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Research Article

Immunocontraception and Increased Longevity in Equids

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Intensive population management by means of fertility control has been shown to change the age profile of a wild horse herd. The primary change has been an increase in the number and percent of older animals, as expected, but also the appearance of new and older age classes. An examination of direct effects of fertility control on two groups of treated animals shows a significant increase in longevity over non-treated animals that is associated with contraceptive treatment. The mean age at death (MAD) was calculated for 128 wild horses for which precise birth and death dates were known, including 56 stallions, 42 untreated mares, 11 mares treated with a porcine zona pellucida contraceptive vaccine for 1–2 years, and 19 mares treated with the same vaccine for ≥ 3 years. The MAD for stallions (10.3 ± 0.84 [SEM] years), and mares treated for 1–2 years (10.2 ± 0.56), was significantly greater ($P < 0.05$) than for untreated mares (6.4 ± 0.85), and significantly $< 19.9 \pm 1.66$ for mares treated ≥ 3 years (19.9 ± 1.66). Zoo Biol 26:237–244, 2007. © 2006 Wiley-Liss, Inc.

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INTRODUCTION

The application of porcine zona pellucida (PZP) immunocontraception to captive and free-ranging wildlife, for population management has increased since its initial use for this purpose in 1988 [Kirkpatrick et al., 1990a]. Previous research has

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shown the PZP vaccine to be efficacious at both the level of the individual animal of many species [Kirkpatrick et al., 1990a, 1995a, 1996; Turner et al., 1996; Shideler et al., 2002; Frank et al., 2005] and in managing entire populations [Turner and Kirkpatrick, 2002; Naugle et al., 2002; Deigert et al., 2003; Rutberg et al., 2004] of captive exotic and native free-ranging species. Additionally, other studies have documented the vaccine's safety with regard to pregnant animals, the reversibility of contraceptive action in equids [Kirkpatrick et al., 1991a, 1992, 1995a; Kirkpatrick and Turner, 2002], cervids [Turner et al., 1996; Naugle et al., 2002; Rutberg et al., 2004], African elephants [Fayrer-Hosken et al., 2000] and the lack of effect on foal survival or out-of-season births in equids [Kirkpatrick and Turner, 2003].

The ability to manage population growth in some manner, either by slowing growth rates, or achieving zero population growth or even through herd reduction has been shown after long-term studies [Kirkpatrick and Turner, 2002; Turner and Kirkpatrick, 2002], but several unanticipated phenomena have modified expectations for managing populations. First, it was not clear at the outset of research with this vaccine, 17 years ago, just what long-term health effects the application of the vaccine might have for individual animals and how these effects might modify population management. Two of the more significant changes that have been noted include a significant improvement in body condition among chronically treated wild mares and an increase in new and older age classes at the population level [Turner and Kirkpatrick, 2002]. It has been hypothesized that these two phenomena were related [Turner and Kirkpatrick, 2002].

A comparison of age classes in a PZP-treated population of wild horses on Assateague Island National Seashore (ASIS), in 1990, 1995, and 2001 [Turner and Kirkpatrick, 2002] showed a steady shift to older age classes in this population. The causes for this increase in older animals were not surprising and were assumed to be the result of fewer pregnancies among treated animals and a resulting increase in body condition, less mortality, and therefore, longer life. The age class shift, however, was apparent only at the population level and not at the level of individual animals.

It was the purpose of this retrospective study to examine the relationship between PZP treatment in two groups of animals of differing treatment protocols and untreated animals, and increased longevity. The working hypothesis was that increasing levels of contraception were associated with increased longevity in the two animal cohorts of the study population, and that both treatment groups would show increased longevity over non-treated animals.

