Chemical Acceleration of Ovarian
Senescence: A CAOS Strategy!

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Relationships to Disclose

Northern Arizona University
- Work performed at NAU has been performed in the laboratories of Loretta P. Mayer, Ph.D., and Cheryl A. Dyer, Ph.D., under a commercial contract with SenesTech, Inc., exclusive licensee of UA technology
- Drs. Dyer and Mayer have a financial interest in SenesTech, Inc.

University of Arizona
- Work performed at UA has been performed in the laboratory of Patricia B. Hoyer, Ph.D., and disclosed under domestic and international patent filings
- Dr. Hoyer has a financial interest in SenesTech, Inc.

Session II: What’s New in Contraceptive Drugs?
4-VHD in Rodents, Dogs and Cats
By Dr. Loretta Mayer
Seminar Objectives

- History of the Chemical Acceleration of Ovarian Senescence (CAOS)
- Characteristics of the physiology of CAOS
- Canine population control
- Future directions
Lesson from the mouse

- Significant body of work done in rats and mice to study a chemical plasticizer used in industrial settings (Hoyer & Sipes).
- Subsequent research done in rodents to further characterize the specific and selective effect on the ovary (Hoyer & Mayer).

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Specific Ovarian Effects
Rodent Data

- NTP studies (1986, 1989)
- No Adverse Observed Effects (Pathology report, Hoyer unpublished data, 2006)
  - Adrenals, kidney, liver, spleen, lung, heart, brain, intestine, stomach, pituitary, lymph node, pancreas, esophagus
- No trauma to the liver (Mayer et al, ATV 2005)
  - AST, ALT in normal range
  - No evidence of histopathology
  - No changes in total cholesterol, triglycerides, or HDL

Design of Mouseopause™

VCD

Single Injection

Follicular Depletion

“Mouseopause™”
U.S. Patent Pending

Mayer et al, Biology of Reproduction, 71 2004
Ovarian Morphology

Correlation of FSH vs. antral follicles

\[ r^2 = 0.87 \]

**Effect of Follicle Depletion on Plasma Gonadotropins and Steroids**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>VCD-treated</th>
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<tbody>
<tr>
<td>LH (ng/ml)</td>
<td>1.50 (±0.21)</td>
<td>3.11 (± 0.23)*</td>
</tr>
<tr>
<td>FSH (ng/ml)</td>
<td>2.17 (± 0.06)</td>
<td>27.57 (± 2.17)*</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>4.54 (± 0.07)</td>
<td>3.39 (± 0.06)*</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>0.44 (± 0.02)</td>
<td>0.29 (± 0.02)*</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>2.21 (± 0.05)</td>
<td>Non detectable*</td>
</tr>
</tbody>
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Female B6C3F1 mice were treated daily with VCD (160 mg/kg/d, 15d, i.p.) or vehicle control.

Plasma samples were collected on d127 following the onset of VCD treatment  n=6

*p<0.05

*Mayer et al, Biology of Reproduction, 71, 2004*
Follicular Depletion by **VCD**

- Repeated exposure results in follicle depletion and ovarian failure in rodents
- Normal follicular depletion occurs via atresia in greater than 99.9% of follicles
- VCD accelerates the natural process of atresia by up-regulating cellular and molecular mechanisms of apoptosis

_Hu, X. et al., 2001a,b, 2002, Mayer et al, 2004_

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**Mammalian ovary**

- Highly conserved physiology
- Same follicle populations
- Same regulatory hormones
- Finite, non-replenishable functional oocyte pool
- All aging mammals have reduced fertility and fecundity

Then.....
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4-VHD in Rodents, Dogs and Cats
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Dr. & Mrs. Balok

Navajo Nation

Proceedings of the Third International Symposium on Non-Surgical Contraceptive Methods for Pet Population Control • www.acc-d.org
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Navajo sheep herding

What is the challenge in our region?

- Navajo Reservation
  - Largest Native American reservation
  - Over 160,000 stray dogs
  - Thousands of dog bites every year
  - Millions of dollars in lost livestock
  - Carry zoonotic diseases
- 136 dogs/cats killed per 1,000 human residents, highest in United States
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Tailgate Medicine

Hypothesis we are testing

- VCD treatment will accelerate elimination of primordial follicles in dog ovaries leading to ovarian failure, eliminate heat behavior and cause permanent sterility
Specific Aims

- Research Objectives
  - Accelerate natural process of atresia → in dogs
  - Hormonal environment mimics that of sterile individuals
  - Treatment is 100% efficacious
  - Sterility is irreversible
  - Delivery can be modified for a continuous release protocol
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Animals - Canine

- Mixed breeds (16)
- Age cohorts
  - (8) 12 weeks (1 set littermates)
  - (8) 6 months
- General Health
  - Good
- Dosing – Vehicle, Low, Medium, High

d30 Spay Surgeries
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**Total Primordial Follicles**
Mixed age, mixed breed dogs were treated daily with compounded VCD (80 mg/kg, 160 mg/kg, or 240 mg/kg S.C.) or vehicle for 6 days. Ovaries were removed on day 30 and processed for histological evaluation. Primordial follicles in every 10th section were counted. Graph represents mean and SEM (n=3-4/group). * indicates significant difference compared to control (p<0.05)

**Reduced primordial follicles in puppy ovaries**
Mixed breed puppies (<1 year) were injected daily with compounded VCD (80 mg/kg, 160 mg/kg, or 240 mg/kg S.C.) or vehicle for 6 days. Ovaries were removed on day 30 and processed for histological evaluation. Graph represents mean and SEM, * indicates significant difference compared to control (p <0.05)
Ovarian Histology

Puppy

Control

High Dose

Adult

Summary of Findings

- Treatment was determined to be safe:
  - All blood chemistry analyses in normal range
  - All animals healthy and active (2.5 years post)
  - No loss in body weights among adults, growth in puppies unaffected
- Primordial Follicles depleted
  - Preferential depletion in puppies
- Hormone Levels
  - FSH – levels not different on d30 post-dose
  - Estrogen -
- Ovarian and Uterine wet weights
  - Uterine weights were reduced in a dose-dependent manner
  - Ovarian weights were not different

Miers et al, SSR Quebec, 2005
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Will Spay For Food

ONE SHOT PLEASE!

Canine Studies Underway

- Domestic and International patents protection for ChemSpay has been filed
- Nanoparticle single injection delivery system (SenesTech, Inc.)
  - Proof of concept in rodents
- Low dose episodic delivery over extended time (USDA)
- Complete follicle depletion formulation (SenesTech & Animal Assistance Foundation)
- Behavior studies (ACC&D)
DDAL: Cohorts of mixed age and mixed breed dogs were treated with ChemSpay containing the active compound VCD at a low, medium, or high concentration for 6-15 days after which a hemi-ovx was performed and primordial follicle numbers determined. (n=2-8, p<0.05).
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Leadership in CAOS!

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