



Original Article

Foaling Rates in Feral Horses Treated With the Immunocontraceptive Porcine Zona Pellucida

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ABSTRACT Locally abundant feral horses (*Equus caballus*) can rapidly deplete available resources. Fertility control agents present promising nonlethal tools for reducing their population growth rates. We tested the effect of 2 forms of the immunocontraceptive porcine zona pellucida (PZP) on foaling rates in 3 populations of feral horses in the western United States. A liquid form requiring annual boosters was administered at Little Book Cliffs Wild Horse Range, Mesa County (CO), and Pryor Mountain Wild Horse Range, Bighorn County (WY) and Carbon County (MT), and a time-release pellet form designed to produce 2 yr of infertility was administered at McCullough Peaks Herd Management Area, Park County (WY). Average foaling rates (foals born/mare-yr) from direct observation of untreated and treated female horses (mares), 2004–2008, were 60.1% ($n = 153$ mare-yr) versus 6.6% ($n = 91$ mare-yr) at Little Book Cliffs, and 62.8% ($n = 129$ mare-yr) versus 17.7% ($n = 79$ mare-yr) at Pryor Mountain, respectively. At McCullough Peaks, mean annual foaling rates from 2006 to 2008 were 75.0% ($n = 48$ mare-yr) for untreated mares and 31.7% ($n = 101$ mare-yr) for treated mares. Controlling for age of mares and pretreatment differences in fertility, PZP reduced foaling rates in all 3 herds. The pellets used at McCullough Peaks (produced by cold evaporation) were less effective than pellets used in a previous trial and produced by heat extrusion. Immunocontraception with PZP may be a useful tool in reducing fertility rates in some western United States feral horse herds, but population growth reduction will depend on timely access to mares for inoculation and the proportion of mares that can be successfully treated. Published 2011. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS *Equus caballus*, feral horse, fertility control, immunocontraception, PZP, western United States, wildlife contraception.

Tools for regulating animal populations are needed when habitat and resources are inadequate to sustain local abundance. Traditionally, regulation of wildlife populations has focused on lethal methods. Fertility control has emerged as an increasingly viable alternative to such methods and may be a particularly valuable tool when managers are faced with regulating protected species that are locally overabundant (Hobbs et al. 2000, Porton 2005). Such is the case with rapidly reproducing feral horses (*Equus caballus*) that roam large areas of public land in the western United States that are managed by the Bureau of Land Management (BLM) and U.S. Forest Service (Eberhardt et al. 1982, Garrott et al. 1991).

Many fertility control techniques have been proposed or tested for managing feral horses in the United States, ranging

from hormone implants (Plotka et al. 1992) and intrauterine devices in females (Daels and Hughes 1995, Killian et al. 2008) to testosterone propionate treatment of males (Turner and Kirkpatrick 1982). These efforts produced tools that were publicly unacceptable, expensive to use, or impractical on a large scale (Kirkpatrick and Frank 2005). In recent decades, immunocontraceptive vaccines incorporating antigens such as porcine zona pellucida (PZP) or gonadotropin-releasing hormone (GnRH) have been used to limit reproduction in feral horses (Killian et al. 2008, Kirkpatrick and Turner 2008, Gray et al. 2010). Manager interest in these agents increased because of the relative ease of their application, expected efficacy and duration, and anticipated lack of side-effects.

Porcine zona pellucida 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

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the zona pellucida and subsequent limited pregnancy in horses (Liu et al. 1989, Kirkpatrick et al. 1990). The most widely used formulations of PZP include a liquid form with a reported effectiveness of 7–10 months (Liu et al. 1989, 2005; Lyda et al. 2005), a time-release, heat-extruded (H/X) pellet form with a reported 22-month effectiveness (Turner et al. 2008), and a liposome-encapsulated liquid form known as SpayVac[®] (Immunovaccine Technologies, Halifax, NS, Canada), targeted for multiple-year effectiveness (Brown et al. 1997, Killian et al. 2008).

Detected physiological side-effects of PZP treatment have included a decrease in ovarian function when treatment is applied for >3 yr (Kirkpatrick et al. 1995), and injection-site reactions that appear to be nondebilitating in feral horses (Roelle and Ransom 2009). Reproductive behaviors were also found to be affected during the breeding season (Ransom et al. 2010) and band fidelity and reproductive behaviors were found to be affected outside of the breeding season (Nuñez et al. 2009).

Access to animals for timely inoculation and other management constraints may affect the utility of PZP as a management tool for western feral horse populations. We hypothesized that effectiveness of the treatments would be less than that previously demonstrated clinically. To evaluate this hypothesis, we applied PZP in liquid and pellet form in 3 western feral horse populations managed by BLM and assessed the effectiveness of the agent on individual female horses (hereafter, mares) from 2002 to 2008. Our objectives were to quantify the effectiveness of multiple years of contraception with liquid PZP and a single application of PZP pellets produced by an alternate, and more efficient, method (cold evaporation; Turner et al. 2008) on individual free-roaming mares in the context of age-specific foaling rates and actual management constraints.

