Biotechnology Input in Fish Breeding

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Pisciculture, to increase the fish production, has a crucial limitation. Fish cultured in land-locked water bodies usually do not breed without the hormonal induction. Technology of fish breeding suffered for a long time due to the lack of suitable commercial product to induce the spawning of economically important fish. Hypophysisation technique, where pituitary extract was used to induce the breeding, faced more failures than success besides the limitation of the source. After the discovery of a brain peptide, gonadotropin releasing hormone (GnRH), which binds to the membrane receptor of pituitary gonadotroph cell and releases gonadotropic hormone (GTH), the situation has changed dramatically. Final maturation and release of germ cells, spermatozoa and oocyte, to the water for fertilization, depends on an acute surge of GTH which is about 10 times greater than the normal circulatory level of GTH. Injection of GnRH causes this acute GTH surge. GnRH is a decapeptide but its gene encodes 92 amino acid containing large molecule. Extensive post-translational processing is necessary to secrete GnRH from the neural cells. For this reason recombinant DNA technology could not be employed for the production of GnRH. Instead, chemical synthesis of this decapeptide is easier and cheaper. Numbers of fish GnRH structure have been elucidated and depending on salmon GnRH peptide sequence, a chemical analogue has now been marketed under the name of “Ovaprim”. There is a strong research background to understand GnRH mechanism of action and signal transduction pathway involved in GnRH mediated GTH function on germ cell maturation and release. These scientific investigations have contributed significantly in designing the superactive GnRH analogues. The only GnRH available in India is from the brain of a freshwater murrel, Channa punctatus. Combination of two murrel GnRH variants, GnRH I and GnRH II, produces far more superior effects than “Ovaprim”. Biotechnology input in fish breeding is no doubt highly appreciable as it provides the cultivators almost a riskless method. However, research in different laboratories is still in progress to have a more potent molecule with the possible addition of some metabolic hormones. Pisciculture is now a booming industry all over the world, which imposes a larger demand for “Ovaprim” like products. Murrel GnRH, therefore is expected to be a highly competitive product in the global market.

Keywords: pisciculture, luteinising hormone (LH) stimulating hormone (FSH) gonadotropin releasing hormone (GnRH), gonadotropic hormone (GTH), ovaprim

Introduction

Gonadotropin Releasing Hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates. It is a decapeptide and was first isolated from pig and sheep hypothalami with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH) (Burgus et al., 1972; Schally et al., 1973). Since then only one form of GnRH has been identified in most placental mammals including human beings as the sole neuropeptide causing the release of LH and FSH. However, in non-mammalian species (except guinea pig) twelve GnRH variants have now been structurally elucidated, among them seven different forms have been isolated from fish species (Sherwood et al., 1993; King & Miller, 1995; Jimenez-Linan et al., 1997). Depending on the structural variants and their biological activities number of chemical analogues have been prepared and one of them is salmon GnRH analogue profusely used now in fish breeding and marketed commercially throughout the world. Question is why fishes require an induction by this neuropeptide hormone to release germ cells from male and female partners so that fertilization of oocytes by sperms may occur in the aquatic ambience.

In fact, most of the economically important culturable fish in land-locked water do not breed until the hormone induces them. Fish in marine or riverine water do not need induction by hormone for their breeding. But to culture fish with the intention of increasing the yield of production in either ocean or river is not possible. Culture requires a controllable...
area, that's why pisciculture to enhance the yield is restricted to land-locked aquatic bodies where fish do not usually breed or spawn without the induction of hormone. Since fish meat has been found to be the best quality food among all animal meats, there is an enormous increase in fish producing industries in global scenario recently where the major rate limiting factor is breeding. The induced breeding of fish is now successfully achieved by the development of GnRH technology. In this overview, a brief description is given to conceive the biotechnology of this highly important area of our food supplement.

