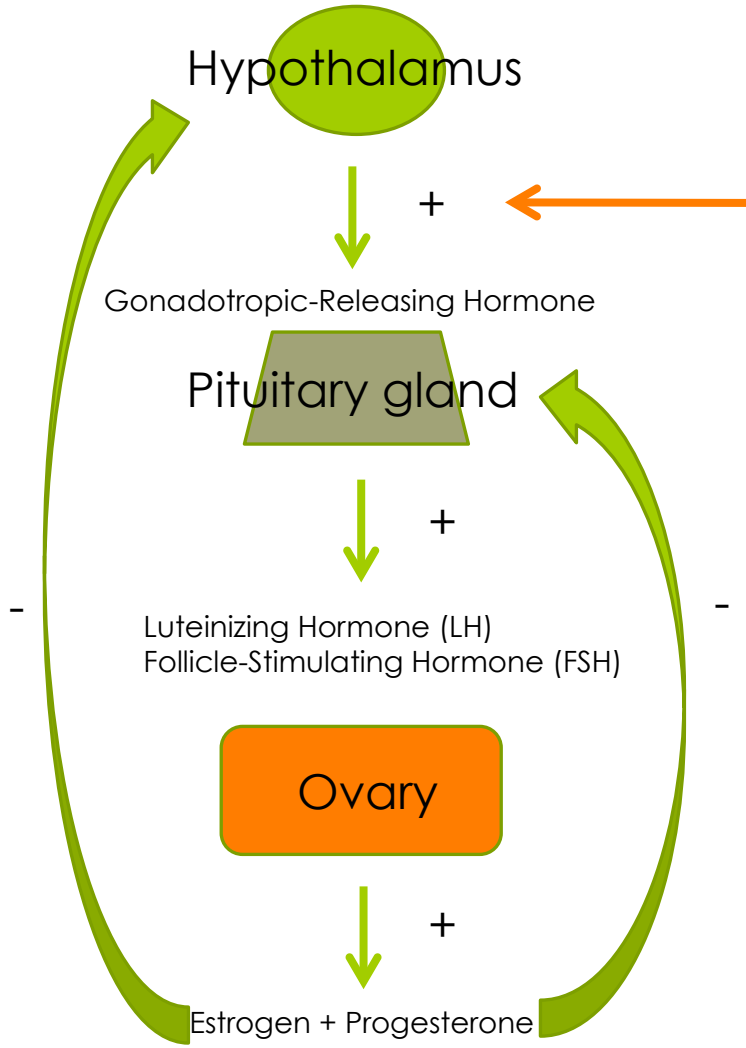
EFFECTIVENESS OF GONACON ™ IN COLONY-HOUSED CATS

Joanne Maki, Amy Fischer, Valerie AW Benka, Joyce Briggs, Marc-Antoine Driancourt, Darcy SO Mora, Kevin Morris, Kayla A Myers, Linda Rhodes, Lindsey M Vansandt, George Robert Weedon, Julie Wolf and Julie K Levy

6TH INTERNATIONAL SYMPOSIUM
on Non-Surgical Contraceptive
Methods of Pet Population Control
ACC&D
BOSTON | JULY 22-24, 2018

GNRH vaccine mechanism - female



Anti-GnRH antibody

- Binding of GnRH to circulating antibody in capillary region of hypothalamus forms large immune-complexes that travel down the hypophysial stalk
- Complexes remain in the venous blood and leave the pituitary without stimulating the release of LH and FSH

GonaCon™:

GnRH Peptide [pEHWWSYGLRPGGC-SH]

+

Carrier Protein (KLH or blue protein)
Coupled at 30:70 ratio

+

Adjuvac™ [modified Mycopar™*]

↓

Emulsion Vaccine

Miller, et al, 2008

*Cattle bacterin containing mineral oil

Pen and Field Studies of GonaCon™

- Recent studies with free-ranging California ground squirrels, captive Norway rats, domestic and feral swine, wild horses, and white-tailed deer have demonstrated the efficacy of the single-shot GnRH vaccine as a contraceptive agent.
- Infertility among treated female swine and white-tailed deer, for example, has lasted up to five years without requiring a booster vaccination.

https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/programs/nwrc/research-areas/sa_reproductive_control/ct_gnrh

So what about cats?

