# Immunocontraception in White-Tailed Deer

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**Abstract:** Immunocontraception may have management application for white-tailed deer populations in parks and preserves, where hunting is illegal or impractical. This study examines physiological aspects of immunocontraception with porcine zonae pellucidae in 53 fertile white-tailed does. In separate studies, we employed protocols of three and two porcine zona pellucida (PZP) injections as well as two different protocols using one injection. Each one-injection vaccination consisted of one dose of porcine zonae pellucidae as in other protocols plus a second controlled-release dose of the material delivered via an osmotic minipump implant or injected, biodegradable polymer microspheres. We monitored fawn production for 1 to 3 years in all does and measured serum PZP antibody titers at various times after treatment in 15 randomly selected PZP does and in 2 control does. All three-injection PZP does were given a single PZP booster inoculation after 1 year. None of the two- or three-injection does and none of the one-injection does with a minipump produced fawns the first year after treatment, whereas two of seven does given a single injection with controlled-release microspheres produced fawns. In 49 control-doe breeding seasons, the pooled incidence of fawn production was 93.8 percent. Regarding reversibility of infertility, the incidence of fawn production was 75 percent within 2 years after treatment was discontinued. Serum anti-PZP antibody titers were present only after PZP treatment, and highest titers occurred in does given two or three separate PZP injections. PZP-treated does showing >50 percent of maximal antibody titers at the onset of a breeding season did not produce fawns; those showing <33 percent of maximal titers did. These data demonstrate in white-tailed does that (1) multiple-injection PZP vaccination can produce complete contraception for at least one breeding season, (2) the contraceptive effect is reversible within 2 years in most does, (3) an elevated anti-PZP antibody titer occurs after PZP vaccination, and (4) multiple-injection PZP vaccination produces a sustained antibody response through at least one breeding season.

In separate field studies of wild, free-roaming deer in three locations, we successfully lured does to within darting range using bait stations. In two locations, we attempted and achieved remote delivery PZP vaccination of 60–90 percent of does. In the one location for which we have data on fawn production, the fawning incidence was 0/10, 6/9, and 8/9 for two-and one-injection porcine zonae pellucidae and untreated controls, respectively. In the same location, we monitored seasonal aspects of activity, behavior, and physical condition. We also determined that there is a good potential for remote assessment of pregnancy in does using measurement of pregnancy-specific elevations of steroid metabolites in fecal samples.

**Keywords:** antibody titers, fertility control, immunocontraception, *Odocoileus virginianus,* reproduction, vaccine, white-tailed deer

## Introduction

The purpose of this paper is to present studies performed by our research team addressing immunocontraception in white-tailed deer *(Odocoileus virginianus).* Our team participated in the 1987 and 1990 "Contraception in Wildlife" conferences in Philadelphia (U.S.A.) and Melbourne (Australia). At the 1987 conference, there were no papers addressing immunocontraception; our own work in this area was embryonic (Turner and Kirkpatrick 1991). At the 1993 conference in Denver, CO, there were 16 papers on immunocontraception! Since the original studies of immunocontraception in feral horses, we have treated 45 mammalian species with the same porcine zona pellucida (PZP) antifertility vaccine. To date, these treatments included 157 individuals across 28 species of captive, exotic ungulates, with 87.7-percent inhibition of fertility in 68 outcomes; and 172 free-roaming equids, with 92.5-percent inhibition of fertility in 133 outcomes. These numbers provide strong encouragement for the exploration of PZP vaccine use for contraception in wildlife.

The first study of immunocontraception in wildlife was in free-roaming feral horses (Kirkpatrick et al. 1990a). We used PZP vaccine, delivered remotely by dart, to produce greater than 95-percent infertility. The effects were reversible after 1 year, did not affect pregnancies in progress, and did not produce behavioral side effects (Liu et al. 1989, Kirkpatrick et al. 1990a and 1991a). Based on this success using PZP vaccine to inhibit fertility in feral horses, we began a series of studies to evaluate the potential for PZP immunocontraception in white-tailed deer.

The PZP vaccine appears to act by inhibiting fertilization (Sacco et al. 1984). In mammals, the egg is surrounded by a noncellular membrane named zona pellucida (ZP). The ZP is made up of three glycoproteins; one of these, ZP3, is the sperm receptor (Florman and Wassarman 1985). Injection of raw porcine ZP protein plus adjuvant (to enhance the immune response) into other species causes the female to raise antibodies against the sperm receptor on the ovum. It is hypothesized that the antibodies bind receptors which prevent sperm binding and, hence, fertilization.

Let us now turn to white-tailed deer. In times past, white-tailed deer populations lived unrestricted in their habitat, regulated by predation, resource limitations, and human hunting. With increasing human settlement and urbanization, predators have been eliminated, and many deer populations and their habitat have become islands surrounded by human communities. While hunting has been the standard approach to controlling North American deer populations, it is illegal or impractical in many parks and preserves. Furthermore, current management practices in these places have not limited populations.

