

## **Current Status of Endocrine Aspects of Fish Reproduction— An Overview**

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Current trend of research to understand the endocrine regulation of fish reproduction is primarily concentrated around brain-pituitary-gonadal axis. However, major emphasis has been given on the brain which secretes a decapeptide, gonadotrophin releasing hormone (GnRH). The structure of GnRH has so far been elucidated in four fish species which clearly shows species variation. GnRH purified from an Indian teleost also indicates a different form. In spite of the difference in structure, salmon GnRH and its chemical analogues are found to be biologically active in a wide variety of species. GnRH binds to the pituitary gonadotroph cell membrane receptor and releases the key hormone of reproduction i.e. gonadotrophin (GtH). This action of GnRH is dependent on the influx of extracellular  $Ca^{2+}$ . Removal of extracellular  $Ca^{2+}$  or blocking of pituitary cell membrane  $Ca^{2+}$  channel completely inhibits GnRH-stimulated GtH release. What precise role  $Ca^{2+}$  plays in GnRH mediated GtH release is yet unknown. On its release from the pituitary cell GtH reaches its target organ, ovary or testis, via the circulation. GtH in fish is different from the gonadotrophins of other vertebrates, not FSH or LH type, but may be of two (GtH I and GtH II) or single type. Both theca and granulosa cells of ovarian follicle have GtH receptor and the amount of receptors varies with the stage of reproductive cycle. Again, affinity of GtH receptors varies widely in different species of fish. Occupation of gonadal cell membrane receptor by GtH results in the induction of steroid hormone synthesis which in turn causes development and maturation of the gonad. Estradiol- $17\beta$  and  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one are two major steroids controlling the growth, final maturation and ovulation in the female while testosterone,  $11$ -ketotestosterone and  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one are responsible for spermatogenesis and spermiation in the male.

**Key Words:** Fish reproduction, Hormonal regulation of reproduction, Gonadotrophin-releasing hormone, Gonadotrophin, Steroid hormones

### **Introduction**

Reproduction in fish, as in other vertebrates, is regulated by hormones from the brain, pituitary and gonads. Hormones originating in sources other than these may help in reproduction in some cases but their influence is not obligatory. In general, reproduction in fish is seasonal, starting from the recrudescence of gonads to the final

maturation usually occurs in a particular time of a year. Endocrine cycle therefore closely corresponds to the seasonal gonadal cycle. Two environmental factors, temperature and photoperiod, play a major role in the release and function of hormones. It is now known that these environmental cues mediate their effect via the release of GnRH, which reaches the pituitary via the

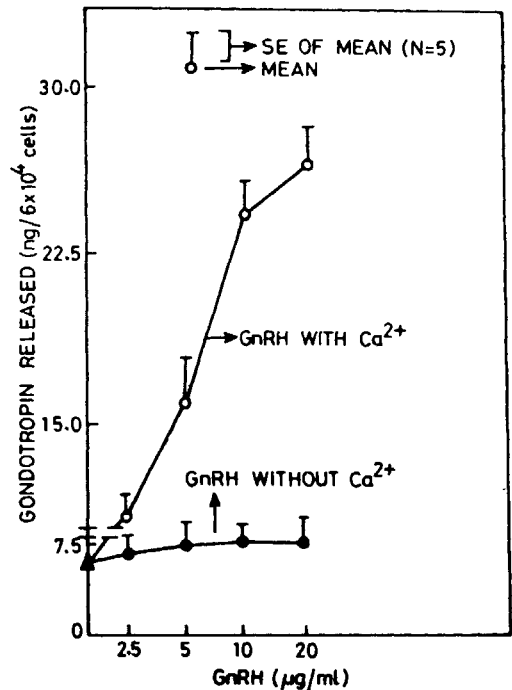
portal circulation where it binds to gonadotroph cell membrane receptor and causes GtH release into the circulation. GtH then travels through the circulation and reaches the ovary or testis where it occupies specific receptor in the cell membrane of theca and granulosa cell of ovary or Leydig cell of testis resulting in the stimulation of steroid hormone synthesis in these cells. Various kinds of steroid hormones from gonads induce development, growth and final maturation of germ cells and their release to the exterior aquatic medium (spawning) effected the final event i.e. fertilization. Although basic plan of fish reproductive regulation appears to be similar to other vertebrates, details of endocrine factors and their control vary greatly. Remarkable progress has been made in last decade which clearly indicates the uniqueness of piscine endocrine regulation of reproduction. This overview provides a brief outline of current research activities in this area.

