

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

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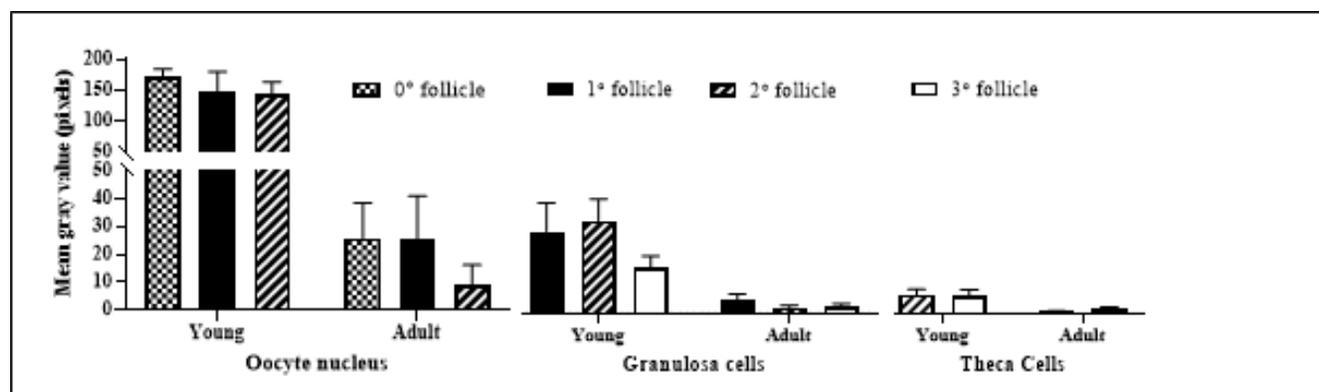
Sperm acrosome associated 3 (SPACA3) is a lysozyme-like protein previously identified in 5 to 7.5-month-old cat ovarian follicles.¹ The objective of this research was to compare SPACA3 expression in young (2 months [n = 3]) and adult (>12 months [n = 3]) queens in different follicular stages. We hypothesized 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

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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

way of enhancing the value of laboratory practical teaching and learning resources within the veterinary theriogenology curriculum. Interestingly, a few of the reflective questions were formulated so as to not have answers that were known by the academic staff. The dissonance was relished by some of the cohort, those excited by investigation and research, but appeared to induce chagrin in a minority that were uncomfortable with the unknown. This was despite the whole group being made aware that this was a safe and fun environment that developed during the session. An important aspect of the peer review process has been the continued discussion between mentors and mentees in relation to enhancing teaching in general, and practical sessions in particular. The vibrancy associated with collegiate interactions between academic staff members and educational designers results in a more enthusiastic and beneficial teaching and learning environment.

Keywords: Abstract reflection, experiential learning, peer support

References

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Agreement level among 4 techniques for analysis of stallion sperm morphology

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Analysis of sperm morphology is important for assessment of sperm quality and prognostication of fertility potential of

stallions. Previous studies have compared various techniques for morphologic analysis of stallion sperm using correlation analysis or mean comparisons. We hypothesized that such approaches might lead to erroneous interpretations when comparing different methods of sperm morphologic analysis. The present study sought to determine the agreement level among four techniques for analysis of stallion sperm morphology: eosin/nigrosin staining (EN); diff-quick staining (DQ); wet mount using phase-contrast microscopy (PH); or wet mount using differential interference contrast microscopy (DIC – gold standard). Ejaculates from 12 sexually active stallions (n = 36) were collected and analyzed using each technique. A total of 100 sperm were observed under 1,000 x magnification and classified as described by the Society for Theriogenology (normal [N], abnormal head [AH], abnormal acrosome [AA], proximal droplet [PD], distal droplet [DD], abnormal midpiece [AMP], bent midpiece [BMP], and coiled tail [CT]). Based on the percent normal sperm observed by DIC microscopy, sperm morphology was categorized in each of 3 categories (n = 12/category) as high (H): > 57% normal sperm, average (A): 23 - 56% normal sperm, or low (L): < 23% normal sperm. Within each morphology category (H, A, L), the agreement level (bias, 95% lower and upper limits of agreement) was determined (Table). The results from this study indicate that the use of EN or DQ leads to an overestimation (negative bias value) of normal sperm, as well as an underestimation (positive bias value) of morphological defects that are known to impact stallion fertility, such as AH or AMP (values in bold). Most discrepancies among methods were observed in categories A and L. These results may affect the interpretation of sperm morphology evaluation and, thus, the estimation of stallion potential fertility.

Keywords: Stallion sperm, sperm morphology, stained smear, wet mount, agreement

Category	Comparison	% N	% AH	% AA	% PD	% DD	% AMP	% BMP	% CT
High	DIC vs. EN	-4 (-15, 7)	11 (7, 16)	4 (-5, 15)	-2 (-10, 7)	-1 (-17, 15)	4 (-3, 11)	4 (-6, 13)	0 (-4, 3)
	DIC vs. DQ	-5 (-14, 5)	12 (3, 20)	4 (-5, 14)	4 (-12, 4)	3 (-11, 18)	5 (-3, 12)	3 (-8, 13)	0 (-3, 3)
	DIC vs. PH	0 (-7, 8)	4 (-19, 10)	4 (-5, 12)	2 (-10, 13)	6 (-10, 22)	-1 (-13, 11)	3 (-6, 11)	0 (-2, 3)
Average	DIC vs. EN	-11 (-29, -6)	12 (6, 18)	3 (-3, 8)	6 (-17, 28)	-1 (-11, 9)	8 (-9, 24)	4 (-7, 15)	0 (-2, 2)
	DIC vs. DQ	-7 (-23, 9)	4 (-4, 12)	3 (-4, 10)	3 (-13, 18)	0 (-14, 14)	3 (-15, 21)	5 (-6, 16)	0 (-3, 3)
	DIC vs. PH	-1 (-15, 13)	3 (-22, 27)	1 (-10, 12)	1 (-15, 16)	0 (-10, 10)	-4 (-20, 13)	1 (-8, 11)	0 (-2, 3)
Low	DIC vs. EN	-11 (-25, -5)	20 (17, 24)	5 (-7, 17)	6 (-22, 34)	-2 (-11, 9)	7 (-7, 20)	7 (-12, 25)	0 (-8, 9)
	DIC vs. DQ	-9 (-20, 3)	19 (14, 24)	3 (-3, 10)	13 (-12, 40)	0 (-20, 21)	2 (-14, 17)	7 (-9, 23)	-1 (-8, 6)
	DIC vs. PH	-2 (-10, 7)	6 (-26, 38)	1 (-16, 17)	5 (-22, 33)	-2 (-17, 12)	-3 (-23, 17)	5 (-14, 24)	-1 (-8, 10)