

8.4.1

Oocyte Maturation

- Reagents required:
 - PBS
 - Dissection medium
 - FCS
 - Maturation medium
 - Oil
- 1. Oocytes are prepared by collection of ovaries in a warm container following slaughter of heifers.
- 2. The ovaries are transported to the laboratory, in warm PBS, within 4 h of slaughter and should be between 25 and 35 °C when they reach the laboratory.
- 3. The ovaries are thoroughly washed in warm saline to remove excess blood before follicles are aspirated with an 18-gauge needle and 10 ml syringe. Note. Only follicles between 4 and 8 mm should be selected for aspiration.
- 4. The follicles are pierced and the cumulus oocyte complexes (COCs) removed in the follicular fluid by applying gentle suction on the piston of the syringe. Each follicle contains one COC, and the number of follicles per ovary is variable, some producing one or two and others up to four or five.
- 5. The aspirated follicular fluid containing the COCs is gently expelled into a universal tube and kept warm in a water bath until the ovaries have been aspirated.
- 6. The COCs are allowed to settle for 10 min before the excess follicular fluid is carefully aspirated from the top of the tube using a sterile plastic pipette.
- 7. The follicular fluid is diluted with an equal volume of dissection medium +10% FCS, then poured into a large Petri dish. The tube is rinsed with a few milliliters of dissection medium +FCS, which is then added to the Petri dish.
- 8. A search is made for COCs that have at least three layers of cumulus cells surrounding the oocyte. COCs with fewer cumulus cells are

either degenerating or have had the cells physically removed during collection.

9. Selected COCs are washed twice in dissection medium and then once in maturation medium. A pipettor is used to move the media around, so that the cumulus cells are not scraped off.
10. Culture ten oocytes/50 μ l drop maturation medium under oil in a 5% CO₂ in air mixture at 39°.

8.4.2

Enucleation of Oocytes

- Reagents required:

- hSOF –Ca
- FCS
- Hyaluronidase
- Nunc four-well plates (www.nalgenenunc.com)
- CB
- Hoechst 33342 dye
- BSA (FAF)

1. After 18–20 h of maturation, gently pipette up and down a few times to remove any free cumulus.
2. The COCs are washed in hSOF –Ca without FCS before transfer to a well with 300 IU hyaluronidase in hSOF –Ca –FCS and then gently pipetted using an automatic pipettor until all of the cumulus cells have been removed. A 100 or 200 μ l volume is sucked into the pipette from a 500 μ l volume in a Nunc four-well plate. Keep the pipette at an angle so as the oocytes are not damaged by being squeezed against the bottom or side of the well.
3. Wash in hSOF –Ca +10% FCS.
4. Oocytes with a first polar body are selected under a dissecting microscope by gently rolling the oocytes until the polar body is seen. These oocytes, which are thought to be mature and at MII, are then stored in hSOF –Ca +10% FCS in a warm box at 37 °C until required.
5. Oocytes put to one side as not having a polar body can be examined again later.
6. Batches of 10–15 oocytes are placed in hSOF –Ca plus 7.5 μ g/ml CB and 10 μ g/ml Hoechst 33342 for 15 min prior to enucleation.

