

A Semen Extender for the Short-Term Storage of Fish Sperm¹

Frank A. Chapman²

Introduction

Artificial or induced spawning of fish is an assisted reproductive technique used worldwide in aquaculture to maximize egg and larval production from fish that cannot normally be bred in captivity. Despite the wide global use of this technique, and much literature published, the success rates of induced spawning are consistently variable.

Based on a comprehensive review of the literature and experience of the author acquired by operating in the field of reproductive sciences since 1981, we have determined that the most overlooked problem is that successful rates of fertilization, hatching, and larval survival are most dependent on high-quality sperm and the surrounding fluid that supports sperm function. In particular, breeding success is affected by difficulties in consistently obtaining good-quality spermations (releases of spermatozoa); poor sperm survival soon after collection and during storage and transport; and the inability to consistently cryopreserve large volumes of semen at one time. Therefore, a successful fish breeding program requiring sperm begins with a source of high-quality semen, and its proper collection, handling, and storage. The objective is to maximize the volume of semen obtained on a per-male basis.

Semen is commonly collected from fish, but the amount obtained varies widely depending on the species of fish, differs from individual to individual within a species, and

could be influenced by a wide variety of environmental factors, such as season. The amount that can be extracted from the same fish will also vary depending on the health of the fish, restraint and handling techniques, and external environmental conditions such as temperature. The amount of semen released at one time can vary from less than a drop (0.05 milliliter or less) in some species to up to several cups (one liter) in others. The number of sperm or “sperm count” per milliliter also varies widely and can range from 0.5×10^6 (million) to 200×10^9 (billion) or more, with the lower density typically representing what may be required as a sufficient number of sperm necessary for obtaining high rates of fertilization. In addition to variable volume, the handling and storage of fish semen is an important consideration. The sperm of most fish are inactive and immotile in the seminal fluid, but will start moving in a forward manner or “swimming” when diluted in water. Once activated, the sperm of most fishes will swim for no more than a couple of minutes, after which they lose their capacity to fertilize. Moreover, the quality of fresh semen from most fishes quickly deteriorates after collection and will last only 1–2 days in the refrigerator (4–5°C), and at most a few hours at room temperature (25°C).

Semen extenders and diluents are water solutions containing mostly salts and sugars that are added to the semen after its collection. They were developed to assist in the handling of semen soon after collection and to temporarily store semen while maintaining sperm viability

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2. Frank A. Chapman, associate professor and Extension aquaculture specialist, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, UF/IFAS Extension, Gainesville, FL 32611.

and fertilizing capacity. The extender helps to prolong the sperm viability from days to weeks after collection. As a diluent it can also serve to increase semen volume when only small amounts are available. Further, it can be used to optimize the number of sperm necessary to fertilize an egg while maintaining a given volume. Raw semen diluted with an extender will prevent inadvertent water activation of the sperm, a common occurrence in the wet environment of a hatchery or in the field.

There are only a few commercial semen extenders for use in animals and none for fish; those developed for fishes are to extend the duration of motility of the sperm. Semen extenders for fish are only described in scientific literature, and thus are not readily available to all fish farmers. Below is a list of selected scientific references that discuss in greater detail aspects of sperm biology and the development of different extenders or diluents. This fact sheet includes a list of reagents used to prepare an extender for fish semen that will greatly facilitate the handling of semen during the artificial spawning of fish. This extender has been used by the author to store raw semen in the refrigerator 2–3 weeks for several species of food and ornamental fishes including catfishes, cichlids, characids, groupers, and sturgeons.

