Developing a Novel Contraceptive Vaccine Against Sperm Protein Reactive with Anti-sperm Antibody (SPRASA) for Use in Horses: Pilot Study

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Introduction
- In the United States, the wild horse population exceeds the recommended carrying capacity of 61,000 horses.¹
- Current contraceptive methods include:
  - Surgical (removal of ovaries)
  - Hormonal (progesterone, GnRH)
  - Immunologic (porcine zona pellucida, GnRH).²
- SPRASA is a sperm surface membrane protein involved in sperm-egg plasma membrane adhesion and fusion during fertilization.³,⁴
- Initial research from our laboratory showed that SPRASA is immunoexpressed in the granulosa cells of primordial, primary, secondary, and tertiary ovarian follicles.⁵

Hypothesis & Objectives
- We hypothesized that immunizing horses against SPRASA will be safe and effective.
- To test this hypothesis, the objectives of this research were to:
  - Monitor behavior and health parameters following vaccination against SPRASA
  - Measure antibody titers following vaccination.

Vaccine development:
- A short SPRASA sequence (Figure 1) was synthesized from a mammalian expression system (Genscript, Piscataway, NJ).
- The SPRASA peptide (1.5 mg) was conjugated with the carrier protein CRM-197 (FinaBio solutions, LLC, Rockville, MD).
  - Aerobic and anaerobic culturing was performed at the OSU VDL to ensure the product was sterile.
- The conjugated peptide was mixed 1:1 with an adjuvant selected for safety in horses (GERBU, V-Biognostics, San Diego, CA).

Methods
- Research was approved by the OSU IACUC (ACUP #0103).
- Horses were housed at the OSU Horse Center.
  - Horses were monitored twice daily for changes in appetite, attitude, temperature, heart rate, and respiration rate (Figure 2).
  - Each vaccine contained 0.5 mg of SPRASA peptide and was administered into both caudal thighs (3 mL on each side) three weeks apart (for a total of three vaccinations).
  - Blood samples were taken before each vaccine was administered and 3 weeks after the last vaccination.
- SPRASA antibody titers will be determined using an enzyme-linked immunosorbent assay.

Results
- The unconjugated SPRASA peptide was 15 kDa (Figure 3A), shown by the yellow arrow.
- The SPRASA peptide conjugated with CRM-197 was also 15 kDa (Figure 3B), shown by the yellow arrow.
  - If the conjugation was successful, the size of the product should have been 70 kDa, shown by the pink arrow.
- No negative side effects or vaccine reactions have occurred.
- Results from the antibody titer testing will be determined at a later time.

Discussion
- Conjugation of the SPRASA peptide to the carrier protein CRM-197 failed in this experiment.
  - This could have been due to the small amount of peptide (1.5 mg) used for conjugation or contamination of the peptide with TRIS buffer.
- This research showed that the SPRASA vaccine is safe in horses.
  - However, because the SPRASA peptide was not conjugated to CRM-197, the vaccine may not be effective in stimulating antibody titers.

Literature Cited

Acknowledgements
We thank the Agricultural Research Foundation for funding this research. In addition, we thank the Branch Experiment Station, the College of Agricultural Sciences, and the Branch Station Research Internship Support Program for support of additional student salary. We also thank Dr. Graham Cox for assistance with vaccine development and Dr. Ryszard Zielke for assistance with Western blotting.