MATERIALS AND METHODS

Study Population

The study population was made up of free-ranging wild horses inhabiting Assateague Island National Seashore in Maryland. This population has inhabited the island for approximately 330 years at varying densities and population numbers. In 1965, the Maryland portion of the island, comprising 16,083 hectares was designated as Assateague Island National Seashore, under the management of the National Park Service (NPS) and at that time the 28 wild horses inhabiting the national Seashore were declared a cultural and historic resource and included among the fauna of the island. In 1988, when research with the PZP vaccine was initiated with 26 mares [Kirkpatrick et al., 1990a], the herd population was 129. From 1988 through 1993, 46 different mares were inoculated for varying periods of time as part of the original research effort, and during that time, the herd size grew to 156. Beginning in 1994, with the herd size at 166, every remaining untreated mare that was 2 years or older was given a single "primer" dose of PZP and in 1995, with the herd size at 173, a contraceptive management plan was developed and initiated, with an immediate goal of reducing the population to 150 animals. For the first several years the management plan called for the inoculation of all 2-year old mares, and booster inoculations of PZP at age 3 and 4, followed by withdrawal of treatment until the mare produced three living offspring or a second generation, after which the mare would be treated with the PZP annually until her death. This provided for a delay in the reproductive age of mares, but a continuous contribution of genes from all mares on the island and this approach resulted in zero population growth almost immediately [Turner and Kirkpatrick, 2002]. This plan was modified in 1998 to allow only two foals after withdrawal from treatment, and again in 2000 to allow only a single foal, largely because there was no apparent decrease in the population as a result of contraception. By March 2006, the population had decreased to 140 (a 19% decrease) without the need for removal of animals.

Vaccine Application

The vaccine was prepared according to the modified method of Dunbar et al. [1980] at the University of California-Davis from 1988–1997 and at the Science and Conservation Center thereafter. Delivery of the vaccine was by 1.0 cc Pneu-Darts, (Pneu-Dart, Inc., Williamsport, PA) at doses of 100 μg, with an appropriate adjuvant, as described by Kirkpatrick et al. (1990a, 1991b) and authorized under the Food and Drug Administration Investigational New Animal Drug Exemption File 8857-G002. No horses were captured or handled during the course of this study.

Pregnancy Testing

To assess pregnancy loss or undetected neonatal losses, 346 horses were pregnancy-tested by means of urinary or fecal steroid conjugates [Kirkpatrick et al., 1990a, 1991b] between 1988–2005. Fall pregnancy rates based on estrone conjugate analysis were compared to foals counted the following spring and summer.

Data Collection

All animals in the Assateague population are monitored every other month by an ASIS employee (AT) and data collected included: 1) identification of individual animals by unique markings, 2) band affiliation, 3) band location, 4) band habitat use, 5) the presence and date of new foals, and 6) the date and death of older animals. Not all carcasses of dead animals are discovered immediately, but the continued absence of animals from their bands is noted on a monthly basis, thus the date of death on a yearly basis is standard and in most cases, the date of death can be noted by month.

The data presented in this retrospective study come from records for 128 horses for whose dates of birth and death are certain, covering the period from 1976–2005. Only horses that lived to be a minimum of 2 years old were included, to normalize data to the treated population; on ASIS, PZP treatment is always initially administered at 2 years of age and contraceptive results would not become apparent until animals were 3 years old. Horses dying from car collisions, accidental death by deer hunters, and drowning by a tidal surge in 1992 were not included in this study, thus, only those horses that died from causes unrelated to human activities and natural disasters were included. The horses were grouped into four cohorts, including: 1) 56 stallions, 2) 42 mares that had never been treated with PZP, 3) 11 mares that had been treated for up to 2 years, and 4) 19 mares treated for 3 or more years. The mean age at death (MAD) was calculated for each group and compared to the MAD of the groups and the differences tested for significance by paired *t*-test.

RESULTS

The results are presented in Table 1. In general, there was increasing longevity among individual animals from untreated mares dying at the youngest ages (6.4 ± 0.85 years), to stallions, and mares treated up to 2 years dying at intermediate ages (10.3 ± 0.84 and 10.2 ± 0.56 years, respectively, to mares treated for ≥ 3 years (19.9 ± 1.66 years). The mean age at death (MAD) of the 56 stallions was not significantly different from the MAD of mares treated for <3 years ($P = 0.973$; $t = 0.032$), but was significantly greater than that of untreated mares ($P = 0.0006$; $t = 3.53$), and significantly less than the MAD of mares treated for ≥ 3 years ($P = 0.005$; $t = 5.51$). The MAD of untreated mares was significantly less than that of mares treated for <3 years ($P = 0.064$; $t = 2.84$), and significantly less than the MAD of mares treated for ≥ 3 years ($P = 0.0001$; $t = 9.67$). The MAD of mares treated for <3 years was significantly less than the MAD of mares treated for ≥ 3 years ($P = 0.005$; $t = 3.92$).

