



Changes in gonadal development in sea bass (*Dicentrarchus labrax*) following intramuscular injection of an FSH single-chain- expression plasmid

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The gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are central players in the control of vertebrate reproduction. In fish, the knowledge on their functions is still too scarce to assign differential roles to these hormones. In the specific case of the FSH, information is very limited in fish species other than salmonids. To undertake functional studies of FSH in sea bass we aimed to evaluate the potential of intramuscular injection of an FSH encoding plasmid as means of hormone delivery in vivo. Immature sea bass males were injected with an expression plasmid containing a scFSH coding gene (pscFSH), or with empty plasmid as control (pControl). Plasmid injections effects were monitored by changes in plasma FSH levels. The biological activity of the FSH produced by fish muscle cells was proved by evaluating plasma 11-ketotestosterone (11-Kt) levels and gonadal histology. Expression changes for LH receptor, Anti-Müllerian Hormone (AMH) and synaptonemal complex protein 3 (SCP3) genes, were also evaluated in gonads by real-time PCR. Increments in plasma FSH levels were detected in the pscFSH injected group 15 days after first injection and lasted until day 23. Up to day 15, plasma levels of 11-Kt were significantly higher in pscFSH group respect to controls. Changes in gonad structure were already observed at day 15, when a significant increase in the percentage of mitosis was observed. mRNA levels of the meiotic cell marker SCP3 rose at day 15, in the pscFSH group, reaching a peak 23 days after the first injection. This increase in SCP3 expression was concordant with the histology, as the pscFSH group showed more advanced germ cells at day 23, while the control group remained immature. The raise of circulating FSH was accompanied by an elevation of LHR transcripts at day 15 and 23 and by a decreasing tendency in AMH gene expression. These findings support the putative role of FSH as the initial signal of spermatogenesis in sea bass, mediating steroid synthesis and germ cell proliferation. Additionally we demonstrate the utility of gene delivery for hormone therapy in fish.

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