Applied Animal Behaviour Science 124 (2010) 51–60

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Influences of immunocontraception on time budgets, social behavior, and body condition in feral horses

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ARTICLE INFO

Article history:
Accepted 22 January 2010
Available online 12 February 2010

Keywords:
Equus caballus
Fertility control
Porcine zona pellucida (PZP)
Wild horse
Wildlife contraception

ABSTRACT

Managers concerned with shrinking habitats and limited resources for wildlife seek effective tools for limiting population growth in some species. Fertility control is one such tool, yet little is known about its impacts on the behavioral ecology of wild, free-roaming animals. We investigated influences of the immunocontraceptive porcine zona pellucida (PZP) on individual and social behavior in bands of feral horses (*Equus caballus*) in three discrete populations and used 14 hierarchical mixed effect models to gain insight into the influences of PZP treatment on feral horse behavior. A model of body condition was the strongest predictor of feeding, resting, maintenance, and social behaviors, with treated females allocating their time similarly to control females. Time spent feeding declined 11.4% from low condition to high condition females ($F_{1,154} = 26.427$, $P < 0.001$) and was partially reciprocated by a 6.0% increase in resting ($F_{1,154} = 7.629$, $P = 0.006$), 0.9% increase in maintenance ($F_{1,154} = 7.028$, $P = 0.009$), and 1.8% increase in social behavior ($F_{1,154} = 15.064$, $P < 0.001$). There was no difference detected in body condition of treated versus control females ($F_{1,154} = 0.033$, $P = 0.856$), but females with a dependent foal had lower body condition than those without a foal ($F_{1,154} = 4.512$, $P = 0.038$). Herding behavior was best explained by a model of treatment and the interaction of band fidelity and foal presence (AIC_c weight = 0.660) which estimated no difference in rate of herding behavior directed toward control versus treated females ($F_{1,102} = 0.196$, $P = 0.659$), but resident females without a dependent foal were herded 50.9% more than resident females with a foal ($F_{3,102} = 8.269$, $P < 0.001$). Treated females received 54.5% more reproductive behaviors from stallions than control mares ($F_{1,105} = 5.155$, $P = 0.025$), with the model containing only treatment being the most-supported (AIC_c weight = 0.530). Treated and control females received harem-tending behaviors from stallions equally ($F_{1,105} = 0.001$, $P = 0.969$) and agonistic behaviors from stallions equally ($F_{1,105} < 0.001$, $P = 0.986$). Direct effects of PZP treatment on the behavior of feral horses appear to be limited primarily to reproductive behaviors and most other differences detected were attributed to the effects of body condition, band fidelity, or foal presence. PZP is a promising alternative to traditional hormone-based contraceptives and appears to contribute few short-term behavioral modifications in feral horses.

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1. Introduction

The size and distribution of human populations has rapidly increased over the last several decades, leading to shrinking habitats and limited resources for many large vertebrates around the world. Managers seek effective tools for limiting population growth in some of these species. Attempts to regulate populations have historically emphasized controlling survival, but more recently, fertility control has emerged as an alternative to lethal methods of population control (Hobbs et al., 2000; Porton,

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0168-1591/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.applanim.2010.01.015

2005). Little is known about impacts of fertility control on the behavioral ecology of wild, free-roaming animals; yet, understanding animal behavior can be an important tool in addressing a species' influences and roles in an ecosystem and can lead to better management and conservation practices (Sutherland, 1998; Buchholz, 2007). The ethical considerations of potentially altering animal behavior and social structure through the use of fertility control agents on free-roaming wildlife are also a growing concern among managers and the public, as are concerns for animal welfare, such as potential negative physiological reactions to treatment (Nettles, 1997; Asa et al., 2005; Porton, 2005).

Large populations of ungulates (white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and feral horses (*Equus caballus*)) are targeted for fertility control management in the United States. Feral horses exhibit the Type I mating system found in the family Equidae, which is characterized by family groups (bands) typically consisting of a polygynous male with females and their young (Klingel, 1975). Such social groups are largely maintained by a suite of behaviors consisting of reproductive, herding, agonistic, and defensive expressions initiated by the polygynous male in the band, as well as hierarchical dominance relationships among females (Feist and McCullough, 1976; McCort, 1984; Klimov, 1988; Pickeral et al., 1993; Linklater et al., 1999). With such complex social organization, artificial changes to behavior may have far-reaching implications for the treated individual and other members of the family group, as well as the social dynamics of the entire herd. An ideal fertility control agent for free-roaming populations of such animals would have little influence on behavior. The immunocontraceptive porcine zona pellucida (PZP) has been proposed as such an agent.

PZP acts as a vaccine against pregnancy by stimulating the production of zona pellucida antibodies in female mammals (Sacco, 1977; Liu et al., 1989). These antibodies provide a barrier that prevents sperm from binding to the surface of an ovum and results in limited penetration of the zona pellucida and subsequent limited pregnancy in many taxa (Kirkpatrick and Turner, 1991; Kirkpatrick et al., 1996). PZP treatment has been shown to reduce fertility in horses with only 0–19% of treated mares producing foals (Liu et al., 1989; Kirkpatrick et al., 1996). Unlike most conventional contraceptives, this immunocontraceptive does not appear to affect ovarian function of the inoculated female horse when used for up to 3 years; however, additional treatments have been reported to decrease ovarian function (Kirkpatrick et al., 1995). Some additional potential side-effects of PZP treatment have also been investigated in feral horses: formerly treated mares have successfully returned to fertility (Kirkpatrick and Turner, 2002) and injection site reactions were minimal and non-debilitating (Roelle and Ransom, 2009). It remains unclear how PZP treatment influences the behavior of feral, free-roaming horses.

