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Abstract

White-tailed deer ($n = 14$ treated, $n = 7$ control) were examined postmortem to identify any possible pathophysiology resulting from PZP immunocontraception vaccination. Deer were treated twice in 1997; given a booster in 1998, with six being revaccinated in September 2000. Granulomas were found at injection sites of most deer, even 2 years post-treatment. Eosinophilic oophoritis occurred in 6 of 8 (75%) deer vaccinated in 1998, and 3 of 6 (50%) revaccinated in 2000. The 2000 revaccinates without oophoritis, had significantly fewer normal secondary follicles than control females ($P = 0.03$), and deer in the1998 treatment group ($P = 0.04$). PZP immunocontraceptive vaccine elicited ovarian pathologies in deer similar to those observed in other species.

Keywords: Immunocontraception; Porcine zona pellucida; White-tailed deer

1. Introduction

Porcine zona pellucida (PZP) antigen has been the most commonly used immunocontraceptive agent for fertility control in female mammals [1,2] and has been administered to 112 species of wildlife, including many ungulate species [3]. Previous investigators have reported the success and reversibility of PZP vaccination with minimal effect on the health of white-tailed deer (*Odocoileus virginianus*) [4–6]. Similarly, Curtis et al. [7] were successful in reducing fawn production of white-tailed deer after treatment with PZP immunocontraceptive vaccine.

Kirkpatrick and Rutberg [8] stated that, "the absence of significant health side effects" is an important characteristic of any contraceptive vaccine. Concerns about ovarian malfunction in early PZP trials on other species [9–14] prompted McShea et al. [4] to conduct histological examinations of ovaries from PZP-vaccinated white-tailed deer, and Miller et al. [15] to monitor progesterone levels throughout their study. No significant ovarian abnormalities were reported for treated deer that recovered to normal fertility. Since that time, a study was conducted on domestic sheep (*Ovis aries*), a cervid species that demonstrated marked changes in estrous cycling and hormone levels associated with severely pathologic ovaries [16]. Species- and breed-specific responses to PZP vaccinations vary both in successful fertility control, and in pathologic symptoms [10,12,13,16–20]. Consequently, we present information that is important for evaluation of potential animal health issues associated with the use of PZP contraceptive vaccines on white-tailed deer.

We conducted detailed necropsy of white-tailed deer to examine potential pathological impacts resulting from a successful immunocontraceptive vaccination regimen [7]. The deer we examined were still under the influence of PZP treatment, had detectable levels of PZP antibodies, and many had not demonstrated full recovery to normal fertility. Pathological effects of PZP treatment 2 years post-vaccination were

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compared to a sub-sample of deer revaccinated within one month of necropsy, and untreated deer. Our objectives were to document ovarian abnormalities or other potential health concerns resulting from the Curtis et al. [7] PZP vaccination protocol compared to control deer.

2. Materials and methods

Postmortem examinations were performed promptly on a sample of white-tailed deer in accordance with a protocol (No. 96-10-99) amendment approved by the Cornell University Institutional Animal Care and Use Committee. The deer had been injected with a porcine zona pellucida vaccine (PZP, $n = 14$), or left untreated (controls; $n = 7$) during the period 1997–2000 as a part of a fertility control study conducted on free-ranging deer contained in a 263-ha fenced, natural area at Seneca Army Depot near Romulus, New York [7].

2.1. Vaccine formulation

Female deer were immunized with native porcine zona pellucida prepared by and purchased from B. Dunbar (Baylor College of Medicine, Houston, TX) [20–22]. A 1-cc prime dose of PZP vaccine consisted of 0.5 cc saline containing 100 μg PZP mixed with 0.5 cc Complete Freund's Adjuvant (CFA). Similarly, the 1-cc booster dose contained 100 μg PZP in 0.5 cc saline, mixed with Incomplete Freund's Adjuvant (IFA) at a 1:1 ratio [5,15].