Levy, et al., 2004 – males

Levy, et al., 2011 – females

Vansandt, et al., 2017 - females

Fischer, et al., 2018 - females

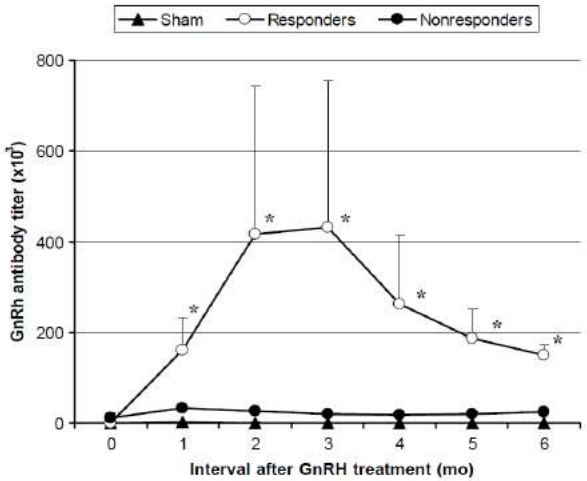


Fig. 1. GnRH antibody titer (mean ± S.E.) following a single treatment with a GnRH immunocontraceptive vaccine ($n = 9$) or sham treatment ($n = 3$). Six treated cats were classified as responders (titer >32,000) and three cats were nonresponders (titer 4000–32,000). Responders had significantly higher antibody titers than nonresponders from 1 to 6 months post-GnRH treatment ($^*P < 0.05$).

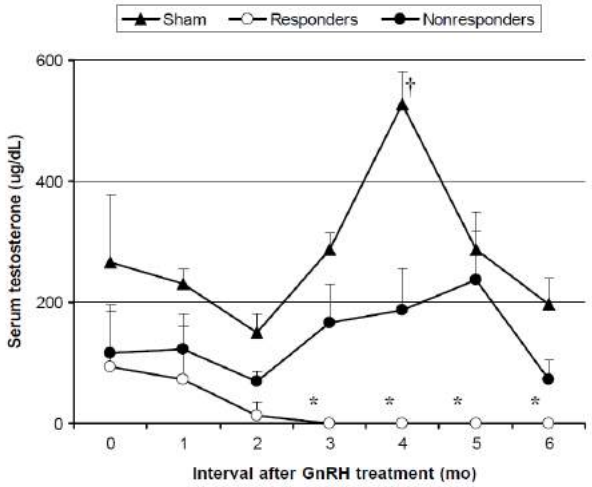


Fig. 2. Serum testosterone concentration (mean ± S.E.) was significantly lower than pretreatment values in responder cats by 3 months post-GnRH treatment ($^*P < 0.05$). Testosterone concentrations did not change significantly in sham-treated ($P = 0.2$) and nonresponder cats ($P = 0.05$), except at 4 months when sham-treated cats has a transient increase in testosterone ($^\dagger P = 0.01$).

GnRH immuno-contraception of male cats (*Levy, et al, 2004*)

- 12 adult male cats/4 groups
- 1 dose i.m.
- 50, 200, or 400 µg GonaCon
- Parameters:
  - 6 cats Ab titer >32,000
- Responders:
  - Testicular size and testosterone levels were decreased
  - Histology confirmed reduced sperm production
  - Fertility was not evaluated

Levy, JK, LA Miller,, PC Crawford, JW Ritchey, MK Ross. GnRH immunocontraception of male cats. Theriogenology 62 (2004) 1116-1130