DISCUSSION

Age-specific reproductive success for the ASIS horses indicates an increasing trend from age 3 through >6 years [Keiper and Houpt, 1984]. This pattern is similar to that seen in other wild horse herds in which reproductive success is low for 1- and 2-year-olds, increasing from 3–5 years and reaching peak reproductive success between 6–15 years [Garrott et al., 1991]. On ASIS, however, mares do not normally breed until they are 3 years old. After that point, fertility rates of 4-year-old mares

TABLE 1. Mean ages at death for Assateague horses as a function of length of PZP treatment

	A	B	C	D
Group	Stallions	Untreated mares	Mares treated <3 years	Mares treated ≥3 years
Number	56	42	11	19
Mean age at death (year)	10.33[b]	6.47[a]	10.27[2]	19.94[c]
SEM	0.845	0.566	1.477	1.666

[a]Differs significantly from Groups A,C,D.
[b]Differs significantly from Groups B,D.
[c]Differs from Groups A–C.

were 46%, that of 5-year-old mares were 53%, and that of 6-year-old mares were 69% [Keiper and Houpt, 1984], which is consistent with the data from the study of Garrott et al. [1991], in western wild horses.

The standard treatment protocol in the ASIS management plan applies initial contraception at 2 years, and booster inoculations at 3 and 4 years of age. Kirkpatrick and Turner [2002] showed that ASIS mares treated for 3 consecutive years would take anywhere from 1 to 4+ years to become fertile again, based on current reversibility data [Kirkpatrick and Turner, 2002], which would cause a significant decrease in foaling from age 5 to beyond 9 years, which are the years of peak reproductive success. On ASIS, a study of 14 mares, all 3 years or older, over an 8-year period and before any contraceptive treatment, showed that they produced a mean of 5.0 foals during that 8-year period [Keiper and Houpt, 1984]. Foals among a different set of 14 ASIS mares, all 3 years of age or older and treated with PZP for varying periods of time, produced a mean of 0.5 foals during their lives. Thus, PZP treatment at 2, 3, and 4 years of age can have a significant negative effect on fertility and foaling. On ASIS, once a treated animal produces a living foal, she is placed back on contraception until extinction, further reducing the number of foals produced in her life.

The increased longevity associated with PZP treatment, in PZP-treated animals, is associated clearly with two phenomena reported earlier [Turner and Kirkpatrick, 2002], including improved body condition and lower mortality. The body condition scores of lactating mares have been shown to be significantly lower than those of non-lactating mares [Rudman and Keiper, 1991]. The energy requirements for a pregnant or lactating mare are significantly greater than for open mares. An open mare requires approximately 17.8 mcal/day, whereas an 11-month pregnant mare increases to 21.4 mcal, and to 30.7 mcal in early lactation [National Research Council, 1989]. These values are for domestic horses in protected environments and do not take into account the greater nutritional needs of wild horses living under harsh conditions or game park animals, where access to food may not be equitable. Thus, it is not surprising that body condition improves in mares as fertility decreases due to contraception. The link between increased body condition and increased longevity in horses is less well understood, but clearly this study suggests some correlation between the two.

In studies of this nature there are several potential sources of error. The first includes mistakes in the aging of animals, which is usually based on tooth wear. This approach to aging is particularly tenuous in older age classes. The second source of error is found in undetected pregnancies because of fetal losses or undetected neonatal losses. The third source of error involves basing reproductive success on pregnancy testing or physical condition of mares, to eliminate the undetected pregnancy and neonatal loss problem. This can include the misclassification of reproductive condition, based on pregnant mares serum (PMSG) or blood progesterone analysis for pregnancy detection or inferred lactational condition based on distended udders.

Pregnancy detection using equine chorionic gonadotropin, or PMSG measurements are relatively accurate through 100 days of pregnancy, but decline after that. In a study of reproductive rates of western wild horses [Wolfe et al., 1989] PMSG analysis was only 53.7% accurate, probably because of the lateness of testing

(August through February), well after PMSG levels had declined below the limits of the test. The use of serum progesterone analysis alone can also lead to inaccurate pregnancy rates. In two studies [Wolfe et al., 1989; Garrott et al., 1991] serum progesterone analyses resulted in accuracy rates of 85% and 82%, respectively. The pitfalls using this approach include a failure to distinguish cycling mares from pregnant mares, and prolonged elevated progesterone concentrations for weeks to months after aborted fetuses [Ginther, 1979].

