Long-term fertility control in female cats with GonaCon™, a GnRH immunocontraceptive

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Abstract

The uncontrolled reproduction of free-roaming feral cats contributes to overpopulation and associated concerns regarding their welfare and impact on public health and the environment. Nonsurgical fertility control that could be administered to feral cats in the field would be a powerful tool for cat population control. The objective was to test the efficacy and duration of activity of a single-dose GnRH immunocontraceptive vaccine (GonaCon™) on the fertility of adult female laboratory cats. Vaccinated cats (n = 15) received a single injection of vaccine containing a GnRH-KLH conjugate (200 μg) emulsified in a mycobacterial and oil adjuvant on study Day 0. Sham-treated cats (n = 5) received a single injection containing all vaccine components except the GnRH-KLH conjugate. A breeding trial started on study Day 120. Vaccinated cats had a longer time to conception (median 39.7 mo) compared to sham-treated cats (4.4 mo; P < 0.001). A total of 93% of vaccinated cats remained infertile for the first year following vaccination, whereas 73, 53, and 40% were infertile for 2, 3, and 4 y, respectively. At study termination (5 y after a single GnRH vaccine was administered), four cats (27%) remained infertile. The GnRH antibody titers declined more rapidly in short-term responding cats with < 2 y of infertility (n = 4), compared to long-term responding cats that experienced fertility control for >2 y (n = 11) (P < 0.05). Non-painful but persistent late-onset granulomatous injection site masses appeared 2 y after initial vaccination in five cats. We concluded that GnRH immunocontraception is an ideal candidate for further development for feral cat control.
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1. Introduction

Unowned free-roaming (feral) cats number in the millions nationally and exist throughout the world [1]. Their uncontrolled reproduction contributes to cat overpopulation and associated concerns regarding their im-pact on public health and the environment [2]. In parts of the world where progressive animal control resources are not available, measures such as poisoning and shooting are still used to control cats [3]. Surgical sterilization followed by return to the colony is an increasingly popular method of controlling feral cat populations, but is expensive, labor-intensive, highly technical, and limited in scale [4,5].

Nonsurgical fertility control that could be administered to feral cats in the field would be a powerful tool

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for cat population control. Control of free-roaming species is often aimed at the level of the population, rather than at specific individual animals. Treatment must be efficacious in a high proportion of the population, but it is not essential that every treated animal be rendered infertile. Feral cats are wary of humans, and once trapped and released, are unlikely to return for repeated trapping episodes. A practical fertility control technique for feral cats would induce permanent or multi-year duration of action following a single treatment in the majority of animals treated.

Vaccines aimed at reproductive targets are one approach to nonlethal control of overabundant free-roaming species. Overcoming tolerance to self antigens and induction of durable immunity without the use of repeated booster vaccines are substantial challenges for the development of immunocontraceptive vaccines. A commonly used contraceptive antigen in wildlife is the zona pellucida. In previous studies, we demonstrated that vaccinating against the zona pellucida was ineffective in preventing pregnancy in cats [6,7]. Subsequently, we showed in a short-term study that a single dose of GnRH vaccine in a mycobacterial and oil emulsion (GonaCon™) effectively blocked testosterone production and spermatogenesis for at least 6 mo in a majority of vaccinated adult male cats [8]. This was apparently the first report to suggest that fertility control may be achievable in cats following a single vaccination against a reproductive self antigen.

We hypothesized that long-term fertility control in cats can be induced by immunological blockade of GnRH activity. The purpose of this study was to test the efficacy and duration of activity of single-dose GnRH immunocontraception on the fertility of adult female cats.

2. Materials and methods

2.1. Cats

Twenty-four 8- to 14-mo-old specific-pathogen-free female domestic shorthair cats were acquired from a commercial vendor (Liberty Research, Waverly, NY, USA). Cats were group-housed in one large enriched room with raised resting benches, maintained at ambient temperatures between 21 and 23 °C, and exposed to controlled lighting (explained later). Food and water were available *ad libitum*. The experimental design was approved by the University of Florida Institutional Animal Care and Use Committee, and facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All cats and

their offspring underwent surgical sterilization and were adopted to private homes at the conclusion of the study.

