

PRESENTATION SUMMARY & POWERPOINT

Multi-year Study of Single-Dose GnRH Immunocontraceptive in Cats

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Unowned, free-roaming (feral) cat populations exist throughout the world. The number of such cats in the United States is unknown, but is suspected to rival that of pet cats (greater than 90 million) and to contribute substantially to cat overpopulation. Feral cats are reported to pose substantial risks to human and animal health as a reservoir of infectious diseases. They are also believed to have a negative impact on the environment via competition with and predation of native wildlife. Of primary concern is the welfare of the cats themselves, particularly of kittens, which suffer >50% mortality prior to maturity.

The most common control method for unwanted cats in the United States is euthanasia, and poisoned baits are used in many parts of the world. Regardless of the method selected, to be effective, control programs must accommodate considerations for environmental safety of nontarget animals and humans, be affordable for municipal agencies or charitable organizations, include plans to mitigate continuous cat immigration and reproduction, and be aesthetically acceptable to the public.

Trap-Neuter-Return (TNR) programs offer a solution that many animal welfare advocates consider more humane than lethal control methods. However, trapping cats, transporting them to a central facility for sterilization, and returning them to the trapping site are very resource-intensive activities, and veterinarians are required to perform the surgeries. Although TNR programs have many benefits, including improving the health of the free-roaming cats and controlling cat populations on a local basis, it is unlikely that TNR programs could be implemented on a large enough scale to eliminate the homeless cat problem alone. This is particularly true in areas of the world where veterinary care for cats is not widely available. Non-surgical contraception methods offer the promise of a lower cost, lower tech solution that does not require the cats to undergo surgery. In contrast to pet cats, in which owners expect a contraceptive technique (generally surgery) that is 100% reliable in each individual animal, feral cat control is treated as a herd management issue. Thus, a contraceptive treatment would not have to be effective in each animal, but a predictable effect on a population level would be required. Most feral cat control models suggest that approximately 70-80% of female cats in a population must be rendered infertile to induce a negative growth rate.

Control of feral cats, like other wildlife species, is made challenging because practicality dictates that most cats are captured and handled only once in their lives. This means that the best feline contraceptive would induce long-term, or ideally, permanent contraception following a single treatment. From a population biology perspective, controlling reproduction in females is the most critical factor in regulating populations; an equivalent

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level of infertility in males has little effect. However, in addition to controlling reproduction, a secondary goal of contraception is to reduce health impacts and objectionable behaviors associated with hormone sex production in both sexes. This benefits the welfare of the treated animals and also makes them more acceptable in their environments. The difficulty in targeting specific cats for trapping means that captured cats are likely to be both males and females and of all ages. For efficiency, an ideal contraceptive agent would be effective in all of these groups. Other characteristics of an ideal contraceptive strategy would include rapid onset of activity, efficacy in a high proportion of treated animals, remote delivery, ability to detect treated animals, product stability under field conditions, safety for the target animal and the environment, efficient manufacturing, and low cost to the user.

For the past 7 years, we have been investigating immunocontraceptive vaccines in cats. In our first studies, we tested zona pellucida as a contraceptive antigen because it is effective in several species, including horses, deer, and seals. Unfortunately, it was ineffective in cats and all vaccinated cats became pregnant. We next turned to GnRH, which was developed by wildlife biologists for the humane control of overabundant species such as deer, bison, horses, and swine. Targeting GnRH has the advantage that it would be contraceptive in both males and females and that hormone production would be suppressed in addition to fertility. We have performed multi-year studies in collaboration with the USDA Wildlife Research Center in both male and female cats to determine the efficacy, duration of immunity, and safety of GnRH immunocontraception in cats.

In an initial pilot study, 12 male cats were divided into groups of three and were immunized once intramuscularly with 0 (sham), 50, 200, or 400g synthetic GnRH coupled to keyhole limpet hemocyanin and combined with a mycobacterial adjuvant to enhance immunogenicity. GnRH antibody titer, serum testosterone concentration, and scrotal size were determined monthly. At 6 months, semen was collected by electroejaculation and testes were examined histologically. GnRH antibodies were detected in all cats receiving GnRH vaccine by 1 month post-treatment and persisted throughout the study. No dose effect of GnRH was observed as titers were not significantly different between cats treated with 50, 200, or 400g GnRH. Six of 9 treated cats were classified as responders based on high GnRH antibody titers (>32,000).