Principle

Sperm Biology

The sperm, short for spermatozoon (singular) or spermatozoa (plural), is the cell of the male that contains the genome and other materials to accomplish fertilization of the egg. Together the sperm and egg provide the genetic information of heritable traits passed from parent to offspring which are needed to build and maintain the animal throughout life. The shape of the sperm is similar to that of a tadpole with head and tail, but the sperm has a much smaller body. The sperm head (typically 5 to 10 microns) encloses a compact nucleus, which contains the chromosomes that carry the genes or genetic information in the form of DNA. The tail or flagellum (whip) is used to propel the sperm forward through water. Millions of sperm are produced in the testis (gonad) and found suspended in a fluid medium called seminal plasma that assists in providing the sperm with nourishment and facilitates their transport to fertilize the egg. The sperm plus the seminal fluid is called semen or milt in fishes. It is characteristically grayish-white or cream colored. While immersed in the seminal fluid, the sperm of most fishes are immotile and believed unable to successfully fertilize the egg. Potassium (K^+) and high osmotic pressure have been shown to prevent sperm motility among the most studied fish

species. Osmotic pressure of the surrounding fluid provides turgidity to the sperm cell to prevent it from swelling or shrinking. Once the semen is diluted with the surrounding water, the sperm are activated and start their swimming.

The sperm swims towards the egg, and both attract each other using chemical signals. Each egg is surrounded by a thick extracellular protective coating called a chorion composed of a carbohydrate- and protein-rich matrix. To gain access to the inside of the egg, the sperm swims through a narrow canal that runs across the thickness of the chorion; this canal is called a micropyle. The sperm then reaches and fuses with the outer surface of the egg-membrane proper. The nucleus containing the male genome is then released inside the egg, eventually combining with the female genome and completing the process of fertilization. Soon after, the formation of the embryo begins, and it hatches out of its shell and continues development.

Semen Extender

The most important objective for a semen extender is to prevent the initiation of sperm activation and motility during the collection, handling, and storage of the sperm. Therefore, the extender should contain salts with high potassium content and other ions like calcium and sodium. The addition of phosphates, carbonates, and bicarbonates may harden the water in the extender and serve as a buffer to neutralize sperm cell waste products while in storage. At the same time they exert high osmotic pressure on the sperm.

The extender solution should also provide nutrients and energy to the sperm, which are essential to maintain their viability during storage. Sugars like glucose or sucrose are used not only to create high osmotic pressure to the solution, but also to provide nourishment to the sperm.

Protocol

Only semen of high quality should be used and stored for induced spawning of fish. Semen should be evaluated soon after collection. Sperm motility typically should be above 80%. It is the author's recommendation that if sperm motility is less than 40–30% or densities are below 0.5×10^6 sperm per milliliter, the semen should be discarded.

The semen extender should be prepared with clean water with few impurities (e.g., through distillation, deionization, or ultrafiltration). Commercially available distilled or deionized water can be used, but always check the source because wide variations exist in their preparation

and dispensing. The safest containers to hold water are polyethylene-based plastics, or plastics no. 1, 2, and 4.

The recipe in Table 1 is for the preparation of 1 liter (1000 milliliters) of solution. The osmolality of the resulting solution will be around 100 mosmol/kg with a pH of 7.3–7.5, even though the osmolality of the extender solution is lower than the plasma of most fishes, which is typically between 181 and 377 mosmol/kg.

The solution must be stored in the refrigerator and used within 3 weeks of preparation or until the sperm fails to initiate motility when diluted again with water.

Use the extender by mixing one part semen with two to three parts extender. The extended semen samples should be shaken several times daily by gently tilting and moving the holding container from side to side. The use of a laboratory rocker will greatly facilitate this task and significantly prolongs the period of semen storage.

Laboratory grade reagents for preparing a one-liter stock solution of a semen extender for fish sperm; distilled water is the diluent.

Bibliography of Supporting Readings

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Table 1 Semen Extender Solution. Semen Extender Solution

Reagent	Quantity, grams (g)
Potassium chloride	0.20
Calcium chloride (anhydrous)	0.05
Magnesium sulfate	0.05
Sodium bicarbonate	0.50
Sodium chloride	1.00
Sodium phosphate, monobasic	0.15
Sodium phosphate, dibasic (anhydrous)	0.15
Glucose	9.00
Or can replace glucose with sucrose	17.20
Dissolve reagents in one liter of water.	