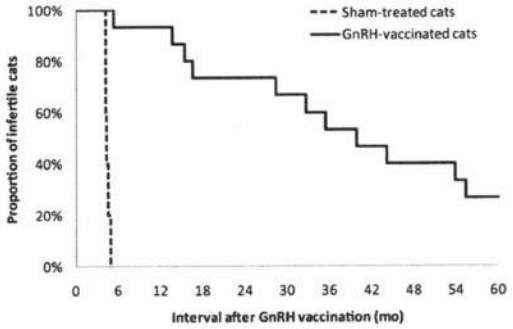
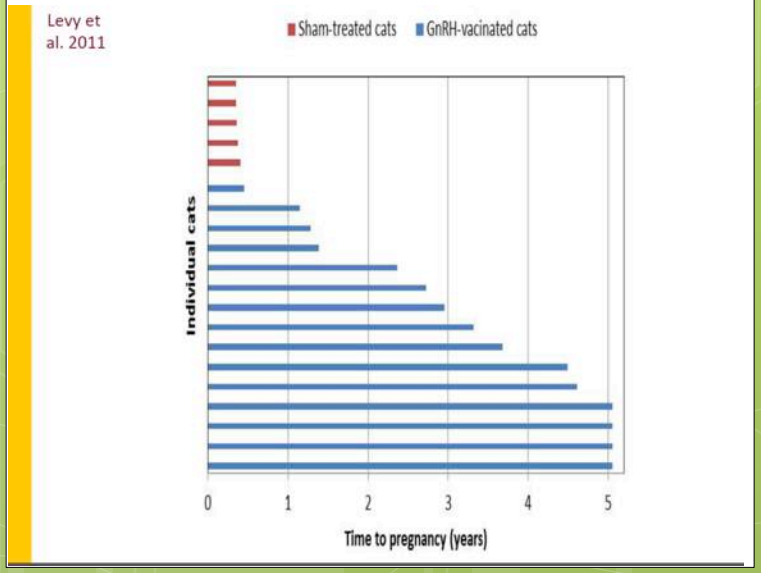


Fig. 3. Fertility control in cats. Vaccinated cats (n = 15) received a single injection of vaccine containing a GnRH-KLH conjugate (200 μg) in a mycobacterial and oil emulsion on study Day 0. Sham-treated cats (n = 5) received a single injection containing all vaccine components except the GnRH-KLH conjugate (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; $P < 0.001$).



GonaCon single dose in SPF female cats under laboratory conditions (*Levy, et al 2011*)

- One i.m. dose GnRH-KLH
- Males introduced 120 post-vax
- Mean Time to conception:
  - Controls (n=5) 4.4 months
  - Vaccinates (n= 15) 39.7 months
- Conception
  - Long-term responders (n = 11) > 2yr
  - Short-term responders (n = 4) < 2yr
- Percent infertile:
  - 1 year = 93% (14)
  - 2 years = 73% (11)
  - 3 years = 53% (8)
  - 4 years = 40% (6)
  - 5 years = 27% (4)

Levy, JK, JA Friary, LA Miller, SJ Tucker, KA Fagerstone. Long-term fertility control in female cats with GonaConTM, a GnRH immunocontraceptive. Theriogenology 76 (2011) 1517-1525

Levy et al, 2011:

- Vaccinates had longer time to conception
  - Infertility of 93% for 1yr
  - 53% for 3yrs
- GnRH antibodies declined more rapidly in cats with short term infertility (< 2yrs)
- No absolute GnRH titer was predictive but antibody persistence was associated with infertility
- Non-painful but persistent granulomas at the injection site appeared 2 yrs after injection in 5 cats
- Multiple factors influence the immunocontraceptive effect in female cats; however, the GnRH pathway is an ideal candidate for further development

Dr. Julie Levy's studies demonstrated **safety, efficacy, and suppression of sexual behaviors** in laboratory cats.

- Activity more durable and predictable in females than males
- Median time to pregnancy for females ~3+ years
- Contraceptive effect directly correlated with antibody titers
- Long-term injection-site reactions observed

Slide by Amy Fisher-Brown, 2016 ACC&D Council of Stakeholders

GonaCon™ formulation comparison:

- USDA-National Wildlife Research Center, Fort Collins CO
- Formulation identical to:
  - GonaCon Immunocontraceptive Vaccine (EPA 56228-40) and
  - GonaCon-Equine (EPA 56228-41)

	Levy, et al, 2004 Levy, et al, 2011	Vansandt, et al, 2017 Fischer, et al, 2018
Route	i.m.; quadriceps	i.m.; quadriceps
volume	0.5 ml	0.5 ml
Carrier protein	KLH	Blue protein
GnRH-protein concentration	50, 200 or 400 µg/ml	1,000 µg/ml
Adjuvant	Adjuvac	Adjuvac
M. avium concentration	166 µg/ml	166 µg/ml

(modified from: Vansandt, et al, 2017)

