Zoo Biology 24:349–358 (2005)

RESEARCH ARTICLE

Immunocontraception of Captive Exotic Species

IV. Species Differences in Response to the Porcine Zona Pellucida Vaccine, Timing of Booster Inoculations, and Procedural Failures

Kimberly M. Frank*, **Robin O. Lyda, and Jay F. Kirkpatrick**

Science and Conservation Center, ZooMontana, Billings, Montana

The biological efficacy of porcine zona pellucida (PZP) immunocontraception and the timing of booster inoculations were determined in 24 species of ungulates and five species of non-ungulates across 10 years of treatment and for which no technical problems were identified. The collective contraceptive efficacy for 265 ungulates, 25 bears, and 11 sea lions, across 517 contraceptive intervals was 93.3%, and contraceptive efficacy ranged from a low of 60% in nyala (*Taurotragus angasi*) to 100% in 16 other species. The timing of annual booster inoculations was adjusted, between 6 months and 12 months, depending on the length of the breeding season and the species-specific immune responses. The high degree of efficacy in bears and sea lions suggests that the PZP vaccine has some application in certain non-ungulate species. Technical problems in the timing and delivery of the vaccine can cause non-biological failures and were identified in an additional 20 animals, not included above, and included inoculating pregnant animals, the use of needles too short for effective intramuscular injections, failure to complete the initial inoculation series, and failure to give booster inoculations at the prescribed time. Zoo Biol 24:349–358, 2005. © 2005 Wiley-Liss, Inc.

Key words: contraception; fertility control; immunocontraception; porcine zona pellucida

*Correspondence to: Kimberly M. Frank, Science and Conservation Center, 2100 South Shiloh Road, Billings, MT 59106. E-mail: zoolzb@wtp.net

Received 23 October 2004; Accepted 29 December 2004

DOI 10.1002/zoo.20060
Published online 27 May 2005 in Wiley InterScience (www.interscience.wiley.com).

INTRODUCTION

The use of porcine zona pellucida (PZP) immunocontraception in zoos has increased to include 112 distinct species in 103 zoos since it was first reported to the American Association of Zoo Veterinarians in 1992. There are 26 distinct species for which five or more animals are on a PZP treatment protocol and complete data have been retrieved from participating institutions. In this study, data from three Malayan sun bears (*Helarctos malayanus*) and two Asian black bears (*Selenarctos thibetanus*) were pooled with that from 12 brown bears (*Ursus arctos*), and eight North American black bears (*Ursus americanus*), and data from 14 Grevy's zebra (*Equus grevyi*), eight Burchelli's zebra (*Equus burchelli*) and three Mountain zebra (*Equus zebra*) were also pooled. Ungulates form the largest single taxon group and have provided the largest body of information regarding effectiveness and safety of PZP treatment [Kirkpatrick et al., 1995b, 1996; Frisbie and Kirkpatrick, 1998; Frank and Kirkpatrick 2002; Deigert et al., 2003]. Previous research reports established the efficacy of PZP immunocontraception in certain primates, including humans [Sacco, 1977; Koyama et al., 1992], cynomolgus monkeys (*Macaca fascicularis*) [Gulyas et al., 1983; Martinez et al., 1996], rhesus monkeys (*Macaca mulatta*), marmosets (*Callithrix jacchus*) [Grootenhuis et al., 1996], baboons (*Papio anubis*) [Dunbar et al., 1989], bonnet monkeys (*Macaca radiata*) [Kolluri et al., 1995], and squirrel monkeys (*Saimiri sciureus*) [Sacco et al., 1983, 1987, 1989, 1991].

The application of PZP immunocontraception to captive zoo species grew out of previous success in its application to wild horses (*Equus caballus*) [Kirkpatrick et al., 1990, 1991, 1992, 1995b; Kirkpatrick and Turner 2002, 2003; Turner and Kirkpatrick, 2002], and white-tailed deer (*Odocoileus virginianus*) [Turner et al., 1992, 1996; McShea et al., 1997; Naugle et al., 2002; Rutberg et al., 2004] in which two initial inoculations and a single annual booster inoculation could cause and maintain contraceptive antibody titers and subsequent infertility. For example, in horses, doses of 65–100 μg of PZP provided effective (≥90%) contraception. The first booster inoculation was required about 3 weeks after the first exposure to the vaccine, and annual booster inoculations sustained contraceptive effects thereafter. Deer and horses are highly seasonal with respect to reproduction and this has a bearing on the contraceptive effectiveness of this protocol.