Upon arrival, the 24 cats were housed for 30 d in a photoperiod regimen (8 h light: 16 h dark) that is inhibitory for estrous cyclicity. Forty-six days prior to treatment (Day −46), the photoperiod regimen was reversed to 16 h light: 8 h dark, which is permissive for estrous cyclicity within 15 d in most cats [9,10]. In cats, behavioral estrus occurs in conjunction with elevated concentrations of estradiol-17β secreted by developing follicles. Progesterone concentrations rise significantly within 4 d after ovulation, regardless of whether or not fertilization has occurred [9,10]. Serum progesterone and estradiol-17β concentrations were measured on Day −64, and then every other day from Day −46 to Day −26. The magnitude and duration (Day > 20 pg/mL) of elevated estradiol-17β concentrations in each cat were used to confirm normal photoperiod sensitivity and estrous cyclicity in cats selected for this study.

2.2. Vaccine construction

A GnRH vaccine was constructed using a synthetic GnRH peptide with the sequence [pEHWSYGLRPGGC-SH] produced by the Fmoc/tBU protection method (Global Peptide Services, Fort Collins, CO, USA). Immunogenicity was enhanced by coupling GnRH peptides to a protein carrier, keyhole limpet hemocyanin (KLH; Pierce Biotechnology, Rockford, IL, USA), in a 1:3 GnRH:KLH mass ratio. The first 10 amino acids represent the native GnRH molecule and "pE" signifies pyroglutamate. One glycine was added at the C-terminus as a spacer and a cysteine was added to ensure consistent alignment of the peptide to the maleimide-activated KLH. The aqueous-based GnRH-KLH conjugate (200 μg) was combined in a 1:1 ratio by volume with an emulsifying adjuvant, AdjuVac™, which was produced by modifying a USDA-licensed Johne's disease vaccine containing inactivated *Mycobacterium avium* in mineral oil (Mycopar®; Fort Dodge Animal Health, Fort Dodge, IA, USA). This GnRH vaccine (GonaCon™, USDA, Pacarello, ID, USA) is licensed as a "restricted-use pesticide" for prevention of estrous cyclicity in white-tail deer.

2.3. Treatment

Twenty cats were selected for use in the GnRH vaccination study based upon estrus induction following an increase in light to 16 h per day. These 20 cats were randomized based upon a similar peak estradiol-

17β concentrations in response to long-day photoperiod into a sham treatment group (n = 5) and a vaccination group (n = 15). The sham-treated group received placebo injections containing all components except the GnRH-KLH conjugate. The treatment group received vaccines containing 200 μg GnRH-KLH. Vaccines were administered intramuscularly under general anesthesia. Brief anesthesia was induced by administration of isoflurane (IsoFlo®; Abbott Laboratories, North Chicago, IL, USA) by face mask. The hair of the right cranial thigh was clipped, and the injection site was cleaned with 70% isopropyl alcohol. The vaccine (0.5 mL) was injected into the quadriceps muscle group. This site was selected to avoid critical structures such as the sciatic nerve in the event of a vaccine reaction. This site is also accessible when cats are resting in a crouched position, making it one of the likely injection targets in nonsedated cats in the field. The right pinna was tattooed with a treatment code for permanent identification.

Potential reactions to vaccination were evaluated by daily physical examination, including inspection of the injection site, and measurement of body temperature for 1 wk following treatment. The injection site was inspected at the time of each monthly blood sample collection throughout the study.

2.4. Breeding trial

The effects of treatment on fertility were evaluated in a breeding trial beginning 4 mo after treatment and continuing until each cat became pregnant or until 60 mo post-treatment, whichever came first. The daily photoperiod was alternated between the estrus-inhibiting short-day regimen (16 h dark:8 h light) and the estrus-inducing long-day regimen (8 h dark:16 h light). The cats were exposed to short-day photoperiod from Days 0 to 120, and from Days 330 to 360 (8% of post-treatment time). For the remainder of the study, the cats were exposed to long-day photoperiod. The switch from short-day photoperiod to long-day photoperiod was intended to induce estrus in any of the cats that were capable of an estrous response. A breeding male was housed continuously with the females during the long-day photoperiods beginning on Day 120. Four breeding males were alternated, to reduce the chance that inter-cat behavioral incompatibility would affect breeding success. Time-lapse videography was used to monitor breeding activity during lighted hours. The recordings were reviewed daily and the number of attempted and successful breedings was recorded.

