Journal of Reproduction and Fertility Supplement **50**, 183–189

Applications of pig zona pellucida immunocontraception to wildlife fertility control

J. F. Kirkpatrick[1], J. W. Turner, Jr[2], I. K. M. Liu[3] and R. Fayrer-Hosken[4]

[1]*ZooMontana, PO Box 80905, Billings, MT 59108, USA;* [2]*Department of Physiology, Medical College of Ohio, Toledo, OH 43699, USA;* [3]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; and* [4]*Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA*

A unique application of pig zona pellucida (PZP) immunocontraception is the control of wildlife populations. A native PZP vaccine has been successfully applied to wild horse and donkey populations. A single annual booster inoculation was capable of maintaining contraception. Seven consecutive years of PZP treatment in wild mares resulted in no detectable debilitating side effects, and reversibility of contraception has been documented among mares treated for up to 4 consecutive years. Long-term treatment (5–7 years) is associated with some ovulation failure and depressed urinary oestrogen concentrations. Complex social behaviours in horses were unaffected by treatment. PZP immunocontraception has also been successfully applied to white-tailed deer, with no detectable changes in ovarian histology after 2 years of treatment. Seventy-four species of captive zoo animals have been treated with the PZP vaccine, with documented success in 27 species, including members of the orders Perissodactyla (Equidae), Artiodactyla (Cervidae, Capridae, Giraffidae, Bovidae), and Carnivora (Ursidae, Mustelidae, Felidae). Immunocytochemistry studies have demonstrated a high degree of crossreactivity between anti-PZP antibodies and African elephant zona pellucida. The need for a one-inoculation form of the vaccine has led to the incorporation of PZP into lactide–glycolide microspheres, which cause a delayed release of the PZP. PZP immunocontraception of wildlife has potential because of (1) > 90% effectiveness, (2) the ability for remote delivery, via darts, (3) reversibility after short-term use, (4) a wide breadth of effectiveness across many species, (5) a lack of debilitating side-effects even after long-term treatment, and (6) minimal effects upon social behaviours.

Introduction

The majority of research devoted to zona pellucida fertilization-blockers has been directed at potential contraception in humans. A unique application of zona pellucida (ZP) vaccines is contraception of certain wildlife populations, where legal restrictions or social objections to lethal control have made fertility control the only remaining option (Kirkpatrick and Turner, 1985). Steroid-based wildlife contraception has proved unacceptable because of difficulties with delivery (Kirkpatrick, 1995), various associated pathologies (Linnehan and Edwards, 1991), and the ability of steroids to pass through food chains (Kirkpatrick and Turner, 1991).

Table 1. Foaling rates for pig zona pellucida-treated, sham-treated and untreated Assateague mares

Group	Inoculations per horse	*n*	Percentage of mares producing foals: Pretreatment 1987	Pretreatment 1988	Post-treatment 1989
Treated	3	18	50.0	51.1	0.0
Treated	2	8	62.4	37.4	0.0
Sham-treated	0	6	33.3	33.3	50.0
Untreated	0	11	—	—	45.4

Modified from Kirkpatrick *et al.*, 1990.

There are certain similarities in the concerns of human-directed and wildlife-directed ZP contraceptive research. These include reversibility (although in some species, like white-tailed deer, this is not an issue), safety in pregnant animals, health side effects and effects upon behaviour. Despite these similarities, there are two issues that are unique to wildlife-directed ZP contraceptive research. The first is the need for remote delivery, either by dart injection or oral administration, and the second is the need for a one-inoculation, long-acting form of the vaccine (Turner and Kirkpatrick, 1991). The remainder of this review examines progress to date with ZP wildlife immunocontraceptive research in wild and feral equids, white-tailed deer, captive exotic zoo animals, and free-ranging African elephants, and directions for future research.

