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GnRH immunocontraception of male and female white-tailed deer fawns

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Abstract. Immunocontraceptive vaccines based on gonadotropin-releasing hormone (GnRH) have been tested in adult white-tailed deer (*Odocoileus virginianus*), but their effects on fawns are unknown. The purpose of this study was to determine if early immunization against GnRH would induce a long-lasting immune response in fawns, and if it would delay or prevent sexual development. We gave primary and subsequent booster injections of a KLH-GnRH/Freund's vaccine to 6 male and 6 female fawns. This vaccine contained the same active ingredients as GonaCon™ vaccine, but it contained Freund's adjuvant instead of AdjuVac™ adjuvant. Two 450- μ g injections were given 1 month apart when fawns were 3 and 4 months of age. Although 1 female fawn produced a multiyear contraceptive response similar to that observed previously in vaccinated adults, the immune response lasted only 1 year in the other 11 fawns. Antibody titers in those 11 fawns declined sharply to near zero during the second and third years after vaccination. Our results indicate that treating 3- to 4-month-old white-tailed deer with the GnRH vaccine did not induce contraception or sterilization, and it did not delay or prevent sexual development. No adverse health effects were apparent in GnRH-treated fawns.

Key words: contraception, deer–vehicle collision, fawn, GnRH, gonadotropin-releasing hormone, human–wildlife conflict, *Odocoileus virginianus*, white-tailed deer

DEER–VEHICLE COLLISIONS (DVCs) and other deer–human conflicts are becoming more frequent in rural areas (Storm et al. 2007), suburbia (Hussain et al. 2007), and urban areas. DVCs cause damage to vehicles and injuries and fatalities to humans (Conover 1997, 2001). There are no panaceas for reducing the frequencies of DVCs, but reducing deer densities may be 1 option. There is great interest in developing an effective method to contracept deer as a way to lower local deer densities and DVCs.

Adult white-tailed deer (*Odocoileus virginianus*) of both sexes have been temporarily rendered infertile by vaccination with an immunocontraceptive vaccine consisting of a gonadotropin-releasing hormone (GnRH) conjugate combined with Freund's adjuvant (Miller et al. 2000). Vaccines used in early studies consisted of GnRH conjugated to a mollusk carrier protein (keyhole limpet

hemocyanin, or KLH). The large size and foreign (non-self) nature of KLH greatly increased the immunogenicity of GnRH, which (if unconjugated) is a weak antigen. Multiple (primary and subsequent booster) injections of vaccine were used to induce strong immune responses in adult white-tailed deer (Miller et al. 2000, Miller and Killian 2001). A different mollusk protein (blue protein) has been used in place of KLH since 2004 as a carrier molecule to which synthetic GnRH is attached. Both KLH and blue protein formulations of GonaCon™ are emulsified with a newly developed adjuvant called AdjuVac™. AdjuVac replaced Freund's adjuvant in the vaccine at the urging of the U.S. Food and Drug Administration due to concerns over Freund's safety.

An application was submitted to the U.S. Environmental Protection Agency during 2007 for registration of GonaCon as a contraceptive

agent for use in female white-tailed deer. We anticipate that GonaCon will be registered as a restricted-use product that will be labeled for use by state and federal wildlife or natural resource management personnel or persons working under their authority. GonaCon users also will be required to follow state authorization processes and regulations.

GonaCon vaccine has been tested as a single-injection contraceptive agent in domestic hogs (*Sus scrofa*; Miller et al. 2004b), feral hogs (Killian et al. 2004b, 2006c), bison (*Bison bison*; Miller et al. 2004a), California ground squirrels (*Spermophilus beecheyi*; Nash et al. 2004), feral cats (*Felis catus*; Levy et al. 2004), black-tailed deer (*Odocoileus hemionus*; Perry et al. 2006), and free-ranging horses (*Equus caballus*; Killian et al. 2004a, 2006b). GonaCon has also been tested for toxicity in white-tailed deer (Killian et al. 2006a). The results of these and other studies (Miller et al. 2004c) suggested that GonaCon vaccine is both safe and potentially useful as a wildlife contraceptive agent in a wide range of mammalian species.

