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The Use of Dairy Protocols for Sperm Cryopreservation of Blue Catfish *Ictalurus furcatus*

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Abstract.—The commercial-scale production of fish by use of artificial (induced) spawning would require reliable, large-volume sources of sperm. Cryopreservation can be used to preserve and store sperm within commercial and research germplasm repositories, but is limited in its application to aquaculture. Straw volume and cooling chamber size restrict the quantity of sperm that can be frozen, and straws must be filled by hand. In contrast, the dairy industry has refined methods for freezing of bull sperm, including automation of straw filling and the use of large cooling chambers. These methods could be used for commercial-scale cryopreservation of fish sperm, although application would require testing. To supply sperm in large volumes, bags originally developed for swine semen could be cooled using dairy protocols and used as a container for fish sperm. The current study documented the use of commercial-scale dairy cryopreservation techniques for the production of hybrids of channel catfish *Ictalurus punctatus* (female) by blue catfish *Ictalurus furcatus*. Four cryoprotectants (methanol, dimethyl sulfoxide, dimethyl acetamide, and glycerol) were initially evaluated for use with blue catfish sperm. During May 2000 and March to April 2001, suspensions of blue catfish sperm were cryopreserved with 10% methanol in 0.5-mL French straws and in commercial swine semen bags (Cochette® bags, IMV International, Minneapolis, Minnesota, USA). Cryopreservation took place at a dairy breeding cooperative, using technology employed for bull semen. Sperm motility before freezing was 26 ± 18% during Year 1 (2000) and 62 ± 30% during 2001. Sperm were thawed at 40 C and used to fertilize the eggs of channel catfish (yielding hybrids). Motility after thawing for sperm frozen in 0.5-mL straws was 11 ± 10% during 2000 and 50 ± 24% during 2001. Motility after thawing was 41 ± 17% for sperm frozen in swine semen bags in 5-mL aliquots and 43 ± 10% for sperm frozen in 10-mL aliquots. Neurulation of eggs fertilized with thawed sperm from straws was 83 ± 13% during 2000 and 54 ± 27% during 2001. Neurulation was 57 ± 24% using sperm frozen in swine semen bags in 5-mL aliquots and 55 ± 10% using sperm frozen in 10-mL aliquots. There was no correlation between sperm motility before freezing (in 0.5-mL straws) and after thawing during 2000 (r = 0.52) or during 2001 (r = 0.49). In addition, there was no correlation between initial motility and neurulation of channel catfish eggs fertilized using thawed sperm during 2000 (r = 0.14) or during 2001 (r = 0.29). Sperm of blue catfish can thus be cryopreserved at a commercial scale using dairy protocols and can be made available for the production of hybrid catfish when viable eggs are available.

Many cultured fish species have a seasonal pattern of reproduction (Bye 1984; Zohar 1989; Redding and Patiño 1993). Manipulation of the environmental factors that influence spawning (such as photoperiod or water temperature) may be required to bring broodfish into reproductive condition outside of their natural spawning season (Bromage 1995). The ability to store sperm is useful for fish cultivation because it minimizes the need to maintain males for artificial spawning (e.g., manual collection of gametes), allowing increased effort and resources to be directed towards females and offspring. Furthermore, sperm storage facilitates genetic improvement of cultured stocks by enabling identification and use of superior males.

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The refrigerated storage (4 C) of fish sperm in extenders (buffers) that suppress motility is useful for short-term retention of motility, although storage time can be limited by factors such as bacterial growth (Jenkins and Tiersch 1997). Another approach is cryopreservation, in which sperm are exposed to chemicals (cryoprotectants) that protect the cells during freezing and thawing before cooling to temperatures as low as −130 C, and plunging into liquid nitrogen (−196 C) for storage. Sperm stored in this way can theoretically be held for hundreds of years (Lueng 1991).

Artificial spawning can benefit culture of channel catfish *Ictalurus punctatus* because it allows genetic improvement of stocks through repeated use of superior males, collection of unfertilized eggs for research, and is the only reliable method for producing the hybrid of channel catfish (female) and blue catfish *Ictalurus furcatus* (Bart et al. 1998). This hybrid is regarded as a superior production animal because of its fast growth rate (Yant et al. 1975), resistance to disease (Dunham and Smitherman 1987), tolerance of low dissolved oxygen (Dunham et al. 1983), and high dress-out percentage (Brooks et al. 1982; Dunham et al. 1982).

