

The Science and Conservation Center

2100 S. Shiloh Road

Billings, MT 59106

(406) 652-9718

Fax: (406)-652-9733

SCCPZP@hotmail.com

www.sccpzp.org

TO: Zoo Staff

FROM: Kimberly M. Frank
Wildlife Contraceptive Program

RE: PZP Immunocontraception of Captive Exotic Species

DATE: 4/13/2011

Thanks for your recent inquiry regarding porcine zona pellucida (PZP) immunocontraception of captive exotic species. As you consider immunocontraception, I feel that it is important for you to familiarize yourself with the procedure we follow with zoo animals.

BACKGROUND INFORMATION FOR THE IMMUNOCONTRACEPTION OF CAPTIVE EXOTIC SPECIES WITH PORCINE ZONAE PELLUCIDAE

PURPOSES:

1. To test the contraceptive effectiveness of active immunization with porcine zonae pellucidae (PZP) in captive exotic species,
2. To develop immunization schedules that will provide maximum contraceptive efficiency with a minimum of inoculations,
3. To document reversibility of fertility inhibition,
4. To determine the effects of PZP immunization upon ovarian function,
5. To determine if PZP immunization will lead to histopathological changes in the ovary and other tissues.

BACKGROUND:

Currently a well-used approach to fertility control in some mammalian species is immunocontraception with porcine zonae pellucidae (PZP). The zona is a non-cellular layer of acidic glycoprotein, which envelops the mammalian oocyte and ovum up until the time of implantation (Sacco 1987). The glycoprotein membrane is produced by the oocyte (Leveille et al. 1987) and is composed of several protein fractions. A 55,000 MW fraction has been shown to be the primary candidate antigen. This particular fraction, referred to as ZP3, has been shown to be the specific zona receptor for sperm recognition, attachment, and acrosome reaction (Sacco et al. 1984; Arns et al. 1990); although one or more of the other zona proteins may also play roles in fertilization (Hasegawa et al. 1992). The contraceptive efficacy of ZP glycoprotein and PZP was originally demonstrated in a wide variety of mammals, including the hamster (Gwatkin et al. 1977), rats (Tsunoda and Chang 1976a), mice (Tsunoda and Chang 1976b), the marmoset (Aitken et al. 1984), cynomolgus monkeys (Gulyas et al. 1983), squirrel monkeys (Sacco et al. 1983,1987), bonnet monkeys (Bamezai et al. 1986), and baboons (Dunbar et al. 1989).

Among the ungulates, PZP immunocontraception has been shown to be effective in the domestic horse (Liu et al. 1989), the wild horse (Kirkpatrick and Turner 2002; 2003, 2007, 2008; Turner and Kirkpatrick 2002; Kirkpatrick et al. 1990,1991, 1992, 1995a, 1997), white-tailed deer (Turner et al. 1992, 1996; McShea et al. 1997; Naugle et al. 2002; Rutberg et al. 2004), Przewalski horses, banteng (Kirkpatrick et al. 1995b), sika deer, axis deer, muntjac deer, Himalayan tahr, and West Caucasian tur (Kirkpatrick et al. 1996). Successful contraception has also been carried out with a large number of either ungulates (see Table 1) (Frank and Kirkpatrick 2002; Shideler et al. 2002; Deigert et al. 2003; Frank et al. 2005) and African elephants (Fayrer-Hosken et al. 1999, 2000; Delsink et al. 2002, 2007) and several species of bears (Frank et al. 2005). IN GENERAL, WE HAVE HAD SOME HIGH DEGREE OF SUCCESS WITH ALMOST ALL UNGULATES, AS WELL AS BEARS AND PINNIPEDS. RESULTS WITH CARNIVORES IN GENERAL ARE INCONCLUSIVE AT THIS TIME (Frisbie and Kirkpatrick 1998).

Table 1.

SCIENCE AND CONSERVATION CENTER DATA BASE
SPECIES SUCCESSFULLY TREATED WITH PORCINE ZONA PELLUCIDA
(Common names in alphabetic order)

Ungulates

Perissodactyla

Horse (*Equus caballus*)
Donkey (*E. asinus*)
Przewalski's Horse (*E. przewalskii*)

Grevy's Zebra (*E. grevyi*)
Plains Zebra (*E. burchelli*)
Mountain Zebra (*E. zebra*)
Black Rhinoceros (*Diceros bicornus*)
Tapir (*Tapirus indicus*)

