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Seven years of white-tailed deer immunocontraceptive research at Penn State University: A comparison of two vaccines.

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Abstract: PZP and GnRH immunocontraceptive vaccines were each tested in white-tailed deer *(Odocoileus virginianus).* Vaccination with PZP produced reversible infertility lasting 1 to 4 years. The first two years of active immunization resulted in an 89% reduction in fawning. Reduction in fawning for the 7-year study containing 4 years of no boosting was 72%. PZP immunization resulted in multi-estrus behavior, with contracepted deer returning to estrus up to 7 times. A five year study of GnRH immunization was conducted in both male and female deer. Treatment of does led to reduced fawning rates, reduced estrus behavior and reduced concentrations of progesterone. During active immunization GnRH does bred to untreated bucks had an 88% reduction in fawning caused by either immunocontraception or immunocontragestion. Reduction in fawning for the 5 year study containing 2 years of no boosting was 74%. The vaccine effect was reversible and directly related to the antibody titer. Infertility lasted up to two years without boosting. GnRH immunized bucks had no interest in sexual activity when paired with control females. Depending on the immunization schedule, antlers either dropped early or remained in velvet.

Key words: immunocontraceptive vaccine, *Odocoileus virginianus,* white-tailed deer

Introduction

A growing interest in nonlethal methods for population control of nuisance or damaging species of wildlife has fostered research in reducing fertility of these pest wildlife species. Fertility may be reduced by interfering with the fertilization of the egg (Contraception) or interfering with the implantation or development of the fertilized egg (Contragestion) (Miller et al. 1998).

Reproduction can be blocked at many sites in the reproductive process (Figure 1). Two contraceptive vaccines discussed in this paper are PZP and GnRH.

The zonapellucida (ZP) is an acellular glycoprotein layer surrounding the mammalian oocyte (egg). During fertilization, sperm must bind to zona pellucida receptors on the outer surface of the oocyte and penetrate the ZP for conception to take place (Miller 2000a). Immunization with PZP inhibits fertilization. Native porcine zona pellucida (PZP) vaccines have been used to produce sterilization in horses, burros, deer, baboons and other species (Kirpatrick et al. 1990; Garrott et al. 1992; Turner 1996a, 1996b; Miller 1999b).

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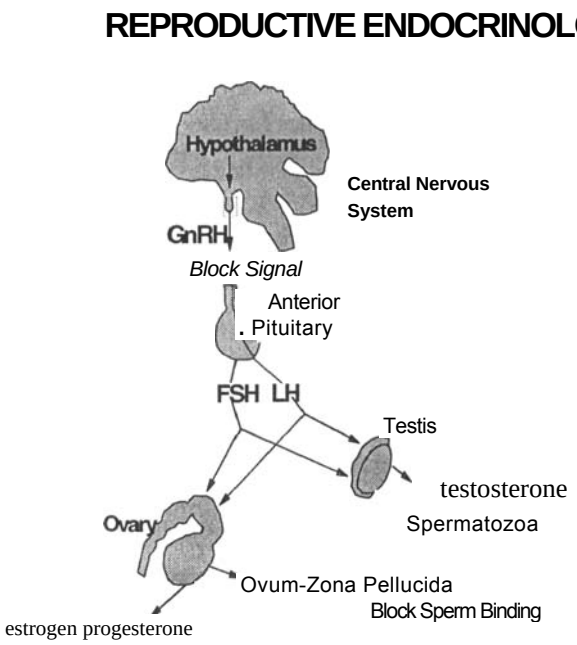

Figure 1. Mammalian Reproductive Endocrinology; showing blocking sites of the 2 immunocontraceptive vaccines (GnRH and PZP) presented in this paper.

GnRH is a small peptide hormone that is not naturally immunogenic, but can be made immunogenic by coupling it to a carrier such as keyhole limpet hemocyanin (KLH). GnRH antibodies bind to endogenous GnRH and prevent the release of FSH and LH (Ferro et al.1996; Ferro and Stimson 1998).

