Viral Particle-Based Display of Multiple Antigens for Companion Animal Immunosterilization

Scott Coonrod, Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University

Immunocontraception – a reversible birth control method that uses the body's immune response to prevent pregnancy

Immmunosterilization – Use of immune system to permanently sterilize animal.

How does immunocontraception/immunosterilization work?

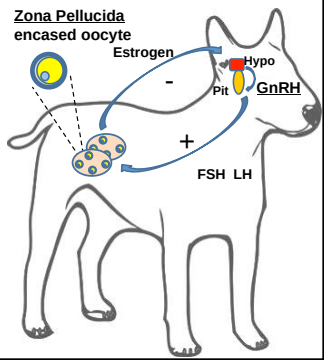
- ❖ Molecules from the reproductive axis that are essential for fertility are identified
- ❖ Recombinant (synthetic) forms of these molecules are produced using bacterial or eukaryotic systems
- ❖Inject reproductive antigen into host animal to generate an immune reaction that either neutralizes antigen or removes cell type that produces the antigen

Primary target for immunocontraception: The hypothalamic-pituitary-gonadal axis

Hypothalamus
❖ Secretes GnRH
❖Many other functions

Pituitary Gland
❖Secretes FSH LH which stimulates follicular maturation and ovulation

Ovary
❖Produces germ cells
❖Synthesizes estrogen and progesterone with estrogen feeding back and suppressing GnRH release



Two main immunological approaches to removing fertility antigens from animal to induce contraception.

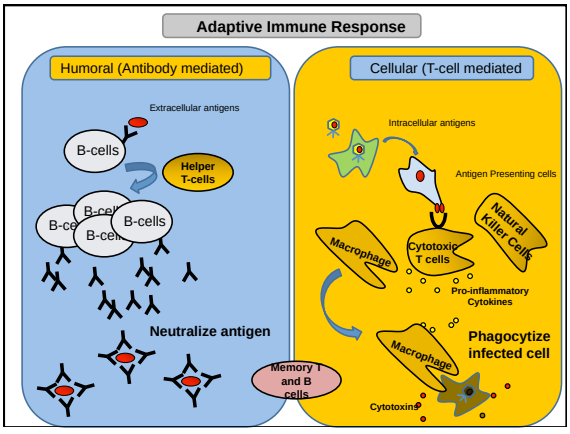
Antibody-based

Antibody removes target antigen (and possibly associated cells) by binding to the molecule rendering it functionally inactive. This technique can potentially be reversible once antibody titers go down

T-cell based

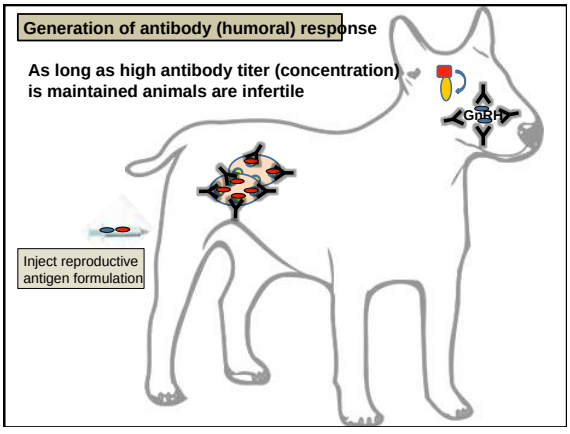
T-cells (such as Macrophages) attack and destroy cells containing target antigen. Technique can be permanent.

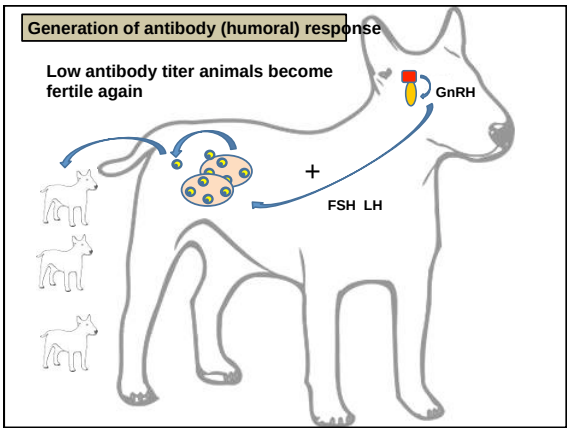
Immune response can be pushed toward antibody (B-cell) or T-cell based response depending on how the antigen is presented to the immune system.

