

Enhancing Fish Reproduction through Recombinant Protein Technology

Prachi Asgolkar, Kiran D. Rasal, Jebarson Solomon, Sheetal Vanjre

ICAR-Central Institute of Fisheries Education, Mumbai-400061

Email: prachi.fbtpb204@cife.edu.in

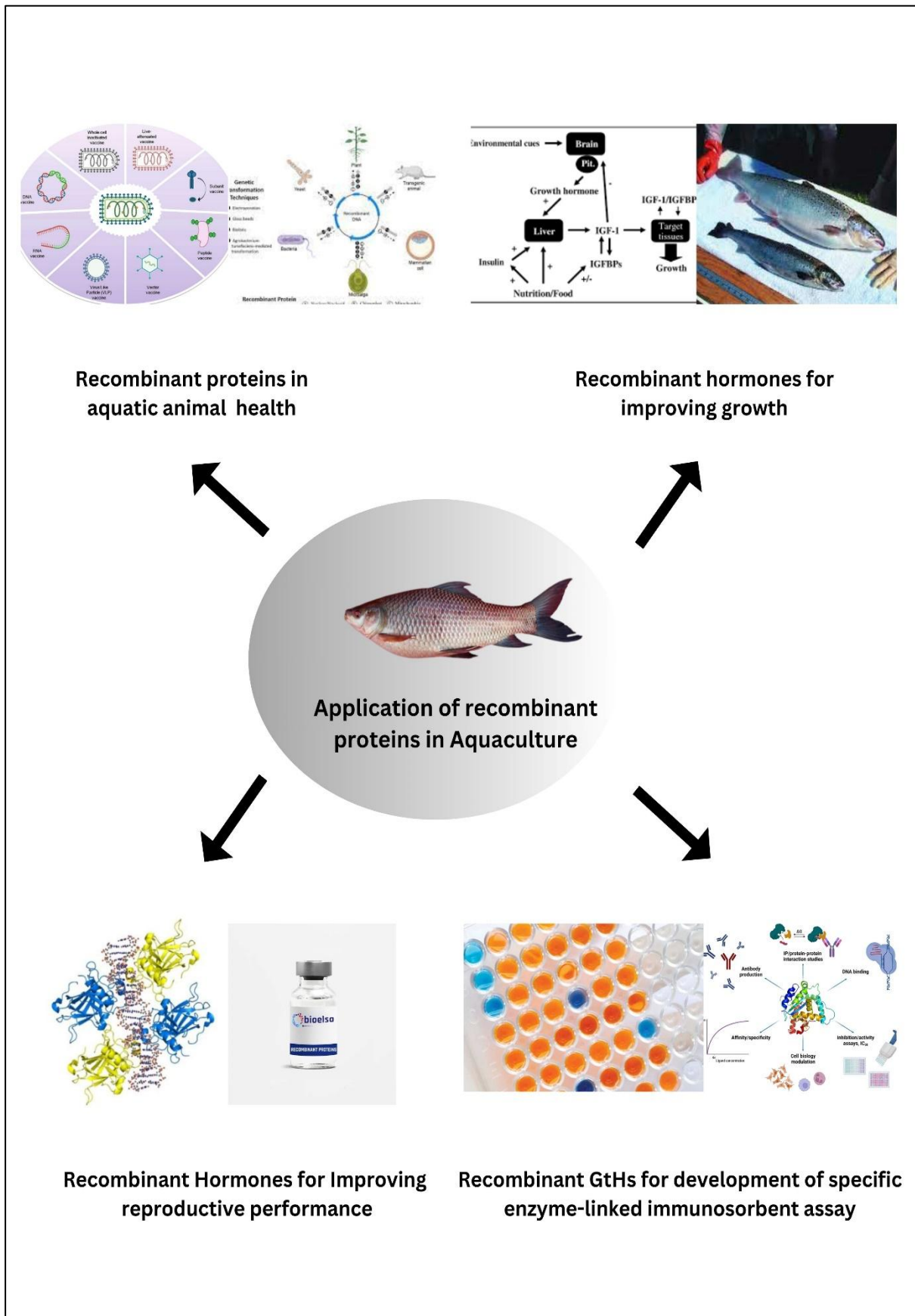
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Abstract

The event of recombinant DNA technology in the 1970s made it possible to produce recombinant proteins. Because of this technology, it is possible to produce the majority of desirable proteins, which were previously isolated from their natural sources using time-consuming procedures that produced poor outcomes (Rosano & Ceccarelli, 2014). However, nowadays, genes can be synthesised and cloned into various cell factories like bacteria, yeast, fungi, and mammalian cells, for large-scale production (Saccardo, Corchero, & Ferrer-Miralles, 2016). Advances in expression systems have enabled large-scale production of essential fish hormones and growth factors, reducing the need for natural sources (Kim, Yoo, & Kang, 2015). The first recombinant protein, somatostatin, was produced using *Escherichia coli* in 1977, followed by recombinant human insulin (Itakura et al., 1977). Commercially available recombinant hormones and enzymes are currently used to enhance reproduction, feed efficiency, and overall health of livestock. These products are also being employed in aquaculture practices (Mohammadzadeh et al., 2022). Various recombinant reproductive hormones for fish, such as FSH, LH, and GnRH, have been developed and tested for biological activity (Huang et al., 1991; Mohammadzadeh, Milla, Ahmadifar, Karimi, & Dawood, 2021; Yeganeh, Mohammadzadeh, Moradian, & Milla, 2022).

