October 2000

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The Ninth Wildlife Damage Management Conference Proceedings. Edited by Margaret C. Brittingham, Jonathan Kays and Rebecka McPeake. Oct 5-8, 2000 State College, PA USA

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Abstract: The results of a 6-year behavioral study of white-tailed deer *(Odocoileus Virginianus)* treated with two immunocontraceptive vaccines, porcine zona pellucida (PZP) and gonadotropin releasing hormone (GnRH) are summarized. Does were immunized late summer and early fall. In November, does were exposed to bucks and observations were made 2-3 times daily for one half hour through the end of February. Does were considered to be in estrus when they were the object of intensive sexual interest by the buck. Compared to controls, PZP-treated does had a significantly lower fawning rate and an increase in the average number of estrous cycles observed per doe. The average breeding days each year for the control group was 45, whereas in some years some PZP treated does were breeding more than 150 days. When previously treated PZP does were not treated in subsequent years, antibody titers declined and fertility was gradually restored. GnRH immunization also induced a decrease in fawning rate, although the average estrous cycles observed per doe were comparable to averages observed for control animals. There was no indication that the GnRH treatment caused repeated estrous cycling as with the PZP treated does. Based on limited observations GnRH treatment has a negative impact on males, resulting in immunological castration, compromised libido and abnormal antler development.

Key words: contraception, estrous cycles, GnRH vaccine, PZP vaccine

Introduction

Two immunocontraceptives that have been shown to be effective antifertility agents in several wildlife species target the egg zona pellucida or gonadotropin- releasing hormone (GnRH). This contraceptive strategy utilizes the immune system of the animal to produce antibodies against naturally occurring proteins that are essential in the chain of events for successful reproduction. In the case of the zona pellucida vaccine, porcine zona pellucida proteins (PZP) harvested from porcine ova are used as an immunogen to stimulate antibody production against the eggs of the target animal. It is believed that the resulting antibodies prevent normal sperm-egg interaction and conception. For the GnRH vaccine, a modified molecule is prepared which also has the capacity when used as an immunogen to stimulate antibodies against native GnRH. The antibodies produced inactivate GnRH secreted from the hypothalamus and prevent the normal cascade of hormone secretions that is essential for gonad regulation and gamete production. Although the potential and efficacy of immunocontraceptive vaccines for wildlife and feral populations has been demonstrated in many earlier studies for PZP (Kirkpatrick et al. 1996, Turner et al. 1996, McShea et al. 1997, Warren et al. 1997, Turner et al. 1997,

Miller et al. 1999) and in some studies involving GnRH (Arimura et al. 1973, Robertson 1982, Ladd et al. 1989, Awoniyi et al. 1993, Brown et al. 1993, 1994, Meloen et al. 1994, Miller et al. 1997) many questions remain about the long term effects of their use regarding the reversibility of infertility and behavioral and physiological effects (Nettles 1997).

There is little doubt that the emerging technologies involving wildlife contraception have been, and will continue to be, the subject of consideration and debate for use in controlling population growth of wildlife. Reliable and fundamental information is needed to better understand how these agents affect the biology of the target species beyond making them infertile, so that regulatory agencies are able to make informed decisions about their use. As a means to this end, we have pursued research on immunocontraception on white-tailed deer *(Odocoileus virginianus)* for 6 years to better understand the long-term effects on fertility, as well as on the behavior and physiology of treated individuals.

Materials and methods

Studies were conducted at the Deer Research Center of the Pennsylvania State University, a 8.9 ha site specifically design for management and observation of deer on research trials. The methodology for the long-term studies we used involving PZP contraceptive treatment (Miller et al. 1999, Miller et al. 2000a) and GnRH treatment (Miller et al. 2000b) of white-tailed deer has been described previously. Studies involving PZP that were conducted from 1992-1997 and for GnRH during 1994-1998 involved both physiological and behavioral observations.

Fawning data were collected for does in the study through 1999. An overview of the experimental chronology for each year of this study is presented in Figure 1.

