

Z315 **The studies on the separation of X- and Y- bearing porcine spermatozoa by discontinuous percoll gradient centrifugation**

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Predetermination of sex in livestock offspring is in great demand and is of critical importance to providing for the most efficient production of the animal agriculture. Such a sexing technology would also enhance the economy of conventional artificial insemination (AI) and aid the porcine industry. The purpose of this study was to evaluate the efficacy of enriching X-bearing porcine sperm using discontinuous percoll gradients. Semen was collected from mature boars of proven fertility (AI center KimHae). Sperm was loaded on the isotonic discontinuous percoll gradient and then it was centrifuged at $120\times g$ for 20 minutes. After centrifugation, sperm included in each fraction were recovered (7×10^6 sperms/ml) and then sperm genomic DNA was extracted for the study. A SRY (sex determining region of Y chromosome) gene was used to evaluate the ratio between X and Y sperm in the separated fractions. In vitro fertilization was carried out by adding the not separated sperm (control) or separated (experiment) to the matured oocytes in TCM-199. Embryos for sex determination were obtained at 2 or 4 cell stage and then was used for SRY gene amplification. After centrifugation of discontinuous percoll gradient, the most motile sperm was obtained at 95% fraction ($94.4\% \pm 5.1\%$, $P < 0.01$). The PCR experiment evaluated that 0%, 50% and 65% fraction were a Y sperm rich fraction, whereas 80% and 95% were a Y sperm poor one. These results suggested that upper fraction was a Y sperm rich, whereas low fraction a X sperm rich. In vitro matured oocytes inseminated with sperm prepared by percoll gradient centrifugation had high fertilization rates than did oocytes inseminated with control. PCR analysis with whole porcine embryo showed that control group was 30% and experiment group was 66.7% as female. In this study, following centrifugation of discontinuous percoll gradient, the population of X sperm increased and enhanced motility in 95% percoll fraction.