We have undertaken studies of immunocontraception in white-tailed deer in hope of developing an alternative tool for management of unhuntable populations (Kirkpatrick and Turner 1993). We will present data from our several studies of captive, unrestrained deer and from three ongoing studies of wild, free-roaming deer. Major issues which must be addressed in considering the potential for PZP vaccine in deer contraception essentially comprise the list of requirements for a management-useful contraceptive vaccine for deer: (1) accessibility to the deer, (2) reliable treatment-delivery technology, (3) high efficacy of treatment, (4) appropriate duration of treatment effect, (5) reversibility of treatment effect, (6) safety of treatment to deer and environment, (7) safety of treatment to deer consumer, (8) cost effectiveness of treatment, and (9) public acceptance of method and treatment.

In our studies, we have attempted to bring together the most important elements of successful wildlife contraception: scientifically sound basic research and successful application to wild animals in the field. This union has included collaborations with the Smithsonian Institution's Conservation and Research Center (Front Royal, VA) on deer behavior and social dynamics; Canada's Pt. Pelee National Park and the Ontario Ministry of Natural Resources on access to wild deer; Fire Island National Seashore, NY, on treatment in the field; and The Humane Society of the United States (Washington, DC) on education regarding urban deer issues.

## Studies in Captive Deer

### Materials and Methods

**General**—We examined the effects of a PZP vaccine on fertility and plasma antibody titers in studies consisting of four experiments testing various protocols of PZP contraceptive vaccine, each with its own controls. Assessment of contraceptive effectiveness and reversibility was determined by comparison of fawn counts in PZP-treated and control does. Serum anti-PZP antibody titers were measured by specific enzyme-linked immunosorbent assay (ELISA).

The PZP vaccine, an emulsion of porcine zonae pellucidae and adjuvant, was prepared from porcine ovaries as described (Liu et al. 1989). It was stored at –20 °C until used in the field. An emulsion of 0.5 $cm^3$ vaccine (equivalent to approximately 5,000 zonae or 64.3 µg of protein) in phosphate buffer and 0.5 $cm^3$ of Freund's Complete Adjuvant (FCA) was prepared and injected remotely (Turner et al. 1992). All inoculations subsequent to the first one contained Freund's Incomplete Adjuvant (FIA) in place of FCA. In a previous study, the use of incomplete adjuvant in subsequent injections produced effective contraception with no visible signs of abscess at the injection site (Turner et al. 1992).

The studies were conducted at a private, U.S. Department of Agriculture-approved deer facility in west-central Ohio (lat. 41° N., long. 84° W.). We used 53 does ranging in age from 2 to 7 years and in mass from 47 to 65 kg. All does had fawned previously. They were maintained in two adjoining 0.5-ha, grass-covered enclosures with a 3-m chain-link fence. The west end of the enclosures was a solid wooden wall with an overhang to provide the deer shelter from cold, wind, and precipitation. They were fed alfalfa, corn, oats, and commercial food pellets (Purina Lamb

Conditioner®, Purina Corp., St. Louis, MO). Food and water were provided ad libitum. We weighed the does and noted their condition when the study began. Health and condition of each doe were subjectively assessed monthly during the study in terms of vigor, demeanor, and physical appearance. Each doe was eartagged to facilitate remote identification and randomly assigned to a PZP or control group.

**Effects of PZP Vaccine on Fawn Production**—For each of four experiments, does were vaccinated prior to the breeding season, placed with a fertile buck, observed for breeding, and observed for fawn production the following spring and summer. Details of these activities are presented below.

Antifertility effectiveness in each experiment was determined by comparing fawn production in PZP-treated and control does. The PZP vaccine was given as two or three injections, 3 weeks apart, or as a single injection combined with a 4-week controlled-release delivery of porcine zonae pellucidae. A three-injection experiment (begun 1989), a two-injection experiment (begun 1990), and two one-injection experiments (begun 1991 and 1992) were performed separately, with respective controls. Untreated controls or a combination of does that received sham injections (saline and adjuvant) and untreated controls were used in each experiment.

Each year, injections were begun in October. In November or early December (3–4 weeks after the end of PZP exposure), PZP-treated and control does were placed together in groups of no more than 18. A single breeder buck was placed with each group. The bucks were healthy 3-year-olds that had previously sired fawns. We observed breeding within 3 days after placing the buck and does together, and the male was permitted to remain with the does thereafter.

Although details of estrus and breeding behavior were not recorded, increased activity and restlessness of a given doe accompanied by increased attention to the doe by the buck, indicated the onset of estrus. A breeding was considered to have occurred when the buck mounted a receptive doe and, after 7–15 seconds, the doe and buck separated vigorously (Halls 1984). Brief mountings (3–4 seconds) were not considered a successful breeding. During the breeding season, observations were usually made daily for 15–30 minutes during feed replenishment, around 6 A.M. and 5 P. M.

After the breeding season, deer were observed for health and physical condition as described previously. Fawning was usually complete by mid-June. Different incidences of fawning among groups in a given experiment were tested for significance using binomial probability distribution (Zar 1984).

In the three-injection experiment treated does ($n$=5) received three separate PZP treatments, three weeks apart, beginning October 1989. Control does ($n$=6) were untreated. In October 1990, the above PZP-treated does were given a booster inoculation (porcine zonae pellucidae + FIA), and the above control does were given a sham (saline + FIA) booster ($n$=4) or remained untreated ($n$=2). In order to determine reversibility of treatment effect, the above control and previously treated (1989; 1990 booster) does received no treatment in October 1991 but were bred as usual during November–December 1991.