CREW STUDY

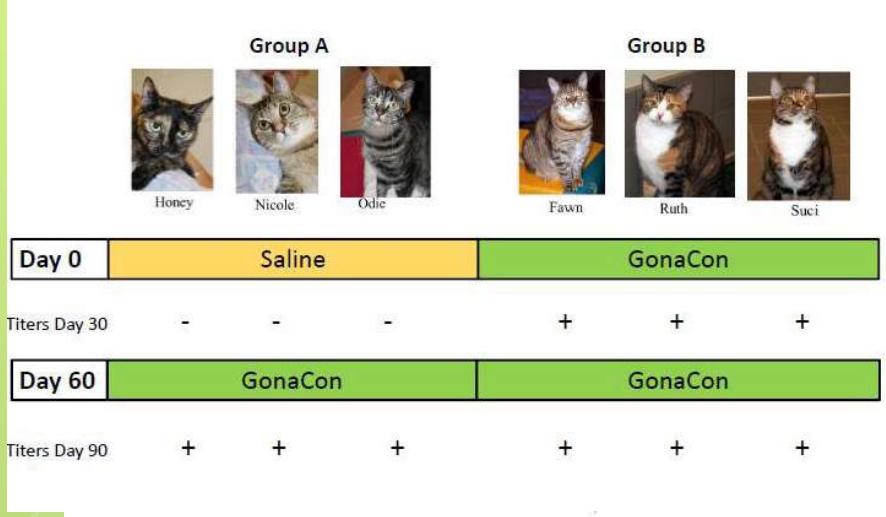


TABLE 3 Adverse vaccine reactions following a single or double treatment with GonaCon

	Day					
Cat[a]	0–29	30–59	60–89	90–119	120–149	≥150
D1	–	–	+	+++	++	–
D2	–	–	–	–	–	–
D3	–	–	++	++++	+++	+++
S1	±	–	–	–	–	–
S2	++	–	+	–	–	+
S3	–	–	–	–	–	++

Days are numbered relative to first GonaCon injection. Cats in Group A (D1–3) received their second GonaCon injection at Day 60.
Longest dimension of mass: (+) <1 cm diameter, (++) 1–1.9 cm diameter, (+++) 2–2.9 cm, (++++) >3 cm, (±) inflammation without palpable mass.
[a]D: cat receiving double injection, Group A. S: cat receiving single injection, Group B.

Graphic by Amy Fisher-Brown, 2016 ACC&D Council of Stakeholders

Single and repeat intramuscular injection of (GonaCon™) in adult female domestic cats (*Vansandt, et al, 2017*)

- Short-term safety
- Six spayed female cats
- One versus two i.m. doses
- Formula: 1,000 µg/ml BP

- All cats had GnRH antibodies
- Granulomas in 4/6 cats
  - Mean appearance = 110 days
- Safety OK for further study

Vansandt, LM, MA Kutzler, AE Fischer, KN Morris, WF Swanson. Safety and effectiveness of a single and repeat intramuscular injection of a GnRH vaccine (GonaCon™) in adult female domestic cats. Reprod Dom Anim 2017; 52 (Suppl. 2) 348-353

Hypothesis

A single intramuscular injection of GonaCon will result in **prolonged** (population median ≥ 3 years) and **safe** contraception of female cats under simulated free-roaming cat colony conditions.

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Slide by Amy Fisher-Brown, 2016 ACC&D Council of Stakeholders

Clowder Concepts, LLC:
USDA APHIS inspected research facility

Contraceptive research: a laboratory and clinical perspective
Tue Jul 24, 2018
2:05 PM - 3:30 PM
Theater