Applications in Wildlife

Wild and feral equids

The first application of immunocontraception in wildlife occurred in wild horses (*Equus caballus*) in 1988. A native pig zona pellucida vaccine (PZP) was administered remotely in 65 µg doses by means of darts to 26 mares on Assateague Island National Seashore (ASIS). Mares received either two or three inoculations over 8 weeks, and contraception was 100% successful (Table 1) (Kirkpatrick *et al.*, 1990). Fourteen of the mares were pregnant at the time of PZP treatment and all delivered healthy foals whose survival rates were not different from those of foals from untreated mares. Complex social organization and behaviours were unaffected. A year later a single booster inoculation (65 µg) was given to 14 of the 26 mares and only a single foal was born (Kirkpatrick *et al.*, 1991a). The remaining 12 mares were not treated and five produced foals (41.6%), a rate not different from untreated mares on ASIS. Over 7 years (120 mare-years) only four foals were born to treated mares and the difference in fertility between treated and untreated mares was significant ($P < 0.001$) (Kirkpatrick *et al.*, in press). Two of the four foals born to treated mares were produced by the same mare. During that 7 year period, a total of 27 mares received their initial PZP inoculations during the later stages of pregnancy and all 27 delivered foals. Three mares from the foals delivered in 1988 have reached maturity and have produced foals of their own (Kirkpatrick *et al.*, 1995a). The use of PZP contraception for the control of ASIS wild horse populations has now reached the management stage.

In a second, larger study of PZP contraception of wild horses, animals were rounded up in Nevada, in December 1992, and hand-injected as they passed through chutes. During the summer of 1994, they were relocated and observed for the presence of foals. Untreated mares ($n = 63$) produced 34 foals (53.9%), sham-injected mares ($n = 20$) produced 11 foals (55%), and mares receiving two inoculations about 1 month apart ($n = 44$) produced two foals (4.5%) (J. W. Turner, personal communication). Larger scale trials are in the planning stage for Nevada horses.

An initial two-inoculation protocol results in effective contraception in wild mares for up to a year; however, a one-inoculation treatment is more practicable for use in the field. The timing of a single PZP inoculation with wild horses is important. In the northern hemisphere, wild mares breed between April and July. A single inoculation of PZP plus Freund's complete adjuvant (FCA) was given to 20 mares in Nevada, in December 1992, approximately 4 months before the onset of the breeding season. In 1994, 14 of those 20 mares were located and four mares (28.6%) had foals at their sides (J. W. Turner, personal communication). In a second test of a one-inoculation PZP trial, a single 65 µg PZP inoculation was given to 42 wild ASIS mares in March 1994, immediately before the onset of the breeding season. This treatment resulted in eight foals (19%), a fertility rate which was significantly different ($P < 0.05$) from the normal 45–55% foaling rate (J. F. Kirkpatrick, unpublished).

Purified PZP has also been administered to horses using a biodegradable bullet (Willis *et al.*, 1994). The PZP was delivered at an initial dose of 400 µg. The mares received a booster dose of 200 µg 3–4 weeks later. All mares developed significant concentrations of anti-PZP antibodies and mares were infertile for 2 years without an annual booster (R. Fayrer-Hosken, personal communication).

In a trial with a non-seasonally breeding equid, 16 feral donkeys in the Virgin Islands National Park were immunized with PZP remotely. Each jenny received 65 µg PZP followed by a second inoculation 3 weeks later and a third inoculation 10–12 months later. Results were not calculated until 12 months after the initial inoculation, to allow for any pregnancies in progress at the time of inoculations. On the basis of observed foals and pregnancy detection by means of faecal steroids (Kirkpatrick *et al.*, 1991b,c), only a single treated female (6.2%) produced a foal or was pregnant, while 6 of 11 (54.5%) control donkeys produced foals or were pregnant (J. W. Turner, unpublished).