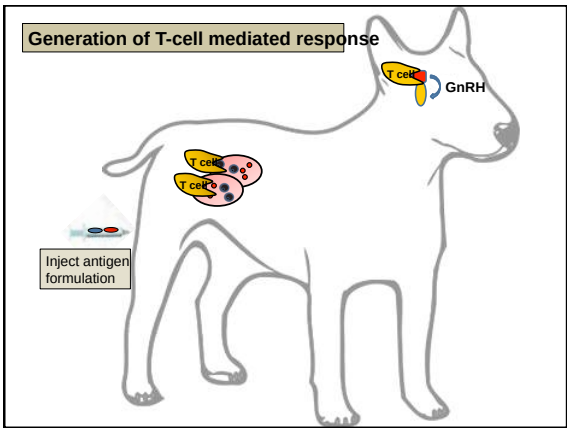


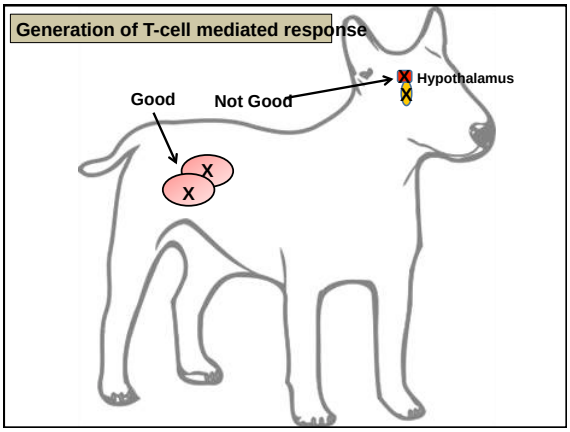
Generation of antibody (humoral) response

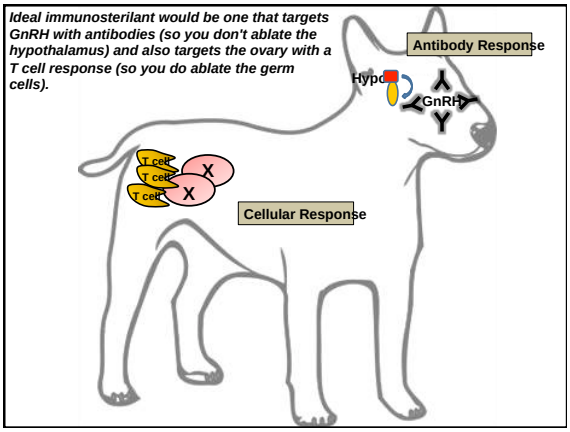
As long as high antibody titer (concentration) is maintained animals are infertile











How would such a immunosterilant be produced

❖State of the art method for targeting GnRH will be described
in next talk

❖Here I will focus on some strong candidate ovarian antigens
for T cell targeting and also discuss how that antigens could be delivered.

Tolerance, Regulatory T-cells (T Regs), and Autoimmunity

❖Tolerance - The ability of the immune system to distinguish self from non-self and not mount a response to self antigens. Autoreactive T cells are deleted and autoreactive T cells that escape deletion are controlled by Tregs

❖Regulatory T-cells - Tregs prevent immune responses from becoming too strong by "training" immune system to tolerate self antigens.

❖Autoimmunity – Induced by an overactive immune response which mistakes certain self molecules as foreign pathogens. Tregs are "overwhelmed" by response and cannot prevent attack of tissues expressing these self antigens by the immune system.

❖During autoimmune response, proinflammatory T cells , macrophages, and proinflammatory cytokines (TNF, IL-1B, GM-CSF) can be activated leading to recruitment of inflammatory cells to self antigen expression tissues leading to tissue destruction.

