

A257 SUPPORTIVE BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, IMAGE ANALYSIS AND DIAGNOSIS, MOLECULAR BIOLOGY AND "OMICS"

#### RELATIVE ABUNDANCE OF MATERNAL TRANSCRIPTS IN OOCYTES WITH AND WITHOUT POLAR BODY AFTER *IN VITRO* MATURATION

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The oocyte ability to undergo maturation and early cleavages during the maternal-zygotic transition (MZT) is associated to transcript levels stored in its cytoplasm. The extrusion of the polar body is an indicative of oocyte ability to undergo maturation. However, to our knowledge, no data comparing mRNA levels among bovine oocytes matured *in vitro* presenting polar body (PB) and oocytes matured *in vitro* without the presence of polar body (NPB) are available. The aim of the present study was to compare the relative abundance of maternal transcripts of Maternal Antigen That Embryo Requires (MATER), Zygote Arrest (ZAR1), TEA domain family member 2 (TEAD2) and High Mobility Group N (HMGN1) genes between oocytes with or without PB (PB and NPB groups, respectively) following *in vitro* maturation. Immature bovine oocytes were obtained by follicular aspiration and matured in TCM-199 199 (Gibco Life Technologies, New York, USA) containing 10% estrus cow serum and 20 µg/mL FSH (Pluset, Serono, Italy), for 24 h under 5% CO<sub>2</sub> in air at 38.5°C. Oocytes separated according to the presence or absence of PB and then they were denuded and frozen in liquid nitrogen. Three pools of ten oocytes for each group were subject to RNA extraction. Reverse transcription and cDNA amplification were performed using the TransPlex Complete Whole Transcriptome Amplification Kit (WTA2 – Sigma Aldrich) according to the manufacturer's instructions. Relative abundance of the target transcripts was performed by real-time PCR (Applied Biosystems Prism 7300 Sequence Detection Systems, Foster City, EUA) using the beta-actin gene as the endogenous reference. The relative expression of MATER (0.81±0.11), ZAR1 (0.82±0.09), TEAD2 (0.83±0.07) and HMGN1 (0.90±0.08) was downregulated ( $P < 0.05$ ) for oocytes NPB after *in vitro* maturation. We conclude that the presence of polar body may be related to abundance of maternal mRNA and can contribute to predict quality of *in vitro* maturation conditions. [Financial support: Embrapa – Animal Genomics Network Project (01.06.9.01.01.00) and Innovations in Animal Reproduction Network Project (01.07.01.002), CNPq, CAPES and Fapemig].

**Keywords:** gene expression, oocytes, *in vitro* maturation.

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#### RELATIONSHIP BETWEEN ACROSSOME REACTION, POST-THAW SPERM EVALUATION AND TOTAL PROTEIN IN MOXOTÓ GOAT SEMEN

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Even been able to fertilize, sperm cells from epididymis can present alterations when in contact with the seminal plasma during the ejaculation, losing its capacity of suffering the acrossome reaction (AR) and to penetrate the oocyte. This study aimed to evaluate the relation between (AR) with the post-thaw semen evaluation and the total proteins (TP) in the seminal plasma of Moxotó bucks in Brazilian Northeast. The semen was collected from five males from January to March/2011, totalizing three samples per animal, being one week of the month used for semen freezing and the other for total protein analysis. For semen freezing, Tris-glycerol at 2% was used, being the sperm stored in 0.25 mL straws, using the portable system Tetakon, TK 3000. The post-thawed spermatic motility (%) was evaluated and the TP analysis followed the Bradford method (1976, M Analyt Biochem 72:248-254). In the AR evaluation, the Naftol yellow/erythrosine B was used aiming to identify the percent of post-frozen/thawed sperm reaction. The Pearson correlation was run between post-thawed spermatic motility and total plasma protein. The mean spermatic motility and the post-thawed AR were 20% and 35%, respectively. It was verified a positive and high intensity correlation ( $r = 0.40$ ) between AR and progressive post-thaw motility, according to studies that are in agreement with these results (Correa *et al.*, 1997, Theriogenology 48:721-731). It was not observed a correlation ( $P > 0.05$ ) between AR and seminal TP. It is concluded that probably Moxotó animals that showed higher spermatic motility can also be able to suffer good rate of AR and, in consequence, can probably show higher fertility. However, it is recommended more studies on this subject.

**Keywords:** cryopreservation, fertilization, proteomics.