Sperm characteristics and motility in *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Pangasius djambal* Bleeker, 1846 (Pangasiidae, Siluriformes)

by

Marc LEGENDRE (1), Jacky COSSON (2) & Jojo SUBAGJA (3)

ABSTRACT. - A drop in osmotic pressure appeared as the main trigger for initiating spermatozoa motility in the two catfish species studied. Motility duration was particularly short in both species (20 to 30 s). Sperm preserved at 4°C in a saline medium (NaCl 155-175 mM, pH 7.5-8.5) survived longer than in any of the other media tested.

Key words. - Sperm - Pangasiidae - Catfishes - Aquaculture - Asia.

Introduction

Although Pangasiid catfishes are now produced at a large scale in S.E. Asia (more than 1,000,000 tons/year), accurate knowledge on their sperm physiology is still needed for defining optimized methods of artificial fertilization and gamete management in hatcheries. This study presents data on sperm characteristics and motility in *Pangasianodon hypophthalmus* and *Pangasius djambal*, two catfish species of great economic importance in Indonesia and other Asian countries.

Methods

Males of *P. hypophthalmus* and *P. djambal* were injected with 0.3 mL kg^{-1} Ovaprim in order to increase the volume of milt collected by stripping 20-24 h later (Cacot *et al*., 2003; Kwantong and Bart, 2003). Sperm was stored in tubes at 4°C, either fresh or diluted (1: 5) in an immobilizing solution (NaCl 155-200 mM). Sperm characteristics were assessed in 2-18 males, depending on species and observation. The variations of the percentage of motile cells, flagellar beat frequency and sperm head velocity were assessed versus time after motility initiation, using still video images recorded through a microscope equipped with a dark field condenser, a stroboscopic illuminator and a video camera. A double dilution procedure was applied: first a 1:50 dilution in an immobilizing medium allowing the dispersion of spermatozoa, then a 1:100 dilution in a drop of swimming medium (distilled or tap water) set on a microscope slide. This resulted in the immediate, synchronous and full activation of spermatozoa. The same procedure was used to assess the percentage of motile cells when using NaCl or sucrose solutions of different osmotic pressures as immobilization or swimming media.

Results and discussion

Effects of sperm collection and storing conditions

Osmolality of seminal fluid from stripped sperm was lower and more variable than that from intratesticular sperm. This indicated a possible pollution of sperm by urine during stripping that may have activated spermatozoa at collection. Hence, in both species, potentiality for movement of sperm was much shorter for semen collected and stored undiluted than for semen collected directly and stored in an immobilizing solution (290-350 mOsm kg^{-1}, dilution 1:5).

Spermatozoa motility parameters

After activation in fresh water, the motility parameters followed similar patterns in both species, with a rapid, sigmoid-shaped decrease for the flagellar beat frequency and percentage of motile cells, and a continuous and rapid decrease of spermatozoa velocity. The total duration of motility (forward movement) was very short in both species, ≤ 20 s in *P. hypophthalmus* and ≤ 30 s in *P. djambal*.

Effects of osmolality of activation and immobilization media

Swimming media (either NaCl or sucrose solutions) of osmolality *circa* 120 mOsm kg^{-1} led to high percentages of motile spermatozoa (90-100 %) and, in comparison to freshwater, reduced damages to flagellum resulting from osmotic shock. In both species, sperm storage in immobilizing (saline) solution of osmolalities slightly higher than that of seminal fluid allowed a longer period of potential spermatozoa motility.

Conclusion

A drop in osmotic pressure appeared as the main trigger for initiating spermatozoa motility, the duration of which was particularly short in both species (20 to 30 s). Sperm preserved at 4°C in a saline medium (NaCl 155-175 mM, pH 7.5-8.5) survived longer than in any of the other media tested here. However, even in these conditions, potentiality for movement of sperm of *P. hypophthalmus* and *P. djambal* could not be preserved for more than 1 and 2 days, respectively.

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(1) IRD, UR 175, GAMET, BP 5095, 34196 Montpellier CEDEX 05, France. [Marc.Legendre@ird.fr]
(2) UMR7009 CNRS/UPMC, Quai de la Darse, 06234 Villefranche-sur-Mer CEDEX, France.
(3) Pusat Riset Perikanan Budidaya, Pasar Minggu, Jakarta, Indonesia.

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