

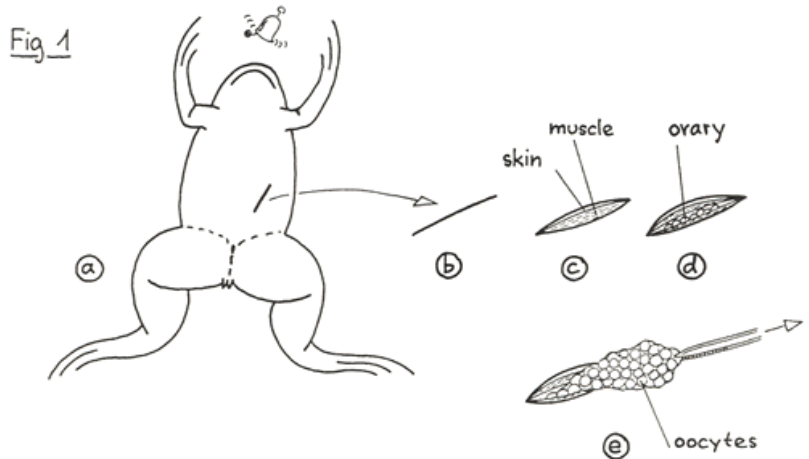
OBTAINING OOCYTES FROM FROGS BY SURGERY

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**Please note that we no longer are authorized
to perform surgery in the USA**

- Place a suitable frog in a small box with 0.2% MS222 (ETHYL-m-AMINOBENZOATE; Methan Sulfonic acid salt) in tap water. Caution: MS222 is a powerful mutagen. Wear gloves!
- Place lid on box and secure it so that the frog cannot jump out.
- After 15-30 minutes the frog should be anesthetized. Check by turning it on its back. The frog should show no reflex, i.e., should not move anymore.
- Wash the frog under tapwater and place it on paper towels.

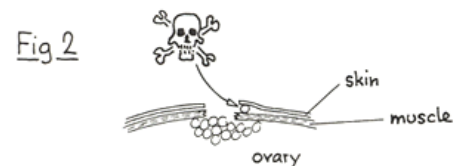
- Make a cut of about 1 cm with a sterile scalpel as shown in Fig. 1a.



- Under the skin is the muscle layer (Fig. 1c). Cut it with scissors. (Keep the scissors and the forceps sterile in 70% EtOH).

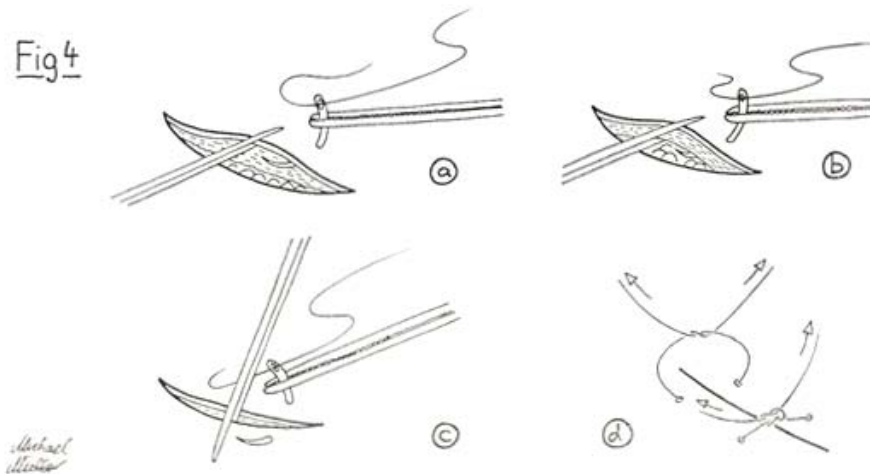
- You should see the ovary now (Fig. 1d). Pull out parts of the ovary with small forceps (Fig. 1e).

- Once you have the desired amount of oocytes, cut off all the oocytes that are on the outside. Never pull out the whole ovary. Check that no oocytes remain between the skin and the muscle layer (Fig. 2).



- The oocytes should be placed in 1x Barth solution. Tear the ovary connective tissue bags apart so that the oocytes can get oxygen from the saline solution.
- Prepare a sewing needle, preferably curved, with enough silk thread for two stitches.

- The closed cut should look as in Fig. 3. Fig 3 ✱
- Hold the skin on one side with forceps and push the needle, which you hold with forceps, through the skin as in Fig. 4a.



- In a second step push the needle through the muscle (Fig. 4b).
- Hold the muscle and the skin (check that you hold both) on the opposite side with the forceps and push the needle through.
- Cut off enough of the thread so that you can easily make a knot.
- Make the second stitch in the same way.
- Close the cut as in Fig. 4d. Do not pull too hard, it is enough, when the cut is nicely closed. Cut off the excess thread.
- Place the frog in a small tank with a little water. Incline the tank so that the frog's nostrils are out of the water. Cover the frog's back with some Kleenex to keep it wet.
- Remove the stitches after 10 days.