductive interest

Contracepted mares received and exhibited more reproductive interest (see Section 2.5) than did control mares (analysis of deviance, overall GLM: $F_{2,27} = 6.46$, $P = 0.005$; PZP treatment: estimate = 2.04, $t = 2.03$, $P = 0.05$; mare age: estimate = −0.13, $t = -1.91$, $P = 0.07$, see Fig. 4). Spearman rank correlations within treatment groups show that harem male age, group size, the presence of a foal, and the percentage of contracepted mares in the group had no effect on the occurrence of reproductive

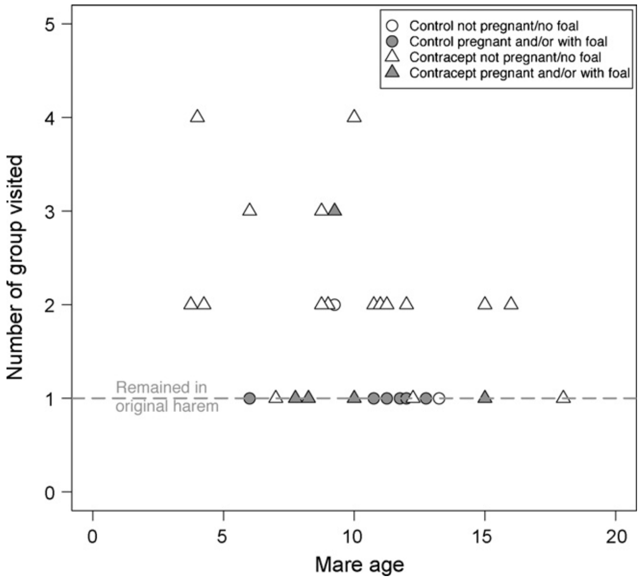

Fig. 2. Number of different groups visited during the study period by mare age for control (n = 8) and contracepted mares (n = 22). Even when controlling for the effect of age, contracepted mares visit more groups than do controls. Individuals on the dotted line did not change groups during the study. Filled symbols represent mares that were either pregnant or nursing a foal at the time of the study. In the events of ties, mare age has been jittered by 0.2 years to allow clear visualization of every individual.

interest (see Table 4). Pregnancy may have had a marginal effect on the reproductive interest received by control mares (Spearman rank correlation: $\rho = -0.66$, $P = 0.08$). This result is not conclusive however, since only one non-pregnant control mare received any reproductive interest. Pregnancy had no effect on the reproductive interest received or initiated by contracepted mares (Spearman rank correlation: $\rho = -0.12$, $P = 0.58$).

4. Discussion

According to past research, contraception with PZP has little to no effect on the behavior of wild horses (Kirkpatrick et al., 1996, 1997; Powell and Monfort, 2001). The results of this study refute that assertion. Much of the aforementioned research has been based on a single island population, all studies have been conducted

