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Long-term effects of PZP immunization on reproduction in white-tailed deer

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Abstract

A 6-year study was conducted to determine the long-term effects of porcine zona pellucida (PZP) vaccine on the immune and hormonal responses, and reproduction of the white-tailed deer. The first 2 years of active immunization resulted in an 89% reduction in fawning. Vaccination with PZP produced reversible infertility lasting 1–4 years. Infertility was directly related to immune titers to PZP. Doe fertility was restored when the antibody titer dropped to minimal levels, but following re-immunization, infertility was reestablished. Reduction in fawning throughout the 6-year study was 76%. It was also observed that immune responses among deer were variable, especially in the first year of treatment. Variability was also observed among deer for the duration of infertility following the initial vaccination.

Keywords: Fertility control; Immunocontraception; Porcine zona pellucida; PZP; White-tailed deer

1. Introduction

Immunocontraceptive vaccines prevent conception by stimulating the production of antibodies that bio-neutralize proteins or hormones essential for reproduction [1]. The zona pellucida (ZP) is an acellular glycoprotein layer surrounding the mammalian oocyte (egg). During fertilization, sperm must bind to ZP receptors on the outer surface and penetrate the ZP for conception to take place. Native porcine zona pellucida (PZP) vaccines have been used to produce sterilization in rabbits, dogs, monkeys, horses, burros, baboons and other species [2–9]. Dunbar et al. demonstrated that, in addition to the commonly held theory that PZP antibodies block sperm penetration into the ovum, antibodies may also interfere with follicle maturation and ovulation [10,11]. The glycosylation associated with PZP appears to have a major role in sperm receptor function on the surface of the oocytes [12].

Despite the fact that PZP has been used to produce infertility in numerous species, virtually nothing has been reported on the long-term effects of PZP immunization on fertility. The present study was undertaken to better understand the long-term response of white-tailed deer to PZP immunization and to gain insight into the mechanisms contributing to the induced infertility. The specific objectives were to observe the titer responses following immunization and boosting of does with PZP over a 6-year period and correlate these observations with serum progesterone profiles, estrus activity and fawning data.

2. Materials and methods

2.1. Immunizations and blood samples

This study was conducted at the Deer Research Center of The Pennsylvania State University, University Park, PA. During handling, deer were restrained mechanically and chemically with 0.5–1.0 ml of xylazine. Deer were immunized with purified PZP which was prepared by and purchased from Dr

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Dunbar at the Baylor College of Medicine, Houston, TX [13,14].

The deer in the start of this study were proven breeders, 3–4 years of age, having a mean weight of 131 ± 15 (SE) pounds. The deer were fed free choice of a pellet prepared for the Penn State deer herd and given free choice of alfalfa hay.

Eleven deer were injected with 1 ml of PZP vaccine distributed subcutaneously and intradermally among several sites above the vertebrae of the back between the scapulae. The 1 ml prime dose consisted of 0.5 ml of saline containing 500 μg of PZP mixed with 0.5 ml of complete Freund's adjuvant (CFA). The booster doses contained 300 μg PZP in 0.5 ml saline mixed with 0.5 ml of incomplete Freund's adjuvant (IFA). Eight control deer were sham injected with saline mixed 1:1 as above with CFA in the prime dose or IFA in each boost dose.

All of the PZP treated deer were immunized the first year, eight of 11 were boosted the second year, and four of nine deer were boosted the third year. None of the deer were boosted after the third year. The decision to boost deer in the second or third years was determined by the antibody titer. Those deer that appeared to have a rapidly dropping antibody titer in the previous year were boosted.

Jugular blood samples were taken immediately before the prime injection and each boost and several times after the vaccinations. Following clotting, samples were centrifuged at 1000 × g. Serum was harvested and samples were stored at −20°C for subsequent enzyme-linked immunosorbent assays (ELISA) and progesterone assays.

2.2. ELISA and progesterone assays

To determine antibody titers, 100 ng of PZP antigen was placed in each well of a micro titer plate. Deer serum was serially diluted from 1:1000 to 1:4,096,000 in phosphate-buffered saline. Antibodies in the deer serum to the native PZP antigen on the plate were directed with the following linkage: deer anti-PZP binds to PZP on the plate, rabbit anti-deer IgG binds to the deer IgG, goat anti-rabbit-peroxidase binds to the rabbit IgG. The chromogen tetramethylbenzidine was used to develop the color and 2 M H_2SO_4 was used to stop the reaction. The color intensity of the sample was read at 450 nm with a Dynatech MR 5000 ELISA plate reader.

