INTRACYTOPLASMIC SPERM INJECTION IN BOVINE OOCYTES AS POTENCIAL REPRODUCTIVE STRATEGY FOR ENDANGERED BREEDS

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Introduction

Conservation of animal genetic diversity allows farmers to select stock or develop new breeds in response to changes in the environment, threats of disease, new knowledge of human nutrition requirements, changing market conditions and societal needs, all of which are largely unpredictable. So, disappearance of any breed will reduce the diversity and thus the option by man and nature. The alternative is an effective conservation program.

Cryogenic preservation is one way for *ex-situ* conservation. However, some animals are irresponsible to cryogenic process. One possibility is the use of advanced reproductive techniques such as Intracytoplasmic Sperm Injection (ICSI), witch one can utilize immobile dead spermatozoa and spermatids for microinjection into oocytes to produce embryos (Goto et al., 1990; Goto et al., 1996), although more researches are necessary to obtain better results.

In this way, the present study aimed to define the best time for bovine oocyte maturation to realize ICSI.

Methodology

Immature oocytes aspirated from 2-8 mm follicles were cultured in TCM 199 supplemented with 10% (v/v) fetal calf serum, FSH (10mg/ml), LH (24UI/ml) and gentamicine (5μg/ml) at 39°C, 5% CO_2 in air, with saturated humidity, in different time of culture. For sperm injection, the cumulus cells were removed after oocyte maturation and the oocytes with first polar body were selected. Injected oocytes and sham injected were activated with 50μM of calcium ionophore for 10 min and the activation was stopped with bovine serum albumin (6mg/ml). After this, the structures were co-cultured with granulose cells in TCM-199 for 18-22 hours, when it this time all structures were fixed and stained to evaluate oocyte activation and fertilization rate. In the first experiment 2129 oocytes were cultured for different lengths of time: 22, 24 and 28 hours. In the second experiment, the oocytes were cultured for 22 hours, denuded and some of them cultured for two or four more hours. In every culture groups the oocytes were divided among ICSI and glutathione (GSH) dosage.

Results

In the first experiment the highest maturation rate (P<0.05) was obtained at 22h of culture (52,82%) if was compared with 24 (45,15%) and 28 h (30,65%). In the second experiment no difference was observed between groups (P>0,05) to activation rate after ICSI in oocytes cultured for 22, 24 and 28 hours and sham injected, 48,28% (28/58), 40,0% (26/65), 32,77% (38/72) e 56,82% (25/44), respectively, as well as to fertilization rate, 10,34% (6/58), 10,77% (7/65) e 8,33% (6/72), respectively for ICSI at 22, 24 e 28 h of culture. No differences (P>0,05) were observed either in GSH content among treatments (22, 24 and 28 hours) and among these and immature oocytes, 6,5±2,57; 5,06±1,92; 8,01±3,05 e 7,89±2,68 pmol/oocyte, respectively.

Conclusion

Oocytes matured for 22h have already capacity to sperm decondensation and male pronuclear formation.

References

Goto, K.; Kinoshita, A.; Nakanishi, Y.; Ogawa, K. Blastocyst formation following intracytoplasmic injection of in-vitro derived spermatids into bovine oocytes. Hum. Reprod., v.11, n.4, p.824-829, 1996.

Goto, K.; Kinoshita, A.; Takuma, Y.; Ogawa, K. Fertilization of bovine oocytes by the injection of immobilised, killed spermatozoa. Vet. Rec., v.127, p.517-520, 1990.