

Endocrine and milt response of Senegalese sole, *Solea senegalensis*, males maintained in captivity

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Abstract

Improving fertilization success in captive Senegalese sole broodstocks has been a challenge in the last years. Recent reports suggest that low sperm volume and quality could be one of the reasons leading to poor fertilization rates, although further studies are needed to reach a conclusive explanation. Here, we report on several experiments focused on this issue. Seasonal profiles of plasma androgen levels (testosterone and 11-ketotestosterone) and sperm production and quality parameters were assessed, although no statistical correlations among them were identified. The response of males to female presence/absence was also analyzed. Long-term isolation from females decreased male androgen levels at the peak of the reproductive period, suggesting some kind of disrupting effects on the endocrine system. On the other hand, short-term exposure of previously isolated males to ripe females decreased androgen levels, possibly reflecting a rapid steroidogenic shift promoting final maturation of spermatozoa, and increased sperm viability, motility and velocity, thus, supporting the concept of positive effects of female contact on male sole performance. Further evidence sustaining the relevant female-to-male communication in sole reproduction was obtained after treating the females with progestagen 17 α ,20 β -dihydroxy-4-pregnen-3-one (regarded as pre-ovulatory pheromone in fish) and registering a significant increase in sperm viability, velocity and motility in surrounding males. Finally, we found that a single administration of a 20 μ g/kg GnRH analogue in males was effective in stimulating androgen release and sperm quality, although the effects were transient and thus, the use of sustained hormone delivery methods were suggested for improving efficiency. Our results point to velocity, viability, and motility as the most sensitive parameters in sole sperm, although further studies will have to evaluate whether these parameters have any relation with fertilization success in captive broodstocks of this important aquaculture species.

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1. Introduction

The finding that several hormonal treatments, which were effective in the induction of Senegalese sole ovulation, were basically ineffective enhancing spermia-

tion, sperm production and fertilization success [1,2], have pointed to the male contribution as an important factor in such reproductive problems in this species [3]. Still, little attention has been paid to the systematic evaluation of sperm quality in *Solea* broodstocks and only recently sperm production and quality have been pointed out as one of the problems compromising the fertilization success in captive broodstocks [3–5]. Nev-

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ertheless, further studies characterizing sperm quality traits in relation to other reproductive parameters (plasma steroid levels, pituitary and circulating gonadotropin levels or inter-sex chemical communication) of male breeders maintained under different experimental conditions are required to reach a conclusive explanation about this issue. Annual profiles of the main androgens involved in fish testicular development (i.e., 11-ketotestosterone [11-KT] and testosterone [T]) and sperm production have been characterized in Senegalese sole broodstocks [3,6,7]. However, no information about their possible relations has been reported to date.

Under natural photo-thermal conditions, Senegalese sole males are able to produce expressible milt during almost all the year, even though ripe females are mainly present during spring and autumn [2,3,6,8]. Although such all-year-round sperm production may indicate a low female contribution to the stimulation of spermiation, the fact that the highest proportions of spermiating males matches the periods of highest female maturation indexes may support the opposite view (as reported for other fish species; [9]). To date, no specific studies have been reported regarding this issue.

Administration of GnRH analogues (GnRH_a) has been successfully used to improve sperm production and quality in several wild-caught captive flatfish species [10–15]. In the case of Senegalese sole, recent studies showed that GnRH_a administration in combination with androgens or dopaminergic inhibitors improves sperm production [16,17], although a systematic evaluation of sperm quality parameters has not been reported at present.

The maturation-inducing steroid (MIS, 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20 β P) and its metabolites behave as pre-ovulatory pheromones in some species, increasing milt production and spermatozoa motility [9]. Whether this phenomenon applies to Senegalese sole as well and has any positive effect on sperm quality has not been investigated, even though it has been suggested that dysfunctions in inter-sex pheromonal communication may be another important cause behind low fertilization success in captive sole broodstocks [18].

According to the afore-mentioned knowledge gaps, the present study aimed to analyze sperm production and multiple milt quality parameters in relation to circulating androgen levels in wild-caught captive Senegalese sole male breeders: 1) throughout the seasonal reproductive cycle to establish reference values and to study possible correlations, 2) in the absence/presence of females to evaluate the possible influence of social inter-sex interactions, and 3) after direct (applied to

males) or indirect (applied only to females in contact with males) hormonal treatments to assess the feasibility of such therapies to improve male performance.

2. Material and methods

2.1. Chemicals

All the chemicals were obtained from Sigma-Aldrich (Madrid, Spain), except the steroid standards, antibodies and tracers used for the steroid immunoassays, which were purchased from Cayman Chemical Company (Michigan, USA). The chemicals were of reagent grade or higher.

2.2. Broodstock management

Senegalese sole breeders were captured from the wild, sexed as described previously [19], PIT-tagged and acclimatised to captivity at the Ramalheite Experimental Station (Faro, Portugal) for at least one year prior to the experiments described hereinafter. Breeders were stocked into 3 m³ indoor round fibre glass tanks with sand substrate, each one containing between 10 and 12 fish with mean weights of 1.2 kg for males and 2.3 kg for females (density < 5 kg/m³). The sex ratio was set at 1 to 1 in the tanks containing fish of both genders, while some tanks contained only males. Water exchange was kept at 0.5 m³/h and aeration was provided. Photoperiod was simulated with a clock system according to environmental conditions in the area (37°00'22"N, 7°58'03"W). Temperature and salinity varied according to external conditions (spring-summer temperature: 20.5 \pm 5.2 °C; autumn-winter temperature: 14.2 \pm 3.1, mean salinity: 36.3 \pm 0.8) except during the sampling periods in experiments 2 and 3 (April) when temperature was artificially maintained at 18 \pm 1 °C to avoid interference with data. Broodstocks were fed on frozen mussels (*Mytilus* sp.) and squid (*Loligo vulgaris*) at a daily ration of 3% biomass.

2.3. Sperm collection and assessment

Individuals were anaesthetized with 300 ppm 2-phenoxyethanol during 10 min before sperm collection. The urogenital pore was dried, sperm was collected with a syringe without a needle by gently pressing the testes on the fish blind side and samples were stored on ice until further analysis (urine contaminated samples were discarded). The sperm volume collected was measured using micropipettes. Males were considered as "spermiating" when at least five μ L of sperm was extracted. Cell concentration and motility were deter-

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