Artiodactyla

Addax (*Addax nasomaculatus*)
Antelope, Roan (*Hippotragus equinus*)
Antelope, Sable (*Hippotragus niger*)
Banteng, Javan (*Bos javanicus*)
Bison (*Bison bison*)
Blackbuck (*Antelope cervicapra*)
Bongo (*Taurotragus euryceros*)
Camel, Dromedary (*Camelus dromedarius*)
Camel, Bactrian (*Camelus bactrianus*)
Caribou (*Rangifer tarandus*)
Chamois (*Rupicapra rupicapra*)
Cow, Domestic (*Bos primigenius* [Taurus])
Deer, White-tailed (*Odocoileus virginianus*)
Deer, Mule (*Odocoileus hemionus*)
Deer, Axis (*Cervus axis*)
Deer, Mainland Sika (*Cervus nippon manchuriensis*)
Deer, Formosan Sika (*Cervus nippon taiwanus*)
Deer, Fallow (*Cervus dama*)
Deer, Eld's (*Cervus eldi*)
Deer, Pere David's (*Elaphurus davidianus*)
Deer, Muntjac (*Muntiacus reevesi*)
Deer, Sambar (*Cervus unicolor*)
Dik Dik, Kirk's (*Madoqua kirkii*)
Duiker, Red Flanked (*Cephalophus rufilatus*)
Duiker, Yellow-backed (*Cephalophus sylvicultor*)
Edmi (*Gazella cuvieri*)
Eland (*Taurotragus oryx*)
Gazelle, Thomson's (*Gazella thomsoni*)
Gerenuk (*Litocranius walleri*)
Giraffe (*Giraffa camelopardalis*)
Goat, Mountain (*Oreamnos americanus*)
Goat, Domestic (*Capra capra*)
Hippopotamus (*Hippopotamus amphibious*)
Ibex (*Capra ibex*)
Impala (*Aepyceros melampus*)
Kudu (*Taurotragus strepiceros*)
Llama (*Lama glama*)
Markor (*Capra falconeri*)

Moose (*Alces alces*)
Musk Ox (*Ovibus moschatus*)
Nyala (*Taurotragus angasi*)
Oryx, Sciimitar (*Oryx dammah*)
Pronghorn, North America (*Antilocapra americana*)
Pudu, Southern (*Pudu pudu*)
Serow, Mainland (*Capricornus sumatrensis*)
Sheep, Big Horn (*Ovis Canadensis*)
Sheep, Dall (*Ovis dalli*)
Sheep, Mouflon (*Ovis musimon*)
Tahr, Himalayan (*Hemitragus jemlahicus*)
Wapiti, North American (*Cervus elaphus*)
Wapiti, Roosevelt (*Cervus elaphus roosevelti*)
Wapiti, Altai (*Cervus _____*)
Waterbuck, Defassa (*Kobus ellipsiprymnus*)
Water Buffalo, Asian (*Bubalis arnee*)
Wisent, Lowland (*Bison bonasus*)
Yak (*Bos mutus*)

Elephantidae

African elephant (*Loxodonta africana*)

Carnivora

Bear, American Black (*Ursus americanus*)
Bear, Sun (*Helarctos malayanus*)
Bear, Brown (*Ursus arctos*)
Bear, Asian Black (*Selenarctos thibetanus*)
Sea Lion, California (*Zalophus Californian*)

Among the ungulates, the majority of data has been derived from horses. Tests with this species indicate that fertility inhibition is greater than 90% effective and is reversible after one to five consecutive years of treatment (Kirkpatrick and Turner 2002). PZP treatment is safe for use with pregnant mares (and banteng and Przewalski's horses [Kirkpatrick et al. 1992], and elephants [Delsink et al. 2002, 2007]); pregnancies already underway at the time of inoculation will be carried to term and foals will be healthy, females which were *in utero* at the time of their mothers' inoculations will themselves be fertile, sexual behavior is unaffected, and a single annual booster inoculation will extend contraceptive effects for a second breeding season. In a single study, sambar fawns exposed to PZP antibodies had low birth weights but were otherwise healthy.

One remaining concern regarding the use of PZP, ZP3, or any immunogenic peptide fragment is the possible permanent loss of fertility and disruption of the reproductive