In the two-injection experiment, does ($n$=8) received two injections, 3 weeks apart in November 1990. Control does ($n$=8) were given saline/adjuvant ($n$=4) or were untreated ($n$=4). The 16 does in the experiment were divided into 2 groups (4 receiving porcine zonae pellucidae, 2 receiving sham injections, and 2 left untreated in each group). Each group was placed in a separate pasture with a buck of proven fertility. Fawns were counted in the summer of 1991. In order to determine reversibility of treatment effect, the remaining PZP-treated and control does were bred without further treatment in November 1991 and November 1992, and fawns were counted in the summers of 1992 and 1993, respectively.

In the first single-injection, controlled-release experiment, six does were given one injection of porcine zonae pellucidae (65 μg) and FCA, and on the same day an osmotic minipump containing 65 μg of porcine zonae pellucidae was implanted subcutaneously in the neck of three of these does. The pump was designed to release PZP antigen continuously for 4 weeks at an approximate rate of 2.5 μg/day. The three other does that received just the single PZP injection served as "active" controls: two of these does were implanted with a pump containing saline buffer, and one of these does was not implanted. Another six does served as untreated controls. Does were bred, and fawns were counted as described previously.

In the second single-injection, controlled-release experiment, seven does were each given one injection containing 130 µg of porcine zonae pellucidae emulsified in FCA. The pellucidae in the injection were present in one of two forms: 65 µg unsequestered or 65 µg sequestered in 10- to 50-µ microspheres of bioerodable lactide and glycolide polymers in a 1:1 ratio. The polymer microspheres were prepared by E. Schmitt, R. Linhardt, and D. Flanagan at the University of Iowa using a coacervation method described by Wang et al. (1991a). Release rates were projected to be continuous over approximately 4 weeks, with greater release in weeks 1 and 4 (Wang et al. 1990 and 1991a). Seven does served as untreated controls. Does were bred, and fawns were counted as previously described.

**Effects of Porcine Zonae Pellucidae on Antibody Titers**—Anti-PZP antibody titers were measured in serum obtained from randomly selected does in each of the above experiments. Blood collection was scheduled to provide antibody titer data for prevaccination, peak titer period, and after fawns were produced. Except for prevaccination, the timing of blood collections was partly dictated by accessibility to deer and availability of assistance for their handling. In total, 15 PZP-treated and two control does were sampled. Does were tranquilized with xylazine (1.5 mg/kg) delivered by darting. The tranquilizer induction time was 8–12 minutes. Samples (10 mL) were taken by jugular venipuncture and allowed to clot for 1 hour at approximately 30 °C. Serum obtained from these samples was frozen at –20 °C until assayed for anti-PZP antibody titers. These titers were determined using an ELISA based on the method described by Voller et al. (1986), with the modifications reported previously for horses (Liu et al. 1989) and additional modifications for deer. The assay is described below.

Fifty µL of 5 µg/mL zona antigen solution in 0.1 M glycine buffer (pH 9.5) were placed in each well of a flat-bottom ELISA microplate (Cat. #MS-3496, E & F Scientific Products, Saratoga, CA) and incubated overnight at 4 °C. The plate was washed with PBS-Tween and incubated for 1 hour each with 50 µL of the following PBS-Tween diluted reagents and subsequent washings: deer serum (1:500), biotinylated rabbit antideer immunoglobulin G (IgG) (1:250), or alkaline–phosphatase avidin (Zymed Laboratories, San Francisco, CA) (1:1,000). Finally, 50 µL substrate solution of 1 mg *p*-nitrophenyl phosphate per mL (Sigma Chemical Co., St. Louis, MO) in 10-percent diethanolamine buffer (pH 9.8) was distributed to each well. After incubation of approximately 20 minutes, the plate was scanned for absorbance at 405 nm using an MR 580 Microelisa Auto Reader (Dynatech Laboratories, Alexandria, VA). The rabbit antideer IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD) listed above was biotinylated according to the method described by Bayer et al. (1986) and Gretch et al. (1987).

The experimental sera were tested in duplicate, and their results were expressed as percentage of the positive reference serum, which consisted of a pool of sera that had demonstrated anti-PZP antibody titers in the high positive range. Maximal serum antibody titers usually occur 4–6 weeks after initial PZP injection (Liu et al. 1989). We therefore determined the average value of maximal titers (designated as 100 percent) achieved during this period in six successfully contracepted does. All other titers are reported as a percentage of this positive reference serum. Pooled pre-immunization sera served as negative control.

In the three-injection experiment, four PZP-treated does were blood-sampled for titer testing at 0, 6, and 36 weeks. Samples were also taken from three of these does at 92 weeks (approximately 1 year after a booster inoculation). In the two-injection experiment, four PZP-treated and two sham-treated does were bled at 0, 6, and 40 weeks. Two of the above PZP-treated does were also bled at 52 and 64 weeks. In the single-injection, osmotic-pump experiment, blood samples were taken at 0, 4, 12, and 32 weeks after vaccination from PZP-injected does in the PZP-pump group *(n*=3) and in the saline-pump group *(n*=2) for anti-PZP antibody testing. Untreated and unimplanted does were not bled. In the second single-injection experiment, employing controlled-release microspheres, blood samples were taken at 0, 4, and 36 weeks after vaccination from four PZP-treated does.

