Rat Sperm Cryopreservation and Thawing Protocols using CARD FERTIUP® Rat Sperm Cryopreservation Agent

1． Sperm cryopreservation protocol

A. Place 3mL of sperm cryopreservation agent (CPA:2 tubes) at room temperature into a 35mm culture dish. Transfer two cauda epididymides taken from one male rat into the agent.
※When using one cauda epididymis, put 1.5mL CPA at room temperature into a 35mm culture dish.

B. Cut the cauda epididymides in two using scissors and a pair of forceps as shown in ①. Next, make further cuts in each piece of caudal tissue as shown in ② and ③.

C. Place the culture dish containing the dissected cauda epididymides on a tin tray set on top of crushed ice. Leave the dish to cool and incubate for 10 minutes.

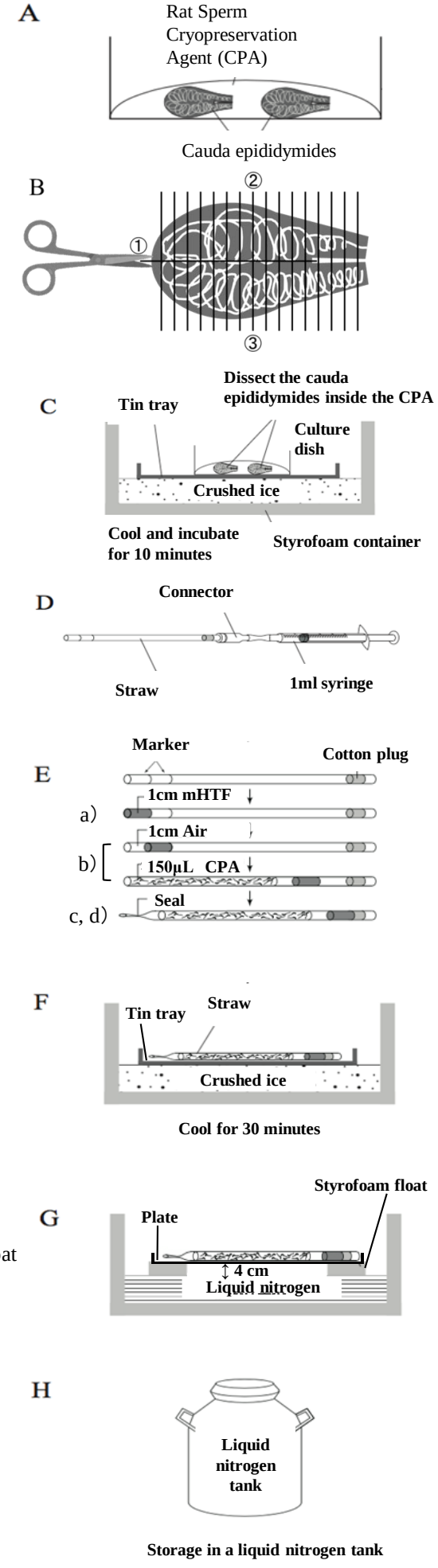
D. Connect the end of a straw to a straw connector attached to a 1mL syringe.

E. Load the sperm suspension into the straw following the method below:
a) Aspirate approx. 1cm of mHTF.
b) Aspirate approx. 1cm of air, then approx. 150µL of the sperm suspension.
c) Pull back the 1mL syringe plunger until the mHTF reaches the cotton plug.
d) Seal the ends of the straw with an impulse sealer.

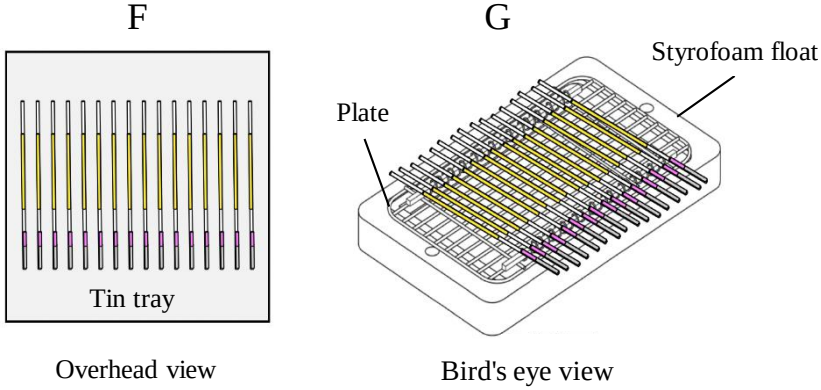
F. Place straws containing the sperm suspension on the tin tray set on top of crushed ice. Leave the suspension to incubate for 30 minutes.