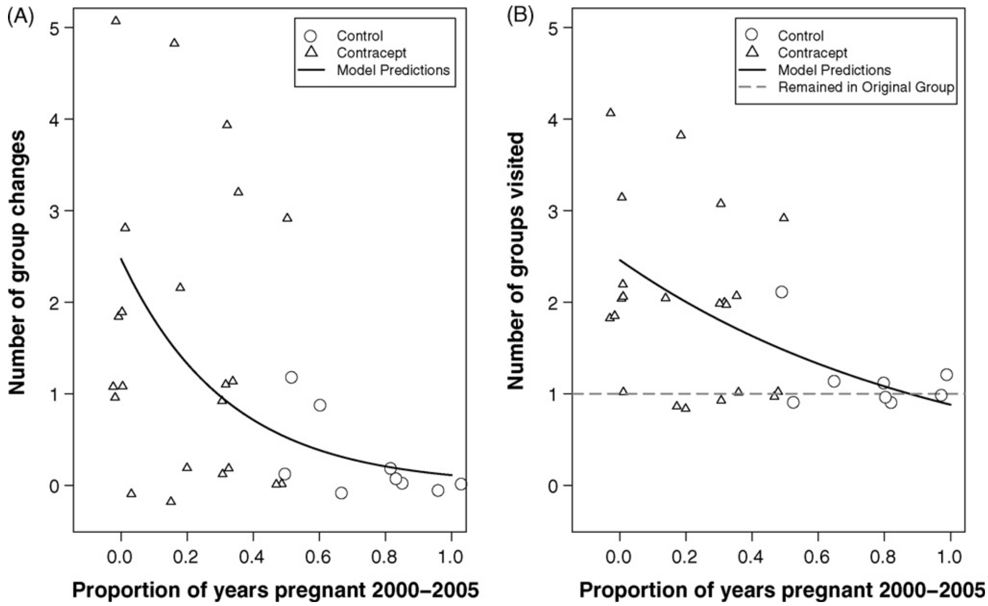

Fig. 3. Number of group changes (A) and groups visited (B) by the proportion of years pregnant from January 2000 to January 2005. Lines show that according to the generalized linear model of the data (see Section 3.3), the number of group changes and groups visited decrease with the proportion of years mares are pregnant. Points have been jittered to allow clear visualization of every individual.

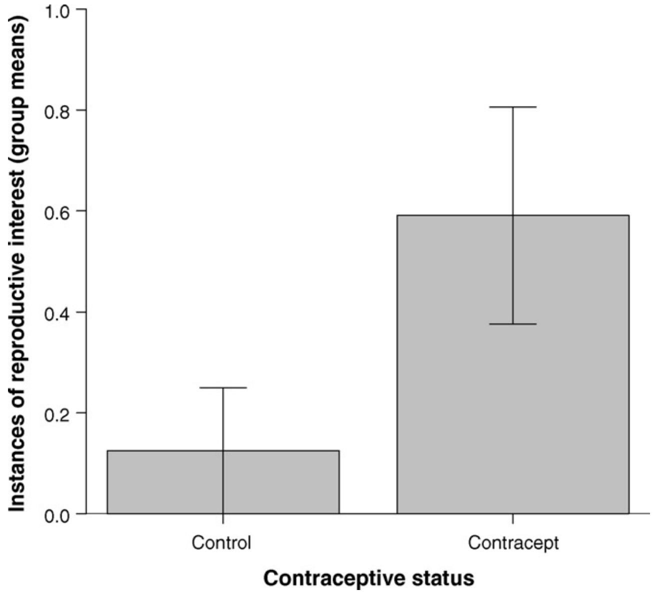

Fig. 4. Instances of reproductive interest during the study period by PZP treatment (means ± S.E.). Contracepted mares exhibit and receive more reproductive interest than do control mares.

solely during the breeding season, and no study has had adequate controls against which to compare PZP-treated females (Kirkpatrick et al., 1997; Powell, 1999). Here, we studied horses during the non-breeding season on Shackleford Banks, North Carolina, making use of animals that had never received contraception as controls. In our study, PZP treatment increased the number of group changes, the number of different groups visited, and the occurrence of reproductive interest, both received and initiated by females. In addition, our results show that even 10 months after PZP inoculation, when anti-PZP antibody titers are likely low, the indirect behavioral effects on recipient animals remain strong. The potential implications of these results for feral horse management are of substantial importance and need to be investigated further.

4.1. Fidelity to the harem male and reproductive interest

Contracepted mares are more likely to switch harem groups and visit more groups than are control mares. Decreases in mare fidelity to the harem male have debilitating consequences for harem stability (see Section 1). Resident females are often disturbed by the addition of new mares, especially if they are strangers (Monard and Duncan, 1996; Parker, 2001), and will become increasingly aggressive in their presence (Rutberg, 1990; Monard and Duncan, 1996). In addition, frequent changes to a harem's composition are likely to prohibit the establishment of a stable female dominance hierarchy, which is paramount to maintaining social cohesion among mares and overall group stability (Berger, 1977; Houpt and Wolski, 1980; Heitor et al., 2006). Moreover, the instability caused by these switching females may adversely affect the resident females' relationship with the harem male, reducing group cohesion even further. Because contracepted females do not simply switch repeatedly between two well-known groups, but rather interact with several different groups, these detrimental effects of harem instability may be felt throughout the entire population.

