

# Research and Investigation

NSTA Assessment 7  
Ashley Grapes





# Improving Clouded Leopard Artificial Insemination

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**Ashley Grapes**

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**Smithsonian**

*National Zoological Park*

# Front Royal, VA



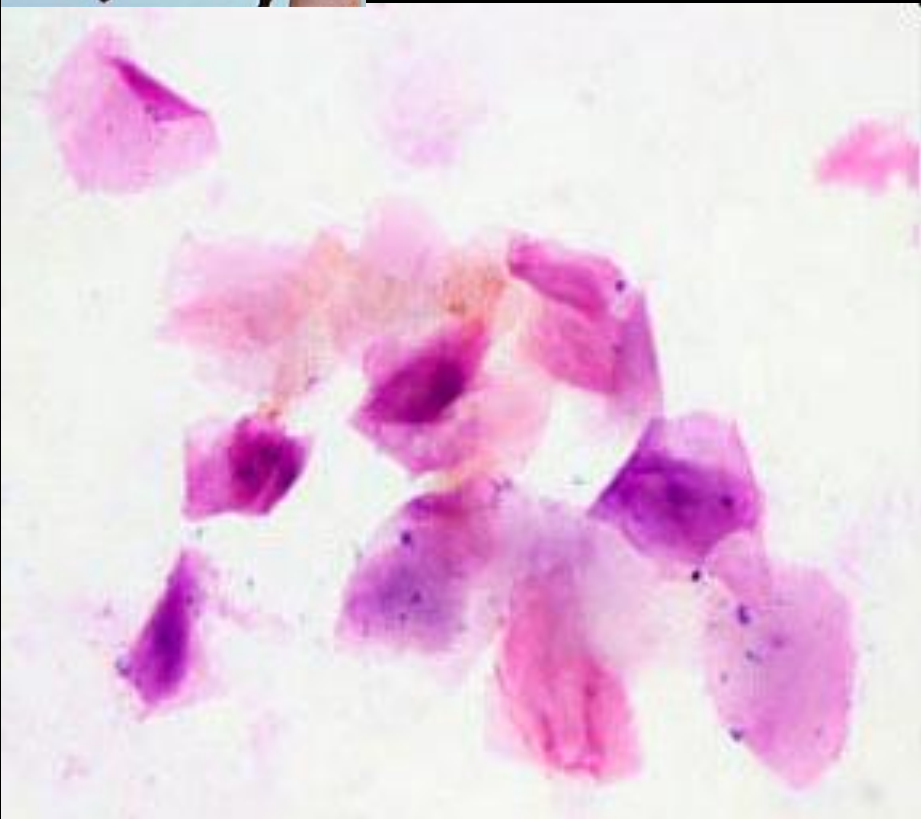




# Side Project...

## Black-Footed Ferret Conservation

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- Successful AI of 20 year old sperm!
- Flush females; >85% keratinized → estrus → AI
- Fate of all animals decided by U.S FWS
- FWS decide based on genetics computer program

