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A METHODOLOGICAL STUDY ON CHROMOSOMAL PREPARATION  
OF MOUSE AND BOVINE EMBRYOS

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A total of 1117 mouse embryos were used to obtain the optimum conditions for chromosomal analysis and to study cytogenetical normality of superovulated embryos.

Chromosomal preparations were made from embryos cultured under three different conditions: culture medium, duration of culture and concentration of colchicine. Criteria used to determine the optimum culture conditions were the number of nuclei, metaphase plates and size of chromosomes. A 2-hour culture by BMOC-3 containing 0.4  $\mu$ g/ml colchicine was the optimum condition for chromosomal preparation in this study.

Immature mice of ddY strain were superovulated by the injection of either 5IU PMSG and 5IU HCG, or 10IU PMSG and 10IU HCG. Normal diploidy was seen in 81.7% (98/120) of the embryos spontaneously ovulated, in 81.3% (208/256) of the embryos superovulated with 5IU PMSG and 5IU HCG, and in 79.2% (217/274) of the embryos superovulated with 10IU PMSG and 10IU HCG, and there was no significant difference among the 3 groups of embryos. Abnormalities of chromosome number included hypotriploidy, triploidy, hypotetraploidy and tetraploidy. These abnormalities increased up to 2.6% with 10IU PMSG and 10IU HCG, but this was not significant. The sex ratio was not affected by superovulation. From these results, it was suggested that the superovulated embryos were cytogenetically normal.

Sex determination of bovine embryos was attempted on 14 blastocysts, and 4 (28.6%) were successfully sexed. When cultured under the optimum condition for chromosomal preparations of mouse embryos, bovine blastocysts showed contraction of chromosomes and a few metaphase plates. Even when cultured with 0.1  $\mu$ g/ml colchicine, it was difficult to differentiate the Y chromosome. These results suggested that more studies are needed on suitable chromosomal preparations of bovine blastocysts for sex determination.