By 3 months post-treatment, responder cats had undetectable testosterone and testicular atrophy. Nonresponder cats had GnRH titers of 4,000 to 32,000 and testosterone concentrations intermediate between responder and sham-treated cats. At 6 months, total sperm counts were similar for sham-treated cats and nonresponder cats. Only one of the 6 responder cats produced sperm, none of which were motile. Combined testicular weights of responder cats were lower than sham-treated controls and nonresponder cats. Histologic evaluation of the testes revealed that in responder cats, the interstitial cells that were present were pale and shrunken compared to the plump, polyhedral eosinophilic cells in sham-treated cats. GnRH responder cats had marked tubular atrophy with vacuolated Sertoli cells and a paucity of germ cells.

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Based on the success of the pilot study, long-term studies in adult male and female cats were initiated. Of 17 cats receiving a placebo vaccine containing adjuvant only and 27 cats receiving a treatment vaccine containing 200g GnRH linked to KLH and mixed with a mycobacterial adjuvant, no acute local or systemic reactions were observed. In female cats, contraceptive GnRH antibody titers (16,000) were achieved by 2 months post-treatment in 80% of cats. The titers were maintained through 24 months despite the lack of a booster vaccine. A breeding male cat was housed with the females continuously beginning 4 months after treatment. Four cats whose titers decreased below 16,000 became pregnant, but the remaining cats remained infertile, resulting in a 2-year efficacy of 73%.

At the end of the observation period, late-onset injection site reactions consisting of a firm, nonpainful subcutaneous lump were observed in a few cats. Histopathology was consistent with a granuloma induced by the vaccine. The response in male cats was less predictable. The GnRH antibody titer required for contraception (128,000) was higher than in female cats. Similar to female cats, the vaccine had a contraceptive effect in 75% of males. This effect was associated with undetectable testosterone, testicular atrophy, and azoospermia. However, the onset and duration of contraception was highly variable, and there was poor correlation between antibody titer and response. In one extreme case, GnRH antibody titer did not increase until 6 months post-vaccination, testosterone was not suppressed until 12 months, and azoospermia did not occur until 14 months. In this cat, the contraceptive effect lasted 14 months, after which normal testosterone concentration and semen characteristics recovered, and the cat sired a litter.

These studies suggest that GnRH immunocontraception meets many of the characteristics of an ideal contraceptive for feral cats, including efficacy with a single treatment in both males and females, safety for target animals and the environment, suppression of sex hormone production, and a simple administration technique practical for use in the field. Still to be determined are whether GnRH immunocontraception is effective in juvenile cats, the significance of injection site reactions, and the duration of immunity in female cats. Since this vaccine can be administered to cats restrained in humane traps without sedation, the issue of how to identify treated cats remains unresolved.

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By Dr. Julie Levy

Multi-year studies Single-dose GnRH immunocontraception in cats

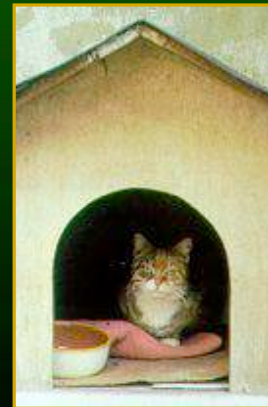


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National Cat Numbers

- 8-12% of households feed stray & feral cats
 - 0.5 cats per household
 - 50 million feral cats
 - 2% are sterilized
- 34% of households own cats
 - 0.7 cats per household
 - 90 million pet cats
 - 82-91% are sterilized

-- Levy 2003, JAVMA



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	Owned Cats	Feral Cats
Percent female	50%	50%
Sterilization rate	85%	2%
Litters per year	1.5	1.5
Kittens per litter	4	4
Kittens per cat annually	0.45	2.9
U.S. cat population	90 million	50 million
Total kittens born annually	40 million	147 million

Call to Action



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Trap-Neuter-Return

- Spay and neuter
- Ear tipping
- Return to colony



Biology of Population Control

- Population control relies on females only
 - 70% of females?
 - Less if targeted on juveniles?
 - Response of individuals less important