During November through January, observations on reproductive behavior were made on deer during 3, one-half hour periods morning, noon and early evening, as well as random observations made throughout the day during herd checks. In February, only morning and evening plus random observations were made, and in the remaining months only random daily observations were made during routine herd checks. Using a scoring sheet (Figure 2), trained observers recorded various interactions occurring between the buck and individual does during each observation period. The behavior criteria used to define these interactions were adapted from those described in earlier studies (Warren et al. 1978, Shumake and Killian 1997). The behaviors described in Figure 2 that were considered to be indicative of a female in estrus and/or that mating had occurred were defined by criteria 7-9. Criterion 6 was also considered a strong indication that a doe was in estrus. Information from individual does was summarized regarding the number of estrous events and an average number of events determined for the does in the experimental and control groups. The total number of days during the season that estrous events were observed for individuals in the group was also determined to assess the duration of breeding activity or "season" within the treatment. Fawning dates and number of fawns born to each doe were recorded and these data were used to determine the breeding date at which conception occurred. Average number of fawns born to does within a treatment group was also determined.

Experimental Chronology

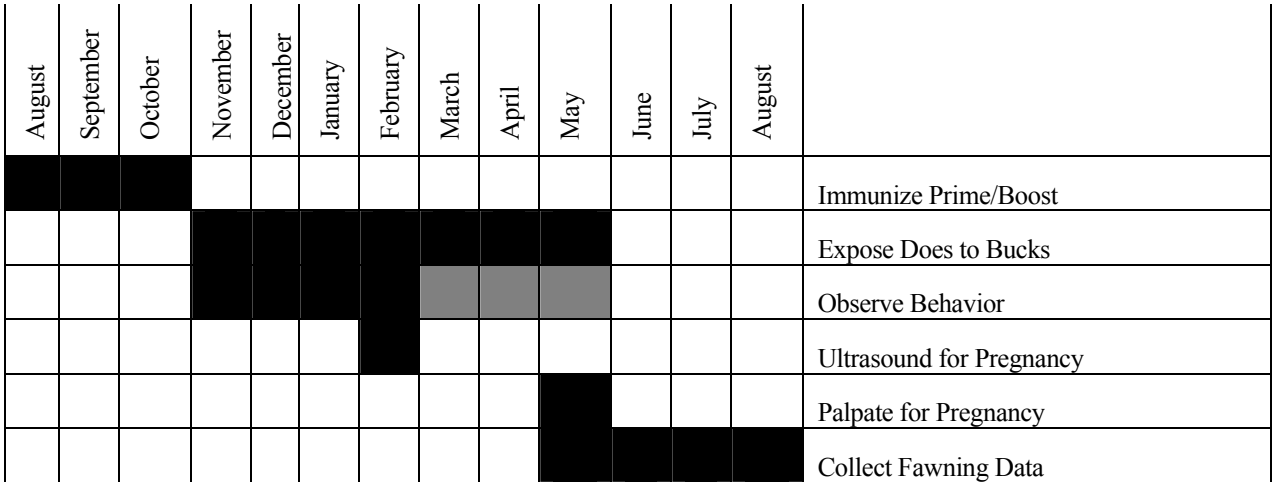


Figure 1. Experimental chronology showing when various aspects of the study were carried out each year.

Sample Observation Sheet

Paddock F

Date
Time
Obsv.____

0 No Interest
1 No pursuit, sniffing lip cur 1
2 Standing/lying with in 8 yds.
3 Short pursuit < 60 sec
4 Extended pursuit > 60 sec
5 Aggressive chasing
6 Aggressive guarding
7 Male licks genital area of female
8 Mounting/Copulation
9 Doe post copulatory posture
10 Buck licks genitalia/urination
11 Does urinating on tarsal gland

Black	703 (#50)	0 12 3 4 5 6 7 8 9 10 1	
	702 (#36)	0 12 3 4 5 6 7 8 9 10 1	- Yellow Collar
	801 (#43)	0 12 3 4 5 6 7 8 9 10 1	
Brown	406 (#23)	0 12 3 4 5 6 7 8 9 10 1	
	510 (#26)	0 12 3 4 5 6 7 8 9 10 1	

Figure 2. Scoring sheet used by trained observers to record observations made on reproductive behavior for various interactions occurring between does and bucks.

Observations were also made on several bucks treated and boosted in 1994-1995 and 1995- 1996, and boosted again in 1997 with GnRH vaccine (Miller et al. 2000b). Observations were made on testicular size, antler development, body form and sexual libido.

Results and discussion

Average fawning rates for does that were treated during the first 3 years of the study with PZP vaccine were significantly less than the average of 1.8 fawns for controls (Figure 3). This dramatic reduction was clearly correlated with high serum antibody titers for PZP and shown to be reversible in subsequent years as antibody titers declined.

