Paper II

Motility characteristics of spermatozoa in cod (*Gadus morhua*) and hake (*Merluccius merluccius*)

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Motility characteristics of spermatozoa in cod (*Gadus morhua*) and hake (*Merluccius merluccius*)

by

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ABSTRACT. - As in many marine fish, motility is triggered by osmotic pressure (OP) in cod and hake spermatozoa and motility ranges several minutes with a high velocity (above 50 μ m/s) period restricted to 100 s. A decrease in the flagellar beat frequency (BF) is partly responsible of this briefness. Detailed observations of the flagella during the motility period showed: 1) sea water provokes OP damages (blebs) which impair the correct wave propagation 2) waves become restricted to the proximal flagellum and tip becomes devoid of wave 3) the wave amplitude decreases. The combination of these factors drastically limits to the earliest period of motility the ability for spermatozoa to efficiently progress towards egg for fertilization.

Key words. - Cod - Hake - Spermatozoa - Sperm motility - Osmotic pressure.

Introduction

Very little is known about activation conditions and motility characteristics of spermatozoa in cod and hake, two species of large fishery and/or aquaculture interests. Optimal conditions for flagellar activation or inactivation were defined; high resolution video microscopy and stroboscopy allowed evaluation of % age of active spermatozoa, beat frequency (BF) or linearity of the sperm tracks.

Methods

Sperm samples were collected by stripping ripe reared cods and wild ripen hakes. Video microscopy and stroboscopy allowed to record spermatozoa at high resolution right after their activation by sea water (SW) and during the motility, lasting several minutes. Images were collected by a video camera and a tape recorder then digitalized (Cosson *et al.*, 1997). Successive images were cumulated for evaluation of velocity of head traces, % age of motility or linearity of tracks. At high magnification, flagellar pattern, BF, wave amplitude, wave length or curvature were evaluated on stroboscopic images.

Results and discussion

Best diluent preventing motility but saving potent motility was SW:DW (1:2) for cod and (1:4) for hake. In cod, motility occurs only above 700 up to 1550 mOsm/kg; by dilution of seminal fluid (SF), motility occurs above a SF:SW of 1:4. BSA 0.1% prevents sticking to glass.

In both species, 80 to 95% spermatozoa are fully activated by SW. The flagellar pattern changes rapidly and traces of head decrease in length due to velocity decrease (initially 130 μ m/s for cod and hake). The swimming period shows a decrease of the % age of swimming cells (initially 95% in cod) down to zero and a decrease in BF (initially 52 Hz for cod, 57 Hz for hake). Immotility occurs at around 8 min for cod and 2.5 min for hake. Flagellar waves decrease in amplitude and number. The diameter of tracks varies from 150 to 50 μ m in cod sperm but from 480 to 40 μ m in hake leading to tight circling of sperm, which restricts their efficient progressiveness.

Motility evolves rapidly from a first period right after contact with SW to a second period where a decrease of all motility parameters appears then to a third period where full immotility is reached. The initial progressive period with high BF and waves progressing all along the flagellum is followed by a BF decreases with waves restricted to the proximal flagellum resulting in velocity decrease. Spermatozoa describe tighter circular tracks, which maintain them locally. Blebs and other abnormalities due to SW osmolality (higher than SF) lead to damages to the flagella and restrict their swimming abilities, which reduce the chances for spermatozoa to fertilize egg.

Conclusions

As already described in other marine fishes (Cosson, 2004), motility is triggered in cod and hake spermatozoa by an OP increase from SF to SW and the motility period is limited to minutes range. The efficient progressive period (velocity above $50 \mu m/s$) is restricted to about 100 sec in cod and hake with decrease in BF and restriction of efficient waves to proximal flagellum. Wave amplitude decrease and abnormalities along the flagella also impair the swimming performances. These factors drastically limit the ability for spermatozoa to efficiently progress towards any egg. Motility features constitute quality factors allowing to better define optimal samples and conditions for artificial propagation of these 2 species.

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