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Physiological effects of gonadotropin-releasing hormone immunocontraception on white-tailed deer

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Abstract: Before immunocontraceptives can be considered safe to use on wildlife species, potential health risks should be assessed. Gonadotropin-releasing hormone (GnRH) immunocontraceptive has successfully reduced fertility of white-tailed deer (*Odocoileus virginianus*); however, associated deer physiology has rarely been examined. We conducted gross necropsy examinations, histology, and blood chemistry comparisons on euthanized deer previously vaccinated with immunogenic GnRH (n = 18 females and n = 4 males), or left as untreated controls (n = 7 females and n = 6 males). Granulomas were found at injection sites of most deer, even 3 years post-treatment. There were no significant differences in ovary ($F_{2,22}$ = 0.31, P = 0.73), or pituitary weights ($F_{2,22}$ = 0.30, P = 0.75) between treatment groups. Ovaries from control females had significantly more secondary follicles ($F_{2,21}$ = 20.56, $P \leq$ 0.001), but not Graafian follicles ($F_{2,22}$ = 2.22, P = 0.13). Immunized males had significantly lower mean testes weights, a number of morphologic abnormalities, and varying degrees of aspermatogenesis with fewer mature spermatozoa. We do not recommend treating male deer with anti-GnRH immunocontraceptive vaccines.

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

POPULATION DENSITIES of white-tailed deer (*Odocoileus virginianus*) in many suburban communities are reaching unprecedented levels (Curtis and Richmond 1992, DeNicola et al. 2008) and continue to escalate throughout North America. In suburban settings, where deer densities are often highest, safety concerns and ethical issues have rendered population regulation via recreational hunting impractical (DeNicola and Williams 2008). Higher deer densities have caused increases in deer–vehicle collisions (DVCs; Hussain et al. 2007, Mastro et al. 2008), and there is no easy way to reduce the frequency of these collisions (Mastro et al. 2008, Ng et al. 2008). In response, efforts to control growth of suburban deer herds with nonlethal methods, such as immunocontraception, have intensified (Warren et al. 1995, Curtis et al. 1997, Cowan et al. 2003, Miller et al. 2008, Rutberg and Naugle 2008). However, recent modeling efforts have shown that it will be very difficult to attain sufficient numbers of treated deer to have measurable reductions in open populations experiencing emigration and immigration (Merrill et al. 2006). Furthermore, community residents' attitudes towards fertility control as a deer management option are not fixed, and after stakeholders received additional information concerning the effectiveness or humaneness of contraception, their perceived acceptance of this technique often declined (Lauber and Knuth 2004). Consequently, it is imperative that communities and wildlife management agencies study all aspects of immunocontraception so that informed decisions can be made about applying these technologies for suburban wildlife management.

Fertility control as a tool in wildlife management has been investigated for many years (Harder and Peterle 1974, Kennelly and Converse 1997) with variable results (Bomford 1990, Turner et al. 1997, Warren et al. 1997).

However, success with effective fertility control of individual deer has been achieved by vaccinating them with the immunocontraceptive antigens gonadotropin-releasing hormone (GnRH; Killian 1998, Miller et al. 2000*a*, Curtis et al. 2002, Killian et al. 2006) or porcine zona pellucida (PZP; Kirkpatrick et al. 1997, McShea et al. 1997, Miller et al. 2000*b*, Rutberg et al. 2004). While many researchers have documented successful fertility suppression, few investigators have considered the effects of immunocontraceptive vaccine treatment on deer physiology or histopathology (McShea et al. 1997, Killian et al. 2006). Kirkpatrick and Rutberg (2001) even stated that, "the absence of significant health side effects" is an important characteristic of any contraceptive vaccine. Hence, in this study, we conducted detailed necropsies on anti-GnRH-immunized deer to document potential pathological impacts.

