Collection of mouse spermatogenic cells

Bioresource Research Center
Bioresource Engineering Division

protocol #2

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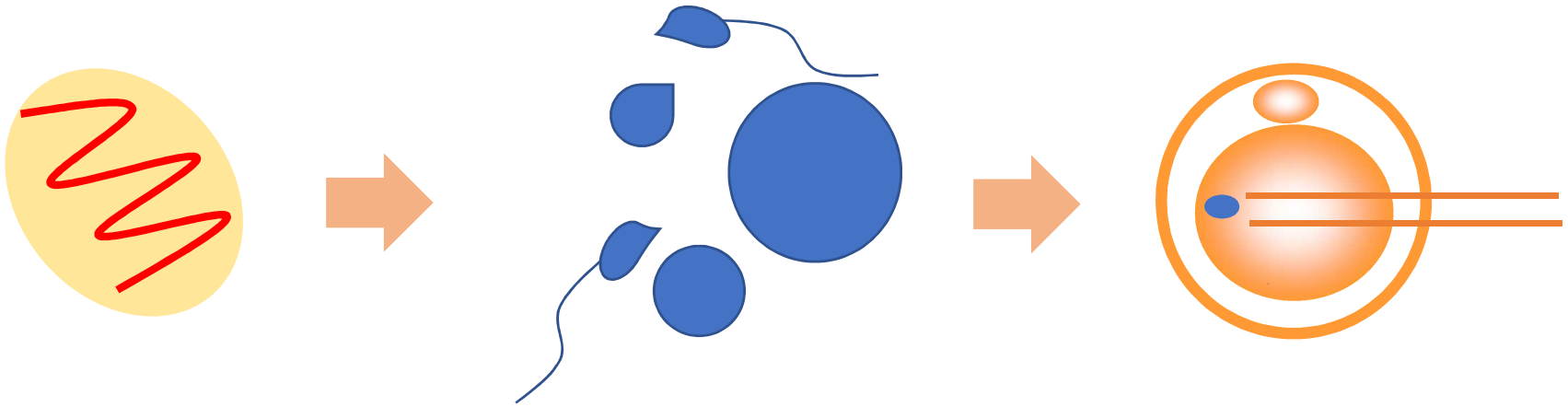
Graphical Abstract



Contents

Introduction

Purpose

Spermatogenic cells for microinjection can be collected from the testes by a mechanical or chemical (enzyme) treatment.

Introduction

In the mechanical collection method, seminiferous tubules are cut into small pieces and pipetted to allow spermatogenic cells released into medium. This method enables collection of spermatocytes, round spermatids, elongated spermatids and spermatozoa, which are located at the luminal side of the seminiferous tubules.

The enzyme-based chemical collection method enables collection of all the testicular cells including spermatogonia and Sertoli cells attached to the basement membrane of the tubules.

Reagents and Equipment

Anatomy Props		
35 mm petri dish	**IWAKI**	**1000-035**
Erythrocyte-lysing buffer	**Home-made**	**Home-made**
GL-PBS	**Home-made**	**Home-made**
Cold pack (4C)		
Pasteur pipette	**Shorten the tip and round the cut surface with a burner**	**Home-made**
Nylon mesh	**NIPPON RIKAGAKU KIKAI**	**I.D. 37-50 µm**
15 ml tube	**IWAKI**	
Centrifuges		
(Chemical methods)		
Collagenase	**SIGMA**	**C-2674**
DNase	**SIGMA**	**DN-25**
Trypsin	**SIGMA**	**T-9201**
Shaker		

Methods

1. Preparation of reagents

- **Erythrocyte-lysing buffer**

NH_4Cl	155 mM	0.829 g /100 ml
$KHCO_3$	10mM	0.1 g /100 ml
EDTA	2 mM	0.0744 g /100 ml
(pH 7.4)		

