Phage-GnRH constructs for population control of feral animals: evaluation in cats

RL Jones, AK Johnson, CJ Kraneburg, AM Cochran, AM Samoylov, CL Barstow, JS Cannon, MS Korbely, JC Wright, RC Cattley, TI Samoylova

SCOTT RITCHEY RESEARCH CENTER

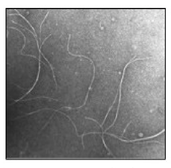
AUBURN UNIVERSITY COLLEGE OF VETERINARY MEDICINE

Abstract

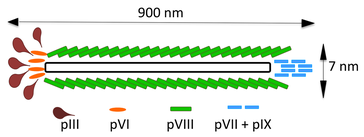
Our research focus is the development of anti-fertility vaccines composed of whole phage particles carrying peptides with contraceptive properties for use in feral animals. The vaccines are designed to trigger antibody production against gonadotropin releasing hormone (GnRH). The antibodies inactivate GnRH, causing reduced release of gonadotropic hormones and gonadal atrophy. Phage-GnRH constructs with potential contraceptive properties were generated via selection from a phage display library using cat and dog GnRH antibodies as selection targets, allowing identification of phages displaying GnRH-like peptides. When tested in mice, these constructs invoked the production of antibodies against GnRH and suppressed serum testosterone. The goal of this study is to evaluate the potential of these vaccines in cats. Sexually mature male cats were characterized as to their reproductive parameters and injected with a phage-GnRH vaccine according to the following treatment groups: single phage-GnRH vaccine with adjuvant (group 1, n=5), a phage-GnRH vaccine without adjuvant and a half-dose booster one month later (group 2, n=5), or a phage-GnRH vaccine with adjuvant and a half-dose booster with adjuvant three months later (group 3, n=5, in progress). Anti-GnRH antibodies and testosterone in serum, testicular volume by ultrasound, and quality and quantity of sperm were evaluated monthly during a 7-month period following immunization. All cats developed anti-GnRH antibodies of varying levels following immunization. Serum antibody levels increased significantly after booster immunization in groups 2 and 3. In group 1, serum testosterone was suppressed in four cats at three time points post-immunization. The total testicular volume (TTV) decreased in four cats in group 1 by a range of 22-42%, indicating potential gonadal atrophy. All experimental cats in groups 1 and 2 produced sperm at month seven with up to a 38% decrease in normal sperm cells. At three months after primary immunization, the TTV in group 3 had decreased by a range of 9-30%. This ongoing study has thus far demonstrated the potential of phage-GnRH vaccines for immunocontraception of cats.

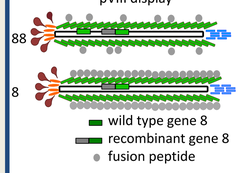
Introduction

Filamentous bacteriophage (phage)



pVIII display





- Bacterial viruses, not pathogenic for animals
- Composed of DNA + 5 coat proteins (4 minor+1 major)
- Can be engineered to display fusion peptides (antigens)
- Major phage vectors 88 and 8

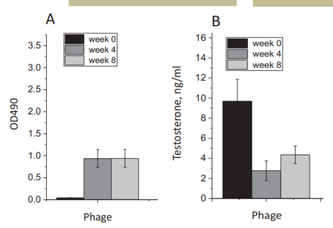
Generation of Phage-GnRH constructs

- Phage-GnRH constructs were selected from an existing type 8 phage display library using GnRH antibodies as selection targets.
- Five phage constructs displaying fusion peptides resembling the amino acid sequence of GnRH were tested in mice as a single dose vaccine.
- Phage-GnRH construct displaying EHPSYGLA fusion peptide stimulated production of anti-GnRH antibodies and suppressed serum testosterone.

Samoylov et al. Humoral immune responses against gonadotropin releasing hormone elicited by immunization with phage-peptide constructs obtained via phage display. J Biotechnol 2015; 216:20-28.