Observations on reproductive behavior during the first 6 years of the study found that while PZP-treated does were infertile, they typically displayed multiple estrous cycles during the season, even though breeding had occurred (Figure 4). These observations support the notion that the ovarian follicular cycle continued to produce ovulatory follicles in PZP-treated does, but conception failed to occur. We previously have shown in vitro that oocytes exposed to immune serum from PZP-treated does are less able to bind spermatozoa (Way et al. 1999) suggesting that a similar mechanism may prevent fertilization in the oviduct of intact animals. However, because serum progesterone concentrations during the

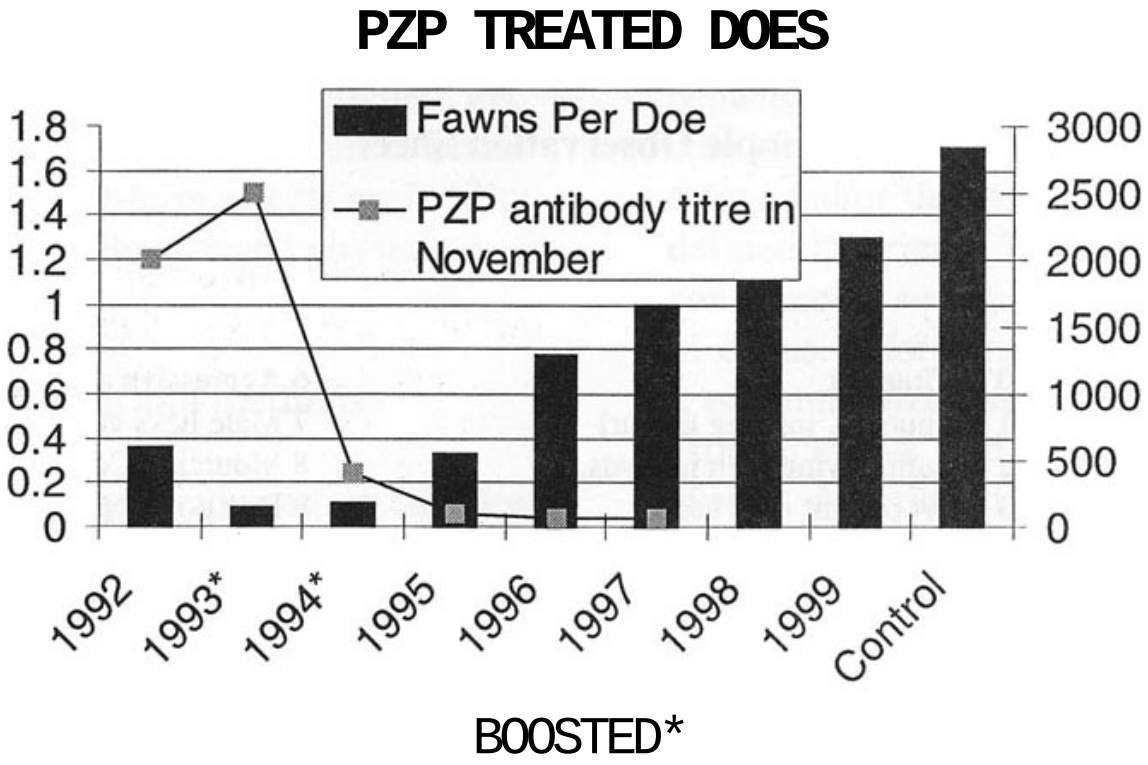


Figure 3. Average fawning rates for PZP treated does from 1992-1999 compared to the average control for does. Also indicated are the PZP serum titers for the PZP-treated does during the first 6 years of the study. The antibody titer scale is 1/serum dilution. The maximum scale number of 3000 equals a titer of 3 million.

