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A225 CLONING, TRANSGENESIS AND STEM CELLS

SUCCESS ON ISOLATION, IMMUNOPHENOTYPICAL CHARACTERIZATION AND DIFFERENTIATION IN OSTEOGENIC, ADIPOGENIC AND CHONDROGENIC LINEAGES OF MESENCHYMAL STEM CELLS (MSCS) FROM THE EQUINE AMNIOTIC LIQUID.

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The bone marrow is the major source of MSCs in equine medicine, however as the amount and differentiation capacity of these cells decrease with age, its therapeutic potential also decrease across the time. So, over the last decades it was observed an increase interest in investigate new sources of MSCs, mainly cells with origin in fetal annexes, which had already demonstrate the capacity of maintain the pluripotency characteristics of the tissues from which its originate (Cremonesi, 2011, Theriogenology, 75, 1400-1415). The aim of the present study was to isolate, characterize immunophenotipically and differentiate the MSCs from equine amniotic liquid (AL) in osteogenic, adipogenic and chondrogenic lineages. Samples were collected from gravidic uterus in slaughterhouse and transported at 5°C to the laboratory in Botutainer® (Botupharma, Botucatu/BR) transport system; where it were centrifuged and pellets were resuspended in culture medium containing DMEM, F12, bovine fetal serum, antibiotic and antimycotic (Gibco® - NY/USA). The culture system was maintained at 37°C with 5% carbon dioxide in air. After the second passage, a portion of the cells were fixed in paraformaldehyde 4% in 24 "well" culture plates. Immunocytochemistry was performed with antibodies anti-vimentine, -cytokeratin, -CD44, -PCNA and -OCT 4. The rest of the cells were maintained in culture system and after the third passage the differentiation process was induce by replacement of support media by another media compound by the differentiation kits (Gibco®-NY/USA). This process was verified by the use of following staining, Alizarin Red (osteogenic strain), Oil Red O (adipogenic strain), Toluidine Blue and Alcian Blue (chondrogenic strain, Sigma®, St. Louis/USA). The results were a positive immunostaining for vimentine, CD44 and PCNA and negative for cytokeratin, confirming the mesenchymal origin of these cells and their proliferation capacity. The absence of OCT-4 immunostaining differs from the results observed in the literature; however a quantitative evaluation was not performed and once the AL population was very heterogeneous the percentage of expression of this marker could vary within the samples. The immunocytochemistry performed directly in the culture plate was efficient to characterize the studied cells population maintaining their fibroblastoid morphology. The staining showed the presence of calcium mineralization, intracytoplasmic lipid and glycosaminoglycans confirming the differentiation potential of the cells obtained from the AL on the three cited lineages. So, we could concluded that the isolation, characterization and differentiation of MSCs from equine AL was successful done. [Acknowledgements: Capes, Fundunesp e FAPESP].

Keywords: mesechymal stem cells, amniotic fluid, equine.

A226 SUPPORTIVE BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, IMAGE ANALYSIS AND DIAGNOSIS, MOLECULAR BIOLOGY AND "OMICS"

ALTERED EXPRESSION OF GENES RELATED TO GLUCOSE METABOLISM IN BOVINE OOCYTES MATURATED *IN VITRO* OR *IN VIVO*

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One of the main challenges of reproductive biotechnology is to understand the metabolic pathway in normal embryo development. Molecular biology methodologies allow the evaluation of metabolic modifications in *in vitro* culture conditions, which could induce epigenetic responses that would finally result in altered gene expression patterns. The aim of this study was to evaluate the expression of genes related to glucose transport and metabolism (GLUT1 – Glucose Transporter; IGF1R – Insulin-Like Growth Factor 1 Receptor, IGF2R – Insulin-Like Growth Factor 2 Receptor) in bovine oocytes obtained from Gyr cattle (*Bos indicus*) maturated *in vivo* (M1 group) and *in vitro* (M2 group). The protocol used to recover the M1 oocytes was: Day 0 (D0) – 2 mg of estradiol benzoate + norgestomet auricular implant; D4- D7 – four administrations of 200mg FSH given every 24h, in decreasing dosages; D6 (morning) – 150 µg of cloprostenol; D7 (afternoon) – auricular implant removal; D8 (12:00h) – 25 mg of LH; D9 (morning) – follicular aspiration (OPU). In the M2 group, the follicular wave was synchronized as follows: D0 – 2 mg of estradiol benzoate + auricular implant + 150 µg of cloprostenol; D5 – auricular implant removal; D6 – OPU. The *in vitro* maturated oocytes (IVM) were cultured in TCM-199 media (Invitrogen, CA, USA) added with 10% of estrous cow serum and 2µg of FSH (Pluset, Callier, Spain) for 24h, at 38.5°C, 5% of CO2 and saturated humidity. Both *in vivo* and *in vitro* maturated oocytes were denuded and stored in 3 pools of 10 oocytes, snap-frozen in liquid N2 and kept at -80°C until use. RNA extraction was performed using the commercial kit RNeasy Micro Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's specifications. The reverse transcription and the cDNA preamplification were performed using the commercial kit TransPlex Complete Whole Transcriptome Amplification Kit (WTA2 – Sigma Aldrich) according to the manufacturer's specifications. The cDNA was submitted to Real-Time PCR, using the β-actin gene as endogenous control and the commercial kit Power SYBR® Green PCR Master Mix (Applied Biosystems) according to the manufacturer's specifications, for expression analysis in the REST® software. The genes GLUT1 (1.37±0.07) and IGF1R (2.01±0.14) were up-regulated ($P < 0.05$) in the *in vitro* group when compared to the *in vivo* group, while in the IGF2R gene (1.29±0.11) there was no difference ($P > 0.05$). In conclusion, the IMV environment can alter the expression pattern of GLUT1 and IGF1R genes. [Financial support: Embrapa – Project Rede Genômica Animal (01.06.9.01.01.00) and Project Rede de Inovação em Reprodução Animal (01.07.01.002), CNPq,CAPES and Fapemig].

Keywords: mRNA, development, gamete.