Many wild ungulate species have complex mating systems maintained by dominance hierarchies within family groups, active recruitment and defensive behaviors by polygynous males, and resource driven behavioral patterns. PZP treatment is not expected to have a direct effect on these behaviors in horses because ovarian function remains intact and artificial hormones are not administered. However, horses are polyestrous and females may exhibit estrous behavior during both ovulatory and anovulatory phases of ovarian follicular development, which is thought to facilitate social structure (Crowell-Davis, 2007). PZP treatment of females has the potential to elevate the exhibition of repetitive estrous behavior beyond that which stallions are accustomed to. We hypothesize that such polyestrous cycling in individual mares may elicit excess reproductive behaviors by both the cycling female and nearby males, prompt more frequent herding and harem-tending behaviors by stallions, and elevate frequency of agonism between stallions and females. We also posit that if individuals allocate more time to reproductive and social behaviors, less time is available for critical feeding and maintenance behaviors and could present long-term concerns for animal health.

Current research on this topic has been limited to a few island populations where time budgets of PZP-treated females were found to be comparable to control females (Powell, 1999; Rogers, 2001); however, it has recently been shown that band fidelity can decrease and rates of reproductive behavior can increase in association with PZP treatment (Nuñez et al., 2009). These results are somewhat conflicting and present concern that animals in disparate populations may exhibit different behavioral responses to PZP treatment, possibly due to percent of females treated, population size, or density. Approximately 37,000 feral horses roam on 13 million ha of public lands in the western United States and represent the vast majority of feral horses in the US. We investigated influences of PZP immunocontraception on the behavior of feral horses in three discrete free-roaming western populations over a 4-year period. Our objective was to quantify effects of immunocontraception on time budgets of individuals and social interactions within family groups, differentiating between control females and effectively contracepted females.

2. Methods

2.1. Study areas

Three study areas, Little Book Cliffs Wild Horse Range, McCullough Peaks Herd Management Area, and Pryor Mountain Wild Horse Range were chosen to represent the wide variety of habitats occupied by feral horses in the western United States. These populations use a mosaic of habitats, including desert, sage steppe, piñon-juniper woodland, coniferous forest, and montane environments (Table 1). A small number of unimproved roads traversed these areas, but the majority of land at all three locations was designated as Wilderness Study Area and closed to motorized vehicles throughout the period of this study.

Little Book Cliffs managers removed 68 horses from the population in 2004 and Pryor Mountain managers removed seven horses in 2003 and 19 horses in 2006, but none of these animals were members of the focal bands

Table 1
Geographic, biologic, climatic, and population data for conditions present during behavioral observations of feral horses (*Equus caballus*) at Little Book Cliffs Wild Horse Range (LBC), McCullough Peaks Herd Management Area (MCP), and Pryor Mountain Wild Horse Range (PRY), USA, April–October, 2003–2006. The number of treated females comprises all individuals vaccinated with the immunocontraceptive porcine zona pellucida (PZP).

Study area	Location	Elevation (m)	Size (ha)	Terrain	Dominant vegetation	Precipitation (% of obs.)	Temperature (mean (°C) ± SE)	No. of treated females	Population size	Band size (mean ± SE)
LBC	39°12′N; 108°27′W	1500–2250	14,614	Sloping plateaus, sagebrush parks, steep shale and sandstone cliffs, 4 major canyon systems	Sagebrush (*Artemisia tridentata*), grasses, Colorado pinon (*Pinus edulis*), Utah juniper (*Juniperus osteosperma*)	4.88	25.12 ± 1.32	27	120–204	4.45 ± 0.16, range = 2–9
MCP	44°35′N; 108°40′W	1200–1964	44,440	Flat, open sagebrush park, rugged badlands along western edge	Sagebrush, grasses, small stands of cottonwood (*Populus* sp.) and willow (*Salix* spp.)	3.78	26.62 ± 1.80	36	112–495	8.01 ± 0.38, range = 2–19
PRY	45°04′N; 108°19′W	1175–2670	16,046	Lowland desert, foothill slopes, forested montane slopes, steep canyons, grassy plateaus	Sagebrush (*A. tridentata*), grasses, Utah juniper, Douglas fir (*Pseudotsuga menziesii*) and limber pine (*Pinus flexilis*)	5.25	20.63 ± 1.17	54	142–168	4.16 ± 0.17, range = 2–11

used in our study. Managers removed 379 animals from the McCullough Peaks population in October 2004. Due to management need, 1-year-old females, and 1- and 2-year-old males were removed from the focal bands used in this study, but all reproductive animals in these bands were captured and released as intact family units. Hansen and Mosley (2000) reported no changes in feeding or reproductive behavior and no effect on fecundity following such removals.

2.2. Study subjects

Horses in the treatment group at all three study areas were given primer injections consisting of 0.5 mL (100 μg) PZP and 0.5 mL (100 μg) Freund's complete adjuvant or Freund's modified adjuvant. Subsequent booster injections at Little Book Cliffs and Pryor Mountain consisted of 0.5 mL (100 μg) PZP and 0.5 mL (100 μg) Freund's incomplete adjuvant. These injections of liquid PZP were designed to provide approximately 1 year of infertility, with annual boosters extending the period of contraception. In addition to the primer dose given to horses at McCullough Peaks, a second (simultaneous) injection delivered booster doses via three time-release PZP pellets (bolus release at 1, 2, and 12 months) designed to provide 2 years of infertility (Turner et al., 2008). A full description of these procedures is described in Roelle and Ransom (2009).