2.2. Vaccination protocol

The primary vaccination (PZP + CFA) was injected by hand into the hip region of female deer during winter 1997 as they were captured for recruitment into the study. At the time of capture, deer were marked with numbered ear tags and neck collars. Age was determined by the extent of tooth wear [23], and deer were released into the fenced study area. The first booster shot (PZP + IFA) was administered remotely via dart rifle with self-injecting 1-cc darts (Pneu Dart Inc., Williamsport, PA) prior to breeding season, 5–7 months later in September 1997. A second booster treatment (PZP + IFA) was delivered via dart rifle in September and October 1998. Booster treatments were suspended until September 2000 when six female deer were revaccinated (PZP + IFA) to provide the comparison of recently-vaccinated deer to those previously treated 2 years earlier.

2.3. Deer collection and gross necropsies

In October 2000, within permit authority from the New York State Department of Environmental Conservation (NYSDEC), deer were humanely killed by a shot to the head or neck from a high-powered rifle fired from a blind or a vehicle [24]. Blood samples were immediately collected via heart puncture at the time of death and were stored in vials on ice for transport. Deer ($n = 21$) were quickly transported within 2 h after collection to the Cornell University College of Veterinary Medicine and placed in a cooler at 4.4 °C. Most necropsies were performed the day the deer were collected. Gross examinations included evaluation of body condition and bone marrow fat [25], examination of injection sites, and documentation of any visible abnormalities. Ovaries, internal iliac lymph node, popliteal lymph node, and thyroid glands were stripped of fat and connective tissue then weighed immediately (nearest mg). Histological analysis was performed on tissues fixed in formalin, embedded in paraffin, cut to 7 μ thickness, and stained with hemotoxylin and eosin, Masson's Trichrome, or acid fast stains.

2.4. Serology

Blood chemistry analysis was performed by the Cornell University College of Veterinary Medicine, Animal Health Diagnostic Center on all of the collected deer. Anti-PZP titers (titer$^{-1} \times 1000$) titers were determined via an enzyme-linked immunosorbent assay (ELISA) as described by Miller et al. [15] and were compared to ovarian weights and histology.

2.5. Follicle classification

Each ovary 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 several 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 two cross-sectional slices from each ovary from each deer. Follicles with greater than 10% apoptotic granulosa cells were classified as atretic. Apoptosis was characterized as granulosa cells with dark, small consolidated (hyperchromatic) nuclei with an absence of heterochromatin. Likewise, any normal appearing follicle having the presence of a corona radiata or total follicular size greater than 0.5 cm was classified as a Graafian follicle.

A single layer of cuboidal epithelia 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. 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.

3. Results

3.1. Average age when euthanized

At the time of necropsy, the mean age for control deer was 5.5 years. The 1998 treatment group also had a mean

age of 5.5 years, and the deer revaccinated in 2000 averaged 6.5 years old. The age differences were not significant ($F_{2,18} = 1.90$, $P = 0.18$).

3.2. Body condition and fat reserves

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 85% or more bone marrow fat. However, there were three exceptions. Deer number 188 (1998 PZP vaccinate) had very good teeth and plenty of rumen ingesta, but she exhibited classic signs of malnutrition normally seen in deer struggling through an extremely harsh winter (poor body condition, <50% fat score, and a gelatinous bone marrow). The other deer with depleted bone marrow fat, number 146 (1998 PZP treatment), and number 323 (control female), had good to excellent body condition.

3.3. Injection sites

Injection sites for 2000 revaccinates were compared to those for 1998 vaccinates to evaluate the persistence of dart-site lesions. While abscesses were more evident and larger in 2000 revaccinates, granulomas could be found at the injection site of nearly all treated deer.

3.4. Lactation

Five of seven (71%) control females, and five of eight deer (63%) receiving their last vaccination in 1998 showed evidence of lactation during summer 2000 prior to the collection. Three of the five (60%) lactating does were also later identified as having eosinophilic oophoritis. None of the 2000 revaccinates showed evidence of lactation during necropsies.

3.5. Blood chemistry

There were few differences in blood chemistry between control and PZP-treated females (Table 1). Some variation occurred for selected blood parameters. However, these values fell within normal ranges and were unremarkable. There were significantly different titer levels between the groups ($F_{2,17} = 42.32$, $P \leq 0.0001$) with the highest titers in the 2000 revaccinates (Table 2).

