

**Zebrafish Egg and Sperm  
Collection  
IACUC Standard Procedure  
Effective Date: April 2022**

**Objectives:**

Eggs and sperm are collected in order to perform in vitro fertilization, sperm cryopreservation for line maintenance and to relieve blocked egg ducts in females that have become egg bound.

**Description of procedure:**

**Egg collection:**

Egg collection can be done on anesthetized or unanesthetized animals depending on whether or not eggs are being collected for fertilization or attempting to relieve egg-bound females with blocked ducts. Gloves may be worn but may also reduce sensitivity to safe handling including sensitivity to the degree of hand pressure on the animal. If gloves are not used, the user should thoroughly wash their hands in warm soapy water and rinse prior to handling the fish.

**Procedure with anesthesia for egg collection:**

- If using anesthetic in order to collect eggs, fish are anesthetized by immersion in 0.02%-0.04% MS-222 (Tricaine) at neutral pH until gill movement is slowed. Stock preparation is 4g/L buffered to pH7 in sodium bicarbonate (at 2:1 bicarb to MS-222). The dosage for anesthesia is 168ug/ml or 4.2 ml stock solution in 100 ml of water.
- Transfer anesthetized fish immediately onto a clean petri dish using a plastic spoon and lay flat on side.
- To release the eggs, the fish is very gently stroked on the side of the belly in the direction of head to tail with a finger wet with system water. Eggs should be immediately expressed, and the fish returned to fresh water. This procedure should take less than one minute. If eggs are not immediately expressed, stop the procedure and return fish to fresh system water. Eggs can then be collected without anesthesia as described in the "block duct" section below.
- After egg collection the anesthetized fish is immediately transferred to a container with fresh system water and monitored continuously until the ability to right itself is regained. Fish should regain swimming ability within 5 minutes.

**Procedure for fish with blocked ducts:**

- If females have blocked ducts, eggs can be expressed without subsequent fertilization to relieve this condition. This procedure can be done without anesthesia, minimizing the possibility of over-exposure to anesthetic and resulting complications. To express eggs without anesthetic, females are picked up with the net, and positioned on their side while holding the fish in the net. In order to release the eggs, the fish is very gently stroked on the belly in the direction of head to tail with a finger wet with system water. Eggs should be immediately expressed, and the fish is returned to fresh water. This procedure should take less than one minute. If eggs are not immediately expressed, the procedure is terminated and fish are returned to fresh water. The fish should not give any indication of distress such as trouble swimming upon return to fresh water.

- After egg collection the females are immediately transferred to a container with fresh system water and monitored continuously until the ability to right itself is regained. Fish should regain swimming ability within 5 minutes.

### **Sperm Collection:**

Sperm collection **must** be done on anesthetized animals.

### **Procedure:**

- Fish are anesthetized as described above by immersion in 0.02%-0.04% MS-222 (Tricaine) at neutral pH until gill movement is slowed. Fish are then transferred immediately into a foam holder in order to stabilize the animal. The area around the cloaca of the fish should be dried with a kim-wipe to prevent activation of the sperm by water. In order to express the sperm, very gently stroke the sides of the fish in the urogenital region using flat forceps or between the thumb and forefinger. Sperm is collected in a capillary tube placed at the urogenital opening as they are expelled using gentle suction. This procedure should take less than 2 minutes. If sperm are not immediately expressed, the procedure is terminated and fish are returned to fresh system water.
- After sperm collection the anesthetized fish is immediately transferred to a container with fresh system water and monitored continuously until the ability to right itself is regained. After immersion in fresh water, fish should regain swimming ability within 5 minutes.

### **IACUC Protocol:**

- **Procedures:**

The maximum number of collections that an individual animal will undergo and the minimum interval between repeat collections in the same animal should be described in the "Procedures" section of the protocol.

- **Agents:**

This procedure requires anesthetic MS-222 (Tricaine) and sodium bicarbonate. All agents administered to animals should be listed in the "Agents" section of the IACUC protocol.

- **Adverse Effects:**

Adverse effects should be listed in the "Adverse Effects" section of the IACUC protocol. Examples include: injury due to handling, failure to exhibit normal swimming movements or eating behavior, over-exposure to anesthetic agents.