PZP Immunocontraception in Coyotes: A Multi-Year Study with Three Vaccine Formulations

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ABSTRACT: The use of poisons for coyote control is controversial because of public opposition to lethal control of pest animals and the perceived environmental risks of pesticide use. The development of immunocontraception for population control of coyotes could result in a more acceptable alternative to poisons. Immunocontraception using porcine zona pellucida (PZP) would allow normal estrus in the female and therefore normal male-female pair-bonding. Coyotes are mon-estrus, therefore PZP contraception during the breeding season of February and March could provide year-round protection. This paper reviews 9 years of research on PZP immunocontraception, starting from a multi-shot PZP vaccine using Freund's adjuvant, to the development and testing of two single-shot preparations combined with a newly developed adjuvant (AdjuVac™). We provide insights into the false assumption that one contraceptive vaccine fits all species and situations.

KEY WORDS: AdjuVac™, *Canis latrans*, coyote, immunocontraception, PZP, single-shot vaccine, SpayVac™

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INTRODUCTION

The coyote (*Canis latrans*) is a seasonally mon-estrus animal that normally breeds in late January and February and whelps in April, with a litter size of 3-9 pups. Adults have a long reproductive life of 3-10 years. They construct and use dens only during whelping of pups. Coyotes are territorial and may live as pairs or in packs of up to 10 individuals. Within each resident pack, a hierarchy exists in which the dominant pair are called the "alphas." Food abundance influences coyote populations by affecting reproduction, survival, dispersal, space-use patterns and territory density (Connolly 1992).

The coyote is highly adaptable in exploiting livestock production. A 1990 survey (GAO 1990) estimated 549,000 lamb deaths occurred from all causes, out of the nearly 6 million lambs born in the 16 western states in that year. Nearly 60% of the lamb deaths were attributable to predators, with approximately 70% of predator damage due to coyotes. The economic impact on producers and consumers in 1990 was approximately $11.4 million. Despite intensive historical control efforts with a variety of methods in livestock production areas, and despite sport hunting and trapping for fur, the coyote continues to thrive and expand its range, such that coyotes are now found in most states in the U.S.

For more than 50 years, the USDA National Wildlife Research Center (NWRC) and its predecessor laboratories have conducted research on coyote reproduction. Hamlett (1938) studied the reproductive cycle of the coyote to support passing better laws involved with the protection and conservation of fur resources. As coyotes began to cause livestock predation problems, an excellent laboratory study by Kennelly and Johns (1976) was undertaken to understand the reproductive cycle of the coyote. Kennelly (1978) continued to study reproduction in the coyote, and Knowlton (1972, 1989) spent many years studying the relationship of coyote population dynamics to livestock depredation management. Techniques developed for managing coyote populations include husbandry practices, shooting, trapping, frightening devices, livestock guarding dogs, and toxicants (Fall 1990, Linhart *et al*. 1992). None of these control methods is completely practical or effective in all of the diverse situations in which coyote predation on livestock occurs; new techniques are needed (Connolly 1992). Coyotes are increasingly viewed as a desirable wildlife species to be fostered in certain situations. Because of this changing view of the coyote, more non-lethal methods are being sought for resolution of predation problems.

Previous research indicates that predation on domestic lambs by coyotes with pups often terminates when the pups are removed; therefore, sterilization of territorial coyotes may reduce predation on nearby sheep flocks (Knowlton 1989). Immunocontraception as a means of sterilizing coyotes has been suggested as one non-lethal technique that could have application for reducing coyote numbers in areas where they are causing predation (Miller 1995).

Scientists with the Product Development Research Program at the NWRC began an infertility project in 1992 to study alternative non-lethal methods of pest animal control. The project began by studying porcine zona pellucida (PZP) contraception of white-tailed deer (*Odocoileus virginianus*) and coyotes (Miller *et al*. 1999, 2001; Miller and Killian 2000). PZP contraception of white-tailed deer resulted in prolonged estrus periods because of multi-cycling (Killian and Miller 2000), a concern because the prolonged cycling could result in increased deer-car collisions. Because the coyote is mon-estrus, this multi-cycling would not be a problem. Also, because the single estrus cycle of the coyote occurs in February, the period needed for contraception can be quite short, in contrast to white-tailed deer that may cycle for up to 5 months if pregnancy does not occur on the

first cycle.

Contraceptive studies on coyotes started in 1995 (Miller 1995), testing both PZP and gonadotrophin releasing hormone (GnRH) contraceptive vaccines. It was decided after the first year study to continue with the PZP contraceptive only, because of the need to maintain pair bonding of the alpha pair and the possibility that the GnRH vaccine may interfere with pair bonding (DeLiberto *et al*. 1998).

