# Methods for freezing mouse sperm in urgent situation

## ～Easy & Quick (EQ) method～

**RIKEN Bioresource Research Center**

**Integrative Developmental Engineering Division**

**Updated technologies　#1**

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# Introduction

## Advantages

- Effective method for preserving sperm from many mice in case of disaster or emergency, and can be used for IVF after thawing.
- No liquid nitrogen required and can be stored in the freezer at -80$^{o}$C.
- Can be performed quickly, even by inexperienced users, and can preserve sperm from 100 males in a short time.

## Key points

- Simple method: Place the epididymis in a microtube with a 20% raffinose solution, cut with scissors and store directly in the -80$^{o}$C freezer.
- If long-term preservation and high fertilization rates are required, standard methods of cryopreservation are preferable.

# Reagents and Equipment

Raffinose: Becton Dickinson, cat#217410

Microtube  (1.5 mL)

Aluminum foil

Fine scissors

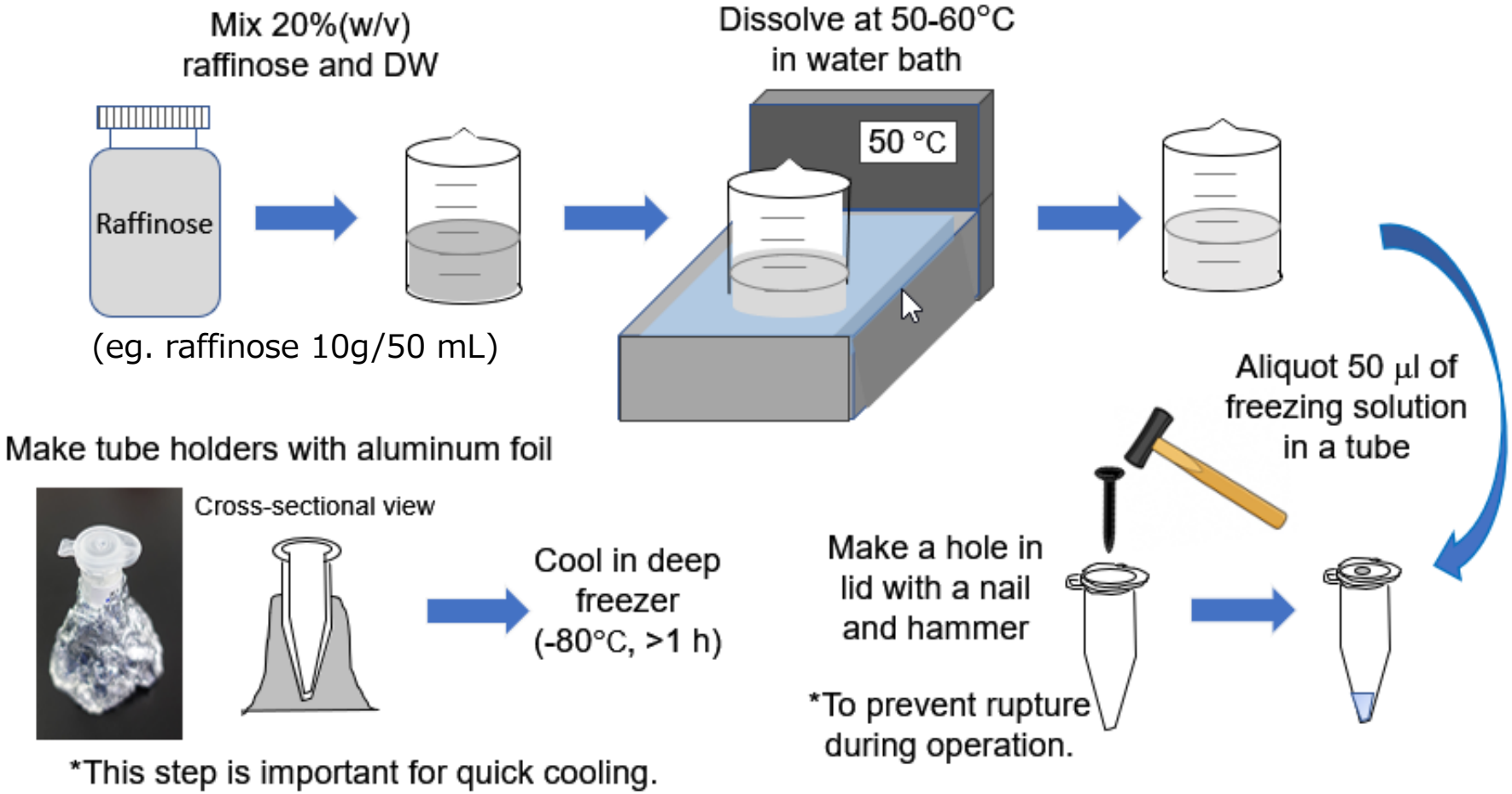
Fine forceps

Tube storage boxes.

