

The Physiological Regulation and Evolution of Male Pregnancy

Prolactin (PRL) is a multifunctional hormone of broad physiological importance, and is involved in many aspects of fish reproduction, including the regulation of live birth (viviparity) and both male and female parental care. Previous research suggests that PRL also plays an important reproductive role in syngnathid fishes (seahorses, pipefish and seadragons), a group with a highly derived reproductive strategy, male pregnancy - how the PRL axis has come to be co-opted for male pregnancy remains unclear. We investigated the molecular evolution and expression of the genes for prolactin and its receptor (PRLR) in an evolutionarily diverse sampling of syngnathid fishes to explore how the co-option of PRL for male pregnancy has impacted its evolution, and to clarify whether the PRL axis is also involved in regulating reproductive function in species with more rudimentary forms of male pregnancy. In contrast to the majority of teleost fishes, all syngnathid fishes tested carry single copies of PRL and PRLR that cluster genetically within the PRL1 and PRLRa lineages of teleosts, respectively. PRL1 gene expression in seahorses and pipefish is restricted to the pituitary, while PRLRa is expressed in all tissues, including the brood pouch of species with both rudimentary and complex brooding structures. Pituitary PRL1 expression remains stable throughout pregnancy, but PRLRa expression is specifically upregulated in the male brood pouch during pregnancy, consistent with the higher affinity of pouch tissues for PRL hormone during embryonic incubation. Finally, immunohistochemistry of brood pouch tissues reveals that both PRL1 protein and PRLRa and Na⁺/K⁺ ATPase-positive cells line the inner pouch epithelium, suggesting that pituitary-derived PRL1 may be involved in brood pouch osmoregulation during pregnancy. Our data provide a unique molecular perspective on the evolution and expression of prolactin and its receptor during male pregnancy, and provide the foundation for further manipulative experiments exploring the role of PRL in this unique form of reproduction.