GnRH peptide: **EHWSYGLRPG**

Phage display peptide: **EHPSYGLA**



A: Antibody ELISA results at dilution 1:6400; absorbance expressed at OD_{490}.
B: Serum testosterone results expressed as ng/mL.

Immunocontraception

Stimulate production of antibodies against GnRH, the master reproductive hormone

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Antibody-bound GnRH cannot bind to its receptor on gonadotrope cells of the pituitary

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Gonadotrope cells do not release gonadotropins

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Without gonadotropins, the gonads do not undergo gamete or sex steroid production

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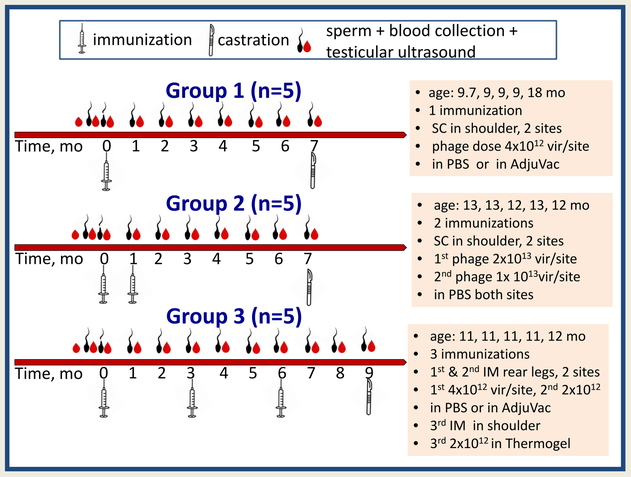
Infertility

Study design



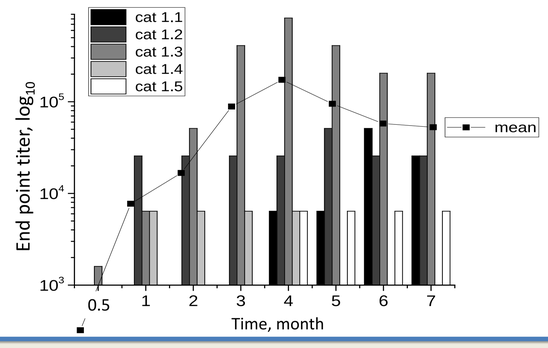
- age: 9.7, 9, 9, 9, 18 mo
- 1 immunization
- SC in shoulder, 2 sites
- phage dose $4x10^{12}$ vir/site
- in PBS or in AdjuVac

- age: 13, 13, 12, 13, 12 mo
- 2 immunizations
- SC in shoulder, 2 sites
- 1st phage $2x10^{13}$ vir/site
- 2nd phage $1x\ 10^{13}$vir/site
- in PBS both sites

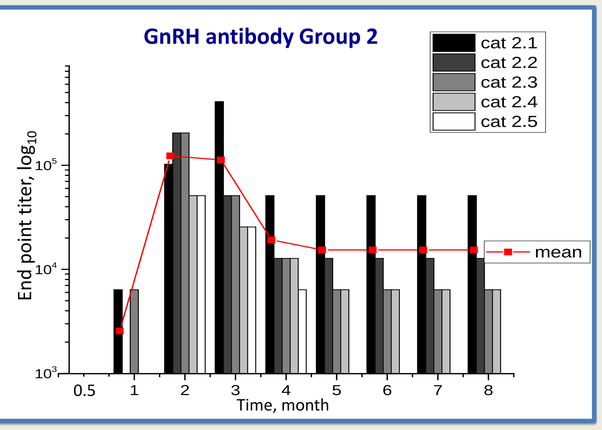
- age: 11, 11, 11, 11, 12 mo
- 3 immunizations
- 1st & 2nd IM rear legs, 2 sites
- 1st $4x10^{12}$ vir/site, 2nd $2x10^{12}$
- in PBS or in AdjuVac
- 3rd IM in shoulder
- 3rd $2x10^{12}$ in Thermogel