3.6. Ovarian weights

There were also no significant differences in ovarian weights between controls, 1998 vaccinates, or 2000 revaccinates (left, $F_{3,17} = 0.53$, $P = 0.67$ and right, $F_{3,17} = 0.78$, $P = 0.52$; Table 2). Furthermore, there was no difference in

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

Blood parameter	Control group	PZP group	*t*-Test	*P*-value
Sodium (mEq/L)	141.00 ± 1.48	141.21 ± 1.33	$t = 0.09$	$P = 0.93$
Potassium (mEq/L)	11.77 ± 0.97	11.14 ± 1.01	$t = 0.37$	$P = 0.72$
Chloride (mEq/L)	103.50 ± 2.11	101.21 ± 1.04	$t = 1.09$	$P = 0.29$
Bicarbonate (mEq/L)	23.00 ± 2.21	23.57 ± 0.74	$t = 0.32$	$P = 0.75$
Anion gap (mEq/L)	26.33 ± 4.18	27.64 ± 1.56	$t = 0.37$	$P = 0.72$
Urea-N (mg/dL)	8.00 ± 1.51	13.14 ± 1.19	$t = 2.48$	$P = 0.02$
Creat-rb (mg/dL)	1.27 ± 0.12	1.72 ± 0.10	$t = 2.53$	$P = 0.02$
Calcium (mg/dL)	9.75 ± 0.21	9.41 ± 0.24	$t = 0.86$	$P = 0.40$
Phosphate (mg/dL)	8.75 ± 1.11	8.70 ± 0.68	$t = 0.04$	$P = 0.97$
Magnes-xb (mg/dL)	2.55 ± 0.09	2.69 ± 0.12	$t = 0.69$	$P = 0.50$
TotProt (g/dL)	6.32 ± 0.36	6.56 ± 0.20	$t = 0.63$	$P = 0.54$
Alb-blk (g/dL)	3.13 ± 0.14	3.13 ± 0.05	$t = 0.04$	$P = 0.97$
Globulin (g/dL)	3.18 ± 0.22	3.43 ± 0.19	$t = 0.74$	$P = 0.47$
A/G	1.00 ± 0.04	0.95 ± 0.05	$t = 0.56$	$P = 0.58$
Glucose (mg/dL)	186.00 ± 82.08	149.86 ± 25.85	$t = 0.55$	$P = 0.59$
AST/PST (U/cL)	11.47 ± 5.29	31.53 ± 13.22	$t = 0.97$	$P = 0.35$
SDH (U/L)	67.18 ± 13.56	125.58 ± 47.91	$t = 0.78$	$P = 0.45$
Alk Phos (U/L)	197.87 ± 132.52	83.71 ± 10.44	$t = 1.34$	$P = 0.20$
GGT (U/L)	45.33 ± 2.80	56.14 ± 3.94	$t = 1.70$	$P = 0.11$
Tot Bili (mg/dL)	0.17 ± 0.02	0.26 ± 0.05	$t = 1.33$	$P = 0.20$
Dir Bili (mg/dL)	0.05 ± 0.03	0.13 ± 0.04	$t = 1.32$	$P = 0.21$
Ind Billi (mg/dL)	0.12 ± 0.03	0.12 ± 0.02	$t = 0.03$	$P = 0.97$
CK (U/cL)	130.10 ± 66.44	459.32 ± 200.61	$t = 1.05$	$P = 0.31$
Iron (μg/dL)	155.67 ± 18.30	182.64 ± 13.38	$t = 1.14$	$P = 0.27$
Tibc (μg/dL)	318.67 ± 28.24	342.00 ± 26.42	$t = 0.52$	$P = 0.61$
Sat (%)	48.50 ± 3.18	79.93 ± 28.11	$t = 0.72$	$P = 0.48$
Lipemia	32.50 ± 8.48	24.57 ± 5.82	$t = 0.76$	$P = 0.46$
Hemolysis	103.83 ± 34.68	196.14 ± 71.17	$t = 0.82$	$P = 0.42$

Note: A Bonferroni-corrected ($n = 28$ tests) alpha level of 0.05 is 0.002.