Photos by Amy Fisher-Brown, 2016 ACC&D Council of Stakeholders

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SAGE

Effectiveness of GonaCon as an immunocontraceptive in colony-housed cats

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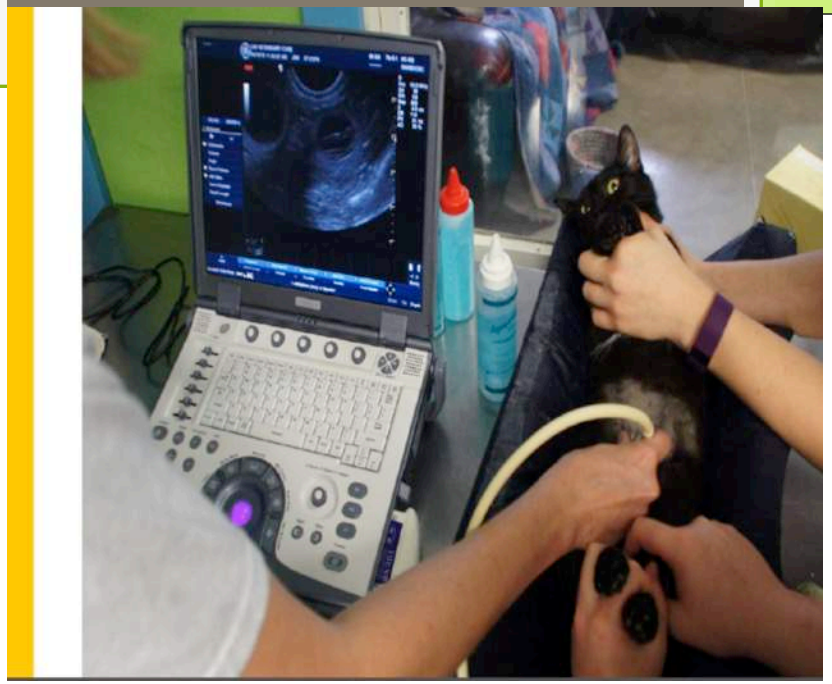
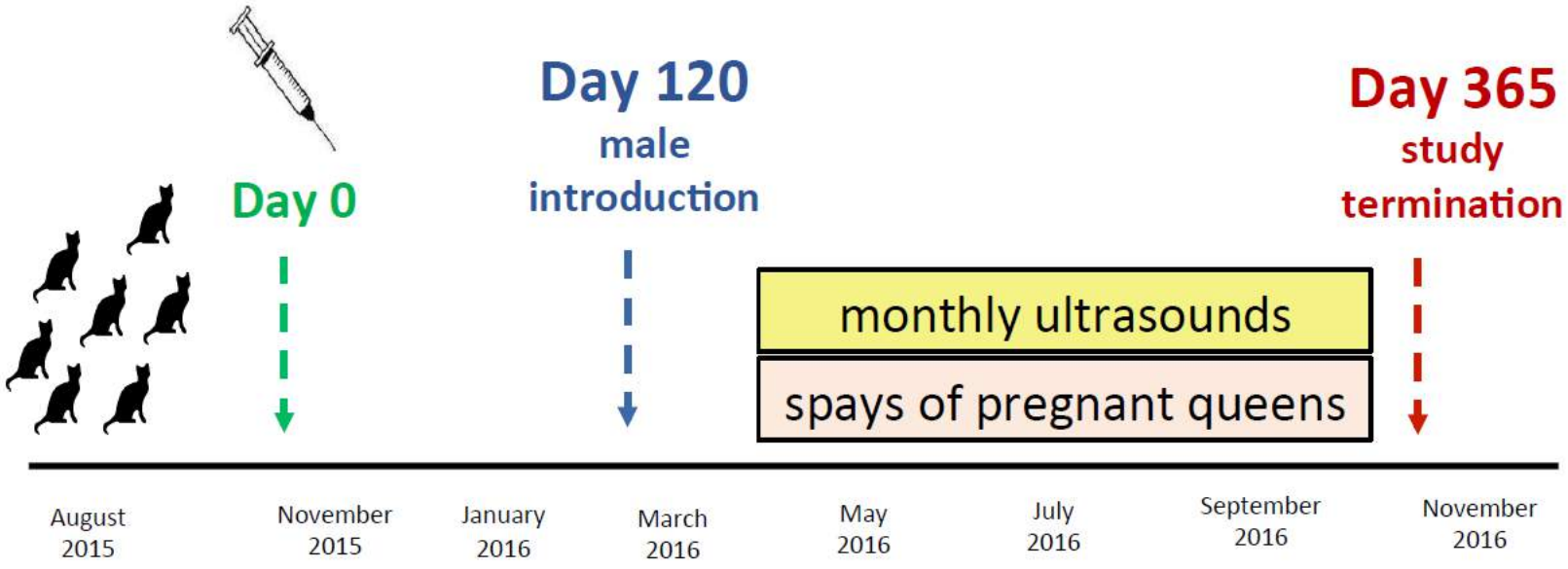
Materials and Methods

- 20 female vaccinates
- 10 female controls
- Day 0 = Vax – 1 dose i.m.

