

PERCOLL GRADIENT VERSUS SWIM-UP: EFFECT OF SPERM PREPARATION METHOD ON SEX RATIO OF IN VITRO-PRODUCED BOVINE EMBRYOS

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The higher production of male embryos from IVF can be considered a problem in the bovine breeds with milk aptitude, where it has a great economic interest in the production of females. As causes that influence this deviation in the sex ratio, the influence of many factors has been considered, like development kinetics according to the sex, culture conditions, higher sensitivity of female embryos to manipulation conditions, Y-linked chromosome gene expression, and the influence of sperm preparation method to IVF. Among the factors that can influence this deviation in the sex ratio, the sperm preparation method to IVF is most easily manipulable, able to be used for reducing the ratio of males determined for other factors that they cannot be manipulated easily, like development kinetics according to the sex and the higher sensitivity of female embryos to manipulation conditions, resulting in a smaller deviation, next to the waited one. The objective of this experiment was to compare the effect of two sperm preparation methods routinely used in IVF technique, swim-up and Percoll gradient, on sex ratio of bovine embryos produced in vitro. Immature oocytes were aspirated from ovaries obtained at a slaughterhouse. COCs were selected and matured in vitro for 24 hours and randomly allocated in 2 groups. Each group was inseminated with sperm selected by swim-up (treatment 1) or Percoll gradient (treatment 2) method. Presumptive zygotes were cultured in SOFaa medium with 5% FCS for 2 to 8 days. After the PCR sexing of the embryos, it was noted that swim-up determined deviations in the sex ratio (Chi-square test), with higher rate of males among the more advanced embryos, in every day analyzed (D-2: 46/73, 63.0%, $P < 0.05$; D-3: 55/81, 67.9% e D-5: 39/58, 67.2%, $P < 0.01$; D-8: 56/88, 63.6%, $P < 0.02$). In the total of produced embryos, swim-up determined rates of males significantly higher in the culture periods more prolonged, that is, in days 5 (110/185, 59.5%, $P < 0.02$) and 8 (206/353, 58.4%, $P < 0.01$). The Percoll method did not determine deviations in the sex ratio, in the total of the produced embryos, in all the culture periods utilized. When the two methods were compared, swim-up determined a higher rate of male embryos than the Percoll, among the total of produced embryos, in every day analyzed (D-2: 55.7% (118/212) vs. 44.1% (94/213), $P < 0.05$; D-3: 53.8% (154/286) vs. 42.6% (120/282), D-5: 59.5% (110/185) vs. 45.3% (81/179) e D-8: 58.4% (206/353) vs. 46.4% (155/334), $P < 0.01$). The fact of swim-up to determine a higher percentage of males among the embryos of more advanced development, supposedly the most viable, since the first cleavages, indicates that this method favored the selection of a higher number of Y-bearing spermatozoa, determining a sex ratio deviation of the bovine embryos in vitro produced.