College of Agricultural Sciences, Department of Animal and Rangeland Sciences

Comparison of Sperm Protein Reactive with Antisperm Antibody Expression in Domestic and Wild Mares

Mikaela Lee, Michelle Kutzler

Figure 1: Original image of secondary equine follicles taken by microscope (Photo by M. Lee)

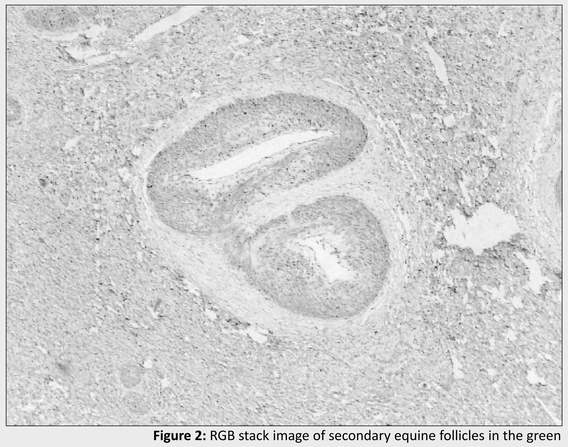


Figure 2: RGB stack image of secondary equine follicles in the green channel image (Photo by M. Lee)

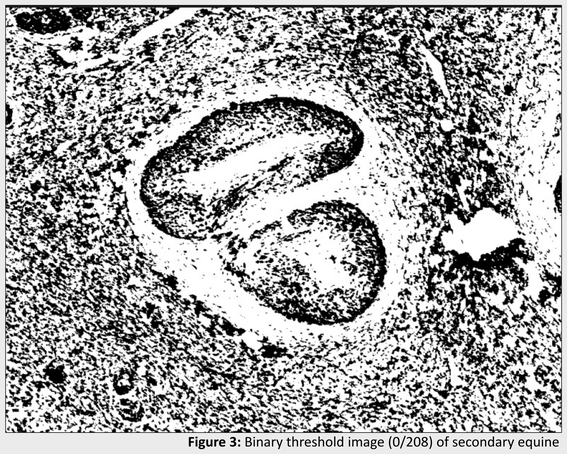


Figure 3: Binary threshold image (0/208) of secondary equine follicles (Photo by M. Lee)

RESEARCH OBJECTIVES

- The objective of this experiment was to quantify SPRASA expression in granulosa and theca cells within different stages of equine follicles
- We hypothesized that:
  1. Ovarian SPRASA expression would not differ significantly between domestic and wild horses
  2. The granulosa cells would have more SPRASA expression than the theca cells

INTRODUCTION & BACKGROUND

- The current nationwide estimate of wild horses and burros is approximately 86,000, which exceeds the appropriate management level of 26,785 animals[1]
- SPRASA, also known as Sperm Protein Reactive with Antisperm Antibody, is known to be expressed in mice, dogs, cats, humans, and horses[2-3]
- SPRASA expression has been localized to the granulosa and theca cells in primordial, primary, secondary, and tertiary follicles[2-3]
- SPRASA is a membrane protein that assists in the process of adhesion of sperm to egg during fertilization[3]

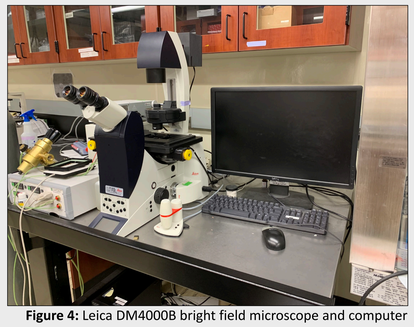
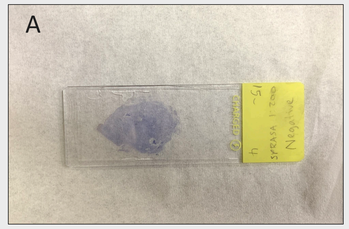


Figure 4: Leica DM4000B bright field microscope and computer used for image collection (Photo by M. Lee)