### Control of Fish Reproduction by Brain Peptide

GnRH is an important mediator for neural control of reproduction. To date, the structure of GnRH has been determined for eleven species of vertebrates, four of which are fish species, viz. salmon (Sherwood et al. 1983), cod (Wu et al. 1986), rat fish (Lovejoy et al. 1991) and lamprey (Sherwood et al. 1986). Recently GnRH has also been isolated from phylogenetically older bony fish (Sherwood et al. 1991) and Indian murrel (Halder et al. 1991) but their detailed structure has not been elucidated. The various GnRH family members appear to have evolved from DNA changes and not from differential splicing of mRNA or differential processing of the translation product. All fish GnRHs are decapeptides like mammalian GnRH but varies in amino acid sequence. The first known structure of

fish GnRH is the salmon GnRH which is Glu - His - Trp - Ser - Tyr - Gly - Trp - Leu - Pro - Gly - NH<sub>2</sub>. There is a structural variation in other teleosts but salmon GnRH induces an increase in GtH level in almost all fishes tested so far. Soon after these findings, synthetic analogues of salmon GnRH have been prepared and a few of them are found to be more active than the native GnRH.

GnRH has been isolated and purified from the hypothalami of two Indian teleosts (Bhattacharya et al. 1990, Halder et al. 1991). Purification procedure involved extraction by 1N acetic acid followed by acetone fractionation, gel filtration through Sephadex G-25, ion-exchange and reverse phase chromatography in FPLC columns. At each step of purification GnRH activity was examined by adding the eluted fraction to an enzymatically dispersed fish pituitary cell incubation and the amount of GtH



**Figure 1** Effect of increasing concentrations of carp GnRH on GtH release in the presence or absence of Ca<sup>2+</sup>. Ca<sup>2+</sup> was added at 2 mM concentration (Bhattacharya et al. 1990).

released into the medium was estimated by GtH-RIA. It has been found that GnRH requires extracellular calcium in GnRH-stimulated GtH release. Figure 1 shows the effect of increasing concentrations of carp GnRH on carp pituitary cell incubation which resulted a linear increase of GtH release in the medium in presence of 2 mM  $Ca^{2+}$ . GnRH effect was not seen in the absence of  $Ca^{2+}$  (Bhattacharya et al. 1990). Similar observation was made earlier by Jamaluddin et al. (1989) with murrel GnRH and murrel pituitary cell. Calcium chelators inhibit GnRH-stimulatory effect on GtH release (table 1) and verapamil, a specific  $Ca^{2+}$ -channel blocker, reduces GnRH action in a dose dependent manner (figure 2). These findings indicate the requirement of extracellular  $Ca^{2+}$  in GnRH-stimulated GtH release. This was further proved by incubating  $^{45}Ca^{2+}$  with fish pituitary cells in the presence of GnRH. Figure 3 demonstrates that there was about 45% increase in  $^{45}Ca^{2+}$  uptake by pituitary cells in response to GnRH while verapamil, a  $Ca^{2+}$  channel blocker, greatly inhibited  $^{45}Ca^{2+}$  incorporation to pituitary cells in the presence of GnRH. All these show that binding of GnRH to pituitary cell possibly results the uptake of  $Ca^{2+}$  by opening  $Ca^{2+}$  channel which is essential for the release of GtH (Jamaluddin et al. 1989, Bhattacharya et al. 1990).

Another aspect of GnRH action on GtH release from the fish pituitary cell has been reported by Peter et al. (1986). They have shown that pituitary GtH secretion in teleostean fish is regulated by a dual neurohormonal system. GnRH stimulates GtH secretion and dopamine serves as an inhibitory factor on the action of GnRH and thus reduces the rate of GtH release. Injection of pimozide, which is a dopamine receptor antagonist, with GnRH or GnRH analogue permitted GnRH augmentation of GtH release. Experiment with goldfish has

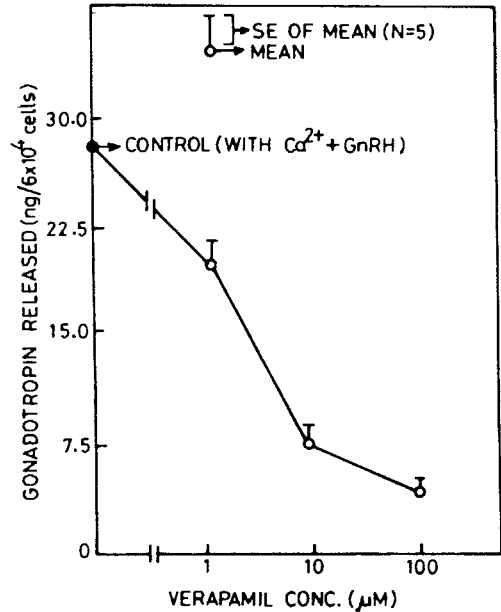


Figure 2 The dose-dependent effect of verapamil on carp GnRH activity in the presence of 2 mM  $Ca^{2+}$  and with 10  $\mu$ g of GnRH (Bhattacharya et al. 1990).

