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DIMETHYL SULFOXIDE / RANA TIGERINA

PIYADA CHITTANON : THE EFFECT OF CRYOPRESERVATION ON  
STRUCTURE AND VIABILITY OF SPERMATOZOA OF RANA TIGERINA.

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This study was undertaken to investigate the motility, viability, fertilizing capacity, and the ultrastructural alteration in *Rana tigerina* spermatozoa brought about by cryopreservation. The spermatozoa were determined by assessment of the percentage of motility, motility score, and the percentage of viability after incubation with an extender containing dimethyl sulfoxide (DMSO) and glycerol. Subsequently, they were frozen to  $-196^{\circ}\text{C}$  in the presence of DMSO for 1 week. The temperature had no effect on the percentage of motile spermatozoa, motility score and sperm viability in spermatozoa stored at  $4^{\circ}\text{C}$  for 0.5, 1, 2, and 3 h. However, the percentages of motile spermatozoa were significantly reduced after incubation in the media containing 4, 5, 6, 8, and 9% DMSO for 3 h. In contrast, the spermatozoa could not survive after incubation in media containing various concentrations of glycerol (4-9%). After 1 week of cryopreservation, the spermatozoa showed a significant decline in the percentages of motility, motility score, live spermatozoa, and fertilization capacity, when compared to the fresh semen. The spermatozoa frozen in media containing 4, 5, 6, 8, and 9 % DMSO did not move as well as those frozen in the medium containing 7 % DMSO. The incubation time in DMSO was found to be an important factor for cryopreservation. The spermatozoa incubated in the media containing 7-8 % DMSO for 1 h before freezing showed a significant difference in the number of motile spermatozoa compared with the other incubation intervals. The extender containing 7 % DMSO offered the best protection of spermatozoa viability. However, a decline in the percentage of live spermatozoa of  $\sim 50\%$  was observed. After thawing, a significant difference in fertilization rates was observed between fresh and frozen-thawed spermatozoa ( $98.80 \pm 0.25$  and  $24.66 \pm 5.33$ , respectively). The ultrastructure of frozen-thawed spermatozoa was observed under scanning and transmission electron microscopes. It was found that the plasma membrane was altered about 50% by freezing and thawing when compared with that of the fresh samples. Under SEM observation, there were fenestrations of sperm plasma membrane at the head region. The middle piece also showed considerable damage to the plasma membrane. Under TEM observation, there were some disruptions of the acrosomal membrane, and relatively low electron density of its interior. The plasma membrane of the middle piece appeared to be damaged. Some mitochondria were swollen and their cristae were reduced. These ultrastructural changes and the changes in motility characteristics were thought to be the important, interrelated factors in fertilization capacity after cryopreservation.