The contraceptive effect of vaccination with GonaCon was greater and longer lasting in females than in males (Killian et al. 2004b, 2006c). Male deer treated with GonaCon exhibited abnormal antler development associated with reduced serum androgens. These findings prompted our recommendation that GonaCon vaccine use in deer be limited to treatment of females (Killian et al. 2005).

Reproductive inhibitors must be tailored to the physiology of the target animal, as no contraceptive agent is effective, practical, and appropriate for use in all species (Fagerstone et al. 2002) or age classes. Although GonaCon safely and effectively causes temporary infertility in adult deer, its effects on fawns are unknown. This paper describes efforts to determine whether injecting 3-month-old white-tailed deer fawns with GonaCon vaccine would induce a long-lasting immune response or affect sexual development.

Methods

White-tailed deer fawns used in this study were born and maintained at the Pennsylvania State University (PSU) Deer Research Center in University Park, Pennsylvania, where they were kept in outdoor paddocks that ranged

in size from 0.12 ha to 1.5 ha (0.3 acres to 3.8 acres). Vegetation in the paddocks consisted of a mixture of clover and orchard grasses and eastern deciduous forest. The study was approved by the Institutional Animal Care and Use Committee of PSU.

Six male and 6 female white-tailed deer fawns each were given 2 injections of 450- μ g KLH-GnRH-Freund's vaccine; a primary injection was given at 3 months, and a booster injection was given at 4 months of age. Blood samples were collected from all fawns in September before the primary injection, in October before the booster injection, and during the following November and February. Blood was centrifuged, and progesterone concentrations and anti-GnRH antibody titers were measured in the recovered serum. Similar collections and evaluations of blood samples occurred during the second and third years of the 3-year study, although no additional GnRH vaccinations were given. During the autumn breeding season each year, adult bucks of proven fertility were kept in the enclosure with the study deer to serve as sires. To determine pregnancy, ultrasonography was performed each February on every female fawn. We documented the development and shedding of antlers by males each year. We assessed the sexual development of the female fawns by measuring serum progesterone concentrations as an indicator of ovarian function. Evaluation of males consisted of measuring serum testosterone concentrations and observing and documenting antler development and scrotal testis length.

Progesterone assay

We used the Coat-A-Coat™ Progesterone In-vitro Diagnostic Test Kit (Diagnostic Products, Los Angeles, Calif.) in these studies, and we followed the manufacturer's recommended procedure.

Antibody response

We used an enzyme-linked immunosorbent assay (ELISA) to assess immune responses to the GnRH vaccine. A 96-well plate was prepared by adding 100 ng of BSA (bovine serum albumin)-GnRH antigen to each well and blocking with SeaBlock™ from Pierce Chemical (Rockford, Ill.). (Blocking prevented binding of antibody to plastic in the ELISA plate.) Because GnRH-KLH

was used in the vaccine, BSA-GnRH was put on the ELISA plate, and, thus, only antibodies to GnRH were detected.

We serially diluted deer blood serum from 1:1,000 to 1: 256,000 in phosphate-buffered saline containing SeaBlock. Antibodies in the deer serum to GnRH on the plate were directed with the following linkage: deer anti-GnRH binds to the GnRH on the plate, rabbit anti-deer immunoglobulin G (IgG) binds

dropped to nondetectable levels by February 1999 (e.g., Figures 1, 2). The immune response of the sixth female fawn (no. 976) also had waned by February 1999, but then the animal appeared to self-boost, as the antibody level increased dramatically and remained elevated throughout the final 2 years of the study (Figure 3).