Male channel catfish and blue catfish cannot be stripped of sperm, and testes must be removed surgically, usually resulting in death of the male (Bart and Dunham 1990). This limits the time available for evaluation of donor males to 2 wk (Christensen and Tiersch 1996). Methods for the cryopreservation of sperm of channel catfish (Tiersch et al. 1994) and blue catfish (Bart et al. 1998) have been developed for research-scale production of fish. However, artificial spawning is only beginning to be employed in commercial settings, and the channel catfish industry has not begun to exploit the benefits of artificial spawning and cryopreserved sperm.

In artificial spawning of channel catfish, females can be stripped of as many as 4,000 eggs per kg of body weight (Goudie et al. 1992; Dunham et al. 1998). In a previous study, 0.5 mL of sperm suspension (diluted at 1 g of testis per 20 mL of extender) was used to fertilize 200 to 300 channel catfish eggs with > 50% fertilization (Bates and Tiersch 1998). In this case, a 10-mL aliquot of sperm suspension would have been required to fertilize the eggs of one 3-kg female, resulting in 6,000 fertilized eggs. Thus, large volumes of sperm would be required for use in artificial spawning to produce fish on a commercial scale. The volume of fish sperm that can be frozen at one time is currently limited by the size of the containers such as the standard 0.5-mL French straws (used for bulls), and the equipment, such as commercially available computer-controlled freezers with maximum chamber capacities of 250 straws (Conget et al. 1996; Wayman et al. 1996). Furthermore, in fish research, sperm have been added to straws manually by use of aspirators (Bart et al. 1998) or syringes (Wayman and Tiersch 2000). Filling of straws in this way is tedious, subject to human error, and can require 30 min to fill 100 straws. Owing in part to such factors, cryopreservation has not yet found application in aquaculture on a commercial scale.

The use of artificial insemination and frozen semen is an integral part of the modern dairy industry, allowing exploitation of superior sires and the achievement of large-scale genetic evaluation and improvement of stocks (Curry 2000). The cryopreservation technology currently employed has resulted from decades of study, refinement, and integration. Optimal estimates of sperm densities have been defined, methods for cooling of straws have been standardized, and filling and labeling procedures are automated. Furthermore, freezing chambers can cool hundreds of straws simultaneously. Large chambers (dewars) capable of holding thousands of straws are used for storage, allowing breeder cooperatives to serve as germplasm repositories. This refinement and integration of cryopreservation has re-

sulted in an industry that efficiently processes, stores, and tracks large quantities of semen, making improved livestock available for dairy breeders.

The adoption of cryopreservation protocols utilized by the dairy industry could improve the cryopreservation of fish sperm and artificial spawning of channel catfish. Potential variability among straws filled by hand and toxicity of cryoprotectants to sperm would be minimized by use of automated straw-filling equipment that would fill straws quickly and uniformly. Large cooling chambers would allow greater volumes of sperm to be frozen during one event, increasing the efficiency of the operation. Adoption of a pre-existing method of cataloguing and tracking sperm would efficiently facilitate the large-scale evaluation of channel catfish families and strains.

Eggs of channel catfish can be produced in bulk by injecting groups of females with gonadotropic hormones and by monitoring them for readiness to ovulate (Bates and Tiersch 1998; Dunham et al. 1998). Commercial-scale production of blue catfish sperm would provide large quantities of sperm to be used with these eggs, and the production of hybrid catfish could expand on a commercial scale. However, freezing protocols utilized by the dairy industry need to be evaluated for the cryopreservation of blue catfish sperm, and no studies have yet addressed this issue.

