



FOLLISTATIN IS EXPRESSED ALONG WITH AROMATASE IN FEMALE GONADS DURING SEX DIFFERENTIATION IN THE RAINBOW TROUT

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Introduction:

Follistatin (Fst) is an inhibitor of some members of the TGF β family that was first identified in mammalian ovary. This gene has been shown to be expressed in granulosa cells and oocyte of mammalian and fish species, and is involved in folliculogenesis and oocyte development. In addition to these functions in ovarian development, *fst* is also expressed in the mouse embryonic ovary, and its inactivation induces partial female-to-male sex reversal, suggesting that *fst* plays a significant role in early ovarian differentiation in mammals. However, only little is known about its potential role in early gonadal differentiation in teleost fish.

Methods:

Rainbow trout (*Oncorhynchus mykiss*) is a teleost fish with a strict male heterogametic sex determination system (XX/XY). Genetic all-male (XY) and all-female (XX) experimental populations can be produced in that species, allowing the sex-specific characterization of the early molecular events that occur before histological differentiation of the gonads. We then used the rainbow trout model to investigate the expression pattern of *fst* in embryonic gonads by In Situ Hybridization and qPCR, and to determine its regulation during sex differentiation in fish.

Results and discussion:

Shortly after hatching that occurs around 30 days post-fertilization (30 dpf), *fst* is already expressed in female gonads in some clusters of somatic cells, whereas no expression is detected in male gonads. Later, its expression becomes restricted to some somatic cells

lining the future ovarian lamellae. This expression pattern is very similar to what is observed for the *aromatase* gene (*cyp19a1a*) coding for a key steroidogenic enzyme needed for ovarian differentiation in fish. Given that *cyp19a1a* is the earliest known gene to be expressed in a sexually dimorphic fashion in female gonads of rainbow trout, we compared *fst* and *cyp19a1a* expressions several days before hatching. Both *fst* and *cyp19a1a* expression begins around 26 dpf in somatic cells, in a punctiform pattern throughout the female gonads. Then, around hatching, this expression becomes continuous throughout the gonads. These results suggest that *fst* is implicated along with *cyp19a1a* in the early steps of ovarian differentiation in teleost fish. As *fst* expression is known to be induced by the Wnt/ β -Catenin pathway in mammals, we treated embryos *in vivo* with IWR-1, an inhibitor of the Wnt pathway, and we analysed by qPCR the effects of this treatment on *fst* expression. We found that the IWR-1 treatment decreases *fst* expression in female gonads, suggesting a role of the Wnt/ β -Catenin pathway in regulating expression of *fst* in rainbow trout.

Conclusion:

These results demonstrate that *follistatin* is one of the earliest markers of ovarian differentiation in rainbow trout and its co-expression with *cyp19a1a* suggests that *fst* could be implicated along with *cyp19a1a* in the ovarian differentiation process. Furthermore, expression of *fst* is downregulated by an inhibitor of the Wnt/ β -Catenin pathway, suggesting a wide conservation of *fst* regulation across vertebrates.