Ultrasonography. As expected, the initial ultrasound examinations during February

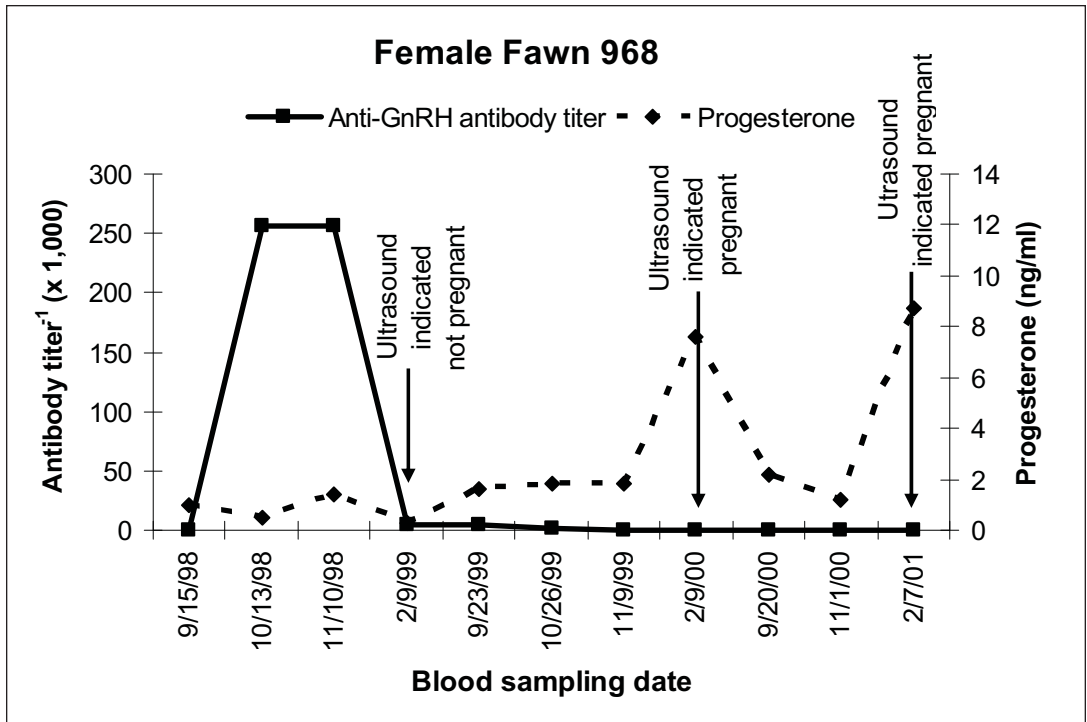


FIGURE 1. (Female no. 968) A very strong initial immune response declined sharply to non-detectable levels within several months, and the doe produced twin fawns in 2000 and 2001. Peaks in serum progesterone concentrations during February 2000 and 2001 indicated pregnancy.

to the deer IgG, goat anti-rabbit-peroxidase binds to the rabbit IgG. We used chromogen (tetramethylbenzidine) to develop the color and 2M H₂SO₄ to stop the reaction. We read the color intensity of the sample at 450 nm with a Dynatech MR 5000 ELISA plate reader (Dynatech Laboratories, Alexandria, Va.).

Results

Females

Antibody response. All female fawns responded to the primary and booster vaccinations by producing anti-GnRH antibody titers of 1:128,000 by October or November 1998. In 5 of these 6 fawns, however, titers subsequently

1999 all indicated non-pregnancy because the fawns had been only 4 to 6 months old during the breeding season in fall 1998 (Figures 1–3). During autumn 1999, 1 of the 6 female fawns died from severe pneumonia, and another died from chronic rumenitis. Both diseases are common causes of death in captive white-tailed deer in Pennsylvania (Hattel et al. 2004) and elsewhere in North America (Haigh et al. 2005). Ultrasonography performed in February 2000 and 2001 indicated pregnancy in 3 of the 4 surviving fawns. All 3 of these fawns had had low anti-GnRH antibody titers 1 year after vaccination, and, thus, they probably no longer exhibited anti-GnRH contraceptive activity (in-