Most female horses were pregnant at the time of their first injection of PZP and some did not exhibit infertility after receiving treatment; therefore, to clarify behaviors associated with effective fertility control, all analyses considered animals to be in the treatment group only when they had received at least the primer and one booster dose, and exhibited non-pregnancy. Three females that received PZP but continued to produce young were omitted from analyses. Observations of treated animals that occurred after prescribed treatment had concluded were omitted from analyses because post-treatment PZP titer levels could not be verified; it was thus unknown if contraception persisted or return to fertility had occurred. Animals in the control group were not injected with PZP and exhibited pregnancy as it naturally occurred.

During the course of this study, 27 horses were treated at Little Book Cliffs, 36 were treated at McCullough Peaks, and 54 were treated at Pryor Mountain, with the number of treatments received by any individual horse varying from 1 to 4. The percentage of treated females >1 year of age in each population therefore varied by year, but averaged 36.0% at Little Book Cliffs, 80.6% at McCullough Peaks, and 25.7% at Pryor Mountain. All focal animals at each site were monitored by trained technicians one to five times per week from April to October each year, 2003–2006. Pregnancy and parturition were primarily determined by visual assessment, with supplemental pregnancy data opportunistically gained through fecal assays of estrone conjugates and progesterone metabolites (Kirkpatrick et al., 1991) or blood estrone sulfate/progesterone analyses (Munro and Stabenfeldt, 1984; Stabenfeldt et al., 1991). All data for this study were collected in accordance with the Colorado State University Animal Care and Use Committee standards (protocol 03-107A-02).

2.3. Behavioral sampling

Eight to ten feral horse bands were chosen in each of the three populations from a stratified random sample consisting of bands with at least one stallion, one treated mare and one control mare, though natural interchange dictated that band compositions were plastic throughout the study. Behavioral observations were separated into three daylight time periods: 08:00–12:00 h, 12:01–16:00 h, and 16:01–20:00 h. We collected behavioral data for 3133 horse hours (total band observation hours times the number of focal animals in each band) at Little Book Cliffs, 4221 horse hours at McCullough Peaks, and 2592 horse hours at Pryor Mountain. Each observation session included collection of climatic (temperature, precipitation, cloud cover, wind speed), geographic (UTM coordinates and elevation), and temporal (time and date) data, a 20-min instantaneous scan sample of time budgets (Altmann, 1974) at 1 min intervals for each band member, and all-occurrence data collection for social interactions (Altmann, 1974).

In order to accurately record behaviors, we defined an ethogram that categorized specific behaviors into a suite of 15 discrete time budget categories (*see* Ransom and Cade, 2009). These categories were agonism, comfort, elimination, feeding, grooming, harem-social (e.g., allogrooming, pair-bonding), harem-tending (e.g., stallion defense of a harem mare or recruitment of a new mare into the harem), herding (driving or snaking behavior by the stallion), interaction-with-humans, locomotion, out-of-sight, reproduction, resting, standing attentive, and submission. The relative infrequency and similar nature of several behaviors in the instantaneous scan samples motivated us to aggregate them into broader categories for time budget analyses. Grooming, comfort, standing attentive, and elimination were combined into *maintenance*; reproductive, harem-tending, harem-social, agonism, submission, and herding were combined into *social*; and interaction-with-humans and out-of-sight were combined into *unknown*. The time spent in the category *unknown* ultimately did not differ between treatment groups in any population.

Instantaneous scan sampling has the potential to underestimate rare events (Houpt, 1991; Doran, 1992); thus, we also collected social behavior data at every occurrence throughout each observation session. These all-occurrence behaviors included intraband social behaviors associated with herding, reproduction, agonism, and harem-tending and were retained as discrete categories for social behavior analyses. We conducted all observations from distances that did not elicit attention of horses to the presence of the observer. McCullough Peaks horses were relatively reactive to the presence of humans and observations were generally conducted approximately 100 m away unless terrain allowed an observer to remain concealed at a closer distance. Horses at Little Book Cliffs and Pryor Mountain were less reactive to human presence and observations were conducted at approximately 50 m or more. All observations utilized 10 mm × 50 mm binoculars or a 16–45 mm × 60 mm spotting scope in situations where unaided observations were not adequate.

2.4. Model covariates

We initially considered age, climate, time of day, band fidelity, presence of dependent young, size of the band, body condition score, and experience of the dominant male within a band as factors that might help explain variation in horse behavior beyond the effects of treatment. We also considered sampling year in the event that behaviors varied temporally due to an effect we did not otherwise explicitly include in our analyses. Age was absolutely known from parturition records for 88.4% of all individuals at Pryor Mountain, 42.1% of all individuals at Little Book Cliffs, and 34.2% of all individuals at McCullough Peaks. An experienced veterinarian estimated ages for the remainder of animals in this study using dental assessments during scheduled management captures. This method provides only approximate age due to the many external factors effecting tooth wear (Martin, 2002).