STUDY AREAS

We inoculated mares with PZP at the Little Book Cliffs Wild Horse Range, the McCullough Peaks Herd Management Area, and the Pryor Mountain Wild Horse Range. The Little Book Cliffs Wild Horse Range, located in Mesa County, Colorado, USA (latitude 39°12'N, longitude 108°25'W), consisted of approximately 14,600 ha of sloping plateaus, sagebrush (*Artemisia* spp.) parks, and steep shale and sandstone cliffs dissected by 4 major canyon systems. Elevations ranged from 1,500 m to 2,250 m. The study area also exhibited dense stands of Colorado piñon (*Pinus edulis*) and Utah juniper (*Juniperus osteosperma*). Population size varied from 122 to 204 horses during the course of this study. Snow accumulation limited access to much of this study area during winter months, though horses that migrated into lower canyons could sometimes be accessed. The horses could only be approached close enough to dart (<60 m) when they were in open sagebrush parks.

Located in Park County, Wyoming, USA (latitude 44°35'N, longitude 108°40'W), the McCullough Peaks Herd Management Area consisted of 44,400 ha of open sagebrush park with badlands along the western edge. Vegetation consisted of large expanses of small shrubs,

grasses, and forbs. Elevations ranged from 1,200 m to 1,964 m. Population size was as high as 495 horses before a large management removal in 2004, and ranged from 112 to 194 in subsequent years. The horses in this population did not allow humans to approach within darting range and remote delivery of PZP was deemed impractical at this site at the time of study.

The Pryor Mountain Wild Horse Range, located in Bighorn County, Wyoming and Carbon County, Montana, USA (latitude 45°04'N, longitude 108°19'W), consisted of slightly >16,000 ha of low desert, foothill slopes, forested montane slopes, steep canyons, and isolated grassy plateaus. Elevations ranged from 1,190 m to 2,625 m. Three undeveloped roads intersected the study area and were impassable from late autumn to early spring due to snow accumulation and seasonal erosion. Horses in this population were more conditioned to humans and could be approached at close range (<40 m) with little or no evasive behavior throughout the summer and when located at accessible low elevations in winter.

METHODS

Vaccine Preparation

We used the liquid form of PZP at Little Book Cliffs and Pryor Mountain and the cold-evaporation (C/V) pellet form at McCullough Peaks. Personnel from the Science and Conservation Center at ZooMontana (Billings, MT) prepared the liquid agent according to the methods of Dunbar et al. (1980) and delivered both agent and adjuvant to us. We emulsified PZP and adjuvant in alcohol-sterilized syringes prior to use, as described by Kirkpatrick et al. (1990). For hand injections, we loaded the emulsion into alcohol-sterilized syringes fitted with single-use sterile needles. For injections by dart, we loaded the emulsion into 1-cm³ barbless darts (Pneu-Dart[®], Williamsport, PA) as they were removed from the manufacturer's packaging and placed a small dab of petroleum jelly on the tip of the dart to prevent leakage. Both primer and booster injections at Little Book Cliffs and Pryor Mountain contained 0.5 mL (100 µg) of PZP. Primer inoculations at Little Book Cliffs also contained 0.5 mL of Freund's complete adjuvant (FCA; Claassen et al. 1992, Kensil et al. 2004), whereas those at Pryor Mountain contained 0.5 mL of either FCA or Freund's modified adjuvant (FMA). Freund's complete adjuvant can produce false-positive tuberculosis test results in treated animals and has been known to contribute to abscessed injection sites (Broderson 1989); therefore, FMA was substituted for FCA after research indicated no difference in efficacy between the 2 adjuvants (Lyda et al. 2005). Booster inoculations were identical except that we substituted Freund's incomplete adjuvant (FIA). Additional details on darts and delivery methods can be found in Roelle and Ransom (2009).

Personnel at the Medical College of Ohio (now the College of Medicine, University of Toledo) prepared the time-release pellets using the cold-evaporation process described by Turner et al. (2008). All pellets contained a saponin adjuvant, QA-21 (Virbac Corporation, Brussels, Belgium), and were

designed to release PZP in bolus fashion at 1 month, 3 months, and 12 months. The 1-month and 3-month pellets each contained 100 µg of PZP and 200 µg of adjuvant; the 12-month pellets contained 250 µg of PZP and 500 µg of adjuvant. The PZP used in these pellets was obtained from ZooMontana.

Contraceptive Treatments

We initiated treatments at each of the 3 study areas in conjunction with gathers (roundups) conducted by BLM (Pryor Mountain in Sep 2001, Little Book Cliffs in Jul 2002, and McCullough Peaks in Oct 2004) and collected all data in accordance with Colorado State University Animal Care and Use Committee Standards (protocol 03-107A-02). Age classes and numbers of horses treated were in accordance with local plans developed by BLM managers. At Little Book Cliffs and McCullough Peaks, these management decisions were based on the number of mares in each class that needed to be contracepted in order to meet specific population growth goals, as predicted by the WinEquus model (Jenkins 1996). Managers at these sites randomly selected mares within each age class to be treated, and we randomly assigned control status to mares within bands to ensure that each band of horses being observed for a concurrent study of PZP effects on behavior contained both treated and untreated mares (Ransom et al. 2010). Treatment decisions at Pryor Mountain were not random across age classes, but rather all animals in given cohorts were treated, as discussed below. We gave all injections intramuscularly in the hip, regardless of delivery method.