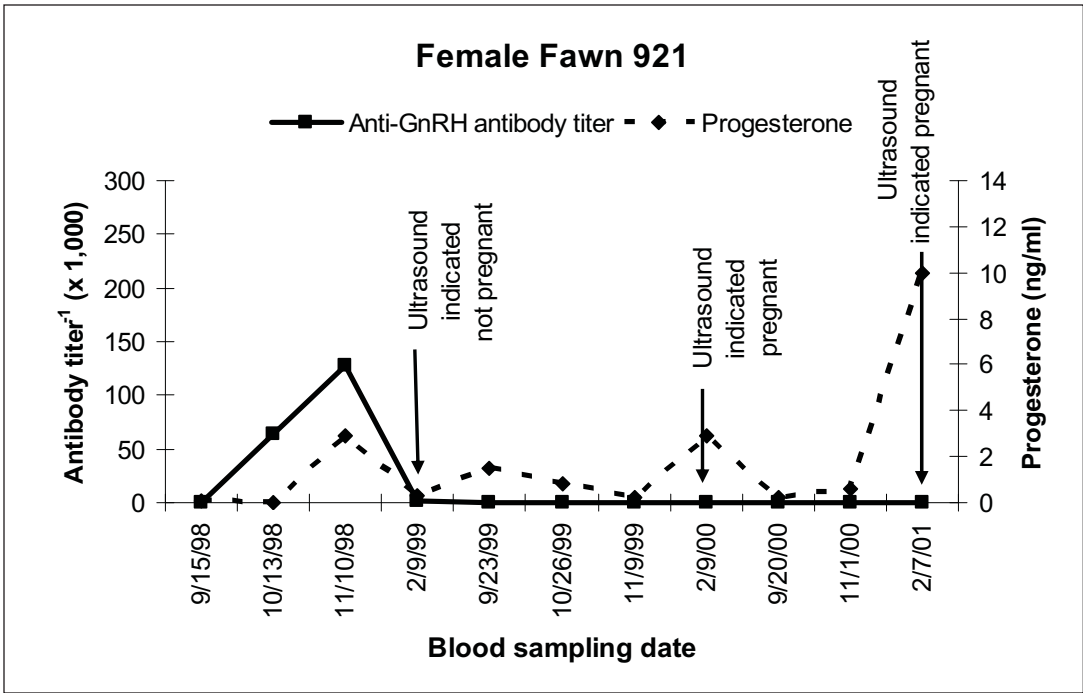


FIGURE 2. (Female no. 921) A strong initial immune response declined to non-detectable levels within several months. Twin fawns were produced in 2000, and triplets were produced in 2001. Minor elevations in serum progesterone concentrations during winters probably indicated estrous cycling and pregnancy.