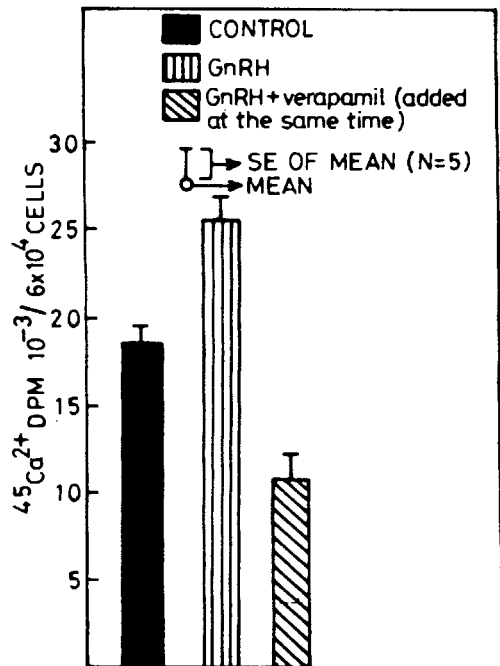


Figure 3 The uptake of  $^{45}Ca^{2+}$  by pituitary cells in the presence of GnRH (10  $\mu$ g) or GnRH plus verapamil (10  $\mu$ M) (Bhattacharya et al. 1990).

**Table 1** Effect of  $Ca^{2+}$  chelator on GnRH activity in the presence or absence of  $Ca^{2+}$ 

Incubation	GtH released (ng/6 × 10 <sup>4</sup> cells) n = 5
Control (pituitary cells alone)	4.5 ± 0.5
Pituitary cells + GnRH	8.6 ± 0.7
Pituitary cells + GnRH + Ca <sup>2+</sup>	27.7 ± 0.4
Pituitary cells + GnRH + Ca <sup>2+</sup> + EDTA	7.4 ± 0.9
Pituitary cells + GnRH + Ca <sup>2+</sup> + EGTA	5.8 ± 0.7
Pituitary cells + GnRH + EDTA	6.4 ± 0.5
Pituitary cells + GnRH + EGTA	4.8 ± 0.7

provided the first clue to overcome the dopamine blockage of GtH release and ovulation in response to GnRH analogue. Injection of pimozide causes potentiation of GtH release in response to GnRH analogue (Chang & Peter 1983, Sokolowska et al. 1985). A recent report on goldfish shows that sex steroids, estradiol-17 $\beta$  or testosterone, increase the pituitary responsiveness to GnRH which enhances serum GtH II level but basal serum GtH II levels are maintained by a concomitant increase in the dopamine turn over in the pituitary (Trudeau et al. 1993).

Extensive studies have been made on the mammalian pituitary in relation to the binding of GnRH to its receptor. These clearly indicate that interaction of GnRH with specific plasma membrane receptors in the pituitary gonadotrophs is the primary event in the stimulation of GtH secretion (Marian & Conn 1979, Conn et al. 1987). Unfortunately very few studies have yet been made with fish pituitary cells. Evaluation of GnRH binding characteristics to the pituitary gonadotrophs is necessary to understand the mechanism of GtH release. Fragmentary reports appeared recently regarding the study of pituitary GnRH receptor in fish. Crim et al. (1987) carried out investigations on the flounder at two stages of reproductive cycle and demonstrated a higher binding of <sup>125</sup>I-Buserelin (GnRH) to

the pituitary fragments of the prespawning and postspawning female. In the goldfish, *Carassius auratus*, two classes of GnRH binding sites have been identified by Habibi et al. (1987) while De Leeuw et al. (1988) found single class of high affinity receptor in the catfish, *Clarias gariepinus*, pituitary. It has also been shown that GnRH binding to its receptor varies with seasons and castration results in a two-fold increase of GnRH binding in catfish which can be reversed by concomitant treatment with androstenedione (Habibi et al. 1989). More recently salmon GnRH analogue binding characteristics in the pituitary of stickleback under different physiological conditions has been studied by Andersson et al. (1992) who reported that temperature is a more important factor than photoperiod for GnRH binding to receptor in the pituitary. It has been shown that GnRH is carried in the circulation by means of a binding protein (GnRH-BP) in goldfish serum. GnRH-BP is a major component of serum protein in goldfish and it is a glycoprotein of 40 Kd molecular weight (Huang et al. 1991).

