

# PRELIMINARY SAFETY STUDY FOR AN EQUINE (*EQUUS CABALLUS*) VACCINE AGAINST SPERM ACROSOME ASSOCIATED 3 PROTEIN

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## INTRODUCTION

- In the United States, the wild horse population exceeds the recommended carrying capacity by about 60,000 horses.<sup>1</sup>
- Current contraceptive methods include: surgical (removal of ovaries), hormonal (progesterone, GnRH), and immunologic (porcine zona pellucida, GnRH).<sup>2</sup>
- SPACA3 is a sperm surface membrane protein involved in sperm-egg plasma membrane adhesion and fusion during fertilization, and has been identified in men, cattle, sheep, and deer.<sup>3,4</sup>
- SPACA3 has also been identified in the granulosa cells and ooplasm in dogs and cats.<sup>4</sup>

## OBJECTIVES & HYPOTHESES

- The objectives were:
  - To determine the expression of SPACA3 in equine ovarian tissue;
  - To determine if a SPACA3 vaccine would be safe to use in mares.
- It was hypothesized that :
  - SPACA3 would be immunoexpressed in the granulosa cells of primordial, primary, secondary, and tertiary equine follicles;
  - SPACA3 vaccine would be safe to use in horses and induce anti-SPACA3 antibodies.

## METHODS

### Ovarian Immunoexpression of SPACA3

- Routine immunohistochemistry was performed on formalin-fixed paraffin-embedded ovarian sections.
- Slides were deparaffinized, rehydrated, blocked, incubated with a SPACA3 primary antibody (1:200), incubated with a secondary antibody, counterstained, dehydrated, and cover slipped.

### Equine SPACA3 Vaccine Development

- A short SPACA3 sequence (**Figure 1**) was synthesized from a mammalian expression system (Genscript, Piscataway, NJ).
- The peptide was mixed 1:1 with an adjuvant selected for safety in horses (GERBU, V-Biogenetics, San Diego, CA).