MATERIALS AND METHODS

The studies were performed at the Millville coyote facilities at the NWRC Logan, Utah Field Station. The initial 3 years of the study were partially funded by the Texas Sheep and Goat Board. The facilities include cloverleaf pens with an observation tower, which are ideal for breeding studies in the coyote. Female and male coyotes of proven fertility were paired and put in the cloverleaf pens for breeding and observations of breeding activities from January through the end of February. Monitoring breeding is an art in many animals; however, in the canine the male-female copulatory tie lasts for a period of time, which allows one to accurately measure breeding activity if sufficient observation time is used.

In all studies, baseline blood samples were drawn, the vaccine was injected sub-cutaneously or intra-muscularly (IM), then periodic blood samples were drawn to assay the immune and hormonal response to the vaccine. Effectiveness of the vaccine was measured by either allowing the female to whelp or by examining the reproductive tract at necropsy.

Porcine Zona Pellucida (PZP)

PZP used in Studies 1 and 2 was purchased from Dr. Bonnie Dunbar of Baylor College of Medicine Houston, TX. PZP in Studies 3 and 4 was purchased from Dr. Irwin Liu University of California at Davis. PZP in both Dunbar and Liu preparations were produced by the method of Wood *et al*. (1981). PZP in Study 5 was SpayVac™ donated by Dr. Bob Brown of Immuno-Vaccine Technologies (IVT), Nova Scotia, Canada. SpayVac™ was produced by the method of Yurewicz *et al*. (1983)

Adjuvants

Freund's adjuvant modified (complete and incomplete) was purchased from Calbiochem (San Diego, CA). Adjumer™ was donated by the Virus Research Institute (Cambridge, MA). AdjuVac™ was developed at the NWRC.

Laboratory Testing

Progesterone and estradiol were assayed at NWRC by Coat-a-tube RIA assay (Diagnostic Products, Los Angeles, CA). Anti-PZP antibody titers were assayed at NWRC by an ELISA method published previously (Miller *et al*. 1999). Antibody titers for Study 5 using the vaccine SpayVac™ was performed at IVT in Nova Scotia, Canada.

Pregnancy Testing

In Study 1, coyotes were tested for pregnancy by x-ray then allowed to whelp. In Study 2, the coyotes were necropsied to test for pregnancy. Study 3 did not proceed to a breeding trial due to a low antibody titer. In Study 4, relaxin hormone levels were used to test for pregnancy status. Relaxin is produced in the pregnant bitch beginning at 20-25 days of gestation with maximal concentrations attained by days 30-35. Confirmation was made by whelping activity (Edqvist and Forsberg 1997). In Study 5 using SpayVac™, pregnancy was tested by whelping activity.

Testing for Fertility in Paired Males

Blood was drawn on all males and testosterone measured to ensure that there was sufficient testosterone for males to be fertile. Viable sperm was determined for each male in at least 1 breeding.

Study 1 (1995-1997)

PZP/Freund's Prime and Boost – coyotes were bred and allowed to whelp

Five females of proven fertility were given a prime dose of 200 µg of PZP/Complete Freund's adjuvant (CFA) on 12/07/95 and a booster dose of PZP/Incomplete Freund's adjuvant (IFA) on 01/05/96. Sham control females were given saline/CFA and saline/IFA. Blood samples were drawn before injection and periodically throughout the study. The coyotes were x-rayed for pregnancy diagnosis and were allowed to whelp in April 1996. The coyotes were boosted with 45 µg of PZP on 11/18/96 and kept through April and allowed to whelp.

Study 2 (1997-1998)

PZP/Freund's Prime and 2 Boosts – coyotes were bred and necropsied

Results in Study 1 showed that PZP had reduced the number of females pregnant, and the females had fewer pups. Mahi-Brown *et al*. (1988) suggested that infertility in PZP-immunized dogs was the result of follicular dysgenesis or cyst formation. This study was performed to understand the mechanism of action of PZP in treated coyotes and to determine whether a second PZP boost would increase contraception to 100%.

Five female coyotes of proven fertility were given a prime dose of 200 µg of PZP/CFA on 12/02/97 and two 100-µg boost doses of PZP/IFA on 12/31/97 and 1/15/98 by subcutaneous injection. The coyotes were exposed to proven males during late January and February 1998 and observations of breeding times and dates were recorded. Thirty days after the last breeding, the females were sacrificed and fertility was determined by examining the reproductive tracts for placental scars, corpora lutea, and fetuses.

