

ALTERNATIVE PROTOCOL FOR PROCESSING OOCYTES

This, the original protocol for washing oocytes after slashing, utilizes more medium than when the cell strainer is used to recover oocytes. It is included here as an alternative protocol for those who are already using this technique.

1. Once a group of ovaries have been processed, place beaker in a water bath at 38.5°C and add medium until the beaker is full. Allow oocytes to settle for 5 min. Using a 25 ml sterile Pasteur pipet, remove all but the bottom 100 µl of medium (Figure 1C).

Be careful here as many oocytes can be lost during this step. An automatic pipettor makes this task quite simple. Aspirate down to the 100 ml mark (refer to Figure 1C). If the settled oocytes become disturbed, STOP IMMEDIATELY and wait a few minutes for the oocytes to settle again.

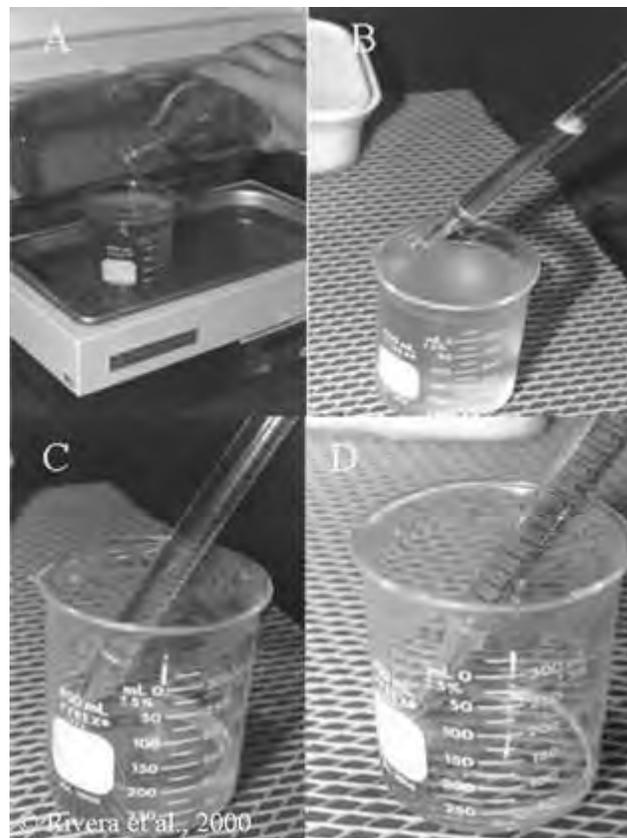


Figure 1. Cleaning up the preparation of oocytes collected by ovary slashing. After slashing ovaries, medium is poured into a sterile 400-ml beaker (panel A). After allowing oocytes to settle, medium is aspirated using a disposable pipette until all but the bottom 100-ml has been removed (panels B-C). The procedure is repeated several times (until the medium is clear) by filling the beaker with fresh OCM. On the last step, the medium is removed slowly until all but the bottom 50-ml has been removed (panel D).

8. While oocytes are settling, add ~ 5 ml of OCM to an X-plate.

9. Add ~350 ml of fresh OCM and repeat process until medium is clear. For the last wash, remove all but the bottom 50 ml of medium and transfer medium into an intergrid culture dish (100 x 15 mm);

Figure 2). Wash the beaker with a small amount of OCM and transfer this medium to the intergrid culture dish also. Place the intergrid dish on plate warmer until ready for searching. *Only go down to the 50 ml mark after the very last wash (it is of extreme importance that this be done very slowly so as to avoid aspirating the settled oocytes).*

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