



LANDSCAPE PHAGE-PEPTIDE CONSTRUCTS FOR LOW-COST IMMUNOCONTRACEPTION OF DOGS



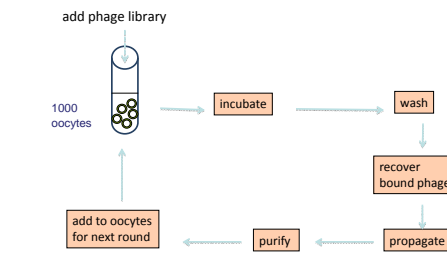
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ABSTRACT

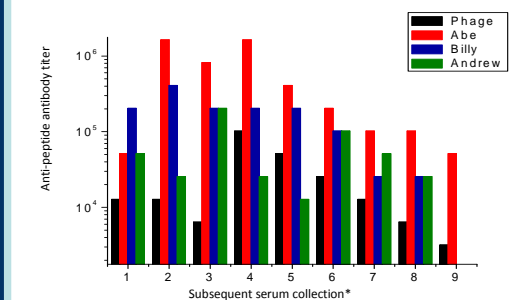
Dog overpopulation remains a serious welfare concern and is a worldwide problem. Our goal is to develop effective, low-cost contraceptive vaccines based on phage-peptide constructs where the peptides mimic sperm that bind to zona pellucida (ZP) proteins at fertilization. Administration of the vaccine would result in an anti-sperm antibody response that would interfere with sperm delivery or function in the male or female genital tract, leading to a contraceptive effect. Due to the natural ability of phage to stimulate B and T cell responses (without adjuvants), the vaccine may also inhibit spermatogenesis and steroidogenesis via induction of cytokine reactions in males. In this study, contraceptive vaccines are composed of landscape phage particles carrying ZP-binding peptides, where phage body plays the role of carrier protein/adjuvant and multiple (4000/phage particle) copies of a ZP-binding peptide stimulate production of anti-peptide antibodies. Multiple candidate phage-peptide constructs were selected from a 9-mer landscape phage display library using our novel selection procedure on intact dog oocytes surrounded by ZP proteins. Four of the candidates were injected intramuscularly into one-year old male dogs. Booster immunizations were given at 3 weeks and again at 7 weeks following initial immunization. Sera collected from immunized dogs were characterized as to the presence of anti-peptide/anti-sperm antibodies as well as testosterone levels and size of testicles. All phage preparations were shown to induce production of high levels of serum IgG antibodies that persisted for at least 5-6 months. Testosterone levels varied during the study showing some decrease (with the lowest testosterone amount of 0.3 ng/ml) in two dogs after booster immunizations. Interestingly, testicular widths in all dogs were decreased when measured 2-3 months after the second booster immunizations. To conclude, the identified phage-peptide constructs may be useful in the design of immunocontraceptive agents for dogs. Importantly, the cost of phage-based vaccines should be much lower than the cost of peptides vectored in mammalian viruses or the cost of production of synthetic peptides fused to a carrier protein because phages are bacterial viruses that can be easily and economically obtained in large quantities from bacterial cultures. Additionally, recombinant phage preparations based on landscape phage are very thermostable, making them very robust during shipping, storage, and operation.

IDENTIFICATION OF ZP-BINDING PHAGE



One selection round is shown
4 rounds required for peptide identification

IMMUNE RESPONSES TO PHAGE-PEPTIDE CONSTRUCTS



*Serum samples were collected every 2-3 weeks
Primary immunizations were followed by 2 boosters, at 3 wk and again at 7 wk

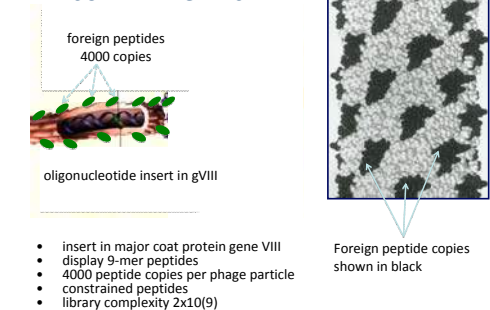
BACKGROUND

The direct use of phage as an immunogen is one of the very attractive approaches in vaccine development. In phage-based vaccines, phage body plays the role of carrier protein/adjuvant while multiple copies of a displayed peptide stimulate production of anti-peptide antibodies. In landscape phage, foreign peptides are displayed in each copy of the phage major coat protein pVIII (4000 peptide copies per phage particle), significantly increasing peptide antigenicity.

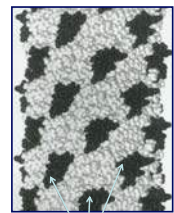
Phages are bacterial viruses that can be easily obtained in large quantities from bacterial cultures which makes the cost of phage preparations much lower than the cost of peptides vectored in mammalian viruses or the cost of production of synthetic peptides. Importantly, landscape phage preparations are very thermostable. They are stable for more than six months at room temperature, more than six weeks at 63° C, and three days at 76° C (Anal Bioanal Chem 2005, 382(6):1346-50), making landscape phage-based preparations very robust during shipping, storage, and use.

