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Observations on the Use of the GnRH Vaccine Gonacon™ in Male White-Tailed Deer (*Odocoileus virginianus*)

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Abstract: Observations made over an 11 year period during the development and evaluation of the GnRH vaccine GonaCon™ use in male white-tailed deer (*Odocoileus virginianus*) are summarized. Sexually mature bucks at the Penn State Deer Research Center were administered a single immunization of GonaCon™ in July. Some males were also given a second boost immunization in September. Compared to similar aged controls, testicular size for treated males was considerably reduced in the first and subsequent years of study, as were testosterone concentrations and sexual libido. During the first year, antler development was relatively normal leading to antler hardening, although shedding occurred early. However, in subsequent years, antlers were generally smaller, remained in velvet throughout the winter, and froze and then broke off. Most males given the single immunization returned to breeding condition in 2-3 years, although they tended to be smaller than similar-aged males. Males given the two immunizations tended to remain in a treated state longer. Treated males that died during the study had a higher incidence of pulmonary disease than non-treated males. We conclude that while GonaCon™ reduces the reproductive capacity of male white-tailed deer, the negative effects on health and antler development make it impractical for field application with bucks.

Key words: antler development, GnRH vaccine, immunocontraceptive, white-tailed bucks

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INTRODUCTION

Gonadotropin releasing hormone (GnRH) vaccines are intended to stimulate an animal's immune system to produce antibodies against GnRH, a small peptide protein produced by the hypothalamus of the brain. Because GnRH is responsible for stimulating anterior pituitary gland hormones which regulate gamete and hormone production by the gonads of both males and females, inactivation of GnRH by an antibody results in an "immunological" castration. Most published studies evaluating anti-fertility effects of a GnRH vaccine in mammals have been directed at livestock and pets (Adams and Adams 1992, Ladd et al. 1994, Meloen et al. 1994, Rabb et al. 1990, Robertson 1982, Thompson, 2000). These have largely been short-term studies to seek alternatives to castration or in the case of pigs, as a means to reduce boar taint (Dunshea et al. 2001). Recently however, we have shown that the GnRH vaccine is effective in impairing reproductive parameters of feral (Killian et

al. 2003) and domestic boars (Miller et al. 2003).

The effectiveness of a GnRH vaccine is dependent on having a sustainable antibody titer which is sufficient to inactivate endogenous GnRH. Small peptides such as GnRH do not provide adequate stimulus to the immune system to mount a response. Peptides are typically coupled to a larger protein which the immune system will recognize as foreign and serve as a stimulus for the immune response. One large protein commonly used in coupling to stimulate an immune response to a small peptide is Keyhole Limpet Hemocyanin (KLH). KLH is a highly glycosilated mollusk protein which is quite immunogenic in mammals. The nature of the coupling protein and the vehicle/adjuvant used to inject the vaccine are the primary determinants of the magnitude of the antibody titer produced and its duration. These are important points to consider during the development of a long-acting contraceptive vaccine.

The development of a contraceptive vaccine for wildlife involves an evolving process by which results of previous experiments shape the next studies in the series. Pen studies with white-tailed deer are expensive, given the initial cost of animals and maintenance expenses. With limited resources and budgets, this by necessity limits the sample size for a given study. Nevertheless, pen studies enable detailed sampling at multiple time points on the same animal which generally provides a fuller understanding of the animal's response to a treatment than is possible in field work.

Our work in this area was initiated in 1994 and subsequently led to the development and testing of the GnRH vaccine, GonaCon™. The primary focus of the effort has been to evaluate the vaccine in female deer. Does were observed for reproductive behavior, animal health and interactions among individuals. Hormonal status, antibody titers, and other blood parameters also were monitered and body measurements taken from all subjects. Early pregnancy was determined by ultrasound and later confirmed with fawning rates. An 86% reduction in fawning was achieved during active immunization and a 74% reduction over 5 years (Miller et al. 2000a, Killian and Miller 2001). Does given the GnRH vaccine had reduced progesterone concentrations, reduced estrous behavior and evidence of both ovulation failure and failure to maintain pregnancy following conception. Infertility lasted up to 2 years without a booster injection. During the past 11 years we also have evaluated the effects of the GnRH vaccine in bucks. Although these studies did not directly evaluate the effect of the vaccine on individual male fertility, indirect indicators of their reproductive status in rut such as plasma testosterone, testis size, body mass and antler development were monitored. We now report the results of these studies with bucks using this GnRH vaccine.

