Cryobiology of Epididymal Cat Sperm: Analysis of Osmotic, Cooling and Centrifugation Stresses

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INTRODUCTION

- Cryopreservation is becoming essential for zoological parks and conservation efforts to preserve the genetic diversity of endangered species.
- Sperm cryopreservation is an effective method of maintaining valuable genetic donors.
  - Example: an animal dies suddenly in captivity or in the wild, the testes can be removed and the sperm collected from the epididymus.
- Domestic cats are used as the research model for exotic species.
- Sperm are extremely sensitive to various physical stresses that occur during the freezing process
- Physical assaults during cryopreservation include:
  - Rapid osmotic changes due to freezing media and cryoprotectants.
  - Cooling of the sperm.
  - Centrifugation to remove extenders and concentrate the sperm.
- The goal of this study was to analyze the effects of osmotic changes, cooling, and centrifugation on cat epididymal sperm motility.
- By analyzing these factors, an appropriate freezing protocol can be formulated and established.

Photo courtesy of Lyons Lab

https://www.google.com/search?q=leopard

MATERIALS AND METHODS

Animals

- Epididymal sperm samples from 3 donor cats

Experimental design

- Epididymal Samples:
  - Sperm was harvested from the cauda epididymus and diluted in Feline Optimized Culture Medium (FOCM). Samples were obtained from mature toms (over 8 months old) from the Central Missouri Humane Society after neuters.

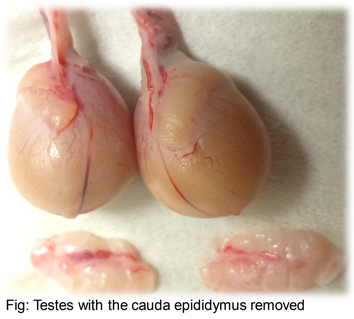

Fig: Testes with the cauda epididymus removed

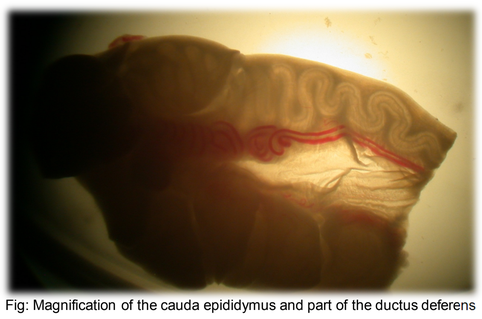

Fig: Magnification of the cauda epididymus and part of the ductus deferens

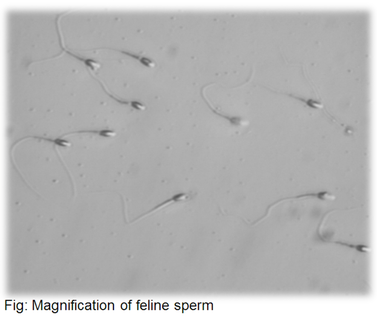

Fig: Magnification of feline sperm

Osmotic Stress

- The sperm were exposed to 0.1 – 0.8M solutions of sucrose for 5 mins
- After 5 mins, the solution was brought back to isosmotic conditions, centrifuged, then resuspended in FOCM.

Cooling Stress

- Sperm were placed in a water bath at 4°C, 10°C, and 20°C for 5 mins then immediately returned to 37°C for 15 mins

Centrifugation Stress

- Sperm was centrifuged for 5 mins at 300xg, 400xg, 500xg, and 600xg. The sample was then resuspended in FOCM.

Motility Analysis

- All samples were analyzed pre-manipulation and post-manipulation on a Computer Aided Sperm Analysis (CASA) machine.
- Parameters measured include Total Motility, Progressive Motility, and Velocity

Statistical analysis

Statistical analysis was performed by using general linear models of SAS to determine the differences and the effects of chilling and anisosmotic conditions on motility of epididymal cat sperm. Mean separation was done by Duncan's–Multiple Range Test. The values were given as the mean±standard error of the mean. The level of statistical significance was chosen as $P < 0.05$.

RESULTS

Osmolality

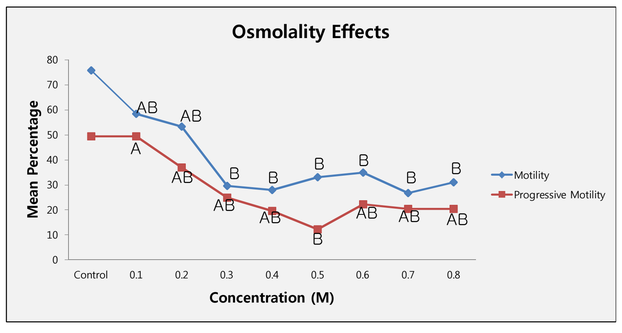

Fig. 1: Motility and Progressive Motility after addition of various sucrose concentration and then returned to isosmotic conditions. B = P < 0.05

