Animal Reproduction

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

OPU-IVF and ET

THE USE OF ASTAXANTHIN IN IN VITRO CULTURE EXERTING POSITIVE EFFECTS ON CRYOPRESERVATION OF BOVINE EMBRYOS

Fernanda Said Aidar [1], Eva Cristiane Doraci Benzi [2], João Vitor Alexandrino Santana [3], Luiz Felipe Fachin Pazianoto [3], Aline Mariana de Carvalho [1], Cláudia Maria Herédias-Ribas [1], Raphaela Gabrielle Brito Sousa [4], Marcílio Nichi [4], Lindsay Unno Gimenes [1]

[1] UNESP - Depto de Patologia, Reprodução e Saúde Única, Faculdade de Ciências Agrárias e Veterinárias (Via de Acesso Prof. Paulo Donato Castellane s/n CEP: 14.884-900 - Bairro Rural - Jaboticabal/SP), [2] Fertvitro - Biotecnologia Aplicada de Rio Preto LTDA (Rod. BR 153, Km 69 - Anexo ao Hospital Veterinário da UNIRP, São José do Rio Preto - SP, 15093-450), [3] UNIRP - Centro Universitário de Rio Preto (R. Ivete Gabriel Atique, 45 - Vila Maria, São José do Rio Preto - SP, 15025-400), [4] Universidade de São Paulo - USP - Depto de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia (Av. Prof. Dr. Orlando Marques de Paiva, 87 – Cidade Universitária Armando de Sales Oliveira, São Paulo/SP - CEP 05508-270)

Resumo

During embryo culture and cryopreservation/warming is recognized that the production of reactive oxygen species (ROS) can cause oxidative stress; therefore, the use of antioxidants as astaxanthin, can mitigate these undesirable effects. In this regard, the objective of this work was to evaluate whether the addition of astaxanthin (AST) to *in vitro* culture medium (IVC) could exert positive effects on the cryopreservation of bovine embryos. For this purpose, presumptive bovine zygotes were assigned for IVC in one of the following treatments: CONT (negative control; n=48), DMSO (0.001% of dimethylsulfoxide; n=49), AST 8 (8 ug/L of AST; n=49), AST 2 (2 ug/L of AST; n=52), and AST 0.5 (0.5 ug/L of AST; n=49). On day 7 of IVC, expanded blastocysts were vitrified with medium composed by TCM 199 + 20% FCS (v/v), ethylene glycol (EG), and DMSO. Vitrified embryos were warmed, cultured in IVC medium, and evaluated after 24 h for reexpansion and 48 h for hatching rate. A subset of embryos of each treatment were analyzed, and malondialdehyde metabolites were measured on day 7 of IVC medium drops by TBARS. Data were analyzed by ANOVA (PROC GLM), including treatment as fixed effect (SAS 9.3). TBARS did not have a normal distribution, therefore, was transformed in 1/ square root, although the means ± SEM be shown not transformed. At 24 h, differences ($P \leq 0.05$) between treatments were found for expanded blastocyst rate (CONT: $67.8^{AB} \pm 8.2$; DMSO: $54.4^{AB} \pm 7.9$; AST 8: $64.9^{AB} \pm 8.5$; AST 2: $73.3^{A} \pm 8.2$; AST 0.5: $47.6^{B} \pm 9.0$), hatching blastocyst rate (CONT: 15.0 ± 6.3^{ABC}; DMSO: $11.5^{BC} \pm 4.3$; AST 8: $20.1^{AB} \pm 7.0$; AST 2: $2.9^{C} \pm 2.0$; AST 0.5: $28.4^{A} \pm 7.6$), hatched blastocyst rate (CONT: $17.2^{AB} \pm 5.5$; DMSO: $34.1^{A} \pm 7.1$; AST 8: $15.0^{B} \pm 6.0$; AST 2: $25.1^{AB} \pm 8.1$; AST 0.5: $24.0^{AB} \pm 6.6$), and TBARS (CONT: $236.3^{AB} \pm 44.3$; DMSO: $244.6^{AB} \pm 91.9$; AST 8: $163.4^{A} \pm 21.4$; AST 2: $461.5^{AB} \pm 203.7$; AST 0.5: $303.2^{B} \pm 64.7$). No differences were found among the treatments for other variables (cleavage rate, expanded blastocyst rate, embryo development rate, expanded blastocyst rate at 48 h, hatching blastocyst rate at 48 h, and hatched blastocyst rate at 48 h). In summary, in our experimental conditions, the supplementation of *in vitro* culture medium with astaxanthin does not have an antioxidant effect protecting embryo during vitrification/ warming.

Keywords: Carotenoids, Cryoprotectants, Vitrification, Reactive Oxygen Species

Acknowledgments

FAMERP - Faculdade de Medicina de São José do Rio Preto, FertVitro - Laboratório de Fertilização *In Vitro*