Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Theriogenology 75 (2011) 354–361

Theriogenology

www.theriojournal.com

Collection of gametes from live axolotl, *Ambystoma mexicanum*, and standardization of *in vitro* fertilization

N. Mansour[1,*], F. Lahnsteiner, R.A. Patzner

Department of Organismic Biology, Faculty of Natural Sciences, University of Salzburg, Hellbrunnerstr. 34, 5020-Salzburg, Austria

Received 26 April 2010; received in revised form 30 August 2010; accepted 9 September 2010

Abstract

This study established the first protocol for collection of gametes from live axolotl, *Ambystoma mexicanum*, by gentle abdominal massage and *in vitro* fertilization. To stimulate spermiation and ovulation, human chorionic gonadotrophin (hCG) and Ovopel pellets, which are commercially used to stimulate spawning in fish, were tested. The hCG was more effective than Ovopel pellets and yielded a higher semen volume in the injected males and a shorter response time in the females. Collected semen by this method was already motile and fertile. Fertile eggs could be collected in 3–4 successive collection times after the female has started the typical spawning behaviour. The fertilization condition that yielded the highest hatching rate was mixing semen with eggs before the addition of a fertilization saline solution (20 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l Mg_2SO_4, 1 mmol Ca_2Cl, 3 mmol $NaHCO_3$, 10 mmol/l Tris, pH 8.5 – Osmolality = 65 mosmol/kg). When the pH of the fertilization solution was increased to ≥ 10, the hatching rate was significantly increased. The use of fertilization solutions with osmolalities of ≥ 150 and ≥ 182 were accompanied with a significant decrease in hatching rates and the appearance of deformed larvae, respectively. In conclusion, a reliable protocol for gamete collection from live axolotl is established as a laboratory model of *in vitro* fertilization for urodele amphibians. This protocol may be transferable to endangered urodeles.
© 2011 Elsevier Inc. All rights reserved.

Keywords: Axolotl; Semen; Fertilization; Urodele; Amphibians

1. Introduction

Amphibians are a vertebrate class with a wide variation in their reproductive strategies. Both external and internal fertilization mechanisms exist [1]. With some exceptions, anurans are oviparous, urodeles are ovoviviparous and caecilians are viviparous. In most urodeles, with exception of Cryptobranchidae, fertilization is internal [2]. Males release spermatozoa surrounded by jelly coats (= spermatophores) in water. Females pick up the spermatophores into their cloaca where sperm are released and stored in the spermatheca at the exit of the oviduct until fertilization [3]. After fertilization females lay the fertilized eggs and attach them to aquatic plants [4].

One of the most alarming crises in recent decades is the sudden decline and disappearance of many amphibian species throughout the world [5]. Therefore, it will become necessary to establish special breeding and re-stocking programs for endangered amphibian species to maintain their populations and counteract total extinction. Today artificial reproduction of urodeles is not appropriate for artificial breeding programs of endangered species. Collection of semen requires sacrificing the males and collecting the sperm directly from

[1] Present address: Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

* Corresponding author. Tel.: (+43)-662-8044-5630; fax: (+43)-662-8044-5698.

E-mail address: nabil.mansour@sbg.ac.at (N. Mansour).

0093-691X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.09.006

ducti deferences [6–8]. Ovulation is stimulated after two injections of gonadotropin and eggs are collected either by a surgical method directly from the oviduct as described for the Japanese newt, *Cynops pyrrhogaster* [7] or by abdominal pressure in newts of the species *Pleurodeles sp.* [9,10]. Due the mode of internal fertilization described above, the sperm-egg interactions are complex and the ideal steps required for successful fertilization have not been standardized [6]. Practical standard techniques for gamete collection and for *in vitro* fertilization are needed for urodeles. In the present study the axolotl, *Ambystoma mexicanum*, a laboratory urodele [6] was used to develop a practicable and reliable protocol for gamete collection and for *in vitro* fertilization.

