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Improved In Vitro Fertilization via Zona Thinning

ABSTRACT

Mouse In Vitro Fertilization (IVF) can be used to rapidly expand mouse lines from a few males that carry the desired genotype or to maintain strains with poor breeding efficiency. This guide describes a detailed method using acidified Tyrode solution to open the zona pellucida, and increase the fertilization capacity of frozen mouse sperm. The embryos generated are suitable for transfer into pseudopregnant recipient females, culture or cryopreservation.



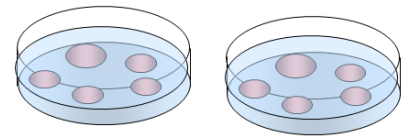
Materials and Equipment

- Frozen sperm sample
- Female mice superovulated with PMSG and hCG
- Water bath at 37°C
- Dissecting microscope
- Timer
- Dissecting instruments (scissors, forceps, Dumont forceps)
- Large Petri dishes 60 x 15mm
- Small Petri dishes 35 x 10mm
- Media
 - Fertilization medium (ex. HTF)
 - Hyaluronidase 10mg/ml
 - Acid Tyrod pH 3.0 (Tyrode's Solution, Acidic T1788 Sigma)
 - Embryo tested mineral oil
 - PBS with BSA (or M2)

Method

1. Prepare one IVF-wash dish for every 5 females.

- In each dish put one 250µl-fertilization drop of HTF and four 150µl-drops of HTF.
- Cover drops with mineral oil and incubate at 37°C, 5%CO₂, 5% O₂, 90% N₂ overnight.



2. Thaw hyaluronidase (500µl for 10 mice) and acid Tyrodes (500µl for 10 mice)

3. Prepare oocyte collection dishes with 2.5ml PBS 37°C(2 dishes for 10 mice)



4. Thaw frozen sperm

- For straws: Place straw containing frozen sperm in a 37°C water bath for ~15-30 seconds, until completely thawed.
- For vials: Remove vial from liquid nitrogen. Check the vial for liquid nitrogen. If there is no liquid nitrogen in the vial, place in a 37°C water bath until thawed. If there is liquid nitrogen in the vial, wait until it dissipates before putting the vial in a 37°C water bath.

5. Add sperm to IVF dish

- Add one 10µl aliquot of the frozen sperm suspension into each 250µl IVF drop
- Incubate the sperm for 45 minutes. During this time, collect and treat the eggs.

6. Collect cumulus oocyte masses

- Collect eggs 13-16 hours post-hCG.
- Dissect ovaries, oviducts, and uteri from 5 mice into dish containing 2.5ml PBS. Remove all cumulus oocyte masses (COMs) from ampulla. Repeat with the remaining 5 females and add to the PBS dish already containing COMs.

7. Remove cumulus cells

- Pick up all of the COMs in 500µl and put in an empty Petri dish.
- Add 500 µl of hyaluronidase to the COMs in the Petri dish

Note: The sensitivity of oocytes to acid Tyrodes is strain dependent and is determined on an individual basis. For C57BL/6J, the optimal time is ~40 seconds. If you lose zonae from more than 10% of the oocytes, try reducing treatment time to 35 seconds. When the ideal treatment time is reached, the zonae should have thinned, expanded and become irregular in shape (i.e., oval rather than round). Exposure time may vary depending on temperature of the acid Tyrodes.

- Pipette (2-3 times) to help break down the COMs.
- Transfer the weakened COMs to 2.5ml of PBS and incubate at 37° C until next step (do not gas).

8. Perform a test treatment to determine optimal time to expose oocytes to acid Tyrodes

- Place 10 oocytes into a 100µl drop of acid Tyrodes. Disperse the oocytes in the drop evenly to achieve consistent thinning. Start a timer immediately and observe the zonae.
- When most of the zonae are thinned, stop the timer. This is the approximate exposure time needed for this group of eggs.

9. Thin zonae with acid Tyrodes

- Using a 20 µl pipettor, pick up all of the hyaluronidase-treated oocytes in 20 µl and transfer to 400µl drop of acid Tyrodes. Dispense the oocytes evenly and swirl the dish to evenly apply the acid Tyrodes.
- Immediately start a timer for the amount of time determined in the test.
- As soon as the time is up, flood the dish with 4 ml of PBS (room temp).
- Swirl the dish to dilute the acid Tyrodes.

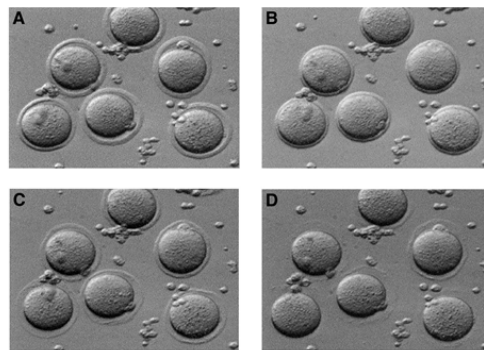


FIG. 1. Landmarks in the thinning of the zona pellucida at low pH. When oocytes are first placed in acid Tyrodes (A), they appear normal. Shortly thereafter (B), the zonae pellucida shrink so that the perivitelline space disappears. As time passes (C), the zonae then begin to swell and become more diffuse, and perivitelline space again appears, until (D) zonae begin to dissolve completely. The diameter of a mouse oocyte is approximately 80 µm.

10. Fertilization

- Transfer the treated oocytes with minimal amount of media to the IVF drop.
- Incubate the IVF dishes at 37°C, 5%CO₂, 5% O₂ & 90% N₂ for 4 hours.

11. Wash fertilized oocytes

- Following four hours of incubation, move oocytes from the fertilization drop through two of the wash drops. Leave behind as much debris as possible. (Caution, treated oocytes may be sticky!)
- Split the healthy oocytes between the two remaining wash drops for overnight culture. Note: After zona thinning, oocytes without zonae are non-viable.

12. Count 2-cell embryos

- About twenty-four hours post-fertilization, check for two-cell embryos.
- Transfer embryos into a pseudopregnant recipient, cryopreserve, or culture.