Materials and methods

In accordance with an amendment approved by the Cornell University Institutional Animal Care and Use Committee (Protocol Number 96-10-99), postmortem examinations were conducted on a sample of euthanized, free-ranging, white-tailed deer (n = 18 females and 4 males) that had been injected with an anti-GnRH vaccine or left as untreated controls (n = 7 females and 6 males) during 1996–2000. The deer were included in a fertility control study contained within a 263-ha fenced, natural area at Seneca Army Depot near Romulus, New York (Curtis et al. 2002).

The immunocontraceptive vaccines used for the study were prepared by L. A. Miller (USDA/APHIS/Wildlife Services' National Wild-life Research Center). A 10-amino acid GnRH complex was made immunogenic by coupling it to the carrier keyhole limpet hemocyanin (KLH; Miller et al. 2000*a*). A glycine residue was added at the C-terminus of GnRH as a spacer, and cysteine residue was added to provide a coupling agent to maleimide on KLH. Maleimide-activated KLH was purchased from Pierce Chemical Company (Rockford, Ill.) and the C-terminal Cys-GnRH was coupled to the activated KLH following the manufacturer's instructions. Both KLH-maleimide and the peptide were lyophilized and rehydrated in a 1:1 (weight:weight) ratio for coupling. Each 1-ml dose of anti-GnRH vaccine contained 0.5 mg of KLH-GnRH in 0.5 ml of phosphate-buffered saline (PBS) solution emulsified with 0.5 ml of Complete Freund's Adjuvant (CFA) or Incomplete Freund's Adjuvant (IFA).

Treated deer had received a primary vaccination (KLH-GnRH + CFA) in the hip region with remotely delivered, self-injecting, 1-ml darts (Pneu Dart Inc., Williamsport, Pa.). We administered the first booster shot (KLH-GnRH + IFA) 3 to 4 weeks later during September and October 1996 prior to the breeding season. A second booster dose (KLH-GnRH + IFA) was delivered by remote injection during September 1997. No other booster treatments were given until 7 female deer were revaccinated in 2000 (KLH-GnRH + IFA) to compare the effects of recent vaccination to deer previously treated 3 years earlier. No males were revaccinated during 2000.

With a permit from the New York State Department of Environmental Conservation (NYSDEC), we administered euthanasia of deer for necropsy in October 2000 using a single, lethal shot from a high-powered rifle fired either out of a blind or from a vehicle (Beaver et al. 2001). We collected blood samples via heart puncture immediately at the time of death, and we stored them in vials on ice for transport. Deer (n = 35) were quickly transported (usually within 1–2 hours after collection) to the Cornell University College of Veterinary Medicine (CUCVM), Necropsy Lab, and placed in a cooler at 4.4°C. Most necropsies were conducted the day the deer were collected.

Blood chemistry analysis was performed by the CUCVM, Animal Health Diagnostic Center, on all of the collected deer. Anti-GnRH titer (1/titer x 1000) levels were determined via an enzyme-linked immunosorbent assay (ELISA), while progesterone and testosterone were determined by radioimmunoassay (RIA; Miller et al. 2000*a*). These values were then compared to the deer's histology and to its ovarian, testes, and pituitary weights.

Gross examinations included an accurate assessment of deer's age by tooth wear (Severinghaus 1949), evaluation of bone marrow fat (Cheatum 1949), examination of injection sites, and documentation of any abnormalities.

Figure 1. Buck treated with GnRH immunocontraceptive vaccine, Romulus, New York, USA. Note malformed antlers covered in velvet during the rut.

Ovaries, testes, pituitary gland, internal iliac lymph node, popliteal lymph node, and thyroid glands were stripped of fat and connective tissue then weighed immediately (to the nearest mg). Morphologic analysis was performed on tissues fixed in formalin, embedded in paraffin, sectioned to 7 microns, and stained with hemotoxylin and eosin, Masson's Trichrome, or acid-fast stains. Each ovary, testis, and pituitary gland was halved before fixation in formalin, and each half was embedded in paraffin. Multiple sections (but not step sections) were made of each surface to be examined with the previously mentioned stains, and if a suspected lesion was observed grossly, a section was also made through the lesion.

