ABSTRACTS

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EFFECT OF PROGESTERONE AND IONOMYCIN ON DOMESTIC CAT SPERM MOTILITY PATTERNS AND ACROSOME REACTION

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Introduction - Sperm capacitation, which involves changes in motility patterns and subsequent acrosome reaction (AR), is an essential step in the process of fertilization [1]. Using the computer-assisted sperm analysis (CASA) these changes in sperm motion, known as hyperactivation, can be assessed in a capacitated population, mainly characterized by alterations in linearity, velocity and head displacement [2]. Progesterone (P4) seems to be involved in the induction of acrosome reaction in many species. Exposed P4 receptors in the sperm surface exhibited correlation with the maturation state of sperm in dogs [3] and with animal fertility in stallions [4], while in bovine P4 showed a potential role in sperm capacitation, but not in AR [5]. Ionomycin, a Ca2+ ionophore, is used to mimic the physiological events during the AR. In cats, the Ca2+ ionophore A23187 was more efficient in inducing AR compared to solubilized zona pellucidae, considered an physiological inducer [6]. The aim of this study was to evaluate changes in domestic cat sperm motility and AR induction efficiency in response to chemical (ionomycin) or physiological (P4) inducers using different concentrations and time-exposure.

Material and methods - Two ejaculates from five mixed-breed tom cats, aged 2-6 years, were collected using an artificial vagina (n=10). Each ejaculate was diluted in 1 mL of a capacitation medium (100 mM NaCl, 3.1 mM KCl, 1.1 mM KH2PO4, 1.1 mM MgSO4, 6 mM glucose, 25 mM NaHCO3, 2 mM CaCl2, 1.2 mM cysteine, 1 mM piruvate, 1 mM glutamine, 25 mM lactate, 20 mM Hapes and 0.6% BSA) (CM), analyzed with CASA and then centrifuged at 300 x g for 10 minutes. Supernadant was discharged and pellet ressuspended with the CM (20 x 10^6 sperm/ mL) and analyzed using CASA and a combination of three fluorescent probes to assess sperm plasma membrane integrity (Iodide Propidium) (PMI), acrosomal membrane integrity (FITC-PSA) (AMI) and mitochondrial transmembrane potential (JC-1) (MTP). After evaluation, samples were divided into seven equal aliquots and maintained at room temperature (23° C) during 2 hours after centrifugation. In group P1, P4 was added after 30 minutes of centrifugation (10 µg/ mL final concentration). After 2 hours at room temperature, groups I1 and I2 were supplemented with ionomycin diluted in ethanol, with final concentration of 4 and 8 µM, respectively. Groups P2 and P3 were supplemented with P4 diluted in ethanol, resulting in final concentration of 10 and 20 µg/ mL, respectively. In group E, a solution of ethanol was added to a final concentration of 0.6% and group C received no supplementation. All samples were incubated for 30 minutes at 38° C in a humidified atmosphere of 5% CO2. After incubation, samples were evaluated using CASA and the fluorescent probes as described above. Data were submitted to statistical analysis by ANOVA and Tukey test, with p < 0.05 taken as significant.

Results and Discussion - Comparing the results obtained by CASA after and before centrifugation and dilution, higher results for percentage of total motility (MT) and progressive motility (MP) and lower straightness (STR) were observed before these procedures. After incubation, the control group was not statistical different from the group prior to incubation for all evaluations. No statistical difference was observed between groups C and E for all results obtained. Compared to the control group, the use of an AR inducer, either P4 or ionomycin, led to sperm motility patterns alterations, which included an
increase in the beat cross frequency (BCF) and amplitude of lateral head displacement (ALH) and a decrease in linearity (LIN) and straight line velocity (VSL), with low values for average path velocity (VAP) only in groups I2 and P3 and no difference in curvilinear velocity (VCL) values among all groups. These changes in motility patterns can characterize a hyperactivation motion for the domestic cat sperm as previously described for other species [7]. For AR induction, the groups treated with AR inducer showed a lower AMI and a higher percentage of sperm showing both reacted acrosome and PMI (ACR) compared to the control group, demonstrating that both P4 and ionomycin were efficient in triggering AR. Among all groups treated with P4, no statistical difference was observed. This way, for the domestic cat sperm, concentrations above 10 µg/mL of P4 and time-exposure higher than 30 minutes did not enhance sperm response to this inducer. Although a lower percentage of AMI and a higher ACR were obtained in the I2 group compared to the P4 groups, a decrease in MT and MP were observed for I2 group. Since I1 group did not showed lost of motility and demonstrated the same efficiency to induce AR as the I2 group, a concentration of 4 µM of ionomycin seems more suitable for the domestic cat sperm. In conclusion, both P4 and ionomycin leaded to hyperactivation-like alterations in sperm motility and were efficient in inducing AR in the domestic cat spermatozoa.

References

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