The goal of this study was to document the use of dairy technology for the cryopreservation of blue catfish sperm. Because previous studies have indicated differences in the toxicity of cryoprotectants to sperm of various catfishes (Tiersch et al. 1994; Mongkonpunya et al. 1995, 2000; Bart et al. 1998), the effect of four common cryoprotectants used for aquatic species was evaluated for blue catfish sperm. In addition, two storage containers were tested: 0.5-mL French straws, commonly used for the cryopreservation of sperm of bulls and aquatic species, and 110-mL swine semen bags (IMV International Corp., Minneapolis, Minnesota, USA). These bags could be used for storing large volumes but have not been tested previously for fish sperm (A. Mirjyn, IMV International, Inc., personal communication). Freezing in larger volumes would potentially reduce the time between stripping of eggs and fertilization. Our specific objectives were to: 1) evaluate motility of blue catfish sperm exposed to four cryoprotectants; 2) evaluate motility of sperm before freezing and after thawing; 3) evaluate the fertilization of eggs of channel catfish by thawed blue catfish sperm; and 4) evaluate the use of dairy technology to cryopreserve blue catfish sperm in different containers and at different volumes. The use of dairy technology for sperm cryopreservation of fishes, including red snapper *Lutjanus campechanus,* common (koi) carp *Cyprinus carpio,* spotted seatrout *Cynosion nebulosus,* and channel catfish, was previously addressed in a master's thesis (Ropollo 2000). We are unaware of any reports that address the use of dairy technology for the cryopreservation of blue catfish sperm and subsequent production of hybrid catfish.

Materials and Methods

Preparation of Sperm Suspensions

During May 2000, sperm suspensions (prepared at 290–300 mOsmol/kg) from blue catfish of a commercial strain (Gold Kist D & B) (N. Chatakondi, Gold Kist Inc., Inverness, Mississippi, USA, personal communication) were collected and shipped from a commercial broodstock supplier (Gold Kist Inc., currently owned by Harvest Select, Inc., Inverness, Mississippi, USA) by overnight delivery to the Louisiana State University Aquaculture Research Station (ARS). Typically ~24 h elapsed between collection and receipt of the suspensions, and ~48 h elapsed between collection and cryopreservation. During February 2001, additional male blue catfish (640–900 mm total length; 4–9 kg total weight) donated by a commercial producer (Gold Kist

Inc.) were held for 2 to 4 wk in heated all-male ponds. Males were collected from ponds by seine and were held in the laboratory for 2 d. For preparation of sperm suspensions, males were killed by administration of a lethal dose of tricaine methanesulfonate (MS-222; Argent Laboratories, Inc., Redmond, Washington, USA), weighed and measured, and their testes surgically removed. Sperm were collected by crushing the testes in 20 mL of Hanks' balanced salt solution (HBSS), prepared at 290 mOsmol/kg (Bates et al. 1996), for each g of testis and straining the suspension with a 26-μ screen (Christensen and Tiersch 1996). All channel catfish sperm suspensions were collected from a research population maintained at the ARS, were stored at 4 C, and were monitored daily for motility. Sperm were cryopreserved within 4 h of collection.

Evaluation of Acute Toxicity

Acute toxicity of cryoprotectants to blue catfish sperm was evaluated during 2000 only. Cryoprotectant was diluted at a ratio of 1:1 of cryoprotectant:HBSS (prepared at 290–300 mOsmol/kg) prior to addition to sperm samples. Sperm from three males were suspended in HBSS and exposed to various final concentrations of the cryoprotectants dimethyl sulfoxide (DMSO) (5%, 10%, 15%, and 20%), methanol (MeOH) (5%, 10%, 15%, 20%, and 25%), glycerol (5% and 10%), or dimethyl acetamide (DMA) (5%, 10%, and 15%). Motility was evaluated at 15-min intervals for 30 min (0, 15, and 30 min) using darkfield microscopy at 200-× magnification.

Cryopreservation and Thawing of Sperm

Sperm of blue catfish were cryopreserved using 10% MeOH by use of commercial protocols developed for the cryopreservation of bull sperm (T. E. Patrick Dairy Improvement Center, Baton Rouge, Louisiana, USA). For samples used during 2000, the entire sperm volume received was cryopreserved (20–100 mL; 40–200 0.5-mL straws). Sperm from each of four males were frozen during each freezing trial. For samples used during 2001, from 100 to 150 straws per male were frozen (200–300 mL per male; ~2.5 L total). Sperm suspensions were placed in 0.5-mL French straws using an automated straw-filler (model MRS 1, IMV International Corp., Minneapolis, Minnesota, USA).