The total number of secondary and Graafian follicles were enumerated from 2 cross-sectional slices from each ovary from each deer. Any normal-appearing follicle with a corona radiata (a single layer of columnar cells anchoring the oocyte) or total follicular size >0.5 cm was classified as a Graafian follicle. Follicles with greater than 10% of their granulose cells (small cells that form the wall of an ovarian follicle) exhibiting cell death (apoptosis) were classified as atretic ovarian follicles (degenerated prior to maturity). Apoptosis was characterized as granulose cells with dark, small consolidated (hyperchromatic) nuclei.

A single layer of cuboidal epithelium (surface) lined several large fluid filled follicles. Because none of these follicles exceeded 1 cm in diameter, they were classified as atretic Graafian follicles; however, they could have been small follicular cysts. Also, some larger Graafian follicles with diminished numbers of granulosa cells may have also been early follicular cysts. However, we found no advanced cyst to confirm this.

Results

Gross necropsy observations

Blood chemistry. There was no significant difference in any of 28 standard blood chemistry measures between control and anti-GnRH-treated female (Table 1) or male deer (Table 2). Although there was some variation for selected blood parameters, these values fell within normal ranges, and patterns were unremarkable.

Body condition. On the basis of body weight, external visibility of individual rib bones, and subcutaneous fat observed during gross necropsy, most animals were judged in good to excellent condition and had a bone marrow fat score of >85%. Two exceptions were: (1) female no. 66, an anti-GnRH revaccinate in year 2000, had poor body condition and was grossly underweight. She also had very poor teeth and had raised twins that summer. (2) Control female no. 323 had good body condition; however, her bone marrow fat score was <50%.

Injection sites. Injection sites for the year 2000 revaccinates were compared to those for deer vaccinated in 1997 to evaluate current and former dart site lesions induced by Freund's adjuvant. While abscesses were more evident and larger in recent revaccinates, granulomas could be found at the injection site of nearly all vaccinated deer.

Lung abscess. A 4x4-cm-circumscribed lung abscess, greenish in color, was noted during the necropsy of deer no. 25 (anti-GnRH-vaccinated female, year 2000). Tissue sections demonstrated this to be a giant cell granuloma containing acid fast bacilli similar to those found at the injection site.

Parasites. Three deer (nos. 13, 19, and 378) had liver cysts, most likely associated with the tapeworm *Echinococcus granulosus*, and 1 deer had an encysted parasite in skeletal muscle consistent with sarcosporidiosis (*Sarcocystis* spp.; no. 20). Each of these animals was in good to excellent body condition.

Table 1. Means, standard errors, and *t*-test statistics for blood parameters of female white-tailed deer in control and anti-GnRH-treated groups at Seneca Army Depot, Romulus, New York, 2000.

