A STUDY ON INTERSPECIFIC *IN VITRO* FERTILIZATION BETWEEN BOVINE SPERMATOZOA AND ZONA-FREE HAMSTER OVA AND ITS POSSIBLE APPLICATION FOR SPERM CHROMOSOME ANALYSIS

Souichi KAGEYAMA

Department of Theriogenology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

Various procedures for inducing capacitation and acrosome reaction were performed on bovine frozen-thawed spermatozoa *in vitro* to examine the optimal conditions that will give a consistent result irrespective of semen from different bulls. Moreover, this was done to establish a procedure for sperm chromosome analysis in bull spermatozoa which had penetrated into zona-free hamster ova.

Spermatozoa from four different bulls were used. In method 1, spermatozoa were first preincubated in Brackett & Oliphant medium without BSA (BF-BO) containing 2mM caffeine (CA) and then treated with 0.1 μM ionophore A23187 (IA) for 1 or 3 minutes. In method 2, spermatozoa were preincubated in BF-BO containing either 10mM CA or 15mM theophylline (TP) followed by IA treatment for 0.5 or 1 minute. The results showed that a high penetration rate (64.5–96.3%) can be obtained when the spermatozoa are preincubated with BF-BO containing 15mM TP followed by IA treatment. However, there was a high incidence of polyspermy. When sperm pretreatment was done with BF-BO containing 10mM CA, a wider variation in the penetration rate (32.7–87.1%) was observed.

The effect of various spermatozoa concentrations at the time of insemination, and washing of eggs after insemination was examined in order to minimize the incidence of polyspermy. A significant decrease in the penetration rate was observed when the spermatozoa concentration was lowered from $1.0–1.25\times10^7$ to $0.5–0.6\times10^7$ cells/ml. The average number of spermatozoa that penetrated into zona-free hamster ova can be lowered by washing the eggs after insemination, but polyspermy still remained relatively high. In one bull, it was observed that a significant decrease in the penetration rate occurred after washing.

Using hamster ova that were fertilized with spermatozoa which were preincubated in BF-BO containing 15mM TP, followed by IA treatment for 1 minute, sperm chromosome analysis was attempted. Out of 93 ova, 39 metaphase plates developed from bull spermatozoa were obtained but only 19 could be sexed. In addition, 1 hyperhaploid karyotype (31,Y) was observed.