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Surgical Sterilization

- Predictable, immediate, and permanent
- Behavioral and medical benefits



Limitations of Surgical Sterilization

- Requires anesthesia and invasive procedure
- Expensive and technical
- Not available in much of the world



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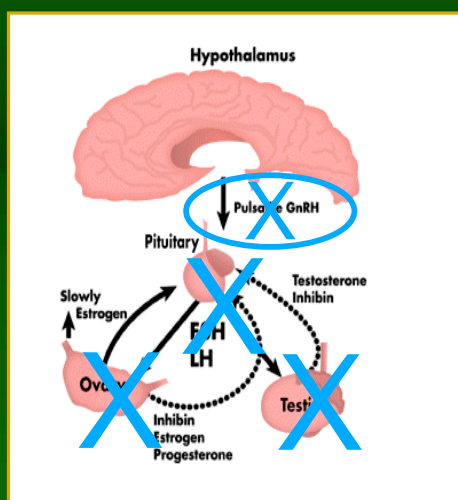
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Considerations for Feline Nonsurgical Contraception

- Delivery and targeting
- Time to contraception
- Duration of contraception
- Hormone production
- Effectiveness
- Sex and age
- Safety
- Cost



Immunocontraceptive Targets



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GonaCon Vaccine

- Synthetic GnRH
- KLH carrier protein
- *Mycobacterium avium* oil in water adjuvant (diluted Mycopar)
- Single intramuscular injection

Pilot Study – Proof of Concept

- Adult male cats
- 4 groups of 3 cats
 - Placebo control
 - 50 ug GnRH
 - 200 ug GnRH
 - 400 ug GnRH



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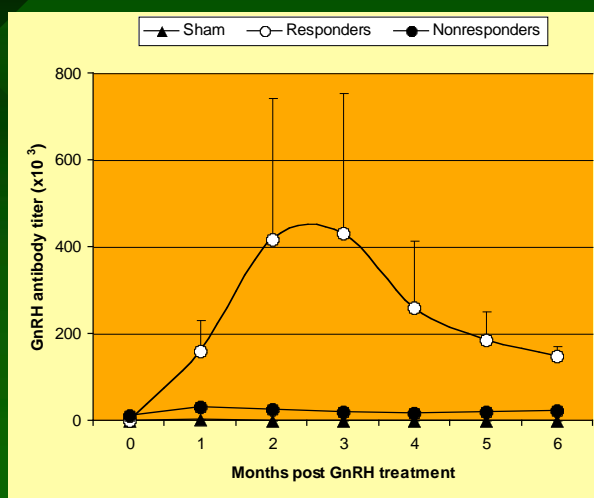
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Monitoring

- Serum (collected monthly)
 - GnRH antibody titer
 - Testosterone
- Semen (collected at 6 months)
 - Concentration
 - Motility
 - Morphology
 - Viability
- Secondary sex characteristics
 - Penile spines
 - Testicular volume
 - Testicular histology