- Males introduced Day = 115
- Rotation of 5 males

- Daily observations
- Palpation of vax site
- Monthly pregnancy exams
- Pregnant cats spayed
  - 0-8 days post-detection





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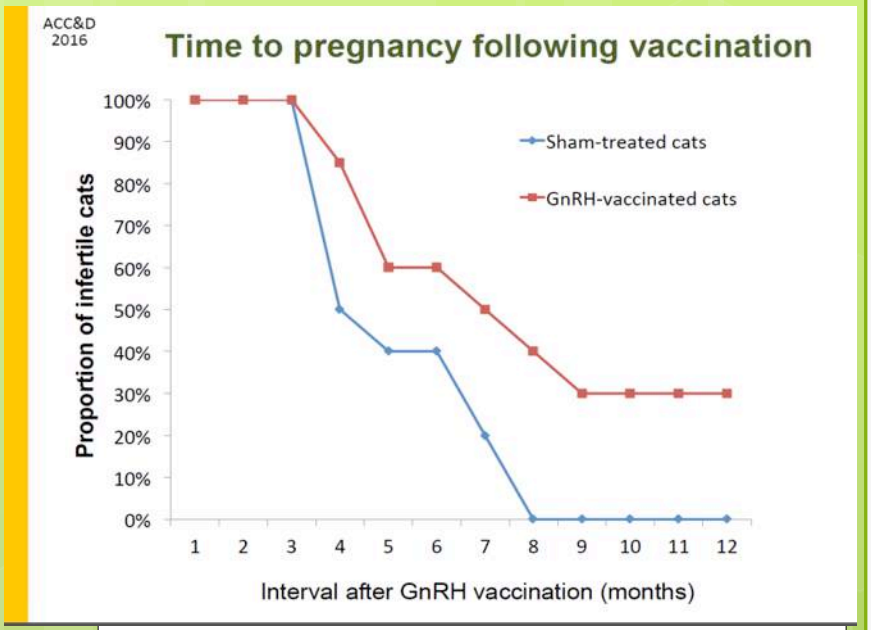
Results:

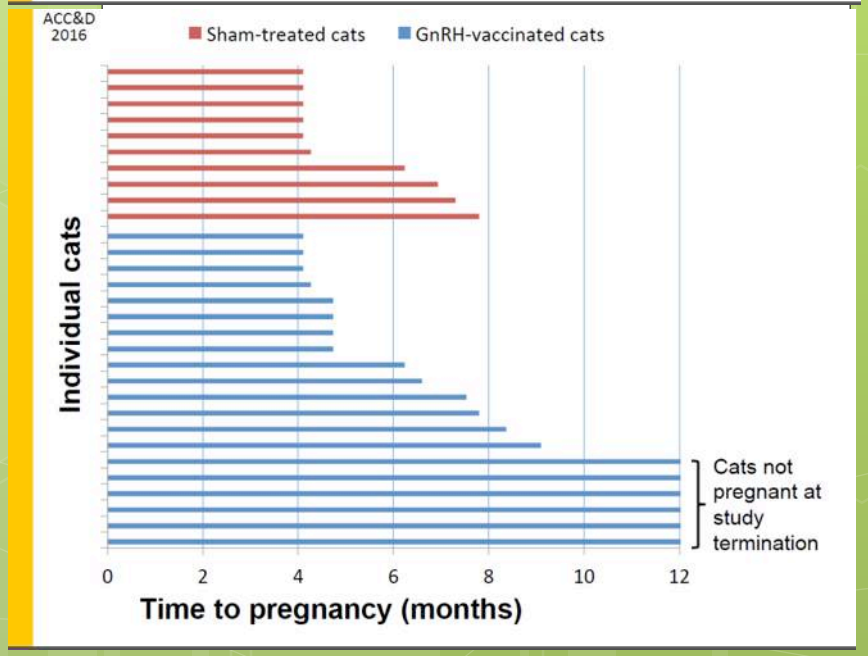
Breeding trial:

- 6 controls (60%) and 8 vaccinates (40%) pregnant 30 days
- 10 controls (100%) and 12 vaccinates (60%) pregnant 4 mos
- 2 additional vaccinates pregnant < 1year
- The remaining 6 vaccinates demonstrated infertility for ~ 1yr

Estrous behaviors:

- Estrous behavior was never observed in 4 of 6 vaccinates that did not become pregnant
- Two queens that did not become pregnant did display estrous behavior but did not conceive





Effectiveness of GonaCon as an immunocontraceptive in colony-housed cats (*Fischer, et al, 2018*)

- One i.m. dose 1000 µg/BP
- Males introduced 115 post-vax
- Study terminated Day 363

- Mean Time to conception:
  - Controls (n=10) 127.5 days
  - Vaccinates (n= 20) 212 days