endocrine sequelae associated with normal ovaries, after prolonged use of the vaccine. Available data suggest there is considerable species variation with regard to the response of the ovaries to the anti-PZP antibodies. In the horse, Liu et al. (1989) demonstrated that serum progesterone concentrations and ovarian histology were normal following treatment over a single year, and reversibility of fertility inhibition can occur after two consecutive years of treatment and ovaries from two mares revealed no histopathological changes after two years of treatment. Ovarian endocrine data indicate that normal ovarian function continues in the majority of animals after three consecutive years of treatment (Kirkpatrick et al. 1992b). However, after six consecutive years of treatment there is evidence of ovarian depression and a decreased number of ovulatory cycles (Kirkpatrick et al 1995a). On the other hand, at the individual and population levels, longevity and body condition have increased and mortality has decreased (Turner and Kirkpatrick 2002; Kirkpatrick and Turner 2007, 2008). Studies in two particular species have indicated significant side effects. Mahi-Brown et al. (1985) found that immunization of bitches led to long-term alterations of estrous cycles and abnormal steroid profiles, however the PZP preparations were extremely impure compared to today's product. Immunization of rabbits with PZP also led to disruption of hormone profiles (Wood et al. 1981; Skinner et al. 1983). Some abnormalities in the menstrual cycle of cynomolgus monkeys also appeared after PZP administration. Menses were interrupted in treated monkeys and the expected mid-cycle E2 elevations were absent for several cycles (Gulyas et al. 1983). Within 3-5 months after the last PZP booster immunization, menses and E2 peaks returned to normal. In the monkey, Sacco et al. (1987) found endocrine and histological abnormalities in response to ZP3 injections. Immunized monkeys demonstrated cyclical E2 and P4, but these cycles were quantitatively different than those of untreated monkeys and they were interpreted as non-ovulatory cycles. By 300 days E2 and P4 values returned to normal levels and patterns. Dunbar et al. (1989) used both ZP1 and ZP3, which share a common epitope, but serious ovarian dysfunction still occurred in baboons after 49 weeks and nine cycles.

With the exception of the work by Mahi-Brown, with the bitch, very little is known about the contraceptive effectiveness of PZP in carnivores. Trials, by other research groups, with wolves, domestic dogs and cats have largely been unsuccessful. One recent study at the University of Florida demonstrated that the PZP-induced antibodies would not cross-react in the cat (Gorman et al 2002).

In summary, almost 20 years of research with PZP immunization indicates no evidence of debilitating side effects in ungulates. However, the possibility of depression of ovarian function exists in some species, and the possibility of permanent infertility, probably because of the depletion of ovarian follicles, exists in all species after long-term (< 7 years) treatment.

STUDY PROTOCOL

The ultimate purpose behind immunocontraception of exotic species is the reduction of unwanted pregnancies and a decrease in surplus animal production. Because PZP immunocontraception has only been used since 1990 in zoo animals, it is vital that we

learn as much as possible about the safety and efficacy of this approach. Some zoos will wish only to reduce pregnancies, but it is important that each zoo attempt to actually participate in a sound research program, to the extent that human and animal resources permit. The protocol for selection of animals, treatment, and collection of materials for analysis outlined below represents the ideal, i.e., under the best of conditions, with all necessary resources, the maximum amount of information that can be collected. It is understood that not all zoos will have the time or personnel resources to be able to collect all samples or make all observations, however, the quality of the science associated with the study of PZP contraception in captive exotic species is directly related to the effort put forward in collecting all potential information.

1. SELECTION OF TEST ANIMALS

Any female mammals which pose a current or potential surplus animal problem, or which should not produce young for genetic, health, or age-related reasons is eligible for PZP immunization. Because of our lack of knowledge regarding the potential effects of long-term immunization in species other than deer and horses, PZP immunization for rare or valuable animals engaged in SSP breeding programs should be undertaken with caution and only after consultation, particularly if treatment will exceed two years.

For the purposes of our research, it is important that all animals selected should be in good health. We recognize that there may be instances where the poor health of an animal makes her a candidate for contraception, to prevent an unnecessary pregnancy, but the data derived from these animals must be kept separate from other, normally healthy animals. That will be our responsibility at The Science and Conservation Center. First priority should be given to animals of known fertility, whose estrous cycles have been documented by behavioral or endocrine parameters and whose fertility has been proven by successful reproduction. Once the contraceptive effectiveness of PZP immunization has been demonstrated in a given species, animals of unknown fertility can be treated.

2. PROTOCOL FOR INITIAL TREATMENT

Initial treatment of each species should be consistent with its seasonal pattern of reproduction. For species with an extremely well defined and short (2-3 months or less) breeding season; a minimum of two inoculations should be given during the first year of treatment. The first inoculation (primer) must be given 1-2 months prior to breeding activity and the second inoculation 2-6 weeks later but no later than 1-2 weeks prior to the onset of breeding activity. In species with a breeding season of 3-4 months or longer, if the vaccine is given at a time other than just prior to the breeding season, then the first inoculation should be given on day 0, the second on day 21 and in cases where contraception is absolutely vital, a third inoculation should be given on day 45.

Until data are obtained that suggest otherwise, single annual booster inoculations may be given during subsequent years in some species. In year-round breeders, recent evidence indicates that booster inoculations should be given every seven to eight months (see Frank et al. 2005).

Thus far, the standard dose of PZP antigen for animals > 100 lbs is 100 µg of protein (equivalent to ≈ 5,000 zonae) in phosphate buffered saline or sterile water. This dose may be adjusted downward for some species and will be determined prior to administration.