Immediately after the discovery of salmon GnRH and synthesis of its superactive analogues, it was used for induced breeding of different fishes. The 'Linpe' method i.e. salmon GnRH analogue plus dopamine antagonist has been used for induced ovulation and spawning of number of fish (Peter et al. 1988). 'Linpe' method has been successfully modified by the addition of Ca<sup>2+</sup> for induced spawning of Indian carps and maturation of Indian perch and catfish (Halder et al. 1991). They have also shown that plasma GtH level is significantly elevated by the addition of Ca<sup>2+</sup> to 'Linpe' method.

### Regulation of Gonadal Function by Pituitary Gonadotrophin

GnRH stimulates the release of pituitary GtH into the circulation and GtH in turn

regulates ovarian and testicular function by inducing steroidogenesis after occupying specific receptor in the gonad. This is true of other vertebrates but in fish both structural and functional details considerably differ from other vertebrates. GtH may be one or two types but not FSH and LH type. Two types of GtHs were isolated and purified from the pituitary of salmon (Kawauchi et al. 1989, Swanson et al. 1991) and from European common carp (Van der Kraak et al. 1992). The molecular weights of chum salmon GtH I and GtH II were estimated by using SDS-PAGE and found to be 50K and 36K dalton respectively. However N-terminal residues of common carp GtH I and GtH II correspond to those of chum and coho salmon GtH I and GtH II (Swanson et al. 1991). Single GtH has been purified from the pituitary of sturgeon (Burzawa-Gerard et al. 1975), rainbow trout (Breton et al. 1976) and silver carp (Kobayashi et al. 1985). The Indian murrel and carp contain a single pituitary GtH, molecular weights of which are 42K and 40K dalton respectively (Banerjee et al. 1989). In all GtHs there are two dissimilar subunits, namely  $\alpha$  and  $\beta$ .

Irrespective of its chemical structure, the function of GtH in fishes is to control oocyte growth including vitellogenesis and maturation, spermatogenesis and

spermiation by inducing steroid hormone synthesis in the gonad. Steroidogenic action of GtH in fish depends on its specific binding to its receptor located in the plasma membrane of the gonadal cell (Nagahama et al. 1985, Jamaluddin & Bhattacharya 1986, Kanamori & Nagahama 1988). It has now been well established in mammals that the quantity of gonadotrophin receptors modifies the sensitivity of gonad to gonadotrophins (Catt et al. 1980, Adashi & Hsueh 1984). Fish is a seasonal breeder and it is expected that GtH circulatory level will rhythmically alter and GtH receptor in the gonad will also fluctuate in tune with the variation of gonadal cyclic activity. In an Indian murrel GtH level in the serum was found to be highest during prespawning and spawning season which declined sharply in the postspawning season (Bhattacharya & Banerjee 1990). Study of GtH receptor seasonal profile in fish has been attempted by few workers and very little is known about the pattern of GtH receptor content at different stages of reproduction. The affinity of GtH receptor in the ovary of different fishes varies considerably (table 2). However, occupation of receptor in the gonad by GtH results stimulation of steroid hormone synthesis (Jamaluddin & Bhattacharya 1986, Kanamori & Nagahama 1988). Steroid hormones then

**Table 2** Affinity of GtH for gonadal receptor in different fishes

Fish	GtH	Kd	Authors
1 Rainbow trout testis	hCG	$0.40 \times 10^{-9}M$	Schlaghecke 1983
2 Rainbow trout ovary	hCG	$0.6 \times 10^{-9}M$	Schulz et al. 1985
3 Indian freshwater murrel, <i>C. punctatus</i> ovary	(a) hCG (b) silver carp GtH	$2.35 \times 10^{-10}M$ $0.78 \times 10^{-10}M$	Jamaluddin and Bhattacharya 1986
4 Goby testis	Salmon GtH	$2.5-200 \times 10^{-9}M$	Aida and Ishii 1985
5 Brown trout ovary	Salmon GtH	$0.3-0.6 \times 10^{-9}M$	Breton et al. 1986
6 Eel ovary	Carp GtH	$0.1 \times 10^{-9}M$	Salmon et al. 1987
7 Amago salmon ovarian follicle	Salmon GtH	$0.2-0.8 \times 10^{-9}M$	Kanamori and Nagahama 1988
8 Indian major carp, <i>Catla catla</i> ovary	Catla GtH	$0.78-0.97 \times 10^{-10}M$	Manna and Bhattacharya 1993

cause final maturation and ovulation or spermiation..