Study 3 (1999-2000)

Single injection PZP/Freund's and single injection PZP/Adjumer™

Because of a desire to reduce immunocontraceptive vaccines to a single injection and to develop a water soluble adjuvant, we tested the water-soluble adjuvant, Adjumer™ (Virus Research Institute, Cambridge, MA), which had shown promise as a single-injection adjuvant in a human viral vaccine trial (Payne *et al*. 1995). PZP

vaccine with Adjumer™ was compared to Freund's/CFA using 1 injection on 9 September 1998. Blood was drawn prior to injection and then periodically thereafter for measurement of antibody titers. The protocol stated that the study would not proceed to breeding if anti-PZP titers dropped to 8,000 before breeding was to begin. Low antibody titers resulted in cancellation of the breeding trial.

Study 4 (2001-2002)
Single injection PZP/AdjuVac™

NWRC scientists developed a new adjuvant that has been found to be more effective than the Freund's adjuvant. The AdjuVac™ preparation is described in Miller *et al*. (2004).

Studies with white-tailed deer had shown that PZP/AdjuVac™ was effective as a single-shot vaccine. However, the single injection in deer was found to be effective only if given several months prior to breeding, such as in July instead of August/September, the normal months for the first injection of a multiple-shot vaccine. Therefore, the single injection for the coyote would similarly need to be given in November instead of December or January.

Five proven female breeders were injected with 200 µg PZP/AdjuVac™ on 23 November 2001. Each female was paired with a proven male and observations of multiple breeding were recorded from 02/08/02 to 2/22/02. Blood samples were drawn in March for pregnancy diagnosis using a relaxin assay; pregnancy was confirmed by whelping data.

Study 5 (2003 to Present)
Single injection SpayVac™/AdjuVac™

Five proven females were injected with 100 µg of SpayVac™/AdjuVac™ on 26 November 2003. Each female was paired with a proven male and breeding was observed throughout the month of February. Blood samples were drawn prior to injection and were drawn periodically to determine antibody titers and hormone levels. Reproductive results were determined by ReproCHEK Canine Pregnancy Test Kit by Synbiotics and confirmed by whelping data.

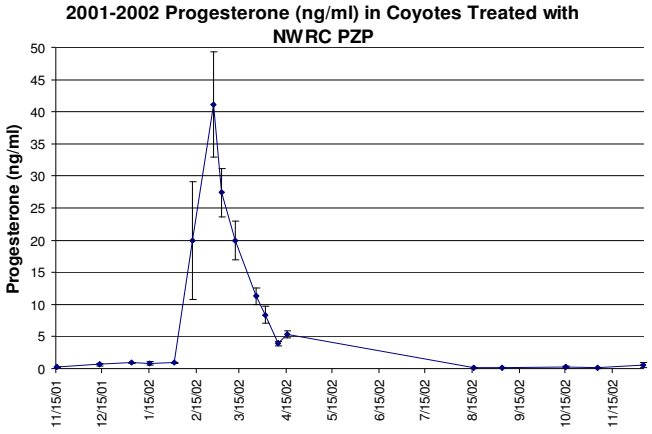

Figure 1. One year, bi-monthly, progesterone data on NWRC PZP-treated coyotes from suggest the coyote has 1 estrus cycle per year.

RESULTS

Estrogen and Progesterone

Progesterone data confirmed that the coyote is a mon-estrus animal (Figure 1). In Study 4, blood samples were collected bi-monthly for 1 year. Progesterone data demonstrated a single synchronous annual peak for the PZP-treated group, beginning the first week of February and lasting through the first week of April. Similar mon-estrus progesterone data were observed by Hodges (1990). This peak represents data from the NWRC/ AdjuVac™ single injection study where coyotes were bred in February, although none of the coyotes became pregnant. The progesterone response in control animals (Figure 2) was similar to that of the PZP-treated animals, which would suggest that, as expected, the PZP vaccine does not interfere with the normal reproductive cycle in the coyote. Gross observation at necropsy indicated that the ovaries appeared healthy with numerous mature corpora lutea. This would suggest that the contraceptive effect in the coyotes tested was due to anti-PZP blocking sperm binding to the zona pellucida.

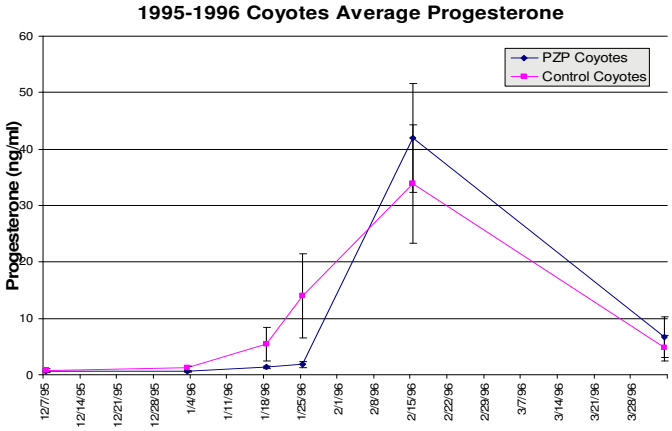

Figure 2. Comparison in the progesterone data in PZP treated coyote to the control coyotes suggest that PZP does not interfere with the estrus cycle of the coyote.