Where appropriate, titer data are presented as $\mu \pm$ the standard error of the means (S.E.M.). Results are presented descriptively, without statistical analysis, because responses to treatment were marked and straightforward in most cases, and differences in sample intervals and the small group sizes limited possibilities for comparisons.

## Results

### *General*

None of the does exhibited a visibly draining abscess at the injection site, but a raised area of 16–24 mm in diameter developed at the final injection site on six does. *This response disappeared after 8–10 days in* three of the does, after 2 months in two other does, and after 6 months in the sixth doe. We observed 60 percent of the does being bred. Subjectively, we observed no differences in breeding behavior between treated and control does. During the breeding seasons monitored in these studies, none of the control does was observed breeding after January, while 26 percent of the PZP-treated does were observed breeding through February. Breeding of two treated does was observed during the first week of March.

Parturition began late in May each year and spanned a 4-week period, with the exception of two fawn births in July and one in August of 1992. All fawns appeared healthy and nursed normally.

Based on criteria described in Methods, does generally appeared to be in good health and physical condition throughout the study period from October 1989 to July 1993. However, during this period two treated and two control does died from causes unrelated to the study as determined by necropsy and bacterial culture. Deaths were associated with a jaw infection *(n*=1), a compound fracture *(n*=1), and *Clostridium* sp. *(n*=2). One treated and one control doe also died of undetermined causes within a 2-week period.

**Effects of Porcine Zonae Pellucidae on Fawn Production**—*Across all experiments, involving* 49 control-doe (no PZP) breeding seasons, 1 sham-treated doe and 2 untreated control does did not produce fawns. The fawning rate for sham (7 of 8) and untreated (39 of 41) control-doe breeding seasons was not different *(p* <0.01), and these control data were pooled. In contrast, the incidence of fawn production for the first breeding season was 0 percent (100-percent contraception) among does given multiple injections and 20 percent (80-percent contraception) among does given a single injection of porcine zonae pellucidae plus additional pellucidae in a controlled-release form of PZP vaccine. The annual incidence of fawning among control does ranged from 83 to 100 percent between 1990 and 1993, and the incidence of twinning was 38 percent. Over all years, 46 or 49 doe-breeding periods associated with control does resulted in production of fawns (reproduction success = 93.8 percent). Thus, the pooled pregnancy failure rate among control does was 6.2 percent.

The fawn-production data for all treatments are presented in table 1. Among does given a three-injection protocol (in October–November 1989), none of five PZP-treated does produced fawns in 1990 while all six control does did. After booster PZP treatment in October–November 1990, none of the above five PZP-treated does produced fawns in 1991. Of the above six control does, four received sham injections and two remained untreated in 1990; all six again produced fawns in 1991. One PZP-treated and one control doe in these groups died in 1991. In both 1990 and 1991, the differences in fawn production between PZP-treated and control groups were significant *(p* <0.01). The remaining PZP-treated *(n*=4) and control *(n*=5) does were bred in the 1991 rut without further manipulation and produced fawns in 1992 as follows: four of five controls and three of four PZP-treated does. There was no difference between groups *(p* >0.1).

**Table 1. Fawn production among white-tailed deer given three, two, or one injections with porcine zonae pellucidae**

| Experiment | Year | Treatment (+ or –) | Years since treatment | Sample size (n) PZP | Control[2] | Placebo[2] | Percent of does producing fawns[1] PZP | Placebo & control[3] |
|---|---|---|---|---|---|---|---|---|
| 3-injection | 1989 | + | 0 | 5 | 6 | 0 | [6]0 | 100 |
| | 1990 | +[4] | 0 | 5 | 2 | 4 | [6]0 | 100 |
| | 1991 | – | 1 | 4 | 5 | 0 | 75 | 80 |
| 2-injection | 1990 | + | 0 | 8 | 4 | 4 | [6]0 | 100 |
| | 1991 | – | 1 | 7 | 7 | 0 | [6]29 | 100 |
| | 1992 | – | 2 | 4 | 4 | 0 | 75 | 100 |
| 1-injection/pump[5] | 1991 | + | 0 | 3 | 6 | 3 | [6]0 | 83 |
| 1-injection/ microspheres | 1992 | + | 0 | 7 | 7 | 0 | [6]29 | 86 |

[1] Fawn production was determined by observation during May–August the year following PZP treatment.

[2] Control = untreated control; placebo = saline and adjuvant.

[3] Placebo and control does were pooled for comparisons with PZP-treated does because fawn production did not differ between placebo and control does (binomial test for equality of proportions, $p < 0.05$).

[4] Treatment was a single-injection booster of PZP + adjuvant.

[5] PZP = PZP injection + PZP pump, placebo = PZP injection + saline pump ($n = 2$) or no pump ($n = 1$).

[6] Different from pooled control, based on binomial test for equality of proportions, $p < 0.05$.