3. ADJUVANTS

The PZP antigen must be given with an adjuvant. We use, exclusively, Freund's Modified adjuvant (product 344289, Calbiochem, PO Box 12087, La Jolla, CA 92039-2087; 800-854-3417; Technical@Calbiochem.com), for the initial or primer inoculation and Freund's Incomplete Adjuvant (FIA) (product no. F5506, Sigma, PO Box 14508, St. Louis, MO 63178; 800-325-5052) for all subsequent inoculations. A recent study (Deigert et al. 2003) has shown that antibody titers resulting from treatment with MFCA in fallow deer are more than adequate for successful contraception, and a similar study with horses (Lyda et al. 2005) has shown the same results.

Our experience has been that the use of the PZP vaccine with the two Freund's adjuvants, FMA and FIA, leads to a very small number of abscesses (< 1% of inoculations); these abscesses have been about 25 mm in diameter and they all drained within a few days without additional untoward effects, and contraceptive efficacy was not compromised. It is not uncommon to see small sterile granulomas (about the size of a marble) form at the injection site. These are merely small scar tissue deposits just under the skin and roughly analogous to the scar we develop after small pox vaccinations, but under the skin instead of on the surface.

The adjuvant will be provided at cost. The FIA \$10.00 per 10ml bottle and the MFCA costs \$10.00 per 5ml bottle and \$10.00 per 10ml bottle. Each 10 ml bottle is good for approximately 18 inoculations.

4. DELIVERY

Preparation of the antigen/adjuvant emulsion is very important for best results. For each inoculation, 0.5 cc of adjuvant should be drawn into a 5 cc glass syringe, followed by drawing the antigen (in 0.5 cc of PBS or sterile water, regardless of the dose of protein) into the same syringe. The syringes should be good quality BD glass syringes, with Luer locks (Yale Hypodermic Syringe, BD # 2311, 5 cc Luer-Lok Tip, graduated 1/5 cc); cheap glass syringes without Luer locks have poor tolerances between barrel and plunger and much of the vaccine is lost in the process of making the emulsion. The syringe should be connected to a second 5 cc glass syringe with a Luer Lock connector and the syringes given 100 brisk strokes. It is important to create a thick emulsion because this has beneficial effects with regard to antibody formation after inoculation. After a thick emulsion has been produced, it can be loaded into a plastic syringe for hand injection, or for delivery with a pole-syringe, or darts, for remote delivery. Nothing smaller than an 18 g needle should be used for injection. If darts are used for remote delivery, we recommend Pneu-Dart (Williamsport, PA phone 570-323-2710) 1.0 cc barbless darts; we

have tried a variety of darts and these work the best. Telinject is the least effective, because of the viscosity of the emulsion and the small bore of the dart needles. The vaccine should be given only in the hip or gluteus, i.m. We use 1.5" needles for animals the size of horses and 1"-1.25" needles for animals the size of white-tailed deer. Also, remember that Pneu-Darts come in two diameters, one size for use in the blowgun and CO₂ pistol or rifle (P-type darts) and another size for use in the cartridge powered Pneu-Dart capture rifle (C-type darts). Dan-Inject also makes a 13 mm barrel for its weapons that will accommodate the Pneu-Darts.

5. CONTROLS

Whenever possible, appropriate control groups should be established. This is particularly important with species in which PZP immunization has not yet been tried. Controls should be selected using the same criteria as experimental animals and should be treated with adjuvant and PBS in place of the PZP emulsion. In cases where a single zoological garden has insufficient animals for sound experimental and control groups, two or more zoos should work together if possible. There will be certain instances where control animals simply will not be available, i.e., an old kudu that we wish to prevent from having more young, or a single animal with some genetic problem.

6. MONITORING OF OVARIAN FUNCTION

When possible, and in certain species, ovarian endocrine function should be monitored by means of fecal or urinary steroid metabolites. This approach, which is well known by most zoo personnel, is desirable because of its non-intrusive nature and the likelihood of avoiding unnecessary stress for animals during the study period. This may not be possible with all species. A wide variety of ungulates have been monitored successfully in this manner, however, there are many species where these techniques have not yet been validated. Ovarian cyclical endocrine changes can be monitored by means of urinary estrone conjugates (E1C) and can be used in a number of species to reflect plasma E₂ concentrations. Similarly, urinary pregnanediol-3-glucuronide (PdG) can be used in a number of species to reflect plasma P₄ concentrations. Among members of Perissodactyla, P₄ concentrations can be indirectly monitored by means of urinary non-specific P₄ metabolites (iPdG). The exact design of the endocrine monitoring procedures will have to be developed for each species. Zoo personnel should be prepared to collect fecal or urine on an every-other-day or every third day schedule during the breeding season, and particularly during periods of clinical estrus and breeding behavior. Each sample should bear the animal number and date (made with a waterproof permanent marker). Samples should be sent frozen. After large batches have been collected, they can be sent, frozen, by Federal Express, to Robin Lyda, The Science and Conservation Center, 2100 South Shiloh Road, Billings, Montana 59106 (406-652-9718). Please call to let us know when to expect the samples. **These tests will not be conducted routinely for all PZP contraceptive trials and unless specific arrangements have been made with us prior to PZP treatment, zoo personnel should not collect fecal or urine samples.**

7. BREEDING BEHAVIOR

It is very important, particularly where urine samples cannot be collected, or where urinary hormone analysis has not been established, to record all breeding behaviors and associated dates, among experimental animals.

