Induced Spawnings and Artificial Insemination in the Axolotl

John B. Armstrong and Laura L. Gillespie
Department of Biology, University of Ottawa
Ottawa, Ontario K1N 6N5

Over the past several years, we have observed that natural spawnings (i.e. when neither the male nor the female have been hormonally stimulated) are frequently unsuccessful. For this reason, we use hormonal stimulation for most of our spawnings.

Newrock and Brothers ('73) first described the use of follicle-stimulating hormone (FSH) to induce spawning. Their procedure involved the injection of the female with 40-60 International units (2-3 mg) of FSH either at the time the male and female were placed together, or 1-3 hours previously. Humphrey ('77) reported that in about half these cases the female laid only infertile eggs, the male having produced no spermatophores. In some cases, where the first male showed no courtship behaviour, a second or third male produced spermatophores and fertile eggs were obtained. Humphrey implies that spermatophores may still be picked up after the female has started laying eggs. We have found that though the female may stimulate the male to produce spermatophores, under such circumstances she rarely picks them up. In these cases we generally resort to artificial insemination.

Spawning procedure

For several years we routinely used 80 IU (4 mg) of FSH plus 50 µg of luteinizing hormone (LH) to induce spawning. More recently, we have used human chorionic gonadotropin (HCG) with similar success (Table 1). Our procedure differs from that of Newrock and Brothers in that the female is normally injected 8-12 hours before being placed with the male: the female is injected in the evening and the animals placed together in the morning.* With this procedure there is some risk that the female may start to lay before the male is added, but this happens only rarely and is balanced by the advantage of being able to observe the courtship. If there is no courtship, or the male produces no spermatophores, a second male can be substituted. We have found that if there is no courtship, or no spermatophores produced within the first hour, then there is normally no advantage to leaving the same animals together.

The animals should be watched continously to ensure that the female picks up the sperm packet immediately; otherwise the spermatophores tend to get knocked off the rocks as the animals swim about. On occasion the female may fail to pick up a spermatophore or may be prevented from doing

* The timing is temperature dependent. If the female is injected in the afternoon and left at room temperature, she will often be laying eggs by the next morning. However, we have not had this problem with females left at 15-16°C overnight.

so by the vigorous courtship of the male. The male may be kept away, or temporarily removed, while the female picks up the spermatophore, or the spermatophore can be presented to the female if she misses it. This is most easily done if the spermatophore is attached to a rock. The rock is placed under the animal just ahead of the cloaca, and she will normally move forward over it. Usually, three average sized spermatophores will be sufficient to fertilize all the eggs that the female will lay, so that once one is sure that she has picked up at least three, the animals may be covered and left alone. Our impression is that the continued presence of the male stimulates the female to lay more rapidly. However, some males eat eggs and obviously should be removed!

Our results also indicate that females stimulated with HCG lay about twice as many eggs in a spawning as FSH-stimulated animals (400-500 compared to 200-250). Spawnings induced with 400 IU FSH for artificial insemination, and natural spawnings, also average about 400 eggs.

Stimulation of the male

Our artificial insemination procedure (see below) originally involved the use of HCG to stimulate the male to produce spermatophores. HCG has been used to stimulate the male in many of our experiments involving normal insemination. However, the success rate of such spawnings (Table 2) indicates that the treatment does not appreciably increase the chance of the male producing spermatophores. Another treatment originally suggested to us by the late R.R. Humphrey is to place the male in ice water for 30 min before placing him in the mating container with the female. This treatment was also without effect on the frequency of successful spawnings.

General comments

Though our impression is that the courtship is more prolonged and that more spermatophores are produced in a successful spawning, our data is not sufficiently quantitative to verify this. However, the hormone treatment of the female definitely does affect the probability of ovulation, which was increased from 28 to 80% (see Table 1). Our results for normal (i.e., noninduced) spawnings support the conclusion of Humphrey ('77) that the female must make contact with the spermatophore and that the spermatophore be taken into the cloaca before ovulation will take place. In both his experiments and ours, the female in a normal spawning never produces only infertile eggs. Our observations also indicate that a female usually will not pick up spermatophores unless she is ready to ovulate. The greater success rate in the hormonally induced spawnings is, therefore, primarily due to a higher percentage of the females ovulating.