As contraceptive data have been accumulated for captive zoo species, it has become clear that there are some similarities between results obtained for horses and deer and other captive species with regard to the effectiveness of the PZP vaccine. The same 65–100 μg PZP dose seems to be effective in the vast majority of captive species and particularly the ungulates [Kirkpatrick et al., 1995a, 1996; Frisbie and Kirkpatrick 1998; Frank and Kirkpatrick 2002; Deigert et al., 2003]. This is not surprising considering the evolutionary conservation of the mammalian zona pellucida sperm receptor site and its molecular homogeneity [Sacco et al., 1981]. Larger doses of PZP (600 μg) have been used only in African elephants (*Loxodonta africana*) [Fayrer-Hosken et al., 2000], but experiments in South Africa have demonstrated that significantly smaller doses also work [Delsink et al., 2002]. In a number of captive exotic species, less seasonal breeding patterns seem to result in the need for more frequent booster inoculations, whereas differences in immune responses in some species may lead to contraceptive effects that are longer in duration than in other species and therefore require less frequent booster inoculations.

This study examines the biological efficacy of PZP immunocontraception for 301 individual captive animals across 29 species for animals treated between 1992 and 2002 (for which complete data has been recovered from participating zoos), the technical problems associated with non-biological failures for an additional 20 animals, and specific recommendations for the timing of booster inoculations in each species.

MATERIALS AND METHODS

PZP Vaccine Preparation

The native PZP antigen used in these studies was prepared from porcine oocytes by the modified method of Dunbar et al. [1980], and was stored and tested for purity and pathogens as described by Deigert et al. [2003]. The antigen was shipped to zoos on dry ice and stored frozen until the time of use, under the authority of The Food and Drug Administration Investigational New Animal Drug Exemption (No. 8840-G003-004) for PZP contraception of zoo animals.

Selection of Animals

Animals treated in the course of this study were selected by individual zoo managers on the basis of population management, genetic management, or health problems that contraindicated pregnancy. The animals used in this study were picked from a larger pool of treated animals and selected on the basis of complete data sheets returned by the zoos. All data reported in this study came from animals of proven fertility that were paired with proven male breeders. No other contraceptives were given to the study animals during the course of this study.

Dose and Adjuvant Protocols

All animals were given intramuscular inoculations of 65–100 μg of PZP (in PBS) in the hip or gluteal regions, either by hand injection or remotely by 1.0 cc dart. The total volume of the inoculation was 1.0 cc and included 0.5 cc of PZP antigen + 0.5 cc of adjuvant. Adjuvants used include Freund's Complete Adjuvant (FCA), Freund's Incomplete Adjuvant (FIA), (Sigma, St. Louis, MO) and Freund's Modified Adjuvant (FMA; Calbiochem, La Jolla, CA). The antigen was emulsified with the adjuvant immediately before immunization as described by Kirkpatrick et al. [1990].

Four different adjuvant protocols have been used over the 10 years reflected in this data review. In equids, species for which no reliable tuberculosis test exists, the adjuvant protocol of choice was an initial inoculation with PZP + FCA followed by one or two subsequent inoculations with PZP + FIA over 6 weeks. In all other ungulates the protocols included three inoculations over 6 weeks with only PZP + FIA: an initial inoculation with FMA, followed by one or two subsequent inoculations with PZP + FIA; or an initial inoculation with PZP + FMA followed by a second inoculation of PZP-FMA. The FMA does not lead to false positive TB tests. A complete treatment was defined as an initial series of two or three inoculations or the completed annual booster inoculation at the specified time (6–12 months) depending on the species. In those species for which 20 or more animals were included (bear, collectively, giraffe [*Giraffa camelopardalis*], ibex [*Capra ibex*], and zebra, collectively) differences in efficacy between the three protocols were tested for significance by Fisher's exact test for contingency tables [Motulsky et al., 2001].

Definition of Contraceptive Interval

A contraceptive interval (CI) is defined as the period of time between a completed initial treatment series until the next scheduled booster treatment, or the time between scheduled annual booster inoculations. For example, for species in which annual booster inoculations were given, a CI would be the time span between the completion of the initial series of inoculations and the time of the annual booster inoculation, i.e., 12 months.