Study 1 (1995-1997)
Reproduction
Year 1 Results (1995-1996)

The 6 vaccinated females produced a total of 6 pups, with a mean of 1 pup/female as compared to 5 pups/female for the untreated coyotes. Antibody titers negatively correlated with whelping success of the females, with higher titers associated with fewer pups. Three of the 6 treated coyotes were 100% contracepted and produced no pups. The remaining 3 females had 1, 2, and 3 pups, respectively. Whelping of both control and PZP-treated coyotes occurred from April 6 to April 17 with no difference in whelping dates noted.

Year 2 Results (1996-1997)

Protective level antibody titers did not last long. By April 1996, there was already a rapid decline in anti-PZP titers, and the pre-boost second year titers were non-detectable at the time coyotes were boosted on 11/18/96. Four of the 5 coyotes responded to the boost with an antibody titer equal to the peak of the previous year. One

coyote responded with a low anti-PZP antibody titer and had 9 pups, an abnormally high number. Two coyotes were contracepted completely with no pups, one had 2 pups, and one had 1 pup.

Females were euthanized after whelping activity ceased. Gross necropsy revealed that the placental scars correlated with the antibody titer and number of pups whelped. The number of corpora lutea did not correlate with the whelping activity, indicating that the females produced multiple ova that were not fertilized. These data are consistent with the concept that anti-PZP antibodies bind to the zona pellucida layer surrounding the ova, preventing sperm penetration and therefore preventing conception.

Hormones

The progesterone results (Figures 1 and 2) of both the control and treated coyotes suggest that the PZP vaccination did not alter the reproductive cycle of the coyotes. Although this had not been tested in the coyote, PZP contraception in other species has been shown not to interfere with normal cycling or breeding activity.

Antibodies

The immune response (Figure 3) to the prime and boost demonstrated an initial titer of over 120,000, a level that has been shown to contracept deer; however, there was a significant drop in titer by February 15, which may be the reason there was not a 100% contraceptive effect in this first study.

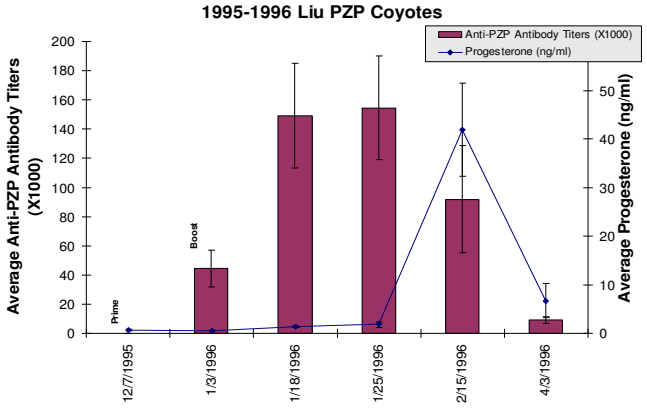

Figure 3. Anti-PZP titers in response to a prime and boost of Liu PZP and its relationship to the estrus cycle of the coyote.

Study 2 (1997-1998)

Receptive Period of Coyotes in Estrus

Many hours spent by the staff at Millville have given us insight into the breeding activity of the coyote. Both control and PZP-treated coyotes were receptive to the male from January 16 to February 15 (Figure 4). Within this 30-day period, the average receptivity in the control group was 8.8 days, with a mean of 11 breeding activities (ties) observed. The average receptivity in the PZP group was 12.6 days with a mean of 12.2 breeding activities (ties) observed. Some females have multiple breedings in 1 day followed by skipped days.

These observations are similar to those of (Morrow 1986), who studied the estrus cycle in the dog. He found that the bitch is receptive to the male during estrus and has a mean receptivity of 9 days with a range of 3-21 days. However, in the dog this cycle is repeated on the average of twice a year as compared to once a year in the coyote.

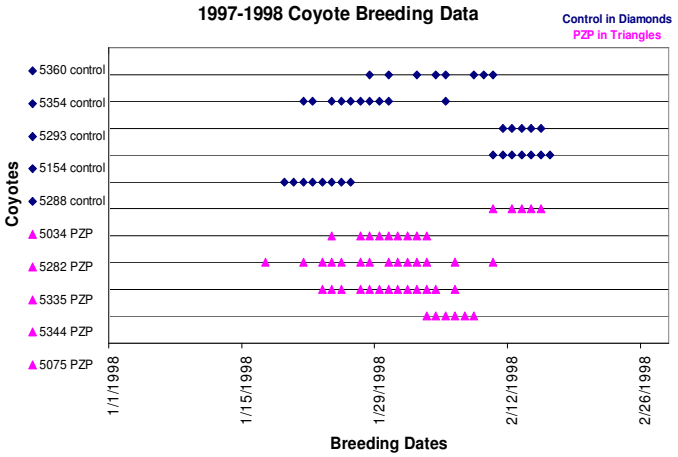

Figure 4. A comparison of the breeding behavior of the PZP treated coyotes as compared to the control coyotes. Each diamond or triangle represents a copulatory tie event.

