Development of a next generation PZP vaccine
Experimental approach

Improved adjuvant
• Potent
• Safe
• Compatible with controlled release formulation

Recombinant PZP
• Well characterized
• Consistent

Controlled release
• Reproducible
• Administered in darts

Next generation PZP vaccine
Adjuvant Development
The immune response to vaccines

- **Dendritic cells**
- **Lymph**
- **Lymph node**
- **T lymphocyte**
- **B lymphocyte**
- **Plasma cells**
Combination adjuvant

Nano-11

Poly (I:C)

CpG ODN

Dendritic cell

immune response
Combination adjuvant – Nano-11 with poly(I:C) and CpG
Antibody response in horses to Nano-11 complex
Recombinant vaccine
Recombinant PZP proteins

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Controlled-Release Development:

PZP Titer Data Analysis
12 month study
Experimental Design

• 12 horses in 3 groups (n = 4 per group) based on PZP delivery system (capsule/emulsion type):
  1. Control (PZP-22)
  2. 1 month (emulsion) + 1 year (PGLA capsule)
  3. 3 month (PCL:Gel capsule) + >1 year (PCL:PET capsule)

• All horses were given an initial primer injection of PZP

• Blood samples periodically across 12 months with Ab titers assayed at Purdue.

• Ultrasound used to test for estrus cycling and to image implanted capsules.
Comparison of PZP from different sources
Experimental Design

~ 9 horses in 3 groups **

~ Groups:
  1. 2016 PZP (n = 2)
  2. 2018 Liu (n = 2)
  3. SCC from Montana 2018 (n = 3)

~ primer Emulsion injection followed in 7 weeks with booster emulsion

~ Blood samples collected at 0, 2, 4, 6, 7, 9, 11, and 13 weeks

**2 horses euthanized during the study for health problems unrelated to the study
Booster injection @ week 7
Significant difference between weeks ($P < 0.0001$).
No difference between PZP sources in titer response ($P = 0.0644$).
No interaction between weeks and the PZP type ($P = 0.3118$).
NOTE:

Outcomes of the above studies indicate that access to increased number of horses per study group (recommend n=10) is necessary to establish the most reliable conclusions.
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Funding:
Sterilization

Non-surgical Sterilization of Mares allowing continued reproductive behavior:

A Preliminary Study
Introduction

~ Sterilization of mares maybe useful in sanctuaries, equine welfare operations and potentially wild horse ranges to limit population growth while allowing intact stallions to co-habitate with mares.

~ Current procedures for sterilization requiring surgical removal of ovaries or ligation of oviducts, are time consuming and require pre/post operative care.

~ Hysteroscopic Hydrotubulation is a non-surgical chemical-based sterilization method that blocks the oviductal lumen.
N-Butyl cyanoacrylate

~ clear liquid, insoluble in water
~ main component of medical cyanoacrylate glues.

Prior studies reported successful use of hysteroscopic cervical-route sterilization using N-butyl cyanoacrylate in the oviducts in a sheep model. (Bigolin et al.)

The objective of this study was to sterilize mares using hysteroscopic hydrotubulation and allow continued estrus cycle post treatment.
The anatomy of the uterotubal junction, oviduct, and ovary of the mare

Ampulla (site of fertilization)

Caudal Isthmus (location of preovulatory sperm storage in other species)

Uterotubal Junction (location of preovulatory sperm storage in other species)

From Sisson & Grossman, 1938
POSSIBLE RESERVOIRS FOR SPERM

- Utero-tubal junction
- entrance into the oviduct (fallopian tube)
- note the folds of the structure
Endoscopic hydrotubation
Method

• **Experiment group**
  Comprised of:
  3 maiden mares (ages 5-7 yrs)
  2 primaparous mares (ages 12-15 yrs)
  1 multiparous mare (age 17 yrs)

• **Control Group**
  Comprised of:
  1 maiden mare (age 23 yrs)
  1 multiparous mare (age 16 yrs)

All reproductive tracts were examined per rectum via ultrasound with no visible signs of abnormalities.

Endometrial cultures were obtained prior to study (performed during estrus phase of cycle)

Mare histories and cultures, indicated fertility prior to inclusion into study.
Equine Sperm at the UTJ 4 Hours after Insemination and are found up to 18 hours after insemination
Results

Treated:
~ No pregnancies occurred in any Treated mares after being mated through an average of 15 estrus cycles (Group I) and 4.5 estrus cycles (Group II) per mare across 3 years.

Untreated:
~ One Untreated (Group III) mare conceived after the first estrus mating and carried a healthy foal to term.
~ The second Untreated mare failed to conceive after three successive estrus cycle matings.
Fig 2. Pair of oviducts from a single mare. Left: oviduct infused with 1 mL of saline. Right: oviduct infused with 0.5 mL of N-Butyl cyanoacrylate. Note disruption of luminal epithelium and distended lumen. 40X
Summary

~ This preliminary study of non-surgical sterilization of mares revealed no pregnancies in any treated mares across 3 years.

~ This hysteroscopic method for cyanoacrylate oviductal occlusion is an attractive alternative to surgical sterilization in view of lesser cost and time with observable adverse conditions post treatment.

~ This procedure may serve as a safe and effective sterilization strategy for management of wild horse populations while allowing continued social behavior, sexual behavior and band integrity.