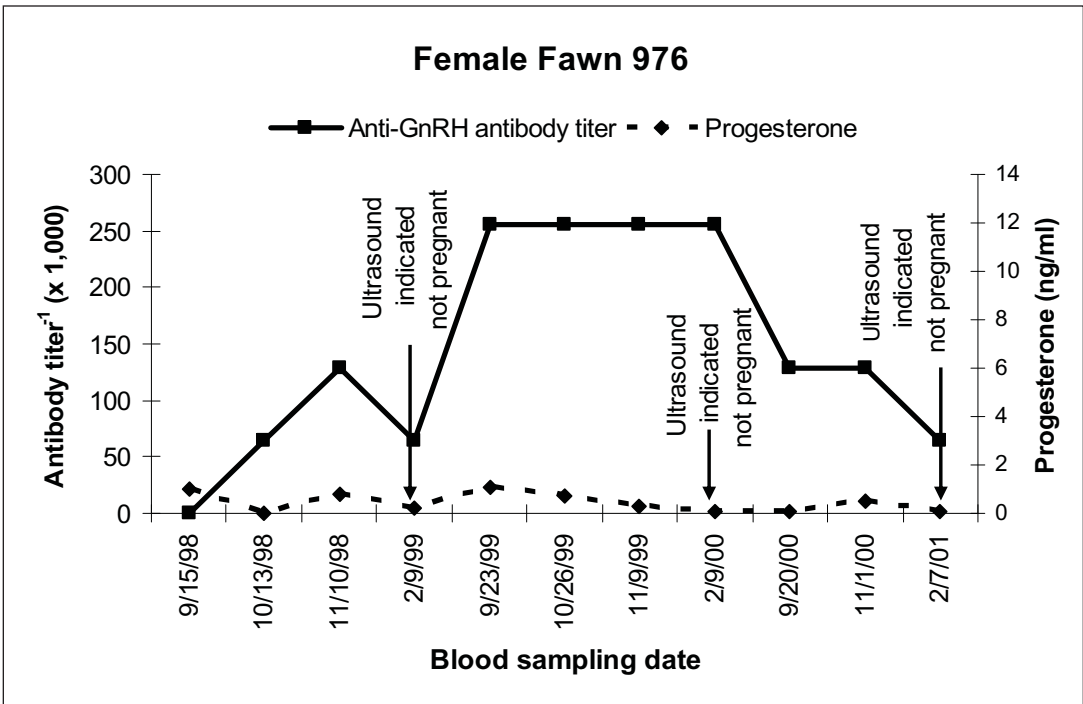


FIGURE 3. (Female no. 976) A strong initial immune response began to decline and then sharply increased to a very high level by September 1999 due to an apparent self-boost. The anti-GnRH antibody level remained elevated and provided a contraceptive effect throughout the remainder of the study.

fertility; Figures 1, 2). In contrast, fawn no. 976 apparently self-boosted between February and September 1999, and then maintained a high level of anti-GnRH antibody, was identified as non-pregnant by ultrasound examination in all 3 years, and remained infertile (Figure 3).

Progesterone. In blood samples collected during the fall breeding seasons in 1998–2000, we observed only slight elevations in serum progesterone concentrations, probably associated with the estrous cycle (Figures 1–3). Mean monthly progesterone concentrations in September, October, and November (all 3 years) ranged from 0.1 to 1.5 ng/ml.

Fawn Production. As noted above, fawn no. 976 was infertile throughout the study. During the second and third years of the study, the 3 remaining fawns bore twins and triplets, with a mean of 2.3 fawns per doe per year.

Males

Antibody response. Anti-GnRH antibody responses generally were strong but of short duration (≤ 1 year). By October or November of the first year (1998), 5 of the 6 males had produced antibody titers of at least 1:128,000. Male fawn 957 produced a titer of 1:32,000 during the first year, but this value declined to a nondetectable

level by February 1999 (Figure 4). In November 1999, one of the males with an initially high (1:128,000) titer died (cause unknown). Titers of the other 4 bucks that had initially produced high anti-GnRH titers dropped to the 1:4,000 to 1:16,000 range during the first year (Figure 5). During the third year, titers of 2 of these 4 males declined to nondetectable levels, while the remaining 2 males maintained titers of 1:2,000 to 1:4,000.

Serum testosterone concentrations. As expected, serum testosterone concentrations in all male fawns were undetectable throughout the first year, regardless of anti-GnRH antibody titers. Throughout the remainder of the study, all surviving males except one (fawn no. 957; Figure 4) maintained abnormally low testosterone concentrations (0.2 to 1.8 ng/ml during the autumn rut). In contrast, during the second and third years of the study, male fawn no. 957 exhibited testosterone concentrations that ranged from 5.2 ng/ml to 13.5 ng/ml.

Antler development. Antler development in fawn number 957 was normal throughout the study. In the other surviving males, antler development was reduced during the second year and was normal during the third year.