Results

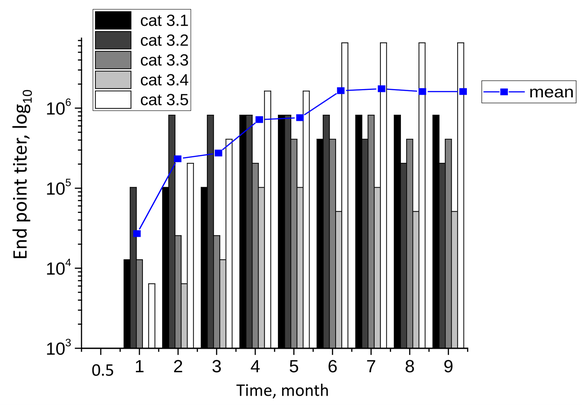
GnRH antibody Group 1



GnRH antibody Group 2

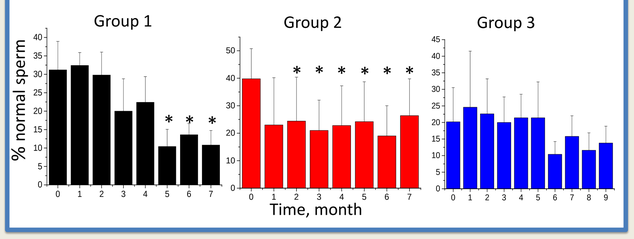


GnRH antibody Group 3



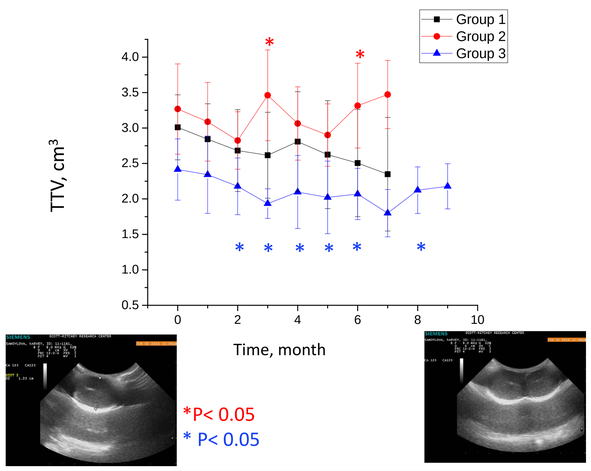
Sperm morphology

* P< 0.05



Results

Total testicular volume (TTV)



*P< 0.05
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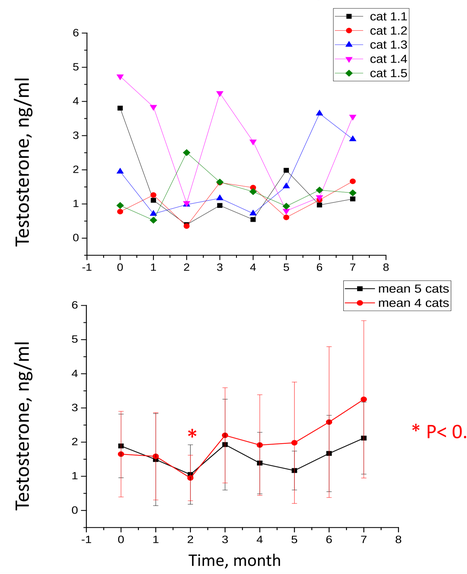
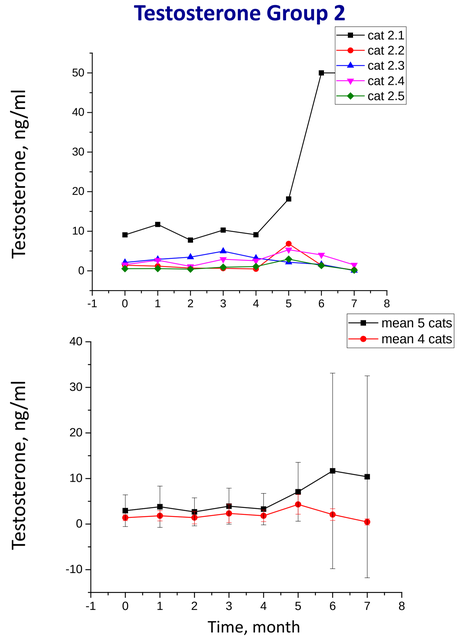
Testosterone Group 1