Contracepted mares both receive and initiate more instances of reproductive interest than do control mares. Reproductive behavior is energetically costly (Galimberti et al., 2000). Repeated bouts of male harassment have been shown to reduce total time foraging in equid species (Rubenstein, 1986; Sundaresan et al., 2007). The relative cost of such behaviors may be especially high during the post-breeding season when resources are scarce (Stevens, 1990). In addition, the costs of this behavior may outweigh the potential benefits, i.e. increased reproductive success. Gestation in wild horses lasts approximately 11–12 months (Asa, 2002). Offspring conceived during the winter months are therefore subject to higher mortality due to the cold temperatures and poor quality forage available at birth.

The differences we observed in harem fidelity and reproductive behavior may result from prolonged estrous cycling into the post-breeding season in response to repeated failures to conceive. This hypothesis has been proposed to explain reproductive behavior during the post-breeding season in both PZP-treated elk (Heilmann et al., 1998) and white-tailed deer (McShea et al., 1997). In equids, reproductive behaviors including copulation, mounting, clitoral winking, and tail raising occur most frequently during estrous when the mare is nearing ovulation (Asa et al., 1979). Additionally, Asa et al. (1979) have shown that mare approaches to, and follows of the harem male are excellent predictors of the transition between estrous and diestrous. We propose that group changes may reflect a similar pattern, with PZP-treated mares approaching non-harem males more frequently during prolonged estrous cycling.

Extended periods of estrous, while relatively rare, have been documented in equids. Tropical species, for example, are less strictly seasonal, with some reproducing throughout the year (Grubb, 1981; Churcher, 1993). In addition, substantial variability in the cycling schedules and receptivity of individual mares (Asa et al., 1979), and the performance of estrous behavior and copulatory activities during the non-breeding season (Asa et al., 1980) have been documented in temperate species. Since the implementation of contraception, at least one winter birth, and therefore winter copulation, has occurred on Shackleford Banks (Susan Stuska, National Park Service, Cape Lookout National Seashore, personal communication). These variations in receptivity, ovulatory schedules, and foaling suggest that the seasonality of reproductive behaviors in *E. caballus* females has the potential to be quite plastic. As contracepted animals have experienced a significant alteration to their physiological state, extended cycling is even more feasible. Future work on Shackleford will test for additional estrous periods in PZP-treated mares by assaying total estrogens and progestins in fecal samples (Asa et al., 2001) during the fall and winter months.

Alternatively, mares may perceive the failure to conceive as a problem with the harem male. This perception alone may be sufficient to cause the observed differences in behavior, regardless of differential estrous cycling. Mares that did not conceive during the prior summer may then switch groups more often during the winter in an effort to prepare for the upcoming breeding season. Such group changes are likely to be less costly in the post-breeding season, as the spacing between band members increases and male herding and aggression decline during this period (Stevens, 1990). This seasonal decrease in harem male attentiveness may have contributed to the observed numbers of group changes and groups visited during this study. Given the strong relationship between contraceptive status and mare fidelity, however, it is unlikely that season is the sole cause of mare behavior. Still, additional study during the breeding season (April–August) is recommended to assess whether the changes in mare behavior result from an interaction between season and contraceptive status.

4.2. The effects of pregnancy

Mare movement between groups is normally rare (see Sections 1 and 2.2). The results of this study strongly suggest that pregnancy and, possibly lactation, are important components to that stability. Regardless of contraception status, pregnant and/or nursing females tend to change groups less often, and over time, mares with a greater proportion of years pregnant are less likely to change groups. These decreases in pregnancy (and possibly lactation) may be the mechanism by which PZP treatment increases the propensity to change groups.