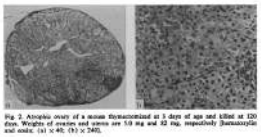
Proof of principle studies demonstrating that T-cell-mediated response targeting the ovary can render animals sterile

Murine autoimmune oophoritis (AOD) identifies germ cell antigens that induce potent sterilizing autoimmune response.

❖***Removal of thymus gland from 3 day old female mice (d3tx) resulted in potent and specific autoimmune responses directed at the ovary at sexual maturity leading to germ cell depletion and infertility.***

Thymus and Reproduction : Sex-Linked Dysgenesia of the Gonad after Neonatal Thymectomy in Mice

Abstract. Neonatal thymectomy of mice, when no ectopic thymus existed, constantly resulted in developmental arrest of the ovary but not of the testis; it also caused sterility in the female. The ovaries of thymectomized mice were extremely small and were characterized by absence of follicles and corpora lutea. Such an ovarian dysgenesis was observed when the mice were thymectomized at 3 days of age, but not at 7 days or later; it was prevented by thymus grafting.

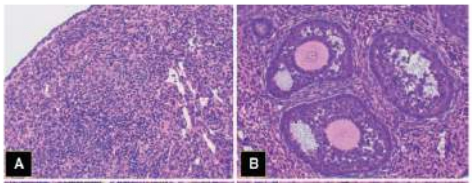


36 of 48 d3tx females were sterile. Ovaries small and atrophic

Science Vol 166, 1969, pp 753

Ovaries from day 3 thymectomy mice have heavy monocytic (T cell) inflammation and are devoid of follicles and oocytes

d3tx **normal**



Kenneth Tung Immunological Reviews

What is the cause of this potent autoimmune respons

❖**Removal of thymus gland at day 3 also removed resident Treg cells thus making female mice incapable of suppressing autoimmune attack when females became sexually mature.**

❖**d3tx phenotype caused by endogenous antigen(s) required for generation and maintenance of the ongoing autoimmune response.**

Endogenous antigens that induce this potent germ-cell depleting immune response would likely make ideal contraceptive vaccine antigens.

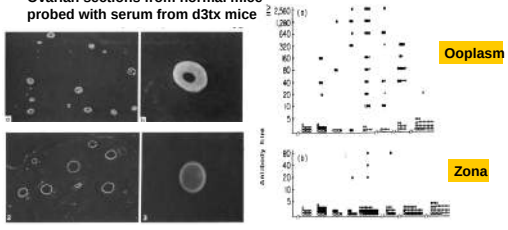
Autoimmune oophoritis in thymectomized mice: detection of circulating antibodies against oocytes

O. TAGUCHI, Y. NISHIZUKA, TERUYO SAKAKURA & A. KOJIMA *Laboratory of Experimental Pathology, Aichi Cancer Centre Research Institute, Chikusa-ku, Nagoya, Japan*

(Accepted for publication 30 November 1979)

Ovarian sections from normal mice probed with serum from d3tx mice

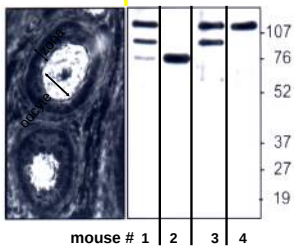
Number of d3tx mice with sera reactive to



Sera from day 3 thymectomized mice specifically reacts with three different mouse oocyte proteins: MW ~ 76, 90, and 125kD

Immunohistochemistry: cross section of mouse ovary probed with pooled sera

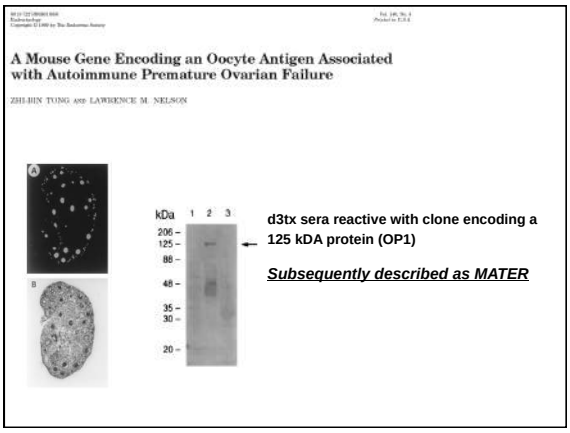
Western blot:mouse egg proteins probed with sera from 4 different mice