GnRH Antibody Responses

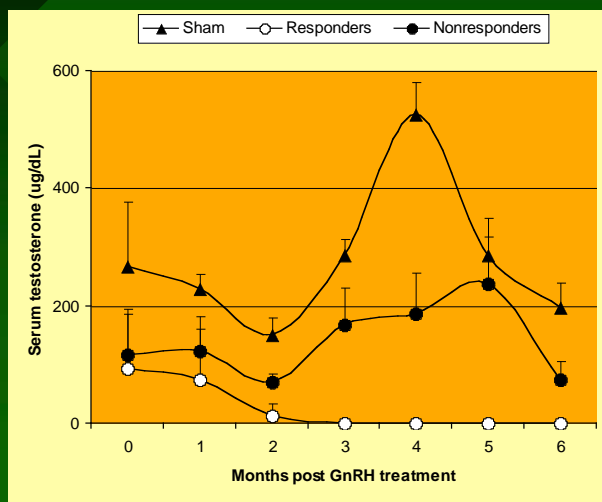


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Serum Testosterone



Results: Semen Analysis

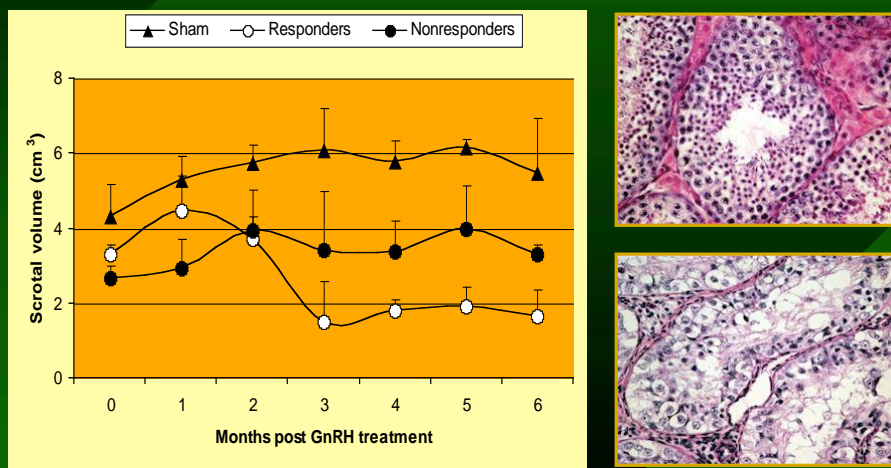
- All 6 responding cats had complete lack of viable sperm
- 3 nonresponders had normal to slightly compromised semen quality
- Control cats had normal semen quality

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Testes Size and Histology



Physiologic Response



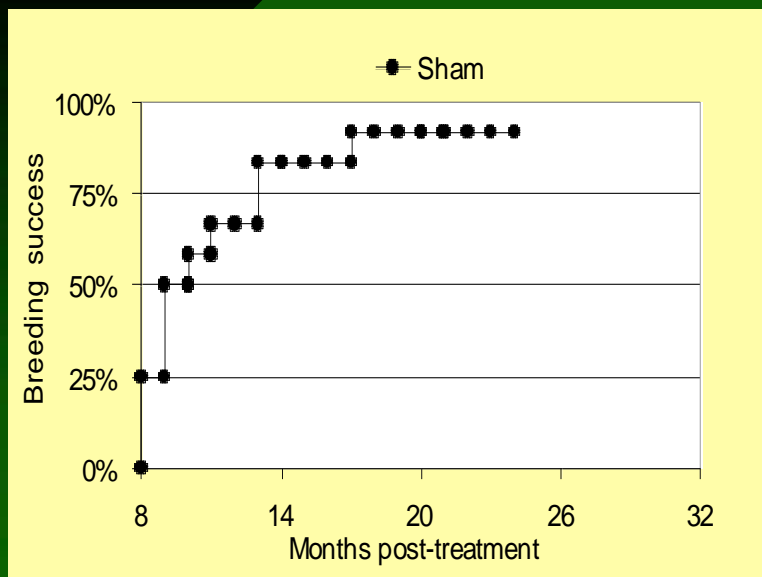
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Pivotal Trial: Duration of Immunity