Non-Biological Causes for Contraceptive Failure

A confounding problem that affects accurate evaluation of the biological efficacy of the PZP vaccine's contraceptive effects was a number of technical problems involving the handling of the vaccine or its delivery, or the timing of its delivery, any of which may interfere with contraception. These technical problems were identified and documented in an additional 20 animals.

RESULTS

Table 1 compares the biological contraceptive efficacy for 29 species (four species of bear and four of zebra given collectively), and the time of booster inoculations for each species. Contraceptive efficacy ranged from a low of 60% to highs of 100% across the 30 species. Based on a total of 517 contraceptive intervals in 301 individual animals of 30 species, mean contraceptive efficacy was 93.8%. The recommended time of booster inoculations was determined by trial and error or by evaluation of antibody titers [Kirkpatrick et al., 1996].

Non-biological technical problems were identified and documented in 20 additional animals and included: initiation of vaccination in already-pregnant animals, failure to complete the initial inoculation series, failure to give booster inoculations at the specified time, placing treated females with males too soon (<2 weeks) after the end of the initial series of inoculations, and the use of inappropriate delivery equipment, such as dart needles that are too short for an intramuscular injection in the particular species [Frank and Kirkpatrick, 2002]. To establish true biological efficacy of the PZP vaccine, contraceptive failures resulting from confirmed technical problems have not been included in the data provided below in Table 1.

Among giraffe for which complete data have been recovered, there were 13 failures during the first year of treatment and one during the second year, and all were attributed to confirmed procedural failures. Of these 13 failures, six animals were already pregnant at the time of first treatment, two animals did not have the three inoculation series completed, one had an inadequate injection because of the dart needle's length (too short), and one was bred before the inoculation series was completed. Presumably, antibody titers had not reached contraceptive levels before the treated animal was reintroduced to the male. Antibody titer data from horses [Liu et al., 1989], fallow deer (*Cervus dama*) [Deigert et al., 2003], white-tailed deer

TABLE 1. Biological contraceptive efficacy and recommended timing for booster inoculations for 29 species

Species	*n*	Contraceptive intervals	Pregnancies	% Efficacy	Recommended booster timing (months)
Order Perissodactyla					
Equidae					
Przewalski's horse (*Equus przewalskii*)	11	11	0	100	7–9
Zebra[a]	26	56	2	96.5	7–9
Order Artiodactyla					
Bovidae					
Addax (*Addax nasomaculatus*)	5	12	1	91.6	7–9
Bison (*Bison bison*)	11	14	0	100	7–9
Bongo (*Taurotragus euryceros*)	7	12	2	83	7–9
Gerenuk (*Litocranius walleri*)	10	10	0	100	7–9
Ibex (*Capra ibex*)	20	57	2	96.4	7–9
Kudu (*Taurotragus strepiceros*)	9	36	1	97.2	7–9
Markhor (*Capra falconeri*)	6	10	0	100	7–9
Nyala (*Taurotragus angasi*)	9	10	4	60	7–9
Bighorn Sheep (*Ovis canadensis*)	11	14	0	100	12
Mountain Goat (*Oreamnos americanus*)	12	13	0	100	12
Himalayan Tahr (*Hemitragus jemlahicus*)	17	21	2	95.2	7–9
Cervidae					
Axis deer (*Cervus axis*)	5	10	2	80.0	7–9
Sika deer (*Cervus Nippon*)	13	32	2	93.7	12
Wapiti (*Cervus elaphus*)	11	11	0	100	7–9
Fallow deer (*Cervus axis*)	9	18	0	100	12
Moose (*Alces alces*)	6	9	0	100	7–9
Muntjac deer (*Muntiacus reevesi*)	6	10	3	70	6
Reindeer (*Rangifer tarandus*)	10	12	3	75	7–9
Sambar deer (*Cervus unicolor*)	16	28	7	75	7–9
Giraffidae giraffe (*Giraffa camelopardalis*)	30	67	2	97	7–9
Order Carnivora					
Ursidae	25	30	0	100	12
Bear[b]					
Otariidae					
California Sea Lion (*Zalophus californian*)	11	11	0	100	12

[a]Includes Grevy's zebra (*Equus grevyi*), plains zebra (*Equus burchelli*), mountain zebra (*Equus zebra*), and hybrids; both pregnancies occurred with *E. grevyi*.
[b]American black bear (*U. americanus*), sun bear (*Helarctos malayanus*), brown bear (*U. arctos*), Asian black bear (*Selenarctos thibetanus*).