**Basic Science in the Utilization of GnRH Technology**

It is known for a long time that an acute surge of pituitary gonadotropic hormone (GTH) is required for final maturation of germ cells followed by ovulation and spermiation. The release of germ cells in water allows the sperms to fertilize the ova or oocyte, zygote thus formed develops further to produce hatchlings or young fish. This high surge of GTH normally never occurs in the fish cultured in land-locked water bodies. Injected GnRH reaches pituitary gonadotroph cells via the circulation and on forming a complex with the surface receptor, it transduces signals through the inositol triphosphate, diacylglycerol, protein kinase C (PKC) and later through protein kinase A (PKA) pathway resulting in GTH synthesis and release. GTH in sudden high concentration (surge) is necessary to occupy large number of receptors in the ovarian (theca and granulosa) and testicular (Leydig cell) somatic cells that initiate signal transduction cascade resulting in the formation of maturation inducing steroid (MIS), 17α, 20βdihydroxy-4-pregnen-3-one (17α,20β-DP), which causes the final maturation, ovulation and spermiation by inducing the generation of maturation promoting factor (MPF), a complex of dimeric protein of two monomers, cyclin B and cdc2 kinase (Fig. 1). MPF affects the oocyte germinal vesicle breakdown (GVBD), a clear sign of final maturation.

When such is the situation, the most critical factor stands out to be the GTH surge. We have shown that the level of circulatory GTH shoots from 30-40 ng/ml to 270-300 ng/ml when GnRH is administered and this ten fold rise is essential to produce maturational effect on germ cells (sperms and oocytes)(Halder et al, 1991). At this point one may argue that instead of GnRH, pituitary GTH can be used in inducing maturation and breeding of fish. In fact this was the earlier practice and proven to be a failure. It was not GTH but pituitary extract containing GTH that was used for induced breeding of fish. GTH being a glycoprotein hormone with two unequal subunits, α and β, poses problem for the employment of recombinant DNA technology. To utilize the natural source i.e. pituitary also faces other crucial problems, viz.(I) each fish has one pituitary gland, hence the source is very limited, (ii) pituitary contains large number of different types of proteins and that makes the extract very unstable, (iii) solvent storage of
pituitary gland destroys GTH activity to a considerable extent, (iv) isolated GTH being a heterodimeric glycoprotein is fairly unstable even in cold conditions, (v) since marketed pituitary glands are usually collected from dead fish (large collection from live fish is difficult and rather not possible), they are often found to be of very bad quality and some contained harmful microbes causing disease to the recipient fish, (vi) because of the poorer stability, their transport by keeping the hormonal activity to the breeding field is a real problem. GnRH, on the other hand, can be easily synthesized chemically as it's a ten amino acid containing peptide, highly stable and its administration confers an acute surge of endogenous pituitary GTH required to produce signals for the formation of MIS from the precursor cholesterol molecule in the somatic cells of ovary (theca and granulosa) and testis (Leydig cell), MIS in turn induces the formation of MPF which effects final maturation of germ cells.

Structure and Synthesis of GnRH
(a) Peptide Structure
GnRH was first isolated from the mammalian hypothalamus as a decapeptide (Burgus et al., 1972; Matsuo et al., 1971). Since then, several structural variants of GnRH have been described (Fig.2). There has been a striking conservation in GnRH peptide length, the first four -NH₂ terminus and two -COOH terminus amino acids are remarkably conserved in different vertebrates. Whatever changes occurred are between 5 and 8 amino acid residues, position 8 is most variable followed by positions 6, 5 and 7. This highly variable position 8 suggests its role in the variation of biological activity between the species and its critical function in recognizing GnRH receptors on the pituitary cell membrane (Millar et al., 1997). To exert its biological function it has to first recognize its receptor on the pituitary gonadotroph cell membrane and when it has to do so its linear structure changes into a loop. The NH₂- and COOH-terminal domain of GnRH are closely opposed when GnRH binds to its receptor and this is the proposed result from a β-II type turn involving residues 5-8. The β turn creates a hairpin loop that aligns N and C termini (Fig. 3)(Sealfon et al., 1997).

(b) Gene Structure
Although GnRH is a decapeptide wherever it has been found but the analysis of the nucleotide sequence of the mRNA reveals that GnRH decapptide is derived from the post-translational processing of a large precursor molecule. Human GnRH gene sequence was first obtained from human genomic DNA library(Seeburg & Adelman, 1984), since then cDNA or genes have been cloned for chicken GnRH I and II, Salmon GnRH, Seabream GnRH and catfish GnRH(Lin et al., 1998) Gene analysis showed the original 92 amino acid containing molecule with tripartite structure, pre-pro-GnRH (Fig. 4). The decapetide is preceded by a signal peptide of 23 amino acids and followed by a Gly-Lys-
Arg sequence (position 11-13) essential for proteolytic processing and carboxy terminal amidation. The last 56 amino acid residues are designated as GnRH-associated peptide (GAP). A single gene is located on the short arm of chromosome 8, encoding the pre-pro-GnRH. This gene contains four exons; exon 1 encodes 5’ untranslated region; exon 2 encodes pro-GnRH including signal peptide; exon 3 and a part of exon 2 and 4 encode the GAP protein and a long 3’ untranslated region is also encoded in exon 4 (Fig. 5) (Radovick et al, 1990).