Scrotal testis length. Scrotal testis length in

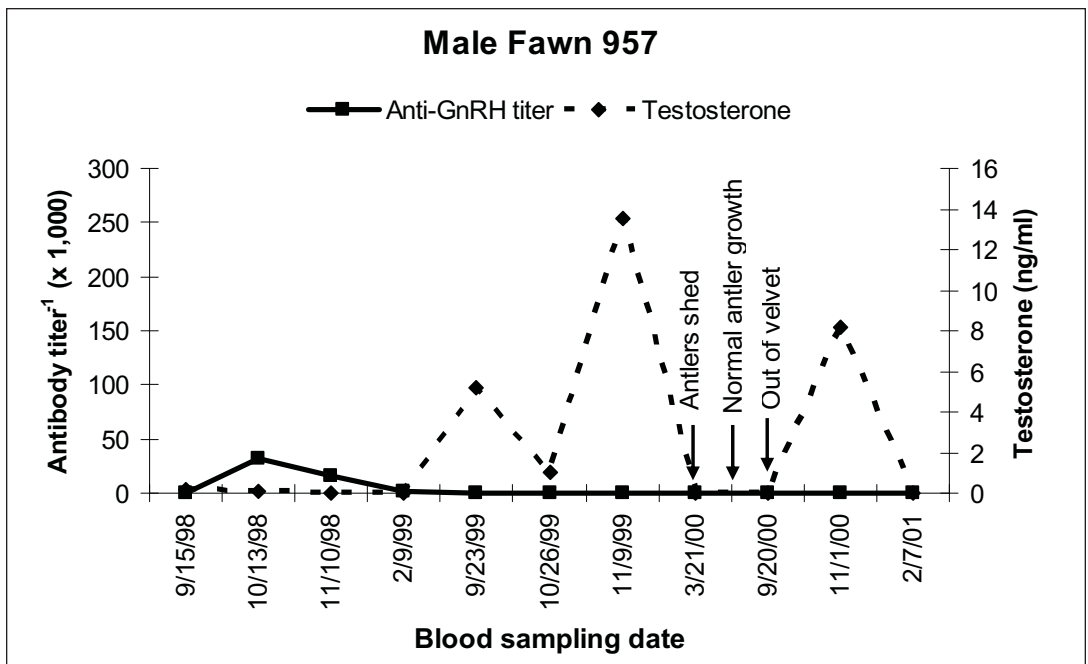


FIGURE 4. (Male no. 957) A moderately strong initial immune response declined to non-detectable levels within several months. Sexual development in this male was not delayed; it followed the pattern typical of unvaccinated males.

