A STUDY ON THE *IN VITRO* CAPACITATION OF FROZEN BOVINE SPERMATOZOA

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Some sperm treatments were examined in order to establish a method for inducing capacitation and acrosome reaction of frozen bovine sperm *in vitro*. Sperm treatment was carried out by the following methods: preincubation at high or low concentration, treatment with 0.1 or 0.5 μM ionophore A23187 (IA), or incubation at high ionic strength solution (HIS) for 5 or 15 minutes.

In experiment 1, a triple-stain technique was used to determine the optimum condition in each of the above treatments on the frozen semen of bulls A and B. The results revealed that there was a difference in the optimum condition for each sperm treatment between the two bulls.

In experiment 2, the sperm from bull A were used to examine the effectiveness of the most suitable sperm treatment determined in experiment 1. *In vitro* fertilization using oocytes matured *in vitro* was employed to determine the effectiveness of each sperm treatment. The highest fertilization rate of 43.3% was obtained when the sperm were treated with 0.1 μM-IA. A high fertilization rate (34.9%) was also obtained when 0.5 μM-IA was used; however, there was a high incidence of polyspermy (26.7%). Fertilization rates of 27.4%, 10.0%, 10.0% and 27.3% were obtained from treatment using preincubation at high or low concentrations and treatment with HIS for 5 or 15 minutes, respectively. By the triple-stain technique, a good correlation between the percentage of live spermatozoa without acrosomes and that of fertilized oocytes inseminated *in vitro* was obtained.