[Turner et al., 1996] suggest that 2 weeks are required from the end of the initial treatment series before antibody titers are adequate to block fertilization. Thus, it is recommended that at least 2 weeks is allowed after the last inoculation of the initial series before females can be safely placed with males. In another two animals boosters were given late, after the 9-month recommendation. It should be noted that the 9-month recommendation emerged as a result of this study, thus these two failures actually preceded that recommendation. The single second year failure was due to a failure to give a booster inoculation. Among seven zebra for which complete data have been retrieved, there were four first-year failures, two second-year failures, and one third-year failure, all attributable to confirmed procedural failures. Three first-year failures were attributable to the initial three inoculation series not being completed. Another animal was already pregnant at the time of initial treatment. Both of the second-year failures and the third-year failure were due to boosters not given on time. There were no significant differences ($P < 0.05$) in contraceptive efficacy among the four study groups that consisted of 20 or more animals and for which different adjuvant protocols were used.

DISCUSSION

The mean 93.8% biological efficacy across all species is similar to the approximate 90% efficacy reported for wild horses [Turner and Kirkpatrick, 2002] and white-tailed deer [Turner et al., 1992]. This is expected, considering the ubiquitous and physiochemical nature of the mammalian zona pellucida and its physiological role in fertilization [Liang and Dean, 1993] and that 24 of 29 species studied were ungulates. There are, however, a group of species such as the felids in which cross reactivity of the PZP antibodies with the zona sperm receptor is poor and contraception does not result [Gorman et al., 2002; Harrenstien et al., 2004].

There are several biological reasons why a contraceptive PZP vaccine might fail. The first is failure of the vaccine to elicit sufficient antibody titers in the target animal, most likely caused by relative homology between PZP and target animals' zona proteins. The best example here is the failure of PZP, derived from porcine protein, to raise significant antibodies in any member of the porcine family where the antigen is identified as "self." The second cause of PZP failure is a failure of resulting antibodies to cross-react with the sperm receptor proteins of the target animal's zona pellucida. A good example of the second type of failure is seen in the differences between the zona epitopes of the cat (*Felis catus*) and the pig (*Sus scrofa*), where, although antibodies can be raised by inoculating cats with PZP, the antibodies will not cause steric hindrance of the cat zona sperm receptor [Jewgenow et al., 2000].

The third reason is failure of the antibodies to remain at adequately high titers to prevent fertilization over the course of the breeding season. The duration of contraceptive action, and by implication, the antibody titers, seem to be quite variable across some species. Over the past 10 years it has become clear that not all captive mammalian species mount identical immune responses with regard to duration of contraceptive effects. Bighorn sheep (*Ovis canadensis*) and Rocky Mountain goats (*Oreamnos americanus*) provide an example of species differences among ungulates. Three big horn sheep inoculated in 1996 with a series of three PZP injections over 6 weeks remained infertile for > 2 years without any subsequent booster inoculations; one lambed in 1999 and again in 2000, and another lambed in 2000. Among eight mountain goats inoculated with a single series in 1996, only one lambed by 1999 (and again in 2000) and one other lambed in 2000. Thus, in goats and sheep, a single initial PZP series will maintain infertility for > 2 years and, in most cases, longer. A single California sea lion (*Zalophus californianus*) that was treated with a single series of PZP inoculations in 1995 and bred with a proven male did not produce a pup until 1998, and despite further breeding, has produced none since that time.

In the case of the big horn sheep, the significantly longer duration of contraception may be attributable to more than sustained antibody titers and interference with fertilization. A recent study indicates that domestic sheep (*Ovis aries*) treated with PZP + FCA demonstrate a high antibody response but also an interference with growing ovarian follicles and a significant reduction in the number of primordial follicles (B.L. Lasley, personal communication). These data from domestic sheep are consistent with a similar destruction of oocytes in the rabbit ovary after exposure to PZP antibodies [Skinner et al., 1984]. In contrast, there is no evidence of this histological phenomenon in horses (L. Munson, personal communication), white-tailed deer [McShea et al., 1997] and wapiti (*Cervus elaphus nannoides*) [Shideler et al., 2002] after treatment with PZP, pointing to still another possible species difference. The absence of histological data from mountain goats and sea lions leaves the question of long-acting effect of treatment in these two species unanswered.

