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Author(s)	MORIYASU, Satoru
Citation	Japanese Journal of Veterinary Research, 36(2), 163-163
Issue Date	1988-05-20
Doc URL	<a href="https://hdl.handle.net/2115/3107">https://hdl.handle.net/2115/3107</a>
Type	departmental bulletin paper
File Information	KJ00002377091.pdf



STUDIES ON *IN VITRO* FERTILIZATION  
USING FROZEN-THAWED BULL SPERMATOZOA  
TREATED WITH IONOPHORE A23187

Satoru MORIYASU

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

In order to examine some factors affecting the *in vitro* fertilization rate, frozen-thawed bovine spermatozoa which were treated with ionophore A23187 (IA) were used on *in vitro* matured bovine oocytes. Bovine follicular oocytes taken from the slaughterhouse were classified into two groups; oocytes with abundant cumulus cells and oocytes with few cumulus cells. Spermatozoa from 2 bulls that had been treated with 0.1  $\mu$ M IA for 1, 3 or 5 minutes were used for *in vitro* fertilization. Using the above-mentioned treated spermatozoa, the relationship between cumulus cell adherence conditions and the fertilization rate (monosperm penetration resulting in two pronuclear formation) was examined. The results showed that oocytes with abundant cumulus cells had a higher fertilization rate than the oocytes with few cumulus cells.

Spermatozoa from 5 different bulls were treated to find out optimum IA treatment time. Spermatozoa that were treated for 3 minutes showed the highest fertilization rate in all bulls. However, significant differences were observed in the fertilization rates of the 5 bulls. Hence, it was suggested that individual differences exist among bull spermatozoa.

Spermatozoa from 3 different bulls were used to study the effect of adding 2mM or 10mM caffeine (CA) and 15mM theophylline (TP) to the medium for fertilization on the fertilization rate and the subsequent development of the oocytes. Following the above procedures, the fertilized oocytes were incubated with cumulus cells for more than 48 hours.

The developmental rates of the ova to the 2-cell stage were 12.7% and 15.9% when 10mM CA and 15mM TP were added to the medium for fertilization, respectively. On the other hand, when 2mM CA was used, a much lower rate (6.6%) was observed. However, there was no significant difference among the developmental rates.