Among does in the two-injection experiment that were bred in the 1990 rut, none of eight PZP-treated and eight of eight controls became pregnant. The between-group difference was significant ($p < 0.01$). One treated and one control doe from these groups died late in March. Necropsy showed that the PZP-treated doe was not pregnant but the control doe was. In order to test possible multiple-year effectiveness of porcine zonae pellucidae, without further treatment, all 14 of the above does were bred in the 1991 rut. Two of seven previously treated with porcine zonae pellucidae and seven of seven control does produced fawns in 1992. The between-group difference was significant ($p < 0.05$). All control animals had fawned by June 25, while the two births to PZP-treated does were June 29 and August 15. Prior to the 1992 rut, three of seven previously PZP-treated (two-injection) and three of seven control does were dropped from the study for economic reasons. The remaining four PZP-treated does, which had not produced fawns since the study had begun, were bred in the 1992 rut and three of these four does produced fawns before June 30, 1993. The other doe did not produce a fawn. All four of the remaining controls produced fawns. The difference between groups was not significant ($p > 0.10$).

Data from the multiple-injection studies in table 1 address reversibility of PZP treatment. Of the treated does monitored for reversibility, 75 percent produced fawns within 2 years of cessation of treatment.

In the single-injection, osmotic-pump experiment, none of the does given a PZP injection produced fawns, regardless of the presence or absence of a pump or of PZP in the pump. Five of six untreated control does produced fawns. Statistical analysis was precluded by the small number of does in the respective PZP treatment groups.

In the single-injection, microsphere experiment, two of seven PZP-treated does and six of seven untreated control does produced fawns. The difference between groups was significant ($p < 0.05$). All control does fawned by June 30 except the one that fawned on July 8. Fawning dates for the two PZP-treated does were June 17 and July 14.

**Effects of Porcine Zonae Pellucidae on Antibody Titers**—Blood samples obtained from PZP-treated does in all cases showed anti-PZP antibody titers in baseline (prevaccination) which were <8 percent of the positive reference serum value, i.e., similar to titers found in negative reference serum. All of these

**Table 2. *Anti-PZP antibody titers during one breeding season in captive white-tailed does given* PZP contraceptive vaccine**[1]

| Experiment | No. of does titer-tested | Prevaccination baseline[5] (week 0) | Anti-PZP antibody titers (% of positive reference serum)[2] Postbreeding season[4] (weeks 32–40) $\bar{X}$ | SE | Prebreeding season (weeks 4–6) $\bar{X}$ | 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*[3] | 2 | <8 | <8 | | <8 | |

[1] Does were treated in October–November; fawning occurred the following summer.

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

[3] Placebo-treated does from 2-injection experiment.

[4] All values were below the lower limit of detection.

[5] No differences between pre- and postbreeding season titers in any treatment group, based on analysis of variance with repeated measures, $p < 0.05$.

PZP-treated does exhibited titers >33 percent of the positive reference serum value after vaccination. No does with titers >50 percent at the onset of a given breeding season produced fawns in the following summer.

Specific antibody titer data are presented in table 2. In the three-injection experiment, average titers among the four tested does were 102.1 ± 1.2 percent by 6 weeks after the initial inoculation and were 72.7 ± 15.7 percent at 36 weeks. In the two-injection experiment, anti-PZP antibody titers in four treated does averaged 100.0 ± 0.0 percent of positive reference serum 6 weeks after vaccination; and 40 weeks after PZP vaccination, titers in these same does averaged 92.0 ± 5.2 percent.

Additional blood samples taken from two of these does showed titers of 70 percent and 57 percent at 52 weeks (prior to placement with a buck), and titers of 86 percent and 75 percent at 64 weeks. Neither of these does fawned. One doe that did produce a fawn had shown a 79-percent anti-PZP antibody titer 12 weeks prior to breeding, but there were no November or February antibody data for this doe. Titers were <8 percent at all times in samples from the two sham-treated does.

In the first single-injection study, prevaccination anti-PZP antibody titers were <8 percent of the positive reference serum titers. Titers in the PZP-pump group ($n$=3) averaged 76.3 ± 7.2 percent, 78.3 ± 9.3 percent, and 69.5 ± 11.8 percent at 4, 12, and 32 weeks, respectively. Titers in active (single PZP injection and saline pump) control does ($n$≈2) averaged 41.5 ± 1.5 percent, 50.0 ± 5.5 percent, and 41.5 ± 12.5 percent at the above sampling times.

In the second single-injection study, employing controlled-release microspheres, average anti-PZP titers in the four treated does sampled were 53.7 ± 15.5 percent at 4 weeks and 19.1 ± 4.3% at 36 weeks. Anti-PZP antibody titers in the two PZP-treated does that produced fawns in this experiment were 32 percent and 27 percent at 4 weeks, i.e., prior to breeding.

## Discussion

In the present study, 100 percent of the does given multiple injections of porcine zonae pellucidae and 80 percent of does given pellucidae as a single injection with additional pellucidae provided in a controlled-release form were effectively contracepted for the first breeding season after treatment. In contrast, does in combined sham and untreated control groups had only a 6.2-percent average annual failure rate in producing fawns. In all cases in which it was applicable, statistical analysis of fawning data within studies showed that the proportion of does producing fawns was lower for PZP-treated does than for control does. These results confirm of our previous report on PZP contraception in white-tailed deer (Turner et al. 1992).