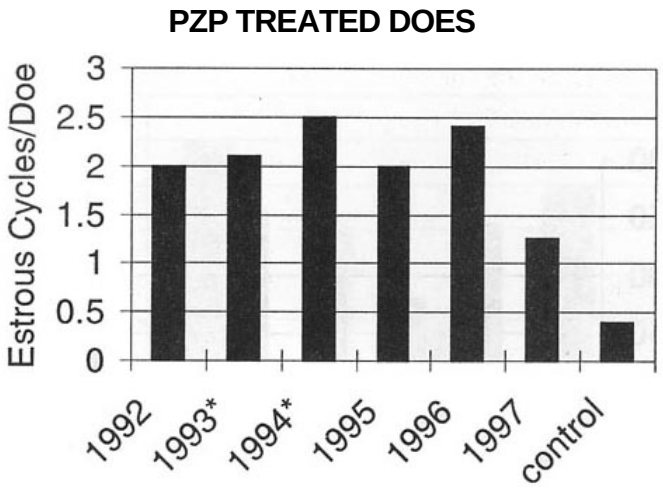
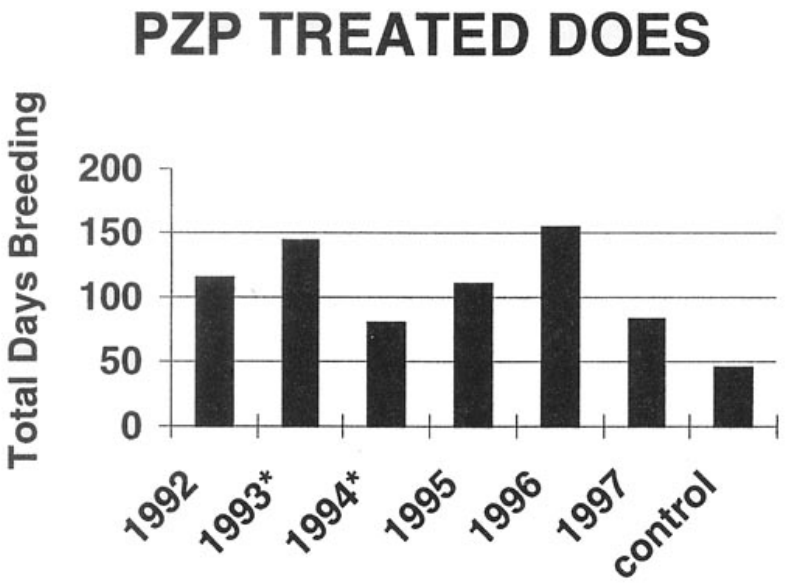


Figure 4. Average number of estrous events observed for PZP treated does from 1992-1997 versus the average for control does. Criteria used for "estrus" are defined in figure 2.

luteal phase of PZP-treated does appear to be lower than that of controls it is also possible that infertility results from the failure of a normal corpus luteum to develop to sustain pregnancy (Miller et al. 1999).

As a result of reproductive failure in PZP-treated does, and the tendency to undergo repeated estrous cycles, the length of the breeding season was extended from an average of more than 40 days in controls to more than 150 days in some years for PZP-treated does (Figure 5). This extended breeding season for some individuals has the potential consequence of fawns being born later in the summer than typically occurs with control does. This later fawning appears to occur in does experiencing a decline in serum PZP antibody titer during the season, resulting in "escape" from immunocontraception enabling conception to occur later than usual. In a few cases we have observed that fawns may be born in July or August to PZP-treated does regaining fertility that season, but the majority of does regaining fertility in a given year will fawn in late May or June, like control does.

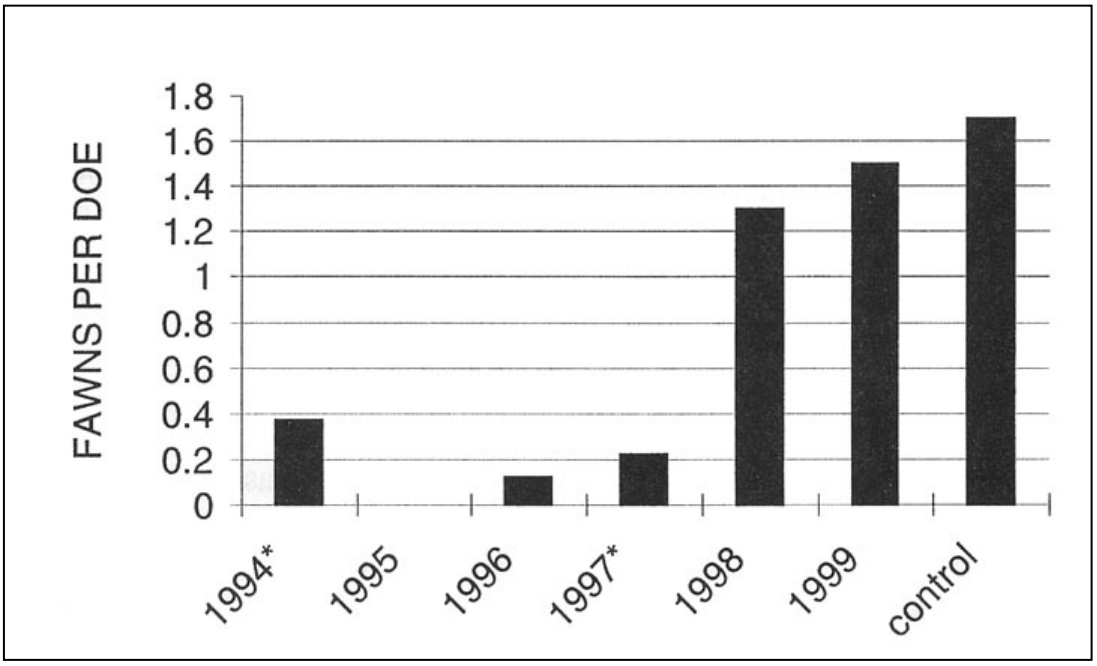
Fertility of does treated with GnRH vaccine was also significantly reduced compared to control does (Figure 6). Does actively immunized with GnRH vaccine averaged 0.0-0.3 fawns per doe compared with a 1.7 average for controls. In contrast to PZP-Treated does however, repeated estrous cycles were not evident in GnRH-treated does (Figure 7) nor was there evidence for an extended breeding season or late fawning in does regaining fertility following a period of contraception. Does in this treatment group showed little or no evidence of sexual libido, and bucks did not attempt to breed them. These observations support the proposed mechanism of action for the GnRH vaccine (reviewed in Miller et al. 1997) inactivating the cascade of reproductive hormones initiated