Cervidae

White-tailed deer (*Odocoileus virginianus*) populations have reached unprecedented numbers in the USA, largely because of the adaptability of the species to human activity and a decline in hunting. Some of the more severe problems are occurring in urban areas, on military bases, and in national parks. Successful PZP contraception of this species, using two inoculations of 65 µg doses, was first demonstrated with captive deer (Turner *et al.*, 1992). Subsequent trials with captive deer examined the effects of (1) one- and two-inoculation protocols, and (2) reversibility of contraceptive effects. Untreated control does ($n = 27$) produced 24 fawns (88.8%). No does ($n = 13$) receiving either two or three inoculations produced fawns, and antibody concentrations ranged from 72.7% to 92.0% of positive reference serum antibody titres 7 to 10 months after treatment (the positive reference serum was composed of a pool of at least ten serum samples from deer in which contraception had occurred after PZP treatment). Seventy-five per cent of does treated in year 1 of the study and given a single booster inoculation in year 2 produced fawns in year 4; of seven does treated in year 1 but not given booster inoculations in year 2, two produced fawns (28.6%) in year 3 and five of the seven produced fawns (71.4%) in year 4 (Turner *et al.*, in press).

After trials with captive deer, a field test was carried out with free-ranging deer at the Smithsonian Institute's Conservation and Research Center in Virginia. Thirty does were live-trapped, weighed, ear-tagged, and released. Ten received a single injection of 65 µg PZP at the time of capture (Group A). A month later, in October 1992, the animals in Group A received a second 65 µg inoculation, remotely, by means of darts. Another ten (Group B) received a single inoculation of PZP. Ten does received a single inoculation of saline plus adjuvant (Group C). A year later 0%, 78% and 82% of does in Groups A, B, and C, respectively, produced fawns. All breeding activity ceased in control animals (Group C) by December, but breeding activity continued on into February for animals in Groups A and B, indicating that their breeding season was extended when pregnancy did not occur earlier. Parturition dates for fawns born to animals in Group B indicated that conception occurred during these extended breeding seasons, presumably when antibody concentrations decreased below contraceptive amounts. Nine of the does in Group B were given a single booster inoculation in 1993, and in 1994, only two (22.2%) produced fawns. This latter experiment was significant because it suggested that, if a single PZP inoculation was given for the purpose of antigen recognition and if contraceptive effects were disregarded in year 1 of the study, a one-inoculation protocol would be effective by the second year. Ovaries from deer in all three groups showed no evidence of autoimmune disease and there were no differences between groups (W. McShea, personal communication).

A second large scale field trial with free-roaming deer is currently being carried out on Fire Island National Seashore, NY. In year one (1993), 74 does were given either one ($n = 6$) or two inoculations of 65 µg PZP remotely. Of these 74 does, 62 were adults in 1992 and of these, 90% produced fawns in May/June of the pretreatment year, 1993. A year later only 39.2% produced fawns. This was a significant reduction over pretreatment fawning rates, but a surprisingly low effectiveness compared with results with captive deer. The primary cause for this relatively low effectiveness was thought to be incomplete injections by the darts. In 1994, of these 73 does, 40 were given a single booster inoculation and another 76 previously untreated does were given two inoculations, using an improved dart. In 1995, of the total deer treated in both years of the study only 20% produced fawns. Among the does given booster inoculations, 15% produced fawns and among the 76 does receiving the initial two inoculations in 1994, only 28% produced fawns (J. F. Kirkpatrick, unpublished). These results are encouraging and have led to the initiation of several additional trials with free-roaming deer.

Exotic zoo animals

A third application of PZP immunocontraception in wildlife has taken place with captive exotic zoo animals. Modern spacious and naturalistic exhibits in zoos have resulted in increased reproductive success among many exotic species, even to the point of producing large numbers of 'surplus' animals. In addition, there is a need to remove some females, with genetic problems or poor health, from reproductive potential without also removing them from their social groups. Contraception in zoos historically has been confined to steroid contraception, sterilization, or both techniques, but associated pathologies (Kollias *et al.*, 1984) and the stresses of surgery for placing implants have led to increased interest in PZP immunocontraception.