### Equine SPACA3 Vaccine Trial

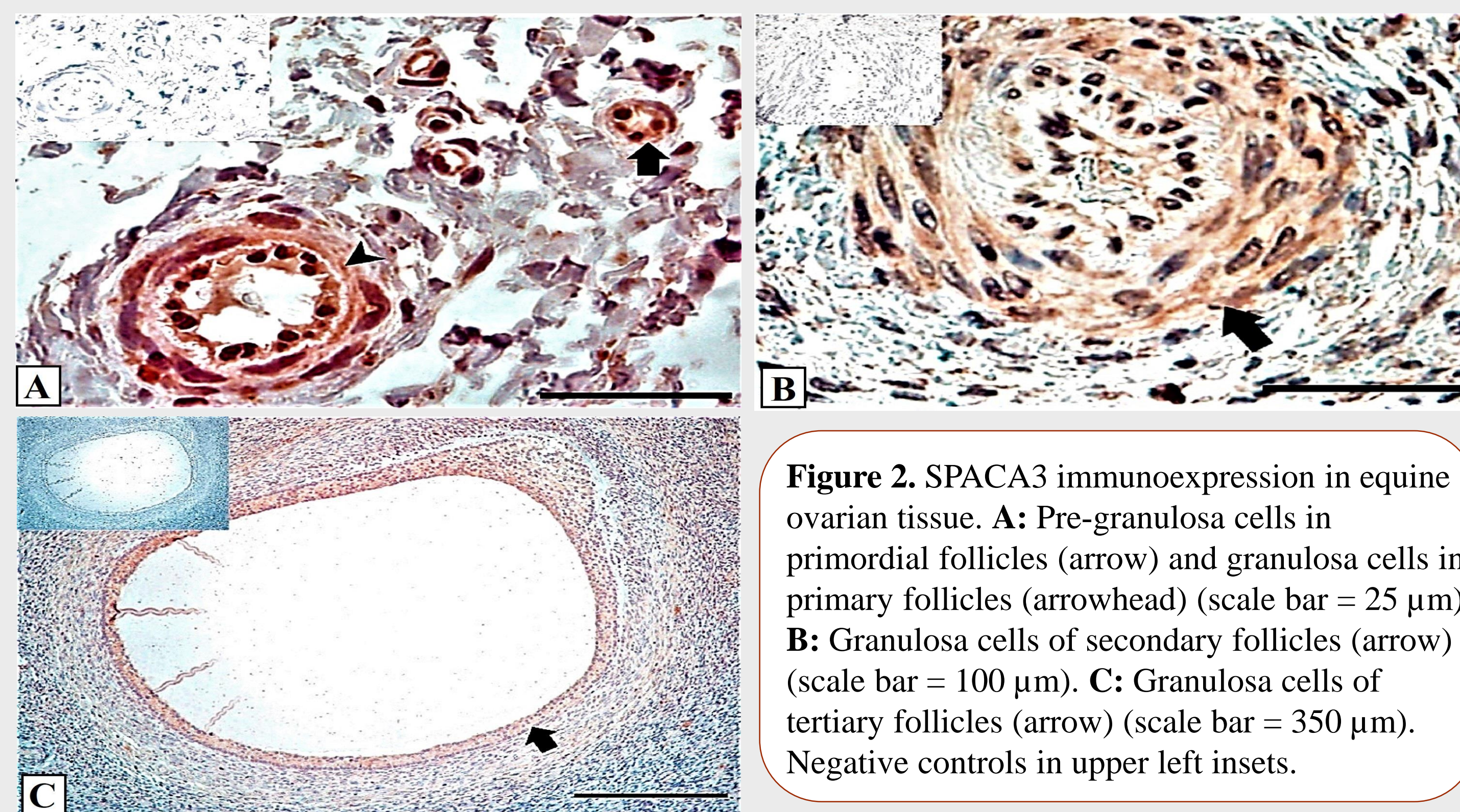
- Research was approved by the OSU IACUC (ACUP #2020-0103).
- Horses were housed at the OSU Horse Center where they were monitored twice daily for changes in appetite, attitude, temperature, heart rate, and respiration rate.
- Each vaccine contained 0.5 mg of SPACA3 peptide and was administered into 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.

### Immunoblotting with SPACA3

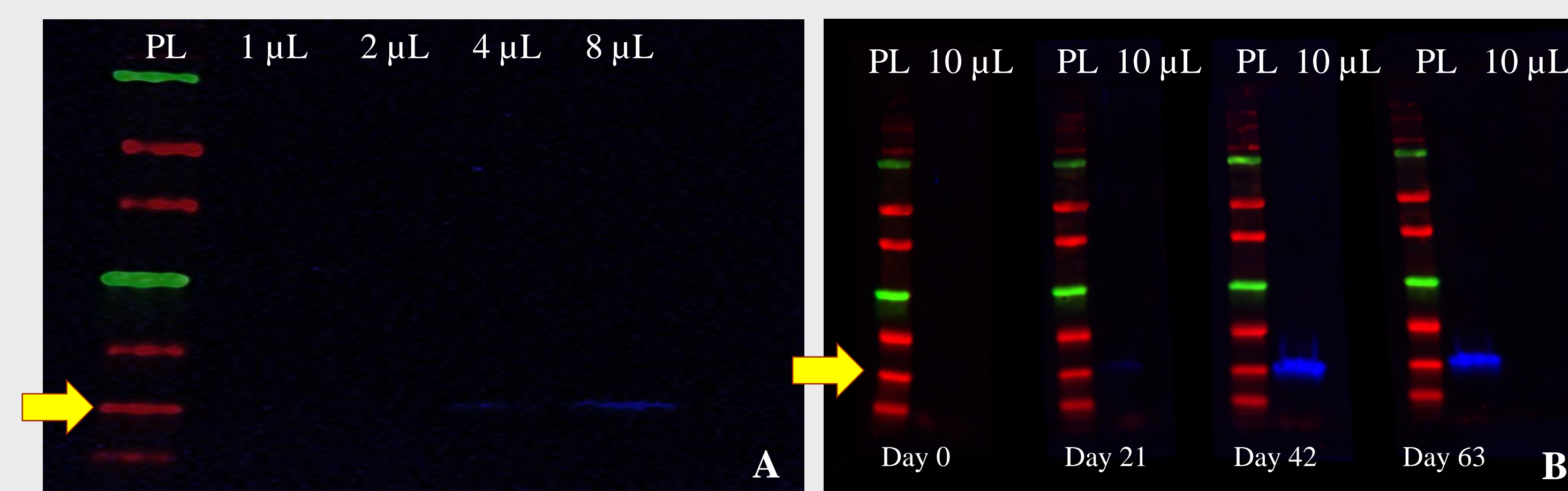
- The unconjugated and conjugated SPACA3 peptide and the horse sera samples were transferred, blocked, incubated with a primary SPACA3 antibody and then a secondary antibody, and subjected to protein detection via Western blotting.

