Paper I

Biological characteristics of European hake (*Merluccius merluccius*) sperm

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Biological characteristics of European hake (*Merluccius merluccius*) sperm

by

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ABSTRACT. - Very little is known on European hake's reproductive biology and especially on biological characteristics of its sperm despite a growing interest in its aquaculture potential. This study reports, for the first time some hake sperm characteristics. After activation, the swimming phase lasts 3 min (8 min when activated with 50% sea water (SW) but lower initial velocity). The initial flagellar beat frequency (BF), velocity and percentage of motile cells decreased after 100 s. After 2 days at 4°C, the mean Adenylate Energy Charge (AEC) level was 0.71. Sperm stored at 4°C still showed motility after 9 days for 2 individuals. When cryopreserved, the motility recovery index of thawed spermatozoa ranged from 11.8% to 29.6%.

Key words. - *Merluccius merluccius* - Sperm Density - Sperm motility - Gamete quality - AEC.

Introduction

European hake has a very important economical value in Europe and its total annual landings have declined considerably since the 1960s. Knowledge of its reproductive biology is required for fisheries management but also for aquaculture diversification. This study reports on several sperm characteristics of *M. merluccius*.

Methods

Sperm were collected from 23 mature males (737 ± 665 g and 44.8 ± 13.0 cm; mean ± SD) caught during winter in the Bay of Biscay (France). Sperm features were assessed in replicate after 48 h at 4°C (time to reach the laboratories). Sperm density was estimated by counting cells in a Malassez chamber. Sperm motility was assessed using a two-step dilution procedure (Fauvel *et al*., 1998). Changes in velocity, flagellar BF and percentage of motile cells with time were recorded using video microscopy combined to stroboscopy. Storage capacities of sperm samples kept at 4°C or cryopreserved, using Modified Mounib extender and 10% DMSO (Mounib et al., 1968), were recorded. Nucleotides were measured using HPLC to calculate AEC = (ATP + ADP / 2) / (ATP + ADP + AMP) for characterizing spermatozoa energy content (Zietara *et al*., 2004).

Results and discussion

The mean sperm volume of collected samples was 3 ± 5 ml and the mean concentration was $8.6 \pm 3.0.10^9$ spermatozoa.ml^{-1}. When adding SW, activation occurred synchronously for all spermatozoa. The total swimming period lasted 3 min. After 100 s the initial flagellar BF decreased from 57 Hz to 30 Hz, the velocity from 130 μm/s to 70 μm/s and the initial percentage of motile cells from 90/70% to 50%. Hake sperm activated with 50% SW showed a lower initial velocity but a longer swimming period (8 min). After 4 days at 4°C, sperm motility was very variable (from 0 to 80% of cells motile); after 9 days, 2 individuals still showed 50 and 65% spermatozoa motility. When cryopreserved, the motility recovery index of the cells at thawing ([percentage of motile thawed spermatozoa/percentage of motile fresh spermatozoa]x100) ranged 11.8% to 29.6%. After 48 h at 4°C, the mean AEC level was 0.71 with a large variability among individual (0.17 to 0.96). A significant positive correlation was found between body length and volume of stripped sperm ($R^2 = 0.47$, $p < 0.01$).

Conclusion

Some European hake sperm characteristics are reported here. Further investigations are needed in order to improve our knowledge of reproductive biology of this species and to establish gamete management methods.

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