Initial successful trials were carried out with Przewalski's horses (*Equus przewalksii*) and banteng (*Bos javanicus*) at the Cologne Zoo (Kirkpatrick *et al.*, 1995b), with West Caucasian tur (*Ibex ibex*) at the Toronto Zoo (Kirkpatrick *et al.*, 1992a), and with sika deer (*Cervus nippon*), sambar deer (*Cervus unicolor*), muntjac deer (*Muntiacus reevesi*), Himalayan tahr (*Hemitragus jemlahicus*), axis deer (*Cervus axis*), and Roosevelt elk (*Cervus elaphus*) at the Wildlife Conservation Center (formerly the Bronx Zoo) (Kirkpatrick *et al.*, 1993). In all cases, except the muntjac deer, the females received either two or three initial inoculations of 65 µg PZP; the muntjac received 43 µg doses. Annual booster inoculations were successful in preventing conception. Together with subsequent trials, 68 species have been inoculated and data are available for 28 species. Contraception has been successful in 27 species, including members of the orders Perissodactyla (Equidae), Artiodactyla (Cervidae, Capridae, Giraffidae, Bovidae), Carnivora (Ursidae, Mustelidae, and Felidae) (Table 2). The only outright contraceptive failure has been in fallow deer (*Dama dama*). Data for the remaining 40 species are not yet available.

One of the greatest challenges for safe yet effective wildlife contraception is the free-ranging African elephant (*Loxodonta africana*). Crossreactivity between PZP and elephant ZP was examined to evaluate the potential use of PZP as a safe, effective contraceptive vaccine in elephants. Polyclonal serum to highly purified PZP was produced in rabbits. The specificity for native PZP was confirmed using one-dimensional and two-dimensional PAGE and western blot analysis. Crossreactivity with heterologous zonae pellucidae (equine) was confirmed with immunocytochemistry using light and transmission electron microscopy. Immunocytochemical evaluation was performed with rabbit anti-PZP antibodies, to test the hypothesis that elephant and pig ZP have similar domains. Significant immunogold staining of the elephant ZP of primary, secondary and tertiary oocytes was present when compared with control oocytes. This crossreactivity suggests that PZP can be used as a contraceptive in elephants (R. Fayrer-Hosken, unpublished).

The contraceptive success across so many taxa indicates a broad spectrum of crossreactivity between anti-PZP antibodies and the mammalian zona pellucida and supports the notion of a high degree of evolutionary conservation of the zona sperm receptor. The contraceptive failure of the PZP vaccine in fallow deer is unexplained, particularly considering that conception was prevented in five other members of Cervidae.

Table 2. Captive exotic zoo species in which conception has been prevented with native pig zonae pellucidae

North American bison (*Bison bison*)
Banteng (*Bos javanicus*)
Przewalski's horse (*Equus przewalskii*)
Grevyi's zebra (*Equus grevyi*)
Common zebra (*Equus burchelli*)
Donkey (*Equus asinus*)
Axid deer (*Cervus axis*)
Muntjac deer (*Muntiacus reevesi*)
Sika deer (*Cervus nippon taiouanus*)
Roosevelt elk (*Cervus elaphus roosevelti*)
Sambar deer (*Cervus unicolor*)
Brown bear (*Ursus arctos*)
White-tailed deer (*Odocoileus virginianus*)
Himalayan tahr (*Hemitragus jemlahicus*)
Rocky Mountain bighorn sheep (*Ovis canadensis*)
Rocky Mountain goat (*Oreamnos americanus*)
Ibex (*Capra ibex*)
West Caucasian tur (*Capra ibex caucasica*)
Greater kudu (*Strepsiceros strepsiceros*)
Barasingha (*Cervus duvauceli*)
Waterbuck (*Kobus defassa*)
Springbok (*Antidorcas marsupialis*)
Impala (*Aepyceros melampus*)
African lion (*Panthera leo*)
Mountain lion (*Felis concolor*)
North American otter (*Lutra canadensis*)
Giraffe (*Giraffa camelopardalis*)