For cryopreservation of sperm suspensions in 110-mL swine semen bags (Cochette® bags, IMV International Corp., Minneapolis, Minnesota, USA), five bags were frozen per male and per aliquot volume. Sperm of 12 males were frozen in 5-mL or 10-mL aliquots (500 mL total). From 3 to 5 min was required to fill each batch of 100 straws, and 5 min was required to add the 5-mL or 10-mL aliquots to the swine semen bags by hand. The filling of each container type was performed in a walk-in cooler at 5 C. Within 30 min, the samples were placed horizontally in a commercial-scale freezing chamber that was cooled at a rate of 16 C per min to −140 C. Once cooled to −140 C, the samples were plunged into liquid nitrogen (−196 C) for storage. Twenty minutes was required to fill and cool the straws of four males (~300 mL sperm solution). Using the available equipment, a maximum of 900 mL of blue catfish sperm solution could be processed in 60 min.

Sperm were evaluated for motility immediately after thawing and during each fertilization trial to collect data for broodstock quality assessment. Sperm in swine semen bags were evaluated for motility only during fertilization trials. The duration of time between sperm cryopreservation and thawing for use in fertilization ranged from 1 to 3 wk. Straws were thawed in a water bath (40 C) for 7 sec (Tiersch et al. 1994). Swine semen bags containing 5 mL of sperm were thawed at 40 C for 10 sec; swine semen bags containing 10 mL were thawed at 40 C for 12 sec. Straws and swine semen bags were dried before opening to avoid contamination of samples with water.

Collection, Fertilization, and Evaluation of Eggs

During 2000, female channel catfish used in fertilization trials for thawed blue catfish sperm were obtained from ponds containing female and male channel catfish (Lang 2001). The females were of a research population maintained at the ARS. To obtain unfertilized eggs, females were injected with 100 μg of leuteinizing hormone-releasing hormone analogue (LH-RHa) per kg of body weight, paired with channel catfish males in 120-L fiberglass tanks equipped with plastic viewing windows (Bates and Tiersch 1998), and monitored for spawning activity. When a female had released ~100 mL eggs, she was removed, and remaining unreleased eggs were stripped into greased bowls (Dow Corning, Midland, Michigan, USA) (Goudie et al. 1992) containing HBSS (290 mOsmol/kg).

During 2001, females used in fertilization trials with thawed blue catfish sperm were obtained from ponds containing only female broodstock of a commercial strain (Gold Kist D; Gold Kist Inc.). Ponds were heated during March and April to induce early out-of-season spawning of channel catfish females (Lang 2001). To obtain unfertilized eggs, females were injected with 100 μg of LH-RHa per kg of body weight and either paired in tanks with males (as described above) or held in 1000-L tanks within a recirculating system, as groups of eight females (Bates and Tiersch 1998). The females were monitored for spawning activity and stripped of eggs when readiness of ovulation was evident. This was indicated by the release of eggs upon netting of the fish and application of gentle abdominal pressure.

Thawed blue catfish sperm suspensions (1 mL) were mixed with aliquots (~100 eggs) of freshly stripped channel catfish eggs to assess the fertilizing capacity of the sperm. Aliquots of eggs from the same females were mixed with fresh channel catfish sperm to assess egg quality. The percentage of embryos to reach neurulation (~27 h) was used as a conservative index of fertilization (Withler 1980; Tiersch et al. 1994). During 2000, one aliquot of eggs from each female was fertilized using thawed blue catfish sperm or fresh channel catfish sperm. During 2001, three aliquots of eggs from each female were fertilized using thawed blue catfish sperm, and neurulation for the aliquots was reported for each male when crossed with each female. One aliquot of eggs per female was fertilized using fresh channel catfish sperm as a control.

