Gel removal and gel extract

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Introduction

In order to protect the oocytes and developing eggs amphibians produce a mucoprotein coating surrounding the oocytes called the gel coat. The gel coat must be removed before the oocyte can be manipulated or microinjected.

De-gelled embryos often have difficulty gastrulating. Coating the dishes with a thin layer of agarose prevents this problem. Once gastrulation is completed, the embryos can be raised in regular petri dishes. A partial de-gel can be very helpful to separate embryos and make their transfer easy or for counting eggs with image analysis.

Gel extract

Some studies have supported the idea of components in amphibian egg gel that are needed to 'capacitate' sperm thus enabling fertilization. To create a fertilization media the extraction of these compounds is accomplished by 'agitating' oocytes in a physiological saline for some time.

Incubate fresh eggs in 0.3 × Modified Amphibian Ringer (MAR; pH 7.8) at the ratio of 8 ml MAR (see Formulations) to 3 grams of eggs for 45 min at 20°C in a Petri dish on a rocker plate at about 15 cycles/min. About 60% of the originally added volume can be recovered.

Right. *Agalychnis callidryas* spawn their highly gelatinous oocytes onto leaves above water. The developed larvae then dissolve the gel with enzymes and drop into ponds. Image Fotographie Artman.

De gelling oocytes or eggs

(adapted from http://tropicalis.berkeley.edu/home/manipulate_embryos/dejelly.html)

Immerse oocytes in 3% cysteine made up in 1/9 x MR (pH to ~7.5-8.0 with NaOH) or water. Swirl the embryos gently and the gel coat will slowly be removed. If the degelling is being done at the one cell stage, very gentle or no swirling is recommended. For embryos past the first cleavage more vigorous swirling is acceptable.

For a partial de-gelling, immerse in cysteine just long enough until the embryos appear to be separate. The gel coat should still be visible around the embryo under the stereomicroscope. Complete degelling is accomplished when the embryos can pack tightly next to each other.

Cysteine can be used for as long as 20 minutes without problem. Once the oocytes or embryos are adequately de-gelled rinse them multiple times with 1/9 x MR to prepare for further manipulation.

In vitro fertilization of degelled oocytes

Fertilisation of degelled oocytes with motile sperm has been achieved by increasing solution viscosity with Ficoll or with gel extract

Ficoll

For *in vitro* fertilization of degelled eggs, solid Ficoll (Sigma 400 DL) is added into the extracted medium to a final concentration of 10% (wt/vol).

Use of gel extract

Degelled oocytes contained in a 35 × 10 mm petri dish are rinsed three times with 0.3 × MR (pH 7.8)(see Formulations). The 0.3 × MR is slowly removed from the dish until the level is just above but not touching the top of the oocytes. Freshly prepared sperm suspension is gently applied to the oocytes. After 5 min another 600 µl of gel extract is added and mixed. To achieve high rates of fertilization, the final sperm concentration must be greater than 10^7 sperm/ml; fertilization usually occurs within 30 min at 18-20°C, as indicated by contraction of the animal hemisphere.