8. ANTIBODY TITERS

Of the major parameters to be evaluated, measurement of antibody titers is the least important. If contraception is successful, antibody production can be implied; if contraception is not successful, antibody production may not be important. However, in certain trials if experimental and control animals are to be handled for reasons other than PZP treatment, i.e., routine examination, treatment of illness, etc., blood samples should be collected, serum harvested, and stored frozen. Ideally, we would like plasma or serum samples from treated animals at the time of their first inoculation, and at 4, 6, and 12 months. Our research team will measure antibody titers. We do not recommend handling the animals during the experiment except for emergency reasons. Plasma samples should be mailed to Robin Lyda, The Science and Conservation Center, ZooMontana, 2100 South Shiloh Road, Billings, MT 59106 (406-652-9718). Please call before shipping samples. **These tests will not be conducted routinely for all PZP contraceptive trials and unless specific arrangements have been made with us prior to PZP treatment, zoo personnel should not collect blood samples.**

9. BOOSTER AND REVERSIBILITY STUDIES

Evaluation of fertility inhibition during the initial year of treatment is only one step in the study of PZP immunocontraception. Ideally, years-two and three should involve booster inoculations of a portion of the experimental population and the other half should be permitted to breed, in order to document reversibility. We realize this may not be desirable or possible for some species in some zoos.

10. HISTOPATHOLOGY

Complete evaluation of ovarian and uterine histology is a sound method for predicting the long-term effects of PZP vaccination on the reproductive system. It is recognized that not all animals placed on PZP contraceptive protocols will be available for hysterectomy/ovariectomy, but in those cases where it is possible, the data derived will be very valuable. In those animals, among PZP vaccinated, adjuvant-treated, or untreated controls, where ovariectomy or ovariohysterectomy is indicated for other reasons, these tissues should be collected (ovariohysterectomy for carnivores and primates; ovariectomy and uterine biopsy for ungulates and equids). Through ovarian histopathology, the degree of follicular and oocyte-mediated destruction can be evaluated, as well as the amount of ovarian atrophy. Uterine evaluations will determine

the long-term effects that lack of steroid stimulation have on the endometrium and myometrium.

Systemic effects have not been noted in other species, but should be evaluated during the preliminary trials in zoo animals. The principal concern is that immunization with an immunogen that is homologous to an endogenous material could result in autoantibody production and autoimmune disease. Presence of autoantibody can be determined by "panning" normal tissues with serum from PZP-vaccinated animals.

For these studies, we will need intact, fixed reproductive tracts including whole uterus or uterine biopsy (including endometrium), ovaries, and serum from any animal in the trial. Reproductive tracts from either necropsies or ovariohysterectomies would be appropriate. The tracts can be fixed in buffered formalin by immersion of the entire tract for 72 hours if a small incision is made into the uterine lumen in each horn (ratio of tissue to formalin = 1:10). Fixed tracts then can be wrapped in formalin-soaked paper towels, enclosed in a leak-proof plastic bag and shipped by ground mail (Federal Express is not necessary) to: Dr. Dalen Agnew, Attn: Histo Research, Diagnostic Center for Population and Animal Health, 4125 Beaumont Rd., Lansing, MI 48910-8104 USA. Phone: 517-353-1683, E-mail: agnewd@dcpah.msu.edu .

ORDERING PROCEDURES

After you have made a decision to use the PZP vaccine you are required to fill out the enclosed order/protocol sheet along with a brief report explaining the management objectives behind the use of the PZP contraceptive vaccine and return it to Kim Frank, at The Science and Conservation Center. This is an FDA requirement and the vaccine cannot be shipped until we are in receipt of the protocol sheet and report. **BE SURE TO INCLUDE YOUR ZOO'S FEDERAL EXPRESS ACCOUNT NUMBER OR APPROPRIATE CREDIT CARD NUMBER -WE CANNOT PAY SHIPPING COSTS.** The protocol form may be faxed ((406) 652-9733) or e-mailed (sccpzp@hotmail.com). The vaccine will be sent to you by Federal Express, on ice packs. It must be kept frozen (ultra-low temperature freezers are not necessary) until use. Vaccine will be 100µg doses in 1.5 ml plastic microvials.

For international shipments the PZP is available lyophilized so if it does get held in customs it will not be damaged. Each dose is \$30.