With the exception of a small number of Przewalski horses (*E. przewalskii*) and zebras (all species) that received FCA for the initial inoculation, all animals in this study received either PZP + FIA × 3, or PZP + FMA followed by PZP + FIA, or PZP + FMA followed by PZP + FMA for adjuvant protocols. Recent data for fallow deer [Deigert et al., 2003] indicate that there is a significantly greater antibody response in animals treated with FMA for the initial inoculation than for those receiving three inoculations of PZP – FIA, and that these responses are similar to those seen in white-tailed deer treated with PZP + FCA [Turner et al., 1996]. There were, however, no differences in contraceptive efficacy between the protocols after the initial inoculation series was completed. All three protocols used provided similar contraceptive efficacy. The change in protocols described in this current study reflected a shift from FCA, to avoid possible tuberculosis-positive testing, and from the three inoculations of FIA to reduce the number of inoculations necessary, rather than any significant differences in contraceptive efficacy.

The issue of timing the "annual" booster inoculations properly is dependent upon the seasonal breeding patterns of target species. Certain free-ranging species, such as wild horses, have a more defined and narrow breeding season than their domestic counterparts [Satoh and Hoshi, 1932; Kenny et al., 1975; Palmer, 1978; Kirkpatrick and Turner, 1982]. Anecdotal evidence from zoo populations of other ungulate species indicates that breeding seasons can be extended in captive populations. In the case of highly seasonal species, a single annual booster inoculation, given 2–4 weeks before the onset of breeding activity will suffice to maintain contraceptive efficacy. In less seasonal species, booster inoculations will have to be given more frequently. In the case of most zoo ungulate populations, booster inoculations given every 7–9 months seem to maintain contraceptive protection. Muntjac deer (*Muntiacus reevesi*) require booster inoculations every 6 months, based on antibody titer profiles [Kirkpatrick et al., 1996]. Contraceptive efficacy in muntjac deer has improved after recommending these shorter intervals for booster inoculations.

Although it is clear that PZP contraception of ungulates is effective, less is known about the vaccine's effectiveness in other orders within Mammalia. There is some evidence that PZP is not effective as a contraceptive in felids [Gorman et al., 2002; Harrenstien et al., 2004] and a number of unpublished reports provide conflicting data for efficacy in canids. The high degree of efficacy in bears, however, suggests that the vaccine has some utility in certain species other than ungulates. A study with grey seals (*Halichoerus grypus*) [Brown et al., 1997] demonstrated highly effective contraception using a liposomal preparation of PZP. These data corroborate the contraceptive action of PZP and the cross-reactivity of the antibodies against PZP and sperm binding proteins in the 11 sea lions in this current study. Among 25 bears treated with the PZP, over 30 CIs, the resulting 100% efficacy supports the effectiveness of this form of immunocontraception among members of Ursidae. Relatively little is known about the effectiveness or long-term consequences of PZP immunocontraception, or any form of immunocontraception for that matter, beyond ungulates and primates. Schwarzenberger et al. [1998] examined the ovarian endocrine cycle of several sun bears, two of which had been treated with PZP and are included in this study. Fecal steroids indicated that 2 years of PZP treatment was associated with missing luteal function and erratic follicular activity, however, there was also missing luteal function in three of four untreated bears as well. The two PZP-treated bears later returned to fertility, thus the ovarian disturbances did not interfere with reversibility of contraceptive effects. Future application of this form of contraception in other species must be conducted carefully and systematically before it can be recommended on a large scale.

CONCLUSIONS

This study indicates that the biological efficacy of PZP contraception in 29 species ranges from 60–100% and averages 93.8%, the timing of booster inoculations must be adjusted to the length of the breeding season and immune response for each species, and treated females should not be placed with males until at least 2 weeks after the completion of the initial series of inoculations.

ACKNOWLEDGMENTS

Special thanks to the many zoo veterinarians and keepers who have been diligent in their efforts to provide us with timely and accurate data, a list too long to recite here. Thanks to I.K.M. Liu for the preparation of PZP before 1998, and to B.L. Lasley and S.E. Shideler for reviewing earlier drafts of this paper.