Table 2
Weights (mg) of left and right ovaries, PZP antibody titers and incidence of oophoritis for control and PZP treated female white-tailed deer at Seneca Army Depot, Romulus, New York, 2000

ID	Treatment group	LT ovary (mg)	RT ovary (mg)	Titer (1/Titer × 1000)	Oophoritis
19	Control	573	606	0	N
21	Control	512	768	0	N
84	Control	507	324	0	NS
98	Control	689	523	0	N
148	Control	503	491	0	N
323	Control	408	306	0	N
378	Control	325	462	0	N
Means		502.4	497.1		
144	1998	870	725	32,000	Y
146	1998	408	476	16,000	N
157	1998	922	1117	64,000	Y
159	1998	362	372	128,000	Y
178	1998	1152	1130	64,000	Y
180	1998	536	545	64,000	Y
182	1998	299	437	16,000	Y
188	1998	451	480	32,000	N
Means		625.0	660.2	52,000	
With eosinophils					
107	2000	819	601	NS	N
109	2000	428	435	>128,000	N
142	2000	367	464	>128,000	N
Means		538.0	500.0		
Without eosinophils					
1	2000	451	669	128,000	Y
55	2000	464	430	128,000	Y
122	2000	477	605	>128,000	Y
Mean		464.0	568.0		
Total 2000 mean		501.0	534.0		

gross ovarian weights between the 2000 revaccinated deer with and without oophoritis ($P = 0.70$ left and $P = 0.71$ right).

3.7. Oophoritis

Eosinophilic oophoritis was frequently observed in atretic follicles of PZP-vaccinated females, and only seen in one solitary follicle from a control female. Among 1998 vaccinates 6 of 8 females (75%) had follicular infiltrates, and among 2000 revaccinates, 3 of 6 (50%) showed similar follicular infiltration (Table 3).

3.8. Follicle counts

There was no obvious trend or difference among the mean count of Graafian follicles between treatments ($F_{2,18} = 0.68$, $P = 0.52$). The only significant difference observed was a lower average number of normal secondary follicles for the deer revaccinated in 2000 when compared to the control females ($P = 0.04$) and 1998 vaccinates ($P = 0.04$; Table 3). When the 2000 revaccinate group was subdivided into those deer exhibiting oophoritis and those without oophoritis, only the group without oophoritis had significant reduction in the number of normal secondary follicles compared to the controls ($P = 0.03$) and the 1998 treatment group ($P = 0.04$). The presence or absence of oophoritis among 2000 vaccinates did not correlate with ovarian weight, nor the total number of normal follicles per ovary.

3.9. Parasites and multifocal lymphocytic infiltrates

One deer was found with meningeal worms (*Parelaphostrongylus tenuis*; no. 178, 1998 treatment group), and two had hepatic cysticerci (most likely *Echinococcus granulosus*; no. 55, 2000 treatment group, and no. 378, control group). These animals were in good to excellent body condition. Focal infiltrates and non-giant cell granulomas were seen in kidney, skeletal muscle, and liver. These lesions were consistent with immune attack against migrating parasites in tissues.

4. Discussion

4.1. Depleted bone marrow fat

Reduced fat content of bone marrow in PZP-treated deer was first observed by pathologists at the Cornell University College of Veterinary Medicine while conducting a necropsy

Table 3
Ovarian follicle development for control and PZP-treated female white-tailed deer at Seneca Army Depot, Romulus, New York, 2000

	Treatment	Secondary follicles			Graffian follicles			Eosinophilic oophoritis	Hyaline arteries
ID	Group	Normal	Atretic	% Atretic	Normal	Atretic	% Atretic		
19	Control	8	8	50	3	1	25	No	Yes
21	Control	16	1	5	1	4	80	No	Yes
84	Control	9	14	60	0	0	–	Yes 1 follicle	Yes
98	Control	11	13	54	0	3	100	No	Yes
148	Control	6	17	73	0	1	100	No	No
323	Control	4	8	66	0	1	100	No	No
378	Control	3	11	78	0	1	100	No	Yes
Means		8.1	10.3	55	0.6	1.6	72	1/7	5/7
144	1998	18	34	65	0	1	100	Yes	No
146	1998	10	15	60	1	4	80	No	No
157	1998	6	16	72	0	4	100	Yes	Yes
159	1998	2	6	60	0	1	100	Yes	Yes
178	1998	5	17	79	1	4	80	Yes	Yes
180	1998	13	13	50	2	0	0	Yes	Yes
182	1998	1	6	66	1	3	75	Yes	Yes
188	1998	7	10	58	1	1	50	No	Yes
Means		7.8	14.6	63.8	0.8	2.3	73.1	6/8	6/8
Without eosinophils									
107	2000	2	12	85	1	4	80	Yes	No
109	2000	8	20	71	0	1	100	Yes	No
142	2000	2	6	75	1	2	66	Yes	Yes
Means		4	12.7	77	0.7	2.3	82	3/3	1/3
Without eosinophils									
1	2000	1	6	88	0	2	100	No	Yes
55	2000	0	7	100	0	0	–	No	Yes
122	2000	1	8	88	0	1	100	No	Yes
Means		0.7	7.0	92	0	1	100	0/3	3/3
Means revaccinates		2.3	9.8	84.5	0.3	1.7	89.2	3/6	4/6