PZP orders to be sent to countries outside the United States must pass through Customs and meet the animal health requirements of that country. It will be the responsibility of foreign zoos to be sure that all necessary arrangements have been made with appropriate officials in the recipient country. It is very helpful if we have copies of necessary permits it enclose with the shipment of vaccine.

VACCINE COSTS

We provide the vaccine to zoos at our cost of production, which is \$24/dose. We will bill you for the vaccine after you receive the vaccine. Do nothing about payment until we send a bill. All payments should be made out to:

The Science and Conservation Center
and should be mailed directly to:
Kimberly Frank
The Science and Conservation Center
ZooMontana
2100 S. Shiloh Road
Billings, MT 59106
USA

Under certain circumstances, which result from financial hardship, philanthropic organizations will subsidize the cost of the vaccine and/or send a member of the research team to the zoo to instruct personnel in its proper administration.

DATA COLLECTION (VERY IMPORTANT....PLEASE DO THIS)

The contraception of zoo animals is conducted under an Investigational New Animal Drug exemption (INAD) (No. 8840). It is an FDA requirement that a data sheet (enclosed) be completed for each animal treated, as well as any control animals. We ask that a photocopy of all data sheets be returned to The Science and Conservation Center after the initial series is complete. The original should be retained by the zoo and updated on an annual basis, again with a photocopy sent to The Science and Conservation Center on an annual basis. Naturally, all zoo personnel who are legitimately involved in this research will be invited to join the research team as co-authors on published papers.

INQUIRIES

All inquiries regarding PZP contraception should be directed to Kimberly Frank, The Science and Conservation Center, ZooMontana, 2100 S. Shiloh Road, Billings, MT 59106, (406) 652-9718 or e-mail **SCCPZP@hotmail.com**. In Kimberly Frank's absence, inquiries can be directed to Dr. Jay Kirkpatrick at The Science and Conservation Center, ZooMontana.

Research with PZP contraceptive vaccine and captive exotic species is a joint venture involving the support of The Science and Conservation center at ZooMontana and many philanthropic organizations. We appreciate your interest in our work and the opportunity to conduct trials with you.

LITERATURE CITED

- Aitken, R. J., D. W. Richardson, and M. Hulme. 1984. Immunological interference with properties of the zona pellucida. in: Crichton, D.B. (ed). Immunological Aspects of Reproduction in Mammals. Boston, Butterworths, pp. 305-326.
- Arns, M. J., G. W. Webb, L. Johnson, J. Martin, T. Welsh, and J. W. Evans. 1990. Zona pellucida-induced acrosome reaction in equine spermatozoa. Proc. Int. Symp. Equine Reprod., Deauville, France, July 1-7, Pp. 70-71.
- Bamezai, A. K., D. C. Suman, and G. P. Talwar. 1986. Effect of immunization against porcine zona pellucida (PZP) on steroid hormone profiles and fertility in primates. J. Reprod. Immunol., Suppl. 85.
- Deigert F.A., A. Duncan., R. Lyda, K. Frank and J.F. Kirkpatrick. 2003. Immunocontraception of captive exotic species. III. Fallow deer (*Cervus dama*). Zoo Biol. 22:261-268.
- Delsink A., D. Grobler, J.J.Van Altena, J.F. Kirkpatrick and R. A. Fayrer-Hosken. 2002. Field applications of immunocontraception in African elephants (*Loxodonta africana*). Reproduction (Suppl. 60):117-124.
- Delsink, A., J. J. van Altena, D. Grobler, H. Bertschinger, J. F. Kirkpatrick, and R. Slotow. 2007. Regulation of a small discrete African elephant population through immunocontraception in the Makalali Conservancy, Limpopo, South Africa. South African J. Sci. 102:1-3.
- Fayrer-Hosken R.A., H. Bertschinger, J. F. Kirkpatrick., D. Grobler, N. Lamberski., G. Honeyman and T. Ulrich. 1999. Contraceptive potential of the porcine zona pellucida vaccine in African elephants. Theriogenology 52:835-846.
- Fayrer-Hosken R.A., D. Grobler, J.J. Van Altena, H. Bertschinger, and J.F.Kirkpatrick 2000. Population Control: African elephants and contraception. Nature 411:766.
- Frank K.M. and J.F. Kirkpatrick. 2002. Porcine zona pellucida immunocontraception in captive exotic species. Species differences, adjuvant protocols and technical problems. Proc. Ann. Conf. Amer. Assoc. Zoo Vet., Milwaukee, WI.
- Frank, K.M., R.O. Lyda, J.F. Kirkpatrick. 2005. Immunocontraception of Captive Exotic Species, IV. Species Differences in Response to the Porcine Zona Pellucida Vaccine, Timing of Booster Inoculations, and Procedural Failures. Zoo Biology 24:349-358.
- Frisbie, K.M. and J.F. Kirkpatrick. 1998. Immunocontraception of Captive Species: A New Approach to Population Management. Animal Keeper's Forum. 25:346-350.
- Gorman S. P., J. K. Levy, A. L. Hampton, W. R. Collante, A. L. Harris, and R. G. Brown. 2002. Evaluation of a porcine zona pellucida vaccine for the immunocontraception of domestic kittens (*Felis catus*). Theriogenology 58:135-149.