We treated mares of all ages ≥ 1 yr at Little Book Cliffs. The primer dose was administered by hand to gathered mares while they were restrained in a squeeze-chute. Additional ungathered mares received the primer by dart in order to make up the desired sample size because an insufficient number of mares were captured. We delivered a booster inoculation by dart when the animals could be relocated (never sooner than 24 days), followed by annual boosters by dart. We recorded delivery distance of dart applications using Leica Rangemaster 1200 Scan laser rangefinders (Leica, Solms, Germany). We attempted to schedule the annual boosters such that mares would be continuously immunized. Duration of effectiveness of PZP has not been studied in detail, especially for multiple years of treatment.

At the time we initiated our experiments, available evidence indicated that PZP antibody titers would be maintained at contraceptive levels in many mares for 10 months following a first booster and that duration of efficacy may be extended following subsequent boosters (Liu et al. 1989, Kirkpatrick and Turner 2002). Based on these studies and consultation with the manufacturers of the drug, we assumed 10 months duration of efficacy for the first booster, 11 months for the second, and 12 months for all subsequent boosters. Using this schedule as a guide, most mares at Little Book Cliffs were treated for 4 consecutive yr, though a few were lost to the study for various reasons and 4 mares received 5 booster inoculations. We did not add young animals to the treatment

group as they reached 1 yr of age, but we did add them to the control group when they reached 3 yr of age. Precise age (observed as a foal) was available for 73.9% of 88 mares (both treatment and control) studied at Little Book Cliffs. Remaining mares were aged by tooth eruption and wear patterns (American Association of Equine Practitioners 2002).

At Pryor Mountain, treatments were similar to those at Little Book Cliffs except that we inoculated mares 1–2 yr of age (2002–2004) and older mares (≥ 15 yr, 2002–2004; ≥ 11 yr after 2004). These age classes were selected by managers to address public input concerning welfare of young and old females. Animals were, thus, added to the treatment group as they reached 1 yr of age (2002–2004) or 15 yr of age (11 yr of age after 2004), and controls consisted of middle-age animals that had never been treated. Treatments at Pryor Mountain also differed from those at Little Book Cliffs with respect to the timing of the first booster. At Pryor Mountain, management constraints (e.g., public appeals timeframe for Environmental Assessments) and weather forced us to deliver a number of first boosters only 14–16 days after the primer, instead of the 3–4 weeks used in previous studies (Kirkpatrick et al. 1997). Of the 83 treated or control mares at Pryor Mountain, ages of 64 (77.1%) were known (observed as a foal) from a long-term demography study (Roelle et al. 2010). We estimated ages of the remaining mares as at Little Book Cliffs.

At McCullough Peaks, we administered all inoculations in October 2004 while gathered mares were individually restrained in a squeeze-chute. We gave each mare a primer inoculation of liquid PZP (FMA as adjuvant), followed immediately by the time-release pellets, which were delivered by jabstick or hand-held trocar syringes and designed to act as boosters. Treated mares were of all ages ≥ 1 yr. We did not add mares to the treatment group in subsequent years, but we did add recruited mares to the control group when they reached 3 yr of age. We collected foaling data for 1 yr beyond the expected contraceptive effect in order to detect a possible residual effect, as was reported in the only similar study of time-release PZP (Turner et al. 2007). Precise ages were known for 34.4% of the 64 mares studied at McCullough Peaks (born during our study). We estimated ages of the remaining mares as at Little Book Cliffs.

Field Observations

U.S. Geological Survey (USGS) and BLM employees and volunteers recorded all field observations from the ground using optical equipment (binoculars, telescope) as necessary. These observers had completed undergraduate degrees in equine science, veterinary or preveterinary medicine, or biology, or had extensive experience working with equines. All mares and foals were identified and cataloged individually on the basis of pelage color, natural markings, and band association. Observers were trained in identifying individuals using these attributes and were provided with a photo database containing images of each individual for reference.

We located $>99\%$ of all mares and foals (when present) at least weekly from April to October of each year, and most

were relocated several times per week. Of those not located weekly, 4–6 mares annually at Little Book Cliffs were difficult to access, but were located at least monthly. Likewise, 1 mare at Pryor Mountain (2004) and 2 mares at McCullough Peaks (2007) were also not found weekly, but were observed at least monthly. Observations during the remainder of the year were opportunistic and largely dictated by weather. It is possible that some foals were born and died without being observed; however, given the intensity of observations, we believe that this rarely occurred. We matched foals with dams based on observations of attachment (e.g., nursing, general proximity) during the early days and weeks of a foal's life (Waring 2003); foaling rate was, thus, defined as the frequency of live births per cohort as detected by direct observation.