G. Line up straws containing sperm suspension on top of a plate (CARD Rat Sperm Freezing Kit) which has been pre-cooled on a bed of ice. Place the plate on top of a Styrofoam float which is floating on liquid nitrogen. Leave the plate there for 10 minutes, then immerse the straws into the liquid nitrogen and transfer them to a triangular cassette for storage.

H. Store the triangular cassette containing the straws in a liquid nitrogen tank.

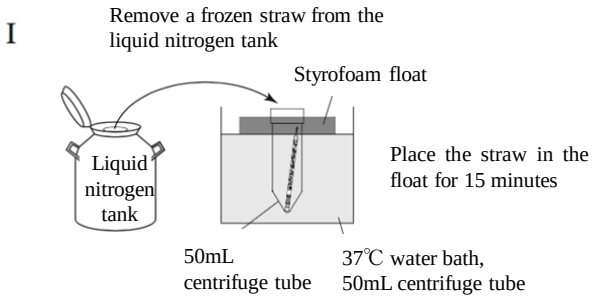


Appendix: Sperm suspension straw cooling diagram

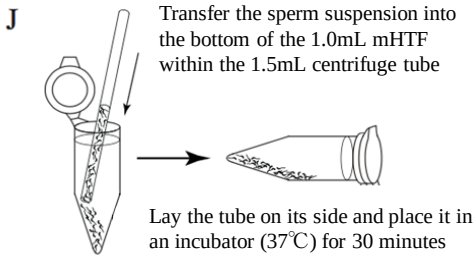


2． Frozen rat sperm thawing protocol

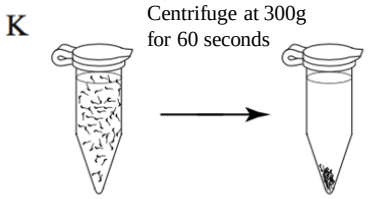
I. Remove a frozen straw from the liquid nitrogen storage tank. Place the straw in a Styrofoam float (in a water bath maintained at 37℃) and leave it for 15 minutes. During this time, dispense 1.0mL of mHTF into a 1.5mL centrifuge tube and equilibrate it at 37℃.



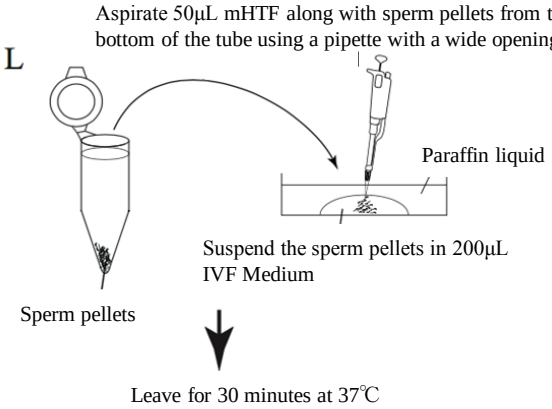
J. Remove the straw from the float. Use fine tissues to wipe any water from the straw. Use a paper towel to wipe any water from the straw. Next, cut the end of the straw closest to the sperm suspension and transfer the suspension into the bottom of the 1.0mL mHTF within the centrifuge tube. Lay the tube on its side and place it in an incubator (37℃) for 30 minutes.



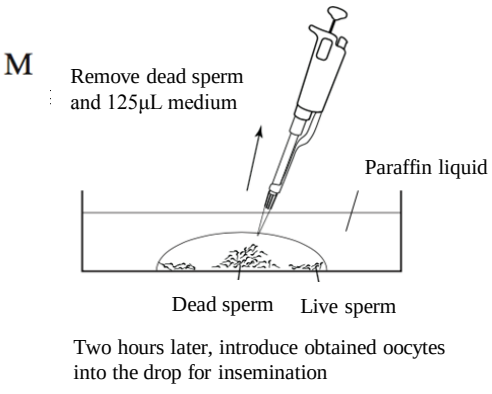
K. After 30 minutes, slowly invert the centrifuge tube 2-3 times to mix the contents, then centrifuge it at 300g for 60 seconds.



L. Take a pipette with a wide opening and gently insert it into the bottom of the centrifuge tube. Aspirate 50μL mHTF along with the sperm pellets at the bottom of the tube, then transfer them to a 200μL drop of CARD Rat In Vitro Fertilization Medium in a culture dish.



M. After leaving the dish in an incubator for 30 minutes, remove dead sperm and 125μL of medium from the mHTF drop using a pipette with a fine opening. Two hours later, introduce obtained oocytes into the drop for insemination.



Thesis title: Establishment of sperm cryopreservation and in vitro fertilisation protocols for rats
Authors: Naomi Nakagata, Nobuyuki Mikoda, Satohiro Nakao, Ena Nakatsukasa& Toru Takeo
Journal : Scientific Reports
doi : https://doi.org/10.1038/s41598-019-57090-7
URL : https://www.nature.com/articles/s41598-019-57090-7