Animal Reproduction

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Treatment of *in vitro* produced embryos with colony-stimulating factor 2 during culture in the presence of serum does not affect pregnancy rates after embryo transfer

André Lopes Cirino[1], João Gabriel Viana de Grázia[2], Rafael Augusto da Anunciação[3], Luany Alves Galvão Martinhão[4,5], Luiz Sérgio de Almeida Camargo[6], João Henrique Moreira Viana[6], Peter James Hansen[7], Luiz Gustavo Bruno Siqueira[6]

[1]Universidade Federal de Juiz de Fora (UFJF)
[2]Apoyar Biotech Ltda
[3]Universidade José do Rosário Vellano (UNIFENAS)
[4]Universidade de Brasília (*UnB)*
[5]Laboratório Norte Embryo
[6]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)
[7]University of Florida (*Department of Animal Sciences, D.H. Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute*)

E-mail: andre.lopes@estudante.ufjf.br

The cytokine colony-stimulating factor 2 (CSF2) is a maternally-derived molecule produced by the endometrium a few days after ovulation in cattle. Previous studies reported a possible role of CSF2 in developmental programming of the preimplantation embryo by altering properties of the blastocyst such as differentiation and pluripotency of blastomeres, gene expression, embryo elongation and secretion of interferon tau (Hansen *et al.*, Animal Reprod Sci, 149:59-66, 2014). More recently, however, we reported that actions of CSF2 on the early embryo may be dependent on whether or not serum was present in the culture medium (Amaral *et al*., Scientific Reports, 12:7503, 2022). Therefore, the objective of this study was to determine pregnancy outcomes after CSF2 supplementation of serum- containing medium from days 5 to 7 of *in vitro* culture (IVC). *In vitro* embryo production was performed using standard procedures of a commercial laboratory (Apoyar Biotech, Alta Floresta, MT). *Cumulus*-oocyte complexes (COCs) were collected from slaughterhouse ovaries (Nelore females) and submitted to *in vitro* maturation for 22 to 24h. Matured COCs were fertilized using Y-sorted sperm from Nelore sires of proven fertility. Embryo culture followed standard procedures in medium supplemented with 3% fetal bovine serum (FBS), in a humidified atmosphere of 5% CO2 and 5.5% O2. On Day 5 of IVC, zygotes were randomly allocated to receive either vehicle (Control group) or 10 ng/mL CSF2 (CSF2 group). A yeast- derived recombinant bovine CSF2 (Kingfisher Biotech, Inc., Saint Paul, MN, USA) was used. Recipients were synchronized for timed embryo transfer (ET) with an estradiol-progesterone based protocol and, on day 7 after anticipated estrus, eligible females (presence of a CL >2 cm^2 in area) were randomly assigned to receive an *in vitro* grade 1 expanded blastocyst from Control (n=173) or CSF2-treated medium (n=180). Pregnancy diagnosis was performed by ultrasonography on Day 23 ± 2 after ET and the presence of an embryo with a heartbeat indicated an ongoing pregnancy. Statistical analysis was performed using Fisher's exact test in a 2x2 contingency table (GraphPad Prism software) to examine for differences in pregnancy rates between the two groups. A *P*-value of <0.05 was considered statistical significance. Data are presented as percent of pregnant recipients relative to total transferred recipients. Pregnancy rates on days 30-32 of gestation did not differ among treatment groups (45.7% versus 48.9% for Control and CSF2 groups, respectively; *P*=0.59). In conclusion, addition of CSF2 to a serum-containing medium from days 5 to 7 of development during IVC did not affect pregnancy rates after embryo transfer. Future studies may investigate further the occurrence of embryonic losses in pregnancies from CSF2-treated embryos cultured in medium with or without serum. Acknowledgements: Financially supported by FAPEMIG (Project APQ-02126-21) and CNPq (INCT 406866/2022-8.)