- **GL-PBS**

Dulbecco-PBS(+) with 0.1 mg/ml polyvinyl alcohol		500 ml
Glucose	5.6 mM	0.5045 g
Sodium lactate	5.4 mM	0.3026 g
BSA	5 mg/ml	2.5 g

Methods

2. Collection of mouse testes

All operations should be performed on a refrigerated (not frozen) cold-pack , taking care not to decrease the temperature below 4°C

① **Testes removed from male mice are placed in erythrocyte-lysing buffer.**

② **Remove *Tunica albuginea* using tweezers, and transfer the seminiferous tubules into GL-PBS.**

③ **Loosen the seminiferous tubules with fine tweezers (be careful not to break the tubules).**

Methods

3. Mechanical collection of spermatogenic cells

① Cut the seminiferous tubules into small pieces using fine scissors (5-6 cuts/testis) and pipette them gently to allow spermatogenic cells released into the medium.

② Filter the cell suspension through a nylon mesh, then centrifuge it at 800 rpm, 4 min at 8°C in a 15 ml tube.

③ Resuspend cells in 3-4 ml GL-PBS and pipette gently. Repeat centrifugation and washing three times.

④ Finally, add 0.5-1 ml GL-PBS to the sediment and store at 4°C until the experiment.

Methods

4. Chemical collection of spermatogenic cells

① After removal of *Tunica albuginea as above* and put the seminiferous tubules into a 15 ml tube containing 1.5 ml GL-PBS +150 µl 1% collagenase +50 µl 0.5% DNase.

② Shake gently in a 37°C shaker until the seminiferous tubules are dispersed (about 30 min).

③ After centrifugation at 800 rpm, 4 min at RT, resuspend cells in 100 µl GL-PBS (BSA free)+200 µl 1% trypsin+20 µl 0.5% DNase and pipette gently.

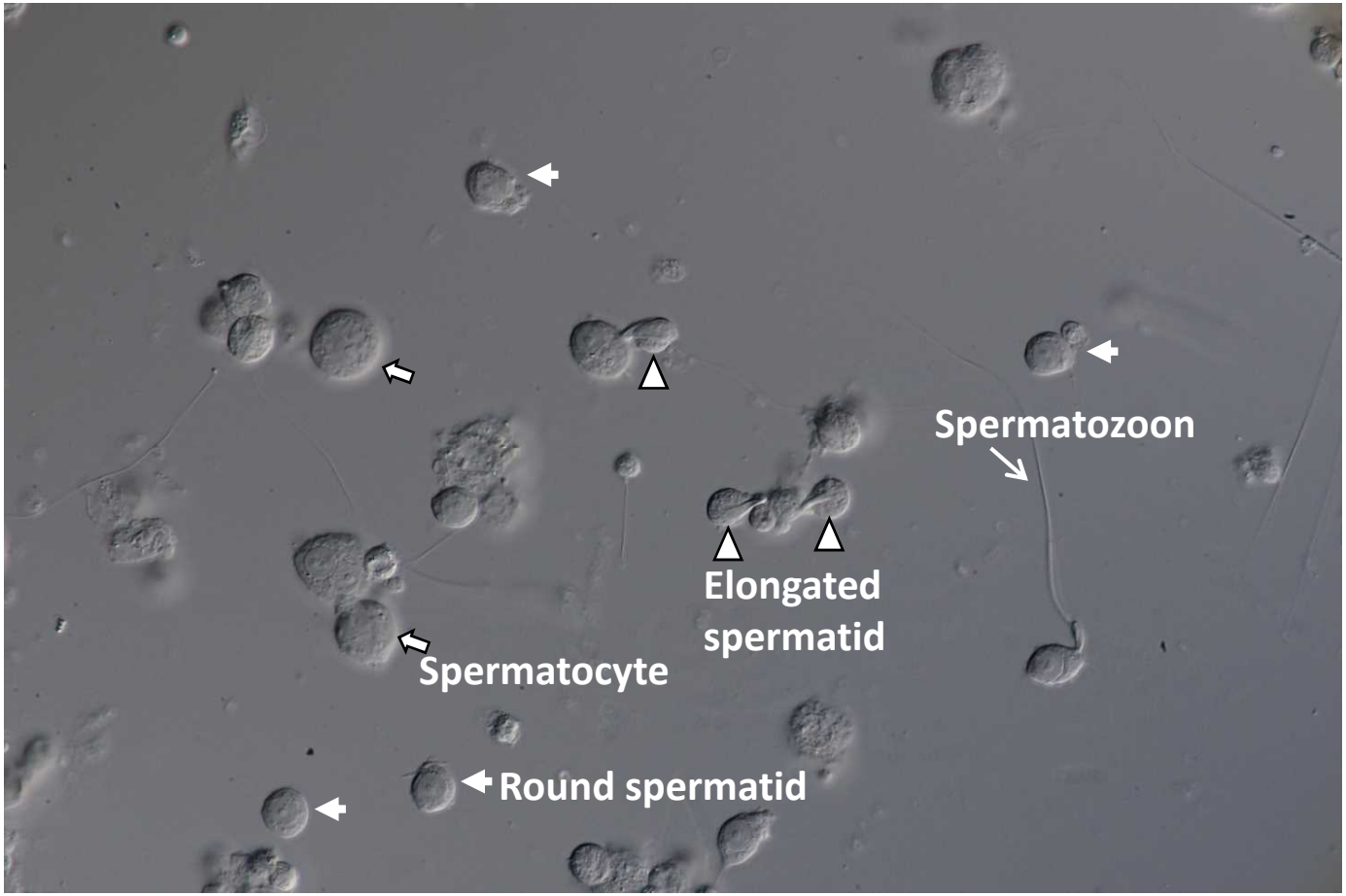
④ Shake gently in a 37°C shaker (about 5-10 min).