Data Analysis

The average gestation period for a horse is 335–342 days (Card and Hillman 1993); thus, mares are often pregnant when they receive a contraceptive agent, and the effect of contraception is not seen until the second foaling season following immunization. For example, a pregnant mare immunized in December of one year will give birth to a foal in the following spring, will be infertile for the subsequent breeding season, and the effect of that contraception will be seen in the next foaling season. We subsequently divided mares into 3 groups: untreated (control) animals that were never immunized; pretreatment animals that had been immunized, but the immunization had not yet taken effect; and treated animals that had been immunized and the immunization had presumptively taken effect. These strata were based on the date of booster treatment for each individual and the presumed duration of efficacy discussed previously.

We modeled foaling probability of mares with mixed-effects logistic regression, where individual mare was included as a random effect on the intercept term to account for the repeated observations (multiple yr) of individuals over time. This was necessary to account for variation that may be present among individuals who were sampled repeatedly, though not always equally over time. Such variation may arise from physiology, behavior, or any number of external factors that may affect conception, pregnancy, and parturition. We used the `lmer()` function in the `lme4` package in R version 2.10.0 (The R Foundation for Statistical Computing 2009) to obtain mixed-effects model estimates. If the variance estimate for the random effect of individual mare converged to zero, we re-estimated the model as a simpler fixed-effects logistic regression using the `glm()` function. Foaling rates of feral horses often increase for the first few years after mares reach sexual maturity, remain high through middle age, and decrease in older mares (Garrott and Taylor 1990, Roelle et al. 2010). We, therefore, included linear and quadratic effects of age in all models. We rescaled age in the quadratic effect by subtracting the mean age of horses (8.89 yr across all populations), so that the intercept term of the model corresponded to probability of foaling at mean age. We fitted separate models for each study area.

To assess effects of the immunocontraceptive, we compared untreated mares to pretreatment mares and untreated mares to treated mares. In both comparisons we used only years in common between the 2 groups so that any environmental effects were constant. We used the first comparison (untreated vs. pretreatment) to isolate any preexisting differences associated with potential nonrandom selection of individuals that could have arisen from allocation of controls per band rather than across the population. We modified the estimated odds ratio of foaling rates in the second comparison (untreated vs. treated mares) by the estimated odds ratio associated with the preexisting differences. The odds ratio of producing foals associated with the immunocontraceptive was then equal to $\exp(\hat{\beta}_{\text{treated}} - \hat{\beta}_{\text{pretreatment}})$ where $\hat{\beta}_{\text{treated}}$ was the estimated difference between treated and untreated mares and $\hat{\beta}_{\text{pretreatment}}$ was the estimated difference between pretreatment and untreated mares. We estimated variance for the combined odds ratio using simple formulas for linear combinations of uncorrelated random variables (Neter et al. 1996:1318). Because the estimated differences for pretreatment and treated groups used the same horses, the assumption of uncorrelated estimates was violated to some degree (le Cessie et al. 2008). Other models combining all 3 treatment groups, which ignored our desired balance across years, suggested that the estimated differences between untreated and pretreatment mares and between untreated and treated mares were positively correlated. Excluding a positive covariance in our variance estimates for the combined odds ratio produced a wider interval than would have resulted had we been able to account for the positive covariance. Thus, we consider our variance estimates for the combined odds ratios assuming zero correlation to be conservative. We estimated degrees of freedom for use with a *t*-distribution for constructing confidence intervals based on Satterthwaite (1946) as provided in Buckland et al. (1993:89).

At Little Book Cliffs and Pryor Mountain, mares received varying numbers of annual boosters: darts did not always function properly (Roelle and Ransom 2009), and it was not always possible to meet the schedule outlined above for keeping mares continuously contracepted. We used mixed-effects logistic regression to estimate probability of foaling among presumptively treated mares as a function of these variables. We included number of boosters received as a continuous variable and assigned a value for dart function (good or poor) based on physical examination of the dart after it was recovered. Darts were classified as poor if the vaccine did not fully discharge from the dart, pieces of the dart separated, or the needle broke on impact, and classified as good if the dart fully discharged and was structurally intact. We assigned a value for booster timing (good or poor) based on the number of days between injections. Consistent with the planned injection schedule for keeping mares continuously contracepted, we considered timing to be poor if >300 days (approx. 10 months) elapsed between the first and second boosters, >330 days (approx. 11 months) between the second and third, and >365 days (12 months) between all subsequent boosters. We also considered timing to be poor if the first booster was delivered during the

breeding season, which we defined based on foaling dates for untreated mares and an average gestation period (approx. 11 months). At Little Book Cliffs, 82.1% of births to untreated mares ($n = 117$ mare-yr) occurred in March–May, so we defined the breeding season as April–June. At Pryor Mountain, 93.5% of births to untreated mares ($n = 108$ mare-yr) occurred in April–June, so we defined the breeding season as May–July. We included linear and quadratic effects of age as in the previous comparisons.

