REMOTELY DELIVERED IMMUNOCONTRACEPTION IN CAPTIVE WHITE-TAILED DEER

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Abstract: We tested a new contraceptive method that offers a potential alternative to white-tailed deer (*Odocoileus virginianus*) population control where hunting is unfeasible. We report the first successful, remotely delivered immunocontraception in captive, unrestrained white-tailed deer using porcine zona pellucida (PZP) antigen. None of 7 PZP-vaccinated does produced fawns, whereas 6 of 7 control does did. No adverse effects of treatment were observed. These results encourage further investigation of immunocontraception in wild white-tailed deer populations.

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Traditionally, sport hunting has been used in an attempt to maintain white-tailed deer (*Odocoileus virginianus*) populations in North America at levels compatible with their habitat. However, in some locations, such as national and state parks, wildlife refuges, and urban parks, hunting is illegal or impractical. Management efforts have been further complicated by continued human encroachment into areas bordering preserves and by increased public pressure to protect the deer from destruction or removal (Kellert 1991).

One potential resolution to this rapidly escalating problem may lie in deer population control through contraception. The feasibility of fertility control in ungulates has been under investigation for more than 20 years (reviewed, Kirkpatrick and Turner 1985; Turner and Kirkpatrick 1991). The earliest deer contraception studies used steroids, primarily diethylstilbestrol and melengestrol acetate administered via implant, injection, or orally in food (Harder and Peterle 1974; Bell and Peterle 1975; Matschke 1977*a*,*b*; Roughton 1979). However, implants require capture or immobilization, which is expensive and potentially dangerous to the animal. Oral administration can be unreliable and requires frequent, often daily, ingestion of the agent. Palatability of treated bait may be a problem, or bait may be eaten by non-target species (Kirkpatrick and Turner 1991).

One antifertility approach that appears to be free of many of the disadvantages of steroids is immunocontraception. Successful application of immunocontraception using a porcine zona pellucida (PZP) antigen in horses has been reported by Liu et al. (1989). More recently, Kirkpatrick et al. (1990*a*) have demonstrated greater than 95% inhibition of fertility in free-roaming feral mares treated with PZP vaccine via remotely delivered dart. The effects of the vaccine were reversible after 1 year, did not affect pregnancies in progress, and did not produce behavioral side effects (Kirkpatrick et al. 1990*a*,*b*). We undertook this study to determine the effectiveness of remotely delivered PZP vaccination in captive, unrestrained white-tailed deer.

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MATERIALS AND METHODS

The study was conducted at a private deer facility in westcentral Ohio (latitude 41°, longitude 84°). We used 14 does ranging in age from 2 to 7 years and in weight from 45 to 60 kg. All does were known to have previously fawned. They were maintained in a 0.5-hectare flat, grassed enclosure with a 3-m chain-link fence. The west end of the enclosure was a solid wooden wall with an overhang to provide the deer shelter from cold, wind, and precipitation. They were fed hay, mixed grain, and commercial food pellets. Food and water were provided *ad libitum*. Each doe was eartagged to facilitate remote identification.

The PZP vaccine was prepared from porcine ovaries as previously described (Liu et al. 1989) and was stored at −5 C until used in the field. An emulsion of 0.5 cc vaccine (equivalent to approximately 5,000 zonae or 64.3 μg of protein) in phosphate buffer and 0.5 cc of Freund's complete adjuvant was prepared. We mixed the 2 components just before injection, using 2 10-cc

glass syringes joined with a plastic connector. After 100 plunger strokes, we loaded the emulsion into a 1.0-cc self-injecting dart (PneuDart Inc., Williamsport, Pa.) tipped with a 2.2-cm, 18-gauge needle. The dart needle was rinsed with 70% ethanol, and the dart was loaded into a standard 1.3-m blow gun and fired through the fence wire into the hip region of each doe. On 4 October 1989, we selected 7 does for the treatment group and gave them an initial inoculation of vaccine. We similarly injected 2 control does with adjuvant-buffer only (no PZP). The remaining control does ($n = 5$) were unavailable for injection and remained untreated. However, PZP studies employing sham controls have been conducted in a number of species, and no inhibition of fertility in sham-control groups has been reported (Paterson and Aitken 1990).

Ages and body masses were similar in the control and treated groups. We gave the second and third inoculations 3 and 6 weeks, respectively, after the first inoculation. These inoculations were identical to the first injection with the exception that incomplete adjuvant (less immunogenic) was used rather than complete adjuvant. In a previous study in mares, the use of incomplete adjuvant after the initial inoculation produced effective contraception with little or no abscessing at the injection site (Kirkpatrick et al. 1990*a*). Therefore, we used this approach in the present study.

In December, approximately 6 weeks after the final injection, a healthy 3-year-old buck, which had previously sired fawns, was placed with the does. We observed breeding within 48 hours after introducing the buck, and he was permitted to remain with the does thereafter. We observed the deer 2–3 times/week periodically through winter and spring to assess health and general physical condition. We recorded the fawning dates and the number of fawns produced. Fawning was complete by mid-June.