***BOOSTED**

Figure 5. Maximum total days some does were observed breeding for the PZP treatment versus that for control does during the breeding season.

GnRH—KLH



***IMMUNIZED**

Figure 6. Fertility of does treated with GnRH vaccine for 1994-1997 versus control does.

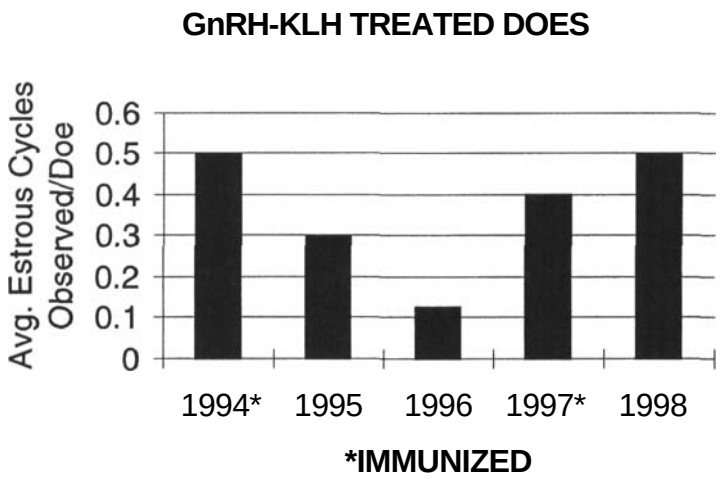


Figure 7. Average number of estrous cycles observed from 1994-1997 for GnRH-treated does versus control does.

at the level of the hypothalamus. This action apparently diminishes or blocks gonadal hormone and gamete production.

Aside from a lack of mating activity during the breeding season and low serum progesterone concentrations associated with GnRH-treated does (Miller et al. 2000b), there were no other treatment effects apparent. For GnRH-treated bucks, however, several effects of the treatment were outwardly evident. GnRH-treated bucks lacked masculine appearance and did not display interest in estrous females. Testicular size was similar to pre-seasonal size and antler development was minimal. Antlers of most GnRH-treated males remained in velvet and failed to undergo hardening associated with "rubbing out"(Miller et al. 2000b). For these males antlers typically broke off in January when colder whether may have frozen the tissue.

Conclusions

Immunocontraception of White-tailed deer with vaccines directed against the zona pellucida and GnRH have been shown to be effective. However, these approaches have also been shown to affect breeding behavior, length of the breeding season and male secondary sex characteristics. Although the use of these vaccines as antifertility agents is feasible for population control of wildlife populations under certain circumstances, the benefits to be gained from limiting reproduction of the target species must be weighed against other affects on the treated animals.

Acknowledgments. Appreciation is expressed to Dr. Stephen Shumake of the National Wildlife Research Center for assistance with defining criteria for making reproductive behavior observations and to the

numerous undergraduates at Penn State who assisted during the seven years of this study.

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