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CHAPTER 94

Contraception in Artiodactylids, Using Porcine Zona Pellucida Vaccination

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The successful use of porcine zona pellucida (PZP) as a contraceptive vaccine was first demonstrated in the domestic equid (*Equus caballus*). The results obtained from field and experimental trials from this species laid the foundation for the application of PZP immunoneutralization in artiodactylids. The investigation in equids demonstrated the effectiveness of the vaccine using a two-inoculation protocol consisting of homogenized PZP as the antigen, with the incorporation of Freund's complete adjuvant in the first inoculation and incomplete adjuvant in the second inoculation as immunizing agent.^{4,7} Experimental data in the equid described the dynamics of antibody response resulting from immunization and its relation to the ability of mares to be protected from conceiving at following matings. In addition, reversibility of the contraceptive effect following diminishing antibody titers, its safe use in pregnant mares, and the continuation of cyclicity with acceptance of the stallion was demonstrated. Further indications from studies in the equid suggested that the antibodies produced as a result of the inoculations exerted their effect at the level of the oocytes, whereby zona receptors to spermatozoa, present on the surface of the oocytes, are being blocked, thus preventing spermatozoa penetration into the oocyte and subsequent fertilization.

In artiodactylia, experimental design to obtain detailed physiologic data as a result of PZP immunization is limited because of the physiologic stresses and high risks for injury associated with physical or chemical restraint of the animals. In particular, frequent and repeated blood collections for determining the dynamics of antibody titers following inoculations, albeit important, can involve major risks.

APPLICATION IN CAPTIVE AND FREE-ROAMING DEER

Successful PZP contraception in artiodactylids was first demonstrated in captive deer (*Odocoileus virginianus*).¹⁰ In this investigation, eight does were given three inoculations of PZP administered remotely with a dart gun, 3 weeks apart. As a result of these inoculations, none of the PZP-treated does produced fawns compared with seven control does that yielded a live fawn rate of 86%. Even though antibody response was not determined in this study, it clearly demonstrated the potential effectiveness of the immunogen as a contraceptive agent for this species. Because populations of white-tailed deer were increasing and because, in some urban areas, parks, and preserves, hunting or killing of excess populations of deer was prohibited, contraception with PZP appeared to be an attractive alternative to population control and management. As a result, further studies on captive and free-roaming deer were performed. In one recent investigation,¹¹ 53 captive white-tailed deer were studied under three different treatment protocols: (1) three PZP inoculations administered 3 weeks apart, (2) two PZP inoculations administered 3 weeks apart, and (3) one PZP inoculation consisting of continuous release vehicles including an osmotic pump or microspheres (biodegradable lactide and glycolide polymers). The results of this study confirmed the effectiveness of PZP as an immunoneutralization in this species when at least two inoculations were administered to the does before the mating season. In this investigation, no does administered the two- and three-inoculation protocols produced fawns during a treatment year. In comparison,

the fawning rate for control does was 93.8%. Even though there appeared to be some contraceptive effect elicited on does receiving only one inoculation with sustained release vehicles, the overall contraceptive effectiveness (80%) was not as significant compared with the two- and three-inoculation protocols (100%). This study further demonstrated that, in all does given PZP, antibody responses developed to the inoculum 4 to 6 weeks after the treatment, regardless of the number of inoculations (Table 94-1). The antibody levels generated represent a significantly elevated increase in antibody titer and is far more than what is believed by the investigators to be the contraceptive titer (>50% of positive reference serum) in this species. The duration of antibodies generated persisted for at least 32 to 40 weeks after inoculation in most does. The protective titers remained elevated for up to at least 64 weeks in two of the four does tested and in those receiving the two-inoculation protocol. Antibody titers for the remaining does in this study were not available. The persistence of antibody titers for such extended periods without additional inoculations in the two does tested is unexplained. Reversibility of the contraceptive effect of the inoculations was substantiated, however, when a majority of the does inoculated became pregnant in subsequent breeding seasons and produced fawns.