Heating devices (e.g. water bath) to dissolve at 50℃

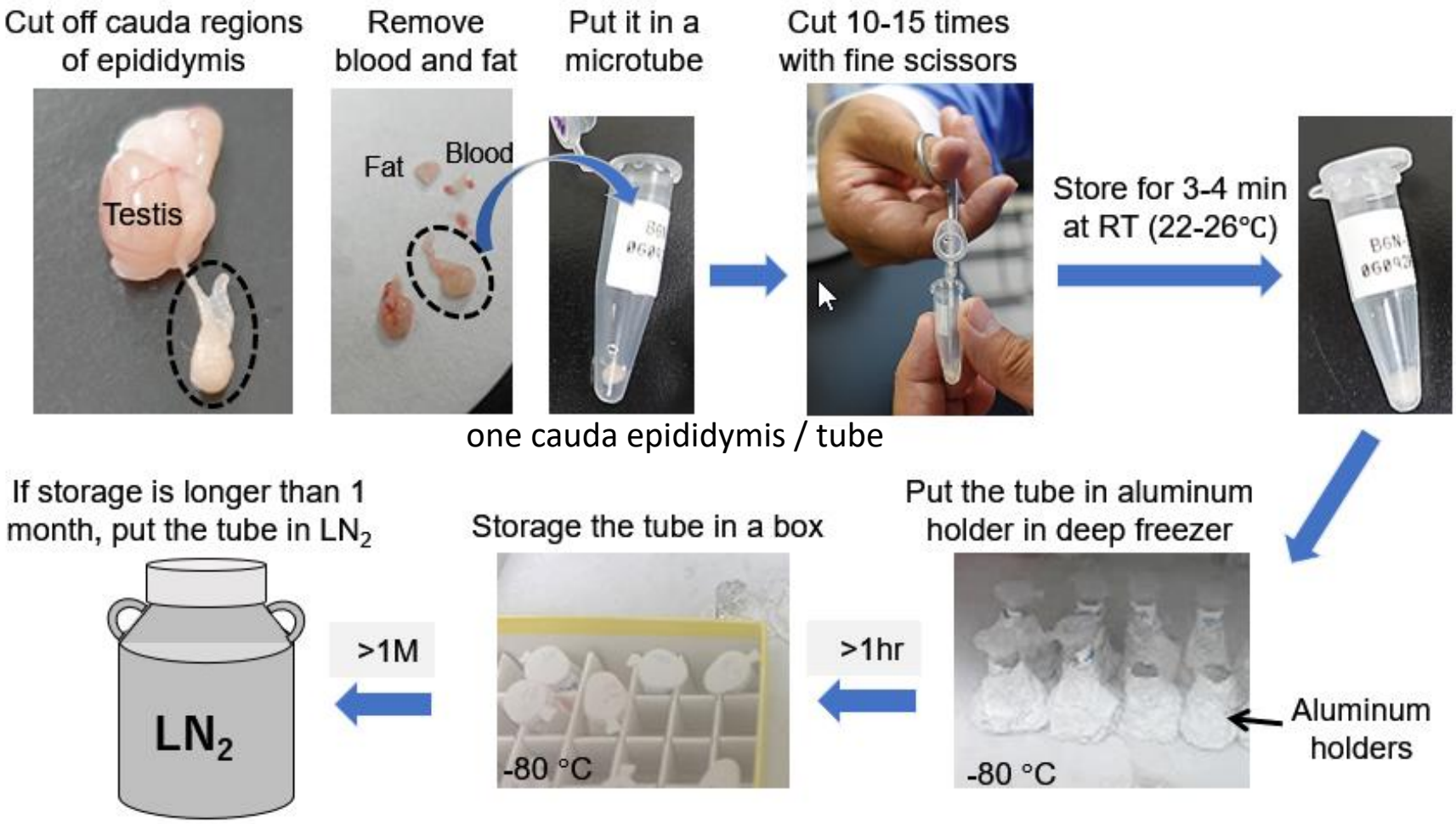
# Methods

## 1. Preparation of freezing solution, microtubes/tube stands



# Methods

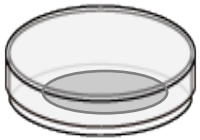
## 2. Collection of cauda epididymis and sperm freezing



