

## THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

## OPU-IVF

## Effects of sexed semen on mitochondrial activity, oxidative stress and *in vitro* development of bovine embryo

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The use of sex-sorted semen for *in vitro* embryo production is an efficient strategy for controlling offspring sex. However, the sex-sorting process can induce structural and functional changes in sperm cells, potentially affecting cellular events during early embryonic development. This study aimed to investigate the effects of semen type (sexed male, sexed female, and conventional) on oxidative stress and mitochondrial activity in bovine embryos on the fifth day of culture (D5), and to evaluate blastocyst development rates on the seventh day (D7). Oocytes were collected from ovaries obtained from abattoirs and, after IVM, conventional and sexed semen from the same bull (the same semen batch from one single bull) were used. To assess mitochondrial activity and intracellular oxidative stress, Mitotracker® Red and CellROX® Green fluorescent probes were used (n=73 embryos, distributed across four replicates). Cleavage and blastocyst rates were evaluated from conventional semen (control embryos; n=137), female-sexed semen (Xembryos; n=226), and male-sexed semen (Yembryos; n=198), also across four replicates. The same IVM, IVF, and IVC protocols were applied for all experimental groups. Data were analyzed in R at 5% of significance. ANOVA was used for normally distributed variables, Kruskal-Wallis for non-normal data, and chi-square test for cleavage and embryo development rates. Embryos produced with conventional semen exhibited higher mitochondrial fluorescence intensity (29.96) compared to Xembryos (20.75;  $P<0.05$ ), while no difference was observed between conventional and Yembryos (25.04) for this parameter. No differences were found among the three groups regarding oxidative stress levels (control=7.55, Yembryos=11.6, Xembryos=6.22;  $P=0.090$ ). However, a higher frequency ( $P<0.001$ ) of embryos with 16 or more cells on D5 was observed in the conventional group (41.6%) compared to Y embryos (20.0%) and X embryos (31.1%), suggesting that the use of sexed semen may impair mitotic progression during early embryonic development. On D7, however, the proportions of embryos at the initial blastocyst (control=33.3%, Yembryos=18.7%, Xembryos=16.7%;  $P=0.45$ ), blastocyst (control=25.0%, Yembryos=25.0%, Xembryos=66.7%;  $P=0.11$ ), and expanded blastocyst (control=41.7%, Yembryos=16.7%, Xembryos=50.0%;  $P=0.37$ ) stages did not differ between groups, indicating similar developmental competence at this stage. In conclusion, the use of sexed semen negatively affects mitochondrial activity and the early cleavage pattern of bovine embryos but does not impair blastocyst development by the seventh day of culture. These results suggest that, although embryos derived from sex-sorted semen present early metabolic alterations, they may retain the ability to reach advanced developmental stages, reinforcing the viability of using this biotechnology in bovine production systems, provided that specific adjustments are considered in reproductive protocols.

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