The mechanism of action of the PZP vaccine appears to be inhibition of fertilization (Sacco et al. 1984). Porcine zona pellucida is comprised of several glycoproteins, at least one of which, ZP3, functions as a receptor for sperm surface molecules (Florman and Wassarman 1985). In the PZP-treated mare, it appears that anti-PZP antibodies block sperm-binding sites on the ovum (Liu et al. 1989).

This study associates the presence of highly elevated, sustained anti-PZP titers with infertility in deer. Some does were monitored for antibody titers across one complete breeding season (6–9 months). Average titers appear to have remained highly elevated (73–92 percent of maximal) during this period in the does given multiple PZP injections while average titers among single-injection does never approached maximal. These results indicate that a minimum of two separate treatments with porcine zonae pellucidae may be necessary to maximize anti-PZP antibody titers across one breeding season.

In a previous study of porcine zonae pellucidae in the mare using similar dosages and protocol, the lowest pellucidae titer associated with contraception was 64 percent of the positive reference serum for mares. In the present study, 3 of 13 does sampled just before or during the breeding period had titers between 40 and 50 percent of positive reference serum for does. None of these three does produced fawns from that breeding season. On the other hand, five of six does with titers <33 percent at the onset of breeding produced fawns the following summer. Although the small number of samples and titer variability across the test animals preclude firm conclusions regarding the contraceptive threshold for anti-PZP titers in white-tailed deer, the present data suggest that the threshold may be in the range of 33 to 50 percent. One doe that had a 79-percent titer 12 weeks before breeding did produce a fawn the following June. Because this doe was not sampled immediately prior to the breeding season, it is possible that her titers were below 50 percent during breeding. In this regard, another doe sampled 12 weeks before breeding had a 93-percent titer at that time, and her titer was 57 percent in November.

The fawning data (table 2) also indicate that a single injection containing unsequestered porcine zonae pellucidae plus controlled-release PZP microspheres was less effective in providing contraception for one breeding season than were two or three separate exposures to the pellucidae (100-percent effective). The fact that the contraceptive effect was less in single-injection protocols than in multiple-injection protocols may reflect that the controlled-release component was not equivalent to a second PZP injection. First, the release pattern of porcine zonae pellucidae was continuous in the controlled-release preparations rather than discrete as in the bolus release provided by a second injection. Second, the multiple-injection protocol included adjuvant in each injection, while in the single-injection protocol, there was no adjuvant in the controlled-release component. Nonetheless, the partial effectiveness of the single-injection PZP preparations encourages further consideration of controlled release of porcine zonae pellucidae for use in immunocontraception (Eldridge et al. 1989, Wang et al. 1991b).

The potential value of controlled-release components is highlighted by the observation that anti-PZP antibody titers were numerically greater in does given a PZP injection plus an osmotic pump containing porcine zonae pellucidae than in does given a PZP injection plus an osmotic pump containing saline. However, the small number of does per group and the lack of fawn production in the saline-pump group make it impossible to draw conclusions regarding a possible role of controlled-release porcine zonae pellucidae in this experiment.

It appears that PZP-mediated infertility is reversible. Eight PZP-treated does in the multiple-injection experiments were monitored for up to 2 years after cessation of PZP treatment. Six of these does produced fawns within this 2-year period. Serum anti-PZP antibody titer data, where available, were consistent with reversibility: none of the titer-tested does that fawned after cessation of PZP treatment showed anti-PZP antibody titers >30 percent at the onset of the breeding season during which they conceived. However, investigation of the possibility of long-term infertility after several consecutive years of PZP treatment appears warranted, based on data which have demonstrated this in horses (Kirkpatrick et al. 1992).

None of the does given a single PZP booster injection prior to year-2 breeding produced fawns the following summer. While it may be that the booster maintained the infertility seen in year 2, it is also possible that the original vaccination effect would have lasted 2 years without the booster. The latter possibility is in part supported by the results of the second experiment. Seven does given a two-injection vaccination in year 1 were not treated in year 2. No fawns were born in the first summer after the vaccination, and only two of these seven does fawned in the second summer after the vaccination, despite the absence of a booster treatment. In any case, these results preclude conclusions regarding the contraceptive effectiveness of a single PZP booster injection.

The maintenance of infertility across two consecutive breeding seasons in five of seven does given the two-injection PZP protocol was an unexpected finding. However, the limited antibody titer data in these does corroborated the fawning data, with titers maintained above 70 percent for 12 to 15 months in both does that were titer-treated at these times. Interestingly, the same PZP preparation and dosage given to does in the present study was effective in the mare for 1 but not 2 years (Kirkpatrick et al. 1992). The fact that the dosage per kg of body weight was severalfold greater in the deer than in the horse may explain why the antibody titer elevations in the deer were sustained longer. Studies of other PZP preparations in various species, using dosages as much as tenfold greater than those used in the horse have reported the occurrence of long-term infertility (Mahi-Brown et al. 1985, Skinner et al. 1984, Dunbar et al. 1989).