Time to conception was defined as the interval between treatment Day 0 and fertilization date, which was estimated by subtracting the average length of cat gestation (63 d) from parturition date. Fertility was defined as full-term pregnancy. Litter size was defined as the number of total births (live and stillborn). Fecundity was defined as the number of live births. Pregnancy was scored as a treatment failure, and the cats were removed from the study after parturition. Short-term response to GnRH immunocontraception was defined as fertility control for two y or less, whereas a long-term response was defined as fertility control lasting more than 2 y after the single vaccination.

2.5. Blood collection

Blood (4 mL) was collected by jugular venipuncture monthly into serum separator tubes for determination of GnRH antibody titer and progesterone concentration. In addition, blood was collected every other day during an interval of 20 d following change from short-day photoperiod to long-day photoperiod beginning on Days 120 and 360 (for determination of serum concentrations of estradiol-17β and progesterone). Serum was separated by centrifugation and stored at -20 °C pending analysis.

2.6. Detection of GnRH antibodies

Serum was tested for GnRH antibodies using an enzyme-linked immunoabsorbant assay (ELISA). The 96-well plates (Thermo Electron Corporation, Waltham, MA, USA) were coated with BSA-GnRH and blocked with PBS buffer containing steelhead salmon serum (SEA BLOCK; Pierce Biotechnology, Rockford, IL, USA). Cat serum was added to wells in two-fold dilutions starting at 1:1,000. Two negative controls (buffer without cat serum and pre-vaccination cat serum) and one positive control (serum from a cat with a known high antibody titer) were included on each plate. The GnRH antibodies were detected with goat anti-cat IgG (Sigma Chemical Co., St. Louis, MO, USA) followed by rabbit anti-goat IgG conjugated with horseradish peroxidase (Sigma Chemical Co.), and color development with tetramethylbenzidine/phosphate-citrate buffer (Sigma Chemical Co.). Sulfuric acid (2M) was added to each well to stop the reaction. Absorbance was read at 450 nm with a Dynatech MR 5000 ELISA plate reader (Dynatech Laboratories Inc., Alexandria, VA, USA). The endpoint titers were defined as the dilution at which the absorbance was twice the absorbance of the pre-vaccination cat serum.
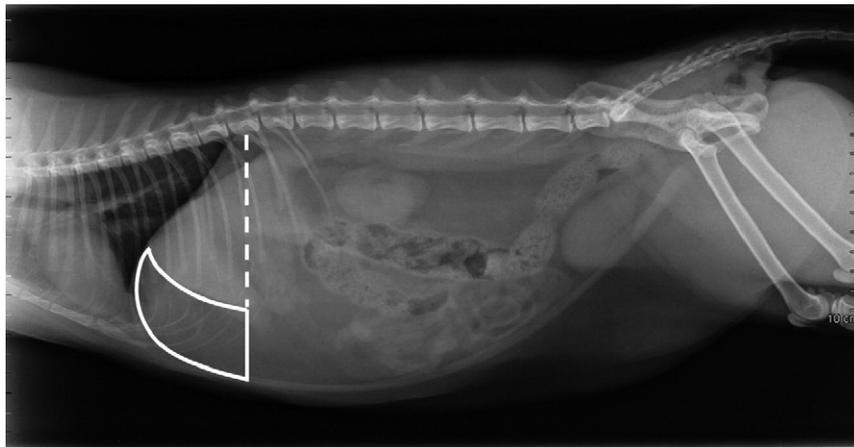
Fig. 1. The size of the falciform fat pad in female cats was assessed radiographically as an estimate of body fat content. The depth of the fat pad was measured at the level of the center of the T12 vertebral body (dashed line) from the ventral border of the liver to the ventral abdominal wall. The area of the fat pad was measured as the area confined by the ventral border of the liver, the caudal surface of the diaphragm, the ventral abdominal wall, and the line used for the depth of the fat pad measurement. The solid line shows the boundaries for measuring the area of the fat pad.