The reduction or absence of serum FSH and LH leads to atrophy of the gonads leading to infertility in both sexes. The immunoneutralization of GnRH through the use of GnRH vaccines appears to be highly specific, and does not appear to affect other hypothalamic releasing hormones; Awoniyi et al. (1993) demonstrated that GnRH immunized rats failed to release FSH and LH, but that other pituitary hormones and their vital non-sexual functions were not impaired.

GnRH contraceptive vaccines have been evaluated as immunocastration agents in pets, cattle, sheep, swine and deer (Ladd et al. 1994; Adams and Adams 1992; Schanbacher 1998; Meloen et al. 1994; Oonk et al. 1998; and Miller et al. 2000b)

Recently, in studies with the Norway rat (Miller et al. 1997a), we found that both males and females immunized with a GnRH vaccine were 100% infertile.

Methods and materials

This study was conducted at the Deer Research Center of The Pennsylvania State University, University Park, PA. Deer were kept in a 17 acre wooded enclosure and provided hay and pellets ad libitum. During handling, deer were restrained mechanically and chemically with 0.5 to 1.0 ml of xylazine.

PZP vaccine

Deer were immunized with purified porcine zona pellucida (PZP) which was prepared by and purchased from Dr. Dunbar at the Baylor College of Medicine, Houston, TX. 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 |ig of PZP mixed with 0.5 ml of complete Freund's adjuvant (CFA). The booster doses contained 300 ^g 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

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each boost dose.

All of the PZP treated deer were immunized the first year, 8 of 11 were boosted the second year, and 4 of 9 deer were boosted the third year. None of the deer were boosted after the third year. The decision to boost deer in the 2nd or 3rd years was determined by the antibody titer. Those deer that appeared to have a rapidly dropping antibody titer in the previous year were boosted.

GnRH Vaccine

The 10 amino acid GnRH peptide hormone which is conserved among all mammals was made immunogenic by coupling the peptide to KLH, a large protein containing numerous T cell epitopes. The GnRH used in this study was synthesized at Macromolecular Resources, Colorado State University (Ft. Collins, CO) with the structure [pEHWSYGLRPGGC-SH]. A glycine was added at the C terminus as a spacer and a cysteine was added to ensure consistent alignment of the peptide to the maleimide activated protein carrier KLH. The underlined amino acids represent the native GnRH molecule (Miller et al. 2000b).

In a 5 year study on the effectiveness of KLH-GnRH vaccine to immunocontracept deer, eight fertile does and 4 fertile bucks were injected subcutaneously with one primary immunization followed later by booster doses all containing 1 ml of vaccine distributed among several sites at the center of the back between the scapulae. All deer received a prime dose containing 500 jag of KLH-GnRH in saline mixed 1:1 with complete Freund's adjuvant (CFA) and approximately one month later were given a boost containing 300 µg of KLH-GnRH mixed 1:1 with incomplete Freund's adjuvant (IFA). Boost injections were given on a yearly basis depending on the current antibody titer. Fawning results of the GnRH treated does were compared to the fawning rates of sham treated does and fawning of the does from the herd not involved in the study (Miller et al. 2000b).

Laboratory studies

In both the PZP and GnRH studies, 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 x g. Serum was harvested and samples were stored at -20 °es C. for subsequent ELISA, progesterone or testosterone assays.

Observations on mating, gestation and fawning

To determine when the does were in estrus for both the PZP and GnRH vaccines, behavioral observations of the bucks toward the treated does were made by Penn State University students three times daily from November 7 through February 12 and then two times daily until February 28. Behavioral activity by the buck included 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 day of the ultrasound and tested for progesterone concentration.

From May through August, does were

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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.

PZP results

Fawning

During the first 3 years, when there was active immunization, 11 treated deer produced 6 fawns, compared with 56 fawns from the same number of control does, or an 89% reduction in fawning ($P < 0.01$). For all 7 years (Table 1), including 4 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 72% 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 antibody titer. As antibody titers dropped and deer regained fertility most does had only 1 fawn, which was born at late as August or September. As the deer regained fertility they could be reboosted and the infertility could last for several years after the boost.

Behavioral observations

Most control deer in the study 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 three 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 7 cycles of sexual activity, with the last observed activity occurring on March 4. This doe was sexually active for 150 days but remained infertile.

