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Quality of Refrigerated Catfish Sperm Declines Because of Bacterial Presence

Artificial spawning is used routinely for study and for genetic improvement of fish. Standard methods include the collection and storage of gametes. Typically eggs are collected from a ripe female, mixed with a sperm suspension, and fertilized by the addition of water or an activating solution. Problems with the collection and viability of gametes limit artificial spawning of the channel catfish, *Ictalurus punctatus*, the most economically important cultured foodfish in the United States. Sperm cannot be stripped from males, and the testis must be removed by dissection. Sperm samples are generally collected and stored in advance to ensure availability when female channel catfish spawn. Catfish sperm samples can be stored at 4°C, although storage seems to be limited by bacterial contamination. We investigated the prevalence of bacteria and its effect on motility of sperm in stored samples. Sperm motility was compared with the occurrence of bacteria during storage at 4°C, and the predominant bacteria found during storage were identified. The study was designed to simulate conditions commonly encountered during the routine storage of fish sperm. This information will assist hatchery managers with appropriate storage of gametes, which is a requisite for the establishment of gene banks for aquaculture or endangered wild stocks.

Healthy 4-year-old channel catfish (2–2.5 kg) were killed and their testes remove. By aseptic technique, adherent tissue was dissected away, and the testes were blotted dry weighed. Three sperm samples were chosen to represent a range of motility values (e.g., 85%, 60%, and 15%) often seen in artificial spawning. Paired samples (1 g) from the testis of each fish were placed into filter-sterilized Hanks' balanced salt solution (HBSS) or nonsterile HBSS prepared with distilled water that had been stored in a plastic carboy for 2 weeks before use. The aged, nonsterile water from the carboy was used because the distilled water used to prepare buffers in fish hatcheries is often stored in a similar manner.

Sperm samples were stored horizontally in 50-mL tissue culture flasks with loosened caps. The refrigerator temperature was maintained at 4°C. Samples were removed aseptically once every 24 h for 10 consecutive days for motility estimates and for bacterial cultures. To estimate the percentage of motile sperm in each sample, a 1-µL aliquot was added to 40 µL of distilled water on a microscope slide, and the mixtures were viewed immediately for 15 s with darkfield microscopy at ×100 magnification. Triplicate readings were taken on each sample.

Bacteria from each sample were cultured at 4°C by spreading 10-µL aliquots onto each of two types of bacteriologic media: tryptic soy agar (TSA; Difco, Detroit, Michigan), a general nutrient medium; and *Pseudomonas* Agar F (PAF; Difco). The PAF medium enhances the production of a fluorescent pigment (fluorescein) that

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differentiates some pseudomonads. Maximal bacterial growth occurred at 5 days, after which identification of morphological and biochemical characteristics of bacteria was made by standard test tube methods with media incubated at 24°C for 24 h. The number of colony-forming units per plate were counted.

DECREASED SPERM QUALITY PARALLELS INCREASED BACTERIAL GROWTH

In the sperm samples stored in nonsterile HBSS, loss of motility was complete within 72 h of storage (Figure). In the samples maintained in sterile HBSS, a decrease in motility was first noted between 48 and 72 h and a complete loss of motility occurred within 10 days (Figure). With either buffer, the decrease in motility was associated with a decrease in the quality of the samples. Initially the samples were consistent liquid suspensions, and sperm that were not motile appeared structurally intact. In as early as 2 days, however, the sperm cells began to clump and became difficult to pipet, aberrant morphologies appeared, and motile bacteria could be observed among the sperm cells. Complete loss of motility was associated with degenerated spermatozoa and a noticeable odor. Loss of motility corresponded to the appearance of bacterial growth on TSA and PAF. Colonies of pseudomonads appeared below the surface of the solid PAF media, indicating a high level of motility.