2.7. Determination of serum concentrations of estradiol-17β and progesterone

Serum samples were analyzed for total estradiol-17β and progesterone by radio-immunoassay (Coat-A-Count®; Diagnostic Products Corporation, Los Angeles, CA, USA) according to the manufacturer's instructions. In each run, controls included human serum with three concentrations of estradiol-17β (57, 127, and 934 pg/mL) and progesterone (1.78, 3.4, and 18.3 ng/mL).The manufacturer reports an assay sensitivity for estradiol-17β of 8 pg/mL with inter-assay CV (coefficient of variation) of 4 to 8% and intra-assay CV of 4 to 7%. Progesterone assay sensitivity was reported to be 0.02 ng/mL with inter-assay CV of 4 to 10% and intra-assay CV of 2 to 9%.

2.8. Body weight and composition

It has been well-documented that cats gain weight and percent body fat after ovariectomy [11,12], but it is unknown if immunocontraception has the same effect. To evaluate for this effect, an estimation of body fat was made by radiographic evaluation of the abdominal falciform fat pad on Day 420. Cats were sedated with medetomidine 40 mcg/kg IM (Domitor; Pfizer Animal Health, New York, NY, USA) and imaged in right lateral recumbency using computed radiography (Kodak, Rochester, New York, USA). The depth (mm) of the falciform fat pad was measured by dropping a perpendicular line from the center of the body of the 12th thoracic vertebra to the ventral body wall and measuring the distance between the caudoventral angle of the liver and the ventral body wall. The area (mm^2) of the fat pad was defined as the area outlined by the line used for the depth measurement, the ventral border of the liver, the caudal aspect of the diaphragm, and the ventral body wall (Fig. 1).

2.9. Statistical analysis

Differences in the median time to conception between the sham-treated group and the GnRH-vaccinated group were determined by the Kaplan-Meier survival analysis log rank test. Mean ± SEM were calculated for normally distributed data (monthly titers, body weight, fat pad measurements, litter size), and groups were compared with the Kruskal-Wallis one way ANOVA. Median and interquartile range (IQR) were calculated for non-normally distributed data (peak titer, titer at conception), and groups were compared with the Mann-Whitney rank sum test. Differences were considered significant when $P < 0.05$. All tests were performed using SigmaStat® statistical software, version 3.1 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Reactions to treatment

Body temperature in all of the cats remained normal after treatment, and there was no inflammation or tenderness at the injection sites. One vaccinated cat died
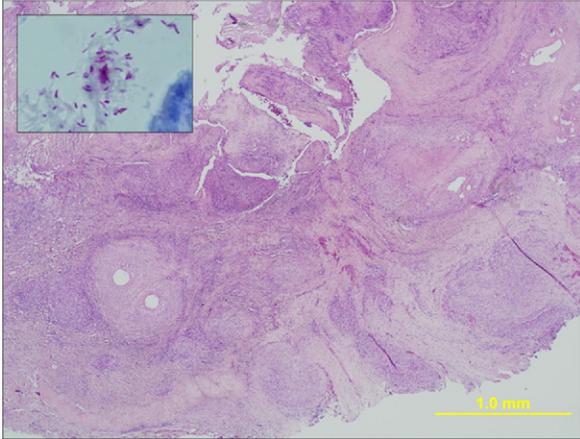
Fig. 2. Biopsy (stained with hematoxylin and eosin) of a subcutaneous injection-site nodule in a cat which appeared 24 mo following a single vaccination against GnRH. There was granulomatous inflammation, with acid fast bacilli evident within the lesion (inset). Five cats developed persistent late-onset, non-painful, injection-site nodules.

suddenly 45 d after treatment; necropsy revealed no gross or histological abnormalities to explain the death. This cat was replaced with a 2 y old cat of proven fertility from the colony and vaccinated with the GnRH vaccine according to the standard protocol.