Progesterone levels

During the normal breeding season of October to December, control deer had serum progesterone concentrations of >4.0 ng/ml at some time during each month representing ovulation. There appeared to be some synchronization of the estrus activity and elevated progesterone levels in the control herd. Progesterone concentrations of >4 ng/ml in late January to early February generally correlated with fawning in the control deer. The 4.0 ng/ml progesterone in the PZP did not correlate with pregnancy since the PZP antibody could protect the ovulated egg from sperm penetration.

Ultrasound and palpation

Ultrasound has been shown to be an excellent predictor of early pregnancy 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 were predictive of fawning.

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Table 1. Fawning data of the PZP treated deer as compared to the Sham controls and the breeding Herd at The Pennsylvania State University, University Park, PA.

Year	Treatment	Fawns/Does	Yearly Average	Cumulative Average
92-93	Primed & Boosted	4/11	.36	.36
93-94	Boosted	1/11	.09	.23
94-95	4/9 Boosted	1/9	.11	.19
95-96	None Boosted	3/9	.33	.23
96-97	None Boosted	7/9	.78	.33
97-98	None Boosted	9/8	1.13	.44
98-99	None Boosted	8/7	1.14	.52
Breeding herd		156/90		1.73
Sham controls				1.84
PZP treated				.52

A reduction in fawns of 89% was achieved during active immunization, and a 72% reduction in fawns was seen over seven years including four years without boosting.

GnRH results

Female deer

Fawning Data. A reduction in fawns of 88% was achieved during active immunization, and a 74% reduction in fawns was observed over five years including two years without boosting (Table 2).

Five fawns were born to the 4 does during the 4 years, or 5 fawns/14 doe years (doe years the number of does times the number years tested), compared to 25 fawns/14 doe years in the control deer during this same period of time.

Of the four additional does given GnRH treatment in the third year, one fawn was born during the first year. With the additional group of does added there were 6 fawns/22 doe years (0.27 fawns/doe/year) as compared with 40 fawns/22 doe years (1.8 fawns/doe/year) in the control group. This represents an 88% reduction ($P<0.01$) in fawning in the GnRH group compared with the controls. The average of 1.83 fawns/doe/year among the control does in the study is representative of the normal breeding herd average at PSU. Fawns born to the GnRH treated deer appeared healthy and normal.

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Table 2. Fawning data of the GnRH treated deer as compared to the Sham controls and the breeding herd at The Pennsylvania State University, University Park, PA.

Year	Treatment	Fawns/Does
94-95	Primed & boosted	3/4
95-96	Primed & boosted	0/6
96-97	Boosted	1/8
97-98	No boost	3/8
98-99	No boost	9/9
Breeding herd		156/90(x=1.7)
Sham controls		35/19 (x=1.8)
GnRH treated		16/35 (x=.46)

A reduction in fawns of 88% was achieved during active immunization, and a 74 % reduction in fawns was seen over five years including two years without boosting.

Behavioral observations. Under normal situation, deer are asexual for the majority of the year. The short day lengths of the autumn turn on the sexual activity for the short breeding season. Some of the GnRH treated deer never develop this seasonal sexual activity and remained asexual throughout the breeding season. Although progesterone data indicated that the does were not cycling, there is some activity from the bucks, ranging from sniffing the does to short pursuits of bucks chasing does. The limited amount of estrus activity in the GnRH treated does was observed over 30 days. The observed estrus events for the control does were typically 1 or 2 per doe over a total period of 44 days.

Male deer

Control bucks in our study had serum testosterone concentrations of less than 100 ng/l00 ml late summer and early fall and developed normal breeding levels of testosterone (350 to > 400 ng/100 ml) in October. Peak breeding activity occurred in November when bucks were introduced to does. Antlers grew slowly early in the summer but rapid growth ensued in August and September. Antlers remained in velvet until September at which time the velvet was rubbed off on nearby trees. Antlers dropped off in March of the following year. The bucks in the GnRH group were treated the first year with a prime dose in September and boosts in

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October. They lost their already hardened antlers two weeks after the second boost (between October 20 to 28). Their testes were less then one half the size of normal bucks, and they did not develop the prominent neck musculature normally observed in the autumn on mature bucks and therefore looked very much like a doe throughout the breeding season. Treated bucks were penned with untreated cycling does but demonstrated little sexual interest in the does. Serum testosterone levels for the GnRH-treated bucks ranged from 11-74 ng/100 ml throughout the breeding season, which were in the range of levels during the non-breeding period.