At Little Book Cliffs and Pryor Mountain, we hypothesized that method of primer injection (dart or syringe) might affect foaling rate of treated mares in the first year of contraception. Also, at Pryor Mountain we hypothesized 2 additional factors might have an effect on foaling rate of treated mares in the first year of contraception: adjuvant used with the primer (FCA or FMA) and time between the primer and first booster (good or poor). We considered time between the primer and first booster to be poor if the booster was delivered <21 days after the primer. We examined foaling rates of treated mares as a function of these factors using fixed-effects logistic regression, again incorporating linear and quadratic terms for age of the mare.

RESULTS

At Little Book Cliffs, 25 individual mares composed the treatment group (received a primer inoculation and ≥ 1 booster inoculation, and were ≥ 3 yr of age at the time of expected parturition), and the untreated group (≥ 3 yr of age at the time of expected parturition) comprised 63 mares. At Pryor Mountain, 40 mares were treated (one additional mare was included in pretreatment analyses but died before a treatment effect could be detected) and 42 were untreated. At McCullough Peaks, 36 mares received the pellet form of the vaccine and 28 were untreated. Most mares were observed for foaling in multiple years and, unless otherwise noted, sample sizes reported hereafter represent mare-years.

Mares at Little Book Cliffs demonstrated a cumulative behavioral aversion to darting with first darts being delivered at an average of 28.6 ± 1.5 m (SE) linearly increasing to fourth darts being delivered at an average of 39.4 ± 3.3 m. Pryor Mountain horses were more conditioned to human presence and most received no >2 dart applications. Behavioral aversion to darting was not evident at this site; these horses received first darts at an average of 17.67 ± 2.1 m and second darts at an average of 12.2 ± 1.6 m.

At both Little Book Cliffs and Pryor Mountain, the original protocol called for boosters to be timed such that mares would always be effectively contracepted. This protocol was followed reasonably well at Little Book Cliffs, but at Pryor Mountain other management considerations and weather often intervened, and 72 of 79 boosters (91.1%) were delivered in August, September, or October, irrespective of when the previous booster was delivered. Delivering vaccinations by dart was seldom possible from November through February at either Little Book Cliffs or Pryor Mountain

because seasonal access to roads and trails was impeded by drifting snow.

Of the 6 foals born to treated mares at Little Book Cliffs, 3 were born to a single mare. At McCullough Peaks, 20 of the 32 foals born to treated mares were attributed to 4 mares that produced a foal in each of the 3 treatment yr and another 4 mares that produced a foal in each of 2 treatment yr. At Pryor Mountain, 4 of the 14 foals born to treated mares were accounted for by 2 mares that produced 2 foals each. Though the number of foals born to treated mares was relatively small, the timing of these births provides some quantitative evidence that second and subsequent boosters may have a longer contraceptive effect than does the first booster (Fig. 1).

In all 6 comparisons of treatment groups (untreated vs. pretreated and untreated vs. treated at 3 study areas) mare age affected foaling (either the linear or quadratic term, or both; $P < 0.05$). Across all populations in our study, there was a nonlinear relationship ($R^2 = 0.80$, $n = 972$ mare-yr) between age of control and pretreatment mares and foaling rate (Fig. 2). After controlling for mare age, foaling rate of untreated mares as compared to pretreatment mares was lower at Little Book Cliffs, and slightly higher at McCullough Peaks and Pryor Mountain; however, the difference only approached significance ($P = 0.09$) at Little Book Cliffs (Table 1).

The combined odds ratios indicate that PZP treatment reduced the foaling rate at all 3 study areas ($P < 0.05$; Table 1); however, effectiveness of the treatment varied by area. At Little Book Cliffs, the mean foaling rate observed among treated animals was 6.6% (range = 0.0–10.0% over 5 yr), whereas the mean annual foaling rate observed at Pryor Mountain was 17.7% (range = 0.0–35.0% over 5 yr). The mean annual foaling rate observed at McCullough Peaks was even higher (31.7%); interestingly, however, foaling rate in the third year of treatment effect at McCullough Peaks

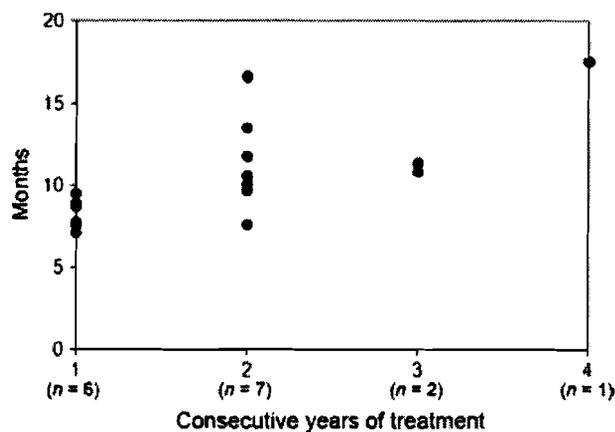


Figure 1. Foals born to feral horse (*Equus caballus*) females treated with the immunocontraceptive porcine zona pellucida (PZP) as a function of time (months) from booster inoculation to conception (calculated from parturition date) and the number of consecutive annual treatments received. Females inhabited Little Book Cliffs Wild Horse Range (Mesa County, CO) and Pryor Mountain Wild Horse Range (SE Carbon County, MT), USA, from 2002 to 2008, and received 1–4 booster inoculations containing a 1-cm³ emulsion of PZP and Freund's incomplete adjuvant.