Field trials on captive and free-ranging deer have also been carried out in other locations within the eastern and midwestern United States, with approximately 250 does being inoculated with differing formulations of the PZP inoculum.⁶ The successful application of PZP was confirmed in these field trials using one- and two-inoculation protocols, with one-inoculation protocols also comprising vehicles for sustained antigen release (osmotic pumps and microspheres). Logistically and economically, the advantage of achieving a slightly lower contraception rate within a breeding season through one inoculation is better than achieving an overall higher conception rate with the administration

of two inoculations to each free-ranging wildlife animal. Although the differences in antibody response, duration of immunity, and contraception between one- and two-inoculation protocols were anticipated before each study, single inoculation protocols were included in the field trials to determine the effectiveness of contraception, if any. The results achieved using single injections of PZP (with or without osmotic pumps and biodegradable microspheres) throughout one breeding season, although moderately effective (70%), did not achieve the contraception rates equal to that of the two-inoculation protocols (100%) in most of the field studies in deer.

In all of the field studies, a limited number of ovaries have been examined from inoculated deer that were accidentally killed. Preliminary evidence indicates that pathologic effects on ovarian tissue have not been detected as a result of PZP inoculations. The continued use of PZP as an immunocontraceptive agent is encouraging. However, the design and release of PZP via a one-inoculation protocol to maintain contraceptive effect in wildlife through one breeding season needs improvement. In addition, the long-term side effects, if any, following yearly PZP inoculations require further investigation.

APPLICATION IN EXOTIC ARTIODACTYLIDS

Application of PZP immunocontraception in other artiodactylids has also taken place in captive zoo animals.⁶ Increased reproductive successes of many species of animals within zoos have resulted in "overcrowded" populations. Translocation of excess animals to their natural habitat is not always feasible, nor is translocation to other zoos. In addition to overcrowding factors, animals whose genetic traits are not desirable have also been subjected to contraceptive studies. Among many agents used for contraception purposes, PZP may have

TABLE 94-1. Anti-PZP Antibody Titers During One Breeding Season in Captive White-Tailed Does Given PZP Contraceptive Vaccine*

Experiment	No. of Does Tested	Anti-PZP Antibody Titers (% of Positive Reference Serum)†				
		Prevaccination Baseline‡ (Week 0)	Prebreeding Season (Weeks 4-6)		Postbreeding Season§ (Weeks 32-40)	
			\bar{x}	SE	\bar{x}	SE
3-Injection	4	<8	102.1	1.2	72.7	15.7
2-Injection	4	<8	100.0	0.0	92.0	5.2
1-Injection/pump	3	<8	76.3	7.2	69.5	11.8
1-Injection/microspheres	4	<8	53.7	15.5	19.1	4.3
Control	2	<8	<8		<8	

*Does were treated in October-November; fawning occurred the following summer.

†Positive reference serum, designated as 100%, was the average value of maximal titers achieved in six PZP-treated does that did not produce fawns.

‡Placebo-treated does from 2-injection experiment.

§All values were below the lower limit of detection.

||No differences between prebreeding and postbreeding season titers in any treatment group, based on analysis of variance with repeated measures, $p < 0.05$.

an important role to play in contraception in zoos. Although the numbers of animals representing each species have been few (5 to 15), of at least 68 different species of animals in which PZP was used, contraception was successful in three families (Cervidae, Bovidae, and Caprinae) of artiodactyla (Table 94-2). The number of blood samples obtained for antibody detection following PZP inoculations in zoo animals has been limited by constrained policies for blood collections; however, of those available, the antibody levels in species (except fallow deer) of the artiodactylids are equivalent to those found in free-roaming and captive white-tailed deer. Reasons for poor antibody response to PZP inoculations and the resulting ineffectiveness in contraception in fallow deer (eight does fawned out of 10 vaccinated does) are unknown.

The use of PZP as a means of contraception in artiodactylids continues to provide results that make this mode of contraception a potentially attractive alternative to population control in this and other wildlife animal species. Current immunocontraception strategies using PZP are directed only at populations of animals in which hunting or killing as a means of culling is prohibited.