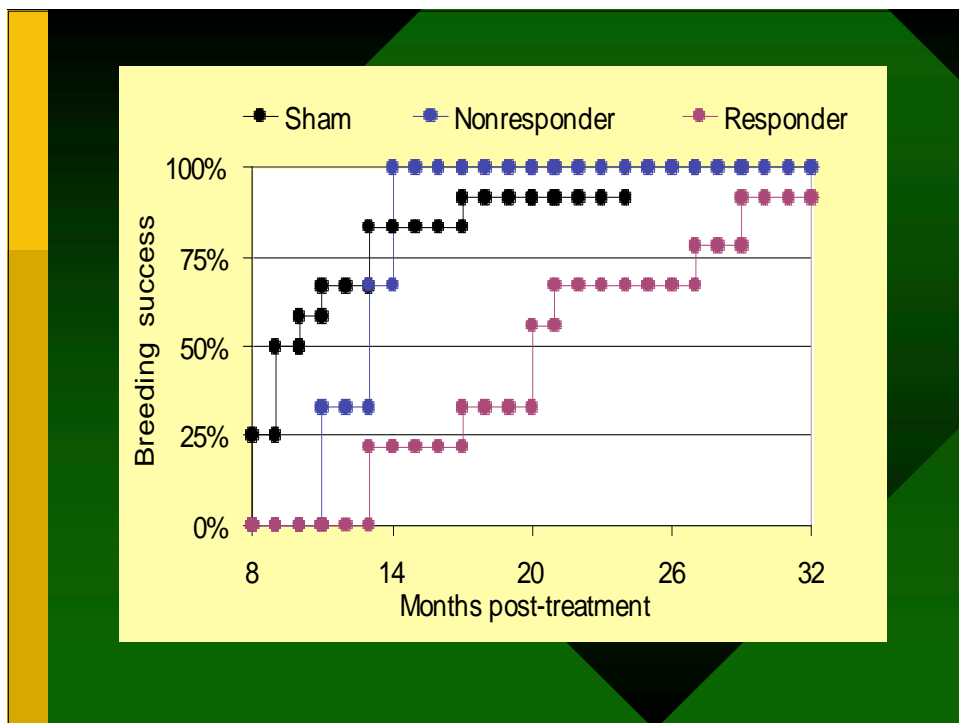
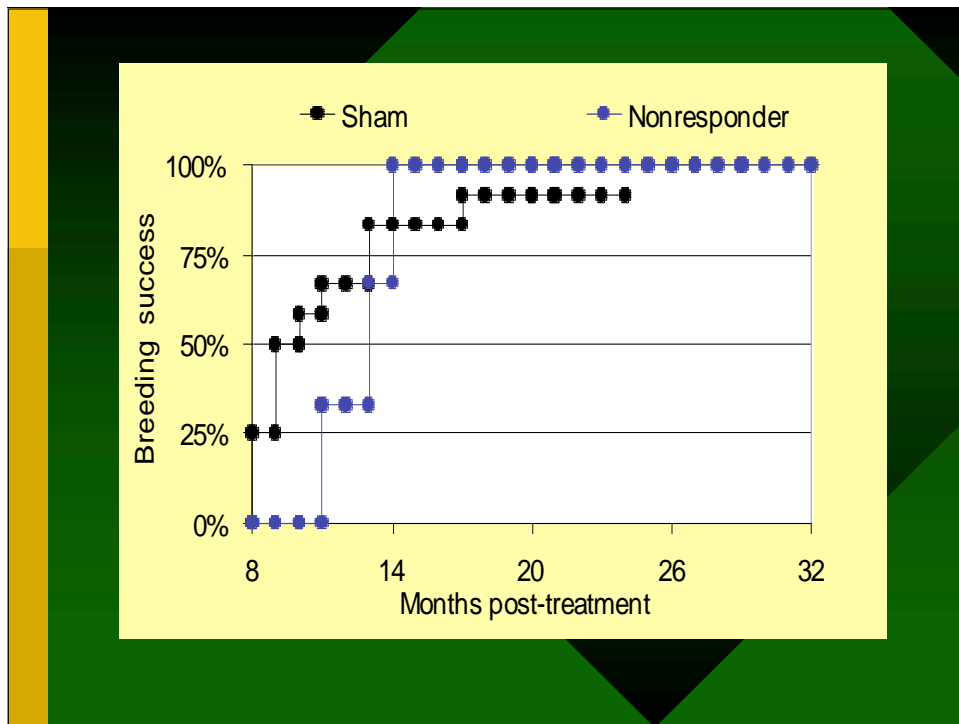
- Adult male cats
 - 12 controls, 12 vaccinates
- 200 ug GnRH-KLH vaccine
- Serum and semen collected monthly for 6 months, then bimonthly
- Breeding trial started at 10 months