- Gulyas, B. J., R. B. L. Gwatkin, and L. C. Yuan. 1983. Active immunization of cynomolgus monkeys (*Macaca fascicularis*) with porcine zona pellucida. *Gamete Research*. 4:299-307.
- Gwatkin, R. B. L., D. T. Williams, and D. J. Carlo. 1977. Immunization of mice with heat solubilized hamster zonae: production of anti-zona antibody and inhibition of fertility. *Fertil. Steril*. 28:871-877.
- Hasegawa, A., Y. Ikeda, Y. Li, M. Shigeta, K. Koyama, and S. Isojima. 1985. Blocking effect of antiserum raised to purified zona pellucida antigen on sperm binding to oocytes in humans. *Acta Obstet. Gynaec. Jap.* 37:2639, Abstr. 17.
- Hasegawa, A., K. Koyama, M. Inoue, T. Takemura, and S. Isojima. 1992. Antifertility effect of active immunization with ZP4 glycoprotein family of porcine zona pellucida in hamsters. *J. Reprod. Immunol.* 22:197-210.
- Kirkpatrick, J.F. and A. Turner 2002. Reversibility and safety during pregnancy in wild mares treated with porcine zona pellucida. *Reproduction (Suppl. 60)*: 197-202
- Kirkpatrick J.F. and A. Turner. 2003. Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island horses. *J Appl Anim. Welfare Sci.* 6:301-308.
- Kirkpatrick J. F. and A. Turner. 2007. Immunocontraception and increased longevity in equids. *Zoo Biol.* 25:1-8.
- Kirkpatrick J.F. and A. Turner. 2008. Achieving population goals in a long-lived wildlife species (*Equus caballus*) with contraception. *Wildl. Res.* 35:513-519.
- Kirkpatrick, J. F., I. K. M. Liu, and J. W. Turner, Jr. 1990. Remotely-delivered immunocontraception in feral horses. *Wildl. Soc. Bull.* 18:326-330.
- Kirkpatrick, J. F., I. K. M. Liu, J. W. Turner, Jr., and M. Bernoco. 1991. Antigen recognition in mares previously immunized with porcine zonae pellucidae. *J. Reprod. Fert. (Suppl. 44)*: 321-325.
- Kirkpatrick, J. F., I. K. M. Liu, J. W. Turner, Jr., R. Naugle, and R. Keiper. 1992. Long-term effects of porcine zonae pellucidae immunocontraception on ovarian function of feral horses (*Equus caballus*). *J. Reprod. Fert.* 94:437-444.
- Kirkpatrick, J.F., R. Naugle, I.K.M. Liu, J. W. Turner, Jr., and M. Bernoco 1995a. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Bio. Reprod. Monograph Series 1: Equine Reproduction VI.* 411-418.

Kirkpatrick, J. F., W. Zimmermann, L. Kolter, I. K. M. Liu, and J. W. Turner, Jr. 1995b. Immunocontraception of Captive Exotic Species. I. Przewalski's horse (*Equus przewalskii*) and banteng (*Bos javanicus*). *Zoo Biol.* 14:403-416

Kirkpatrick, J. F., P. Calle, P. Kalk, I.K.M. Liu, M. Bernoco and J.W. Turner. 1996. Immunocontraception of captive exotic species. II. Formosan sika deer (*Cervus nippon taiouanus*), axis deer (*Cervus axis*), Himalayan tahr (*Hemitragus jemlahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), muntjac deer (*Muntiacus reevesi*), and sambar deer (*Cervus unicolor*). *J. Zoo Wildl. Med.* 27:482-495.

Kirkpatrick, J.F., J. W. Turner, Jr., I. K. M. Liu, R. A. Fayere-Hosken and A. T. Rutberg 1997. Case studies in wildlife immunocontraception: wild and feral equids and white-tailed deer. *Reprod., Fert. Dev.* 9:105-110.

Leveille, M. C., K. D. Roberts, S. Chevalier, A. Chapdelaine, and D. Bleau. 1987. Formation of the hamster zona pellucida in relation to the ovarian differentiation and follicular growth. *J. Reprod. Fert.* 79:173-183.

Liu, I. K. M., M. Bernoco, and M. Feldman. 1989. Contraception in mares heteroimmunized with pig zona pellucida. *J. Reprod. Fert.* 85:19-29.

Lyda, R.O., J.R. Hall, J.F. Kirkpatrick. 2005. A Comparison of Freund's Complete and Freund's Modified Adjuvants Used with a Contraceptive Vaccine in Wild Horses (*Equus caballus*). *Journal of Zoo and Wildlife Medicine* 36(4): 610-616.