Hormones

The female receptive period is related to estrogen and progesterone concentrations (Figure 5). Although the individual coyotes came into estrus at different times throughout the 30-day period, the receptiveness of the individual coyote was quite short– as little as 5 days.

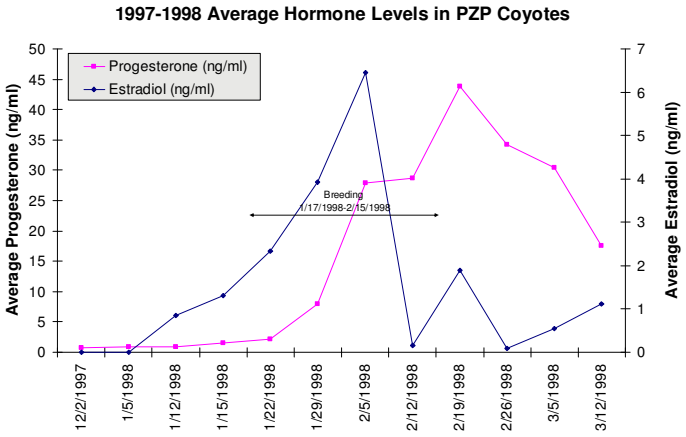

Figure 5. The relationship of the rise in estradiol and progesterone in relation to the breeding activity observed in Figure 4.

Morrow (1986:466) showed a similar hormone response in the dog. He demonstrated that a rising estrogen concentration triggered a spike in FSH and LH, which results in ovulation and the start of estrus. In the dog, receptiveness to the male is typically 1 to 2 weeks after ovulation as the estrogen is dropping and the progesterone is rising. Progesterone will continue to be elevated for 2 months following estrus in either the

pregnant or non-pregnant animal. Although our coyote study does not include the FSH and LH spike, the relationship of the estrogen and progesterone suggests that the same mechanism is responsible for estrus in both the domestic dog and the coyote.

Gross Necropsy

All control females were pregnant with a mean of 5.8 fetuses per female, as compared to zero fetuses in the PZP-treated females. Gross observation at necropsy indicated that the ovaries of PZP-treated animals appeared healthy with numerous mature corpora lutea. This would suggest that the contraceptive effect in the coyotes tested was due to anti-PZP blocking sperm binding to the zona pellucida.

The mean estimated age of the fetus in the control animals was 37.7 days, consistent with breeding observations. There was no difference in the number of corpora lutea in the control as compared to the PZP treated coyotes, indicating that the PZP animals continued to cycle and ovulate. There was no difference in the ovary weights between the two groups.

Antibody Results

The prime and 2 boosts were given to ensure complete contraceptive success and to determine whether 3 injections would induce an inflammatory response in the ovaries. The antibody titer response (Figure 6) to the vaccine was over 100,000 throughout most of the breeding period (12 January to 15 February), with a drop to 70,000 at the 12 February bleed.

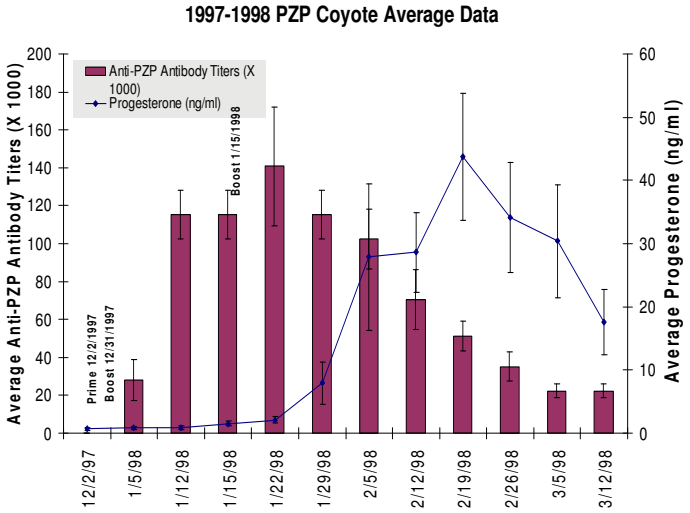

Figure 6. Relationship of the rise in anti-PZP titers following a prime and 2 boosts and the rise in progesterone representing estrus activity.

