**Freezing of rat spermatozoa**

1. Cauda epididymides were collected from males at 22-24°C.
2. Sperm was isolated into freezing medium (23% (v/v) egg yolk, 8% (w/v) lactose, 0.7% (w/v) Equex Stem).
3. Sperm was loaded into 0.25 ml straws.
4. The straws were cooled to 5°C at 0.5°C/min by programmable freezer and held for 5 min.
5. The straws were exposed to LN₂ vapor at 4 cm above level of LN₂ for 10-15 min.
6. The straws were plunged into LN₂ and stored until before use.
Thawing of rat spermatozoa and in vitro fertilization
1. Straws were thawed in 37°C water bath for 10-15 sec.
2. Frozen-thawed sperm was spread on a cell culture dish.
3. Frozen-thawed sperm (2 µl) was collected from the dish.
4. The sperm was directly diluted into drops (oil-covered 200 µl mR1ECM supplemented with 200 µM IBMX and cultured for 5 h.
5. Oviductal ampullae were placed in the oil and COCs were transferred into the drops and cultured for 10 h.
6. Denuded oocytes were cultured in 100 µl drops of culture medium.
References

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If you have any questions, please contact us by e-mail.
J. Ito (itoj@azabu-u.ac.jp) or N. Kashiwazaki (nkashi@azabu-u.ac.jp)