Background:

The application of biotechnology in aquaculture has significantly boosted its production capabilities, with recombinant protein production being one of the most notable advancements. Recombinant proteins play a crucial role in enhancing the growth, health, and reproductive physiology of aquaculture species, thereby addressing key challenges such as disease resistance and feed efficiency. Their production relies on advanced methods and protocols, including comprehensive catalogues of expression plasmids that enable precise and controlled protein expression. These plasmids are employed across various host systems, ranging from bacteria and yeast to insect and mammalian cells, ensuring flexibility and high yield depending on the specific protein requirements. Additionally, genetically engineered species, including transgenic fish and plants, serve as biofactories for producing aquaculture-specific proteins, such as growth hormones and antimicrobial peptides, further reducing the



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Fig 1. Summary of recombinant hormones applied in reproduction therapies.

need for external supplementation. Refined culture strategies such as optimized fermentation processes and sophisticated bioreactor systems ensure consistent and scalable production in laboratory settings. Collectively, these resources and techniques enable efficient production of recombinant proteins, paving the way for their integration into commercial aquaculture practices to enhance productivity and sustainability.

Recombinant hormones in fish reproduction

The reproduction of aquatic invertebrates is controlled by the brain-pituitary-gonadal (BPG) axis, which is achieved by the neuroendocrine and endocrine systems of fish (Levavi-Sivan, 2018). In vertebrates, the brain and gonads do not communicate directly to regulate reproduction. Instead, the main endocrine organ in vertebrates, the pituitary gland, is influenced by neurohormones produced in the brain, particularly in the hypothalamus. Gonadotrophin-releasing hormone (GnRH), a neurohormone produced by the brain, stimulates the pituitary to produce reproductive hormones in vertebrates (Sherwood & Adams, 2005). The pituitary gland produces two reproductive hormones, known as gonadotrophins (GTHs), using cells known as gonadotrophs. These two hormones are follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are transported in the bloodstream to the target cells in the gonads, where they control maturation and spawning (Planas, Swanson, Rocha, Arukwe, & Kapoor, 2008).

GnRH controls the synthesis and release of gonadotropins (GTHs), specifically follicle-stimulating hormone (FSH) and luteinizing hormone (LH), from the fish pituitary, which play key roles in reproduction. FSH is linked to early follicle growth and vitellogenesis, whereas LH drives the final maturation and ovulation (Swanson, Suzuki, Kawauchi, & Dickhoff, 1991). GTHs are heterodimeric glycoproteins composed of a common α -subunit and distinct β -subunits that determine their specific biological activity (Levavi-Sivan, Bogerd, Mañanós, Gómez, & Lareyre, 2010). Owing to their central role in reproduction, GnRH and GTHs have emerged as preferred therapeutic options for addressing infertility and other reproductive disorders. Initially, crude pituitary extracts or human chorionic gonadotropin (hCG) were used in fish reproduction treatments; however, these methods had issues such as batch variation and immune response risk (Levavi-Sivan et al., 2010). The structure of hormones exposes them to various endopeptidases in the body, leading to a reduction in the half-life of the hormone. This results in a short half-life of synthetic hormones, and a high quantity of this peptide is required to cause fish to spawn. Advances in molecular biology have led to the development of recombinant GTHs and recombinant GnRH (rGnRH), which offer higher purity, specificity, consistency, and the

potential for genetic modifications to enhance potency and lifespan (Molés et al., 2020). Recombinant GTHs and rGnRH are now preferred for reproductive therapies due to their renewability, batch uniformity, and reduced immune response risk (Adams & Boime, 2008).

Animal reproduction is one of the areas in which recombinant protein production is mainly used. Induced breeding plays a vital role in increasing aquaculture production. The artificial reproduction of fish using hormones increases the number and synchronized extraction of male and female gametes. Consequently, this reduces costs and simplifies gamete collection and egg incubation. Currently, the most widely utilized hormones for reproduction are extracted from animal sources, such as the pituitary glands, and some synthetic hormones are also used. However, it creates problems related to purity and specificity, limiting its use in induced breeding. Advancements in recombinant DNA technology and the development of novel expression systems have made it possible to obtain large quantities of hormones from different fish species. Otherwise, it would have required the sacrifice of a huge number of fish to obtain only a small amount of the native protein (Funkenstein, 2006). To date recombinant hormones have been developed in various fish including follicle-stimulating hormone in Japanese eel (*Anguilla japonica*) (Kamei, Kaneko, & Aida, 2006), luteinizing hormone in Common carp (*Cyprinus carpio*) (Aizen, Hollander-Cohen, Shpilman, & Levavi-Sivan, 2017), gonadotropin-releasing hormone beluga (*Huso huso*) (Mohammadzadeh, Moradian, Yeganeh, Falahatkar, & Milla, 2020).

Conclusion:

The production and use of recombinant proteins can be used as advanced technology in induced breeding programs. This technology will be helpful for aquaculturists in building out-of-season breeding programs, overcoming reproductive issues in aquaculture, and ultimately enhancing aquaculture production. The recombinant hormones, such as GnRH, FSH, and LH, can be more effective than traditional hormonal treatments in promoting gonad maturation in both sexes, triggering ovulation, and increasing sperm production. It is important to produce inexpensive recombinant proteins, and need to give more attention to this technology as it helps manipulate reproductive physiology in fish without any harmful effects.

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