Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

PRE-FERTILIZATION APPROACH USING α-L-FUCOSIDASE MODULATES ZONA PELLUCIDA HARDENING DURING BOVINE IN VITRO EMBRYO PRODUCTION

Thainá Sallum Bacco Manssur [1], Talita Raquel Cavichioli Sesastião [1], Priscila Helena dos Santos [2], Fernanda Fagali Franchi [2], Sarah Gomes Nunes [2], Anthony César de Souza Castilho [1,2], Patrícia Kubo Fontes [2]

[1] UNOESTE - Universidade do Oeste Paulista (Rod. Raposo Tavares, km 572 - Limoeiro, Pres. Prudente - SP, 19067-175), [2] UNESP - Universidade Estadual Paulista, Júlio de Mesquita Filho, Campus de Botucatu (Rua Prof, R. Dr. Valter Maurício Corrêa, s/n, Botucatu - SP, 18618-681,)

Resumo

Polyspermy incidence during in vitro fertilization is still an important limitation for bovine in vitro embryo production technique advance, resulting in reduced embryo development, early embryonic death, or miscarriage. The glycoprotein α-L-fucosidase (FUCA), a glycosidase found in mammalian oviductal fluid, has been described to be involved in the hydrolytic degradation of fucose as well as to participate in sperm-oocyte binding through interactions with complementary glycans on the surface of the zona pellucida (ZP), acting in the control of polyspermy. Therefore, our objective was to investigate the effect of the addition of FUCA during in vitro pre-fertilization on the modulation of the ZP hardening, embryonic development, and quality of bovine blastocysts produced in vitro. For that, after in vitro maturation, bovine cumulus-oocytes complexes (COCs, at least 100 COCs/treatment/experimental analysis) were incubated for one hour with FUCA at four different concentrations (0; 0.0625; 0.125; and 0.25 IU/mL). Subsequently, the treated COCs were fertilized and the probable zygotes intended for evaluation of embryonic development in vitro. During the experimental analyses we evaluated the embryo production rate, ZP digestion time, and monospermic fertilization rate. Embryonic quality was evaluated by analysis of marker genes of pluripotency, differentiation, implantation, and embryonic development. The effect of FUCA addition was evaluated by ANOVA and, when present, the means were compared by Tukey test. The differences were considered significant when $P<0.05$. There was no difference ($P>0.05$) in the blastocyst rate when comparing the groups 0; 0.0625; 0.125; and 0.25 IU/mL (46,1±17,3; 48,2±7,8; 34,9±6,9; 33,4±10,9%, respectively), however, the number of blastocysts that hatched after treatment with FUCA at the concentration of 0.0625 IU/mL was more than twice (n=15, 21,4±4%) as many blastocysts hatched as the control group (n=6, 10,4±10). Additionally, the addition of 0.0625 IU/mL of FUCA during pre-fertilization of COCs increased ($P<0.05$) the ZP digestion time (344±102 seconds) compared to 0; 0.125; and 0.25 groups (279±68; 300±104, 289±93 seconds, respectively), suggesting an important role of FUCA in the control of the ZP hardening. When analyzing the sperm penetration assay, we did not observe any difference between treatments, but the addition of 0.0625 IU/mL of FUCA during pre-fertilization demonstrated a biological tendency to increase monospermic fertilization ($P=0.10$). When we prospected the quality of blastocysts through the transcription profile, treatment with FUCA did not modulate the abundance of OCT4, PLAC8, CDX2, SOD2, and VEGF mRNA ($P>0.05$). We believe that FUCA does not alter the production of bovine blastocysts in vitro, however, it positively modulates the ZP hardening and seems to improve the performance of monospermic fertilization.

Acknowledgements

Supported by FAPESP (19/12223-0).