Animal Reproduction

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Impact of Sperm Sexing Technique on Quality of In Vitro Produced Bovine Embryos

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Resumo

Although sperm sexing technology has been widely studied, many evidences confirms this kind of environment stressors have serious impacts on reproduction, although its influence on embryonic quality and/or offspring is not very well stablished in domestic animals. Therefore, here was proposed to evaluate the paternal effect on IVP embryos, using flow cytometry sperm sexing as a model. For this purpose, we used conventional (CV) and sexed male (SX-Y) and sexed female (SX-X) semen from 5 different Nellore bulls. Oocytes obtained from slaughterhouse ovaries were submitted to IVM for 24 hours, then were inseminated (D0) with CV and sexed semen (SX-Y and SX-X) from the 5 different bull semen. To eliminate sexed effect but preserve the sexing procedure effect SX-Y and SX-X were pooled to form the sexed (SX) sample. On D1, possible zygotes were transferred to culture medium – SOF with 0.4% BSA, where they remained for 8 days. Cleavage rates at D2, and blastocyst rates at D6, D7 and D8 were evaluated. At D8, expanded blastocyst stage embryos were storage for gene expression analysis. Five genes (OCT4, NANOG, Fematrin-1, DNMT3A, TET1) were chosen to be evaluated by qPCR. Blastocysts rates data were analyzed by Chi-square, and for individual bull rates effect and gene expression ANOVA was used, considering $P \leq 0.05$ for all analysis. Cleavage rate at D2 (77.13% x 70.24%) and blastocyst rate at D6 (6.56% x 2.61%), D7 (21.2% x 9.9%) and D8 (26.64% x 14.1%) were higher ($P<0.05$) for CV than SX. Analyzing developmental kinetics, CV semen had greater ($P<0.05$) percentage of expanded blastocysts than hatched blastocysts on D7 (11.84% x 0.3%) and D8 (14.04% x 0.96%), while SX semen had this difference only on D8 (9.41% x 0.65%). When comparing embryo development rates between semen CV and SX for each bull (B1 – B5), no differences were observed at D2 and D6. However, B2 and B3 presented superior blastocysts rates on D7 and D8 for CV (B2: 22.3%, D7 and 30.4%, D8; B3: 32.9%, D7 and 42.0%, D8) than their SX counterparts (B2: 6.0%, D7 and 9.3%, D8; B3: 16.3%, D7 and 20.6%, D8). Regarding to gene expression evaluation, only NANOG presented difference ($P=0.002$) between CV and SX treatments. Results evidenced that sexing procedure affected bovine embryos production, embryo kinetics and gene expression. In addition, the effect were influenced by the sire used for IVF step. To supplement the founding evidences and determinate embryo quality markers, additional analysis accomplishments are necessary.