THE UNIVERSITY OF QUEENSLAND
STANDARD OPERATING PROCEDURES
LABORATORY ANIMAL RESEARCH

SOP No: **AHP 65**

SUBJECT: **Cryopreservation of mouse sperm**

POLICY: **This procedure may only be performed by a skilled operator.**

PRECAUTIONS:
If the sample is to be placed into a "gassed" incubator the pH may be changed
If the sperm sample is drawn up too quickly, it will spread across the wall of the straw and be lost
Raffinose acts as a weight so that the straw doesn't float in liquid nitrogen

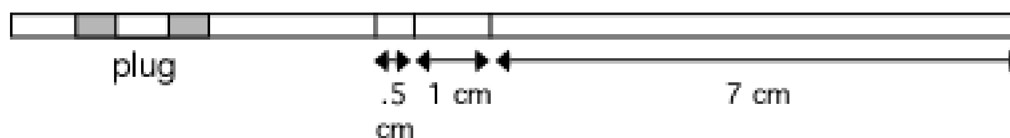
EQUIPMENT:
Protective clothing
One male mouse
Euthanasia of choice
0.25cc straws
Brady LAT-17-361-2.5 straw labels
Cryoprotectant agent (CPA -18% raffinose, 3% non-fat dried milk in water)
70% alcohol
20µl Pipetman
Dissection microscope
Heat sealer
Incubator or warming pad (37°C)
Dewar w/LN2 for freezing

INSTRUMENTS (sterilized):-

120mm sharp scissors x2
Pair Dumont forceps
120mm straight toothed forceps x2

PROCEDURE

1. **Label straws at the plug end and mark with a pen using the figure as a guide.**



2. **Place 100 µl of CPA per male in the small petri dish and place on warming tray or in incubator at 37° C.**
3. **Sacrifice the male mouse. Remove both epididymides and vas deferentia and place in the dish containing CPA.**
4. **Under the dissecting scope, slice the epididymides 3 to 5 times with the needle of a tuberculin syringe to release the sperm using either a pair of syringes or hold the tissue with the Dumont forceps and use a single syringe for slicing.**
5. **Squeeze the sperm out of the vas deferens either by *gently* running a pair of forceps down the vas or by "walking" a pair of 30 gauge needles along the vas.**
6. **Allow the sperm to "swim out" for 4 minutes at 37°C.**
7. **Remove the epididymides and *gently* stir the dish with a pipette tip to mix and distribute the sperm equally, and then load the straws as follows:**
   A. Attach a 1 cc Monoject syringe to the plug end of the tube.

B. Draw 18% raffinose into the straw up to the 1st line at 7 cm.
C. Draw 1 cm of air into the straw (until the raffinose reaches the 2nd line).
D. Wipe the straw with a kimwipe.
E. Slowly draw 0.5 cm of sperm sample (≈10μl) into the straw (the raffinose reaches the 3rd line).
F. *Slowly* draw air into the straw until the raffinose hits the plug. This seals the top of the straw
G. Seal the bottom of the straw with a heat sealer or with Critoseal
H. Place the straw in a goblet or cassette
I. Repeat until all the sample is collected

8. Place the goblet or cassette into the liquid nitrogen vapour at approximately -120°C. Leave for at least 10 minutes
9. Plunge the cassette into LN2

RECOMMENDATIONS:

Expect to get 6-7 straws from a single male. Recovery will be more efficient with larger, pooled samples and it would be possible to collect 16 samples from 2 males and 25 from 3
Cool the straws at around 1°C/min. This can be achieved by placing the straws in a box in the vapour phase of a liquid nitrogen storage tank, by using a controlled-rate freezer or by putting straws in a rack into a Revco at -80°.
Limit the exposure of the frozen sample to room temperatures. Straws will warm up extremely rapidly, thawing and destroying the sample. Transfer straws as rapidly as possible to their storage place in liquid nitrogen.

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REVISED:

REFERENCES

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2. Critser JK, Mobraaten LE. (2000) ***Cryopreservation of murine spermatozoa*****. ILAR J.; 41(4):197-206.**
3. Sztein JM, Noble K, Farley JS, Mobraaten LE. (2001) ***Comparison of permeating and nonpermeating cryoprotectants for mouse sperm cryopreservation*****. Cryobiology Feb; 42(1):28-39**