



A197 Physiology of reproduction in male and semen technology

Embryo production using epididymal sperm submitted to different selection methods and their influence on the embryo sex

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Epididymal spermatozoa and their use in assisted reproductive technologies (RT), such as IVP, have an important role in the multiplication of genetic material from sires that die suddenly and/or have acquired reproductive failure. However, in order to establish an appropriate procedure to use those sperm in embryos IVP, a better knowledge about their physiological behavior facing events involved IVP, such as different methods of sperm selection, is needed. The aims of this study were evaluated different methods of sperm selection for IVP procedures and their influence on the embryo sex. A pool of epididymal (EP) and ejaculated (EJ) cryopreserved spermatozoa, recovered from seven Gir bulls through electroejaculation followed by bilateral orchiectomy were used. The pool of the two groups were selected by three different methods: Percoll gradient 45%90% (GE Healthcare Bio Science, Uppsala, Sweden), PureSperm gradient 40%80% (Nidacon Laboratories AB, Gothenborg, Sweden) and wash in Tyrode's Albumin Lactate and Pyruvate (SpTALP). Four groups were formed: ejaculated on Percoll (EJ-P), control group; epididymal on Percoll (EP-P); epididymal on PureSperm (EP-PS) and epididymal on SpTALP (EP-T). After selection, sperm samples were co-incubated with a total of 759 cumulus-oocyte-complexes (COC's) in fertilization medium in 7 replicates experiment. Embryos were evaluated two days (D2), six days (D6), seven days (D7) and eight days (D8) after fertilization and then, embryos were storage for sex evaluation. Embryo sexing was performed according to Sousa et al (Theriogenology, 90, p.25, 2017), by PCR technique. Embryo rates data were analyzed using Chi-square (mean±SD; P<0.05) and sexing date by Wilcoxon using Prophet 5.0 (mean±SD; P<0.05). Cleavage rates (D2) and blastocyst rates on D6 were higher for EP-PS group (80% and 48%, respectively) than the other groups. At the D7 and D8, blastocyst rates were similar (P>0.05) between EP-P (D7 54%; D8 55%) and EP-PS (D7 37%; D8 37%) groups. EP-T and EJ-P groups showed similar blastocyst rate in D6 (27%; 32%), D7 (37%; 44%) and D8 (37%; 45%), which was lower than the others groups that used EP sperm. Male and female embryos showed differences only in EP-P group (38% and 62%, respectively). For others methods of sperm selection differences were not significant (P>0.05). These results suggest that PureSperm and Percoll were the better methods of sperm selection, for embryos production using EP. Furthermore, the relation between male × female embryos only showed differences when Percoll gradient was used, resulting in more female embryos. Financial support: CNPq and Embrapa.