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CONTROLLED RATE FREEZING OF CANINE SPERMATOZOA[1] USING A PROGRAMMABLE FREEZER

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Optimal freezing rate is an important factor affecting the post-thaw viability of dog semen. Earlier reports on freezing dog semen were of pellet freezing by placing a drop of semen on a dry ice block, where freezing was very rapid (Seager, 1969., Platz and Seager, 1977). Attempts have been made to control freezing rate by exposing straws at different heights above the liquid nitrogen level (Oettle, 1982., Christiansen, 1984., Smith, 1984). Few scientists have attempted to define the freezing rates achieved, but have compared different unknown rates (England, 1993). Dobrinski *et al.* (1993) compared fast, moderate and slow freezing rate and found that a slow freezing rate resulted in higher post-thaw motility. The present study, has formulated a well defined slow freezing rate for freezing canine spermatozoa in plastic straws using a programmable freezer.

Materials and methods

Semen collected from the adult male mongrel dogs were used for the study. The sperm rich second fraction was extended to 1:2 ratio with Egg yolk-Tris diluent (Yubi *et al.*, 1979) containing nine per cent glycerol. Semen was filled in plastic straws and equilibrated for four hours. Then the straws were frozen using a programmable freezer[7] with a slow freezing rate of -2°C min^{-1} from 5°C to -15°C, -5°C min^{-1} from -15°C to -40°C and -10°C min^{-1} from -40°C to -70°C (Fig.). The frozen straws were stored in liquid nitrogen. The samples were thawed and evaluated for viability and fertilizing ability by estimating post-thaw motility, live sperm percentage, abnormal morphology, acrosome integrity and hypo-osmotic swelling response. Live sperm percentage and abnormal morphology were evaluated by eosin nigrosin staining technique (Hancock, 1957). Hypo-osmotic swelling test was performed by mixing 0.1 ml of semen and 1 ml of 150 milliosmol fructose solution in test tube and incubating the mixture for 30 minutes at 37°C (England and Plummer, 1993). After incubation smears were stained with Rose Bengal stain

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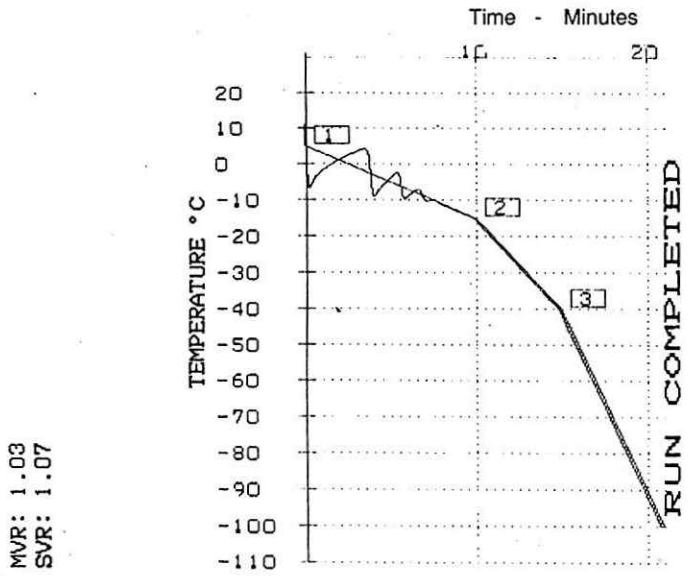

for 15 minutes (Tomar, 1976). Hypo-osmotic swelling response was indicated by various degrees of tail curling. Acrosome integrity was evaluated by Giemsa staining technique (Watson, 1975).

Results and discussion

Post-thaw evaluation of seminal characteristics revealed post-thaw motility of 63.67 ± 1.29 per cent, live sperm percentage of 66.38 ± 1.28, abnormal sperm percentage of 29.02 ± 1.95, acrosome damage of 33.02 ± 2.48 per cent and hypo-osmotic swelling response of 67 ± 1.02 per cent. The slow freezing rate used resulted in satisfactory post-thaw motility. Abnormal morphology, live sperm percentage and acrosome damage showed that slow freezing did not substantially reduce the viability of spermatozoa. Hypo-osmotic swelling response indicated that fertilizing ability of spermatozoa were maintained at a satisfactory level. The results concurred with the findings of Dobrinski *et al.* (1993) who showed maximum post-thaw motility at slow freezing rate of -5.7°C min^{-1} from 3°C to -157°C compared with fast and moderate freezing rates. Olar *et al.* (1989) used a freezing rate of -5°C min^{-1} from 5 to -15°C and then -20°C min^{-1} from -15°C to -100°C which was superior to its faster and slower freezing rates. This well defined slow freezing can be used for cryopreservation of canine spermatozoa.

Summary

A well defined slow freezing rate using a controlled rate freezer was used for cryopreservation of canine spermatozoa using Tris-egg yolk extender. Viability and membrane integrity of spermatozoa were assessed following freez-thawing. The freezing rate of -2°C min^{-1} from 5°C to -15°C and -5°C min^{-1} from -15°C to -40°C and -10°C min^{-1} from -40°C to -70°C resulted in acceptable level of fertile spermatozoa following freez-thawing.

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