Additionally, decreased pregnancy and increased group switching have the potential to feedback on each other, resulting in even lower overall stability. Increased group switching has the potential to decrease mare fertility via male harassment. The more moves females make, the more male harassment they tend to receive (Rubenstein and Nuñez, 2008). Such harassment can lower female reproductive success, as measured by the number of offspring surviving to independence (Rubenstein, 1986; Rubenstein and Nuñez, 2008). As evidenced by our results, such decreases in pregnancy increase the likelihood that females will change groups. These cascading effects have the potential to adversely affect entire populations (see Section 1) and are worth serious consideration when making management decisions.

4.3. Management implications

If feral horse populations are to be maintained in the most natural state possible, we suggest that a small population of mares never be inoculated with PZP. Although the control mares' effects on group structure on Shackleford Banks have yet to be fully determined, the results clearly demonstrate that they are more faithful to their harem males than are contracepted mares. Mare fidelity to the harem male is important to overall harem stability. As such, it is likely, especially when one considers the sociality of these animals, that control females afford a stabilizing influence not only to individual harems, but also to the entire herd.

We also suggest that the subset of animals designated for control status be more fully representative of female demography. For example, at the time of this study, all control mares on Shackleford were between 8 and 15 years of age (see Figs. 1 and 2). This distribution does not currently afford for the behavior of very young or very old animals. Time can always provide for older individuals, but younger controls are needed to approximate the female population's age structure and natural behavior. For instance, contracepted, dispersing, subadult females likely move between more harems than they would naturally. Therefore, this demographic may adversely affect the entire herd's stability level. Allowing some portion of these animals to disperse, join harems, and reproduce normally could help to stabilize population behavior and structure.

Reevaluating the scheduling of PZP administration may also prove beneficial. An inoculation schedule that allows mares to conceive and give birth may help to ameliorate the most deleterious behavioral effects of PZP. Inoculating females every second and even every third year significantly reduces pregnancy in Shackleford Banks horses (see Section 2.3) and other wild populations (Turner et al., 2007). Contraception on such schedules will keep pregnancy rates low, but will allow for the birth of a manageable number of individuals which, according to this study, have a stabilizing influence on female behavior. Additional research is needed to determine if such contraception schedules will limit population size effectively. If so, this could provide a cost-effective means of controlling animal numbers while maintaining their natural behavior.

The broader management implications of this research are substantial. PZP has been reported to have little to no effect on the behavior of wild horses, specifically, but also wild ungulates in general (Kirkpatrick et al., 1996, 1997; Powell, 1999). The results of this study refute those claims, and in fact, highlight the pitfalls of generalizing recipient and group responses to PZP from one population to another. Moreover, these data emphasize the necessity of study during all stages of the animals' reproductive cycle to determine the effects of contraception on social behavior. Managers of feral horse and other ungulate populations must use caution in basing contraceptive decisions upon data collected only during the breeding season and from a few, separate populations. Regardless of the ecological and sociological similarities between sites, subtle differences in factors such as demography, ready access to resources, and, as this paper suggests, seasonality, may prove important. Among different populations, such factors may shape the physiological and behavioral effects of PZP in unique and potentially unpredictable ways. Finally the trade-offs between managing population size and maintaining animal health and well-being are worth serious consideration. For social species such as the horse, such consideration is crucial if managers are to maintain behaviorally functional populations.

5. Conclusion

In this study, mares contracepted with PZP behaved differently from control mares. They changed groups more often, visited more groups, and both exhibited and initiated more reproductive interest. These differences in behavior have the potential to adversely affect the stability not only of individual harems, but the entire population on Shackleford Banks, North Carolina. Additional study into the mechanism behind these behavioral differences and into the scheduling of PZP administration will help ameliorate these effects.

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