⑤ Add 700 µl GL-PBS (with BSA), pipette gently and centrifuge at 800 rpm for 4 min at RT. Repeat centrifugation and washing three times.

⑥ Finally, add 0.5-1 ml GL-PBS to the sediment and store at 4°C until the experiment.

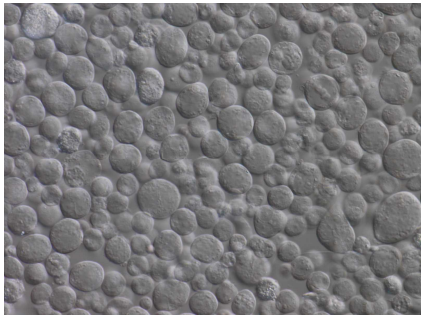
Appendix

Isolated spermatogenic cells (by mechanical collection)



Appendix

- **The isolated cells can be stored for several days at 4-10^{o}C.**
- **For the cell freezing, the pellet of cell are resuspended in GL-PBS containing 7.5% glycerol + 7.5% fetal bovine serum and frozen at -80^{o}C (Ogura et al., 1996).**
- **Spermatogenic cells can be collected from testes that have been refrigerated (4-10^{o}C) for 1 to 2 days.**
- **Sperm or elongated spermatids collected from frozen whole testes (at -80^{o}C) can produce normal offspring after microinsemination (Ogonuki et al., 2006).**
- **For collection of only round cells, cell suspension is treated with 0.1-0.2 mg/ml pronase in GL-PBS and centrifused/washed. This treatment removes most elongating spermatids and spermatozoa from cell suspension as agglutinated sticky masses (Figure below) (Ogura et al. 1993).**



References

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- Ogura et al., 1996, J.Assist. Reprod. Genet. 13, 431-434.
- Ogura et al., 2005, Int. Rev. Cytol., 246, 189-229.
- Ogonuki et al., 2006, PNAS 103, 13098-13103.

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