The Science and Conservation Center

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TO: Zoo Staff

FROM: Kimberly M. Frank
The Science and Conservation Center, Director

RE: PZP Immunocontraception of Captive Exotic Species

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 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 many both ungulates (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).

Among the ungulates, most data have 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 Przewalskís 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 regarding 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 most 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 fewer 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 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.

Apart from 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 30 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 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 in each species. 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. 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 each 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, two inoculations should be given during the first year of treatment. The first inoculation (primer) can be given at any time of year, but the second inoculation (booster) must be given, no later than 1-2 months before breeding activity but **no sooner than 2 weeks after the primer.** Single annual booster inoculations may be given during subsequent years in seasonal breeders 1-2 months before the breeding season.

Year-round breeders, booster inoculations should be given every 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 (MFA) (product 344289, Sigma-Aldrich, St Louis, Missouri), for the initial or primer inoculation and Freund's Incomplete Adjuvant (FIA) (product no. F5506, Sigma-Aldrich, St Louis, Missouri). for all subsequent inoculations. A study (Deigert et al. 2003) has shown that antibody titers resulting from treatment with MFA 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, MFA, 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 smallpox vaccinations, but under the skin instead of on the surface.

The adjuvant will be provided at cost. The FIA $19.00 per 10ml bottle and the MFA costs $27.00 per 10ml bottle. Each 10ml 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 0.5 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; 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 back and forth strokes. It is important to create a thick emulsion because this has beneficial effects regarding 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 18g 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, intramuscularly.

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, but not required, appropriate control groups should be established.

6. 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.

7. 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, to document reversibility. We realize this may not be desirable or possible for some species in some zoos**. Reversibility data is very important, so no matter how long after an animal is removed from the use PZP, please report to the SCC!**

8. 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.

The AZA Reproductive Management Center (RMC) conducts pathology surveillance to identify adverse reactions that might be associated with contraceptive products. For this surveillance, we are requesting reproductive tracts from all male and female mammals treated with contraceptives as well as from *non-contracepted individuals,* so we can compare normal changes with lesions arising from contraceptive use. Please fill out the submission form found on www.sccpzp.org as completely as possible, so the samples can be evaluated in the appropriate context.

Females: Intact, formalin-fixed reproductive tracts (uterus and ovaries plus mammary gland, if possible) obtained from necropsy or ovariohysterectomy. Make a *small* incision into the body of the uterus (for primates) or into each horn (for species with bicornuate uteri).

Males: Half of each testis (include cauda and head of epididymis) obtained at necropsy or castration. Send half of each testis, cut longitudinally plus cauda and head of epididymis.

Immerse tissue in buffered formalin for at least 72 hours (ratio of tissue to formalin = 1:10).

For reproductive tracts that are too large to fix whole and ship (e.g., elephant, rhinoceros, etc.), representative sections may be cut per the protocol on the Wildlife Contraception Center website: www.stlzoo.org/contraception.

SHIPPING: Wrap fixed tracts in formalin-soaked paper towels, enclose in a leak-proof plastic container and ship by ground US 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 USA 48910-8104
Phone: 517-353-1683
agnewd@dcpah.msu.edu
:

ORDERING PROCEDURES

Visit Request PZP Vaccine - The Science & Conservation Center (sccpzp.org) to place an order. You will first be asked to sign up for a user name and password, if you have not already, or email Kim Frank at kfrank@sccpzp.org.

The vaccine will be sent over-night via Federal Express, on ice packs. It must be kept frozen (ultra-low temperature freezers are not necessary) until use. Vaccine is 100µg doses in 1.5 ml plastic micro-vials.

PZP shipped internationally will be lyophilized when shipped. The cost for each does is $35.00US.

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. Provide copies of necessary permits to enclose with the shipment of vaccine.

VACCINE COSTS

We provide the vaccine to zoos and non-profit organizations at our cost of production, which is $30/dose. We will bill you after you receive the vaccine. Do nothing about payment until we send an invoice.

DATA COLLECTION (VERY IMPORTANT)

The contraception of zoo animals is conducted under an Investigational New Animal Drug exemption (INAD) (No. 8840). It is an FDA requirement that data be completed for each animal treated. The data input form can be found on Request PZP Vaccine - The Science & Conservation Center (sccpzp.org).

INQUIRIES

All inquiries regarding PZP contraception should be directed to:
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Research with PZP contraceptive vaccine and captive exotic species is a joint venture involving the support of The Science and Conservation Center and philanthropic organizations. We appreciate your interest in our work.

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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 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