In a previous PZP study in white-tailed deer, we reported that some PZP-treated does continued to be bred for at least 4 weeks longer than control does. The extension of estrus was also observed in the present studies in some PZP-treated does, with the time between end of breeding in control does and treated does ranging from 3 to 5 weeks. More than 90 percent of white-tailed fawns are born in May and June (Halls 1984). In the present studies of 53 does, 3 fawns were born after June 30. A control doe fawned on July 8, and two PZP-treated does fawned, on July 14 and August 15, respectively. While these results do not clearly demonstrate that incomplete contraception causes fawns to be born late in the season, the subject of breeding-period extension due to contraception warrants further study for potential influence on deer bioenergetics and fawn survival.

## Studies in Wild Deer

Based on the encouraging results obtained in the studies with captive deer, we began to address the issues of accessibility to deer and deliverability. Toward this end, we entered into three field studies. The first study addressed accessibility to wild, free-roaming deer and it was carried out at Pt. Pelee National Park in Ontario, Canada, in collaboration with D. Voigt (Ontario Ministry of National Resources) and G. Mouland and D. Rieve (Park employees). This preliminary study examined the accessibility of deer to within darting range.

Point Pelee National Park is a small natural area (16 km$^2$, with only 30 percent dry land) located in the southwest corner of Ontario. It is isolated from other natural areas by urban development and an intense agricultural zone. In recent years, the park has been subject to white-tailed deer densities as high as 38 deer/km$^2$ of dry land. Two population reductions were carried out, in 1991 and 1993, to reduce and maintain the population density at about 7 deer/km$^2$.

Before immunocontraception via injection can be considered, investigators must make sure they have sufficient access to free-ranging does at low population density. To determine this, we established bait stations using apples and whole-kernel corn within 5 m of active deer trails at clearings before and after the 1993 population reduction. Prior to the population reduction (density = 12.5 deer/km$^2$ dry land), 14 bait stations were established throughout the park. Within 5 days, seven stations had daily deer activity. Over a period of 15 days, all bait stations were being used. Random observations showed 44 deer utilizing bait stations (23 does, 2 bucks, 13 deer of unknown sex, and 4 fawns). After the population reduction, bait stations were reestablished at five of the previous locations where deer were known to remain. Within 5 days, all 5 stations had daily deer activity, and over a period of 18 days, 23 deer were sighted (10 does, 6 bucks, and 7 of unknown status). Dusk and dawn were the predominant times of sighting. Because we found that the use of blinds gave us darting access to deer within 15 m, we believed the scenario adequate to attempt remote delivery of immunocontraception. The results of this study suggest that wild deer at Pt. Pelee can effectively be baited into darting range.

The Pt. Pelee deer are among the most wary and least visible of the numerous deer populations that we have observed on our invited visits to various parks during the 1990's. Our results at Pt. Pelee suggest that appropriately operated baiting programs can guarantee access to deer for vaccination in most parks. It is further encouraging that accessibility was only temporarily hindered by the occurrence of the cull. This is particularly important in light of the high probability that already excessive deer populations in many places would be culled before management by immunocontraception would be implemented.

In an effort to further our understanding of the vagaries of field delivery of porcine zonae pellucidae to deer, and in order to examine potential social–behavioral aspects of PZP contraception (such as continued estrus cycling and late fawning), we undertook a second field study. In collaboration with W. McShea and S. Monfort, we remotely vaccinated free-roaming does with porcine zonae pellucidae at the Smithsonian Institution's Conservation and Research Center in Front Royal, VA. The Front Royal field site was a 30-ha fenced area of mixed wood (80 percent) and fields (20 percent). The study population consisted of 28 adult, ear-tagged does, 4 bucks, and an unknown number of fawns.

The PZP vaccine treatment consisted of either two injections (3 weeks apart) of porcine zonae pellucidae plus Freund's adjuvant as described previously ($n$=10) or one injection of porcine zonae pellucidae plus Freund's adjuvant and a second dose of the pellucidae in controlled-release microspheres ($n$=9), as described previously. The nine remaining does, serving as controls, were injected with saline plus adjuvant. All injections were given in September. Access to the does was achieved by stalking, blinds, and bait stations. Most of the deer were extremely wary and nervous. Best success in darting was accomplished using blinds at bait stations. All does were vaccinated with a self-injecting 1-cm$^3$ dart fired from a 22-caliber rifle (Pneu-Dart, Inc., Williamsport, PA). Effectiveness of treatment was determined by counting fawns between June and August the following year. Does and bucks were also monitored for mating behavior and activity budgets between October and March. Details of behavioral and activity monitoring are presented in the paper by McShea et al. elsewhere in this book. Treatment effects on fertility, behavior, and activity budgets are presented below.

Regarding efficacy, none of the 10 does receiving 2 injections of porcine zonae pellucidae produced fawns; 67 percent of the one-injection does and 89 percent of the untreated control does produced fawns. All except one of the fawns produced by the one-injection does were born between mid-July and the end of August. The results demonstrate complete effectiveness of the two-injection PZP treatment. They also suggest that the one-injection PZP treatment inhibited fertility for most of the breeding season but did not prevent conception at the end of the season. Based on titer data from our previous studies and the fawning dates in the present study, it is likely that the anti-PZP antibody titers fell below the contraceptive threshold by the March following treatment.