Plasma progesterone levels were assayed by the coat-a-tube RIA method (Diagnostic Products, Los Angeles, CA).

2.3. Observations on mating, gestation and fawning

To determine when the does were in estrus, behavioral observations of the bucks toward the treated does were made by Penn State students three times daily from November 7 through February 12 and two times daily until February 28. Behavioral activity by the buck was ranked as sniffing or pursuit of the female, aggressive guarding, and mounting and copulation.

Trans-rectal ultrasound was performed in late January or early February, and abdominal palpation was performed in late March or early April. Blood was drawn the same day of the ultrasound and tested for progesterone concentration.

From May through August, does were observed daily for evidence of fawning. Observations on behavioral estrus were used to estimate the date of conception. Ultrasound observations were used to confirm that does were in the first trimester of gestation. Abdominal palpation was used to confirm that does were in the last trimester of gestation.

White-tailed Deer
PZP Immunocontraception

Year	Treatment	Fawns/Does
92-93	Primed & boosted	4/11
93-94	Boosted	1/11
94-95	4/9 boosted	1/9
95-96	None boosted	3/9
96-97	None boosted	7/9
97-98	None boosted	9/8
Fawn/doe years - 92-98		
Breeding herd		156/90 (x=1.7)†
Sham controls		35/19 (x=1.8)†
PZP treated		25/57 (x=.44)*
= 0.76% reduction in fawns in PZP group		

x = Average number of fawns/doe
doe years = does x years tested
† p = >0.05
* p = <0.01

Fig. 1. Fawning results of PZP immunocontracepted deer compared to the fawning results of sham injected controls and fawning results from the deer herd not on study.

3. Results

3.1. Fawning data

Although eleven deer began the PZP study, two died after the second year, and one died after the fifth year. Necropsy results suggest that these deaths were caused by pneumonia, which was considered not to be related to the PZP treatment. The remaining eight does were monitored for the entire 6 year period. During the first 3 years when there was active immunization, treated

