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Morulae aggregation

1. Collect morula (E2.5) by 10am.

2. Preparation of aggregation plate

3. Put 5~6 microdrops (about 40 ml) of M16 into a 6-cm bacterial plate.

4. Cover the whole plate with paraffin oil.

5. Sterilize the Darning needle by wiping with ethanol.

6. Press the Darning needle into the plastic, while making a circular movement with the free end of the needle you are holding. Do not twist.

7. Make three depression (or more) in each microdrop.

8. Return the plate to the incubator while you are preparing embryos.

Removing zona

9. Transfer embryos (no more than 20) with minimum volume to prewarmed Tyrode's solution. (3-ml Acid Tyrode's solution in 3-cm plate. Transfer embryos to different region of the solution if the solution is going to be used multiple times).

10. Transfer embryos back to M16 soon after the zona is removed. Monitor the embryos under the microscope. It normally takes less than 1 min. Rinse pipette with Tyrode's solution three times. (I usually start to pick up embryos at soon as zona is dissolving).

11. Wash embryos with M16. Be aware the embryos without zona are very easy to be damaged and are sticky.

12. Transfer embryos to aggregation plate. Place one embryo by an individual depression.

13. Repeat the same procedure for partner embryos. Place a partner embryo to the aggregation plate.

14. Prime pipette with enough medium. Arrange the pairing embryos into the depression and contact each other by the flow of medium.

15. Incubate on warm plate for at least 30 min and transfer to incubator.