Blood parameter	Control group ($\bar{x}$ ± SE)	GnRH group ($\bar{x}$ ± SE)	*t*-test results	
Sodium (mEq/L)	141.00 ± 1.48	142.56 ± 1.04	$t = 0.81$	$P = 0.43$
Potassium (mEq/L)	11.77 ± 0.97	10.40 ± 0.84	$t = 0.93$	$P = 0.36$
Chloride (mEq/L)	103.50 ± 2.11	101.94 ± 0.92	$t = 0.80$	$P = 0.43$
Bicarbonate (mEq/L)	23.00 ± 2.21	24.06 ± 0.71	$t = 0.61$	$P = 0.55$
Cation-anion difference (mEq/L)	26.33 ± 4.18	27.53 ± 0.94	$t = 0.41$	$P = 0.69$
Urea nitrogen (mg/dL)	8.00 ± 1.51	13.82 ± 2.15	$t = 1.54$	$P = 0.14$
Creatinine (mg/dL)	1.27 ± 0.12	1.42 ± 0.06	$t = 1.24$	$P = 0.23$
Calcium (mg/dL)	9.75 ± 0.21	9.68 ± 0.17	$t = 0.21$	$P = 0.83$
Phosphate (mg/dL)	8.75 ± 1.11	8.16 ± 0.37	$t = 0.66$	$P = 0.52$
Magnesium (mg/dL)	2.55 ± 0.09	2.45 ± 0.07	$t = 0.75$	$P = 0.46$
Total protein (g/dL)	6.32 ± 0.36	6.75 ± 0.16	$t = 1.29$	$P = 0.21$
Albumin (g/dL)	3.13 ± 0.14	3.09 ±0.06	$t = 0.34$	$P = 0.74$
Globulin (g/dL)	3.18 ± 0.22	3.66 ± 0.14	$t = 1.73$	$P = 0.10$
Albumin:globulin ratio (A:G)	1.00 ± 0.04	0.87 ± 0.04	$t = 1.90$	$P = 0.07$
Glucose (mg/dL)	186.00 ± 82.08	155.06 ± 22.14	$t = 0.52$	$P = 0.61$
AST/PST[a] (U/cL)	11.47 ± 5.29	10.85 ± 4.18	$t = 0.08$	$P = 0.94$
SDH[b] (U/L)	67.18 ± 13.56	73.42 ± 20.31	$t = 0.18$	$P = 0.86$
Alkaline phosphatase (U/L)	197.87 ± 132.52	81.71 ± 11.99	$t = 1.49$	$P = 0.15$
GGT[c] (U/L)	45.33 ± 2.80	51.88 ± 6.42	$t = 0.59$	$P = 0.56$
Total bilirubin (mg/dL)	0.17 ± 0.02	0.19 ± 0.04	$t = 0.40$	$P = 0.69$
Direct bilirubin (mg/dL)	0.05 ± 0.03	0.05 ± 0.02	$t = 0.00$	$P = 0.99$
Indirect bilirubin (mg/dL)	0.12 ± 0.03	0.14 ± 0.04	$t = 0.44$	$P = 0.66$
Creatine kinase (U/cL)	130.10 ± 66.44	159.41 ± 74.64	$t = 0.22$	$P = 0.83$
Iron (µg/dL)	155.67 ± 18.30	168.71 ± 10.98	$t = 0.61$	$P = 0.55$
TIBC[d] (µg/dL)	318.67 ± 28.24	353.94 ± 13.71	$t = 1.24$	$P = 0.23$
% saturation	48.50 ± 3.18	48.24 ± 3.01	$t = 0.05$	$P = 0.96$
Lipemia	32.50 ± 8.48	13.18 ± 2.67	$t = 2.91$	$P = 0.01$
Hemolysis	103.83 ± 34.68	115.59 ± 32.12	$t = 0.20$	$P = 0.84$

Note: A Bonferroni-corrected ($n = 28$ tests) alpha level of 0.05 is 0.002.
[a]aspartate aminotransferase; [b]sorbitol dehydrogenase; [c]gamma-glutamyltransferase; [d]total iron-binding capacity

Multifocal lymphocytic infiltrates. Focal infiltrates and nongiant cell granulomas were seen in kidney, skeletal muscle, and liver. These lesions were consistent with immunological action against migrating parasites in tissues.

Female deer

Neither combined ovarian weights ($F_{2,22} = 0.31$, $P = 0.73$) nor pituitary weight ($F_{2,22} = 0.30$, $P = 0.75$) differed significantly between treatment groups and showed no correlation with antibody titer (Table 3). Mean anti-GnRH antibody titers were greater in the year 2000 vaccinates than for control females (Table 3; $t_1 = 2.31$, $P = 0.03$). The average titer for female deer treated in 1997, including 2 of 8 deer that had no detectable titer, was not significantly different from control females ($t_1 = 0.13$, $P = 0.90$).

The average total number of secondary follicles was less in both 1997 and year 2000 vaccinates than for control females ($F_{2,22} = 17.8$, $P \leq 0.001$). The mean number of Graafian follicles ($F_{2,22} = 2.22$, $P = 0.13$), and average total follicle count did not differ between treatment groups ($F_{2,22} = 1.60$, $P = 0.23$).

Male deer

When collected in October 2000, most of the male deer were in breeding condition with hardened antlers and swollen necks. Blood analysis confirmed appreciable levels of testosterone in the bucks sampled. Average testosterone levels of the control males (312 ng [nanograms]/100 ml) were considerably higher

Table 2. Means, standard errors, and *t*-test statistics for blood parameters of male white-tailed deer in control and anti-GnRH-treated groups, Seneca Army Depot, Romulus, New York, USA, 2000.