MATERIALS AND METHODS

The study was conducted at the Deer Research Center at the Pennsylvania State University over an eleven year period from 1994-2004. All animal procedures were approved by the Institutional Animal Care and Use Committee of Penn State. Bucks were chemically restrained during handling with 2.2-4.4mg/kg of xylazine administered IM in the rump. Anesthesia was reversed with Tolazine at 4mg/kg given IV or IM. The GnRH vaccine, consisting of the GnRH peptide conjugated to KLH was administered as a single shot, or a single shot follow by a second immunization. During the development stage of the vaccine, the GnRH-KLH component was administered in a 1 ml dose containing Freund's complete adjuvant and mineral oil

(Miller et al. 2000a). A second immunization was typically given 4-6 weeks later using Freund's incomplete adjuvant. Preliminary studies evaluated several doses of the vaccine and compared subcutaneous versus intra-dermal versus intra-muscular. From these studies we adopted a standard protocol dose that utilized 850-1000ug of GnRH-KLH, injected IM in the rump. The latter protocol was used in the studies reported.

Although not observed in the deer we studied, the formation of granulomatous lesions caused by FCA at the injection site is not uncommon in many species vaccinated with Freund's. These observations have caused the USDA to raise the classification of use of FCA in experimental animals from a Category C to Category D, among their definitions of painful procedures. Therefore we developed an alternative adjuvant called AdjuVac, which was used in the studies reported. When AdjuVac was used with GonaCon^{TM} in place of FCA, sustained antibody titers were observed comparable to FCA, without any evidence of a tissue reaction.

Blood samples were collected 5-6 times from July through February from the jugular vein. After clotting, the serum was harvested by centrifugation and stored frozen at -20C until assay. Serum was used to determine antibody titers to GnRH and concentration of testosterone using methods described elsewhere (Levy et al. 2004). During blood sampling, testis length and width were determined using calipers, and body condition and antler development was noted. If a buck was euthanized during the study, the testes and epididymides were removed and prepared for histological evaluation.

During the 11 year period of study we developed the general impression that bucks given the GnRH vaccine were less healthy than non-treated males in the Penn State deer herd. To explore this notion further, a retrospective analysis was conducted on the causes of death of deceased animals determined by veterinary pathologists at the Pennsylvania State Animal Diagnostic Lab located on the Penn State campus. This study was limited to comparing the causes of death between the groups rather than the incidence of death for the populations.

RESULTS AND DISCUSSION

Antibody titers for most treated bucks reached values of 1:128,000 or greater by 10-12 weeks post vaccination, which coincided with rut. Bucks with titers less than that in general showed less of an effect of the vaccine. Bucks receiving the boost vaccination sustained the 128,000 titer longer, especially into the second breeding season. Based on our experience with does and bucks given the GnRH vaccine in earlier studies (Miller et al. 2000a), the ability to obtain a high titer that is sustainable is key to the vaccine's ability to inhibit reproduction.

The most apparent effect of GnRH vaccine use in bucks was the alteration of antler development. During the first year antlers of treated males typically hardened and the males rubbed out of velvet. However, antlers of GnRH vaccine treated males were shed approximately 4-6 weeks earlier than non-treated controls. If antibody titers were adequate to suppress testosterone production by the testis during the second year following immunization, the antlers grew but failed to harden. Considering the normal antler growth cycle of deer (Figure 1) it is evident that as testicular testosterone increases in mid summer, there is a corresponding increase in antler growth. The rise in testosterone is also associated with antler hardening and shedding of velvet that occurs prior to rut. During first breeding season following GnRH vaccine treatment

in July, there is a time delay before an effect is seen. Antlers typically develop and harden, although they are shed early. This is a result of the lag time necessary for the immune system to develop antibody titers sufficient to complex with most endogenous GnRH and the subsequent "trickle down effect" that eventually removes the stimulus for testicular hormone secretion. During the first year, testosterone levels are apparently adequate to sustain development through hardening, but as the antibody titers rise there is a drop in testosterone and antlers are shed prematurely. If antibody titers are sustained into the second year following vaccination, the seasonal changes associated with breeding are prevented because GnRH secretion is compromised. Consequently, growth and hardening of antlers facilitated by testosterone do not occur, and the antlers tend to be smaller and remain in velvet throughout the season.