Study 3 (1998-1999)

Our first attempt at single injection was using PZP with Freund's adjuvant. However, the injection was given in September. There was a relatively good antibody response for about 45 days (Figure 7). If these 45 days had surrounded the month of February, instead of October, this single injection may have provided a contraceptive effect. However, antibody titers had dropped by December to levels that would not provide a contraceptive effect; therefore, the study did not proceed to breeding. The water-soluble adjuvant, Adjumer™, produced insufficient titers to act as an adjuvant for the contraceptive vaccine, and research on it was discontinued.

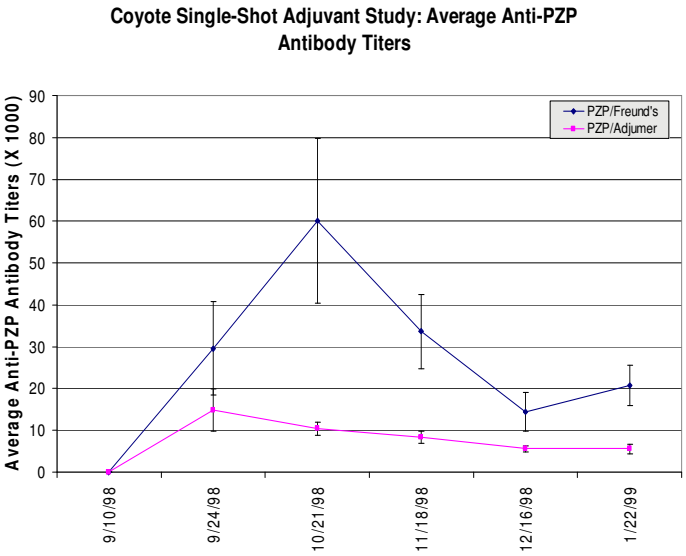

Figure 7. Comparison of the anti-PZP response using 2 adjuvants.

Study 4 (2002-2003)

The results of the previous adjuvant study demonstrated the value of a good adjuvant to the success of the contraceptive response. Contraceptive research in white-tailed deer resulted in the development of the new adjuvant AdjuVac™, which has been shown to be as or more effective than Freund's adjuvant. AdjuVac™ was tested with a single injection of PZP in white-tailed deer and provided 100% contraception for the first year (Miller, NWRC unpubl. data). Therefore, a single injection of PZP with the new adjuvant was tested in a group of proven female breeder coyotes. The single injection was given in November.

Breeding Activity 2002

The following are the dates of the first copulatory tie for each of 5 coyotes in 2002: 20 January, 8 February, 8 February, 9 February, and 19 February. Viable sperm were identified for each observed breeding tie.

Breeding Activity 2003

The following are the dates of the first copulatory tie for each of 5 coyotes in 2003: 28 January, 30 January, 7 February, 9 February, and 11 February.

Whelping Data

All coyotes tested negative for relaxin and had no pups in year 2002. In 2003, 3 coyotes tested positive for relaxin and had 2, 5, and 6 pups respectively. Two coyotes remained contracepted the second year.

Antibody Titer

The single injection of PZP/AdjuVac™ in November provided a sufficient antibody response throughout the breeding season, with anti-PZP titers remaining over 80,000 into April (Figure 8). Continued bleeding of coyotes throughout the year provided us with data on anti-PZP antibody titers and progesterone levels for a year. Average progesterone levels for the PZP group (Figure 8) comprise only 1 peak, and levels are quite synchronous among the group.

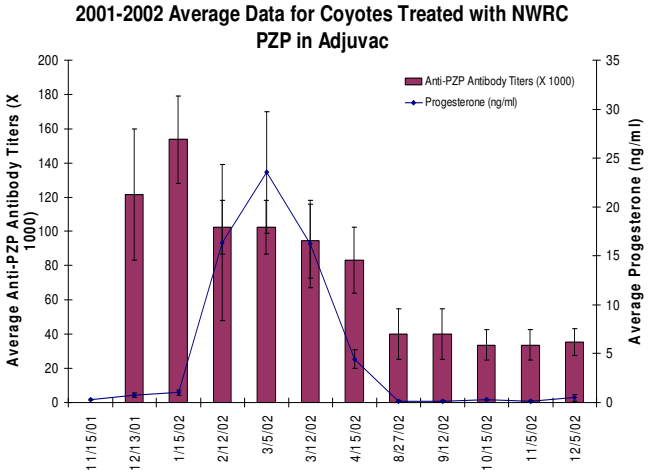

Figure 8. Anti-PZP response follow a single shot in AdjuVac™ adjuvant in relation to the estrus activity represented by the progesterone activity.