Regarding behavior and activity budgets, 38 hours of observation on females revealed that contracepted does were more active and spent less time feeding than control does. However, the average body weight of treated does was greater than that of control does at the end of the winter. These results suggest that for does, the metabolic drain of pregnancy is greater than the metabolic demands of an extended breeding season with greater activity. During 84 hours of observations on males, mating occurred until March, but mating activity shifted from largest to smallest males as the winter progressed. Also, the intensity of the rut fell markedly after December. These conditions considerably moderated the energetic cost to bucks of an extended breeding seasons associated with PZP contraception. While these results suggest that there are no life-threatening behavioral or energetic consequences to a breeding season extended by PZP contraception, the details of PZP effects on these factors nonetheless deserve further exploration.

## Remote Assessment of Reproductive Status

As a corollary to the development of PZP immunocontraception for wildlife management, we have been exploring the remote assessment of reproductive status in free-roaming wildlife via monitoring of sex-hormone metabolites in remotely collected urine and feces (Kirkpatrick et al. 1990b, 1991b, and 1992). Recently, we have begun to evaluate this technology for use in white-tailed deer. Using fecal samples collected from deer studied in the reports presented in this paper, we have performed a preliminary analysis of concentrations of pregnancy-specific steroid metabolites in pregnant and nonpregnant does. The metabolites, measured by specific ELISA, were estrone conjugates ($E_1C$) and pregnanediol-3$\alpha$-glucuronide (PdG). In samples collected between April 25 and May 21 from does that produced fawns ($n$=9) and does that did not produce fawns, there was a 100-percent correlation between pregnancy and elevated $E_1C$, and/or elevated PdG. The $E_1C$ and PdG concentrations averaged $10\times$ and $4\times$ greater, respectively, in pregnant does as compared to nonpregnant does. All does that produced fawns showed an $E_1C$ concentrations >1.0 ng per g of feces, while all nonpregnant does had $E_1C$ levels <0.7 ng/g. All but two does that fawned exhibited PdG >7 ng per g of feces, while only one doe that did not fawn had PdG >5 ng/g. In general, $E_1C$ appears to be more reliable than PdG as an indicator of pregnancy, although it is useful to have both measures to maximize the probability of accurate assessment. These results are generally similar to those reported for equids, and suggest that this methodology can be useful for remote assessment of reproductive status in wild white-tailed deer.

## Summary

For all treatments in which does were exposed to porcine zonae pellucidae plus adjuvant at least twice within 4 weeks, the contraceptive effectiveness was 100 percent. Our combined data for all one-injection protocols shows a 42.1-percent fertility rate. While this represents better than 50-percent reduction in fertility relative to controls, we consider the single-injection efforts to be a failure. Most of the fawns born to one-injection does were delivered in August. This late-fawning phenomenon, with the consequent probable high winter mortality of late-born fawns, makes it essential to complete the development of a single-injection vaccine with a guaranteed minimum of 1-year efficacy. In fact, without denying the importance of issues such as recombinant *v.* natural ZP and dart *v.* biobullet, it is clear that there can be no practical field application of this technology without a single-injection vaccine that is highly effective for at least 1 year. The bioengineering of this PZP preparation is under way.

Regarding our continuation of field studies of wild deer populations, we completed the PZP vaccination of 68 wild, free-roaming does on Fire Island National Seashore in New York State in October 1993. The 68 treated does represent >70 percent of the adult does in the discrete population that we targeted. We employed a two-injection protocol because the single-injection, 1-yr vaccine was still in development. The results of this study should provide useful data toward the potential use of PZP immunocontraception for wild deer management in limited deer populations.

## Conclusions

The studies presented in this paper have clearly demonstrated that PZP immunocontraception can prevent pregnancy in white-tailed deer. A brief reexamination of the requirements for management-useful contraceptive in wild, free-roaming deer reveals that our data also show feasibility of access to the deer, remote delivery of PZP vaccine, and remote assessment of pregnancy. When development of a single-injection capability is completed, management of many urban deer populations with PZP immunocontraception should be technologically realistic. In the meantime, other issues regarding immunocontraception must be addressed in the area of public attitudes and education, administration and policies, and long-term management strategies (Kellert 1991), all of which are influenced by politics, economics, and management goals as discussed in other papers from this conference.

Members of our research team have visited many locations where insular deer populations are presently so large that the deer have already severely compromised their habitat. Many species of plants and animals, not just deer, are now suffering the consequences of ballooning deer numbers. While contraception may be used to limit population growth, it will not reduce the numbers presently in excess. Therefore, initial population reductions by existing means may be necessary where indicated in order to protect habitat for the future and to establish a basal population of deer that thenceforth may be managed by contraception.

While our research team recognizes that wildlife population control is an important issue, the need for such control reflects a much larger and burning issue: expanding human population. We believe that as we continue our work, all of us in the growing field of wildlife contraception have an obligation to help focus public consciousness in this regard.

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