REFERENCES

Brown RG, Bowen WD, Eddington JD, Kimmins WC, Mezei M, Parsons JL, Pohajdak B. 1997. Evidence for a long-lasting single administration contraceptive in wild grey seals. J Reprod Immunol 35:43–51.

Deigert FA, Duncan AE, Frank KM, Lyda RO, Kirkpatrick JF. 2003. Immunocontraception of Captive Exotic Species. III. Contraception and population management of fallow deer (*Cervus dama*). Zoo Biol 22:261–8.

Delsink AK, Van Altena JJ, Kirkpatrick JF, Grobler D, Fayrer-Hosken RA. 2002. Field applications of immunocontraception in African elephants (*Loxodonta africana*). Reprod Suppl 60:117–24.

Dunbar BS, Lo C, Powell J, Stevens VC. 1989. Use of a synthetic peptide adjuvant for the immunization of baboons with denatured and deglycosylated pig zona pellucida glycoproteins. Fertil Steril 52:311–8.

Dunbar BS, Wardrip NJ, Hedrick JL. 1980. Isolation, physicochemical properties, and macromolecular composition of zona pellucida from porcine oocytes. Biochemistry 19:356–65.

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

Frank KM, Kirkpatrick JF. 2002. Porcine zona pellucida immunocontraception in exotic species: species differences, adjuvant protocols, and technical problems. Proceedings of the American Association of Zoos and Veterinarians Annual Meeting, Milwaukee, WI. p 221–3.

Frisbie KM, Kirkpatrick JF. 1998. Immunocontraception of captive exotic species: a new approach to population management. Animal Keepers Forum 25:346–50.

Gorman SP, Levy JK, Hampton AL, Collante WR, Harris AL, Brown RG. 2002. Evaluation of a porcine zona pellucida vaccine for the immunocontraception of domestic kittens (*Felis catus*). Theriogenology 58:135–49.

Grootenhuis AJ, Philipsen HLA, de Breet-Grijsbach JTM, van Duin M. 1996. Immunocytochemical localization of ZP3 in primordial follicles of rabbit, marmoset, rhesus monkey and human ovaries using antibodies against human ZP3. J Reprod Fertil Suppl 50:43–54.

Gulyas BJ, Gwatkin RBL, Yuan LC. 1983. Active immunization of cynomolgus monkeys (*Macaca fascicularis*) with porcine zonae pellucidae. Gamete Res 4:299–307.

Harrenstien LA, Munson L, Chassy LM, Liu IKM, Kirkpatrick JF. 2004. Effects of porcine zona pellucida immunocontraceptives in zoo felids. J Zoo Wildl Med 35:271–9.

Jewgenow K, Rohleder M, Wegner I. 2000. Differences between antigenic determinants of pig and cat zona pellucida proteins. J Reprod Fertil 11:15–23.

Kenny RM, Ganjam VK, Bergman RV. 1975. Non-infectious breeding problems in mares. Vet Scope 19:16–24.

Kirkpatrick JF, Turner JW. 1982. Seasonal ovarian function in feral mares: seasonal patterns of LH, progestins and estrogens in feral mares. J Equine Vet Sci 3:113–8.

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

Kirkpatrick JF, Turner A. 2003. Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island wild horses. J Appl Anim Welfare Sci 6:30–308.

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

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

Kirkpatrick JF, Liu IKM, Turner JW, Naugle R, Keiper R. 1992. Long-term effects of porcine zona pellucida immunocontraception on ovarian function in feral horses (*Equus caballus*). J Reprod Fertil 94:437–44.

Kirkpatrick JF, Zimmermann W, Kolter L, Liu IKM, Turner JW. 1995b. Immunocontraception of captive exotic species. I. Przewalski's horse (*Equus przewalskii*) and banteng (*Bos javanicus*). Zoo Biol 14:403–16.

Kirkpatrick JF, Naugle R, Liu IKM, Turner JW. 1995a. Effects of seven years of porcine zona pellucida contraception on ovarian function in feral mares. Biol Reprod Monogr Ser 1:411–3.