Current and Future Research

There are two important dimensions to the immunocontraception of wildlife that supersede other concerns. The first is the issue of reversibility of contraceptive effects. The public's concern over certain free-roaming wildlife species, and the conservation value of rare and endangered captive exotic species make the issue of reversibility of PZP immunocontraception very important. Thus far, in wildlife, reversibility has been documented in addax, ibex, and muntjac deer, and among white-tailed deer and domestic and wild horses, but most studies have involved only the effects of short-term treatment (1–3 years). Only in the wild horse have reversibility studies been extended to 7 years of treatment. Reversibility has been documented in mares treated for 1, 2, 3 and 4 consecutive years (Kirkpatrick *et al.*, 1992b, 1995b). Ovulation rates for mares treated from 1, 3 and 7 consecutive years declined from 73.3% (11 of 15), to 55.5% (5 of 9), and 10% (1 of 10), respectively. One mare treated for 5 consecutive years ovulated during year 6 and year 7 but has not yet conceived. Urinary oestrogen concentrations declined from a normal range of 100–385 ng mg^{-1} creatinine during 7 years of PZP treatment. The percentage of mares with normal urinary oestrogen concentrations declined to 46%, 33%, 40% and 0% after 2, 3, 6 and 7 years of treatment, respectively. Several mares with depressed urinary oestrogen concentrations continued to demonstrate cyclic oestrogen peaks and nadirs, suggesting continued follicular activity. The results of this study indicate that long-term PZP treatment is associated with a decline in ovarian oestrogen production and ovulation rates. However, the reversibility of low oestrogen production and ovulation failure, and therefore fertility, will require 3–7 additional years to assess.

Western blot analysis of glycoprotein hormones was performed to ensure the safety of the PZP vaccine immunocytochemistry of equine somatic tissues. No immunocytochemical crossreactivity using horse anti-PZP or rabbit anti-PZP was present with heart, kidney, lung, liver, the somatic ovary or testes.

Table 3. Antibody concentrations in response to pig zonae pellucidae (PZP) in individual domestic mares after one inoculation using PZP + PZP-containing microspheres or two inoculations of PZP given at intervals of three weeks

Treatment	Horse	Antibody titres (% of positive reference serum) (days after treatment) 0	20	34	55	97	213
FCA-PZP bolus + PZP microspheres	I	5	99	114	131	106	79
	II	4	70	107	123	82	28
	III	6	33	70	95	98	131
	IV	5	38	76	120	114	39
	V	5	56	108	106	99	26
Mean titres ± SEM		5.0 ± 3.1	59.2 ± 11.9	95.0* ± 9.1	115** ± 6.4	99.8 ± 5.2	60.6 ± 20.0
FCA-PZP bolus + FIA-PZP bolus 3 weeks later	VI	4	90	130	131	152	115
	VII	6	66	145	135	144	58
	VIII	8	58	118	123	91	35
	IX	3	73	116	133	123	79
	X	4	67	147	126	136	94
Mean titres ± SEM		5.0 ± 0.9	70.8 ± 5.3	131.2* ± 5.5	129.6** ± 2.2	129.2 ± 10.6	76.2 ± 13.8

*Values differ significantly between treatment groups ($P < 0.002$; $t = -6.76$); **values differ significantly between treatment groups ($P < 0.03$; $t = -3.14$).
FCA: Freund's complete adjuvant; FIA: Freund's incomplete adjuvant.

Furthermore, rabbit anti-PZP did not crossreact with equine LH, FSH or chorionic gonadotrophin (R. Fayrer-Hosken, personal communication).