Here, we report our progress towards the development of **contraceptive vaccines based on landscape phage-peptide constructs** which, by the nature of its constituents, is (1) of low cost to produce, (2) stable to transport, store and deliver (3) easily administered via parenteral routes, and (4) likely to be quickly translatable into an approved product that is safe and practical. Most importantly, we expect these simple, easily obtainable phage-peptide preparations to be effective because of the expression of high numbers of peptide antigens and the natural immunogenicity of the phage particles without need for additional adjuvants.

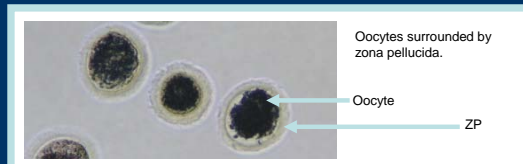
LANDSCAPE PHAGE DISPLAY



- insert in major coat protein gene VIII
- display 9-mer peptides
- 4000 peptide copies per phage particle
- constrained peptides
- library complexity 2x10⁹



Foreign peptide copies shown in black

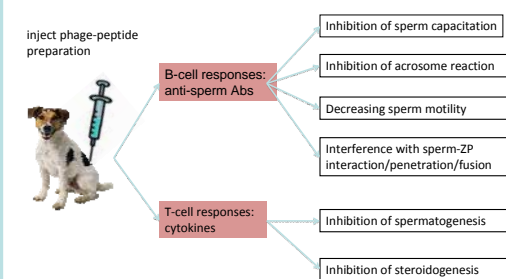


Oocytes surrounded by zona pellucida.

Oocyte

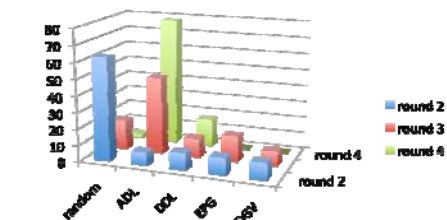
ZP

POTENTIAL CONTRACEPTIVE MECHANISMS



RESULTS

FREQUENCY (%) OF ZP-BINDING PHAGE CLONES IN SELECTION ROUNDS



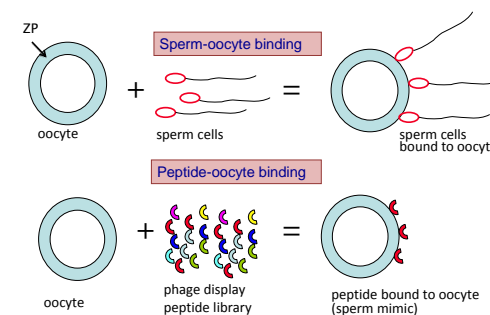
SEMEN QUALITY/TESTOSTERONE LEVEL TESTICLE WIDTH

Dog name	Sperm* count/ejaculate	Sperm motility	Testosterone range [^] , ng/ml	Testicle width, mm before tr/6 month later
Phage	2.9x10 ⁸	85%	0.3-2.4	21.5/18.5
Abe	No libido	No libido	0.8-5.9	18.5/18.9
Billy	2.5x10 ⁸	69%	1.7-6.4	21.3/19.9
Andrew	6.8x10 ⁴	60%	2.0-7.5	19.0/18.3

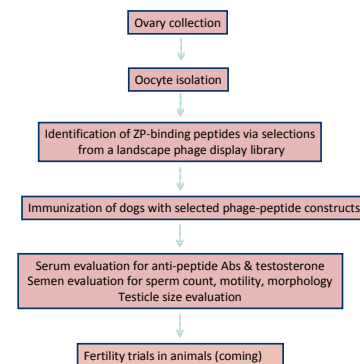
*Normal dog sperm count: ~ 3x10⁸/ejaculate; [^] Blood collected ~ same time of the day.

METHODS

OUR APPROACH – SELECT PHAGE CONSTRUCTS (SPERM MIMICS) BINDING TO ZPs ON OOCYTES



OUTLINE OF PROCEDURES



DOGS/PEPTIDE SEQUENCES USED FOR IMMUNIZATIONS

Name	Breed	DOB	Sex	Peptide sequence
Phage	beagle	4/5/08	male	ADLAIFYDD
Abe	beagle	4/20/08	male	DDLNSFVND
Billy	beagle	6/22/08	male	EPGGMVGD
Andrew	beagle	4/23/08	male	EVNGGSADS

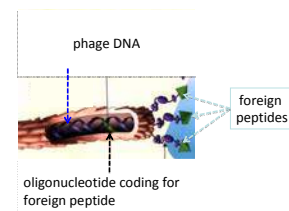


PHAGE DISPLAY PEPTIDE TECHNOLOGY

- Phage: virus that infects bacteria only
- Phage display library: mixture of billions of genetically engineered phages



Phage particle



Structure of phage particle from phage display peptide library

CONCLUSIONS

- We have developed a novel approach for identification of ZP-binding peptides that mimic sperm cells at fertilization
- Using this approach, multiple landscape phage-peptide constructs binding to dog ZPs were identified
- Four of these constructs were shown to be safe, stimulate production of high titers anti-peptide Abs, and affect sperm quality/libido in dogs
- Phage display might provide an alternative approach for identification of antigens with contraceptive potentials
- Phage might be a cheap substitute for protein-based or mammalian virus-based contraceptive vaccines

ACKNOWLEDGEMENTS

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