100% keratinized (orange) → estrus

# Main Project

## Establishing Clouded Leopard AI Protocol

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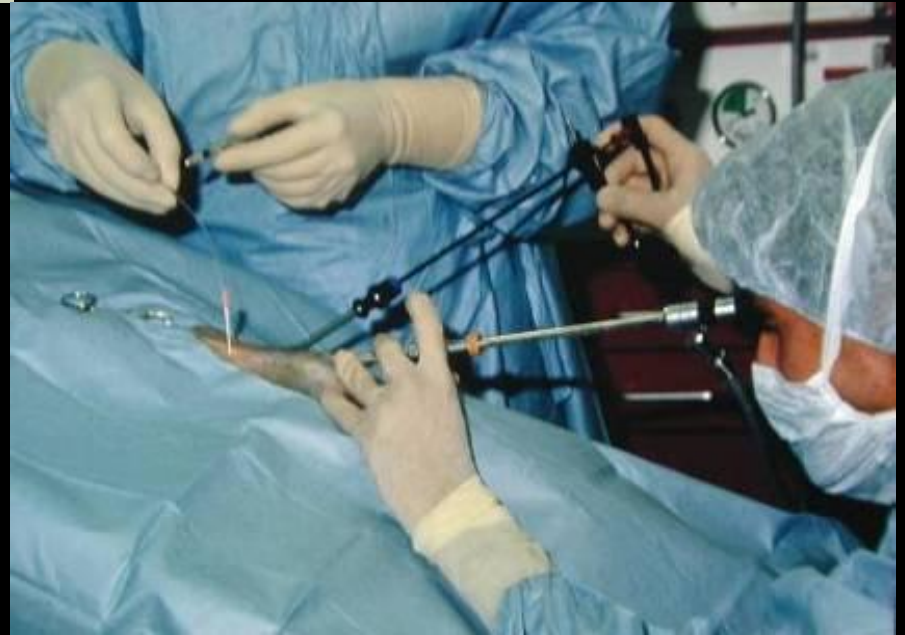
### Why do we AI?

- Improve genetic variation!
  - Old sperm from sperm banks
  - Sperm shipped from other countries
- May be only option for certain animals
  - Animals that form strong pair bonds (cloudeds!)
  - Extremely rare animals





# Laparoscopic Intrauterine Insemination



# Traditional Felid AI Protocol

## INDUCE ESTRUS

**200 IU equine chorionic gonadotropin (eCG)**

## INDUCE OVULATION

**100 IU human chorionic gonadotropin (hCG)**

**AI at ~46 h post hCG**

**Laparoscopic Intrauterine AI**



**Cheetah AI**  
**11 Pregnancies**  
**20 cubs**

# Variable AI Success in Felids

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- Cheetah:

**23 attempts, 11 pregnancies, ~50% success; 20 cubs; fresh or frozen sperm**

- Clouded leopard:

**10 attempts, 1 pregnancy, 10% success; 2 cubs 1992 at Nashville Zoo**

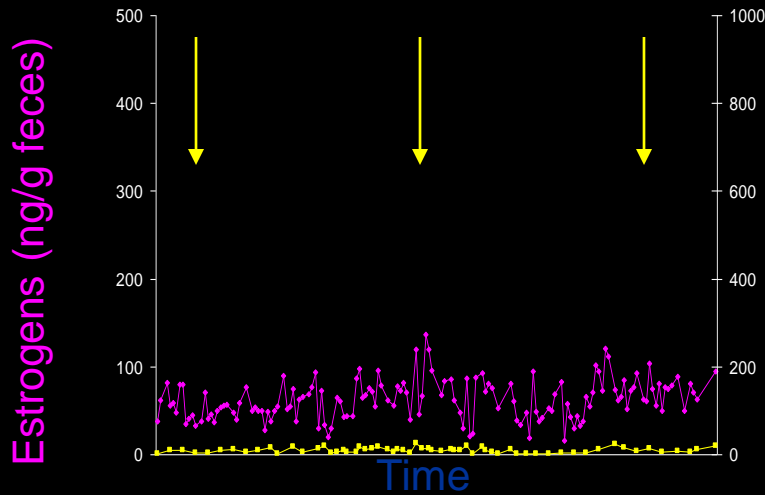




# Cause of AI Failure

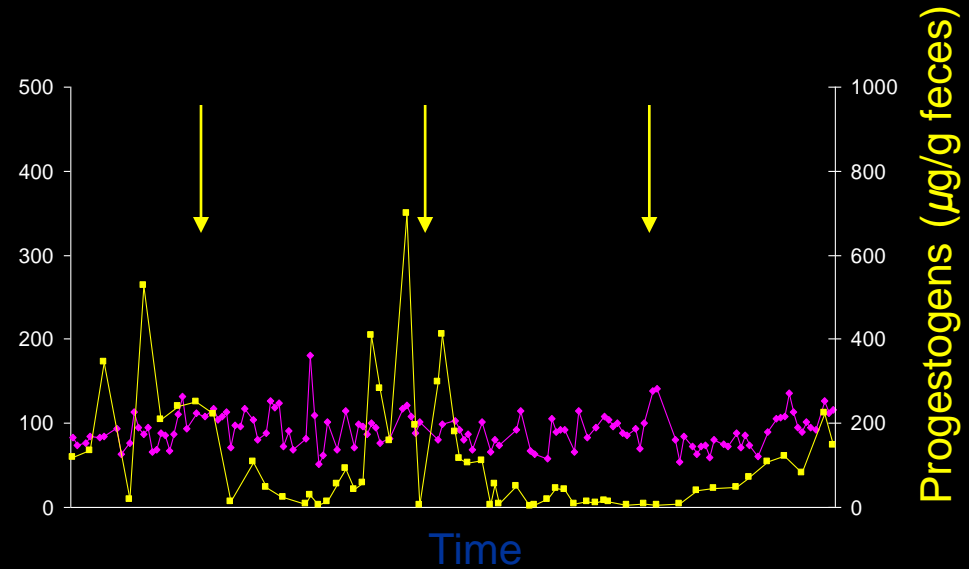
## Inconsistent Ovarian Response

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**Induced Ovulator**

*cheetah, ocelot, tigrina*



**Spontaneous Ovulator**

*clouded leopard, fishing cat, lion,  
caracal, margay*

# Suppression of Estrus and Ovarian Cycle Control

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- **Improves AI and IVF in humans, non-human primates and domestic livestock**
- **Uniform and predictable ovarian response to gonadotropic hormones for AI and IVF**

# METHODS

## Ovarian Suppression Prior to Ovulation Induction in Clouded Leopards

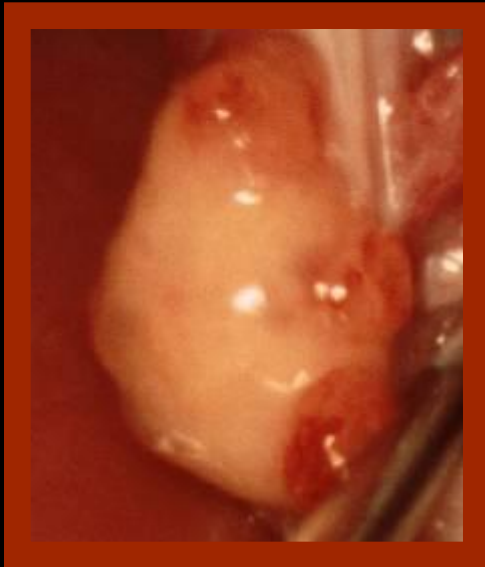
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- Collect daily fecal samples 60 days before, during and for 100 days following treatment
- **Altrenogest (Regumate®): Progestin oral**
  - 0.6 mg/kg daily oral dose for 51 days
- **LOW dose gonadotropins**
  - 300 IU equine chorionic gonadotropin (eCG)
  - 225 IU human chorionic gonadotropin (hCG)
- **HIGH dose gonadotropins**
  - 600 IU equine chorionic gonadotropin (eCG)
  - 450 IU human chorionic gonadotropin (hCG)



# Altrenogest (Oral) + eCG + hCG Effective in Clouded Leopards

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**Excellent Response !!!!!**

**Consistent Ovarian Response for Artificial Insemination!!**

# 2008-2009 Clouded Leopard AI Study

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**NO PREGNANCIES !!!**

**Next step:**

- **Assess fecal hormones to determine cause of AI failure**
- **Assess oocyte quality after ALT/eCG/hCG (Rebecca Hobbs-new project)**



# Experimental Design

- Partnerships with zoos all over the world
- Collect and freeze poop every day
- Lyo machine to freeze dry (no water)
- Smash feces into powder
- Go through hormone extracting process
- Check to see if hormones extracted
- Test to see what hormone levels are in each sample
- Develop graphs: hormone concentration over time

