Estrogen-inducible yolk precursors: Characterization of the multiple vitellogenin system in European sea bass (Dicentrachus labrax)

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This study explored multiplicity of the estrogen-inducible yolk precursor protein, vitellogenin (Vtg), in the European sea bass (Sbs), a major aquaculture species and leading model in fish endocrinology. Three full-length Sbs *vtg* cDNAs were assembled from partial cDNAs that were cloned and sequenced from total RNA isolated from estradiol-induced male livers. Homology analyses of the deduced polypeptides via BLAST and ClustalW alignments with available Vtg sequences for teleosts from a broad array of taxa identified them as as Sbs VtgAa, VtgAb and VtgC. Analyses of conservation by Vtg-type of the key structural residues, cysteine (C), proline (P) and glycine (G) using Evotrace and their localization in the 3-D polypeptide structures modelled using Cn3D with a lamprey lipovitellin (Lv) template indicated that the N-sheet of the Lv domain of SbsVtgC, which bears the binding surface for the 'classical' Vtg receptor (Vtgr), has undergone massive alteration of its structure relative to the A-type Sbs Vtgs that may explain the limited Vtgr-binding reported for this form of Vtg. The presence and relative concentrations of each form of Sbs Vtg or product YP in postvitellogenic female liver, plasma and ovary were measured by nanoLC-MS/MS as ProteoIQ-normalized spectral counts. VtgAb tryptic peptide spectra were two- to several-fold more abundant than for the other Vtgs, and VtgC spectra were very limited except in ovary where they were a third of VtgAb spectral counts. Western blotting performed using antisera raised against purified grey mullet (*Mugil cephalus*) Lvs revealed limited degradation of all three forms of Lv during oocyte maturation, unlike the case in other marine pelagic spawners where only the LvAa undergoes almost complete proteolysis to free amino acids (FAA), which are important osmotic effectors of oocyte hydration and egg buoyancy. The nearly identical Vtgs and patterns of YP degradation during oocyte maturation in sea bass and *Moronidae* spawning demersal or pelagic eggs in freshwater indicate that Vtg system structure and function cannot be inferred solely from reproductive life history.

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Expression of P450arom mRNA in the ovary and brain of Indian teleosts, *Labeo rohita* and *Anabas testudineus*: seasonal variation and effects of gonadotropin

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The *cyp19* encodes cytochrome P450 aromatase, the key enzyme catalyzing the conversion of androgens to estrogens. Estrogens play a critical role in controlling functional, behavioural and physiological aspects of sexual development both in males and females. Except in *Anguilla anguilla*, most teleosts studied so far possess two *cyp19* genes namely, *cyp19a* and *cyp19b*; ovary- and brain-specific respectively. The present study in two fishes, *Labeo rohita,* a major carp and *Anabas testudineus,* a climbing perch, show a very high expression of ovary-specific *cyp19a* mRNA in the ovary of both the fishes. Partial sequencing of cDNA product of *cyp19a* genes of both the teleosts revealed a high degree of homology with *cyp19a* cDNA sequences of other teleosts. Comparative seasonal analyses of the expression of *cyp19a* mRNA and its protein in these two teleosts showed increased expression of this gene in the ovary of vitellogenic and post-vitellogenic stages fish. HCG induced a profound stimulatory effect on the expression of ovarian *cyp19a* mRNA and its protein in both the fishes. Interestingly, *cyp19b*-specific primers used in semi-quantitative RT-PCR analyses gave ~300bp fragment in the brain of both the fishes. Results showed that *cyp19a* mRNA expression in the ovary was dependent on the seasonal reproductive variation, however it was not so in the brain. Overlapping expression of *cyp19a* and *cyp19b* mRNAs were also detected in ovary and brain of both the teleosts. The physiological and functional significance of the presence of *cyp19a* and *cyp19b* in the ovary and brain of these two fishes has been discussed.