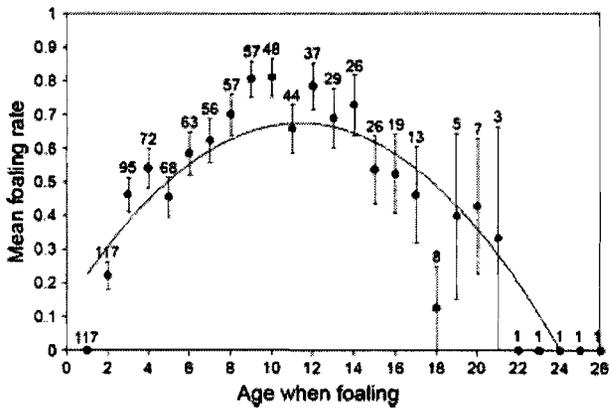


Figure 2. Mean foaling rates (\pm SE of the mean) for all ages of untreated and pretreatment feral horse (*Equus caballus*) females (n given at the top of each error bar) at Little Book Cliffs Wild Horse Range (Mesa County, CO, USA) 2002–2008, McCullough Peaks Wild Horse Management Area (Park County, WY, USA) 2003–2008, and Pryor Mountain Wild Horse Range (SE Carbon County, MT, USA) 2002–2008.

(29.0%, $n = 31$ mares) was nearly identical to that in the first year (27.8%, $n = 36$ mares), even though the pellets used at McCullough Peaks were designed for only 2 yr of efficacy.

When combined in a mixed-effects model with linear and quadratic effects of age and a random effect of individual mare, neither dart function ($z = 1.30$, $P = 0.20$), consecutive years of treatment ($z = 0.49$, $P = 0.62$), nor booster timing ($z = 1.04$, $P = 0.30$) affected foaling at Little Book Cliffs ($n = 91$; Table 2). Similarly, none of these covariates had an effect at Pryor Mountain (dart function, $z = 0.71$, $P = 0.48$; consecutive years of treatment, $z = -0.04$, $P = 0.97$; booster timing, $z = -0.32$, $P = 0.75$; $n = 79$). When combined in a fixed-effects model with linear and quadratic effects of age, time between the primer and first

booster also had no effect ($z = 0.38$, $P = 0.70$) at Pryor Mountain, nor did adjuvant used with the primer ($z = 0.15$, $P = 0.88$; $n = 40$ mares). There was no random effect of individual mare in this model because we considered only the first booster for each mare. Lack of significance for the primer adjuvant term, despite the relatively large difference in foaling rates (FCA = 7.4%, FMA = 46.2%; Table 2) may be explained by the fact that all (17) of the mares ≤ 6 yr of age, most of which would have a low foaling rate in the absence of contraception, received FCA as the primer adjuvant, and none produced foals. An exact Chi-square test on the remaining 23 mares (all ≥ 12 yr of age) also showed no effect of the primer adjuvant (FCA, 2 of 10 mares foaled; FMA, 6 of 13 foaled; $P = 0.38$). No models could be properly fitted for foaling rate in response to method of primer delivery because of the uneven distribution of delivery methods at each site (Table 2).

DISCUSSION

Feral horses treated using 2 different forms of the immunocontraceptive PZP exhibited decreased foaling rates compared to untreated mares. Fertility was strongly related to age of mares and proved to be critical in understanding the effectiveness of treatment. Both the liquid and C/V forms of PZP were robust in terms of timing and the various management constraints encountered during application periods, though we suspect the difference in foaling rates between the 2 sites where liquid PZP was used relate to such challenges. Few treated mares produced foals in our study, but there is some evidence that repeated annual boosters increased the effective duration of each treatment.

Several investigators have noted that individual mares vary greatly in their ability to raise antibody titers against PZP and that some mares may simply not respond (Liu et al. 1989,

Table 1. Logistic regression comparisons of foaling rates (%) for untreated, pretreatment, and treated females at mean age in 3 feral horse herds in the western United States, 2002–2008. Contraceptive treatments consisted of liquid porcine zona pellucida (PZP) followed by annual boosters at Little Book Cliffs Wild Horse Range (Mesa County, CO) and Pryor Mountain Wild Horse Range (SE Carbon County, MT), and a liquid primer plus time-release PZP pellets at McCullough Peaks Herd Management Area (Park County, WY).