Most reactive mouse oocyte protein is 125 kDA

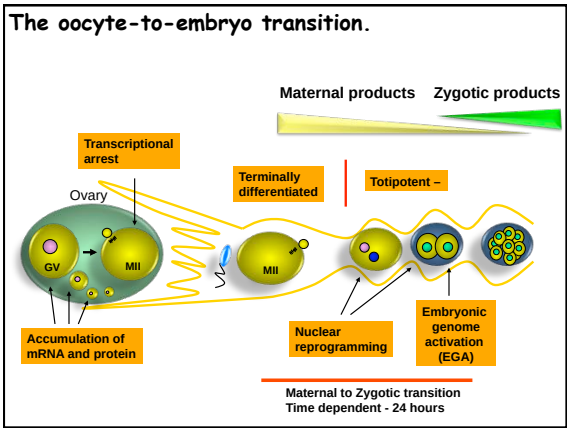
Pascale et al, and Tung

J. Immunology, 2001, 166: 4363-4369.



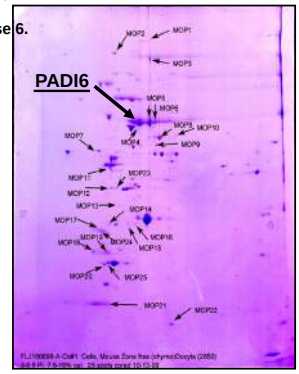
MATER – Maternal Antigen That Embryos Require.

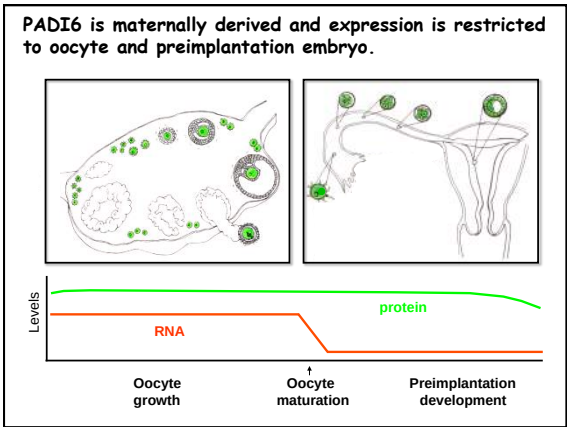
MATER is an oocyte and embryo abundant protein that is required for oocyte-to embryo transition.

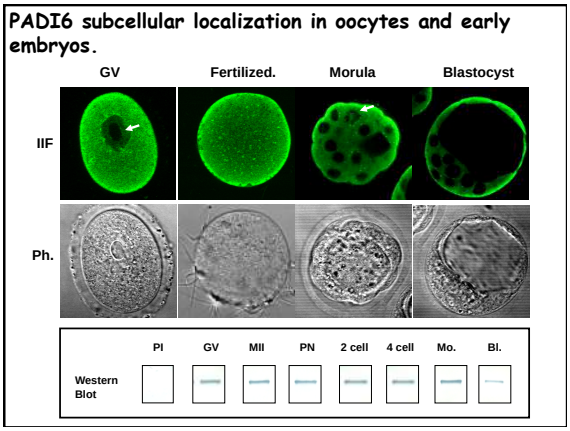


Used proteomics to identify abundant oocyte-restricted factors in the mature mouse oocyte that may play a role in the OET

PADI6 – Peptidylarginine deiminase 6.



