Immunocontraception of Captive Species: A New Approach to Population Management

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There are several rationales for fertility control in captive populations. First, the problem of surplus captive animals and acceptable management of these animals has become a serious issue for zoos (Lacy, 1991). Second, fertility inhibition may be indicated for specific animals for reasons of poor health, undesirable genetic traits, age, or behavioral problems (Kirkpatick and Turner, 1991). Because of the need for contraception in some captive populations, the American Zoo and Aquarium Association (AZA) formed the Contraception Advisory Group (CAG). Some of the goals of the CAG are to: (1) make recommendations regarding what form of contraception to use for specific species, (2) educate the public regarding this issue and (3) educate the zoo community about the importance of contraception for responsible management of zoo collections (Asa, 1993).

One of the relatively new tools for fertility control is Porcine Zona Pellucida (PZP) immunocontraception. A non-cellular membrane known as the zona pellucida (ZP) surrounds all mammalian ova. The ZP consists of several glycoproteins one of which, ZP3 is thought to be the sperm receptor. The PZP vaccine is derived from porcine ovaries; the ZP are separated from the ova and the glycoprotein is solubilized and is delivered to target animals intramuscularly. The principle of PZP immunocontraception involves stimulating the animal's immune system to produce antibodies, which will interfere with sperm attachment on the protein receptor on the ovum. When the PZP is injected into the target animal, the animal produces antibodies against the antigen (PZP). These antibodies also attach to the target animal's own ZP3 thus distorting the receptor, and thereby blocking the sperm from fertilizing the ovum (Kirkpatrick, 1995)

Porcine Zona Pellucida has been proven to be a promising form of birth control in captive exotic species and free-roaming wildlife because: (1) it has a very high contraceptive efficacy, (2) it can be delivered remotely, via darts, (3) its effects are reversible after short-term use, (4) it is effective across many species, (5) it lacks debilitating health side effects, even after long-term treatment, (6) it has minimal effects on social behaviors, (7) the vaccine and the antibodies induced in response to it cannot be passed through food chains, and (8) it is safe to give to pregnant or lactating animals (Kirkpatrick et. al., 1997).

Not only are there management concerns for captive species, but there are also management concerns for wild, free-roaming populations where non-lethal management methods are required or advisable. The PZP vaccine is being used successfully to control reproduction in wild horses (*Equus caballus*) (Kirkpatrick, 1995), white-tailed deer (*Odocoileus virginianus*) (Turner et. al., 1996a), feral donkeys (*Equus asinus*) (Turner et. al., 1996b), water buffalo (*Bubalus arnee*), elk (*Cervus canadensis*) (Kirkpatrick et. al., 1996a) and African elephants (*Loxodonta africana*) (Fayrer-Hosken et. al., 1997). In fact, it was successful trials in wild horses that stimulated the idea to try the PZP vaccine in zoo animals.

The PZP vaccine is currently being used in zoo animals worldwide to control captive animal populations. The first project began at the Cologne Zoo in Germany in 1990. The species tested were the Przewalski's horse (*Equus przewalskii)* and the banteng *(Bos javanicus)* and the PZP vaccine was shown to be an effective form of contraception for both species (Kirkpatrick et. al., 1995). In 1990 a larger project, involving six different ungulates (Kirkpatrick et. al., 1996b), was initiated at the Wildlife Conservation Center - Bronx Zoo. By 1993, 17 zoos were participating in the study (Kirkpatrick et. al., 1993) and to date approximately 90 species in 76 zoos have been treated with PZP in a collaborative program with ZooMontana and the Humane Society of the United States. Table 1 indicates animals that have been treated with PZP.

During the initial year of treatment the PZP vaccine is given either two or three times and a booster inoculation is given every year thereafter. If the animal has a well-defined and short breeding season, a two-inoculation protocol may be sufficient. The first inoculation is given 1-2 months prior to breeding activity and the second inoculation 1-2 weeks prior to the onset of breeding activity, however there is some latitude in the timing of these inoculations. If the animal does not have a specific breeding season then the first dose can be given anytime, followed by two more inoculations given two weeks apart. The important point in the timing of the inoculations is to achieve maximum antibody titers during the peak breeding season.

The reason the PZP vaccine works in so many different species of mammals is because the sperm receptor (ZP3) has been highly conserved in the course of evolution. However, the cost of this molecular evolutionary conservation is that the PZP vaccine is a very poor immunogen. Thus, in order to be effective the vaccine must be given with an adjuvant, which is a general immunostimulent (Kirkpatrick et. al., 1996a). The initial research has shown that the use of Freund's Complete adjuvant (FCA) (Sigma Chemical Company) for the first inoculation and Freund's Incomplete adjuvant (FIA) (Sigma Chemical Company) for all subsequent inoculations results in the highest antibody (Ab) titers and the greatest contraceptive success. Although FCA is the most effective adjuvant it may cause a positive tuberculosis (TB) test. All vaccinations may be given with FIA, which will avoid the problem of positive TB tests but extra inoculations will have to be given because the Ab titers will not be as great as when FCA is given for the initial inoculation. In those species for which a TB test does not exist, as in the case of equids, the FCA can be used routinely.