- Conception
  - 60% of vaccinates pregnant
  - 100% of controls within 4 months

- Infertility:
  - 6 cats at ~ 1 year post-vax

Fischer, A, VAW Benka, J Briggs, <-A Driancourt, J Maki, D SO Mora, K Morris, KA Myers, L Rhodes, LM Vansandt, GR Weedon, J Wolf,JK Levy. Effectiveness of GonaCon as an immunocontraceptive in colony-housed cats. Journal of Feline Medicine and Surgery, February 2018, 1-7

Safety: 9 vaccinates with delayed site reactions

Cat	Days												
	0-29	30-59	60-89	90-119	120-149	150-179	180-209	210-239-	240-269	270-299	300-329	330-359	360-389
3	-	-	-	-	-	-	-	-	-	-	+	A	+
8	-	-	-	-	-	-	-	+++ +++	+++ +	+	+	A	A
9	-	+++ +	+++	-	-	-	-	-	-	-	-	-	-
12	-	++	+/-	-	-	-	-	-	-	-	-	-	-
15	-	+++ ++	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	+++ ++	+++	+++	A	A	++
31	-	+/-	-	-	-	-	-	-	-	-	-	-	-
36	-	+++ +++	+++ ++	-	-	-	-	-	-	-	-	-	-
38	-	+/-	-	-	-	-	-	-	-	-	-	-	-

+ = <1 cm; ++ = 1-1.9 cm; ++ 2-2.9 cm; +++ = 3-3.9 cm; ++++ = 4-4.9 cm; +++++ = 5-5.9 cm;
+ = inflammation without mass; A = adopted cat not assessed

Results:

- Injection site reactions ranged from swelling to masses in 45% (9/20) vaccinates
- Two cats with masses did not become pregnant
- Vaccinates had slightly longer (P = 0.0120) median time to conception (212 days) compared to controls (127.5 days)
- Litter size was significantly smaller in vaccinates (3.9 $\pm$ 1.7) compared to controls 5.6 $\pm$ 1.8); P = 0.04
- All cats were sterilized and adopted

Conclusions:

- A single dose of GonaCon provided contraception in 30% (6/20) cats housed under colony conditions in this experiment
- The level of contraception was not sufficiently effective
- The level of contraception was significantly less than previous trials (Levy, et al, 2011)

Hypotheses

- Vaccine batch variability
- Individual cat variation
- Environmental conditions

Vaccine batch variability

- The formulation was the same as the CREW study but a different batch
- A retrospective review of manufacturing and handling conditions was conducted
- Post-study comparison of batches was not conducted

Individual cat variation

- Feline studies (n = 3) have shown variable GnRH antibody levels per study and per individual
- Previous studies have also had variable time of onset and duration of site reactions
- Variability was also observed in days to conception in vaccinates that became pregnant: 6 within 1 month; 4 within 119 days

Environmental conditions

- Variables between laboratory housed and free-roaming cats are many
- Wild white tail deer vaccinated with GonaCon showed less effect that captive deer but cats in this study were acclimatized, well fed and under veterinary care
- This study's environmental conditions were similar to anticipated field use conditions

Take away thoughts…

- The GnRH pathway remains a viable target for feline immunocontraception
- Multiple factors will continue to interplay and impact vaccine effectiveness under various field environments
- Persistence of serum GnRH antibodies overtime appears to be a key factor in males and females
- The cat colony model is an excellent environment to evaluate potential immunocontraceptive vaccine candidates

The Human Team

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USDA National Wildlife Research Center (NWRC)

Doug Eckery, PhD

Darcy Orahood Mora, MS

And many more invaluable contributors…

The Feline Team

Amaretto
Black Russian
Bobwhite Quail
Bon Bon
Figment
Frangelico
Gherkin
Hermione
Hostess Cupcake
Hot Toddy
Jello Shot
Klondike
Macchiato
Mariska Hargitay
Marlene Walker
Mimosa
Mojito
Monkey
Moonshine
Oyster
Ozzie
Pearl
Polly Pancakes
Princess Carolyn
Ruby Port
Samoa
Sassafras
Stella Artois
Tawny Port
Thirteen

Toms

Bathroom Kitty
Captain Obvious
Conway Kitty
Maxx
Sirius Black
Wicken
Wolfgang

Queens with cameos

Birdie
Gimlet
Mabel
Margarita
Maude
Ruby Port
Svetlana
Yo-Ho Tokyo Black

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Thank you