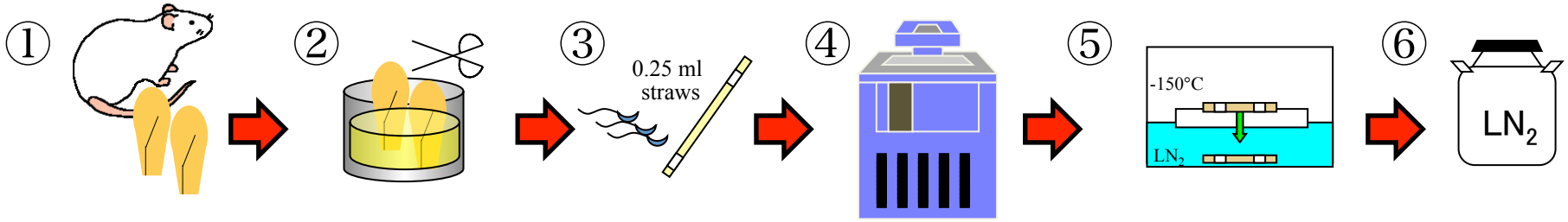
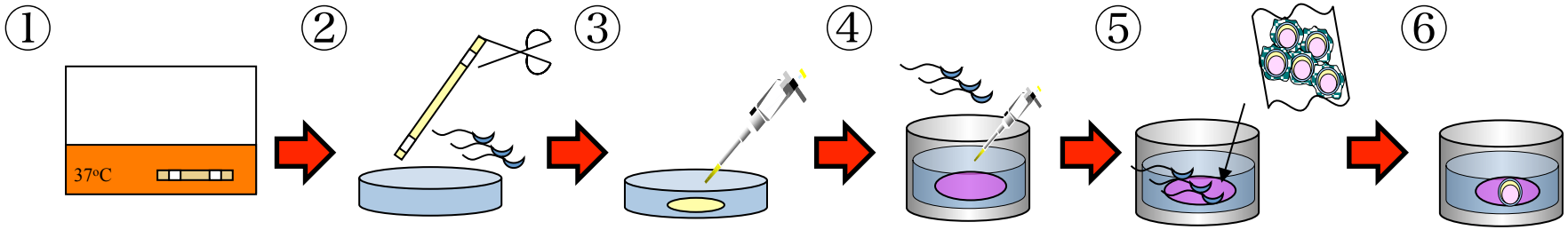
Rat *in vitro* fertilization protocol using cryopreserved sperm

Laboratory of Animal Reproduction, Azabu University
Junya Ito and Naomi Kashiwazaki



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 LN_2 vapor at 4 cm above level of LN_2 for 10-15 min.
6. The straws were plunged into LN_2 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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2. Seita Y, Fujiwara K, Takizawa A, Furukawa K, Inomata T, Ito J, Kashiwazaki N. (2011)
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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)