on a single PZP-vaccinated deer from Irondequoit, New York [26]. The total sample of treated deer examined was small, and these results were not published.

Bone marrow fat is normally the last energy reserve used in severe cases of malnutrition during winter [25,27]. Even fawns confined to a restricted energy diet for 10 weeks during the fall showed no significant difference in reserves of bone marrow fat compared to fawns offered feed ad libitum during the same time period [28,29]. Therefore, it is very unusual to see marrow fat depleted while the deer are otherwise in good to excellent body condition. The small number of PZP-treated deer examined to date makes it difficult to isolate a possible cause. Poor marrow fat content was also observed in one control deer, further complicating possible explanations. Additional investigation of the frequency and possible causes for marrow fat depletion should be conducted, considering that all cellular elements of blood develop from hematopic stem cells in the bone marrow, including leukocytes, and T and B lymphocytes of the adaptive immune system [30].

4.2. Injection site and other abscesses

A variety of infectious, parasitic, and non-inflammatory lesions were described in these deer. Most remarkable 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 tuberculosis. Likewise, giant cell inflammation was seen in the regional (popliteal) and deep (internal iliac) lymph nodes draining these sites. Typical acid fast bacilli were documented in these giant cells and were still apparent in the lesions and lymph nodes 2 years following injections. These types of inflammations, including the production of multinucleated giant cells associated with the presence of *Mycobacteria* in the adjuvant, have been previously described in other species vaccinated with Freund's Complete Adjuvant [19,31–34].

4.3. Lactation

Residual milk, in even a completely involuted udder, is considered evidence that a doe had raised fawns during the previous summer [35,36]. Our findings corroborated the Curtis et al. [7] report that five PZP-vaccinated deer delivered fawns in 2000. The fact that none of the six deer revaccinated in 2000 had lactated was a result of the selection of deer receiving the revaccination, not the treatment itself. The lac-

tation evidence further confirms the return to normal fertility for PZP-vaccinated deer, even as some continue exhibiting oophoritis.

4.4. Histological findings

Ovarian inflammation resulting from PZP immunocontraceptive vaccinations have been extensively studied for many species [9–11,13,14,16–18,37–39]. However this is the first report of eosinophilic oophoritis in ovaries from PZP-treated white-tailed deer. We documented both B-cell humoral and T-cell response from the same injection formula, and we were also able identify reproductive recovery in deer still experiencing eosinophilic oophoritis. The two most striking results from the necropsied deer were: (1) the widespread documentation of eosinophilic oophoritis in PZP-vaccinated deer, and (2) the reduced number of normal secondary follicles in females without oophoritis that were revaccinated in 2000.

Oophoritic inflammation of the ovary, in our study, specifically describes the infiltration of eosinophils (and to lesser extent neutrophils) into the theca interna across the basement membrane, and into the cumulus oophorus, or zona granulosa layer. However, it appears that apoptosis (a hallmark of follicular atresia) is necessary before eosinophils progress through the theca interna. Eosinophils were never seen within the lumen of the follicle until the glassy membrane was first formed. Also, this inflammation was restricted to secondary and Graafian follicles; which was particularly interesting because the oocytes of the primary follicles were likewise surrounded by the zona pellucida (ZP). Multifocal aggregates of small lymphocytes were also commonly seen in the ovarian parenchyma (but not in the follicles). Although ovaries are not considered to provide a distinct morphologic barrier from the immune system [18,40], it appeared that the ZP was protected by the ovary from eosinophilic attack until ovulation or atresia. This supports the observations by McShea et al. [4] who noted neutrophils in atretic follicles, but not eosinophils.