c) GnRH- Synthesis
GnRH gene therefore translate 92 amino acid protein which is post-translationally processed in the brain neural cells to produce GnRH decapetide. For this reason production of GnRH by recombinant DNA technology is avoided as it would be extremely difficult. On the other hand, chemical synthesis of this decapetide is much easier and considerably cheaper for commercial viability. Chemical synthesis of superactive analogues with a greater stability in the circulation has given rise more effective molecules for the purpose of induced breeding of fish (Sherwood et al, 1993).

GnRH-mediated Signal Transduction Cascades
The downstream ultimate target of GnRH-mediated signals is the release of GTH from the pituitary gonadotroph cells. In fish, like other vertebrates, two types of GTHs — GTH I and GTH II — are active at different stages of germ cell maturation. The GTHs are heterodimeric glycoprotein, each consisting of α and β subunits, latter confers biological specificity to the hormone (Kawauchi et al, 1989; Tanaka et al, 1993; Schultz et al, 1995; Elizur et al, 1996; Yoshiura et al, 1997). Current observations suggest not only release but also synthesis of GTH is effected by GnRH-mediated signals (Levavi-Sivan & Yaron, 1993; Mukhopadhyay et al, 1995).

The cellular mechanisms mediating GnRH action in stimulating GTH release was reported in teleosts mainly in tilapia, Oreochromis niloticus (Levavi-Sivan & Yaron, 1993), in murrel, Channa punctatus (Mukhopadhyay et al, 1995, 1997), in European Carp, Cyprinus carpio (Mukhopadhyay et al, 1994) and in goldfish, Carassius auratus (Chang et al, 1995; Jobin...
et al, 1996) Briefly, occupation of GnRH receptors on fish pituitary gonadotroph cell membrane by GnRH follows several intracellular signal cascades, ultimately leading to the release of GTH. However, unlike mammalian GnRH mediated signal cascades, major part of these cascades is still unknown. There are evidences of four possible pathways: (i) Ca$^{2+}$-calmodulin-kinase II, (ii) adenylate cyclase-cAMP-protein kinase A (PKA), (iii) Ca$^{2+}$-protein kinase C (PKC) and (iv) combination of both these pathways where Ca$^{2+}$ and/or cAMP behaves as second messenger independently or in a dependent manner (Bhattacharya, 1999).

The presence of voltage sensitive Na$^+$, K$^+$ and Ca$^{2+}$ channels have been shown in goldfish pituitary cells (Price et al, 1993). Involvement of voltage sensitive calcium channels (VSCC) in GnRH augmented GTH release has been implicated in a number of teleostean fish, e.g. the goldfish (Jobin et al, 1996), hybrid tilapia (Levavi-Sivan & Yaron, 1993), and Indian murrel (Mukhopadhyay et al, 1995, 1997; Jamaluddin et al, 1989). The presence of VSCC indicates the requirement of extracellular calcium influx in GnRH action. Studies have shown that GnRH augmented GTH release from Indian carp and murrel is dependent on the influx of extracellular Ca$^{2+}$ through VSCC (Jamaluddin et al, 1989; Bhattacharya et al, 1990). In GnRH stimulated GTH release from fish pituitary cells, Ca$^{2+}$ appears to play an obligatory role. A correlation between medium calcium and calcium uptake by the pituitary cell is noticed between 5 and 15 min of GnRH addition. Medium calcium decreases with concomitant increase in cells, the peak was observed at 15 min. The influx of calcium into the cell coincides with GTH release. Addition of verapamil, a calcium channel blocker, blocks calcium uptake and also stops GTH release simultaneously (Mukhopadhyay et al, 1997).