Twenty-four months after immunization, it was discovered that one of the cats had a firm 4 cm × 5 cm mass at the vaccination site. No pain reaction was elicited on palpation of the mass. A biopsy revealed granulomatous inflammation with interlesional acid-fast bacteria (Fig. 2). Aerobic, anaerobic, and myco-bacterial cultures were negative. Smaller single or multiple masses < 1 cm in diameter developed at the injection site of four other cats. The size of the masses in all five cats waxed and waned for the duration of the study, but the cats never appeared painful and the lesions never drained. Three of the cats with masses never became pregnant, and the other two became pregnant at 863 and 1212 d, respectively. No masses were observed in the sham-treated cats.

3.2. Breeding trial

All four breeding male cats sired litters during the study. The first male cat was introduced on Day 120 post-treatment at the beginning of a second long-day photoperiod. All sham-treated cats displayed behavioral signs of estrus and were receptive to breeding attempts by the male within 10 days of the change to long-day photoperiod. Time-lapse videography revealed that the male bred each of the sham-treated cats

at least 15 times between Days 122 and 155. One sham-treated cat was observed breeding in two intervals during gestation (Days 7 to 11 and Days 25 to 26 of gestation). Four (27%) vaccinated cats that displayed estrous behavior and began breeding on Days 128, 418, 469, and 504 were classified as having a short-term response to GnRH vaccination.

Based on an average gestation period of 63 d, parturition date was used to estimate the date of conception. The GnRH-vaccinated group had a longer median time (P < 0.001) to conception following treatment (39.7 mo) compared to the sham-treated group (4.4 mo; Fig 3). All five sham-treated cats became pregnant 7–28 d (study Days 127–148) after the male cat was introduced. One short-term responder cat was bred in three successive periods of estrus between Days 129–164, before becoming pregnant on Day 164, whereas the other three short-term responder cats became pregnant during their first estrus following a prolonged vaccine-induced anestrous period (Days 418, 469, and 504, respectively).

Eleven (73%) cats were classified as long-term responders with fertility control lasting greater than two y following a single GnRH vaccination. A total of 93% of vaccinated cats remained infertile for the first year following vaccination, 73% for 2 y, 53% for 3 y, and 40% for 4 y (Fig. 3). At the time the study was terminated (5 y after a single GnRH vaccine was administered), four cats (27%) had never become pregnant.

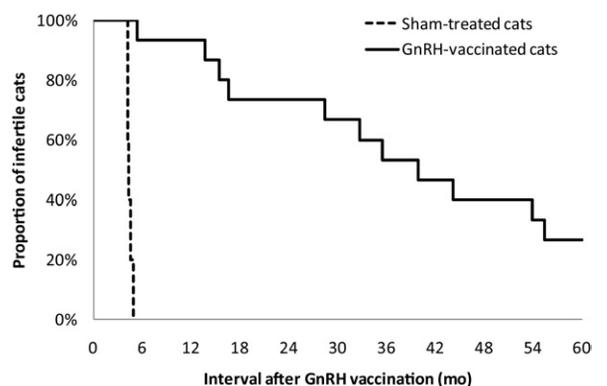There was a small but significant difference in fecundity (live births) in the GnRH-vaccinated group (2.6 ±

Fig. 3. Fertility control in cats. Vaccinated cats (n = 15) received a single injection of vaccine containing a GnRH-KLH conjugate (200 μg) in a mycobacterial and oil emulsion on study Day 0. Sham-treated cats (n = 5) received a single injection containing all vaccine components except the GnRH-KLH conjugate (n = 5). A breeding trial was commenced on study Day 120. Vaccinated cats had longer time to conception (median 39.7 mo) compared to sham-treated cats (4.4 mo; P < 0.001).

Table 1
Time to conception, litter size, and GnRH antibody titers in individual sham-treated cats (n = 5), short-term responding cats (n = 4), and long-term responding cats (n = 11). Four cats remained infertile for the entire duration of the 5 y study.