In humans, eosinophils are inflammatory cells which develop in the bone marrow under the influence of several T-lymphocyte produced cytokines (IL-3, IL-5) [41,42]. They are activated by platelet-activating factor and the split products of the complement system (C5a, C3a, C4a) [43–45]. These eosinophils then migrate to sites of inflammation within specific tissues under the influence of various chemokines including eotaxin 1–3 (CCL11, CCL 24, CCL 26) secreted by fibroblasts [41,46,47]. At the site of inflammation, eosinophils release cationic and basic proteins, or reactive oxygen species, which leads to cellular death and simultaneous release of various chemokines, including MIP-1a (CCL3), RANTES (CCL5), and IL-8 (CXCL8) [41,48,49]. Thus eosinophils, in addition to an effector function, control their own accumulation as well as the recruitment of other leukocytes such as T cells, monocytes, neutrophils, and basophils [42]. Observations made on deer in this study indicated that ovarian ZP antigens presented to the immune system stimulated release of chemokines, which attracted eosinophils. The eosinophils then modulated the subsequent inflammatory response in the ovary.

We observed that 2000 PZP revaccinates had the greatest reduction in secondary follicles for those deer that lacked eosinophilic infiltration. This is best explained by an antibody-mediated attack against the ZP antigens, resulting in complement-mediated destruction, where T-helper cells secreted IL-4 and IL-10, enhanced B-cell production of antibodies, and interfered with the T-cell production of cytokines necessary for an inflammatory response. Thus, cellular events occurring within the ovary following PZP vaccination set the stage for primarily antibody-mediated activity, or cellular inflammation, but not both at the same time [30].

The observation that the eosinophils only occurred during atresia signified that oophoritis is not a sign of impeded ovarian function, but rather indicative of normal ovulation. Such an observation could be expected in successful contraception of deer when females display typical cyclic behavior. The question then would be: does the oophoritis cause pain or distress to the deer? Observations of good to excellent body condition for most deer in our study would imply that they were not impaired by PZP vaccination.

Conversely, half of the 2000 PZP revaccinates had significantly fewer normal secondary follicles and no eosinophilic invasion. Such follicular deterioration has been associated with inhibited ovarian function, abnormal cycling, or suppressed progesterone levels observed in many species [9,11,14,16,18,38].

4.5. Anomalies

As for any species, reproductive anomalies can arise. A small number of women experience infertility due to premature ovarian failure from autoimmune oophoritis distinguished by ovarian lymphocyte infiltrates [17,18,50]. Similarly, we identified a control female (deer no. 84) maintained in the same environment with exposure to reproductively active males similar to other female deer on trial, yet failed to produce fawns during the course of the field study. The human case described by Page et al. [50] was determined to be eosinophilic perifolliculitis and tested positive for serum antiovarian antibodies. Similar to the human case, this infertile doe had an eosinophilic infiltrate in one of her ovaries without any treatment or sham injection, indicating self-induced autoimmune activity.

It is noteworthy that deer number 159 maintained a titer of 1:128,000 2 years post-vaccination, and had no injection site lesions. Miller et al. [15] observed similar prolonged immunocontraceptive efficacy and high titers, indicating the potential for continued self-inoculation each fall as normal ovulation resumed. Miller et al. [51] offered that variations in the residual immune response and physiological, morphological, or pathological differences among deer receiving identical treatments are likely due to genetic differences among individual animals from the same population.

5. Summary

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.

Deer vaccinated with PZP in this study continued to exhibit eosinophilic oophoritis 2 years after the last booster injection, including some deer that had already returned to normal fertility. The long-term health implications resulting from the Curtis et al. [7] vaccination protocol and associated pathologies are still unknown, and further study is advisable.

Observation of deer with bone marrow fat depletion, at a time of year when they should have their maximum energy reserves prior to winter, is an important concern. In climates with severe winters and deep snow, deer exhibiting reduced bone marrow fat during late summer and fall could be at risk for malnutrition and death if harsh conditions are prolonged. Hence, regardless of any possible connection to the PZP vaccine, continued effort to find the cause and extent of this condition should be a research priority, especially for deer herds in northern climates.

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