Experimental Design

On 11 May 2000, sperm of four blue catfish males were cryopreserved in 0.5-mL French straws. Sperm from each male were evaluated for motility and used to fertilize eggs of five females. On 15 May 2000, sperm from four additional males were frozen in the same manner and used to fertilize the eggs of two females. From 18 March 2001, to 15 May 2001, sperm of 12 males were frozen in 0.5-mL French straws, and in swine semen bags in 5-mL aliquots. Additionally, sperm of four of the 12 males were cryopreserved in the swine semen bags in 10-mL aliquots. On 26 April 2001, sperm of eight of the 12 males (frozen in straws, or in 5-mL aliquots in swine semen bags) were used to fertilize the eggs of two females. On 3 May 2001, sperm of the remaining four males (frozen in straws or in 5-mL or 10-mL aliquots in swine semen bags) were used to fertilize the eggs of two females.

Statistical Analysis

All percentage data were arcsine-root transformed prior to statistical analysis. The general linear model procedure of the SAS system (Statistical Analysis Software system version 8 for Windows®; SAS Institute Inc., Cary, North Carolina, USA) was used to detect differences in mean motility of sperm samples exposed to selected concentrations of cryoprotectants for 30 min. To

TABLE 1. *Toxicity of various concentrations of the cryoprotectants dimethyl acetamide (DMA), dimethyl sulfoxide (DMSO), and methanol (MeOH) to sperm of blue catfish. Only concentrations with ≥20% mean motility at 30 min are shown. Motility (%) was assessed at 15-min intervals for 30 min.*

Cryoprotectant concentration	Motility (mean % ± SD)		
	0 min	15 min	30 min
5% DMA	50 ± 0	43 ± 18	23 ± 15
5% DMSO	43 ± 5	33 ± 8	22 ± 3
5% MeOH	50 ± 0	43 ± 10	38 ± 3
10% MeOH	43 ± 13	40 ± 10	37 ± 18
15% MeOH	40 ± 13	27 ± 15	25 ± 19
Undiluted (control)	50 ± 0	50 ± 0	50 ± 0

indicate the time interval at which motility was significantly reduced, Tukey's studentized range test was used to make pairwise comparisons.

The SAS system was used to test for significant correlations between initial motility and thawed motility of sperm frozen in 0.5-mL straws and between initial sperm motility and neurulation of channel catfish eggs. Because different males were used, tests were performed separately for each year.

Results

Acute Toxicity

Within 30 min, sperm motility was reduced to below 5% in samples diluted with 20% and 25% concentrations of MeOH and DMSO. After 30 min, motility in samples diluted with 5% of cryoprotectant ranged from 38 ± 5% (mean ± SD) for MeOH to 5 ± 0% for glycerol, while motility of sperm without cryoprotectant was 50 ± 0% (Table 1). After 30 min of exposure to cryoprotectants, mean motility was highest in samples containing 5%, 10%, and 15% MeOH, in comparison to other cryoprotectant concentrations. Further analysis indicated no significant decrease in motility between any of the three sampling points in samples diluted with 5% MeOH ($P = 0.44$) or in samples diluted with 10% MeOH ($P = 0.36$). Based on these results 10% MeOH was chosen for use in subsequent cryopreservation trials.

Cryopreservation and Neurulation

Motility of blue catfish sperm before freezing was 26 ± 18% during 2000 ($N = 8$ males) and 62 ± 30% during 2001 ($N = 12$ males), and after thawing was 11 ± 10% during 2000 and 50 ± 24% during 2001 (Table 2). Motility of fresh channel catfish sperm (control) was 65 ± 7% during 2000 ($N = 4$ males) and 40 ± 0% during 2001 ($N = 2$ males). There was no correlation between sperm motility before freezing and after thawing during 2000 ($r = 0.52$) (Fig. 1) and during 2001 ($r = 0.49$) (Fig. 2). Neurulation for eggs fertilized with thawed sperm was 83 ± 13% during 2000 and 54 ± 27% during 2001. There was no correlation between initial motility and neurulation in channel catfish eggs fertilized using thawed sperm during 2000 ($r = 0.14$) or during 2001 ($r = 0.29$). Motility after thawing was 41 ± 17% for sperm frozen in

TABLE 2. *During 2000 and 2001, blue catfish sperm diluted with 10% methanol were frozen in 0.5-mL French straws or in 5-mL or 10-mL aliquots in swine semen bags. Sperm were evaluated for percent motility upon receipt or collection at Louisiana State University, and after thawing. Thawed sperm were used to fertilize channel catfish eggs. Percent neurulation (mean ± SD) was evaluated at 27 h. Control neurulation refers to eggs fertilized with fresh channel catfish sperm.*