In the second year, three of the bucks were boosted with the KLH-GnRH vaccine in July and August. The fourth buck died before the start of the second year of research. The anti-GnRH titers peaked in September. The testosterone of these bucks remained less than 30 ng/100 ml throughout the breeding season. In contrast to the first year, the antlers remained in velvet throughout the fall. The antlers never cleaved normally at the base of the pedicel, but instead broke off 1 to 4 inches above the pedicel between January 10 and February 13. This breakage was likely a result of freezing temperatures killing the unhardened antler, thereby resulting in reduced antler strength.

The sexual responses of the bucks were directly related to the anti-GnRH titers throughout the three years. When GnRH treated bucks were put in the same pen with control cycling females they demonstrated no sexual interest in the does that were in estrous. At the same time the GnRH treated bucks were casually eating next to the doe in estrus, the fertile buck in the next paddock was trying to break down the fence. Three out of the four bucks returned to fertility in 2 years; however, 1 buck remained infertile with regressed testicles (even though the antibody titer had dropped) until he died several years later.

Discussion

Both the PZP and GnRH vaccines have advantages and disadvantages. Both vaccines change the behavior of the deer in reproductive season. The PZP vaccine induced infertility in does is associated with increased sexual activity for an extended period, as evidenced by an average increase in the number of estrous cycles observed per doe. Although the multiple estrous cycles observed with PZP-treated does have been reported by others (Turner et al. 1992), the present study clearly demonstrates that if the PZP antibody titer drops to an unprotecting level late in the breeding season, conception may occur resulting in fawns born in August or September, which places them at considerable risk for winter survival. The increased duration of the rutting season may increase deer-car collisions because of the restlessness of the herd. In our study, the control herd majority had become pregnant in 44 days, but the PZP treated deer were still cycling after this period. There is little or no observable difference in the appearance of the PZP treated deer as compared to the control does.

The does treated with the GnRH vaccine have a reduced level of estrus activity. The GnRH treated bucks have little sexual interest in the control does, and the effects of reduced testosterone in the bucks either causes the antlers to drop off in the fall the year of treatment or remain in velvet in the succeeding years.

The GnRH vaccine has potential as a management technique that could control

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population size as well as reduce deer movements during rutting season, thereby reducing deer/car collisions. Additionally, the vaccine could reduce the aggressiveness of a "pet buck" in a park setting during the fall rutting season.

Although both PZP and GnRH demonstrate potential as contraceptives, improved delivery methods still need more research (Miller 1997 b; Miller et al. 1999a) In addition to the immunocontraceptive technology, research in other methods of interrupting reproduction to control population of various pest species need more research (Miller and Fagerstone 2000)

Conclusion

Both vaccines are effective in reducing the fertility of white-tailed deer. In PZP immunocontracepted deer, fertility was reduced as much as 89% during active immunization, and for the entire 7-year study, with 4 years of no immunization, was reduced by 72%. PZP immunization stimulates high antibody titers that can reduce fertility for one to four 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 contraceptive effects of the PZP vaccine are desirable aspects 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.

The GnRH vaccine is effective during active immunization in reducing fawning rates by 88%. After 2 years of no immunization the overall reduction in fawning for the 5 years was 74%. The GnRH conjugate produced high antibody titers resulting in infertility for up to two years without boosting. It appears that this vaccine is reversible; that is, when the antibody titer drops, fertility is restored. Furthermore, the deer could be boosted again causing the deer to again become infertile. There was little difference in the observed behavioral effects between the GnRH treated does and the control does. GnRH treated bucks, on the other hand, lost most sexual interest in the does coming into estrus. Early antler loss and the absence of antler hardening may be considered negative side effects of treating bucks.

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