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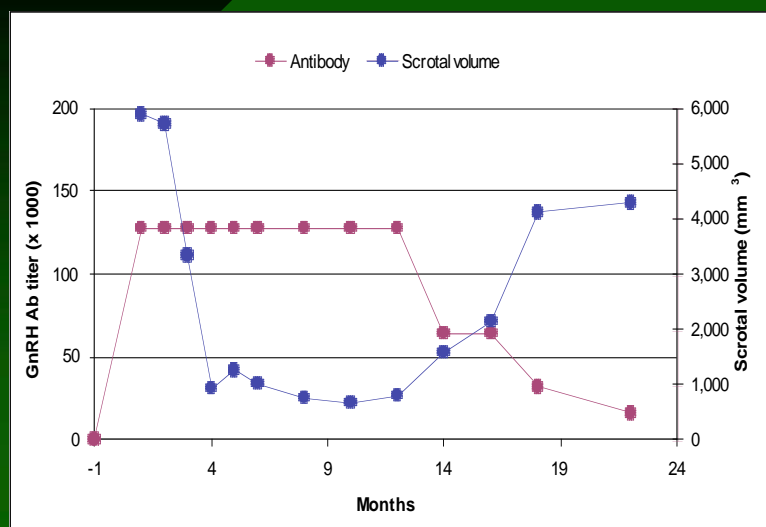
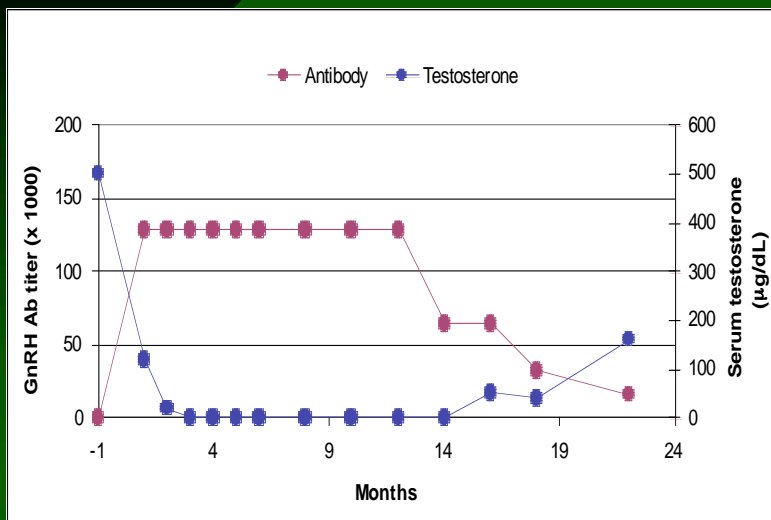
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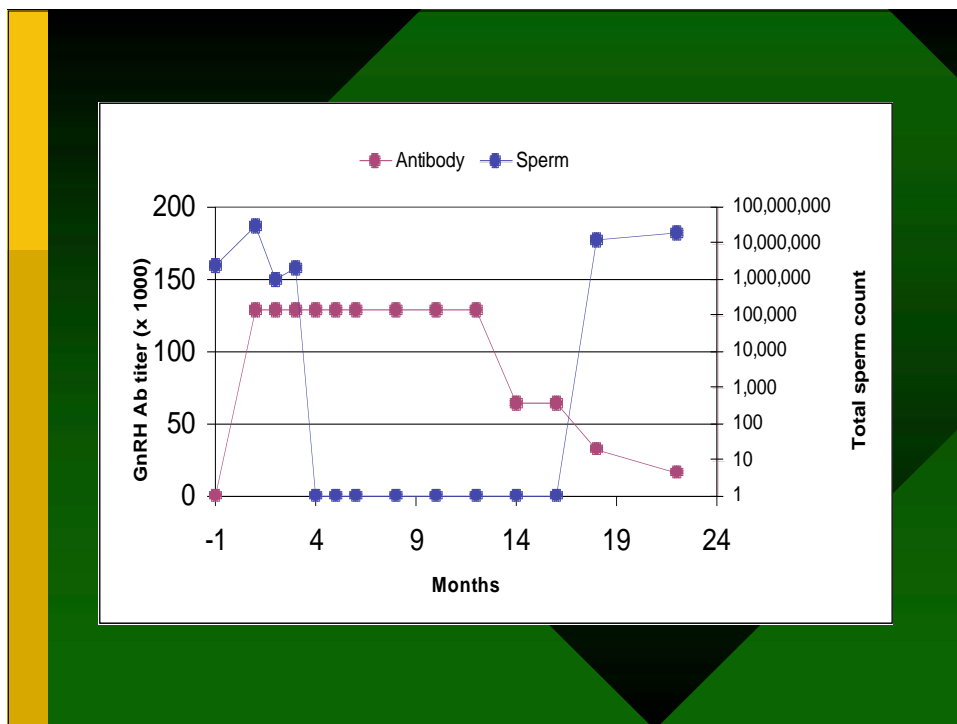
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Female Cats