Perhaps the single most important issue in wildlife immunocontraception is the need for a one-inoculation form of the vaccine. While it has been clearly established that two inoculations given 3–6 weeks apart will provide contraception for about a year, the need to give two inoculations reduces the usefulness of the PZP vaccine in wildlife.

In an initial trial to determine whether continuous release of PZP would cause contraceptive antibody concentrations, as opposed to tolerance, three does were given an initial inoculation of 65 µg native PZP and on the same day an osmotic mini-pump containing 65 µg PZP was implanted subcutaneously in the neck of each animal. The pump was designed to release PZP continuously for 28 days at approximately 2.5 µg day^{-1}. Anti-PZP antibody titres were 76.3% of positive reference serum several weeks after placement of the pumps, and 69.5% 7–10 months later, indicating that continuous release will raise titres to contraceptive concentrations (Turner *et al.*, in press).

In an attempt to develop a one-inoculation PZP vaccine, the PZP antigen was incorporated into non-toxic, biodegradable 50 µm homogenous lactide–glycolide microspheres. Upon intramuscular injection the lactide–glycolide material erodes, releasing the antigen over predetermined periods (Eldridge *et al.*, 1989). The lactide–glycolide is metabolized to lactic acid and carbon dioxide. Five mares were inoculated with an initial bolus of 65 µg PZP plus another 65 µg PZP contained in microspheres. Antibody concentrations were compared in mares inoculated with two 65 µg doses of PZP given 3 weeks apart. Over 31 weeks antibody concentrations did not differ significantly between the two treatment groups at weeks 3, 14, and 31, but were significantly higher in the two-inoculation group at weeks 5 and 8 than in the one-inoculation group (Table 3).

This same preparation was administered to 14 wild ASIS mares. One dart failed to inject. This mare, plus one other mare produced foals following treatment. The differences in fertility were significant ($P < 0.05$) comparing either two foals per 14 mares or one foal per 13 mares with foaling rates for untreated mares (Kirkpatrick *et al.*, in press). White-tail does receiving a single inoculation of

PZP + PZP-containing microspheres ($n = 7$) produced two fawns (28.6%) and had antibody concentrations of 19.1 ± 4.3% 7–10 months after treatment. These results confirmed that a single inoculation of PZP + microspheres produced a significant contraceptive effect but not as well as a two-inoculation protocol. Research is underway to produce a one-inoculation vaccine using microcapsules, which are made from the same lactide–glycolide material but which release the PZP antigen in pulses rather than continuously.

Pig zona pellucida immunocontraception appears to have great potential in certain populations of wildlife, because of (1) > 90% contraceptive effectiveness, (2) the ability to deliver the vaccine remotely, via darts, (3) reversibility after short-term use, (4) a wide breadth of effectiveness across many species, (5) a lack of debilitating side-effects even after long-term treatment, (6) minimal effects upon social behaviours, and (7) the inability of the vaccine or its resulting antibodies to pass through food chains.

The work reported in this paper was supported by the National Park Service, National Institutes of Health, The Humane Society of the US, the Bureau of Land Management, PNC Corporation, the American Association of Zoos and Aquariums, the Geraldine R Dodge Foundation, and the Eppley Foundation. The Humane Society of the US provided travel funds for this conference.

References

Eldridge JH, Gilly RM, Staas JK, Moldoveanu Z, Muelbroek JK and Tice TR (1989) Biodegradable microcapsules: vaccine delivery systems for oral immunization *Current Topics in Microbiology and Immunology* **146** 59–66

Kirkpatrick JF (1995) *Management of Wild Horses by Fertility Control: The Assateague Experience* National Park Service Scientific Monograph, National Park Service, Denver, CO

Kirkpatrick JF and Turner JW, Jr (1985) Chemical fertility control and wildlife management *BioScience* **35** 485–491

Kirkpatrick JF and Turner JW, Jr (1991) Reversible contraception in nondomestic animals *Journal of Zoo and Wildlife Medicine* **22** 392–408