There are of course, many factors that should be considered in planning a spawning: the age and health of the animal, past records of performance (some animals are born duds!), how many spermatophores or eggs were laid in the previous spawning, etc. For example, if a healthy young male produced less than 5 spermatophores in a particular spawning, but had a good record, he could be used again in a week. However, if he had laid more than 12 spermatophores, we would not use him again for another 3 weeks.

Artificial insemination

In 1975 we first described an artificial insemination procedure (Trottier and Armstrong, '75) utilizing spermatophores that had been obtained by stimulation of the male rather than by maceration of the vas deferens. Since then we have tried various modifications of the procedure. The most important factor appears to be preventing the jelly coats on the egg from imbibing water and swelling. The "dummy" females, used to stimulate the male, are not essential. If the animals are watched carefully, the experimental female can be used to stimulate the male and spermatophores collected before the female picks them up. Two males placed together will also frequently stimulate each other, and we have had considerable success stimulating males by stroking them in the cloacal region with a rubber bulb. The revised procedure is as follows:

1) The female is given a dose of 20 mg FSH + 50 µg LH, or 500 IU HCG, 8-12 hours before the planned insemination.
2) When the experimental female begins to lay eggs, or preferably shortly before (as judged by her behaviour and/or the production of jelly), spermatophores are collected from the male.
3) Each spermatophore is placed in a small tube, on ice, in about 250 µl of 50% Leibovitz L-15 culture medium (GIBCO), or Wolf and Quimby's amphibian culture medium, undiluted. The former is less expensive. Sperm preserved in this manner will remain viable for up to 24 hours.
4) Just before use the spermatophore is gently broken up by triturating it 2 or 3 times with a Pasteur pipette.
5) Eggs are collected as laid and dried immediately on a paper towel for 1-2 min to remove the excess water. For maximum fertility it is important that the jelly coats have not begun to swell and have been well dried (the jelly coats should be somewhat sticky). The eggs are then placed in a dry petri plate and inseminated with a drop of sperm suspension. After 10 min 25% Holtfreter's saline is added. Eggs are generally collected over a 10 min period. Thus, some are actually inseminated for closer to 20 min.

One average sized spermatophore can be used to inseminate 50-75 eggs with 90% fertility; the limiting factor is the rate at which the female lays her eggs as the sperm remain viable for only 30-60 min once the sperm packet has been broken up.

References

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Table 1

Effect of hormonal stimulation of the female on spawning success.

Treatment	Male produced sperm		No sperm	
	Fertile eggs	no eggs	Inf. eggs	no eggs
None (18)*	28% (403)+	33%	0%	39%
FSH + LH (199)	70.9 (239)	6.0	9.5 (143)	13.6
250 IU HCG (29)	75.9 (512)	10.3	6.9 (258)	6.9
500 IU HCG (130)	57.7	6.2	26.2	10.0

* number of spawnings
\+ average number of eggs laid

Table 2

Effect of attempted stimulation of the male on spawning success.

Treatment*	Male produced sperm		No sperm	
	Fertile eggs	no eggs	Inf. eggs	no eggs
None (43)**	72.1% (246)+	4.7%	14.0% (115)+	9.3%
Ice (50)	60.0 (235)	6.0	14.0 (117)	20.0
350 IU HCG (23)	82.6 (224)	4.3	8.7 (300)	4.3
500 IU HCG (83)	73.5 (243)	7.2	4.8 (150)	14.5

* All females treated with FSH + LH. Results are similar for HCG treated females

** Number of spawnings

\+ Average number of eggs laid