Cat	Conception (Day)	Peak GnRH titer	GnRH titer at conception	Live births	Still births	Injection-site reaction
Sham 1	127	0	0	3	0	No
Sham 2	128	1000	1000	3	1	No
Sham 3	131	0	0	4	0	No
Sham 4	137	0	0	5	1	No
Sham 5	148	4000	4000	4	0	No
Short-term 1	164	32,000	8000	3	0	No
Short-term 2	418	32,000	4000	2	0	No
Short-term 3	469	128,000	4000	3	0	No
Short-term 4	504	128,000	4000	1	0	No
Long-term 1	863	128,000	16,000	3	0	Yes
Long-term 2	995	128,000	64,000	2	1	No
Long-term 3	1078	128,000	64,000	3	1	No
Long-term 4	1212	256,000	128,000	4	0	Yes
Long-term 5	1343	256,000	128,000	4	0	No
Long-term 6	1640	128,000	0	3	1	No
Long-term 7	1684	256,000	0	1	3	No
Long-term 8	NA	256,000	NA	NA	NA	No
Long-term 9	NA	128,000	NA	NA	NA	Yes
Long-term 10	NA	256,000	NA	NA	NA	Yes
Long-term 11	NA	256,000	NA	NA	NA	Yes

0.3) compared to the sham-treated group (3.8 ± 0.4; P = 0.04; Table 1). There was no difference in the number of stillborn kittens between the groups (P = 0.9).

3.3. GnRH antibody titers

All vaccinated cats developed detectable GnRH antibodies (Table 1). The magnitude of GnRH antibody titers 1 mo after vaccination was similar in cats that responded to vaccination for < 2 y (short-term responding group) and those that responded for > 2 y (long-term responding group; Fig. 4). However, antibody titers decreased more quickly in the short-term responding group and remained lower at each monthly point beginning 5 mo post-vaccination (P < 0.05). Peak
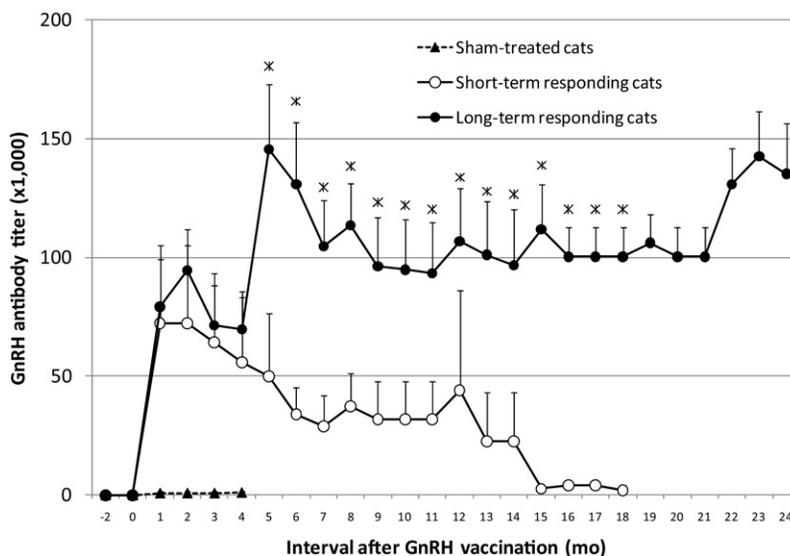

Fig. 4. GnRH antibody titers (mean ± SEM) in sham-treated cats (n = 5), short-term responding cats (n = 4), and long-term responding cats (n = 11) during the first 2 y following treatment with a single sham or GnRH vaccine. The GnRH antibody titers declined more rapidly in short-term responding cats with < 2 y of infertility compared to long-term responding cats that experienced fertility control for >2 y (*P < 0.05).

GnRH antibody titers were higher in the long-term responding group (median 256,000, IQR 128,000–256,000) than in the short-term responding group (median 80,000, IQR 32,000–128,000; P = 0.02; Table 1). Most cats that became pregnant had antibody titers of 16,000 or less at the time of conception, but four cats had antibody titers of 64,000–128,000. Antibody titers in the four cats that never became pregnant were 128,000–256,000 at the end of the study, 5 y after the single GnRH vaccination.