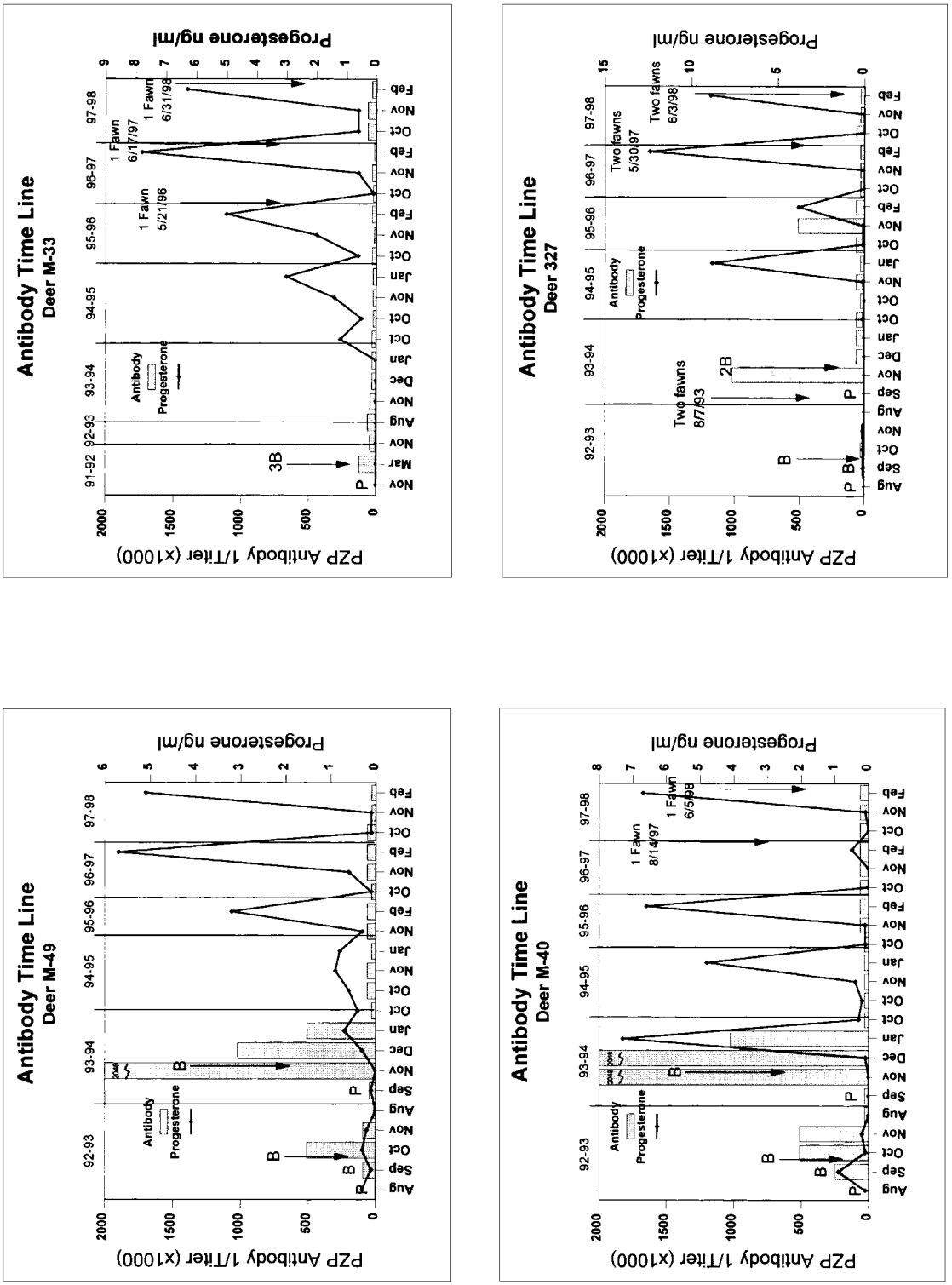
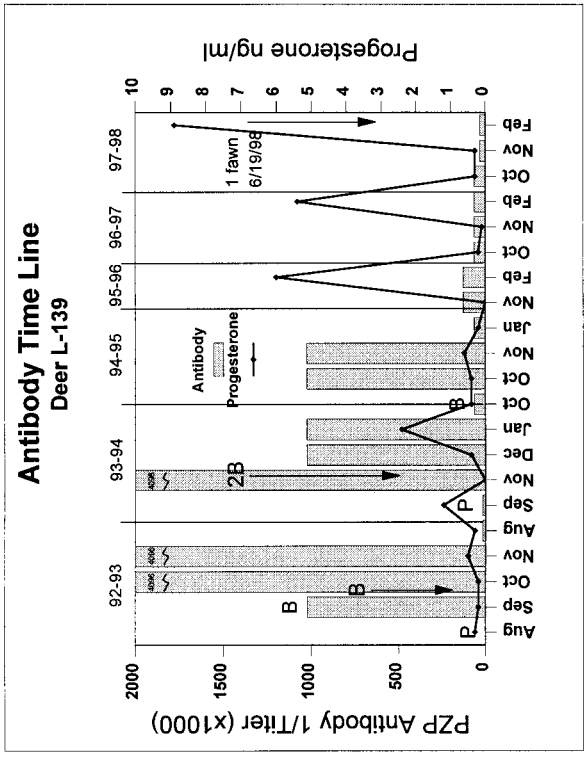
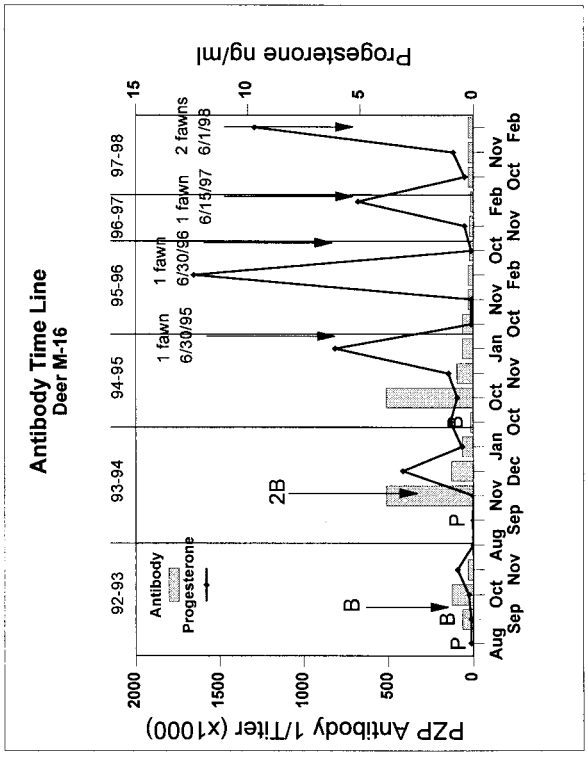
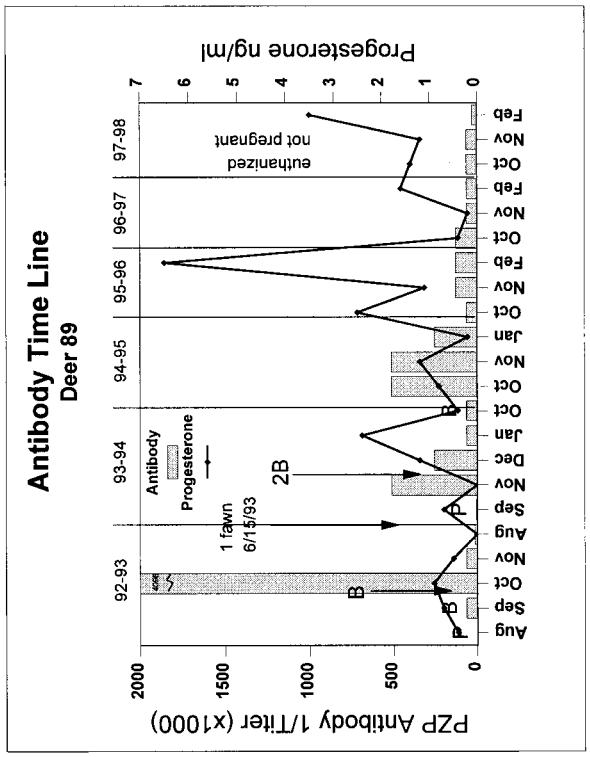