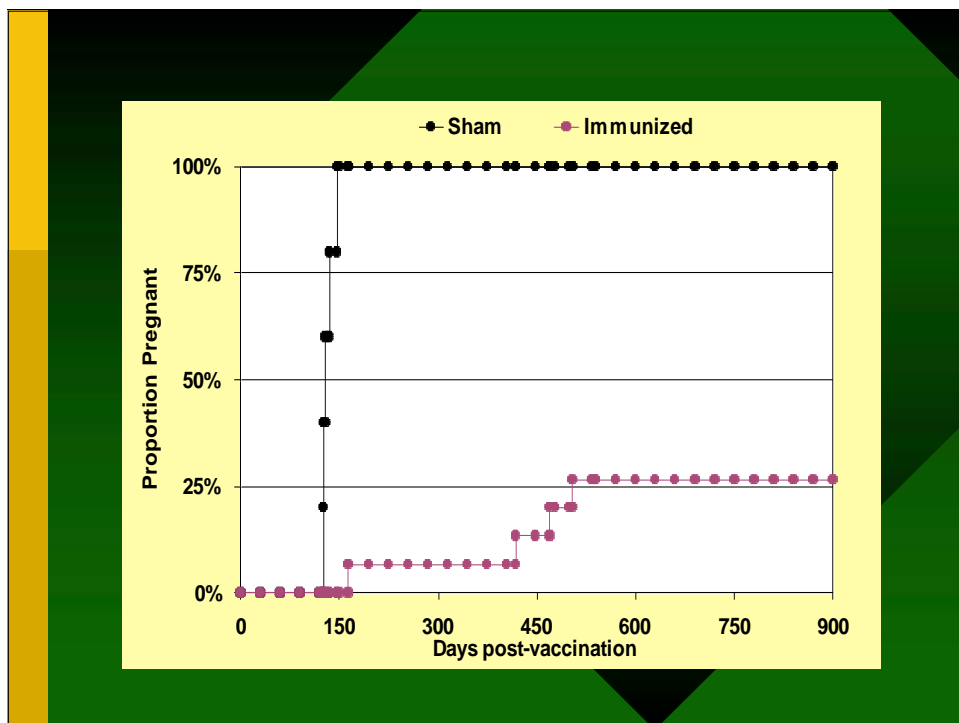
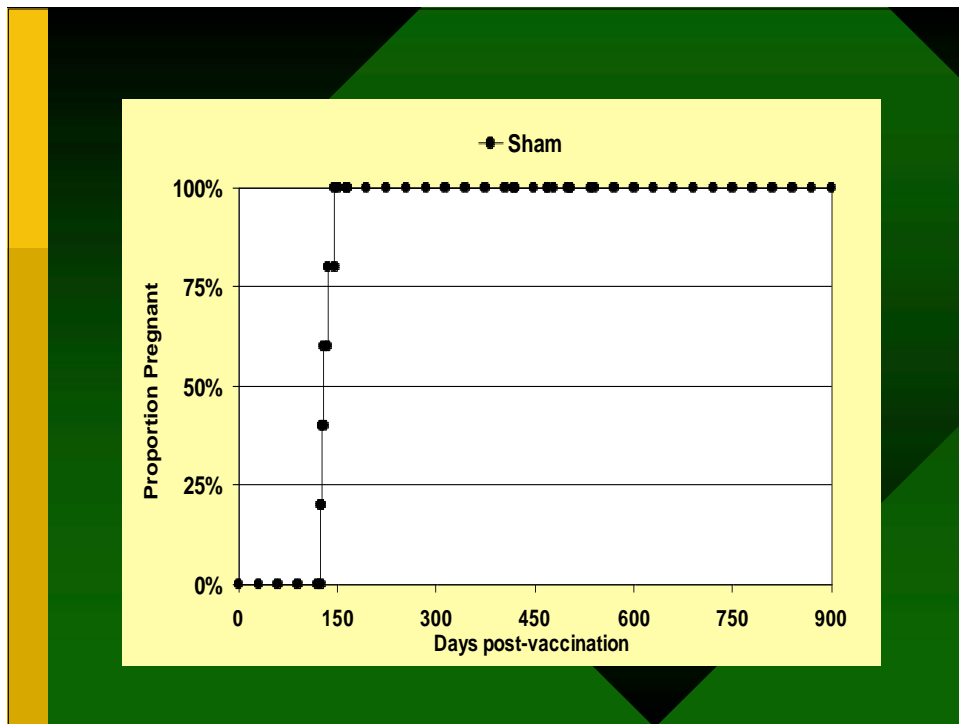
- 5 controls
- 15 vaccinates
- Monthly: GnRH titers, estrogen, progesterone
- Change to long day length light cycle at 4 months post-immunization to induce estrus and begin breeding trial