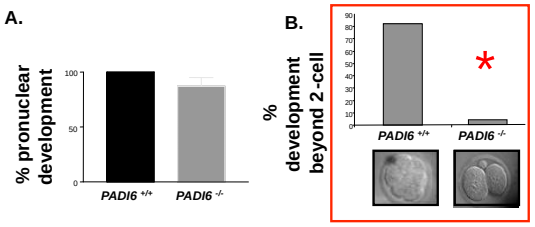




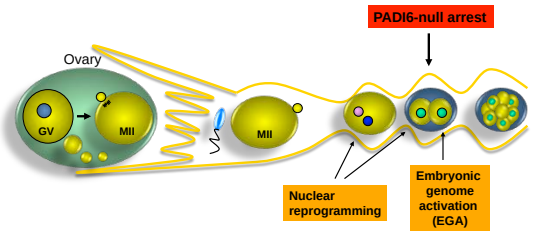
Characterization of the PADI6 $^{-/-}$ Phenotype.

Alejandra Vitale/Piraye Yurttas

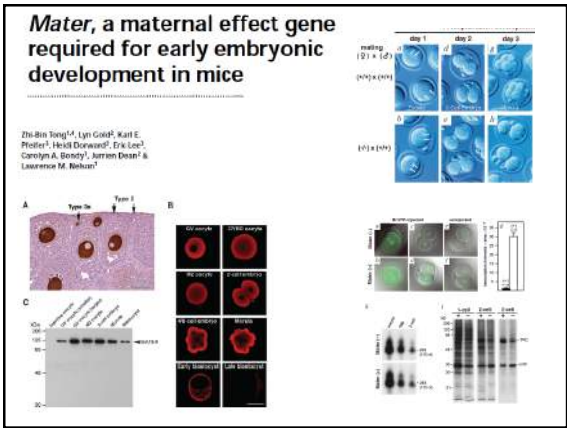
PADI6 $^{-/-}$ developmental arrest occurs at the two-cell stage.



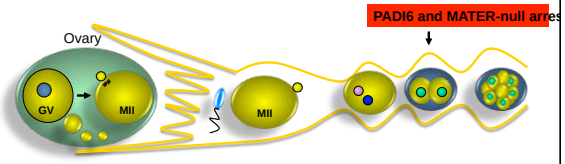
PADI6-null two-cell developmental arrest likely due to failure to activate embryonic genome.



Mater, a maternal effect gene required for early embryonic development in mice



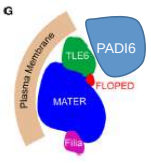
MATER and PADI6-null two-cell developmental arrest likely due to failure to activate embryonic genome.



MATER and PADI6 are part of the same complex in mouse oocytes

Developmental Cell Article

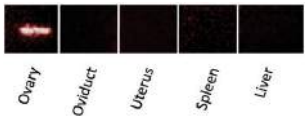
A Subcortical Maternal Complex Essential for Preimplantation Mouse Embryogenesis



Is MATER expressed in companion animals.

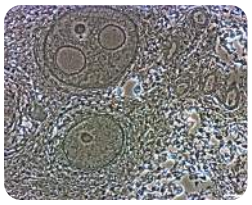
MATER RNA expression is restricted to the ovary in the dog

PCR with MATER primers

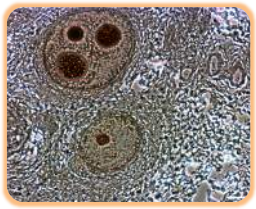


Dog ovary

preimmune rabbit serum



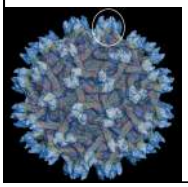
anti-mouse-MATER peptide antiserum

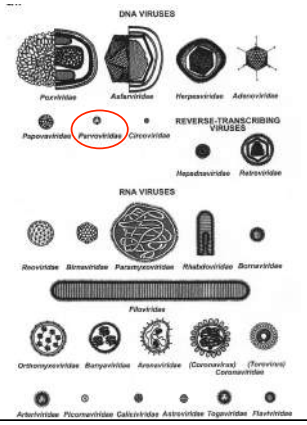


How can MATER, GnRH, and possibly other autoimmunogenic maternal genes be incorporated into an immunosterilant formulation for companion animals.