Calmodulin (CaM), a calcium binding protein, clearly augmented GnRH+Ca$^{2+}$ effect on GTH release from pituitary cells (Mukhopadhyay et al, 1995). It is rather puzzling to find that CaM, whose molecular weight is 17 kDa, is stimulating GTH release when added in the presence of GnRH and Ca$^{2+}$. Calmidazolium, a specific inhibitor of CaM could effectively inhibit the CaM stimulatory effect. Detection of CaM binding protein in murrel pituitary cell plasma membrane indicates the rationality of CaM effect (Mukhopadhyay et al, 1995). However, Ca$^{2+}$-CaM-GnRH interaction in GTH release is still unclear and requires further investigations. The second part of the signal transduction by GnRH in fish appears to be adenylate cyclase-cAMP-PKA. GnRH clearly stimulates PKA activity in murrel pituitary cell. This profile of PKA activity corresponds to cellular cAMP level. During the same time, GTH synthesis in the cell also increases (Mukhopadhyay et al, 1997). The simultaneous formation of diacylglycerol also activates calcium, phospholipid dependent enzyme, PKC and this process is facilitated by elevations of [Ca$^{2+}$]. Activation of PKC results in translocation of PKC from the cytosol to the plasma membrane. Ca$^{2+}$ and PKC act in parallel during exocytosis (Ben-Menahem et al, 1994) Ca$^{2+}$ and PKC are reported to be involved in the diverse effects of GnRH upon GTH secretion and synthesis (Naor, 1997).

Final Maturation and Release of Germ Cells
Final maturation of oocytes and sperms is induced by sequential action of the following major mediators: GnRH $\rightarrow$ GTH $\rightarrow$ MIS $\rightarrow$ MPF and Mos. Meiotic process in germ cells faces a halt at diplotene. This diplotene arrest is withdrawn by MPF. In teleostean fish, like other vertebrates, MPF consists of two components, p34$^{\text{cdc2}}$ and cyclin B. Mos, a protooncogene protein, is a threonine kinase, plays an important role during final maturation (Yamashita, 1998). All these act in a synchrony resulting in final maturation and release of germ cells.

GnRH from Indian Fish
Although India has several varieties of indigenous culturable bony fish, practically nothing is known about their GnRH. It is really a very unfortunate situation for which we still have to depend on the supply from other countries to investigate the reproductive biology of our economically important culturable fish as GnRH is the key regulator of reproductive control mechanism. For the isolation of this peptide an assay system to identify the desired molecule at each step of purification is needed. First piscine GnRH isolated purified and structure determined was Salmon GnRH (Sherwood et al, 1983), followed by isolation of a number of GnRH variants depending on the immunoreactivity of this molecule, and the most current GnRH purified and characterized was by Carolsfeld et al (2000) and Robinson et al (2000). Sherwood et al (1983) took the help of anti-GnRH antibody from mammalian source, later they developed Salmon anti-GnRH antibody which permitted to isolate different GnRH forms from
other sources. During several attempts to isolate GnRH from the brain of Indian freshwater murrel (*Channa punctatus*) at Indian Institute of Chemical Biology (ICB), we had to overcome a number of constraints, the most crucial was the identification the GnRH molecule. Jamaluddin *et al* at ICB (1989) developed a very dependable GnRH bioassay. Basic principle of this bioassay is to get the release of GTH from murrel primary pituitary cell culture and determine the amount of GTH released by GTH – RIA. That was a tremendous boost to our endeavor to avail GnRH from at least one Indian fish. We started purification with novel solvent extraction through which a large chunk of extraneous protein and peptide could be eliminated, then employed Sephadex G-25 gel filtration, FPLC Mono Q and Mono S column chromatography and finally purified through pep-RPC hydrophobic column. A preliminary report was first made in 1991 (Halder *et al*, 1991), we then observed the activity and mechanism of action (Mukhopadhyay *et al*, 1994, 1995, 1997; Bhattacharya *et al*, 1990), and were surprised to find the activity to be several fold greater than the salmon GnRH or its superactive analogues. We obtained two forms of GnRH from Indian murrel brain, GnRH I and GnRH II, both are highly active, and interestingly, combination of GnRH I and GnRH II accelerate the activity to a highly significant extent. It is, therefore, clear that two murrel GnRHs are immunologically distinct from Salmon GnRH but their biological activity i.e. to release GTH from the pituitary cell, which is the prime function of GnRH, is far greater than salmon GnRH or superactive analogues (Table I).