MGWSCILFLVATATGVHSKIYSRCELTRTLRNFGLGYRGYSLADWVCLAYYTSGFNTAAVDH  
EADGSTNNGIFQVNSRKWCQNLDPAPNLCQMYCSDLLNPNLKNVICAMKITQEPGRMGGS  
WEAWRHHCQGKDLRDWVDGDFHHHHHH

**Figure 1.** Peptide abbreviations for the SPACA3 sequence used in this vaccine.



**Figure 2.** SPACA3 immunoexpression in equine ovarian tissue. **A:** Pre-granulosa cells in primordial follicles (arrow) and granulosa cells in primary follicles (arrowhead) (scale bar = 25 µm). **B:** Granulosa cells of secondary follicles (arrow) (scale bar = 100 µm). **C:** Granulosa cells of tertiary follicles (arrow) (scale bar = 350 µm). Negative controls in upper left insets.



**Figure 3.** Western blotting results for the SPACA3 peptide (**A**) and horse sera samples following immunization against SPACA3 (**B**). PL: protein ladder; yellow arrow: 15 kDa

## RESULTS

### Ovarian Immunoexpression of SPACA3

- SPACA3 was localized to the pre-granulosa cells of primordial follicles (**Figure 2A**) and the granulosa cells of primary (**Figure 2A**), secondary (**Figure 2B**), and tertiary follicles (**Figure 2C**) of all equine ovaries examined.
- SPACA3 was weakly localized to theca cells in tertiary follicles.
- There was no staining in the negative controls (**Figure 2**).

### Equine SPACA3 Vaccine Development

- The SPACA3 peptide was 15 kDa (**Figure 3A**), shown by the yellow arrow.

### Equine SPACA3 Vaccine Trial & Immunoblotting

- No long-term negative side effects or vaccine reactions have occurred
- Antibodies were successfully raised against the vaccine (**Figure 3B**).
- There were no antibodies against SPACA3 detected by Western blot at the time of the initial vaccination (Day 0) (**Figure 3B**).
- There was a small amount of antibodies detected 3 weeks following the initial vaccination (Day 21) (**Figure 3B**).
- The SPACA3 vaccine elicited a strong immune reaction 3 weeks following the second and third vaccinations (Day 42 and 63) (**Figure 3B**).

## CONCLUSION

- To the best of our knowledge, this is the first report of SPACA3 immunoexpression in horses.
- Mice immunized against SPACA3 saw a 70% reduction in female mice fertility.<sup>4</sup>
- Immunizing horses against SPACA3 may induce permanent sterilization because antibodies would be directed against all stages of follicles, including primordial follicles.

## LITERATURE CITED

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## ACKNOWLEDGEMENTS

We thank The United States Department of Agriculture and Agricultural Research Foundation funded this research