Assessing pregnancy status on the basis of lactating mares or mares with distended udders has also been shown to be relatively inaccurate. A number of investigators have reported the majority of foal mortality during the first 1–2 neonatal months in wild horses [Welsh, 1973; Boyd, 1979; Keiper, 1979], thus mares examined after that period would show no distension of the udders and be misclassified as not having foaled.

None of these potential errors were a factor in the current study, where birth and death dates were absolutely correct and the presence of foals was confirmed by direct observation. Additionally, pregnancy testing on ASIS is based on urinary or fecal estrone conjugate analysis rather than progesterone or progesterone metabolites alone [Kirkpatrick et al., 1986; Daels et al., 1991]. Of 346 horses tested for pregnancy, 334 proved accurate based on the absence or presence of foals, for a 96.5% accuracy rate. Of 12 horses whose pregnancy status was not predicted accurately on the basis of urinary or fecal steroid metabolite analysis, 7 were false positives and 5 were false negatives. The 7 false positive horses may in fact have been accurately pregnant at the time of testing, and experienced fetal loss later in the year. Urinary estrone conjugate concentrations, however, decline precipitously within 12 hr of loss of the fetus [Daels et al., 1991]. Additionally, urinary estrone conjugate concentrations can distinguish between pregnant animals and cycling animals, and are useful from approximately Day 40 of pregnancy to parturition [Daels et al., 1991; Lasley and Kirkpatrick, 1991]. Previous studies using either urinary estrone conjugates alone [Kirkpatrick et al., 1990b] or with fecal estrone conjugates [Kirkpatrick and Turner, 1991a,b; Kirkpatrick et al., 1991b] have shown accuracy approaching 100%. In this retrospective study, pregnancy testing accuracy, over 18 years, was a minimum of 96.5% accurate. Thus, in this study, error has been minimized or possibly even eliminated.

CONCLUSIONS

Contraception can be effective in managing free-ranging wildlife or captive game park populations [Deigert et al., 2003], and in the process, longevity of individual animals will be increased significantly, probably across all species. This, in turn, will have profound effects on the age profile of the herd, and the intensity of fertility control application necessary to achieve any given population or growth rate goal. At the same time, contraception will improve the health of individual animals and consequently, the target herd.

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REFERENCES

Boyd LE. 1979. The mare-foal demography of feral horses in Wyoming's Red Desert. In: Denniston RH, editor. Symposium on the Ecology and Behavior of Wild and Feral Equids, University of Wyoming Press, Laramie, WY.

Daels PF, Ammon DC, Stabenfeldt GH, Liu IKM, Hughes JP, Lasley BL. 1991. Urinary and plasma estrogen conjugates, estradiol and estrone concentrations in nonpregnant and early pregnant mares. Theriogenology 35:1001–17.

Deigert FA, Duncan A, Lyda RO, Frank KM, Kirkpatrick JF. 2003. Immunocontraception of captive exotic species. III. Fallow deer (Cervus dama). Zoo Biol 22:261–8.

Dunbar BS, Waldrip NJ, Hedrick J. 1980. Isolation, physiochemical properties and macromolecular composition of zona pellucida from porcine ovaries. Biochemistry 19:356–65.

Fayrer-Hosken RA, Grobler D, Van Altena JJ, Kirkpatrick JF, Bertschinger H. 2000. Immunocontraception of free-roaming African elephants. Nature 407:149.

Frank KM, Lyda RO, Kirkpatrick JF. 2005. Immunocontraception of captive exotic species. IV. Species differences in response to the porcine zona pellucida vaccine and the timing of booster inoculations. Zoo Biol 24:349–58.

Garrott RA, Eagle TC, Plotka ED. 1991. Age-specific reproduction in feral horses. Can J Zool 69:738–43.

Ginther OJ. 1979. Reproductive biology of the mare. Cross Plains, WI: Equiserve, Inc. p 334–8.

Keiper RR. 1979. Population dynamics of feral ponies. In: Denniston RH, editor. Symposium on the Ecology and Behavior of Wild and Feral Equids, University of Wyoming Press, Laramie, WY.

Keiper RR, Houpt K. 1984. Reproduction in feral horses: an eight-year study. Am J Vet Res 45: 991–5.

Kirkpatrick JF, Turner JW. 1991a. Changes in herd stallions among feral horse bands and the absence of forced copulation and induced abortion. Behav Ecol Sociobiol 29:217–20.