Blood parameter	Control group ($\bar{x}$ ± SE)	GnRH group ($\bar{x}$ ± SE)	*t*-test results
Sodium (mEq/L)	140.67 ± 1.98	143.50 ± 1.44	$t = 1.04$ $P = 0.33$
Potassium (mEq/L)	9.47 ± 0.84	10.22 ± 1.26	$t = 0.52$ $P = 0.61$
Chloride (mEq/L)	101.17 ± 1.17	102.00 ± 0.91	$t = 0.51$ $P = 0.62$
Bicarbonate (mEq/L)	25.00 ± 1.46	27.00 ± 0.41	$t = 1.08$ $P = 0.31$
Cation-anion difference (mEq/L)	23.83 ± 1.74	25.00 ±2.52	$t = 0.40$ $P = 0.70$
Urea nitrogen (mg/dL)	9.67 ± 2.59	6.25 ± 0.85	$t = 1.03$ $P = 0.33$
Creatinine (mg/dL)	1.57 ± 0.13	1.52 ± 0.20	$t = 0.18$ $P = 0.86$
Calcium (mg/dL)	9.98 ± 0.20	10.18 ± 0.44	$t = 0.44$ $P = 0.67$
Phosphate (mg/dL)	7.48 ± 0.52	9.10 ± 1.14	$t = 1.46$ $P = 0.18$
Magnesium (mg/dL)	2.30 ± 0.08	2.30 ± 0.16	$t = 0.00$ $P = 0.99$
Total protein (g/dL)	7.87 ± 0.29	7.82±0.57	$t = 0.07$ $P = 0.94$
Albumin (g/dL)	3.43 ± 0.10	3.18 ± 0.10	$t = 1.74$ $P = 0.12$
Globulin (g/dL)	4.43 ± 0.32	4.65 ± 0.65	$t = 0.33$ $P = 0.75$
Albumin:Globulin ratio (A:G)	0.80 ± 0.07	0.72 ± 0.10	$t = 0.64$ $P = 0.54$
Glucose (mg/dL)	184.83 ± 46.46	79.50 ±123.89	$t = 1.72$ $P = 0.12$
AST/PST[a] (U/cL)	5.17 ± 2.16	4.96 ± 2.35	$t = 0.06$ $P = 0.95$
SDH[b] (U/L)	39.30± 7.48	40.58 ± 12.41	$t = 0.09$ $P = 0.93$
Alkaline phosphatase (U/L)	61.17 ± 16.86	67.25 ± 8.78	$t = 0.27$ $P = 0.79$
GGT[c] (U/L)	57.00 ± 6.35	51.50 ± 4.56	$t = 0.63$ $P = 0.55$
Total bilirubin (mg/dL)	0.25 ± 0.05	0.20 ± 0.04	$t = 0.65$ $P = 0.54$
Direct bilirubin (mg/dL)	0.10 ± 0.03	0.10 ± 0.00	$t = 0.00$ $P = 0.99$
Indirect bilirubin (mg/dL)	0.15 ± 0.03	0.10 ± 0.04	$t = 0.93$ $P = 0.38$
Creatine kinase (U/cL)	53.24 ± 25.89	49.26 ± 32.48	$t = 0.10$ $P = 0.93$
Iron (μg/dL)	115.67 ± 36.96	159.75 ± 29.50	$t = 0.85$ $P = 0.42$
TIBC[d] (μg/dL)	336.00 ± 16.59	417.75 ± 56.64	$t = 1.66$ $P = 0.14$
% saturation	39.50 ± 8.42	39.00 ± 7.45	$t = 0.04$ $P = 0.97$
Lipemia	14.50 ± 4.28	16.00 ± 8.09	$t = 0.18$ $P = 0.86$
Hemolysis	41.50 ± 16.71	46.75 ± 19.17	$t = 0.20$ $P = 0.84$

Note: A Bonferroni-corrected ($n = 28$ tests) alpha level of 0.05 is 0.002.
[a]aspartate aminotransferase; [b]sorbitol dehydrogenase; [c]gamma-glutamyltransferase; [d]total iron binding capacity

than the vaccinated males (100 ng/100 ml) even 3 years post-treatment.