# Methods

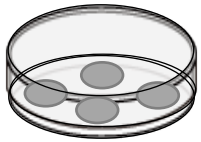
## 3. Preparation of preincubation and fertilization dishes for IVF

Sperm preincubation dish



1 drop/ dish (300 μl)

Fertilization dish



4 drops/ dish (80 μl)

Cover the drops with mineral oil

Incubate for at least 30 min at 37 °C in $CO_2$ incubator

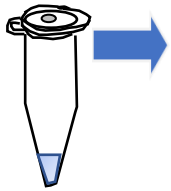
***For more information on culture media and IVF conditions, see reference**

# Methods

## 4. Thawing of frozen sperm and performing of IVF

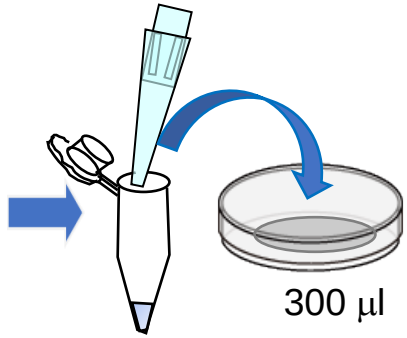
Remove the tube from deep freezer


Keep in air for 10 sec

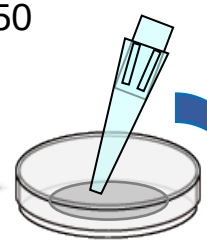


Warm for 15 min at 37 °C in water bath

Transfer 23-25 µl content into a drop of sperm preincubation dish




Incubate for 40-50 min at 37 °C in $CO_2$ incubator



Transfer 15 µl to each drop

Collect COCs and moved into drop of fertilization medium (20-30 oocytes/ drop)

Incubate for 30 min< at 37 °C in $CO_2$ incubator

***Insemination***

# Results

## 1. Fertilazation rates using sperm frozen by standard and EQ methods.

| Strain of males | Method | $Ca^{2+}$ concentration (mM) | No. of epididymides used | Total no. (%) of oocytes fertilized/inseminated | Mean % of fertilization (±SEM) |
|---|---|---|---|---|---|
| B6N | Standard | 2 | 8 | 427/469 (91.0) | 91.4 (±1.5) |
| | EQ | 2 | 7 | 209/401 (52.1) | 49.9 (±7.0)** |
| | | 5 | 7 | 176/307 (57.3) | 56.7 (±4.9)** |
| B6J | Standard | 2 | 10 | 583/754 (77.3) | 77.3 (±1.4) |
| | EQ | 2 | 7 | 118/380 (31.1) | 30.8 (±2.0)** |
| | | 5 | 7 | 150/301 (49.3) | 50.9 (±10.5)* |

Result:

Even with the simple EQ method,
fertilization rates of about 50% have been achieved.

# Results

2. **Full-term development of fertilized embryos with sperm stored for one month using the EQ method.**

| Strain of males | No. (%) of females used | | | No. (%) of embryos | | | | |
|---|---|---|---|---|---|---|---|---|
| | Transferred | Pregnant | (%) | Transferred | Implanted | (%) | Developed to offspring | (%) |
| B6N | 3 | 3 | (100) | 45 | 39 | (87) | 26 | (58) |
| B6J | 4 | 4 | (100) | 60 | 49 | (82) | 33 | (55) |

Result:
Offspring were obtained at high developmental rates (>50%) in both strains.

# Reference


- Mochida K, Hasegawa A, Shikata D, Itami N, Hada M, Watanabe N, Tomishima T, Ogura A. Easy and quick (EQ) sperm freezing method for urgent preservation of mouse strains. Sci. Rep. 2021, 11:14149. https://doi.org/10.1038/s41598-021-93604-y

# Appendix

## Tips for sperm freezing

- It is important to cool the tube quickly. First, an aluminium foil holder should be made to fit the shape of the tube.
- This simple method can be used to preserve the sperm of a large number of mice in a short time. It is better to store in $LN_2$ as soon as possible rather than in the freezer for a longer period to reduce freezing damage.
- Under usual situation, we recommend using standard freezing methods for IVF that are easy to use.。
- Sperm cannot be preserved at temperatures beyond -80°C.

# Contact

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