Comparisons and parameter values	Little Book Cliffs	Pryor Mountain	McCullough Peaks
Pretreatment vs. untreated			
Years of comparison	2002–2004	2002–2007	2003–2005
n pretreatment	41	116	88
n untreated	92	153	23
Estimated foaling rate, pretreatment (95% CI)	82.9 (67.3–92.0)	65.9 (51.2–78.0)	83.1 (69.3–91.4)
Estimated foaling rate, untreated (95% CI)	70.3 (57.0–80.9)	71.0 (60.5–79.7)	87.0 (66.3–95.8)
Odds ratio	2.051	0.786	0.734
95% CI for odds ratio	0.878–4.790	0.376–1.647	0.230–2.337
P -value (H_0 : foaling rates are equal)	0.094	0.522	0.596
Treated vs. untreated			
Years of comparison	2004–2008	2004–2008	2006–2008
n treated	91	79	101
n untreated	153	129	48
Estimated foaling rate, treated (95% CI)	8.0 (3.4–17.5)	34.0 (13.8–62.3)	30.2 (17.4–47.0)
Estimated foaling rate, untreated (95% CI)	73.7 (62.5–82.5)	73.4 (62.2–82.2)	89.7 (68.8–97.2)
Odds ratio	0.031	0.187	0.049
95% CI for odds ratio	0.011–0.083	0.059–0.598	0.013–0.189
P -value (H_0 : foaling rates are equal)	<0.001	0.004	<0.001
Treated vs. untreated (age adjusted)			
Combined odds ratio	0.015	0.238	0.067
95% CI for combined odds ratio	0.004–0.056	0.060–0.950	0.011–0.403

Table 2. Comparisons of covariates potentially affecting foaling rates (%) among treated females in 2 feral horse herds in the western United States, 2002–2008. Rates given are calculated from the observed data and are not controlled for age. Contraceptive treatments consisted of liquid porcine zona pellucida (PZP) followed by annual boosters at both Little Book Cliffs Wild Horse Range (Mesa County, CO) and Pryor Mountain Wild Horse Range (SE Carbon County, MT). FCA = Freund's complete adjuvant, FMA = Freund's modified adjuvant.

Covariate	Little Book Cliffs		Pryor Mountain	
	Foaling rate (%)	<i>n</i>	Foaling rate (%)	<i>n</i>
Booster timing				
Good	5.1	59	19.4	31
Poor	9.4	32	16.7	48
Dart function				
Good	5.8	86	16.9	71
Poor	20.0	5	25.0	8
Consecutive years of treatment				
1	8.0	25	20.0	40
2	4.4	23	21.4	28
3	9.1	22	0.0	6
4	5.9	17	0.0	5
5	0.0	4	—	—
Time between primer and first booster				
<21 days	—	—	26.7	15
≥21 days	8.0	25	16.0	25
Primer adjuvant				
FCA	8.0	25	7.4	27
FMA	—	—	46.2	13
Primer delivery method				
Hand injection	10.5	19	0.0	3
Dart	0.0	6	21.6	37

Turner et al. 2002, Lyda et al. 2005). Our results, particularly at Little Book Cliffs and McCullough Peaks, where multiple births to individual mares accounted for a high proportion of the foals born to treated animals, seem to confirm this conclusion. Early investigations also showed that application of PZP does not affect pregnancies in progress (Liu et al. 1989, Kirkpatrick et al. 1990). Our results support this conclusion; foaling rates among pretreatment mares at all 3 study areas were greater than or equal to foaling rates among untreated mares.

Our results at Little Book Cliffs (6.6% observed foaling rate) for mares treated with liquid PZP are similar to those reported previously. Turner and Kirkpatrick (2002) reported that 34 of 340 mares (10.0%) that received a primer followed by annual boosters produced foals on Assateague Island National Seashore, Maryland, USA. Later in the same multiyear study, the authors reported a foaling rate of 3.9% ($n = 861$) among treated mares (Kirkpatrick and Turner 2008; table 1). In a composite sample from multiple western horse herds, Turner et al. (2002) found an 8% foaling rate ($n = 376$) among treated mares. In contrast to the multiyear study on Assateague Island, however, mares in these western herds received only a single booster inoculation.

At Pryor Mountain, the foaling rate observed among treated mares (17.7%) was somewhat higher than that found at Little Book Cliffs and reported in other studies (Turner and Kirkpatrick 2002, Turner et al. 2002, Kirkpatrick and Turner 2008). Although none of the covariates we included in the models were significant at either Little Book Cliffs or Pryor Mountain, we suspect that timing of the boosters, especially in the first and second years, may be responsible for the higher foaling rate at Pryor Mountain. In the only other multiyear study of PZP efficacy in feral horses

(Assateague Island), researchers delivered boosters in the spring to maximize the probability that anti-PZP titers would remain high and mares would remain contracepted throughout the following breeding season, rather than attempting to keep mares contracepted throughout the year (Kirkpatrick et al. 1990). At some (unspecified) point in time, however, investigators at Assateague began delivering boosters in August or September if the mares had already been contracepted for ≥ 2 yr (Turner and Kirkpatrick 2002). Delivery of the booster in August or September allowed more efficient access to the mares and apparently did not affect efficacy of the treatments (Turner and Kirkpatrick 2002). Actual booster timing at Pryor Mountain was, thus, similar to the revised approach at Assateague, except that mares received boosters in August–October irrespective of the number of years contracepted. If the first PZP booster is effective for only 7–10 months (Liu et al. 1989, 2005; Lyda et al. 2005), then first boosters delivered in August–October may not result in effective contraception throughout the following breeding season. The same may be true for second boosters, for which duration of efficacy is not well-known. Apart from this potential effect at Pryor Mountain, however, effectiveness of the liquid form of PZP appears to have been quite robust with respect to timing of boosters.