Kirkpatrick JF, Calle PP, Kalk P, Liu IKM, Turner JW. 1996. Immunocontraception of captive exotic species. II. Formosa sika deer (*Cervis nippon nippon*), Axis deer (*Cervus axis*), Himalayan tahr (*Hemitragus jemlahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), Reeve's muntjac (*Muntiacus reevesi*) and Sambar deer (*Cervus unicolor*). J Zoo Wildl Med 27:482–95.

Kolluri SK, Kaul R, Banerjee K, Gupta SK. 1995. Nucleotide sequence of cDNA encoding bonnet monkey (*Macaca radiata*) zona pellucida glycoprotein-ZP3. Reprod Fertil Dev 7:1209–12.

Koyama K, Hasegawa A, Inoue M, Isojima S. 1992. Blocking of human sperm-zona interaction by monoclonal antibodies to a glycoprotein family (ZP4) of porcine zona pellucida. Biol Reprod 45:727–35.

Liang L, Dean J. 1993. Conservation of mammalian secondary sperm receptor gene enables the promoter of the human gene to function in mouse oocytes. Dev Biol 156:399–408.

Liu IKM, Bernoco M, Feldman M. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. J Reprod Fertil 85:19–29.

Martinez ML, Fontenot GK, Harris JD. 1996. The expression and localization of zona pellucida glycoproteins and mRNA in cynomolgus monkeys (*Macaca fascicularis*). J Reprod Fertil Suppl 50:35–41.

McShea WJ, Monfort SL, Hakim S, Kirkpatrick JF, Liu IKM, Turner JW, Chassy L, Munson L. 1997. The effect of immunocontraception on the behavior and reproduction of white-tailed deer. J Wildl Manage 61:560–9.

Motulsky HJ, Searle P, Platt M, Pilkington J. 2001. GraftPad Software, Inc., San Diego, Ca.

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.

Palmer E. 1978. Control of the estrous cycle of the mare. J Reprod Fertil 54:495–505.

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.

Sacco AG. 1977. Antigenic cross-reactivity between human and pig zona pellucida. Biol Reprod 16:164–73.

Sacco AT, Yurewicz EC, Subramanian MG, DeMayo FJ. 1981. Zona pellucida composition: species cross reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA). Biol Reprod 25:997–1008.

Sacco AG, Subramanian MG, Yurewicz EM, DeMayo J, Dukelow WR. 1983. Heteroimmunization of squirrel monkeys (*Saimiri sciureus*) with a purified porcine zona pellucida antigen (PPZA): immune response and biologic activity of antiserum. Fertil Steril 39:350–8.

Sacco AG, Pierce DL, Subramanian MG, Yurewicz EC, Dukelow WR. 1987. Ovaries remain functional in squirrel monkeys (*Saimiri sciureus*) immunized with a purified porcine zona 55,000 macromolecule. Biol Reprod 36:481–90.

Sacco AG, Yurewicz EC, Subramanian MG. 1989. Effects of varying dosages and adjuvants on antibody response in squirrel monkeys (*Saimiri sciureus*) immunized with the porcine zona pellucida M_r = 55,000 glycoprotein (ZP3). Am J Reprod Immunol 21:1–8.

Sacco AG, Yurewicz EC, Subramanian MG, Ye L, Dukelow WR. 1991. Immunologic response and ovarian histology of squirrel monkeys (*Saimiri sciureus*) immunized with porcine zona pellucida ZP3 (M_r = 55,000) macromolecules. Am J Primatol 24:15–28.

Satoh S, Hoshi S. 1932. A study of reproduction in the mare. J Japan Soc Vet Sci 11:257–67.

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 tule elk (*Cervus elaphus nannodes*). Reprod Suppl 60:169–76.

Skinner SM, Mills T, Kirchick HJ, Dunbar BS. 1984. Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. Endocrinology 115: 2418–32.

Schwarzenberger F, Schaller K, Chadwick Y, Pagan O, Kolter L. 1998. Faecal steroid analysis for monitoring ovarian function and the effect of PZP (porcine zona pellucida protein) in the sun bear (*Helarctos malayanus*). Proceedings of the European Association of Zoos and Wildlife Veterinarians. Chester, UK. p 387–95.

Turner A, Kirkpatrick JF. 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). Reprod Suppl 60:187–95.

Turner JW, Kirkpatrick JF, Liu IKM. 1992. Remotely-delivered immunocontraception of captive white-tailed deer. J Wildl Manage 56: 154–7.

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.