Oocyte Transfer in the Axolotl

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Introduction. It was known that a heat-shock acting on fertilized axolotl eggs (Benford and Namenwirth 1974) or on tilted maturing oocytes (Beetschen and Gautier 1987), i.e., combining the effect of heat with that of a gravity vector, could immediately elicit the appearance of a gray crescent. The significance of that gray crescent for subsequent development had been established for the fertilized egg, but remained unknown for the maturing oocyte. Therefore, transfer experiments were performed, in which treated coelomic oocytes were reintroduced into the general cavity of a recipient female, previously inseminated after mating with a normal male. Such transfer experiments were inspired from earlier results on *Xenopus* and *Rana* oocytes (Holwill et al. 1987, Elinson and Pasceri 1989).

The technical procedures leading to fertilization and development of transferred normal axolotl oocytes are described herein, and the results that we obtained with heated oocytes will be published separately.

Methods. It has proven difficult to obtain a reliable synchronization between donor and recipient females. We describe the best conditions which were established after numerous trials.

The donor females, producing pigmented oocytes, were from dark or white (*d/d*) strains. The host females were from albino (*a/a* or *ad/ad*) strains. They were anesthetized with MS 222 1°/°°.

1. The anterior part of each oviduct of the donor female was ligated with two tightened knots of surgical silk thread a few days prior to induction of ovulation. Body wall incisions were sewn up with surgical thread.

2. On **day 1**, at 9 a.m., the donor female was injected with 12-15 µg of an LH-RH analogue (des-Gly10, [D-Ala6]-LH-RH ethylamide, ref. L4513, Sigma) and was subsequently kept at 11-12°C.

   At 6 p.m., the recipient female was similarly injected and kept at 11-12°C.

3. On **day 2**, at 9 a.m., the recipient female was mated with a male, at 13°-14°C. If spermatophores were not laid within 20 minutes, a second male replaced the first one. The animals remained together for 2-3 hours.

   At 2 p.m., the donor female was killed and the maturing oocytes (1st polar body was conspicuous but not yet separated) were collected into OR_2 medium, pH 7.6 (Wallace et al., 1973). Heating experiments took place in the afternoon. Control, nonheated oocytes were kept in OR2 medium at 18°C during 3-4 hours, until they were transferred into the host female. At 6 p.m., the recipient female was operated on. A small aperture was opened through the lateral-ventral area of the body wall (laparotomy), in the middle part of the trunk length. The pigmented oocytes were introduced with a large-mouthed pipette, inserted forwards into the abdominal cavity. The available space is limited in the axolotl, as compared to *Rana* or *Xenopus*. The pipette was inserted several times. The oocytes could be distributed on both sides with one aperture, but it was necessary to hold the female vertical to help the oocytes find their way far from the incision. Normal oocytes were vitally stained (Neutral red or Nile blue) to distinguish them from heated oocytes. A total of 100-120 oocytes could be transferred into one recipient female. This means that two recipient females were used for one donor female.

   The host female was kept at 11°C during the following night.

4. On **day 3**, most of the transferred pigmented oocytes were laid first in the morning, usually prior to the albino oocytes. When the recipient female had not been inseminated, artificial fertilization was attempted, according to

Armstrong and Duhon (1989). Artificial fertilization worked on normal oocytes but gave very poor results on heated oocytes. For that reason, natural insemination of recipient females was compulsory, since heated oocytes thus could be fertilized and subsequently developed.

Results. The development of transferred normal oocytes from four different donor females is summarized in Table 1.

Comments. A high proportion (nearly 80%) of the transferred oocytes were actually laid. The results are still better appreciated when the percentages of fertilized eggs and of developing embryos are calculated in relation to the number of laid eggs.

As compared to the usual results from normal spawnings in the axolotl, it can be observed that the transfer diminished the proportion of viable offspring, but nevertheless allowed a significant number of embryos to develop normally.

The transfer procedures must avoid at least two pitfalls: 1) oocytes must be kept *in vitro* for as short a time as possible; 2) oocytes must be embedded in jelly layers in the oviducts as soon as they are engulfed through the ostium, hence the need for synchronizing the recipient female that we mentioned earlier. When the oocytes are transferred too early, jelly layers are very thin, and this may prevent fertilization.

The time-table that we propose might be modified by changing the temperature. Nevertheless, we believe that slower processes occurring at a relatively low temperature are better for successful results.

The oocyte transfer technique might be useful for *in vitro* manipulation of maturing oocytes, especially when a homogeneous distribution of injected molecules is desired. In that respect, manipulation of injected oocytes might lead to better results than that of fertilized eggs.

Table 1. Development of transferred normal oocytes

	Transferred oocytes	Laid oocytes	Fertilized cleaving eggs	Gastrulae stage	Hatching stage	Feeding
Number	135	106	78	48	31	30
%	*100*	*78.5*	*57.7*	*35.5*	*22.9*	*22.2*
%	—	*100*	*73.6*	*45.2*	*29.2*	*28.3*

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