### Global Market

The above mentioned results placed Indian murrel GnRHs (I plus II) as a highly promising global competitor since Salmon GnRH analogue (Ovaprim) prepared by Syndel Laboratory, Canada is dominating the market and extensively sold all over the world to breed culturable fish. We have now completely sequenced murrel GnRH I and II, which are novel, and no naturally occurring GnRH discovered till date has such sequences. Indian murrel GnRHs (I plus II) are now synthesized chemically by Dr. S. Pasha, Centre for Biochemical Technology (CSIR), New Delhi and the activity of the synthetic GnRHs has been examined in the laboratory (pituitary cell culture) and field to breed carps. Both these synthesized GnRHs showed greater activity in the laboratory and field experiments as compared to salmon GnRH, its analogues and other superactive analogues and commercial product "Ovaprim". Amino acid sequence of these two decapeptides (murrel GnRH I and II) could not be given here as we have applied for patent. This Indian product (murrel GnRH I and II), therefore, has great promise for national and international market.

### GnRH-Technology and Indian Scenario

Unfortunately, in a number of urban and rural centres of fish production, pituitary gland extract or hypophysation technique is still in practice although failure in breeding by this method is strikingly more than success. Such a risk of success has created a depressing and frustrating situation for the poor rural fish cultivators of our country. On one hand, requirement for carp and other fish seed production has enormously increased while on the other, hypophysation technique poses several problems including conspicuous limitation of pituitary as a resource. For various reasons (as briefly stated above) hypophysation technique has been completely abandoned in developed countries. Induced breeding of fish in these countries is now carried out by synthetic GnRH peptide or its chemical analogues prepared by amino acid substitution of naturally occurring brain peptides. GnRH peptide in suitable dose will cause the required high surge of endogenous circulatory GTH by inducing the pituitary. There is practically no risk in this technology if fish farmers can assess proper maturation of brood fish, select suitable dose and appropriate time of injection. Our experience in field experiments with murrel GnRH I plus II gave a clear notion that by making proper conditions, there can not be any failure (Fig. 6). For the risk less fish production, Syndel Laboratory, Canada is marketing "Ovaprim", a salmon GnRH analogue with other components. In India, "Ovaprim" is marketed by Glaxo Laboratory Ltd and it is now a

<p>| Table 1 — Comparison of murrel GnRH I and II and their combinations with salmon GnRH and superactive analogue. |</p>
<table>
<thead>
<tr>
<th>Incubations (2 μg GnRH fraction)</th>
<th>GTH released (ng/6×10^4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>2.6 ± 0.18</td>
</tr>
<tr>
<td>C + muGnRH I</td>
<td>74.5 ± 2.32*</td>
</tr>
<tr>
<td>C + muGnRH II</td>
<td>82.6 ± 3.02*</td>
</tr>
<tr>
<td>C + muGnRH I + muGnRH II</td>
<td>118.2 ± 3.21**</td>
</tr>
<tr>
<td>Salmon GnRH (synthetic)</td>
<td>58.5 ± 2.15</td>
</tr>
<tr>
<td>GnRH superactive analogue (des-Gly10)</td>
<td>68.0 ± 2.2</td>
</tr>
<tr>
<td>[D-Ala6]-LHRH</td>
<td></td>
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</table>
popular item for induced breeding of carps. There is another product named as “Ovatide”, its use in southern and western India is known but nothing is known about its peptide nature, source for preparation or information on research and development.

Ovaprim, however, is expensive to the rural fish farmers who earn out of seed production; its cost is about Rs. 500 per vial, which can be efficiently used for spawning of about 5-7 fish having an average body weight of 2 to 2.5 kg. Field trials with mammalian and salmon GnRH analogues containing domperidone or dopamine antagonist by taking carp, goldfish and loach have shown to be very successful (Lin et al., 1991; Volkoff & Peter, 1999; Lin & Peter, 1996). In comparison to “Ovaprim”, which is the most dominating biotechnology product in the global market for fish breeding, our murrel GnRH I plus II in combination with suitable amount of Ca\(^{2+}\) and domperidone showed significantly better results (Table 2). The experiments were conducted in summer i.e. early May (unusual time for breeding) and monsoon i.e. July (usual breeding season). In both seasons starting from egg size, rate of fertilization, rate of hatching to spawn production, at every step murrel GnRHs have shown significantly greater advantages. Best advantage with the Indian murrel GnRH is its effect on egg size; carps bred by the induction of this GnRH I plus II always produced remarkably greater size of eggs leading to significantly large hatching size. Ovaprim, on the other hand, cannot produce such large eggs. Many cultivators, who used both murrel GnRHs and “Ovaprim”, also informed about this fact. The reason appears to be related to the molecular variations between salmon and Indian murrel GnRHs. Ovaprim is a salmon GnRH analogue and murrel GnRH I plus II are native form of hormones with different amino acid sequences between 5-8 residues. We are presently investigating the receptor recognition capability of salmon GnRH analogue and Indian murrel GnRHs and then will compare the quantum of transduced signals responsible for GTH release. This information will strengthen our claim for advantages and will make murrel GnRH a potentially stronger
Table 2—Comparison of Ovaprim and murrel GnRHs (mu GnRH) in field trials