Year	Container type	Number of		Initial motility	Post-thaw motility	Percent neurulation	Control neurulation
		Males	Females				
2000	French straws	8	7	26 ± 18	11 ± 10	83 ± 13	85 ± 17
2001	French straws	12	4	62 ± 30	50 ± 24	54 ± 27	70 ± 16
2001	Swine bag (5-mL)	12	4	62 ± 30	41 ± 17	57 ± 24	76 ± 12
2001	Swine bag (10-mL)	4	2	70 ± 18	43 ± 10	55 ± 10	80 ± 14

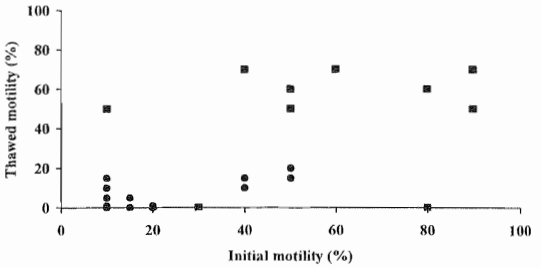

FIGURE 1. *Motility after thawing of blue catfish sperm cryopreserved with 10% methanol versus initial sperm motility for the years 2000 (represented by circles) and 2001 (squares). Regression analysis indicated no correlation between post-thaw motility and initial motility (2000, r = 0.52; 2001, r = 0.49).*

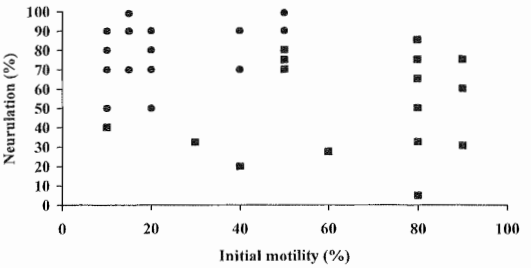

FIGURE 2. *Neurulation of channel catfish eggs fertilized with thawed blue catfish sperm cryopreserved with 10% methanol versus initial sperm motility for 2000 (represented by circles) and 2001 (squares). Regression analysis indicated no correlation between neurulation and initial motility (2000, r = 0.14; 2001, r = 0.29).*

swine semen bags in 5-mL aliquots and 43 ± 10% for sperm frozen in 10-mL aliquots. Neurulation was 57 ± 24% for eggs fertilized with thawed sperm frozen in 5-mL aliquots and 55 ± 10% for eggs fertilized with sperm frozen in 10-mL aliquots.

Discussion

This study documents the first application of dairy cryopreservation technology for production of the hybrid of channel catfish by blue catfish. Large volumes of sperm (approaching 1 L of extended samples) were cryopreserved in a small amount of time (60 min) and were made available for use as needed or for shipping. This study also demonstrated that swine semen bags were effective containers for cyopreservation of 5-mL and 10-mL aliquots of sperm. Future studies should evaluate freezing larger volumes in the swine semen bags to increase the usefulness for artificial spawning and commercial-scale production of hybrid catfish. Additionally, techniques for collecting eggs in bulk should be refined to maximize egg production in all-female production systems that utilize frozen sperm.

Methanol was practical and effective for cryopreservation of sperm of ictalurid catfishes in this and prior studies, because it allowed the greatest amount of time for equilibration of sperm without substantial reduction of motility and typically produced satisfactory (> 50%) rates of neurulation in channel catfish eggs fertilized with thawed sperm. In a prior study of channel catfish sperm cryopreservation, the use of 5% and 10% MeOH as a cryoprotectant resulted in the highest percentage of motile sperm (from 5% to 10%) after thawing (Tiersch et al. 1994). Neurulation was 72 ± 12% for eggs fertilized using thawed sperm with 5% MeOH and 82 ± 13% for fresh sperm. In another study, motility of thawed channel catfish sperm that were frozen with 5% MeOH using dairy methods was 6 ± 3% and was 3 ± 2% using a computer-controlled freezer (Ropollo 2000). Neurulation of channel catfish eggs fertilized using thawed sperm ranged from 70% to 79% for the dairy method and from 21% to 71% for the computer-controlled freezer. In another study, however, motility of blue catfish sperm diluted with 5% methanol and 15% skim milk was less than 10% and resulted in no fertilized eggs (Bart et al. 1998).