RESULTS

None of the does exhibited a visibly draining abscess at the injection site, but a raised area of about 20 mm in diameter developed in the final injection site on 3 does. This response disappeared after 8–10 days in 2 of the does and after 5 weeks in the third doe. We observed 10 of the 14 does being bred. Some treated does continued to cycle and breed periodically into early March. Three treated does were bred in February, and 1 treated doe was bred during the first week of March. None of the control does were observed breeding after January. All does appeared to be in good health and physical condition throughout the study.

Parturition began late in May and spanned a 3-week period. None of the PZP-treated does produced fawns, whereas 6 of the 7 control does (86%), including both injected controls, did. All fawns appeared healthy and nursed normally. The incidence of fawning for all does at the facility was 82% in 1988 ($n = 12$) and 93% in 1989 ($n = 14$). Analysis of fawning data via binomial probability distribution (Zar 1984) revealed that the proportion of does giving birth to fawns was lower ($P < 0.01$) for PZP-treated does than for control does. The incidence of twinning at the facility averaged 42% for 1988 and 1989 and was 33% for the control does in 1990.

DISCUSSION AND MANAGEMENT IMPLICATIONS

We present the first successful inhibition of fertility in white-tailed deer using remotely delivered immunocontraception. Successful inhibition of fertility using PZP vaccination has been reported for a number of mammals, including non-human primates (Sacco 1987). In previous studies of horses (Liu et al. 1989, Kirkpatrick et al. 1990*a*), PZP successfully suppressed mare fertility. The PZP vaccine appears to act by inhibiting fertilization or possibly implantation (Sacco et al. 1984). The zona pellucida of the pig is comprised of 3 glycoproteins, one of which (ZP3) acts as a receptor for sperm surface molecules (Florman and Wassarman 1985). Liu et al. (1989) suggested that PZP antibodies generated in the horse may prevent fertilization by blocking sperm receptor sites on the ovum.

We observed small open abscesses in 12% of vaccinated horses in a previous immunocontraception study (Kirkpatrick et al. 1990*a*). Although there were no open abscesses observed at the injection site in our does, the localized swelling suggested a significant tissue response to the adjuvant. We used Freund's adjuvant in our study because of previous success with it in mare immunocontraception (Kirkpatrick et al. 1990*a*). Less tissue reactivity has been reported for some other adjuvants such as a muramyl dipeptide analog, which has been used successfully in immunocontraception studies (Sacco et

al. 1989). However, the effectiveness of such alternative adjuvants in deer is unknown.

We did not determine the duration of infertility in does after vaccination. Antibody titers in horses remained elevated up to 8 months (Liu et al. 1989), and we have found persistence of infertility through a second year in mares given a single annual booster PZP injection (Kirkpatrick et al. 1990b). Also, none of our does were pregnant at the time of vaccination, so possible effects on pregnancy and the fetus remain unknown. The gestation period of the white-tailed deer is approximately 7 months (Halls 1984), so if vaccinations are given during the autumn prior to the rut, the possible pregnancy-related side effects would be minimized.

Although it is not known if the PZP treatment effect in deer is reversible, such treatment in the horse is reversible if no annual booster inoculation is given (Kirkpatrick et al. 1990b, Liu et al. 1989). However, there are data in several species, including the rabbit (*Oryctolagus cuniculus*) (Wood et al. 1981), the dog (*Canis familiaris*) (Mahi-Brown et al. 1985), and the baboon (*Papio* sp.) (Dunbar et al. 1989) that show changes in ovarian morphology and function associated with immunocontraception which may lead to long-term, possibly irreversible, infertility.

The proteinaceous nature of the PZP vaccine provides several distinct advantages in terms of its potential use as a deer contraceptive. First, it is water soluble and can be delivered in a small volume. This enables remote delivery using a 1-cc dart, as in this study. In addition, these darts can be delivered accurately at greater distances than larger darts commonly being used now. Finally, the PZP protein is metabolized prior to excretion or if ingested, and therefore cannot be passed through the food chain via excrement or via consumption by hunters, predators, or scavengers. Because deer are prey for several species, steroid contraceptives, especially synthetics which resist biodegradation, are particularly unattractive as contraceptive agents for this species.

Another disadvantage of using steroid contraceptives in wildlife is their potential for behavioral side effects. PZP immunocontraception did not directly affect breeding behavior in the present study. However, some PZP-treated does did continue to cycle after not becoming pregnant. Although our limited estrus and breeding data are inconclusive, these events may have continued at least a month longer in some treated does than in control does. Thus, treatment may affect long-term patterns of behavior and social organization. Furthermore, possible consequences of altered patterns of energy expenditure in bucks and does during this time deserve consideration.

Liu et al. (1989) reported successful contraception of mares using 3 PZP inoculations. Although we produced successful immunocontraception in feral mares using either 2 or 3 PZP inoculations (Kirkpatrick et al. 1990a), we used the 3-injection protocol in our study to maximize effectiveness in this untested species. However, development of a single-dose vaccination is essential to pursue this pathway for contraception in wild deer. Microencapsulation technology may offer the means to develop such a vaccination. This process, which provides a timed release of the agent, has been used successfully with contraceptive steroid (Turner and Kirkpatrick 1982) and antigenic protein (Eldridge et al. 1989). Even if this issue can be resolved, other considerations of remote delivery in the field, such as accessibility to does and identification of treated versus untreated individuals, remain to be addressed.

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