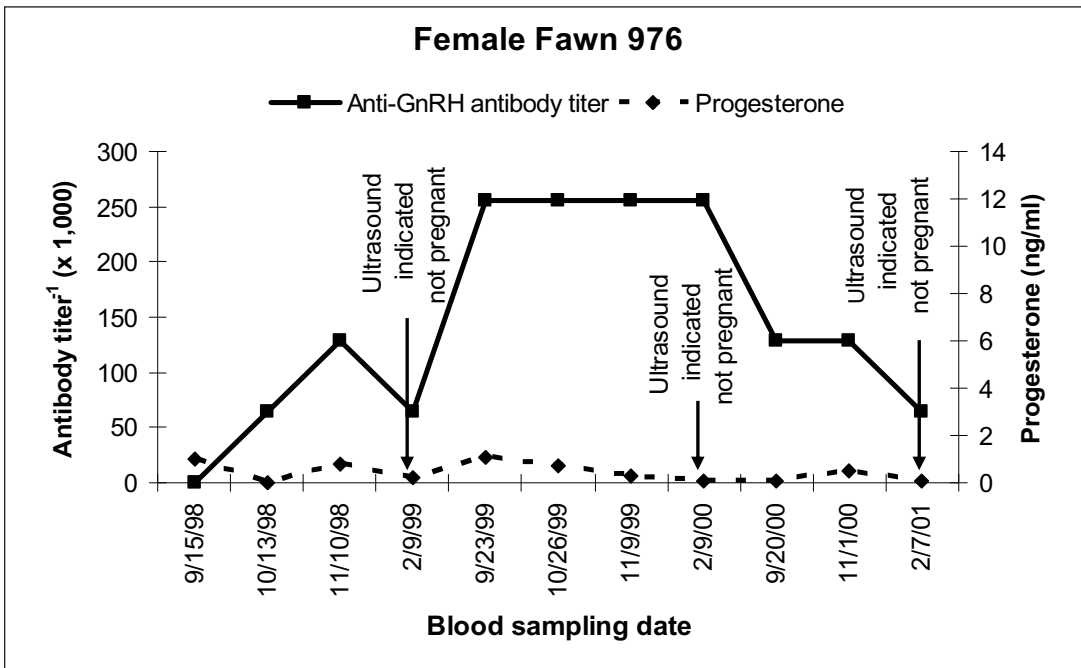


FIGURE 5. (Female no. 976) A very strong initial immune response dissipated within a year. Serum testosterone concentrations and antler growth were reduced through February 2001. By November 2001, the serum testosterone concentration had returned to the normal range.

male fawn no. 957, which had the weakest immune response to the vaccine, was 70 mm and 84 mm, respectively, in the second and third years. Scrotal testis lengths among the remaining males ranged from 1.0 to 5.6 mm in the second year and from 5.1 to 8.3 mm in the third year.

Discussion

The strong initial immune responses of female and male fawns to vaccination with the KLH-GnRH/Freund's formulation of GonaCon vaccine used in this study were similar to those observed previously in adult deer injected with that vaccine formulation or with a more recently-developed GonaCon vaccine formulation that incorporated blue protein and AdjuVac adjuvant. Anti-GnRH antibody titers in the fawns, however, declined relatively rapidly. During the 3-month period before February 1999, high initial antibody levels dropped sharply to nondetectable levels in 5 of the 6 female fawns. Titers in male fawns declined more slowly than in females, but at a much more rapid rate than was observed previously in adults (Miller et al. 2000). An exception to the general pattern exhibited by the fawns in this

study was the anti-GnRH antibody pattern observed in female fawn number 976 (Figure 3), which was typical of that of adult does vaccinated with GonaCon (Miller et al. 2000).

Anti-GnRH antibody levels $\geq 1:64,000$ generally are associated with infertility in adult white-tailed deer (Miller et al. 2000). In earlier studies, adult white-tailed does given primary and booster (1,000 μg per dose, given 2 months apart) injections of KLH-GnRH-AdjuVac vaccine typically remained infertile for up to 4 years, with an apparent self-boosting effect often occurring during the fall of the third year (L. A. Miller, unpublished data). The failure of female and male fawns in this study to sustain an initially strong antibody response may be related to a lack of "immune maturity" (Tizard 2000).

Elevated serum progesterone concentrations in 2 female fawns during November 1998 (6.0 ng/ml in fawn no. 905 [not illustrated]; 2.9 ng/ml in fawn no. 921 [Figure 2]) may have indicated that these deer were cycling. As these fawns were about 5 months old at the time of blood sampling, these elevated concentrations may have been associated with estrus at puberty. All other elevated progesterone concentrations

observed in this study were associated both with ultrasound examinations that indicated pregnancy and with subsequent fawning.

Although fawn no. 976 was infertile throughout the 3-year study, the other 3 surviving female fawns each produced an average of 2.3 fawns per year during the last 2 years of the study. Mean fawn production among adult does in the PSU deer herd was 1.7 fawns per doe per year (Miller et al. 2000), with single fawns typically resulting from first pregnancies. The relatively high rates of twin and triplet production in the present study may be due to a rebound phenomenon related to the decrease in anti-GnRH antibody levels. Similar rates of twin and triplet production have not been observed in GnRH-vaccinated adult does. In fact, GnRH-treated adult deer often produce single fawns during the first year after anti-GnRH antibody titers have declined, and then twins are produced during subsequent years (G. J. Killian, unpublished data).

