Animal Reproduction

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Male reproductive physiology and sperm technology

A preliminary analysis of commercial trans-anethole effects during ram semen cryopreservation

Rachel Ferreira Braga [1], Nathalia Oliveira Barbosa [1], Lucas Francisco Leodido Correia [1], Mariana Pedrosa de Paula Guimarães [1], Erlandia Marcia Vasconcelos [1], Thais Gomes de Oliveira [1], Caio Cezar de Carvalho Cunha [1], Bruna Rios Coelho Alves [1], Felipe Zandonadi Brandão [1], Joanna Maria Gonçalves Souza-Fabjan [1]

[1] UFF - Universidade Federal Fluminense (Av. Alm. Ary Parreiras, 507 - Icaraí, Niterói - RJ)

Resumo

Cryopreserved semen is used in artificial insemination, positively impacting the genetic improvement of many species. Oxidative stress, a major event caused by cryopreservation, can cause cellular damage and reduce sperm viability. Trans-anethole, a natural antioxidant found in plants (e.g. fennel), appears to improve sperm cell survival during semen cryopreservation. This study assessed the effects of different concentrations of trans-anethole (117870, Sigma) added to sheep semen extender on post cryopreservation survival, kinetic parameters, and sperm binding to egg perivitelline membrane. Semen was collected from six rams using an artificial vagina, on five different days. Ejaculates with sperm motility >70% were used (n=22) and diluted individually with Tris-Egg Yolk-Glycerol extender, with trans-anethole, according to the treatment: CONT (control group, 0 µM), AN10 (10 µM), AN50 (50 µM) and AN100 (100 µM), with a final concentration of 400 x 106 spermatozoa/mL. Cryopreservation was carried out using a freezing machine. A cooling rate of 0.25 °C/min was applied until 5 °C, and this temperature was maintained for 4 h. The freezing rate used was −20 °C/min from 5 to −120 °C, when the straws were immersed in liquid nitrogen (−196 °C) and stored. Thawing was performed at 37 °C for 30 s. Samples were evaluated regarding sperm kinetics (by objective Computer Assisted Semen Analysis, CASA), plasma membrane integrity (PMI, staining with acridine orange and propidium iodide), and functionality (PMF, hypoosmotic swelling test) as well as the sperm binding to egg perivitelline membrane test. Data were analyzed using a generalized linear mixed model, and the results are presented as mean ± SEM. After thawing, the progressive motility of AN100 was higher ($P<0.05$) than AN50 (13.8 ± 1.8 vs 10.2 ± 1.8%), and both were similar ($P>0.05$) to CONT (11.5 ± 1.8%). The medium velocity of AN100 (11.3 ± 1.4%) was higher ($P<0.05$) than AN50 (8.5 ± 1.4%) and CONT (8.1 ± 1.4%). Differences ($P<0.05$) were also observed in average path velocity between AN100 (33.6 ± 2.6 µm/s) and AN50 (29.5 ± 2.6 µm/s), both being similar to CONT (32.1 ± 2.7 µm/s); and straight-line velocity between AN100 (24.0 ± 2.0 µm/s) and AN50 (20.7 ± 2.0 µm/s), with CONT presenting an intermediary value (23.3 ± 2.1 µm/s). The number of sperm bound to the egg perivitelline membrane was higher ($P=0.05$) between AN100 and CONT groups (4293.0 ± 483.9 vs 3076.2 ± 483.9 sperm/mm2, respectively). There was no difference ($P>0.05$) among groups regarding other kinetics parameters, PMI and PMF. In conclusion, the addition of 100 µM trans-anethole to cryopreservation media leads to an improvement in sperm kinetics as well as its fertilizing capacity assessed by the binding test, without disrupting their membrane integrity and functionality.

Keywords: sperm, antioxidant, freezing, fennel

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