ABSTRACTS

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Sperm nuclear decondensation after in vitro and in vivo matured dog oocytes

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OBJECTIVES AND METHODS: The fertilizing spermatozoa must undergo changes after its penetration to ovum cytoplasm, these changes involve protamine disulfide bond reduction and protamine replacement by oocyte histones [1], leading to sperm nuclear decondensation. One of the criteria for oocyte cytoplasmic maturation is the ability to decondense the sperm chromatin, which depends upon the maturational state of the oocyte [2,3]. Therefore, the objectives of this study were to evaluate the sperm nuclear decondensation capacity of ovulated and in vitro matured (IVM) canine oocytes during different culture times and correlate this decondensation ability with the state of oocyte nuclear maturity in vitro and in vivo. Cumulus-oocyte complexes (COC) for IVM were obtained from bitch ovaries after ovariohysterectomy, selecting those COC according compact cumulus cells and a homogeneous dark cytoplasm. The COC were matured in vitro in TCM-199 with Earle’s salt supplemented with 25 mM HEPES, 10% FCS, 0.25 mM pyruvate, 10 IU mL–1 of hCG, 300 IU mL–1 of penicillin, and 20 mg mL–1 of streptomycin for 0, 48, 72, and 96 h at 38.5°C and 5% CO2 [4]. In vivo matured oocytes (ovulated) were obtained from bitches 50-72 h after ovulation. These oocytes were collected by flushing the oviducts with 15 mL of TCM 199 supplemented with 10% FCS [4]. Fresh ejaculates from 3 adult dogs were use for IVF experiments, semen specimens were centrifuged and the sperm pellets were re-diluted in Fert-TALP medium. After each culture period of IVM, 100-μL fert-TALP drops containing 2.5 × 10⁶ spermatozoa mL –1 were co-culture with around 12 IVM or 6 ovulated oocytes for 24 h, under 38.5°C and 5% CO₂ in humidity. After co-cultures, the oocytes were denuded from cumulus cells and fixed in 3% paraformaldehyde. The nuclear stage of the oocytes and the appearance of the sperm nucleus were determined by DAPI staining with fluorescence microscope. At least four replicates were performed and the data were analyzed with a Chi-square (X²) test.

RESULTS: The rates of decondensation were the sum of both, moderately and grossly decondensed sperm heads. These rates in IVM oocytes (N= 150) significantly increased up to 72 h of culture, showing 12.2, 65.7, 80.5 and 80%, with 0, 6.5, 20.3, and 27% of second metaphase (MII) stage at 0, 48, 72 and 96h respectively (P <0.05). In contrast, all in vivo matured oocytes (N= 30), showed MII nuclear stage and had a total of 96.7% of sperm chromatin decondensation. The percentages of MII stage were much lower (P <0.05) in all in vitro maturing groups oocytes in comparison to those ovulated; in the same way, the rate of chromatin decondensation was higher in ovulated oocytes than all those matured in vitro.

CONCLUSION: These results suggest that canine oocytes matured in vitro are able to decondense the sperm chromatin during IVF, and this ability increases with time up to 72 h of culture. Nevertheless, cytoplasmic maturation, as evaluated by sperm chromatin decondensation, is less efficient than ovulated oocytes and may not be completely connected with nuclear development as occur in vivo.


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