FISH GONADOTROPIN AGONISTS: APPLICATIONS IN ASSISTED REPRODUCTIVE TECHNOLOGIES

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Introduction:

Over the years, hormonal approaches employing GnRH, freshly ground pituitaries of reproductively mature fish, and human chorionic gonadotropins, have been used to overcome problems halting final stages of gamete maturation and spawning [1]. Nevertheless, the available therapeutic agents fall short of dealing with induction of early stages of gonadal growth (i.e., vitellogenesis and spermatogenesis). As a result, many commercially important fish, exhibiting a complete failure to undergo gametogenesis, are not at hand for aquaculture uses, and their production is based merely on fishery of wild stocks.

The pituitary gonadotropins (GtHs), FSH and LH are the key regulators of ovarian and testicular function. Therefore , much research efforts have focused so far on developing heterologous eukaryotic systems (i.e., insect cells, mammalian cell lines, and yeast) for the production of fish recombinant GtHs [2]. These have circumvented the need to purify the native hormones from thousands of fish, and provided an important tool for studying the differential functions of LH and FSH. Recently, using yeast (*Pichia pastoris*) fermentation, our group at IOLR-NCM scaled-up the production of biologically active fish recombinant GtHs for therapeutic purposes, providing fine-tuning agents for the regulation of fish sexual maturity and reproduction.

Experimentation with fish LH agonists:

LH, also known as the maturation hormone, is the main GtH responsible for stimulating events leading to final oocyte maturation and ovulation in females and spermiation in males. In an effort to establish fish LH agonist for *in vitro* and *in vivo* experimentation, a recombinant LH chimera, based on a translational fusion of the sea bream (*Sparus aurata*) LH beta and alpha subunits [rsbLH], was produced in the *P. pastoris* expression system. The yeast culture has produced over 100 mg rsbLH per liter of culture supernatant, a quantity that is about 1 to 2 orders of magnitude higher when compared to yields reported for cognate systems [2]. The produced rsbLH stimulated steroidogenesis in gonadal fragments derived from sea bream as well as in those derived from other Perciformes, including the European seabass (*Dicentrarchus labrax*), and the grey mullet (*Mugil cephalus*), pointing to its generic nature. Further *in vitro* studies demonstrated that physiological rsbLH levels (50-100 ng ml^{-1}) increased the frequency of sea bream oocytes undergoing germinal vesicle breakdown, and significantly attenuated the occurrence of atresia among the cultured oocytes. As it has been suggested for the mammalian model, the atretic degeneration could be suppressed directly by LH, or indirectly by paracrine factors, from the somatic cells that mediate the LH signals [3]. *In vivo* trials have demonstrated the hormone's ability to induce spawning in fully mature captive grey mullet, replacing our conventional treatment of GnRH analogue combined with dopamine antagonist [4].

Experimentation with fish FSH agonists:

In most vertebrates FSH has a dominant role in the initiation of gametogenesis and regulation of gonadal growth (i.e., spermatogenesis in males and follicle growth in females). Thus far, therapeutic preparations of FSH are available only for the treatment of human infertility. Using the aforementioned yeast expression system, we produced fish recombinant FSH chimera, based on a translational fusion of the Atlantic bluefin tuna (BFT, *Thunnus thynnus*) FSH beta and alpha subunits [rbftFSH]. Despite the relatively low evolutionary conservation of the FSH molecule among the Perciform species [5], the rbftFSH succeeded to stimulate steroidogenesis in gonadal fragments of heterologous fish species such as sea bream, European seabass, and grey mullet. Exposure of testicular fragments of sexually immature BFT to graded doses of rbftFSH resulted in a dose-dependent increase in the diameter of the seminiferous lobules, and in the proliferation of germ/somatic cells, as compared to untreated controls. These results further confirm previous notion that FSH is an important regulator of spermatogonial proliferation [6]. In addition, our *in vivo* experiments indicate that following injection, elevated rbftFSH levels are sustained over 9 hours in the circulation of the grey mullet. Furthermore, two consecutive rbftFSH injections (150 ng rbftFSH per kg in the grey mullet, spaced two weeks apart) at the onset of the reproductive season (i.e. mid July), significantly improve milt production, in terms of volume and fluidity. This protocol is recently utilized in commercial hatcheries in Israel for a scale-up production of mullet fingerling.

Conclusions:

Our work exemplifies the potential of fish GtH agonists, in therapeutic application to alleviate reproductive dysfunctions and expedite the onset of puberty in captive fish. Future studies should focus on optimizing the delivery system for sustained release of GtHs, circumventing the need to use repeated injections, which are labor intensive and stressful to the fish. Thus, industrial production of clinical grade GtHs and development of efficient delivery system(s) for their administration are expected to be the two most relevant improvements to be developed for an intensive aquaculture industry that needs to fill the increasing supply/demand gap for marine fishes.

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