

Sperm acrosome associated 3 protein expression in the feline ovary decreases with age

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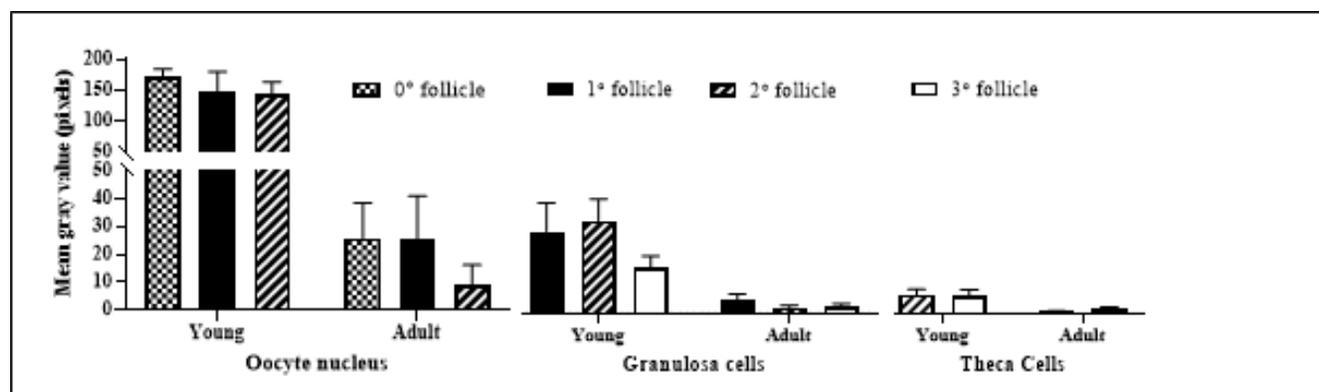
Sperm acrosome associated 3 (SPACA3) is a lysozyme-like protein previously identified in 5 to 7.5-month-old cat ovarian follicles.¹ The objective of this research was to compare SPACA3 expression in young (2 months [n = 3]) and adult (>12 months [n = 3]) queens in different follicular stages. We hypothesized that SPACA3 expression does not differ by age. Routine immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections, followed by image analysis using FIJI software. Heat-induced epitope retrieval with sodium citrate (pH 6.1) was used. AntiSPACA3 polyclonal antibody (#HPA023633, Atlas Antibodies) was applied at 1:200 dilution and immunostaining specificity was verified by replacing the primary antibody with negative control rabbit serum on adjacent sections. Sections were then reacted with one-step horseradish peroxidase-conjugated polymer antirabbit IgG (IH-8064-custom-OrSU, ImmunoBioScience), followed by a NovaRED peroxidase substrate (#SK-4800, Vector Labs). Representative images of each follicle stage from each ovary

were digitally captured using QCapturePro image capture software by a single observer at 200 x magnification. Cellular expression of SPACA3 was then quantified in primordial (0°), primary (1°), secondary (2°), and tertiary (3°) follicles using FIJI software with RGB stack and manual thresholding to isolate areas of staining. The oocyte nucleus, granulosa cells, and theca cells were outlined using the freehand selection tool and mean gray value was measured. Results (mean ± SEM) were compared between young and adult queens using a Student's *t*-test and significance was defined as $p < 0.05$. There was greater SPACA3 expression in young compared to adult queens (Figure) in the oocyte nucleus of 0° ($p < 0.001$), 1° ($p = 0.006$), 2° follicles ($p < 0.001$), in granulosa cells of 1° ($p = 0.016$), 2° ($p < 0.001$), 3° follicles ($p = 0.004$), in theca cells of 2° ($p = 0.006$), and in 3° follicles ($p = 0.043$). This is the first study to evaluate differences in SPACA3 expression by age in any species. More research is needed on the mechanisms that regulate ovarian SPACA3 expression and its role in female fertility.

Keywords: Cat, granulosa cell, oocyte nucleus, ovary, theca cell

Reference

1. Wagner A, Holland OJ, Tong M, et al: The role of SPASA in female fertility. *Reprod Sci* 2015;22:452-461.



Using reflection to optimize student engagement during theriogenology practical classes: the benefits of mentoring and peer support in teaching

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Veterinary training is a resource consuming process. Practical sessions throughout the curriculum are considered vital aspects of professional training and consume large amounts of time, space and human resources and are not without risks to human

and animal health.¹ It is therefore, important that the educational experience is optimized during practical sessions. Mentee dialogue with mentors, as part of institution peer teaching support, is aimed to optimize the practical sessions in order to develop professional expertise (knowledge and skills) of veterinary students learning theriogenology. We used the theory of experiential learning and reflective observation as a means of enhancing learning during the practical session.² We attempted to do this by discussing abstract concepts associated with the authentic learning tasks covered in each practical session. Anonymous end-of-course student feed-back revealed that the process encouraged in depth and alternative critical thinking and discussion in the groups that was a fun way for them to embed the knowledge and develop the skills being taught. The use of 'abstract reflection' appears to be a really useful and efficient