Climatic data (remperature, cloud cover, wind speed, and precipitation) was relatively homogeneous across locations and observations so we did not further consider climate in behavior analyses. Behavioral observations were distributed equally across daylight time periods so we did not further consider time of day. The total number of all horses present in the band at the time of the observation constituted the band size. Band composirion data collected every 7 days, from April to October of each year, derermined band fidelity: females were considered transient if they left their band and went to another band at least once during the season and resident if they never lefr during the season. Presence of young was recorded as a dependent foal from birth to the end of that sampling season or documented death that season. Body condition of individuals was determined using a 10-step index similar to the system of Henneke et al. (1983); however, since these scores were assigned solely by visual assessment (rather than palpation) in the field, the scale was adapted to a 0.0–5.0 score with half-step intervals similar to that described by Rudman and Keiper (1991). This scale facilitated more consistent estimation of body condition in the field. Tenure of males as band stallions in this study was unkuown, so we assumed age was a reasonable indicator of band stallion experience. Age range of the observed band stallions was 4–26 years, with mean age = 11.88 (95% CI: 10.43–13.33).

2.5. Statistical analyses

Some horses were born into focal bands, died, or immigrated/emigrated to/from focal bands during the study; therefore, some horses were observed repeatedly over the course 4 years while others were only observed in a single year. As a result, samples were not equally distributed across individuals or across years. Repeated measures of individuals were accounted for by averaging data for each individual or unique pair of individuals per year and including the influence of individual identification on the random effect intercept of each model. This design allowed analyses to include a measure of variability among individuals as well as among years. Observations of individual horses were not averaged over the entire 4-year

period because potentially important variables such as age, band fidelity, presence of a dependent foal, and treatment status often varied annually. Five extreme outlier values were detected in the residual values for time budget behaviors and these observations were omitted from analyses. The final dataset considered in all analyses consisted of 184 control female and 103 treated female observations that were comprised of 99 individual control horses (32 at Little Book Cliffs, 34 at McCullough Peaks, 33 at Pryor Mountain) and 56 individual treated horses (18 at Little Book Cliffs, 16 at McCullough Peaks, 22 at Pryor Mountain).

We formulated a candidate set of 14 hierarchical mixed effects models, which, in the notation of Gelman and Hill (2007), took the form of $y_{hjk[i]} = \alpha_{0hj[i]} + \alpha_{1hk[i]} + \beta_0 + \beta_x x_{[i]} + \varepsilon_{hjk[i]}$, where the random effect intercept of $\alpha_{0hj[i]} \sim N(\mu = 0, \sigma^2_{hj})$ for treatment h (treated or control) in population j (Little Book Cliffs, McCullough Peaks, or Pryor Mountain), for $i = 1, \ldots, n$ observations, and the random effect intercept $\alpha_{1hk[i]} \sim N(\mu = 0, \sigma^2_{hk})$ for h in $k = 1, \ldots, n$ individual horses for i observations. This equation identifies the fixed effect intercept as β_0, with ε = statistical error, and y = dependent variable. The candidate model set included models with the fixed effect parameter (β_x) for fixed effects (x) of age (model 1), treatment (model 2), body condition (model 3), foal presence (model 4), band fidelity (model 5), band size (model 6), sampling year (model 7), and the interactions of treatment and band fidelity (model 8), treatment and body condition (model 9), band fidelity and body condition (model 10), band fidelity and foal presence × treatment (model 11), age and treatment (model 12). Social behavior involved pairs of animals, and the fixed effect of stallion age (model 13) and average distance between the stallion and focal mare (model 14) were also considered for those analyses.

Analyses of individual time budget behaviors and social behavior data were performed using the Hierarchical Linear Mixed Model function of SYSTAT 12® (SYSTAT Software Inc., 2007) with estimates obtained by restricted maximum likelihood (Harville, 1977). We also analyzed body condition as a dependent variable using the candidate model set, excluding Models 3, 9, and 10 because they contained body condition as an independent variable. Probabilities for all tests are reported with a 95% level of confidence.

We used minimum corrected Akaike's Information Criteria (AIC_c) to evaluate the strength of evidence for all mixed models (Burnham and Anderson, 2002). Models achieving the greatest AIC_c weight were considered the best model for the predicted behavior, and all models exhibiting a ΔAIC_c of <2 were considered plausible (Burnham and Anderson, 2002). Time budget behaviors were modeled individually to investigate the influences of fixed effects on each behavior; however, the strength of evidence for each model was ultimately evaluated based on the composition of all behaviors in the time budget (feeding, resting, locomotion, maintenance, and social). This was performed by summing the minimum AIC_c scores from each individually modeled constituent behavior and using the aggregate AIC_c score ($aAIC_c$) as strength of evidence for the overall composition (Ransom and Cade, 2009). This procedure allowed detailed analyses of the individual behaviors without violation of the inherent compositional dependence. Time budgets were analyzed as mean percent of time allocated to each behavior in the composition, per horse, per year and social behavior models were evaluated individually because the data represent frequency-of-occurrence.

3. Results

3.1. Time budgets

Feeding behavior for all horses occupied the largest percent of daylight time budgets and there was no difference detected between treatment groups, with control females feeding an estimated 57.7% ± 3.2 SE of daylight hours and treated females feeding an estimated 55.0% ± 3.3 SE of daylight hours ($F_{1,154} = 0.351$, $P = 0.554$). The most-supported model for feeding behavior was Model 3 (body condition) with an AIC_c weight of 0.947, and no other models were considered plausible. This model estimated an 11.4% decrease in time spent feeding as body condition score increased from 1.0 to 4.0 (Fig. 1, Table 2).