The mean combined testicular weight for anti-GnRH-treated male deer ($\bar{x}$ = 70.3) was significantly lower ($t_1 = 3.56$, $P = 0.007$) than for the control group ($\bar{x}$ = 130.5). Similarly, the average testes:pituitary index for anti-GnRH-treated ($\bar{x}$ = 69.2) male deer was less ($t_1 = 2.52$, $P = 0.04$) than for the control group ($\bar{x}$ = 130.0). However, there was no difference in pituitary weights ($t_1 = 0.30$, $P = 0.77$) for anti-GnRH-treated males ($\bar{x}$ = 1.0) and control ($\bar{x}$ = 1.0) bucks.

Morphologic alterations were observed in the testes of anti-GnRH-treated males (Table 4). The pathologies included diffuse lymphocytic infiltrate with associated seminiferous tubule degeneration, segmental tubular aspermatogenesis (where large contiguous areas of the testes were seen with only sertoli-

Table 3. Mean left and right ovary weights (milligrams), pituitary gland weights, anti-GnRH antibody titers, and follicle counts for control does vaccinated during 1977 or 2000 with anti-GnRH at Seneca Army Depot, Romulus, New York, 2000.

	Control does	Anti-GnRH does				
		1997	2000	*F*	*df*	*P*
	n = 7	*n* = 11	*n* = 7			
Left ovary	502.4	421.2	505.4	0.71	2,22	0.50
Right ovary	497.1	446.4	475.2	0.15	2,21	0.86
Pituitary gland	788.1	729.0	733.9	0.30	2,22	0.75
Titer (1/titer x 1,000)	0.0	1,700	31,100	3.37	2,18	0.06
Secondary follicles						
Normal	8.1	3.4	5.9	3.38	2,22	0.05
Atretic	10.3	3.1	3.3	11.2	2,22	0.0004
% Atretic	55.1	55.3	38.5			
Graafian follicles						
Normal	0.6	3.2	5.7	2.65	2,22	0.09
Atretic	1.6	4.4	2.1	2.34	2,22	0.12
% Atretic	84.2	42.3	32.2			

cell-lined tubules); subepithelial concretions in the collecting tubules, decreased cross-sectional diameter of seminiferous tubules, decreased cytoplasmic volume of Leydig cells, interstitial fibrosis, and total aspermatogenesis. Histology of testes from control males revealed none of these abnormalities. Among the treated males, one had only segmental aspermatogenesis with none of the associated pathologies. The remaining 3 males all had ≥2 of the abnormalities noted above.

Male no. 31, had malformed (atypical) antlers still covered in velvet (Figure 1), exhibited total aspermatogenesis with small fibrotic testes, and vacant epididymi. This same male had no detectable anti-GnRH antibody titer, but also lacked any measurable blood testosterone level. The 3 other anti-GnRH vaccinated males did have some epididymal spermatozoa.

Discussion

Observations for both male and female deer

Lesions. A variety of infectious, parasitic, and noninflammatory lesions were observed in our study animals. Most noteworthy was the formation of granulomas at the injection site with all the characteristics of tuberculosis. These granulomas were characterized by the presence of a necrotic core surrounded by mixed inflammatory cells and fibrous connective tissue. All granulomas had Langerhans giant cells typical of a tuberculosis condition. Likewise, giant cell inflammation was seen in the regional (popliteal) and deep (intral iliac) lymph nodes draining these sites. Acid fast bacilli were documented in these giant cells.

These injection site lesions and reactions, including the production of multi-nucleated giant cells associated with the presence of *Mycobacteria* in the adjuvant in other species vaccinated with CFA have been previously described (Stills and Bailey 1991, Kleinman et al. 1993, Munson et al. 2005, Stills 2005). In other studies, remote lesions comparable in composition to the Freund's injection sites had also been documented in lungs and other organs (Rigdon and Schadewald 1972, Broderson 1989, Kleinman et al. 1993, Malaga et al. 2004).