ADVANTAGES OF IMMUNOCONTRACEPTION

The advantages of using PZP as an immunocontraceptive agent over other available contraceptive agents are compelling. The effectiveness of PZP as an immunocontraceptive agent covers a broad range of animal species. Although PZP cross-reactivity studies are limited to a few animal species, it appears that macromolecules within the zona pellucida are highly conserved across species. Thus, PZP is highly tissue specific but not species specific.³ The safety of PZP in pregnant mares is documented, but further studies are necessary to evaluate its safety in pregnant artiodactylids. The

effects of PZP are reversible, in that antibodies generated as a result of inoculations eventually decay if the animal is not re-inoculated, thus diminishing protection against pregnancy. There is convincing evidence that there are threshold levels of antibodies that correlate with contraception and that this level varies according to the animal species inoculated. So, the mere presence of antibodies does not necessarily indicate protection against fertilization. Once antibodies decay to levels below the threshold, fertilization resumes. PZP can be delivered remotely with a darting rifle, making it unnecessary for highly stressful, physical restraint of the animal for inoculation purposes. Immunocontraception with PZP in the herd animals investigated does not disrupt the social behavior nor does it disrupt the band integrity. Most importantly, PZP and the antibodies generated as a result of an inoculation are not passed on to other wildlife species during scavenging of carcasses.

DISADVANTAGES OF IMMUNOCONTRACEPTION

One disadvantage of immunocontraception with PZP is the use of Freund's adjuvant in wildlife. It results in seroconversion to *Mycobacterium tuberculosis* antigen and cross-reactivity with active infections of *M. tuberculosis* and potentially other mycobacterial agents. Attempts to incorporate alternative adjuvants with PZP vaccines are being investigated.¹ There is also conclusive evidence of development of ovarian malfunction following the use of PZP in laboratory animals studied—the canine species and nonhuman primates.^{2,4,5,12} The primary side effects were the disruption of folliculogenesis and oocyte atresia. In a small number of ovaries obtained from both inoculated equids and Cervidae, evidence of short-term effects on ovarian function caused by PZP inoculations has not been found nor is there evidence of histologic abnormalities associated with the use of PZP.^{6,7} The disparity in side effects

TABLE 94-2. Captive Exotic Zoo Artiodactylids Inoculated with Porcine Zona Pellucida

Family	Common Name	Contracepted	Not Contracepted	Results Pending
Bovidae	North American bison	3	2	1
	Bangiang	2	0	2
	Siberian ibex	16	0	—
	Greater kudu	6	0	8
	Water buck	4	1	2
	Impala	3	0	—
Cervidae	Barasingha	1	0	2
	Axis deer	3	2	—
	Muntjac deer	2	0	—
	Sika deer	9	1	—
	Roosevelt elk	7	1	—
	Sambar deer	9	1	—
	White-tailed deer	5	0	8
Caprinae	Himalayan tahr	6	0	9
	Rocky Mountain big horn sheep	3	0	3
	Rocky Mountain goat	2	0	8
	West Caucasian tahr	6	0	—
Giraffidae	Reticulated giraffe	4	2	—

found in other mammalian species studied may be related to the amount of antigen used for each inoculum.² Indeed, following long-term use of PZP immunizations (5 to 6 years of annual inoculations), ovarian function ceases temporarily in the equid.⁵ While the cause of impaired ovarian function is not understood, cessation of yearly inoculations allows for resumption of ovarian function in the equid. Investigations of long-term effects of PZP immunization in artiodactylids is not available. Other criticisms directed against PZP immunization include the extension of breeding activity outside of the natural mating season and concerns of the survival of newborns born late in the season as a result of this procedure.

For immunocontraception of wildlife, a single inoculation program that ensures contraception for one or two mating seasons is most desirable. The incorporation of various forms of matrices with PZP agents is being investigated in continuous or controlled release patterns to achieve effectiveness following single inoculations of PZP. Preliminary evidence of matrix-incorporated PZP inoculations in a field study of a small number of inoculated horses (28) indicates that the method can be applied to large-scale field studies in wildlife and that the method shows promise of being as successful as the standard two-inoculation protocol. In this investigation, 2 of 28 mares were tentatively determined to be pregnant by microtiter enzyme immunoassays for estrone conjugates and nonspecific progesterone metabolites of feces collected 8 months after a single inoculation of PZP containing Freund's adjuvant and the controlled-release matrix.

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CHAPTER 95

Parelaphostrongylus tenuis and Elaphostrongylus cervi in Free-Ranging Artiodactylids

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Throughout the world, the capture and translocation of free-ranging wild ungulates not only has become a common wildlife management tool but also is an essential component of game farming and ranching. Translocation of wildlife has been used to restock historic

ranges, supplement dwindling populations, and reduce undesirably high densities of wildlife in urban areas.²³ Among the many issues associated with translocation events are concerns that introduced animals do not carry diseases or parasites that could establish new endemic