Study 5 (2004-2005)

Single injection SpayVac™/AdjuVac™

Year 1

Of the 6 control coyotes, 5 whelped a total of 25 pups and 1 had no pups, for an overall average of 4.2 pups/female. Of the 6 treated females, 2 had pups (2 pups and 3 pups, respectively), while the other 4 females appeared to be contracepted, producing an overall average of 0.8 pups/female for the group of 6 treated females.

Year 2

In the second year of the study, the 6 control females delivered 27 pups or 4.5 pups/female; all females whelped, with a range of 2 to 7 pups/female. The SpayVac™-treated group surprised us by delivering 30 pups or 5.0 pups/female; all females whelped, with a range of 3 to 8 pups/female. None of the treated coyotes were contracepted the second year.

It was later learned that the SpayVac™ used in Study 5 was part of a lot that was heated to 85°C, instead of the 75°C that was the normal procedure. This increased temperature was used to sterilize the vaccine to meet FDA sterility requirements. However, the process denatured the PZP antigen as well as killed the bacteria. It is not totally understood why the second year gave no contraceptive effect. Probably, the endogenous PZP did not match the denatured SpayVac™ form, and therefore had no endogenous boosting effect.

Injection Site Reactions

All coyote studies from 1995 to 2002 used Freund's complete adjuvant FCA (containing *Mycobacterium*) for a prime dose and Freund's incomplete adjuvant (without bacteria) in the booster dose. The presence of cell-mediated skin lesions at the site of the injection has caused controversy in the use of Freund's adjuvant. Our previous studies had suggested that these lesions only became problem when FCA was used twice in the same animal. The coyote was unique to all the animals tested with a PZP vaccine, in that the lesion developed after a single injection. In all studies started before 2004, which included both the Freund's and AdjuVac™ adjuvant, the injection site developed a weeping sterile lesion. These lesions measured 2 inches in diameter and resulted in hair loss around the injection site. The lesions would last about 2 months and then heal, with resultant regrowth of hair. The site did not appear to cause pain to the coyote. The same type of injection site reaction was observed in domestic dogs in a small study. However, in a large cat study using GnRH/AdjuVac™, there has not been any observed injection site reaction, even though reactions to vaccines are relatively common in cats.

Our research suggests that the canine injection problem stems from the series of puppy shots given to both the domestic dogs and the coyotes. The standard puppy shots are given at 8, 12, and 16 weeks and then the dog is given a yearly boost. The vaccines commonly include 5 modified live viruses and a killed *Leptospira* bacterin. In contrast, cat vaccines only contain modified live viruses. It was suspected that multiple vaccinations with the *Leptospria* bacterin were causing a cell mediated cross-reaction with the *Mycobacterium avian* in the AdjuVac™. Since the puppy shot can be purchased with or without the *Leptospira* bacterin, it was decided to switch to the vaccine containing only the 5 modified live viruses. The last *Leptospira* bacterin injection was given in 2002. All 6 of the coyotes receiving the single PZP/AdjuVac™ injection in 2002 developed an injection site lesion. However, only 1 of 6 SpayVac™/AdjuVac™ coyotes started in 2004 had an injection site lesion. It appears that the 2-year period between the last bacterin injection in 2002 and the SpayVac™ vaccination in 2004 reduced the cross-reaction and lesion. Further studies are being conducted, looking at the *Leptospira* antibodies in Studies 4 and 5 to confirm this hypothesis.

Use of *M. avium* Antibody Titer as Supportive Data for Cause of Coyote and Dog Lesions

The *M. avium* bacteria is ubiquitous in nature; therefore, most animals have been exposed to the organism and will have some antibody titer. Since AdjuVac™ contains *M. avium*, post contracepted animals should have a much higher titer. In a small study conducted with 3 domestic dogs injected with GnRH/ AdjuVac™, the pre-injection *M. avium* titers were higher than most post injection titers in other animals contracepted. This high pre-injection titer suggests the dogs were primed for a cross-injection to *M. avium* because of a recent *Leptospira* vaccination.

DISCUSSION

The above data represent studies that date back to 1996 conducted with coyotes housed in unique clover pens specifically designed for breeding studies in coyotes. The initial 2 studies showed that PZP/Freund's adjuvant contraceptive vaccines were effective in reducing fertility in the coyote. However, coyotes in these studies were given 2 injections of the vaccine, which is impractical in free-ranging animals. Also, the FDA has determined that the use of Freund's as an adjuvant is not acceptable in a final contraceptive product.

During the past 8 years, the PZP vaccine has evolved from a multiple-shot vaccine including Complete Freund's Adjuvant to a single shot with the newly developed NWRC adjuvant, AdjuVac™. The evolution of the immunocontraceptive vaccine into a single-shot product marks a breakthrough in wildlife contraception. This single injection PZP/AdjuVac™ vaccine was shown to be successful in white-tailed deer and was therefore tested in coyotes.

