



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

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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  $\mu$ M 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 pre-treatment 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  $1.0\text{--}1.25 \times 10^7$  to  $0.5\text{--}0.6 \times 10^7$  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.