Kirkpatrick JF, Turner JW. 1991b. Compensatory reproduction among feral horses. J Wildl Manage 55:649–52.

Kirkpatrick JF, Turner A. 2002. Reversibility of action and safety during pregnancy of immunizing against porcine zona pellucida in wild mares (*Equus caballus*). Reprod Suppl 60:197–202.

Kirkpatrick JF, Turner A. 2003. Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island horses. J Appl Anim Welfare Sci 6: 301–8.

Kirkpatrick JF, Liu IKM, Turner JW. 1990a. Remotely-delivered immunocontraception in feral horses. Wildl Soc Bull 18:326–30.

Kirkpatrick JF, Shideler SE, Turner JW. 1990b. Pregnancy determination in uncaptured feral horses based on free steroids in feces and steroid metabolites in urine-soaked snow. Can J Zool 68:2576–9.

Kirkpatrick JF, Liu IKM, Turner JW, Bernoco M. 1991a. Antigen recognition in mares previously immunized with porcine zona pellucida. J Reprod Fertil Suppl 44:321–5.

Kirkpatrick JF, Shideler SE, Lasley BL, Turner JW. 1991b. Pregnancy determination in uncaptured feral horses by means of fecal steroid conjugates. Theriogenology 35:753–9.

Kirkpatrick JF, Liu IKM, Turner JW, Naugle R, Keiper R. 1992. Long-term effects of porcine zona pellucida immunocontraception on ovarian function of feral horses (*Equus caballus*). J Reprod Fertil 93:437–44.

Kirkpatrick JF, Naugle R, Liu IKM, Bernoco M, Turner JW. 1995a. Effects of seven consecutive years of porcine zona pellucida immunocontraception in feral mares. Bio Reprod Monogr Series 1: Equine Reproduction VI. p 411–8.

Kirkpatrick JF, Zimmermann W, Kolter L, Liu IKM, Turner JW. 1995b. Immunocontraception of captive exotic species: I. Przewalski's horses (*Equus przewalskii*) and banteng (*Bos javanacus*). Zoo Biol 14:403–13.

Kirkpatrick JF, Calle PP, Kalk P, Liu IKM, Bernoco M, Turner JW. 1996. Immunocontraception of captive exotic species. II. Formosan sika deer (*Cervus nippon taiouanus*), Axis deer (*Cervis axis*), Himalayan tahr (*Hemitragus jem lahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), muntjac deer (*Muntiacus reevesi*), and sambar deer (*Cervus unicolor*). J Zoo Wildl Med 27: 482–95.

Lasley BL, Kirkpatrick JF. 1991. Monitoring ovarian function in captive exotic species and free-roaming wildlife by means of urinary and fecal steroids. J Zoo Wildl Med 22:23–31.

National Research Council. 1989. Nutrient requirements of horses. Washington, DC: National Academy of Sciences.

Naugle RE, Rutberg AT, Underwood HB, Turner JW, Liu IKM. 2002. Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, New York, USA. Reprod Suppl 60: 143–53.

Rudman R, Keiper RR. 1991. The body condition of feral ponies on Assateague Island. Equine Vet J 21:453–6.

Rutberg AT, Naugle RE, Thiele LA, Liu IKM. 2004. Effects of immunocontraception on a suburban population of white-tailed deer *Odocoileus virginianus*. Biol Conserv 116: 243–50.

Shideler SE, Stoops MA, Gee NA, Howell JA, Lasley BL. 2002. Use of porcine zona pellucida (PZP) vaccine as a contraceptive agent in free-ranging mule elk (*Cervus elaphus nanodes*). Reprod Suppl 60:169–76.

Turner JW, Kirkpatrick JF, Liu IKM. 1996. Effectiveness, reversibility and serum antibody

titers associated with immunocontraception in captive white-tailed deer. J Wildl Manage 60: 45–51.
Turner A, Kirkpatrick JF. 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). Reprod Suppl 60:187–95.
Welsh AD. 1973. Population, behavioral and grazing ecology of the horse of Sable Island, Nova Scotia. PhD Thesis. Halifax, Nova Scotia: Dalhousie University.
Wolfe ML, Ellis LC, Macmullen R. 1989. Reproductive rates of feral horses and burros. J Wildl Manage 53:916–924.