Kirkpatrick JF, Liu IKM and Turner JW, Jr (1990) Remotely-delivered immunocontraception in feral horses *Wildlife Society Bulletin* **18** 326–330

Kirkpatrick JF, Liu IKM, Turner JW, Jr and Bernoco M (1991a) Antigen recognition in feral mares previously immunized with porcine zonae pellucidae *Journal of Reproduction and Fertility Supplement* **44** 321–325

Kirkpatrick JF, Shideler SE and Turner JW, Jr (1991b) Pregnancy determination in uncaptured feral horses based on free steroids in feces and steroid metabolites in urine-soaked snow *Canadian Journal of Zoology* **68** 2576–2579

Kirkpatrick JF, Shideler SE, Lasley BL and Turner JW, Jr (1991c) Pregnancy determination in uncaptured feral horses by means of fecal steroid metabolites *Theriogenology* **35** 753–759

Kirkpatrick JF, Calle PP, Kalk P, Kolter L, Zimmermann W, Goodrowe K, Turner JW, Jr, Liu IKM and Bernoco M (1992a) Immunocontraception of female captive exotic ungulates *Proceedings of the American Association of Zoo Veterinarians* 100–101

Kirkpatrick JF, Liu IKM, Turner JW, Jr, Naugle R and Keiper RR (1992b) Long-term effects of porcine zonae pellucidae immunocontraception on ovarian function of feral horses *Journal of Reproduction and Fertility* **94** 437–444

Kirkpatrick JF, Calle PP, Kalk P, Kolter L, Zimmermann W, Goodrowe K, Liu IKM, Turner JW, Jr, Bernoco M and Rutberg AT (1993) Immunocontraception in zoo animals: vaccinating against pregnancy *Proceedings of the American Association of Zoo Veterinarians* 290–291

Kirkpatrick JF, Naugle R, Liu IKM, Bernoco M and Turner JW, Jr (1995a) Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Biology of Reproduction Monograph Series 1: Equine Reproduction VI* 411–418

Kirkpatrick JF, Zimmermann W, Kolter L, Liu IKM and Turner JW, Jr (1995b) Immunocontraception of captive exotic species. I. Przewalski's horse (*Equus przewalskii*) and banteng (*Bos javanicus*) *Zoo Biology* **14** 403–416

Kirkpatrick JF, Liu IKM and Turner JW, Jr Contraception of wild and feral equids. In *Contraception in Wildlife Management* Ed. TJ Kreeger. US Government Printing Office, Washington, DC (in press)

Kollias GV, Calderwood-Mays MB and Short BG (1984) Diabetes mellitus and abdominal adenocarcinoma in a jaguar receiving megestrol acetate *Journal of the American Veterinary Medical Association* **185** 1383–1386

Linnehan RM and Edwards JL (1991) Endometrial adenocarcinoma in a Bengal tiger (*Panthera tigris bengalensis*) implanted with melengestrol acetate *Journal of Zoo and Wildlife Medicine* **22** 130–134

Turner JW, Jr and Kirkpatrick JF (1991) New developments in feral horse contraception and their potential application to wildlife *Wildlife Society Bulletin* **19** 350–359

Turner JW, Jr, Liu IKM and Kirkpatrick JF (1992) Remotely-delivered immunocontraception of captive white-tailed deer *Journal of Wildlife Management* **56** 154–157

Turner JW, Jr, Kirkpatrick JF and Liu IKM Effectiveness, reversibility and serum antibody titers associated with immunocontraception in white-tailed deer *Journal of Wildlife Management* (in press)

Willis P, Heusner GL, Warren RJ, Kessler D and Fayrer-Hosken RA (1994) Equine immunocontraception using porcine zona pellucida: a new method for remote delivery and characterization of the immune response *Journal of Equine Veterinary Science* **14** 364–370