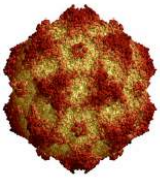
Animal viruses:

- **Small packages of protein or membrane.**
- **DNA or RNA genome.**
- **Replicate only inside cells.**
- **Damage host cells and tissues.**
- **Many controlled by vaccines – induce host immunity.**

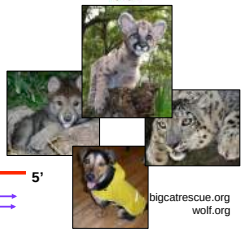


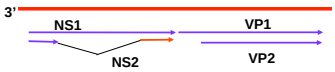


Canine parvovirus (CPV)



- Non-enveloped, 26nm diameter capsid
- Single-stranded DNA genome ~5kb
- 2 genes – 4 overlapping proteins
- Capsid - 60 copies of VP2 and VP1 (90% VP2; 10% VP1).
- VP2 forms capsids; VP1 required for cell infection
- Infects rapidly dividing cells
- Evolved from feline panleukopenia virus
- Binds the transferrin receptor
- Infects young dogs and wild carnivores

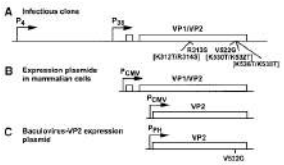




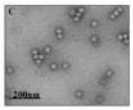
bigcatrescue.org
wolf.org

Production of CPV virus-like particles (VLPs)

- Contains only the VP2 capsid protein
- Can be generated in mammalian or insect cells
- Can bind and enter permissive cells (but are non-infectious)
- Have similar stability and morphology to complete viruses w/o pathology



Yuan and Parrish, *Virology* 2001

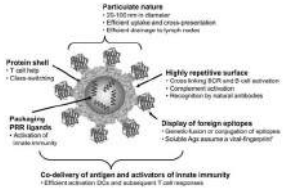


Singh, P. et al. *J Nanobiotech.* 2006

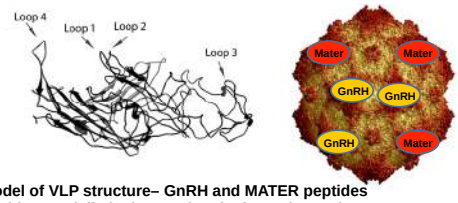
The CPV-derived VLPs present two opportunities to generate enhanced immune responses.

1) **They are multivalent carriers: An incorporated antigen can be displayed as 60 copies/capsid with spacing sufficient to cross-link receptors on lymphocytes, thus effectively inducing activation.**

2) **Most dogs and cats of reproductive age have been exposed to CPV/FPV, thus existing immune surveillance system will mount a classical immunological memory response to the immunosterilant vaccine. Therefore, even though the specific attached antigen may not have been the target of an immune response before, this still results in an enhanced immune response.**



VLP containing GnRH and MATER epitopes.



Model of VLP structure– GnRH and MATER peptides could potentially be inserted at the loop sites. These sites are antigenic and recognized by antibodies

(Courtesy of C. Parrish)

Final Immunosterilant Vaccine

❖A combination of GnRH-VPL and MATER-VLP

❖Would likely rapidly reduce GnRH levels, leading to reduced hormone levels and infertility

❖Would get slower cellular immune attack against MATER thus permanently eliminating oocytes and follicular cells

Acknowledgements

Baker Institute, Cornell University
❖ Vicki-Meyers Wallen
❖ Collin Parrish

What if a dog or cat has not been exposed to CPV?

- **Juvenile animals may not have been exposed to CPV infection or vaccination**
- **For these, we may need to give a booster dose of vaccine after the initial dose**
- **But ideally we would like a single-injection vaccine**

How does immunocontraceptive vaccination formulation "trick" immune system into destroying reproductive antigens

Immunology 101.

Innate immune response – A non-specific response to infection. Does not confer immunity to host. Provides immediate cellular defense against infection

Adaptive (acquired) immune response – A learned response by the immune system (B and T cells with defined antigenic specificity) whereby resistance to pathogen is achieved by previous exposure (vaccination).