Resting behavior occupied an estimated 27.1% ± 2.0 SE of control female daylight time budgets and 27.9% ± 2.2 SE of treated female daylight time budgets ($F_{1,154} = 0.075$, $P = 0.785$). Models 3 (body condition) and 4 (foal presence) were both plausible for explaining resting behavior, with Model 3 carrying an AIC_c weight of 0.705 and Model 4 an AIC_c weight of 0.209. Body condition in model 3 estimated an increase of 6.0% in time spent resting as body condition increased from 1.0 to 4.0, which partially compensated for the negative trend detected in feeding behavior (Table 2). Model 4 estimated that foal presence best explained resting behavior with independent females resting an average of 29.3% ± 1.6 SE of the time and females with a dependent foal resting 12.9% less ($\bar{x}$ = 25.5% ± 1.6 SE).

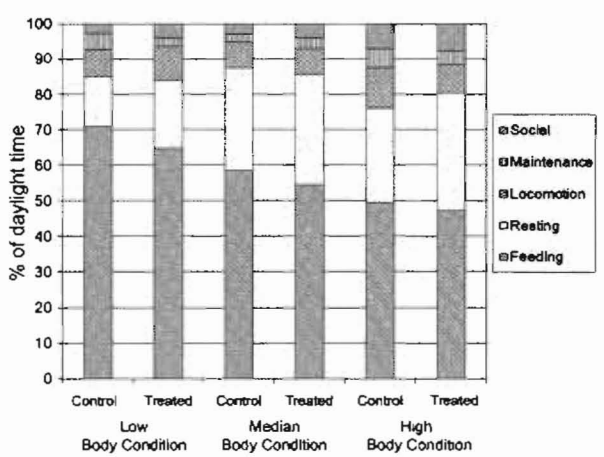

Fig. 1. Mean allocations of daylight time budget behaviors as a function of low (<2.5), median (3.0) and high (>3.5) body condition scores for repeated observations of 99 control female and 56 treated female feral horses (*Equus caballus*) at Little Book Cliffs Wild Horse Range, McCullough Peaks Herd Management Area, and Pryor Monntain Wild Horse Range, USA, 2003–2006.

Table 2
Hierarchical linear mixed model estimates of the difference in means for each fixed effect in the most-supported models of daylight time budget behaviors (feeding, resting, locomotion, maintenance, social) and all-occurrence social behaviors (reproductive, herding, harem-tending, agonism) for repeated observations of 99 control and 56 treated feral horses (*Equus caballus*) at Little Book Cliffs Wild Horse Range, McCullough Peaks Herd Management Area, and Pryor Mountain Wild Horse Range, USA, 2003–2006. Random effect variance is denoted as σ_p^2 for the horse population and σ_i^2 for the individual horse.

Behavior	Model	AIC_c weight	Effect	df_{NUM}	df_{DENOM}	F	P	Difference	95% LCL	95% UCL	σ_p^2	σ_i^2
Feeding	3	0.947	Body condition	1	154	26.427	<0.001	−0.114	−0.158	−0.070	0.0013	0.0001
Resting	3	0.705	Body condition	1	154	7.629	0.006	0.060	0.017	0.104	0.0003	0.0005
	4	0.209	Foal presence	1	154	5.374	0.022	0.038	0.006	0.070	0.0008	0.0007
Locomotion	2	0.461	Treatment	1	154	0.006	0.941	0.002	−0.043	0.046	0.0007	<0.0001
	4	0.250	Foal presence	1	154	2.020	0.157	0.006	−0.002	0.014	0.0006	<0.0001
Maintenance	3	0.782	Body condition	1	154	7.028	0.009	0.008	0.002	0.015	0.0002	<0.0001
Social	3	0.947	Body condition	1	154	15.064	<0.001	0.018	0.009	0.028	0.0002	0.0001
Reproductive	2	0.530	Treatment	1	105	5.155	0.025	−0.060	−0.113	−0.008	0.0005	0.0051
Herding	11	0.660	Treatment	1	102	0.196	0.659	−0.123	−0.443	0.659	0.1091	0.0211
			Band fidelity × foal presence	3	102	8.269	<0.001	–[a]				
Harem-tending	3	0.930	Body condition	1	105	20.608	<0.001	0.141	0.079	0.203	0.0075	<0.0001
Agonism	6	0.925	Band size	1	105	12.080	<0.001	−0.007	−0.011	−0.003	<0.0001	<0.0001

[a] This variable produces a vector of interaction estimates, as described in the text.

Locomotion occupied a relatively small amount of female daylight time budgets, and while model 2 (treatment) best explained this behavior (AIC_c weight = 0.461), it estimated no difference between treatment groups (Table 2). Control females in this model were in locomotion an estimated 6.6% ± 1.6 SE of the time and treated females were in locomotion an estimated 6.4% ± 1.6 SE of the time. Model 4 (foal presence) was also plausible for explaining locomotion behavior and was supported by 25.0% of the AIC_c weight. The effect of foal presence was weak and the model estimated females with a dependent foal were in locomotion an average of 6.2% ± 1.1 SE of the time and independent females were in locomotion an average of 6.8% ± 1.1 SE of the time (Table 2).

Maintenance behavior was expressed by control females an estimated 1.7% ± 0.8 SE of the time and by treated females an estimated of 2.4% ± 0.8 SE of the time ($F_{1,154} = 0.420$, $P = 0.518$). This behavior was best explained by Model 3 (body condition) and while the effect was significant, the amount of time spent in expression of maintenance behavior only increased 0.8% as body condition increased from 1.0 to 4.0 (Table 2). Likewise, social behavior was also best explained by Model 3 (body condition) and this behavior increased 1.8% as body condition score increased from 1.0 to 4.0. No other models were supported for maintenance or social behaviors.

