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

Cassandra M.V. Nuñez [a,*], James S. Adelman [a], Carolyn Mason [b], Daniel I. Rubenstein [a]

[a] 106A Guyot Hall, Ecology and Evolutionary Biology Department, Princeton University, Princeton, NJ 08544-1003, USA
[b] Foundation for Shackleford Horses, Inc., 306 Golden Farm Road, Beaufort, NC 28516, USA

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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

* Corresponding author. Tel.: +1 609 258 3836; fax: +1 609 258 1334.
E-mail addresses: cmvnunez@princeton.edu, cmvnunez@gmail.com (Cassandra M.V. Nuñez).

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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 ± 1 S.E. for the past 20 years for December, January, and February were 7.93 ± 0.40 °C, 7.08 ± 0.28 °C, and 7.95 ± 0.26 °C, respectively. During the present study average daily temperatures ± 1 S.E. in Beaufort, NC for December, January, and February were 6.86 ± 1.65 °C, 7.19 ± 0.48 °C, and 7.26 ± 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