2. Materials and methods

2.1. Animals

Mature axolotls, *A. mexicanum* (total length, 18 ± 3 cm, weight 109.1 ± 17.8 g) were obtained from a breeder in Switzerland and were kept in Fiberglas channels of 220 × 40 × 50 cm (length × width × height). The bottom of the channels was covered with fine sand and aquatic plants and PVC pipes about 25 cm long were added to offer the animals possibilities to retreat. Water temperature was 18 ± 1 °C and approximately 50% of the water was changed in two week intervals. Males and females were kept separately and fed with earthworms or bovine meat 3 times per week.

2.2. Hormone treatment

The two treatments tested were: Ovopel pellets which are originally used to induce gamete maturation in fish (one pellet consists of 10–15 μg artificial GnRH, D-ala6, pro9 Net, and 2.5–3 mg of water soluble dopamine antagonist metoclopramide; Interfish, Hungary), and human chorionic gonadotrophin (hCG, Pregnyl®, Organon, Vienna, Austria). For the Ovopel treatment, half a pellet was used for the female and a third pellet for the male. The Ovopel pellets were ground and dissolved in 0.7% NaCl before injection. For hCG treatment, a dose of 200–300 IU was used for a female, and 150–200 IU for a male. These concentrations were derived from a preliminary experiment in which some doses from each treatment were tested. For Ovopel pellets, one fourth or half a pellet were tested for the females and one sixth or one third were tested for the males. Also, three doses of hCG were tested: 100, 200 or 300 IU for the females and 50, 100 or 200 IU for the males. For both treatments, the highest tested doses were the most effective to produce the highest semen volume in the males and a shorter response time for the females to release their eggs. Hormones were injected in the muscle dorsal to the hind limb after anaesthetization of the animals by immersion in 200 μg/L MS-222 for 20 min. To compare the success of each treatment, 10 males and 10 females were used for each treatment.

2.3. Collection of gametes

To determine the optimal time point of gamete collection, several trials were carried out to collect the maximum amount of semen and fertile eggs. For gamete collection animals were anaesthetized by immersion in 200 μg/L MS-222 for 20 min. As shown in Figures 1 and 2, the anaesthetized animal was held in the right hand with its head and back on the palm of the hand. With the thumb and index fingers of left hand, a gentle successive massage was made on the lower abdomen starting from 2–3 cm cranial to the genital papilla till the genital papilla itself. Semen was released and collected into graduated Eppendorf tubes to determine the semen volume. Eggs were released and collected into Petri-dish or in 50 ml beaker. Semen was stored at 4 °C, eggs at room temperature. For fertilization experiments, eggs were used immediately after collection but the semen was usually used within 1 h after collection.

To determine the quality of the collected semen, sperm density and sperm motility were evaluated. To

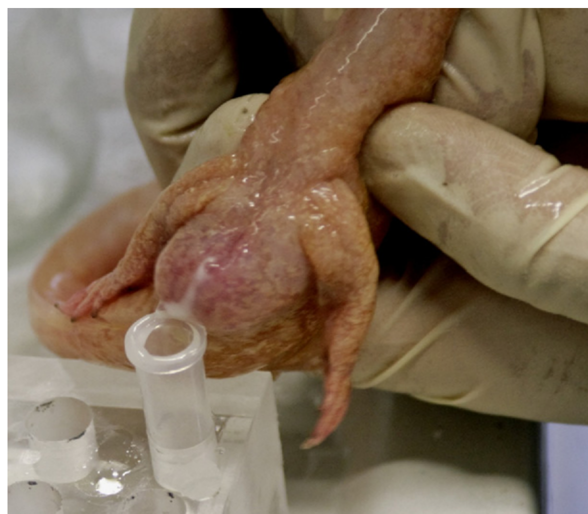

Fig. 1. Collection of semen from live axolotl, *A. mexicanum,* by abdominal massage.

Download English Version:

https://daneshyari.com/en/article/2096073

Download Persian Version:

https://daneshyari.com/article/2096073

Daneshyari.com