MATERIALS AND METHODOLOGY

- Procedure was broken into two parts: image collection and image analysis
- Image collection:
  - Each slide was placed onto the Leica DM4000B bright field microscope
  - Using coarse and fine adjustments, desired structures were found
  - Several images of primordial, primary, secondary, and tertiary follicles from each horse ovary were taken with a QImaging camera (QICAM 12-BIT, #QIC-F-M-12-C, Surrey, BC, Canada), saved into a folder on a computer, and exported onto both an external hard drive and google drive
- Image analysis
  - Image analysis was performed using Image J Fiji software
  - Photos of structures were opened in the software and converted into RGB stack files **(Figures 1 & 2)**
  - RGB stack files were then binarized using an auto threshold determined by the FIJI program **(Figure 3)**
  - Using an iPad with the sidecar function, images were dragged onto the iPad screen to be traced with a stylus for a more accurate measurement
  - Binary images were then traced for structures of interest and measurements of mean grey value were taken and exported into a Microsoft Excel spreadsheet
  - Data were expressed as Mean ± SEM (standard error of the means). SPRASA expression was compared between domestic and wild horses for each follicle type using t-tests
  - Significance was defined as a p-value < 0.05



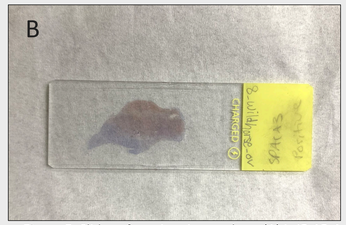


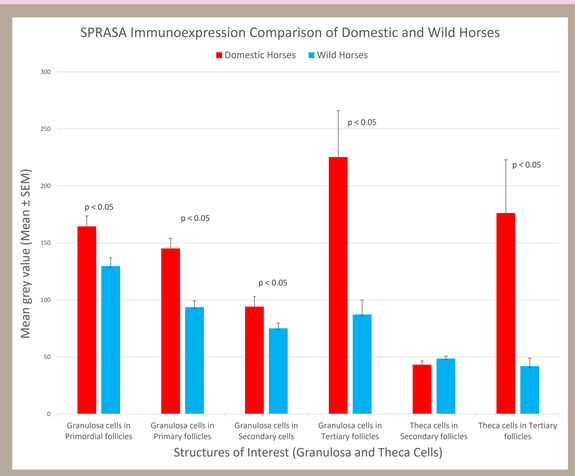
Figure 5: Slides of ovarian tissue where (A) is ID 15-4 for a domestic horse and (B) is ID 8-WH for a wild horse (Photos by M. Lee)

ACKNOWLEDGEMENTS

A special thanks to:

- Beginning Undergraduate Researchers Support Program
- College of Agricultural Sciences
- E.R. Jackman Alumni & Friends
- Dr. Michelle Kutzler
- Dr. Christina Walsh
- Brynley Cozzi & Ann Ramsey for helping me through the process of data collection and analysis

Oregon State University



RESULTS

- The highest SPRASA expression was observed in the granulosa cells of tertiary follicles in domestic horses
- Domestic horses had higher SPRASA expression than wild horses in all follicles ($p < 0.05$) except for the theca cells in secondary follicles

OBSTACLES FACED

- Images were all taken under the same conditions besides magnification to see the desired structures
- This created different colored backgrounds ranging from blue to white, causing threshold values to vary depending on the background color
- The issue was resolved by allowing the Fiji software to pick a threshold value that best represented the expression in the image and helped reduce human bias

DISCUSSION & FUTURE RESEARCH

- The age of the domestic horses used in this study ranged from 3-13 years old while the age of all the wild horses used in this study were two years old
- It is not known if age could have made a difference in the SPRASA expression in horse ovarian tissue. However, research in cats comparing SPRASA expression between kittens and adults has shown that SPRASA expression decreases with age[4]
- Additional research is planned to create and test a novel contraceptive vaccine that targets SPRASA in all stages of equine follicles

REFERENCES

1. Bureau of Land Management. (2021, March). *Herd Area and Herd Management Area Statistics.* https://www.blm.gov/sites/blm.gov/files/docs/2021-06/wildhorse_2021PopulationEstaimtes.pdf
2. Cozzi, B., Habeeb, H., & Kutzler, M. 2020. Sperm protein reactive with antisperm antibody is immunoexpressed in equine primordial, primary, secondary, and tertiary follicles. *Clinical Theriogenology, 12*(3), 362.
3. Wagner, A., Holland, O. J., Tong, M., Shelling, A. N., & Chamley, L. W. (2015). The role of SPRASA in female fertility. *Reproductive sciences, 22*(4), 452–461.
4. Ramsey, A & Kutzler, M. (2021). Sperm acrosome associated 3 protein expression in the feline ovary decreases with age. *Clinical Theriogenology, 13*, 329.