Fig. 2. Four of the PZP immunized deer carried throughout the 6 year study. The data indicates the date of vaccinations, fawning results, anti-PZP titers and serum progesterone concentrations. P = Prime Immunization dose. B = Boost dose.







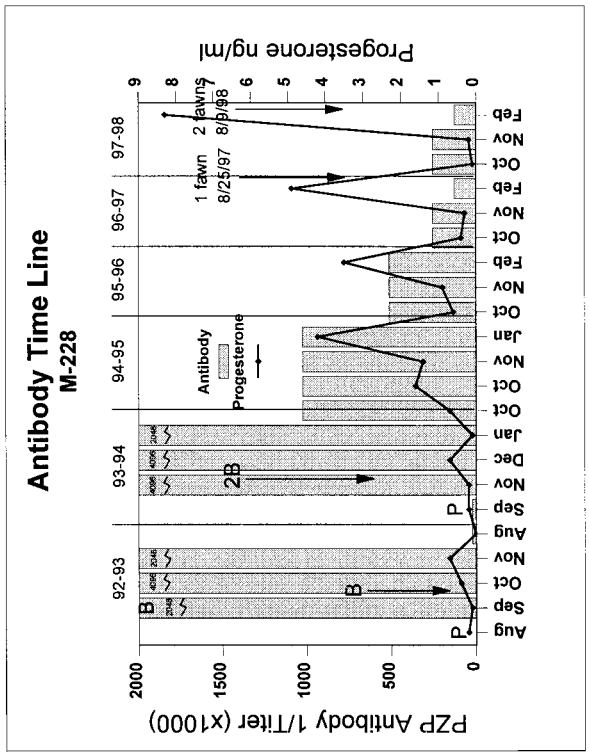


Fig. 3. Four of the PZP immunized deer carried throughout the 6 year study. The data indicates the date of vaccinations anti-PZP titers and serum progesterone concentrations. P = Prime Immunization dose. B = Boost dose.

deer produced six fawns, compared with 56 fawns from the same number of control does, or an 89% reduction in fawning ($P < 0.01$). For all 6 years, (Fig. 1.) including 3 years when deer were not boosted, 25 fawns were born to the PZP treated does compared with 103 fawns born to the same number of control does or a 76% reduction in fawning ($P < 0.01$). The sham injected does had a normal fawning rate similar to the control herd ($P > 0.05$).