Previous companion animal immunocontraception research: Contraceptive vaccines targeting the Zona Pellucida oocyte antigens.

Native Porcine Zona Pellucida preparation.

Hamster (Mahi Brown)
❖Females immunized with soluble pZP generated antibodies reactive with hamster ZP.
❖Females became permanently infertile.
❖Ovarian follicles completely destroyed.

Wildlife
❖ SpayVac is a contraceptive vaccine formulation containing native porcine ZP and CFA
packaged in liposomes
❖SpayVac induced long-term contraception in seals and deer (and other wildlife species).

Cats (Levy)
❖In cats, SpayVac induced long-lasting high-titer anti-pZP antibodies but the antisera was
not reactive with feline ZP.

Dogs (Mahi Brown)
❖Immunization of bitches with crude and purified native pZP with CFA or Alum induced infertility, abnormal estrous cycles, follicular cysts and degenerating oocytes.

Recombinant ZP2 and ZP3

Mice

❖Studies by Tung showed that either B or T cell response can lead to either neutralizing response or oophoritis.

Dog
❖Immunization of female dogs with recombinant ZP2 and ZP3 conjugated to diphtheria toxin led to the generation of canine ZP-specific antibodies. All females that were immunized with ZP2 became pregnant while 3 of 4 females immunized with ZP3 did not conceive. Ovarian histology revealed inhibition of follicular development in treated animals

❖Several other studies using recombinant ZP proteins showed negative results.

❖Study in rabbits showed that while native porcine ZP induced infertility in rabbits when the porcine zona prep was deglycosylated the immunized females did not

GnRH

❖GonaCon, synthetic GnRH bound to adjuvant

❖In swine and deer, infertility has lasted up to five years after a single treatment.

❖GonaCon induces GnRH antibodies in cats and a recent publication demonstrated the induction of GnRH antibodies in female dogs. However, problems with adjuvant mediated side effects.

Summary

❖Results with GnRH suggest that, once optimized, immunization with this reproductive antigen could generate a long term B-cell mediated immunocontraceptive response (more later on this from Dr. Schiller)

❖Results with native zona pellucida preparations provide a proof of principle that immunization of animals with this reproductive antigen can generate both a B and T cell mediated response neutralizing the zona pellicida and also stimulating follicular degeneration /oophoritis

❖However, results from zona deglycosylation and recombinant zona studies suggest that carbohydrate configuration is important for immunoreactivity.

Previous Research

Human Immunocontraception Research: Persistent problem with the immune system becoming "overstimulated" generating a T-cell mediated inflammatory response targeting ovary.

What's bad for people may be good for companion animal sterilization!!

Immunocontraception with Zona pellucida Proteins: Review

The prospect of an immunological approach to contraception that would disrupt the process of fertilisation itself has resulted in a considerable interest into research in this area. It has been known for some time that antibodies raised against the zona pellucida (ZP) can suppress fertility very effectively. **However, the initial optimism of this approach has been marred by the appearance of an ovarian pathology characterized by disruption of folliculogenesis and depletion of the primordial follicle pool. Adverse auto-immune reactions have been observed in the ovaries of mice after the induction of immunity with mouse ZP3 epitopes. However, this was associated with lymphocytic infiltration of the ovarian stroma, which could be circumvented by careful selection of B-cell epitopes to induce reversible infertility.** In order to identify similar epitopes on primate ZP3, epitope-mapping studies were performed and incorporated into chimeric vaccines that included a promiscuous T-helper cell epitope. Both single and triple peptide vaccines have been evaluated in vivo and no detrimental effects on ovarian function were observed. The resulting high titre antibodies bound exclusively to the ZP of marmoset and human ovarian sections and could suppress in vitro human sperm-egg binding by approximately 60%, but did not prevent pregnancy in actively immunised female marmosets. Thus, considerable research is still required to identify a combination of ZP3 epitopes that will induce infertility free of any unwanted side effects.

Aitken, Cells Tissue Organs 166

Ideal immunosterilant would be one that targets GnRH with an antibody response and the ovary with a T cell response