Development of testes and antlers in cervids are directly related to testosterone levels (Verme and Ullrey 1984). Vaccination of adult male deer with GonaCon vaccine indirectly affects testicular and antler development by reducing testosterone concentrations (Killian et al. 2005). Mean scrotal testis length in adult deer in the PSU deer herd is 74 mm, in contrast to a mean length of 40 mm in GnRH-treated adult bucks (Killian et al. 2005). Although testicular development in male fawn no. 957 was normal, it was greatly suppressed in the other male fawns in which testes remained substantially smaller than normal during the third year of the study. The comparatively weak and short-lived immune response by male fawn no. 957 suppressed serum testosterone concentrations only briefly, and it appeared to have no long-term contraceptive or developmental effects. On the other hand, the other male fawns showed stronger and longer-lasting immune responses to vaccination with GonaCon. In those males, testosterone suppression persisted into the second and third years, with a resultant reduction in the developmental rates of testes and antlers.

GonaCon's multiyear contraceptive effect appears to be due to antigen retention caused by a water-in-oil emulsion that provides a slow release of the antigen from the injection depot

site. The retained antigen (GnRH) is present long enough that the antibody combines with GnRH to form GnRH-specific immune complexes (ICs). Retention of these ICs through their binding to follicular dendritic cells (FDCs) provides 2 functions: (1) it increases the duration of the antigen's stimulatory effects by protecting the antigen from destruction by macrophages, and (2) it dramatically increases the strength of the immune response through self-boosting. Bound FDCs and ICs settle into the draining lymph node where they are closely surrounded by B-cells to form a germinal center. Due to this proximity, 1 ng of GnRH released from the FDC in the draining lymph node can provide a self-boost to the immune system similar in strength to the boosting effect of μg quantities of the same antigen in the original vaccine injection. The boosting effect from the draining lymph node could, therefore, be 1,000 times the strength of the initial reaction. The release of GnRH from the ICs in the lymph node is increased as the serum anti-GnRH antibody drops, whereas it is suppressed in the presence of high antibody levels (Burton et al. 1991, 1994).

In the treated fawns in this study, the mechanism that supports FDCs and ICs with the formation of the germinal center may not have been sufficiently developed to produce a normal, adult-like, multiyear contraceptive response, with the exception of female fawn no. 976. Endogenous GnRH from the hypothalamus of these immature fawns may not have been released in quantities sufficient to produce immune complexes with the circulating, induced antibody. Without the binding of FDCs to ICs, a long-term immune response cannot develop (Burton et al. 1991, 1994).

Perhaps if the fawns in this study had been several months older when vaccinated, sexual development already would have been underway, and the associated release of endogenous GnRH from the hypothalamus already would have occurred. Had that been the case, the injection of GnRH vaccine might have caused the formation of GnRH and anti-GnRH ICs and prompted the self-boosting immunity previously observed in GonaCon-treated, adult white-tailed deer. Our results indicate that treating 3-month-old white-tailed deer fawns with GnRH vaccine, although safe for the fawns, is not an effective means of inducing temporary

contraception, permanent sterilization, or the delay or prevention of sexual development.

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JAMES P. GIONFRIDDO is a wildlife research biologist with the USDA's National Wildlife Research Center in Fort Collins, Colorado. He received B.S. and M.S. degrees in wildlife ecology from the University of Arizona and a Ph.D. degree in ecology and evolutionary biology from Iowa State University. His research interests include wildlife contraception and vertebrate behavioral ecology.

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DONALD C. WAGNER obtained his B.S. degree in wildlife and fisheries science at Pennsylvania State University in 1997. He has managed the Penn State Deer Research Center since 2000. He has been involved with immunocontraceptive studies at the facility since 1996.

GARY J. KILLIAN received his Ph.D. degree in reproductive physiology from Pennsylvania State University. Until 2006, he was a distinguished university professor at Penn State, where he collaborated with scientists at the National Wildlife Research Center since 1991 evaluating and testing immunocontraceptive vaccines in white-tailed deer, domestic and feral swine, and wild horses. Recently, he left Penn State to focus exclusively on collaborations with Lowell Miller and colleagues at the National Wildlife Research Center. His interests are fertility and disease management in overabundant wildlife and feral species. He is currently a reproductive physiologist with USDA/APHIS/Wildlife Services and resides in New Mexico.