# The Enzymeimmunoassay (EIA)

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- Used to measure small [ ] of hormones
- Comparing labeled antigen (tracer) binding to unlabeled antigen binding (hormone in sample) to the antibody
- Assumption that an antigen can be linked to an enzyme and retain both immunological and enzymatic activity in the resultant conjugate
- The soluble antigen/antibody must also be linked to an insoluble phase in a way in which the reactivity of the immunological component is retained



# Extract the Hormones

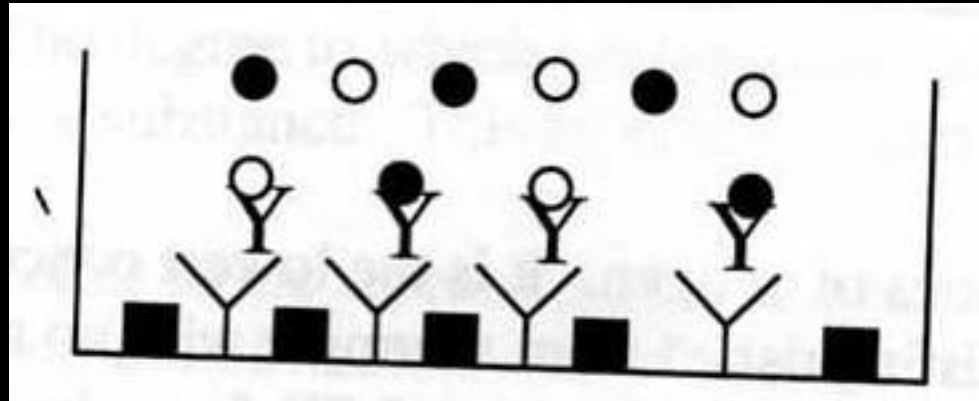
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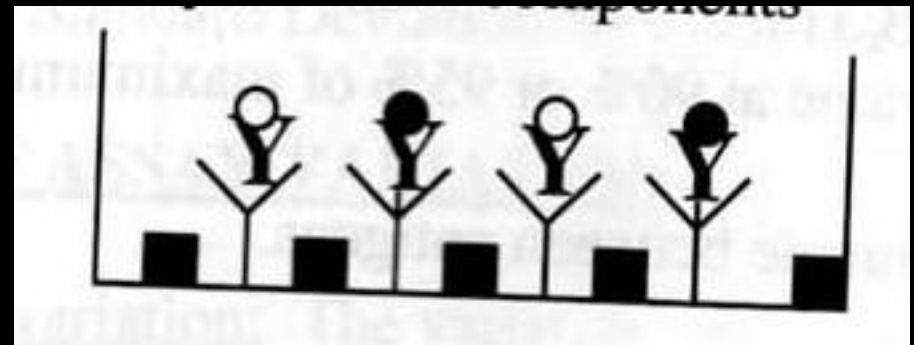
Efficiency measurement

Did we extract the hormones? PHEW we can move on...

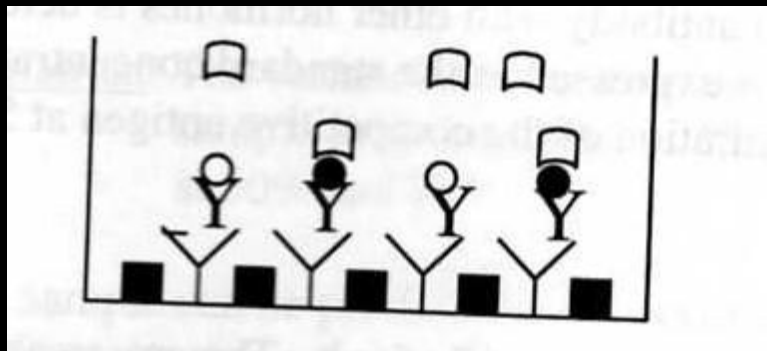
Addition of hormone-specific, first antibody, biotin-labeled hormone, and free hormone