Body condition was clearly the most-supported effect for explaining daylight time budget behaviors, with an $aAIC_c$ weight of 0.996 among the candidate models. Body condition scores of the animals observed ranged from 1.0 to 4.0 with a median score of 3.0. Model 2 (treatment) and Model 4 (foal presence) were the most plausible models for predicting body condition, with AIC_c weights of 0.390 and 0.285, respectively. There was no difference detected in body condition of control females ($\bar{x} = 2.99 \pm 0.08$ SE) compared to treated females ($\bar{x} = 2.97 \pm 0.08$ SE) ($F_{1,154} = 0.033$, $P = 0.856$) in Model 2; however, females with dependent foals in Model 4 exhibited lower body condition ($\bar{x} = 2.91 \pm 0.04$ SE) than females without dependent foals ($\bar{x} = 3.02 \pm 0.03$ SE) ($F_{1,154} = 4.512$, $P = 0.038$). The estimated decrease in time feeding was compensated by an increase in resting, maintenance, and social behavior as body condition increased (Fig. 1); however, body condition data exhibited considerable variation suggesting that other factors we did not measure may also influence time budget behaviors.

3.2. Herding behavior

Herding was the most commonly observed intraband social behavior at all three sites. Stallions were observed herding females at Little Book Cliffs at a mean rate of 0.20 (95% CI: 0.16–0.25) times per hour (tph), McCullough Peaks 0.17 (95% CI: 0.13–0.22) tph, and Pryor Mountain 0.68 (95% CI: 0.52–0.85) tph. Band fidelity contributed strongly to the models of herding behavior and models containing this variable totaled 79.7% of the AIC_c weight. The most-supported model for herding behavior was Model 11 (treatment + the interaction of band fidelity and foal presence), and it attained an AIC_c weight of 0.660 with no other candidate models being plausible.

The effect of treatment in Model 11 was not significant and was accompanied by considerable variation, but the interaction effect of band fidelity and foal presence was strong (Table 2). Resident females without a foal were herded by stallions 0.57 tph ± 0.15 SE whereas resident females with a dependent foal were herded by stallions 50.9% less at an average of 0.29 tph ± 0.15 SE. Transient females without foals were herded by stallions least often, at an average of 0.18 tph ± 0.15 SE, and transient females with a dependent foal were herded similarly to resident females with a foal at an average of 0.27 tph ± 0.16 SE.

3.3. Reproductive behavior

Reproductive behavior was the second most frequently observed intraband social behavior at all three sites. Little Book Cliffs stallions were observed exhibiting reproductive

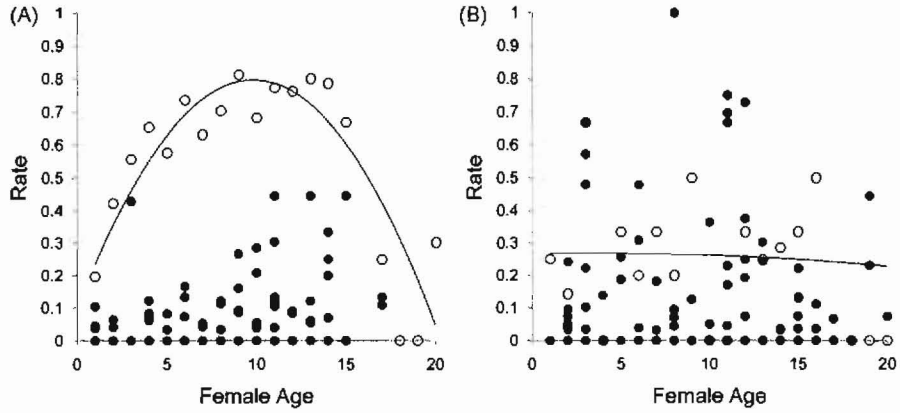

Fig. 2. Rate of feral horse (*Equus caballus*) reproductive behaviors exhibited per hour (●) as a function of female age, and mean rate of viable foal production (○) as a function of female age at conception, for repeated observations of 99 control females (A: $y = -0.007x^2 + 0.142x + 0.101$, $R^2 = 0.89$) and 56 treated females (B: $y = -0.0002x^2 + 0.001x + 0.267$, $R^2 = 0.002$) at Little Book Cliffs Wild Horse Range, McCullough Peaks Herd Management Area, and Pryor Mountain Wild Horse Range, USA, 2003–2006.

behavior toward females at a mean rate of 0.05 (95% CI: 0.02–0.09) tph, with McCullough Peaks stallions exhibiting the same rate of 0.05 (95% CI: 0.03–0.08) tph. Pryor Mountain stallions exhibited this behavior toward females at the rate of 0.10 (95% CI: 0.07–0.13) tph. Copulation (successful and unsuccessful) comprised 34.2% of the reproductive behaviors observed and pre-copulatory behaviors such as reproductive tending, flank-rubbing, chin-resting, flehmen response, and penis extension comprised 65.8% of the observations. The most-supported model for reproductive behavior was Model 2 (treatment), with an AIC_c weight of 0.530, and no other candidate models were plausible.

The effect of treatment was highly significant with control females receiving an average of 0.05 ± 0.02 SE reproductive behaviors from stallions per hour and treated females receiving 54.5% more reproductive behaviors at an average of 0.11 ± 0.02 tph (Table 2). The rate of reproductive behavior expressed toward females generally followed the relationship between viable foal production and age of the female at the time of conception (Fig. 2A), but this relationship deteriorated in the treatment group and the rate of reproductive behaviors expressed was both higher than control females and exhibited no clear pattern among age cohorts (Fig. 2B).