Mahi-Brown, C. A., R. Yanagimachi, J. C. Hoffmann, and T. T. Huang. 1985. Fertility control in the bitch by active immunization with porcine zona pellucida: use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. *Biol. Reprod.* 32:761-772.

McShea W.J., S. L. Monfort, S. Hakim, J.F. Kirkpatrick, I.K.M. Liu, J.W. Turner, L. Chassy, and L. Mujnson. 1997. Immunocontraceptive efficacy and the impact of contraception on the reproductive behaviors of white-tailed deer. *J. Wildl. Manage.* 61:560-569.

Naugle R.E., A.T. Rutberg, H.B. Underwood., J.W. Turner and I.K.M. Liu. 2002. Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, New York, USA. *Reproduction (Suppl.60)*:143-153.

Rutberg A.T., R. E. Naugle, L.A. Thiele, I.K.M. Liu. 2004. Effects of immunocontraception on a suburban population of white-tailed deer *Odocoileus virginianus*. *Biological Conservation* 116:243-250.

Sacco, A, G. 1977. Antigenic cross-reactivity between human and pig zona pellucida. *Biol. Reprod.* 16:164-173.

Sacco, A. G., M. G. Subramanian, E. C. Yurewicz, F. J. DeMayo and W. R. Dukelow. 1983. Heteroimmunization of squirrel monkeys (*Saimiri sciureus*) with a purified porcine zona antigen (PPZA): immune response and biological activity of antiserum. *Fertil. Steril.* 39:350-358.

Sacco, A. G. 1987. Zona Pellucida: Current status as a candidate antigen for contraceptive vaccine development. *Am. J. Reprod. Immunol. Microbiol.* 15:122-130.

Sacco, A. G., E. C. Yurewicz, and M. Subramanian. 1989. Effect of varying doses and adjuvants on antibody response in squirrel monkeys (*Saimiri sciureus*) immunized with the porcine zona pellucida Mr = 55,000 glycoprotein (ZP3). *Am. J. Reprod. Immunol.* 21:1-8.

Shideler S.E., M.A. Stoops, N.A. Gee, J.A. Howell, and B.L. Lasley. 2002. Use of porcine zona pellucida (PZP) vaccine as a contraceptive agent in free-ranging Tule elk (*Cervus elaphus nannodes*). *Reproduction (Suppl. 60)*: 169-176.

Skinner, S. M., H. J. Kirchick, and B. S. Dunbar. 1983. Abnormal cellular differentiation of ovarian follicles is induced by immunization with zona pellucida (ZP). *J. Cell Biol.* 97:182a.

Trounson, A. O., C. A. Shivers, R. McMaster, and A. Lopata. 1980. Inhibition of sperm binding and fertilization of human ova by antibody to porcine zona pellucida and human sera. *Archs. Androl.* 4:29-35.

Tsunoda, Y., and M. C. Chang. 1976a. Effect of anti-rat ovary antiserum on the fertilization of the mouse and hamster eggs in vivo and in vitro. *Biol. Reprod.* 14:354-361.

Tsunoda, Y., and M. C. Chang. 1976b. In vivo and in vitro fertilization of hamster, rat and mouse eggs after treatment with anti-hamster ovary antiserum. *J. Exp. Zool.* 195:409-416.

Turner, A. and J. F. Kirkpatrick 2002. Population effects, increased longevity and condition among immunocontracepted wild mares. *Reproduction (Suppl. 60)*: 187-195.

Turner, J. W. Jr., I. K. M. Liu, and J. F. Kirkpatrick. 1992. Remotely-delivered immunocontraception in white-tailed deer. *J. Wildl. Manage.* 56:154-157.

Turner J.W., J.F. Kirkpatrick and I.K.M. Liu. 1996. Effectiveness, reversibility and serum antibody titers associated with immunocontraception of captive white-tailed deer. *J. Wildl. Manage.* 60:45-51.

Wood, D. M., C. Liu, and B. S. Dunbar. 1981. Effect of alloimmunization and heteroimmunization with zona pellucida on fertility in rabbits. *Biol. Reprod.* 25:439-450.

Revised 4-17-06

Liability Statement
(please return with order)

I understand that this request is being initiated for an experimental product. The FDA through an Investigational New Animal Drug (INAD) exemption authorizes porcine Zona Pellucida (PZP) use in animals, including horses, deer and captive exotic species. The vaccine has not been extensively tested in many of the species for which it is requested. I agree to participate in this study of PZP for contraceptive purposes and will provide information of efficacy and side effects, and reversals of this vaccine. I also agree that copies of all pathology reports can be sent to the Science and Conservation Center for FDA reporting.

Signed _____
Attending/Chief Veterinarian/Owner