3.4. Hormone concentrations

Nineteen of the cats selected for the study responded to long-day photoperiod prior to treatment, with a surge in estradiol-17β > 20 pg/mL for at least four consecutive days and peak estradiol-17β > 40 pg/mL for at least 1 d. The remaining cat responded to long-day photoperiod with elevation of estradiol-17β from baseline, but exceeded 20 pg/mL on 1 d only. Six of 20 cats had an increase in serum progesterone > 2 ng/mL following the change to long-day photoperiod prior to treatment, indicating pseudopregnancy following estrus, even though the male cat had not yet been admitted to the colony. A cycle of 1 mo of short-photoperiod followed by return to long-day photoperiod on Day 360 failed to trigger behavioral estrus, breeding attempts, or increases in serum progesterone in any of the 14 vaccinated cats that remained in the study at that time.

The first elevation of progesterone > 2 ng/mL during monthly testing was associated with pregnancy in 14 of the cats (5 sham-treated cats and 9 vaccinated cats). In two other cats, both long-term responders, episodic non-fertile progesterone increases were detected during Days 1500 to 1590 and Days 1410 to 1650 before conception followed by term pregnancy occurred on Days 1640 and 1684, respectively. Progesterone elevation was also observed on Days 1230, 1650 to 1680, and 1830, respectively, in three of the cats that never became pregnant during the 5 y study period. Elevated progesterone was never detected in one of the cats that never became pregnant.

3.5. Body weight and composition

At the time of treatment on Day 0, there were no significant differences (P = 0.5) in body weight among the sham-treated (3.5 ± 0.2 kg), short-term responder (3.2 ± 0.2 kg), and long-term responder (3.3 ± 0.1 kg) groups. Similar to cats undergoing surgical sterilization, long-term responder cats had a higher percentage gain in body weight than sham-treated cats and short-term responder cats (P = 0.02; Table 2). Weight gain

Table 2
Body weight gain and abdominal fat (mean ± SEM) in cats as a function of response to GnRH vaccination. Body weight was measured at the time of treatment on Day 0 and again on Day 420 and the percentage change in weight calculated. Abdominal falciform fat pad depth and area were measured from lateral radiographs made on Day 420. Long-term responding cats (n = 11) gained more weight and had greater fat pad depth than sham-treated (n = 5) and short-term responding cats (n = 4).

Response group	Body weight gain (%)	Fat pad depth (mm)	Fat pad area (mm^2)
Sham (n = 5)	15 ± 7	19 ± 2	728 ± 145
Short-term responses (n = 4)	4 ± 10	19 ± 2	839 ± 172
Long-term responses (n = 11)	40 ± 6*	28 ± 3*	1193 ± 148

* P ≤ 0.02.

was also reflected in abdominal fat content. The mean falciform fat pad depth for the long-term responder group was greater than the mean for the sham-treated group (P = 0.02) and the short-term responder group (P = 0.03). The area of the falciform ligament was not significantly different among groups (P = 0.1).

4. Discussion

The vaccine used in this study induced fertility control in 100% of cats following a single injection, but duration of contraception varied from 5 mo to > 5 y. Infertility was maintained for 3 y in approximately half of the cats and for more than 5 y in a quarter of the cats. Response to vaccination was accompanied by a cessation in estrous cyclicity and with weight gain, similar to cats undergoing surgical sterilization via ovariectomy.

This is apparently the first study to demonstrate multi-year fertility control following a single vaccination in cats. Although permanent sterilization is ideal, the relatively short life-span of many free-roaming feral cats suggests that a contraceptive that blocks fertility for several years in a high proportion of cats may be successful in reducing the population. Most models propose that more than 70% of the female cats in a population must be rendered infertile to trigger a negative population growth rate [13–15], but one model found that focusing a control program on juvenile cats and utilizing a contraceptive having 3 y duration of activity would stop population growth when just 60% of the female cats were treated [16].