3.4. Harem-tending behavior

Harem-tending behavior was observed with greater frequency at Pryor Mountain than at the other two sites, but was still relatively infrequent in all populations. Stallions exhibited harem-tending behavior toward females at Pryor Mountain 0.17 (95% CI: 0.12–0.22) tph, whereas this was observed at Little Book Cliffs only 0.04 (95% CI: 0.03–0.05) tph and at McCullough Peaks only 0.02 (95% CI: 0.01–0.03) tph. Model selection among harem-tending models revealed body condition as an important effect, with Model 3 attaining 93.0% of the AIC_c weight. Harem-tending behaviors by stallions toward females increased 14.1% as body condition increased from 1.0 to 4.0 (Table 2). There was no difference detected in harem-tending behaviors by stallions toward control females ($\bar{x} = 0.08$ tph $\pm$ 0.04 SE) as compared to treated females ($\bar{x} = 0.08$ tph $\pm$ 0.05 SE) ($F_{1,105} = 0.001$, $P = 0.969$).

3.5. Agonism

Agonistic behaviors from stallions toward female band members were observed 0.03 (95% CI: 0.01–0.05) tph at Little Book Cliffs, 0.01 (95% CI: 0.01–0.02) tph at McCullough Peaks, and 0.04 (95% CI: 0.03–0.06) tph at Pryor Mountain. In order of increasing intensity, 67.7% of these agonistic expressions were in the form of a threat, 1.7% were bumps or pushes, 26.8% were kicks or bites, and 3.8% were chases. Model 6 (band size) best explained the observed agonistic expressions and was supported by 92.5% of the AIC_c weight. The effect of band size was statistically significant, but only resulted in agonistic behavior decreasing 0.7% as band size increased from 3 to 14 horses. There was no difference in rates of agonistic behavior exhibited by stallions toward control females ($\bar{x} = 0.03$ tph $\pm$ 0.01 SE) compared to treated females ($\bar{x} = 0.03$ tph $\pm$ 0.01 SE) ($F_{1,105} < 0.001$, $P = 0.986$). Agonism between females occurred too infrequently to conduct meaningful analyses.

4. Discussion

Previous research indicated that immunocontraception with PZP did not lead to behavioral changes in feral horses (Kirkpatrick et al., 1996; Powell and Monfort, 2001); however, more recent work by Nuñez et al. (2009) refuted these assertions with evidence that PZP immunocontraception led to a decrease in band fidelity and an increase in reproductive behaviors during the non-breeding season. Our findings are similar to those of Nuñez et al. (2009), indicating that PZP treatment can affect behavior in feral horses. We found that treated females received significantly more reproductive behaviors from stallions than did control females, and the most-supported model of this behavior was that of treatment. Models containing the fixed effect of treatment were also supported for

locomotion and herding behavior, but no differences were detected between treatment groups. All other behaviors investigated were better explained by models of body condition, foal presence, or band size.

4.1. Time budgets

Estimates of daylight time budget allocations for all age and treatment groups fell within the broad ranges previously reported for equids (Mayes and Duncan, 1986; Boyd, 1988; Berger et al., 1999). The differences detected in time budget behaviors were primarily a function of body condition and are comparable to expected differences in time budgets between barren and pregnant/lactating females. Boyd (1988) found pregnant female horses spent more time feeding than barren females, which corresponds to the respective metabolic needs of animals in these conditions (Henneke et al., 1984; National Research Council, 2007).

These results largely concur with Powell's (1999) finding of no difference in time allocation between PZP-treated and control horses; however, data were not completely comparable because control females in Powell's study were previously treated and PZP titer levels were unknown. Rogers (2001) found that PZP had no effect on time spent walking, grooming, or standing in feral horses. Likewise, Heilmann et al. (1998) found no differences in time spent moving or idling between PZP-treated and untreated free-ranging female elk. Our results are consistent with those from these studies, and it appears that the complex social organization of feral horses does not greatly influence the time allocated to these basic maintenance behaviors. McShea et al. (1997) did note that PZP-treated female white-tailed deer spent more time running or walking than untreated females, but we did not detect this effect in horses.

Models that were most strongly supported for time budget compositions attributed primary differences between treatment groups to body condition and foal presence effects. Body condition contributed to individual time budget behavior with females in lower body condition feeding more than those in higher condition. Few animals exhibited extremely low or high body condition in our study and investigation into this effect may require a wider range of conditions to elucidate more in-depth results. Social dominance in relation to body condition has been attributed to food gain and energy expenditure (Hogstad, 1987; Cuthill and Houston, 1997; Fischoff et al., 2007) and stochastic climate events could cause depressed or elevated states of body condition and lead to greater differences between treatment groups. Turner and Kirkpatrick (horses: 2002) and McShea et al. (white-tailed deer: 1997) found that contracepted females exhibited higher body condition than control females, and Conner et al. (2007) found a seasonal difference in winter body condition of females, but no overall difference between fertility control treatment groups. Likewise, we found no difference in body condition of treated versus control females in our study.

van Dierendonck et al. (2004) reported that mares without foals spent less time resting and a greater percent of time in social interaction than either pregnant or post-partum mares. One plausible model of resting behavior in our study exhibited support for the effect of foal presence, but with contrary results. Mares with dependent foals rested less than their barren counterparts. This conflicting result may be attributed to the social structure (absence of stallions) in van Dierendonck et al.'s (2004) study, compared to the free-roaming natural populations that experienced threats of predation and competition among stallions in our study. Females with dependent young simply had to be more vigilant when faced with these natural constraints on their time.