The length of time that the deer remained infertile after they were given a vaccine boost, ranged from 1 to 4 years. There was a direct correlation between reduced fawning and the elevated antibody titer. As deer regained fertility most does had only one fawn, which was born late in the season.

3.2. Antibody titers

During the 6 year study, assays of antibody titers to PZP detected many titers at more than one million dilution. Although antibody titers were high after boosting during the first year, titers of most of the deer declined by the fall of the first year. Boosting in the second year produced the highest anti-PZP titers which lasted for several years. Titers of 128,000 or greater occurred for every deer at some time during the 6-year study.

There is some evidence that self-boosting may have occurred in several deer in the months of October and November. However, the increases in titer were generally never more than two-fold, as compared with at least eight-fold increases in titer from vaccine boosts. Non-vaccine boosts in titer were not observed at any other time of the year. Fawning occurred in previously infertile does when the PZP antibody titers fell to or below 32,000 to 64,000.

3.3. Behavioral observations

For control deer in the study, most were bred at the first observed estrus, and breeding was completed during the month of November. One or two estrus events were observed on average for each control doe during the breeding period lasting 44 days. During the first 3 years of active immunization, PZP treated does were observed to have one to four sexual encounters per doe (mean = 3.6), and remain sexually active over 98 days. Several does exhibited sexual activity in January and February. For all 6 years, including years when fertility was regained by some of the does, the mean number of estrus events in the PZP treated deer was 2.4. One doe was observed with seven cycles of sexual activity, with the last observed activity occurring on March 4. This doe was sexually active for 150 days but remained infertile.

3.4. Progesterone levels

During the normal breeding season of October to December, control deer had serum progesterone concentrations of $\geq$4.0 ng/ml at some time during each month. There appeared to be some synchronization of the estrus activity and elevated progesterone levels in the control herd. Progesterone concentrations of $\geq$4 ng/ml in late January to early February generally resulted in fawning in the control deer.

In contrast, serum progesterone concentrations in the PZP deer did not increase in the breeding during the first 2 years, when the deer were being actively immunized. During the following 4 years, evidence for a rise in serum progesterone in January and February began to become apparent. However, the rise in serum progesterone concentrations of $\geq$4 ng/ml in the PZP treated does did not correlate with fawning success (Figs. 2a–d and 3a–d).

3.5. Ultrasound and palpation

Ultrasound has been shown to be an excellent predictor of early pregnancy [15]. In control animals and the PZP treated does, a positive ultrasound observation in late January or early February, and a positive abdominal palpation in April or May was related with fawning. Observation, and serum progesterone concentrations were used to assess whether does treated with PZP had evidence of embryo reabsorption or abortion. A positive ultrasound and positive palpation were generally good indicators that fawning would occur. Two PZP does were exceptions to this trend, however. These does had sufficient antibody titers in early fall to prevent conception, but coincident with titer decline, they conceived in late fall and fawned in August and September. In these cases, ultrasound and palpation evaluations were performed too early to reliably detect pregnancy. The relationship observed among elevated progesterone concentrations and positive ultrasound and positive palpations was consistent with a 200 day gestation period.

4. Discussion

Native PZP is a large complex glycoprotein and an excellent immunogen for producing high, long-lasting antibody titers. Based on our observations, the PZP glycoprotein stimulated high titers needed to maintain infertility for up to 4 years. However, this long-lasting antibody response was not initiated until the deer had been boosted several times. It can also be concluded that as the serum antibody titers dropped to 32,000 to 64,000 or less, fertility was restored.

The immune response associated with the PZP vac-

cine was different from what we have observed for a KLH-GnRH immunocontraceptive vaccine also used in does (unpublished data). The antibody titer which developed in KLH-GnRH treated does was not sustained for more than two seasons without boosting. In practical terms, the use of the GnRH vaccine would require the does to be boosted each fall or every other fall to maintain GnRH titers sufficient to maintain infertility. In contrast, infertility resulting from the use of the PZP vaccine could be maintained by vaccinating every 2–4 years. These observations relate to the multi-epitope immunogenic properties of PZP compared with the single epitope property of GnRH.