* P< 0.05

Testosterone Group 2

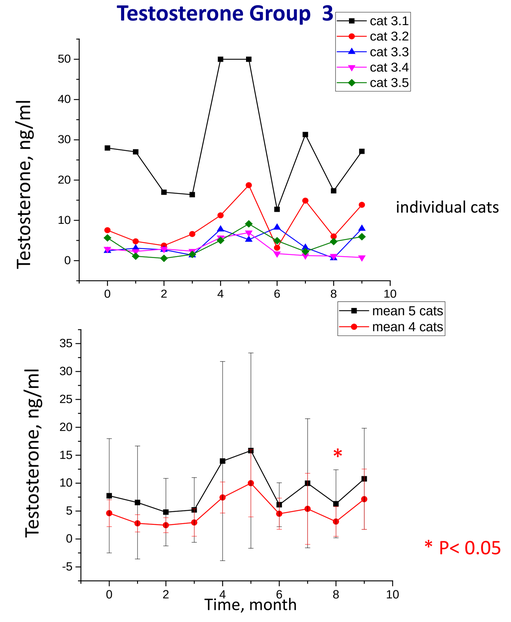


Sperm abnormalities

- Normally, sperm morphology improves, if cats collected regularly
- In cats groups 1 & 2 sperm morphology declines after immunization
- Many sperm cells with curled tails, probably, released prematurely
- Decrease in normal sperm cells usually indicates disruption of normal spermatogenesis

Results

Testosterone Group 3



* P< 0.05

Injection site reaction

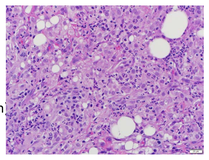
Group 1 (SC, AdjuVac)
- All cats developed inflammatory masses at AdjuVac injection site
- Firm, raised nodule
- Tended to increase for couple months, then slowly decreased
- Masses did not appear painful
- Biopsied at time of neuter (7 months after immunization)
- Not neoplastic
- 3/5 cats did not have masses 8 months after immunization

Raised nodule at AdjuVac SC injection site

Group 2 (SC, no adjuvant)
- No reaction to phage

Group 3 (IM, AdjuVac, Thermogel)
- No reaction to Thermogel
- 4/5 cats developed inflammatory masses at the site of 1st injection with AdjuVac
- All developed inflammatory masses at the site of 2nd injection with AdjuVac (opposite leg)
- Firm pulpable masses
- Tended to increase for couple months, then slowly decreased
- Masses did not appear painful, not interfere with movement
- Biopsied at time of neuter (9 months after initial immunization)
- 2/5 cats did not have masses at time of neuter
- 1 mass abscessed and was removed at time of neuter
- No histological results as of now

Histology of inflammatory masses



Inflammatory masses in muscle

AdjuVac

Thermogel

Statistical analysis

Data were analyzed using the general linear model (GLM) for repeated measures analysis of variance (ANOVA). For each group of cats each time point post-immunization was compared to pre-immunization month 0 using contrasts. P<0.05 shown by asterisks.

Conclusions

NOW
- Multiple phage-GnRH injections are required to suppress reproductive parameters. Not acceptable for feral cats, might be applicable for zoo animals.
- Immunization regimen appeared to be very important.
- Higher phage doses produced higher antibody responses.
- Booster immunizations increased antibody responses significantly.
- AdjuVac supported high-level antibody responses for extended time.
- Testosterone suppressed in cat Groups 1 & 3 at one time point.
- Size of testes decreased significantly in Group 3 at multiple time points.
- AdjuVac produced adverse injection site reaction in some cats

NEXT

Optimize immunization regimen:
- phage doses above 5×10^{13} vir/animal;
- multiple immunizations, less than 1 month apart;
- novel adjuvants that are potent/well tolerated by cats;
- test in young (prepubertal) cats.

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