Our first attempt at testing a single shot PZP using Freund's adjuvant resulted in a marginal antibody response that was short lived. Freund's adjuvant produced a good but short 45-day response. Since the vaccine was given in September, the response declined before the breeding period started, and the study was stopped. The water-soluble adjuvant (Adjumer™) gave disappointing results, producing only a weak immune response. Research by the NWRC Infertility Project has shown that water-soluble adjuvants have to be designed to chemically match the vaccine antigen. This chemical matching was not done for this vaccine.

Complete necropsies performed in Study 2 allowed in-depth examination of the PZP contraceptive effect. Observations included determination of the number of placental scars, corpora lutea, and fetuses formed during pregnancy. The scientist reporting the gross necropsy stated "Observation at necropsy indicated that the ovaries appeared healthy with numerous mature corpora lutea. This would suggest that the contraceptive effect in the coyotes tested was due to anti-PZP blocking sperm binding to the zona pellucida." There was no inflammation or cyst formation, as suggested by Mahi-Brown *et al.* (1988), who hypothesized that infertility in PZP immunized dogs was the result of follicular dysgenesis or cyst formation. However, Mahi-Brown injected dogs with 500 µg crude or partially purified PZP 3 to 6 times in CFA and IFA. It is likely that repeated injections of up to 3.6 mg of crude PZP plus cellular debris would likely cause reproductive cellular damage in the dog.

The development of the new adjuvant (AdjuVac™) has shown great promise for enhancing effectiveness of a single injection immunocontraceptive vaccine for both PZP and GnRH in several species. The importance of the adjuvant in the immune response is demonstrated by the response curves in Figure 9, where in 3 studies coyotes were given a single injection of 200 µg PZP combined with 3 different adjuvants. The data demonstrate that the 3 adjuvants produce greatly different response titers, both in titer amount and the length of the response. The comparison shows that the new adjuvant AdjuVac™ results in higher antibody titer and an increased length of immune response.

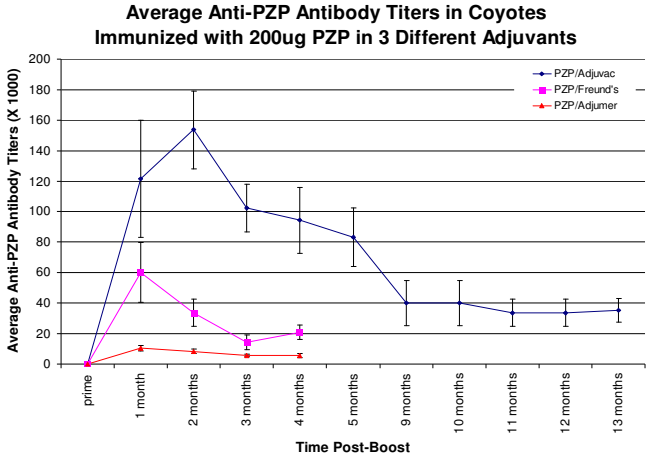

Figure 9. Anti-PZP response following a single injection using 3 adjuvant preparations.

Using PZP to Contracept the Poly-Estrus Deer vs. the Mon-Estrus Coyote

We have compared contraceptive responses of NWRC PZP/AdjuVac™ with SpayVac™/AdjuVac™ in white-tailed deer. Both the vaccine preparations gave 100% contraceptive effect the first year. However, in the second year the deer injected with NWRC PZP preparation began to have fawns, whereas the SpayVac™ preparation has contracepted deer for 4 years. It is unknown if the same long-term contraceptive response of SpayVac™ will be present in the coyote.

Both species responded to the single injection during the first year because of the *M. avium* in the AdjuVac™ adjuvant. However, the long term response in the deer appears to be due to reboosting, which may be the result of the fact that the PZP-contracepted deer recycle during the rut season. Miller *et al.* (2000) demonstrated that the PZP contracepted deer cycle 4 to 6 times during rut, as compared to the control deer which were settled or bred during the first heat.

It is possible that because the coyote is mon-estrus, there will be no seasonal endogenous boosting, reducing the long term contraceptive effect. However, there are several oral agents being developed at NWRC that would be effective for a 2-month period and that may be effective in contracepting the coyote.

CONCLUSION

Scientists at NWRC have made progress in the production and testing of new immunocontraceptive technology. Early contraceptive vaccines required 2 to 3 injections to obtain contraceptive titers. Improvements include the new adjuvant, AdjuVac™, to replace Freund's adjuvant, and an effective single-injection PZP technology called SpayVac™. A single injection of an alpha female coyote involved in sheep predation may provide the reduction in pups needed to reduce local sheep damage. The short breeding period of the coyote makes it a good candidate for testing contraceptives, including oral vaccines or contraceptive agents that may provide a short duration of effective contraception.

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