Duration of infertility was associated with persistence of high GnRH antibody titers. Most cats with an antibody titer > 16,000 remained infertile. However, several cats, all long-term responders, successfully re-

produced in the presence of antibody titers as high as 256,000. We inferred that there is no absolute contraceptive antibody titer that is predictive of infertility and that other factors also influence the contraceptive effect of GnRH immunization in female cats. In a previous study in male cats, antibody titers of $> 32,000$ were associated with suppression of serum testosterone concentrations and spermatogenesis consistent with immunocastration [8]. Immunocontraception targeting GnRH appeared to be more effective in female cats than in male cats. All female cats in the current study experienced a delay in conception compared to sham-treated cats, whereas only 67% of male cats demonstrated infertility as determined by semen analysis in the previous study [8]. Higher efficacy in females has been observed in other species including feral swine [17], white-tailed deer [18], and domestic dogs (L. Miller, unpublished).

In addition to suppression of estrus, GnRH immunocontraception may have additional effects on reproduction. In this study, litter sizes in cats that recovered fertility were slightly smaller than litter sizes in sham-treated cats. Whether this is a transient phenomenon in the first pregnancy following recovery of fertility, is related to the older age of the vaccinated cats at the time of pregnancy, or is a persistent effect possibly related to the continued presence of GnRH antibodies is unknown, as cats were removed from the study following delivery of their first litter.

Granulomatous injection-site reactions containing acid fast bacteria, presumably from the adjuvant, were identified in five long-term responding cats. This evidence of an ongoing immune response may explain the ability of this depot vaccine to create durable immunity against a self antigen in a large proportion of cats in the absence of a booster vaccination protocol. Granulomatous and neoplastic masses at the injection site and systemic inflammatory responses have been previously reported in cats receiving zona pellucida antigens in the presence of mycobacteria and oil (Freund's complete or incomplete adjuvants) [6,19]. The production of durable immunity using non-replicating vaccines is a great challenge, particularly against a small self antigen such as GnRH [20]. Adjuvants and carrier proteins with T-cell epitopes that stimulate the immune system to react against the target antigen do so in the context of inducing a local inflammatory response. As a species, cats are at increased risk for development of life-threatening sarcomas at the site of inflammation resulting from vaccination and other injections [21]. In another study of GnRH immunocontraception in cats, all kittens vaccinated with a GnRH vaccine or placebo containing oil developed acute transient injection-site masses [22]. The initial reactions resolved, but examination of injection sites following booster vaccines up to 20 mo later was not reported. Consideration should be given to the development of vaccine formulations that reduce the risk of injection-site reactions and possible sarcoma formation in cats. However this concern should be balanced against the very real threats to the welfare of cats associated with their uncontrolled reproduction and with control programs that rely on long-term confinement or culling. Risk assessments associated with nonsurgical fertility control should also include a comparison with the known morbidity and mortality associated with surgical sterilization, particularly in owned cats in which the benefit to the individual is a higher priority than population control.

GnRH has many of the characteristics of an ideal contraceptive target. Hypothalamic GnRH is a decapeptide "master hormone" that controls downstream pituitary and gonadal responses in both male and female mammals. In the absence of GnRH stimulation, the gonadal hormones estrogen, progesterone, and testosterone are suppressed and gamete maturation does not occur [8,22,23]. This results in infertility, elimination of repeated estrous cycles that can lead to uterine disease and mammary carcinoma, and reduction of undesirable nuisance behaviors such as fighting, roaming, spraying, and estrous calling. This is an advantage over contraceptive targets (such as zona pellucida) that block fertility, but not necessarily estrous cyclicity. The availability of a single product effective in both males and females following a single dose is also an advantage, because it eliminates the need to re-trap animals for repeat treatments, to maintain an inventory of multiple different products, to target trapping campaigns to one sex, or even to determine what sex a trapped cat is. The vaccine used in this study was approved in September 2009 for control of white-tail deer by the Environmental Protection Agency, which regulates the use of "pesticides," including nonlethal products for use in feral and "pest" species [24]. The vaccine has not been approved for use in any other species at this time, including feral cats.

Many feral cats are too unsocialized for safe handling. In contrast to surgical sterilization, the GnRH vaccine used in this study could possibly be administered without sedation to cats confined in a humane trap. This obviates the need for veterinarians to be directly involved with each cat targeted in a population control campaign, a distinct advantage in parts of the

world where veterinary services for companion animal species are not universally available. These findings suggest that GnRH immunocontraception is an ideal candidate for further development for the control of feral cats.

Acknowledgments

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