4.2. Social behavior

Frequency of some social behavior was heterogeneous across the populations we studied, likely resulting from local densities of bands on the landscape. This proximity of groups to one another introduces different levels of social pressure between bands and illustrates that some populations may be more sensitive to fertility control management strategies than others. All-occurrence analyses of social behavior detected 54.5% more reproductive behaviors were received by treated females than by control animals. This is not surprising given the higher rates of estrous reported in PZP-treated females of other species as compared to control females (Mahi-Brown et al., 1985; Shumake and Wilhelm, 1995; Heilmann et al., 1998; Curtis et al., 2002). Nuñez et al. (2009) reported more frequent reproductive behaviors exhibited and received by treated female horses outside of the breeding season; likewise, Heilmann et al. (1998) found that PZP-treated female elk exhibited higher rates of sexual interaction outside of the breeding season than did untreated females. Powell (1999) also found that reproductive tending and mating duration by stallions lasted longer as a result of mares continuing their estrous cycles into the fall, which he proposed may contribute to a disruption in band stability. Similarly, McShea et al. (1997) found that the breeding season behavior of white-tailed deer males was extended, and started earlier in the subsequent year after females were treated. Our study examined only the frequency of reproductive behaviors and did not examine seasonality. The difference between treatment groups in pattern and relationship to age-specific fecundity we detected appears to directly correspond to the treatment applications across age cohorts and efficacy of the vaccine. It may be important to consider additional research to assess the scope of this potential effect as it could have implications for stallion body condition and consequent lifetime fitness.

Herding behavior may be important because of the high energetic cost of locomotion for both the male and female(s) involved and may have biological meaning in terms of stallion body condition and fidelity of females to the band. We detected no significant difference in herding between treatment groups, but there was a strong effect of the interaction between band fidelity and foal presence in predicting this behavior. Rogers (2001) found that the percentage of time females were herded was different between treatment groups and detected significant effects of foal presence and band fidelity. Even when generalized

across 3 populations, we also detected this difference and found that absence of a dependent foal was associated with an increase in herding behavior. Linklater et al. (1999) found that transient mares had poorer body condition than resident mares as a result of greater harassment by stallions and Nuñez et al. (2009) found that immunocontracepted female feral horses exhibited a decrease in band fidelity during the non-breeding season. These findings suggest further investigation into the effects of fertility control on band fidelity would help determine the scope and extent of this relationship in immunocontracepted female horses during the breeding season.

Harem-tending and agonistic behaviors by stallions did not differ among treatment groups, but stallions invested more harem-tending behaviors in mares with higher body condition. Optimality theory may explain this finding by the perceived fitness pay-off the stallion would receive from investing in animals more likely to contribute offspring (MacArthur and Pianka, 1966), but the true nature of this relationship has not been explained by our analyses. Stallions also expressed no difference in frequency of agonistic behaviors roward control and treated females. This is not surprising since agonistic expressions within feral horse bands are often expressed between females in order to establish and facilitate dominance (Houpt et al., 1978; Waring, 1983; Keiper and Receveur, 1992; Weeks et al., 2000). The stallion is generally not the most dominant member of the band, perhaps as a result of greater time spent away from the group while defending or recruiting females (Keiper and Receveur, 1992).

While few studies have investigated the relationship of agonism and contraception in feral horses, agonism related to presence of a foal and band fidelity has been described. Estep et al. (1993) found no change in agonistic behaviors associated with presence or absence of a dependent foal, and Rutberg and Greenberg (1990) found thar frequency of aggression did not vary between females of transient and resident status. Our study concurs with these results as the covariates of interest were not present in the most-supported models of agonistic behavior, and they were not significant effects in the candidate models that contained them.

5. Conclusion

Our study found that reproductive behavior was the only acute short-term response attributable to PZP treatment. Most behaviors we measured were better explained by body condition and those findings are consistent with the metabolic needs of barren versus pregnant/lactating females. Foal presence was also an important effect in some behaviors, and while no differences in treatment groups were detected, the efficacy of treatment inherently influences this effect making it important in the long-term consideration of fertility control use. The long-term influence of changes to band fidelity and foal presence on band structure, social networks, dominance hierarchies, pair-bonding, and socialization requires further study. Such effects have broader implications than the results presented in this study and may imply more dynamic changes to the ecology of feral horses.

Acknowledgments

Thank you to the guiding forces of the research advisory team that provided oversight for this project: F. Singer, J. Roelle, A. Kane, L. Coates-Markle, D. Bolstad, and A. Shepherd. F. Singer and L. Coates-Markle were instrumental in developing the field trial plan, treatmenr straregies and initiating the larger fertility control study. Thanks to W. Andelt, P. Cryan, T. Holcombe, J. MacLeay, J. Roelle and two anonymous referees for reviewing earlier versions of this manuscript. Special thanks to T. Hatle and J. Dollerschell with the US Bureau of Land Management (BLM) and C. Bromley with the National Park Service for logistical assistance on the ranges. A generous thank you to the outstanding field technicians that made this work a reality: H. Abouelezz, L. Caldwell, M. Esser, M. Felix, K. Grams, P. Grigsby, S. Hahn, B. Hutchings, G. Manus, J. Nibler, C. Petrandis, P. Preator, J. Severude, and G. Thygerson. This study was funded by the U.S. Geological Survey Wildlife Program, in collaboration with the BLM, USDA Animal and Plant Health Inspection Service, and Colorado State University. Any use of trade, product, or firm names is for descriptive purposes and does not imply endorsement by the U.S. Government or Colorado State University.

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