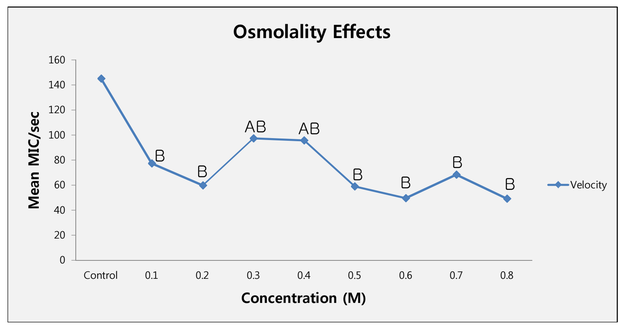

Fig. 2: Sperm Velocity after addition of various sucrose concentration and then returned to isosmotic conditions. B = P < 0.05

- Changes in osmolality cause a decrease in motility, progressive motility and velocity due to hypotonic and hypertonic conditions which cause swelling and dehydration respectively.
- Most semen extenders have an osmolality around 300mOSM but the addition of cryoprotectants increases osmolality to 800-900 mOSM.

Cooling

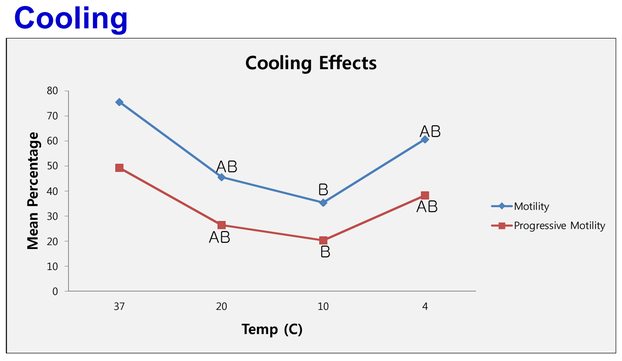

Fig. 3: Motility and Progressive Motility after cooling for 5 mins then returning to normal body temperature (37℃). B = P < 0.05

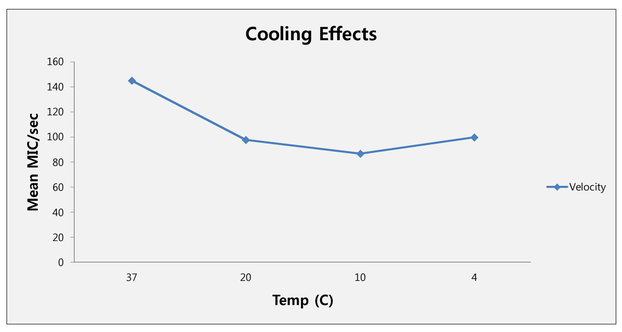

Fig. 4: Sperm Velocity after cooling for 5 mins then returning to normal body temperature (37℃).

- Cooling to 20℃ and 10℃ causes a decrease in motility, progressive motility, and velocity. Feline sperm is very temperature sensitive.
- Sperm is cooled during freezing to allow cells to adjust to osmotically to cryoprotectants.

Centrifugation

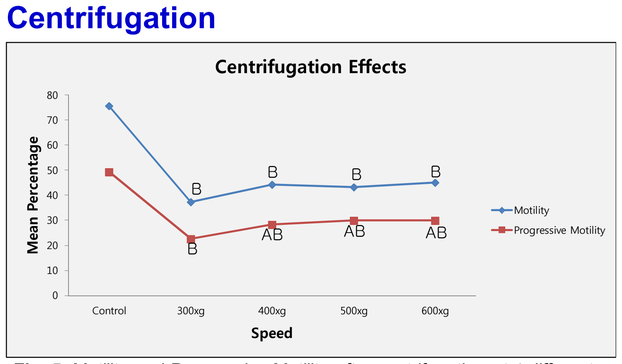

Fig. 5: Motility and Progressive Motility after centrifugation at 4 different speeds. B = P < 0.05

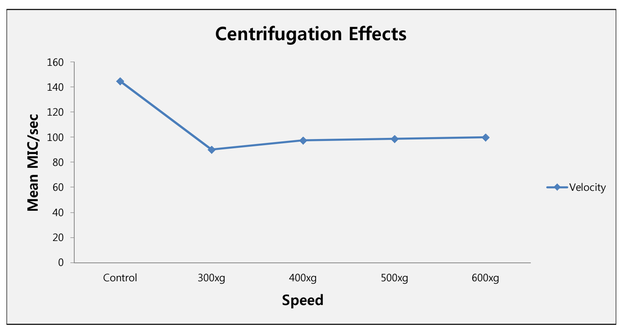

Fig. 6: Sperm Velocity after centrifugation at 4 different speeds.

- Centrifugation causes a decrease in motility, progressive motility, and velocity
- Centrifugation is used to concentrate sperm and to remove the freezing media post-thaw.

CONCLUSIONS

- Cat sperm are highly sensitive and go through many insults during cryopreservation.
- Osmolality changes, cooling, and centrifugation damage the sperm and decrease motility, progressive motility, and velocity.
- The goal is to minimize the damage and find the best parameters to design a protocol for freezing.
- More samples are needed in the future to draw more concise conclusions
- Further investigations should look at electroejaculate sperm vs. epididymal sperm.
  - Epididymal sperm may have a higher tolerance to stress than ejaculate sperm just based on observations.

ACKNOWLEDGEMENT

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