PSEUDOMONADS THRIVED IN THE SPERM SAMPLES

The predominant bacteria from the cultured sperm samples were members of the genus *Pseudomonas*, representing 70% (7 of 10) of the bacterial isolates, and occurring in 5 of 6 sperm samples. Bacteria (and the number of samples in which the bacteria occurred) that were isolated from the samples in sterile HBSS were *Pseudomonas* spp. (3/10), *P. paucimobilis* (1/10), *P. fluorescens/putida* (1/10), *Pantoea agglomerans* (1/10), *Aeromonas hydrophila* (1/10), and *Klebsiella oxytoca* (1/10). The dominant pseudomonad cultured from each of these sperm samples produced lecithinase, β-hemolysin, and caseinase. Bacteria (and number of samples in which the bacteria occurred) that were isolated from sperm samples stored in nonsterile HBSS were *P. fluorescens/P. putida* (3/10) and *P. fluorescens* (1/10). In all instances, pseudomonads were numerically dominant. *Pseudomonas* was the sole genus observed by day 10.
Additional sperm samples were collected aseptically from three fish and asssayed over 10 days as before for motility and for the presence of *Pseudomonas* (identified biochemically to the genus level). Starting motilities were 80%, 80%, and 5%, and two of these additional sampl (5% and 80%) contained pseudomonads from the time collection. Therefore, *Pseudomonas* spp. were commensal to five of the six catfish sperm samples analyzed. Pseudomonads may derive from the natural environment because they are motile, free-living bacteria ubiquitous in aquatic situations.

USE ASEPTIC METHODS

Unlike abundant data on domesticated farm animals, few data are available on the qualitative assessment of bacterial contamination of sperm from fishes. In our study, morphologic changes of channel catfish spermatozoa and decreased motility were associated with the increased prevalence of bacteria. Growth of microbes can be suppressed, but not stopped, by refrigeration, and microorganisms are not necessarily killed by freezing. To control infectious agents that might be transmissible in sperm, the addition of antibiotics is common in breeding practices with farm animals. There is a need to identify nonspermicidal antibiotics that inhibit the microorganisms in fish sperm.

This is especially important because cryopreservation of sperm has become an accepted technique for selective breeding and genetic improvement in livestock industries.

As cryopreservation techniques are developed for sperm of aquatic species, procedures will be needed to reduce the possibility of transmission of disease agents among hatcheries.

Suggested procedures for sperm collection and refrigerated storage include the aseptic removal of testes, exposure to adequate oxygen, and the use of freshly prepared buffers that are sterile. The osmotic pressure of the buffer must be above 270 mOsm (Tiersch, unpublished observations) to prevent the activation of stored spermatozoa of channel catfish. Stored sperm samples should be checked daily and discarded when infected to reduce the possibility of transferring bacteria to other samples. Training of personnel is suggested for estimating sperm quality before use for successful fertilization. Protocols developed for the aseptic collection and storage of sperm will reduce vertical transmission of microorganisms and improve fertilization success and larval survival in intensive culture in hatcheries.

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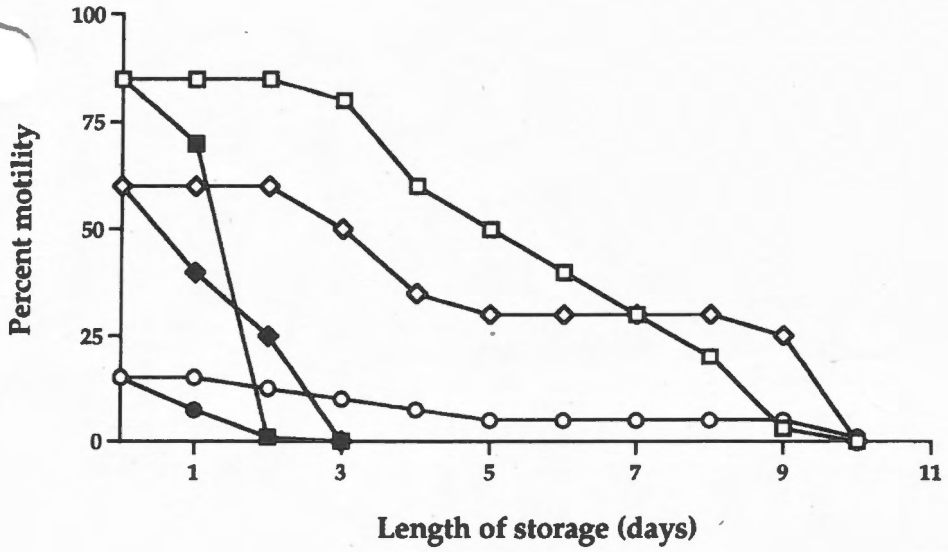

Figure. Percent motility of spermatozoa collected from three channel catfish (*Ictalurus punctatus*) and stored at 4°C in sterile Hanks' balanced salt solution, HBSS (*open symbols*), or in HBSS prepared with aged distilled water (*closed symbols*). The initial motilities were 85%, 60% and 15%.