The optimal numbers of sperm used for fertilization is an important consideration in the commercial use of cryopreserved catfish sperm, because collection involves the destruction of the testes and usually the male (Steyn and Van Vuren 1987; Tiersch et al. 1994). Proper dilution of sperm will maximize the usefulness of individual males and will determine how many fish can be processed per hour using dairy cen-

ter protocols. In the present study, sperm concentrations were not measured during 2000, although samples with low motility (~1%) resulted in fertilization of eggs, which agrees with other studies (Tiersch et al. 1994; Ropollo 2000). During 2001, sperm concentrations ranged from 1.8×10^6 to 2.9×10^6 sperm per egg (1.8×10^8 to 2.9×10^8 sperm per mL) and yielded neurulation rates of 5% to 75%. However, these numbers of sperm may have been excessive. In prior studies, concentrations of 2×10^3 to 2×10^4 motile channel catfish sperm per egg ($\sim 5 \times 10^5$ to 2.5×10^6 motile sperm per straw; total straw concentration of $\sim 5 \times 10^7$) yielded 72 ± 19% neurulation in 200-mL to 300-mL aliquots of channel catfish eggs (Tiersch et al. 1994). To maximize the efficiency of sperm processing and the return from investments by producers, studies should seek to further optimize sperm concentrations for fertilization of eggs. At least initially, until standardized techniques become available, these studies should also address the use of sperm of minimal quality.

Motility is the most-used estimator of sperm quality, and in general, motile sperm are required to fertilize eggs (Billiard et al. 1995). However, motility of thawed sperm is often influenced by damage occurring during cryopreservation. In channel catfish, the response of sperm to the stress caused by cryopreservation varied from male to male (Christensen 1994). During the present study, there was no direct relationship between initial sperm motility and thawed motility or between initial sperm motility and neurulation. Therefore, this variation may also exist among blue catfish males. Cryoprotectants can be toxic to fish sperm, and prolonged exposure to cryoprotectants can reduce motility before freezing and after thawing, as demonstrated in the present study and previous studies (Tiersch et al. 1994). Improper rates of cooling and thawing can also cause injury to sperm (Lueng 1991; Denniston et al. 2000; Leibo 2000). Large ice crystals can form during cryopreservation. When cooling is too rapid, these crystals can form internally and damage the sperm cells. Alternatively, when cooling is too slow, excessive water can be removed from cells, and changes in intracellular conditions such as pH can cause the denaturation of proteins and loss of buffering capability. Upon thawing and addition to water (for fertilization), sperm cells can undergo rapid changes in volume as equilibrium with the surrounding medium is reestablished, and during this time lysing of cells can occur (Lueng 1991; Leibo 2000).

Beyond the effects of cryopreservation on sperm viability, several factors have also been suggested to influence the quality of eggs, including broodstock nutrition, husbandry conditions (e.g. water quality), time of ovulation, and the genetic composition of the broodstock (Billiard et al. 1995; Donaldson et al. 2000). Any of these factors could decrease egg quality, resulting in reduced rates of fertilization despite high sperm quality. Given the variability possible in quality of sperm and eggs, initial sperm quality can be a poor predictor of post-thaw motility and fertilizing ability. Thus, although motility is the most practical means for assessing sperm quality, care should be taken to ensure that each step involved in artificial spawning maximizes the quality of gametes.

Another important consideration for the use of dairy technology in the commercial-scale utilization of catfish sperm is the shipping of sperm samples to a cryopreservation facility. Care must be taken when shipping to prevent free movement of the samples within the container, to avoid direct contact of samples with cooling gels, and to ensure that samples do not inadvertently freeze during shipping (Tiersch 2000). Practical methods should also be employed for the maximization of sperm quality and biosecurity in refrigerated catfish sperm. These include the aseptic removal of testes, exposure of sperm to adequate oxygen, and the addition of antibiotics or methanol prior

to refrigerated storage (Tiersch et al. 1994; Christensen and Tiersch 1996).

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