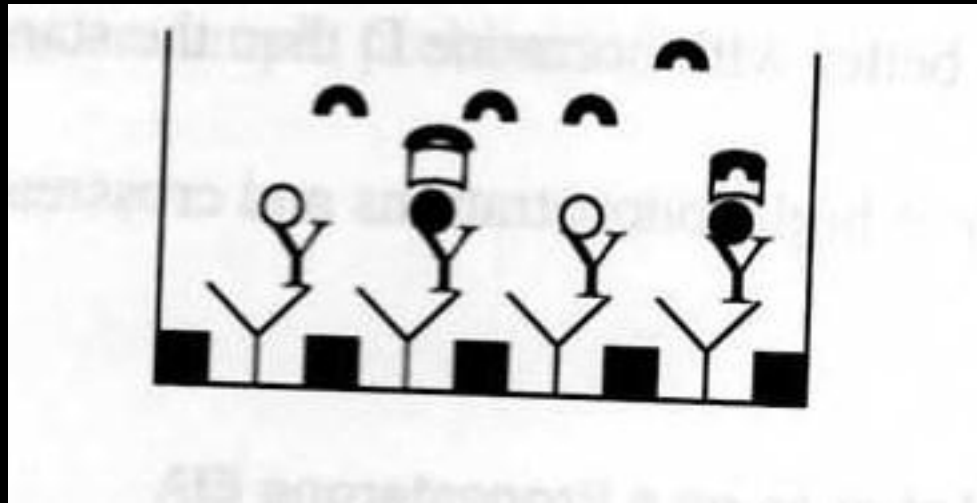
Wash away unbound components



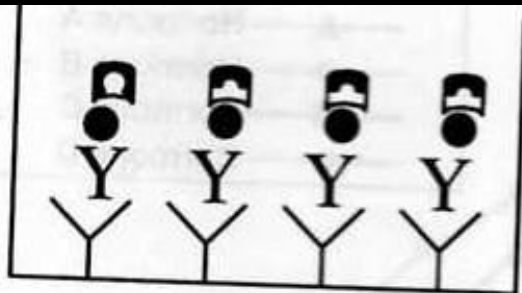
Addition of enzyme



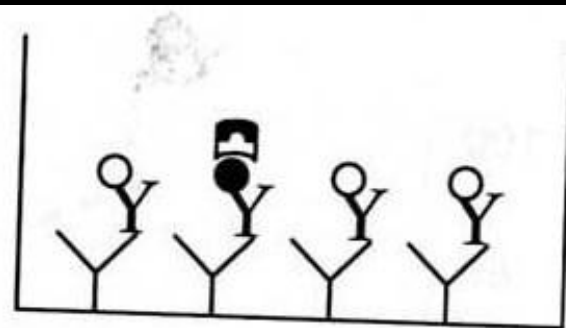
# Addition of substrate



## Results



Zero  
Maximum color



High standard  
Minimum color



# NSTA 1.d

Understand research and can successfully design, conduct, report and evaluate investigations in science.

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- Full-time job for 4 months
- Worked under senior Smithsonian Scientist, JoGayle Howard, Head of Reproductive Sciences
- Responsibilities included all steps in fully-funded project although I came in half-way through and did not finish as it was an internship

# NSTA 1.e

Understand and can successfully use mathematics to process and report data, and solve problems, in their field(s) of licensure.

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- Math needed to count keratinized ferret cells
- Math needed for entire procedure: ex. to measure radioactive quantities & pipette EIA quantities
- Excel used to create graphs and data table
- Dr. Howard not on premises, responsibility for data collection given to me



# Thank you

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Dr. JoGayle Howard, Ph.D.

Dr. Katie Pelican, Ph.D.

Smithsonian Women's Committee

Dr. Rebecca Hobbs, Ph.D.

Lawrence Layman

CRC Staff