PZP induced infertility in does is associated with increased sexual activity for an extended period as evidenced by an average increase in the number of estrus cycles observed per doe. Although the multiple estrus cycles observed with PZP-treated does have been reported by others [16], the present study clearly demonstrates that if the PZP antibody titer drops to an unprotecting level late in the season, breeding and conception occur. As a result, fawns are born in August or September placing them at considerable risk for winter survival.

Plotka and co-workers [17] demonstrated that progesterone was the key hormone for the maintenance of pregnancy, and that serum progesterone concentrations associated with pregnancy are similar to concentrations in the luteal phase of the non-pregnant deer. For control does in the present study, data indicate that serum progesterone concentrations of $\geq$4 ng/ml in late January or early February are correlated with fawning in the spring. For PZP treated deer, however, elevated progesterone in early February does not necessarily indicate pregnancy, but may reflect that the doe has ovulated and is in the luteal phase. If titers to PZP are sufficiently high at this time, serum antibodies will block sperm penetration of the ovum and prevent conception [10].

A possible exception to this proposed mechanism of PZP contraception may have occurred during the first 2 to 3 years of our study when does were immunized. During this period, a rise in serum progesterone was not observed in any of the blood samples, suggesting that the deer failed to develop functional corpora lutea during an estrus cycle (Figs. 2a–d and 3a–d).

It has been reported that active immunization with PZP may result in dramatic alteration of ovarian development and function [7,9]. Rabbits immunized with ZP proteins failed to form functional corpora lutea or induce elevated serum progesterone concentrations in response to HCG administration, as observed with control rabbits. Microscopic analysis of ovaries from immunized rabbits indicated that the numbers of primary, secondary and tertiary follicles were markedly reduced within 7 weeks, and by 23 weeks there were few if any growing follicles [9].

Baboons immunized with ZP proteins, that produced high titers, had lower concentrations of estrogen and became amenorrheic for several months with no sign of ovulation [7]. In contrast, baboons which responded with low antibody titers exhibited normal oocyte and follicular development. High antibody titers to ZP may interfere with the follicle–oocyte communication needed to regulate follicular development [7].

Taken together, our results suggest that antibodies to PZP may interfere with deer fertility by two distinctly different mechanisms. High titers of antibody or PZP/anti-PZP immune complexes may alter ovarian function by interfering with follicle–oocyte communication involved in the regulation of follicular development. If this occurs during follicular differentiation, when ZP proteins are synthesized and secreted, ovulation or normal corpus luteum development may be compromised. Alternatively, lower serum titers of PZP antibody or the lack of PZP/anti-PZP immune complexes may not interfere with follicular development, but induce infertility by inhibition of sperm binding or penetration of the ZP.

The immune response of the doe to the PZP antigen is unusual in that high titers were maintained for up to 4 years without external boosting. Typically, an immunogen stimulates an antibody response lasting no more than several months because the antibody-secreting plasma cells are short-lived.

It is possible that the ZP protein, produced by the does each summer and fall as their ovaries are activated for the breeding season, may have provided a mechanism for yearly re-immunization. This re-immunization may have occurred in the last 4 years of the study as the antibody titers began to decline (Figs. 2a–d and 3a–d).

Prolonged immune responses are traditionally thought to be due to the depot effect of Freund's adjuvant at the injection site [18]. It has also been suggested that follicular dendritic cells may play a role in providing a long-lasting immune response [19].

The contraceptive results of this study demonstrate that this immunocontraceptive technology could be effective in reducing populations of deer in small confined settings. However, because the present technology requires injection of the vaccine, it is not practical in a field setting until an oral delivery system could be developed [20].

5. Summary

PZP immunocontraception appears to be effective in controlling deer fertility. Fertility was reduced as much

as 89% during active immunization, and for the entire 6-year study was reduced by 79%. However, an extended breeding season and late fawning dates are disadvantages. PZP immunization stimulates high antibody titers that can reduce fertility for 1–4 years without boosting. Thereafter, as anti-PZP titers declined, doe fertility was restored but at a reduced fawning rate. Previously infertile does that regained fertility were again made infertile by a single boost before the next breeding season. The prolonged immune and contraceptive effects in deer to the PZP vaccine are desirable aspects of using PZP as an agent for deer population control. However, these benefits should be weighed against the extended breeding season and late fawning associated with its use.

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