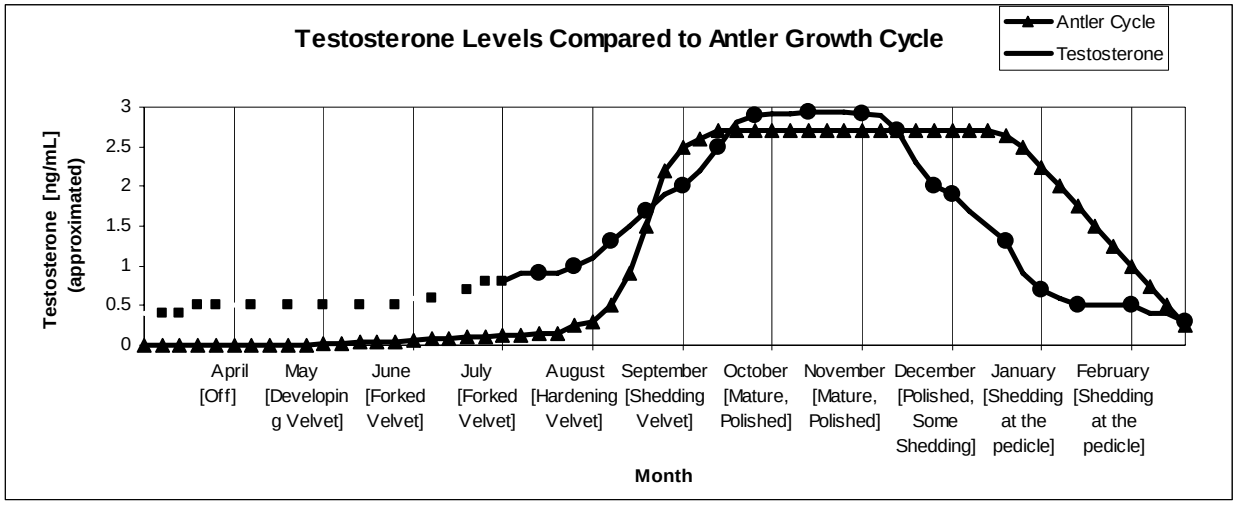

Figure 1. Serum testosterone during the normal antler growth cycle of white-tailed deer. From March through early summer, the primary source of testosterone is the adrenal glands. From mid summer through February, the primary source of testosterone is the testis.

Another notable effect of the GnRH vaccine on males was their failure to develop the muscular appearance typical of mature bucks in rut, analogous to a weight lifter on steroids (Figure 2). Removal of testosterone in GnRH-treated males leaves them with the body form of a female with antlers. These males show little or no interest in estrus does that are penned with them or in adjacent pens. Infrequently, GnRH-treated males may attempt to mount a doe in heat in the absence of control males, but the mount is not complete. Although we did not test fertility of the GnRH males in a breeding trial per se, these observations suggest that GnRH treated males would not reproduce successfully.

The effects seen in GnRH-treated males on antler development, behavior and body mass are the direct consequence of a reduction of serum testosterone, which modulates these characteristics. For treated bucks in our study, there was a significant drop in testosterone compared to non-treated controls (Figure. 3). This drop in testosterone corresponded to histological changes in the testis which resembled the testis of males during the non-breeding season. In the treated males, the Leydig cells which produce testosterone appear inactive, and the seminiferous tubules are regressed and do not contain mature sperm. These observations support the conclusion that these males were infertile.

Figure 2. A comparison of body mass of males in the breeding season a GnRH vaccine treated male (left) and a control male of similar age.

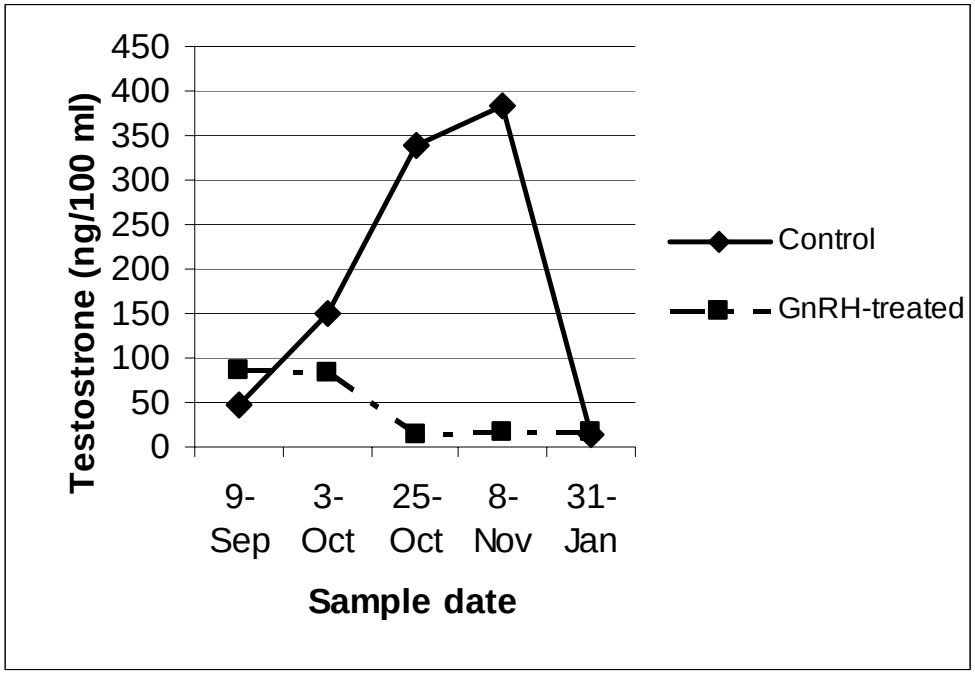

Figure 3. Average plasma testosterone concentrations (n=5 males per group) during the breeding season for control males and males vaccinated with the GnRH vaccine in July.