7. Oocytes are placed in the manipulation chamber with hSOF -Ca +FCS with CB.
8. Move the holding pipette to the oocyte and apply gentle suction until an oocyte is picked up.
9. This oocyte is then raised above the rest of the oocytes, and the microscope stage moved to an area of the chamber away from any other oocytes.
10. Turn the oocyte using the enucleation pipette until the polar body is in a position where the pipette can be pushed through the zona pellucida, the polar body and the adjacent cytoplasm within the body of the oocyte (where the metaphase plate is located), such that the polar body and metaphase plate can be aspirated gently into the pipette (Fig. 8.4). An 18 μm pipette ground at 35° with a pipette beveller and a spike put on the end with a hot glass bead on a microforge to allow it to go easily through the zona pellucida, is used.
11. The holding pipette with oocyte attached is removed from the field of view; the white light is turned off before the enucleation pipette is exposed to UV light. The DNA in the polar body will be seen to fluoresce brightly, with the maternal DNA fluorescing less brightly (Fig. 8.4). Depending on the orientation of the metaphase plate, the chromosomes can be seen as circular, linear or any configuration in between. *Note:* it is important that the enucleated oocyte is not exposed to UV light.
12. Successfully enucleated oocytes are deposited to the right of the chamber so that they can be easily removed, using a small hand-drawn glass pipette.
13. Wash enucleated oocytes in hSOF -Ca +FCS. Store in the incubator in SOF -Ca +BSA (0.8 mg/ml FAF BSA). *Note:* enucleation of the oocyte is most easily carried out as soon as the polar body is expelled, as the metaphase lies near the polar body at this time. Therefore the polar body can be used as a marker for enucleation. Polar bodies are expelled from around 18 h of maturation onwards, so enucleation can begin at around 20 h post maturation. Electrofusion can then be carried out at 24 h.

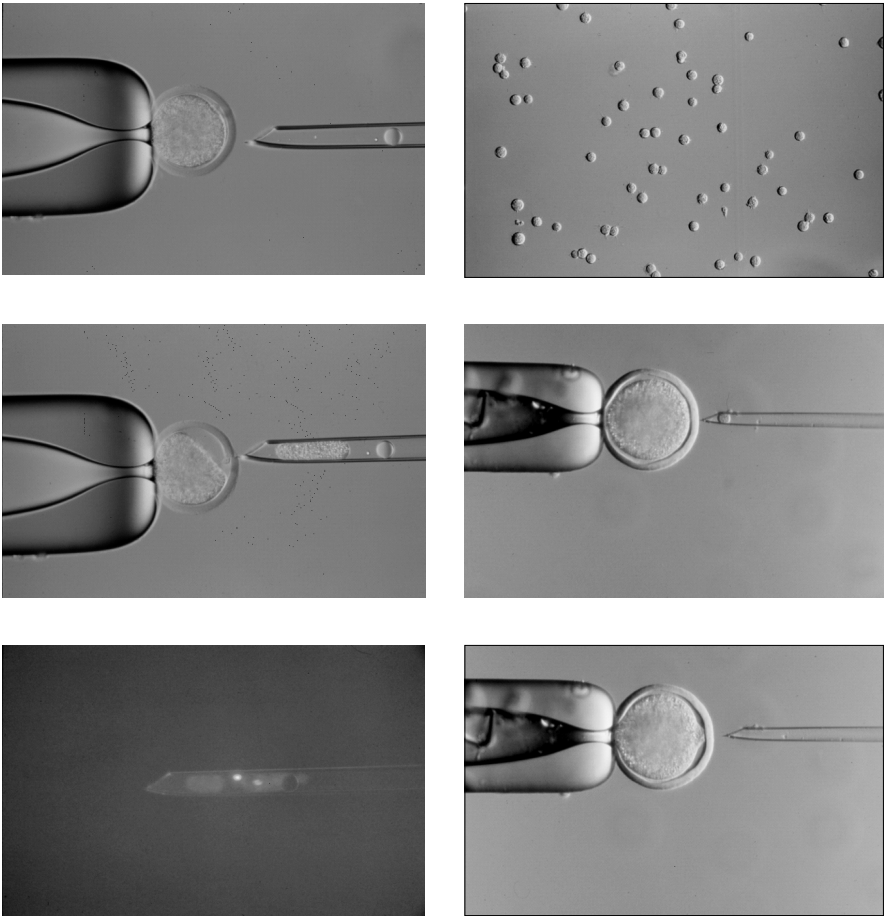


Fig. 8.4. Enucleation and cell transfer. Left: *Top* Oocyte orientated ready for enucleation, *middle* cytoplasm and polar body removed from the oocyte, *bottom* pipette exposed to UV light to show metaphase chromosomes and polar body. Right: *Top* Individual cultured cells ready for transfer, *middle* cell in transfer pipette, *bottom* cell transfer to enucleated oocyte

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