Addax	Cougar*	Leopard	Reindeer
Addra Gazelle	Cuvier Gazelle	Llama	Reticulated Giraffe
African Lion	Dall Sheep	Lynx	River Hippo
American Bison*	Domestic Ass	Malayan Sun Bear*	River Otter
Angolan Springbok	Domestic Goat	Mandarin Sika Deer*	Roan Antelope
Arabian Wildcat	Dromedary	Mandrill	Rock Hyrax
Asian Black Bear*	Elk*	Markhor	Rocky Mt. Bighorn Sheep*
Axis Deer	European Wolf	Mountain Goat	Rocky Mt. Goat*
Babirusa	Fallow Deer	Mule Deer*	Ruffed Lemur
Banteng	Fennec Fox	Muntjac	Sambar Deer
Barasingha	Gerenuk*	Nilgiri Tahr*	Siberian Ibex*
Binturong	Grant's Zebra*	Northern fur seal	Snow Leopard
Black Buck	Greater Kudu*	Nubian Giraffe*	Sri Lanka Elephant
Black Lemur	Grevyi's Zebra*	Nubian Ibex*	Taiwanese Sika Deer*
Brazilian Tapir	Hamadryas baboon	Nyala	Tiger
Brown Bear*	Hartman's Zebra*	Onager	Topi
Bush Baby	Hippopotamus	Oryx	Tur*
Calif. Sea Lion*	Impala*	Persian Leopard	Water Buffalo*
Chapman's Zebra*	Jaguar	Plains Zebra*	Waterbuck*
Coatimundi	Kirk's Dik Dik	Przewalski's Horse*	White-tailed Deer*
Colobus Monkey	Kodiak Bear*	Reeve's Muntjac	Wolf

Table 1: List of species treated with the PZP vaccine. Those species marked with an asterisk (*) are species for which there are sufficient data to indicate successful contraception. The database for those species not so marked is still insufficient for drawing conclusions about successful contraception.

Although there have been very positive results in most animals in which PZP has been tested, several problems have been encountered. The mixing of the thick PZP-adjuvant emulsion can be a challenge. Good quality glass syringes attached with Luer locks (Yale Hypodermic) work very well at mixing the thick emulsion. Using plastic syringes, for example, tends to cause binding of the emulsion. The proper delivery system is also essential. If darts are used for remote delivery Pneu-Dart® darts are recommended, because of the ease with which the thick emulsion passes through the 14-gauge needle. If other equipment is used the needle should not be smaller than 16 g. A good intramuscular injection is essential. Thus, the length of the needle used must be selected with the skin thickness and fat pads in mind.

On occasion an abscess will form at the injection site. The few that have occurred have been small and healed without incidence. The cause of these abscesses may involve the adjuvant, and in particular FCA, or the intrusion of foreign material by the dart or syringe needle.

In animals in which a sustained contraceptive effect is desired, the timing of the annual booster inoculation is also important. Some animals such as zebra (*Equus ssp.*) and muntjac (*Muntiacus ssp.*) have become pregnant because the titers have dropped sooner than a year after the initial inoculations, but this can be easily rectified by booster inoculations every 7-9 months. In most other species however, a single annual booster inoculation is sufficient.

Reversibility of contraception is often an important consideration in valuable captive exotic species where the goal is to delay reproduction rather than prevent it permanently. Most of the data regarding reversibility of PZP contraception has been derived from wild horses and white-tailed deer. In horses, reversibility usually occurs after three or four consecutive years of treatment, and there is limited data to indicate that five consecutive years of treatment inhibits fertilization but not ovulation. However, PZP treatment longer than five years may render the animal incapable of ovulation by destroying ovarian oocytes (Kirkpatrick, 1995). In any case, reversibility of contraceptive action after short-term use (1-3 years) has been documented in horses, white-tailed deer, muntjac, and zebra.

The maintenance of social behaviors in zoo animals is extremely important if we are serious about our mission as educational institutions. Because the PZP vaccine blocks fertilization and does not alter endocrine patterns, behaviors are not seriously affected. Again, the largest body of data has come from wild horses, and treatment over many years has not altered band fidelity by mares or stallions nor has it changed social hierarchy ranks among animals (Kirkpatrick, 1995). Although systematic studies have not been carried out, the same appears to be true among white-tailed deer maternal groups.

The PZP vaccine is a new contraceptive tool that is not suitable for every situation, but along with other contraceptive methods, it increases our ability to manage captive populations. The PZP vaccine has many advantages. The foremost advantage is that it does not cause any debilitating health problems.

Additionally, the PZP vaccine can be delivered remotely so there is no need to immobilize or anesthetize the animal, thereby reducing stress. Finally, the contraceptive effects of the PZP vaccine are reversible after short-term use (up to three years) and this provides management flexibility.

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