A245 SUPPORTIVE BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, IMAGE ANALYSIS AND DIAGNOSIS, MOLECULAR BIOLOGY AND "OMICS"

STUDY OF FOLICULAR VASCULARIZATION USING TRIDIMENSIONAL IMAGES: A NEW APPROACH

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The vascularization is important to the follicle development and it is directly related to fluid follicular composition, which in turns is critical to oocyte maturation. Because of the reduced dimension of the blood vessels the assessment of vascularization by pulsatility and resistance indexes is frequently unfeasible, and quantification is represented by a percentage subjectively obtained by visualization. The aim of the present study was to develop a new methodology to assess the follicular vascularization using color Doppler technology and a software for image analysis (Image J) to recreate tridimensional images of the blood vessels found on the follicle. For tridimensional images generation it is important to determine the number of frames necessary to maintain the spatial resolution of the structure. Therefore, latex spheres of different diameters (12, 11, 10, 9, 8, 7, 6, 5 and 4 mm) were filled up with a volume of PBS similar to the expected volume of follicle of same diameters (approximately 900, 700, 520, 380, 270, 180, 110, 70 and 30 mm^3, respectively). Subsequently, sequences of ultrasonographic images of these mimetic follicles were recorded and evaluated with Image J to calculate the volume. The number of frames necessary to maintain the spatial resolution was 120, 11, 99, 78, 70, 60, 50 and 31 for the mimetic follicles of 12, 11, 10, 9, 8, 7, 6, 5 and 4 mm in diameter, respectively. With these numbers of frames, the volume calculated by Image J varied less than 5% compared to the expected value (921.55; 712.70; 514.16; 385.99; 277.34; 182.93; 112.73; 69.74 e 29.71 mm^3, respectively). Thereafter, the follicular wave was synchronized (beginning of protocol = D0) in two cows (Gyr and Holstein). The growth of dominant follicle was daily evaluated by ultrasonography since its emergence, and its vascularization visualized using color doppler technology. Tridimensional images were generated using the number of frames previously determined, and the volume of vascularization was calculated. In both cows, the presence of vascularization was first detected on D5 (2.2 *vs.* 9.9 mm^3, Gyr and Holstein respectively), and progressively grew until dominance phase (54.9 *vs.* 83.2 mm^3, Gyr and Holstein respectively). After dominance a sharp decrease on vascularization volume was observed (4.1 *vs.* 49.3 mm^3, Gyr and Holstein respectively). Although this is a preliminary study with a limited number of observations, the results obtained are consistent with the expected variation during the follicle development, showing the potential of this new approach as an alternative and less subjective methodology to assess follicular vascularization. [Acknowledgment: To FAPEMIG and MP1 of Embrapa (01.07.01.002)].

Keywords: ultrasonography, color-doppler, ovary.

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QUALITATIVE STUDY OF THE INTERACTION OF BSPS (BINDER OF SPERM PROTEINS) WITH THE SPERM CELLS IN RUMINANTS

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BSPs (Binder of Sperm Protein) are the most abundant proteins of the seminal plasma of several ruminants and play important roles during sperm capacitation and interaction between sperm cells and the oviductal epithelium. In the ram, RSVP 14 and 22 kDa represent the BSP family and BSP 1 and 5 are their homologues in the bovine. Thus, the present study was conducted to evaluate the expression of BSPs in fluids of the reproductive tract and their interactions with sperm of ruminants. Seminal plasma and sperm were obtained by centrifugation of semen from Morada Nova rams and cauda epididymal sperm, collected from slaughtered animals. After the first centrifugation of semen samples, sperm were washed three times in PBS, homogenized and the resulting pellet was washed three more times in PBS. The pellet was resuspended in PBS 1% triton X-100 and kept a 4°C for two h, with homogenizations every 15 min. The solution was sonicated at 4°C for 30 min and centrifuged for one h (5.000 x *g*, 4°C). The supernatant and seminal plasma were then precipitated with acetone and resuspended in a buffer (urea, thiurea, chaps, dtt, Ipg buffer). Samples containing 400 µg of total protein were subjected to 2-D electrophoresis and the maps, analyzed using PDQuest software (BioRad, USA). A similar protocol was used to process seminal plasma and sperm membrane proteins from Saanen goats and Holstein bulls. Two-dimensional maps were also constructed using fluid from the cauda epididymis (CEF) and accessory sex glands (AGF). In rams, we detected RSVP 14 as the major component in seminal plasma maps and in gels of proteins extracted from membranes of ejaculated sperm, but not from epididymal sperm. However, RSVP 22 did not appear in gels of ejaculated sperm as the same pattern detected for the RSVP 14. In goats, proteins with kDa and pI similar to those of RSVP 14 were detected in the seminal plasma and in gels containing proteins from ejaculated sperm. It is possible, thus, that 14-kDa BSPs, in comparison with 22-kDa BSPs, have stronger affinity for sperm membranes after ejaculation. In the case of bulls, BSP1 was also detected as the major component of seminal plasma, AGF and in gels of membrane proteins extracted from ejaculated sperm, but absent in the CEF. In conclusion, we suggest that there is a conserved mechanism of secretion of BSPs and of interaction of these proteins with sperm cells in different ruminant species.

Keywords: proteomics, semen, ruminants.