In practice, the use of any contraceptive approach with wildlife should be effective for multiple years, since opportunities to retreat the same animal will be unlikely. Contraceptive vaccines using porcine zona pellucida (PZP) as the immunogen have been reported to be effective in does for 1-2 years (Turner et al. 1992; Miller et al, 2000b). In ongoing studies in our deer herd testing the effect of the PZP vaccine SpayVac on does, we have observed 80% contraceptive efficacy after 4 years. Although we have not conducted extensive long-term studies with the GnRH

vaccine in bucks, there is evidence that the inhibitory effects of the vaccine are present for multiple years based on testicular size (Table 1) and plasma testosterone concentration (Table 2) during rut. Compared to controls, males receiving the single or two shot regimen on average had reduced testicular size and plasma testosterone for at least three breeding seasons. In the study with the single shot vaccine where data were collected in the fourth year post vaccination, there was evidence that testicular function was being restored.

Table 1. Average scrotal testis length in mm for (n) bucks treated with a single vaccination of GnRH vaccine (GnRH-1X) or a single vaccination followed by a boost (GnRH-2X) compared to untreated control males.

Treatment	Year 1	Year 2	Year 3	Year 4
Controls	74(3)	-	-	-
GnRH-1X	44(5)	38(5)	58(3)	65(2)
GnRH-2X	40(5)	46(3)	39(3)	-

Table 2. Average ng of serum testosterone for (n) bucks treated with a single vaccination of GnRH vaccine (GnRH-1X) or a single vaccination followed by a boost (GnRH-2X) compared to untreated control males.

Treatment	Year 1	Year 2	Year 3	Year 4
Control	603(3)	-	-	-
GnRH-1X	6(5)	28(5)	10(3)	175(2)
GnRH-2X	30(5)	23(3)	50(3)	

Data on testicular function must be interpreted somewhat cautiously however, when predicting male fertility. It is possible that while average testicular size and plasma testosterone were reduced, some level of sperm production may have occurred in some of the bucks. Unfortunately, we did not examine histology of the testes of all of the treated males, but based on studies we have conducted with feral swine (Killian et. al. 2003, Killian et al, unpublished), it is certainly possible that some sperm production could occur. However, the reduced testicular function of GnRH treated bucks may not be adequate to assume successful reproductive function. Moreover, the failure of antlers to harden, the lack of interest in estrus does and the female like body mass suggest that GnRH vaccine-treated males would not be reproductively active.

Our ability to closely monitor individual males during the course of these studies led us to believe that GnRH-treated males may be less healthy than non-treated males in the Penn State herd. To explore this possibility, necropsy reports were examined, if available, to assess the causes of death for GnRH-treated and untreated males that died during the 11 years of study. Causes of death included bone fractures and associated infections, enterocolitis and pulmonary diseases including pneumonia, pleuropneumonia, pulmonary abscession, and bronchopneumonia. For both the control and GnRH-treated bucks, pulmonary diseases were the single greatest cause of death (Table 3). This agrees with a published study indicating that in Pennsylvania captive white-tailed deer, pulmonary disease was the most common cause of death (Hattel et al. 2004). For our study, however, the relative incidence of death resulting from pulmonary disease in the treated males was significantly greater than that for control bucks. We can only speculate on the reason for this observation. Microbes associated with pneumonia appear to be endemic in captive deer herds and GnRH-treated bucks appear to be less

resistant to infections caused by these microbes.

Table 3. Causes of Mortality for Bucks at PSU Deer Research Center 1994-2004. Pulmonary diseases included pneumonia, plueropneumonia, pulmonary abscession, and bronchopneumonia. Other causes included enterocolitis and bone fractures and associated infections as the primary causes of mortality.

	Controls n=22	GnRH-treated n=13
Pulmonary Disease	**41%**	**62%****
Other	**59%**	**38%**

****Significant p <.05 by Chi square.**

CONCLUSIONS

We have demonstrated that both single and two injection protocols of GonaCon™ were effective in altering reproductive function of male white-tailed deer for multiple years. Plasma testosterone, testis size, breeding behavior and body mass were all reduced in GnRH vaccine treated males compared to non-treated males. In addition, antler development was reduced, antlers failed to harden and mortality due to pulmonary disease was greater in treated males. Although GonaCon™ was effective in impairing reproductive function, given the latter considerations we do not recommend its use for controlling fertility of bucks.

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