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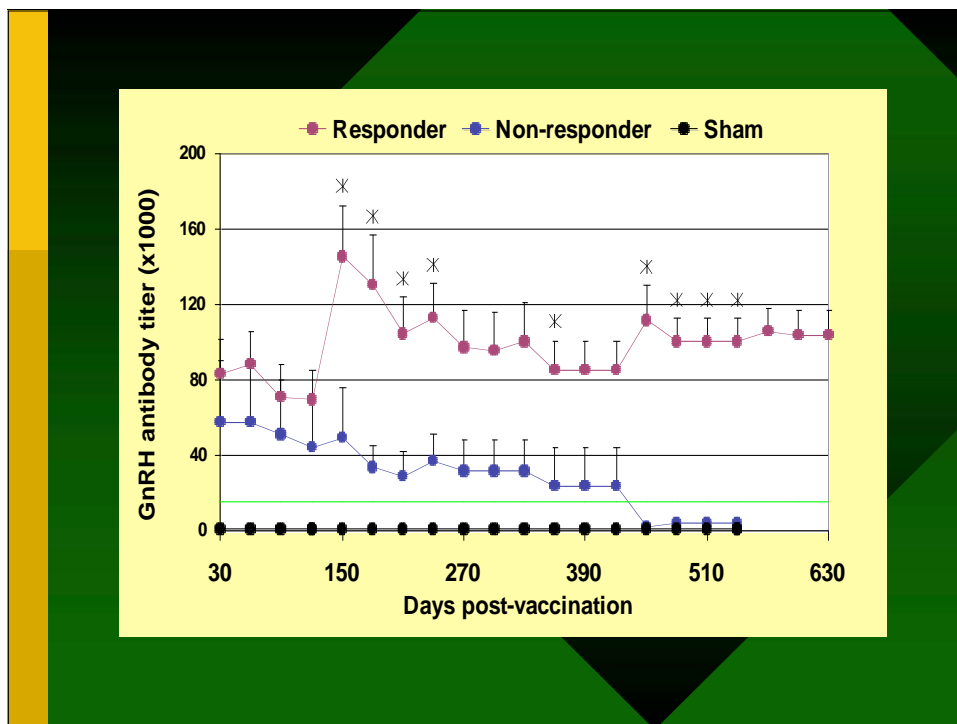
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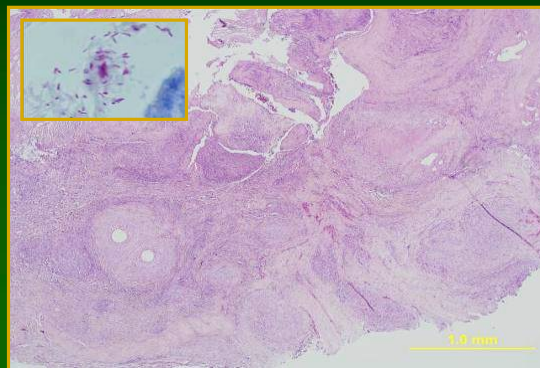
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Injection-Site Reactions

- Late onset – 24 months
- 6/20 cats – all responders



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Potential for Feral Cat Immunocontraception?

- Easily administered to unsedated cats in the field
- Activity in females >> males
- Effective in $\frac{3}{4}$ of female cats for >30 months
- Probably safe but significance of injection site reactions uncertain

Acknowledgements



*Morris Animal Foundation
PETsMART Charities
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