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Summer</th>
<th>Ovaprim</th>
<th>Monsoon</th>
<th>Ovaprim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weight per fish (kg)</td>
<td>2.5±0.012</td>
<td>2.4±0.015</td>
<td>2.7±0.02</td>
<td>2.3±0.015</td>
</tr>
<tr>
<td>Spawning rate (%)</td>
<td>81.5±2.3</td>
<td>72.1±2.1</td>
<td>100</td>
<td>93.3±2.18</td>
</tr>
<tr>
<td>Latency period (h)</td>
<td>7.0-7.5</td>
<td>7.0-7.5</td>
<td>6-6.5</td>
<td>6-6.5</td>
</tr>
<tr>
<td>Egg production (10^5/kg body weight)</td>
<td>0.96±0.13</td>
<td>1.05±0.07</td>
<td>1.60±0.09</td>
<td>1.65±0.14</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>84.1±2.79</td>
<td>70.1±1.85</td>
<td>98.2±0.23</td>
<td>90.9±1.86</td>
</tr>
<tr>
<td>Hatching rate (%)</td>
<td>88.3±2.72</td>
<td>78.4±3.1</td>
<td>96.8±0.73</td>
<td>85.0±0.49</td>
</tr>
<tr>
<td>Spawn production (10^5/kg body weight)</td>
<td>0.70±0.09</td>
<td>0.56±0.05</td>
<td>1.5±0.15</td>
<td>1.25±0.21</td>
</tr>
<tr>
<td>Mean egg size (mm)</td>
<td>4.8±0.09</td>
<td>4.02±0.12</td>
<td>5.1±0.05</td>
<td>4.5±0.06</td>
</tr>
</tbody>
</table>

Equal dose (10 µg/kg body weight) of muGnRHs (I plus II) or Ovaprim was injected into the male and female fish when water temperature was between 31-34°C (summer) or 26-28°C (monsoon).

product in the global market. However, there is no end of improvement in scientific products. It has been found that IGF-I (Maestro et al., 1995) and insulin (Dasgupta et al., 2001) can induce ovulation in fish, these two components can be efficiently combined to produce further better effect in future.

Conclusion
There is no doubt that the use of GnRH for maturation and release of germ cells (resulting spawning) is a far superior technology as compared to the traditional hypophysation technique. Many Indian fish cultivators are now using ‘Ovaprim’ for carp seed production. We are spending a considerable amount of foreign exchange to import ‘Ovaprim’, a Salmon GnRH analogue. India has several hundred species of teleostean fish, many of them are highly commercially important food fish, and their culture for greater yield should be our mandate. To achieve this objective, utilization of GnRH-technology for induced breeding is rather obligatory. A technology, which has to be used regularly, cannot depend for long on a supply from a foreign country. Moreover, it is extremely important to know the amino acid sequences of naturally occurring GnRH peptide in our fish which will offer a better choice to design a superactive analogue suitable to breed our fish for higher and healthy production in future. However, murrel GnRH I plus II combination is strikingly more active than ‘Ovaprim’. Chemical synthesis of murrel GnRH I and II has retained original activity, hence commercial marketing would not be a problem. ‘Ovaprim’ came out from a long-standing work of Dr. Nancy Sherwood and her associates who published several good papers on different aspects of Salmon GnRI. We are working for more than twelve years and published number of papers on murrel GnRHs, except its sequence for the sake of secrecy required for patent acceptance. No product should be given for marketing without proper scientific investigations. Unfortunately, that sometimes happens in our country, which weakens our credibility in the world market. Otherwise India has genuine strength in biotechnology which will emerge as the most dependable and sustainable one in the world market of this millennium.

Acknowledgement
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