When evaluating the safety of immunocontraceptive vaccines, potential complications associated with treatment should be considered. Killian et al. (2006) reported that pulmonary disease was the most common cause of death in their captive deer herd and that males treated with anti-GnRH vaccine had significantly higher mortality than did control deer. Microbes associated with pneumonia were endemic in their captive herd and anti-GnRH-treated bucks appeared less resistant to infection.

Table 4. Presence (+) or absence (-) of different features in the testes of control (n = 6) and anti-GnRH-treated bucks at Seneca Army Depot, Romulus, New York, 2000.

	Deer identification number	Estimated age at start of trial	Diffuse lymphocytic infiltrate genesis (sertoli cells only)	Segmental tubular aspermatogenesis	Subepithelial concretions	Total aspermatogenesis seminiferous tubules	Decreased cross-sectional diameter	Decreased cytoplasm in Leydig cells	Interstitial fibrosis
Controls		3 ½	-	-	-	-	-	-	-
GnRH	22	2 ½	-	+	-	-	-	-	-
	31	3 ½	+	+	+	+	+	+	+
	37	3 ½	-	-	+	-	+	-	-
	4	3 ½	+	-	+	-	+	-	+

Likewise, Miller et al. (2000*b*) reported that 3 female deer died of complications resulting from pneumonia during a study of porcine zona pellucida (PZP) immunocontraceptive with Freund's adjuvant. It was unclear if there was an association between the pneumonia cases and treatment with Freund's adjuvant. Bearing in mind the warning by Billiau and Matthys (2001) that too few studies consider the biological effects of using CFA when evaluating experimental vaccines, we feel that future researchers should examine the possibility that injections with CFA could predispose deer to pneumonia.

Hypothalamic-pituitary response. Normally GnRH released from the hypothalamus circulates into the anterior pituitary gland where it activates production of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by gonadotrophs. Antibodies to GnRH interacted with neurons secreting GnRH from the hypothalamus at terminals within the median eminence. These GnRH release points are accessible because they are not within the blood-brain barrier that otherwise protects the hypothalamus from circulating antibodies. One study demonstrated degeneration of the neuron cell bodies (perikarya) and a sharp decrease of the GnRH immunochemical reactivity in the terminals within the median eminence of hyperimmunized hogs (*sus scrofa*; Molenaar et al. 1993). With hymotoxylin and eosin stain on fixed pituitary gland tissue, gonadotrophs appear as large cells with secretory granules of moderate and variable size. Cells with little or no cytoplasmic staining (chromophobes) are likely degranulated chromophilic cells. Thus, we classified large granule-bearing cells as gonadotrophs in this study, and found large variation in their numbers among females in each treatment group. A more precise staining method would involve immunoperoxidase staining with anti-LH/FSH. This would allow simultaneous quantification of cell numbers and the relative intensity of intracytoplasmic LH/FSH stores.

Female deer

Titers in 5 of the 7 deer revaccinated in 2000, and 6 of the 8 deer last vaccinated in 1997 were well below the level of 64,000 to 128,000 identified in deer with successful contraception, or the 16,000 to 32,000 level for contragestation (Miller et al. 2000*a*). Because the revaccinates in 2000 were retreated <4 weeks prior to collection, it is possible that the immune response was incomplete and that a peak anti-GnRH titer would be greater. Lower anti-GnRH titer in females vaccinated in 1997 than those vaccinated in the 2000 treatment group validates the assumption that, if not boosted, the anti-GnRH vaccine treatment is reversible for most deer. There were no identifiable health concerns associated with anti-GnRH treatment for female deer.