Foaling rates among mares at McCullough Peaks treated with the C/V pellets were higher than those reported in the only other study where a presumptive multiple-year, time-release form of PZP was used on horses. Using H/X pellets on the Clan Alpine herd in Nevada, USA, investigators found foaling rates of 5.2% ($n = 58$) in the first year of treatment, 14.9% ($n = 68$) in the second year, and 31.6% ($n = 19$) in the third year (Turner et al. 2007:table 4). Foaling rates in the first 2 yr were significantly different

from rates among untreated mares ($P < 0.001$), and investigators felt that there was some residual, though statistically nonsignificant, effect in the third year (31.6% foaling, $n = 19$; Turner et al. 2007). Observed foaling rate in the third year of our study (29.0%) was remarkably similar, suggesting no advantage of the C/V pellets in terms of increased duration of efficacy.

Differences between our results and those from Clan Alpine appear to be related to the pellet formulation (C/V vs. H/X). Subsequent to the initiation of our study, Turner et al. (2008) reported that C/V pellets performed poorly in vitro; >80% of the total PZP was released before the desired window. In white-tailed deer (*Odocoileus virginianus*), C/V pellets were also less effective in the first year (2 of 8 F produced fawns) than H/X pellets (0 of 9 F produced fawns), though sample sizes were small (Turner et al. 2008).

The primer doses for both liquid and pellet applications contained either FCA or FMA, which are widely recognized as being highly immunogenic (Kensil et al. 2004): the PZP dosage in the liquid boosters and the 1-month and 3-month pellets was equal and the 12-month pellet contained 2.5 times more PZP. However, whereas the booster doses of liquid PZP contained FIA, the pellet doses contained QA-21. The adjuvant dosage was much higher for QA-21 than for FIA, and QA-21 is also highly immunogenic (Kensil et al. 2004), but it is possible this difference in booster adjuvants could have contributed to the effectiveness of treatment observed.

Our study also illustrated the importance of considering age-specific differences in foal production when evaluating the effects of a contraceptive. In most previous studies of which we are aware, age has been addressed by selecting only mares in prime reproductive status, usually ages 6–15. This is a reasonable approach in situations where investigators are able to select specific mares for treatment; however, this is not the case in most western United States feral horse populations, where treatments necessarily span a broader range of ages. Age was a significant covariate in all of the models we used to compare treatment groups. Furthermore, we might well have reached an incorrect conclusion regarding importance of the primer adjuvant had we not included mare age in our comparisons. Equivalence of FCA and FMA in raising antibody titers has also been demonstrated in vitro (Lyda et al. 2005) and in vivo at Assateague Island, where the switch from FCA to FMA has apparently not affected effectiveness of the treatments.

Liquid doses of PZP can be delivered by dart or by hand injection. Pellet forms of PZP can currently only be delivered by hand injection in conjunction with gathers, but researchers are developing the technology to apply pellets via dart. The liquid form of PZP appears to reduce foaling rates more effectively than the C/V pellet form, but considerable time can be expended in delivering the drug by dart, depending on wariness of the animals. The additional challenge of cumulative behavioral aversion to dart application may be a long-term concern for repeated vaccinations in areas where horses are not accustomed to human presence. Feral horses in the

western United States are often inaccessible at the optimal time for an annual inoculation (late winter); therefore, if PZP is applied remotely in the liquid form, scheduling boosters such that mares are continuously contracepted appears to be a more effective strategy than a static annual date of inoculation.

To date, time-release (pellet) technology appears to offer the greatest promise for a multiyear immunization using PZP and, based on the 2 studies completed thus far, pellets produced by the H/X process appear to be superior to those produced by the C/V process. Both types, however, significantly reduced foaling in treated mares as compared to controls. Pellets produced by a third process referred to as pressure-molding have not yet been tested in vivo in feral horses (Turner et al. 2008). The liposome-encapsulated form of PZP, SpayVac[®], may also be useful as a multiyear contraceptive agent, but to date has been tested in only a small number of horses (Killian et al. 2008, Gray et al. 2010).

MANAGEMENT IMPLICATIONS

In herds where feral horse mares can be accessed annually for booster inoculations, the liquid form of PZP delivered by dart can reduce foaling rates and the effective duration of each treatment may increase with repeated annual applications. However, a multiyear contraceptive agent would be preferable for use in most cases, even if horses can be accessed for annual boosters, because of increased efficiency in application and decreased problems with behavioral aversion to treatment and ability to locate individual animals on the range. Ultimate success in limiting population growth will depend not only on the efficacy of the agent, but also on the proportion of mares that can be accessed for treatment (Garrott 1991, 1995; Kirkpatrick and Turner 2008) and the age of mares treated. Porcine zona pellucida appears to be a robust form of fertility control in feral horses and able to withstand a wide array of application challenges in the field, though effectiveness may vary considerably by drug form and application strategy. Managers must also consider the scope and magnitudes of potential side-effects associated with use of the drug and weigh the known effects against the benefit of reducing population growth.

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