Male deer

Little has been reported about the effects of anti-GnRH vaccination on the reproductive physiology of male deer. Miller et al. (2000*a*) injected 4 male deer with the anti-GnRH vaccine, and testes size observed for treated bucks was <50% testes size in control bucks. Killian et al. (2006) also observed similar physical differences in testicular size and qualified those observations reporting histological changes in the testes "which resembled the testes of males during the non-breeding season." They further noted that the Leydig cells, which produce testosterone, appeared inactive in anti-GnRH treated males. Similar testicular pathologies have also been documented in research conducted on hogs (Molenaar et al. 1993, Meloen et al. 1994).

It is clear from testes weights and histology that sperm production was reduced, even 3 years after the last anti-GnRH vaccination. The significantly lower average weight of testes in this study correlated with a number of persistent morphologic abnormalities and decreased (qualitative assessment) spermatozoa in epididymi. Observing these effects 3 years after vaccination suggested that anti-GnRH vaccination early in the adult lives of deer may be sustained over several years. Similarly, in a study on 3- to 4-year-old sheep vaccinated with anti-GnRH antigen as neonates, Clarke et al. (1998) reported that in these study animals males had small gonads and females had no large follicles or corpora lutea, and titers had dropped to undetectable levels.

Immunized bucks had reduced cross-sectional diameter of seminiferious tubules and varying degrees of aspermatogenesis, including

buck no. 31 with total aspermatogenesis that was vaccinated at 3.5 years of age. This latter animal had no detectable anti-GnRH titer, suggesting a possible permanent alteration in the hypothalamic-pituitary-testes axis. The other 3 anti-GnRH treated bucks had normal outward physical traits and detectible mature spermatozoa, although the latter were fewer than normal. While it was not possible to assess their actual fertility from this study, this evidence suggests that anti-GnRH contraception is reversible; however, some portion of treated males may become permanently sterilized.

Variations in the residual immune response and the extent of physiological, morphological, or pathological differences among the treated deer are thought to be due to genetic differences among individual animals (Miller et al. 2000*a*). In fact, substantial research has been conducted using anti-GnRH vaccines to chemically castrate hogs as an alternative to surgical castration typically used to control boar taint, with considerable variations in efficacy documented (Molenaar et al. 1993, 1994; Oonk et al. 1995, 1998).

Management implications

Many factors affect the success and potential pathology associated with an immunocontraceptive vaccine, including formulation of the immunogen, concentration of the immunogen, adjuvant used, and species treated. Therefore, before judgments can be made concerning the effectiveness and health risks associated with a given vaccine for a particular species, adequate evaluation must be conducted.

Curtis et al. (2002) demonstrated that vaccinating free-ranging female white-tailed deer with anti-GnRH immunocontraceptive vaccines can be effective at reducing fawn production. We further evaluated anti-GnRH vaccinated deer and identified no detrimental, morphologic effects that could jeopardize the normal health of vaccinated females. Vaccinating female deer with anti-GnRH vaccines eliminates the potential problems associated with repeated estrous cycl-ing commonly observed in deer treated with PZP immunocontraceptive vaccines (McShea et al. 1997, Miller et al. 2000*b*, Kirkpatrick and Rutberg 2001). Baker et al. (2004) studying leuprolide, a GnRH agonist, which would cause similar effects as the anti-GnRH immunocontraceptive vaccine used in this study, also reported no significant adverse health impacts to female deer resulting from the reduced systemic GnRH. Furthermore, if GnRH could be administered to newborn female fawns, as Clarke et al. (1998) were able to do with neonatal lambs, considerable long-term efficacy could be achieved.

Morphologically, anti-GnRH-treated males essentially became neutered and exhibited behavioral and physiological traits consistent with castrated males (Pooler 2001, Killian et al. 2006). Antlers on treated males did not harden or shed velvet and became atrophied (Killian 1998, Miller et al. 2000*a*, Curtis et al. 2002, Killian et al. 2006). Such antlers are spindly, atypical, and possibly pose a health risk to deer from frost bite when they freeze and break off during winter. Treated male deer also could develop adverse health effects including increased risk for pneumonia. Killian et al. (2006) recommended that anti-GnRH vaccines should not be used on male deer due to these concerns. Our results were consistent with these previous studies, and we also do not recommend treating male deer with anti-GnRH vaccines.

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