### Endocrine Control of Gonadal Development and Maturation by Steroid Hormones

Development and maturation of fish gonad is controlled by two or more biologically important steroids, which are induced by GtH, estradiol-17 $\beta$  and 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-2-one (17 $\alpha$ , 20 $\beta$ -diOH prog) in the ovary and testosterone, 11-kitotesterone and 17 $\alpha$ , 20 $\beta$ -diOH prog in the testis. Ovarian maturation consists of different stages and the first stage is growth which is the active vitellogenic stage when estradiol-17 $\beta$  causes the synthesis of a yolk precursor protein, vitellogenin, in the liver of fish which is transported via the circulation to the ovary and taken up by the developing ovarian follicles or oocytes through micropinocytosis (Nath & Sundararaj 1981, Sundararaj & Nath 1981, Wallace 1985, Mukherjee et al. 1989). Estradiol-17 $\beta$  induces vitellogenesis in trout by expressing vitellogenin gene (Guellec et al. 1988). Next step in the process of maturation is chromosome condensation and extrusion of first polar body and then germinal vesicle breakdown (GVBD). GtH triggers all these processes but not directly. Maturation action is mediated by the follicular production of maturation inducing substances (MIS) which is 17 $\alpha$ , 20 $\beta$ -diOH prog. This is one of the most potent steroids for inducing final maturation of oocytes in a number of teleostean species whose presence during the time of GVBD has been reported (Nagahama et al. 1983, Upadhyaya & Haider 1986, Scott & Canario 1987, Haider & Inbaraj 1989a, Kime & Bieniarz 1987). Evidences are there that GtH stimulates MIS synthesis. Two specific inhibitors of 3 $\beta$ -hydroxysteroid dehydrogenase, cyanoketone and epostane, which inhibited GtH-induced final maturation of oocyte,

had no effect on the maturation induced by progesterone or 17 $\alpha$ , 20 $\beta$ -diOH-prog (Young et al. 1982, Iwamatsu & Onitake 1983, Haider & Inbaraj 1989b, Haider (1990). 17 $\alpha$ , 20 $\beta$ -diOH-prog has recently been purified from the oocytes of an Indian catfish, *Clarias batrachus* and this appears to be the major naturally occurring MIS in this fish (Haider & Rao 1992). This unusual steroid in fish then form a major cytoplasmic mediator which is the maturation promoting factor (MPF) and MPF induces meiotic maturation (Nagahama 1987a, b, Nagahama & Yamashita 1989). 17 $\alpha$ , 20 $\beta$ -diOH prog has been identified recently during the oocyte growth and gestation of the guppy (Venkatesh et al. 1992) and injection of GtH increased the sensitivity to 17 $\alpha$ , 20 $\beta$ -diOH prog in blue gourami (Degani & Boker 1992). Nagahama (1987c) proposed a two-cell-type model for the production of estradiol-17 $\beta$  during vitellogenic phase and 17 $\alpha$ , 20 $\beta$ -diOH prog during post vitellogenic phase in the ovarian follicle of salmonid fishes. In the vitellogenic phase oocytes thecal layer secretes testosterone which then passes to granulosa layer where it is converted to estradiol-17 $\beta$  under the influence of GtH. In the post vitellogenic oocytes binding of GtH to thecal cells produces 17 $\alpha$ -hydroxy progesterone which traverses to the granulosa layer where it is converted to 17 $\alpha$ , 20 $\beta$ -diOH prog under the influence of GtH. However, it may not be uniformly true in all species of fishes as Kobayashi et al. (1988) detected four stages of sensitivity to GtH and steroids in the daily spawning of kisu (*Sillago japonica*). Stage A, in which oocytes are insensitive to hormones; stage B, in which GtH induces GVBD but steroids do not, stage C, in which either GtH or steroids induce GVBD and stage D, in which oocytes undergo GVBD spontaneously without additional treatment with hormones. Information about the steroid involvement in male is rather scanty.

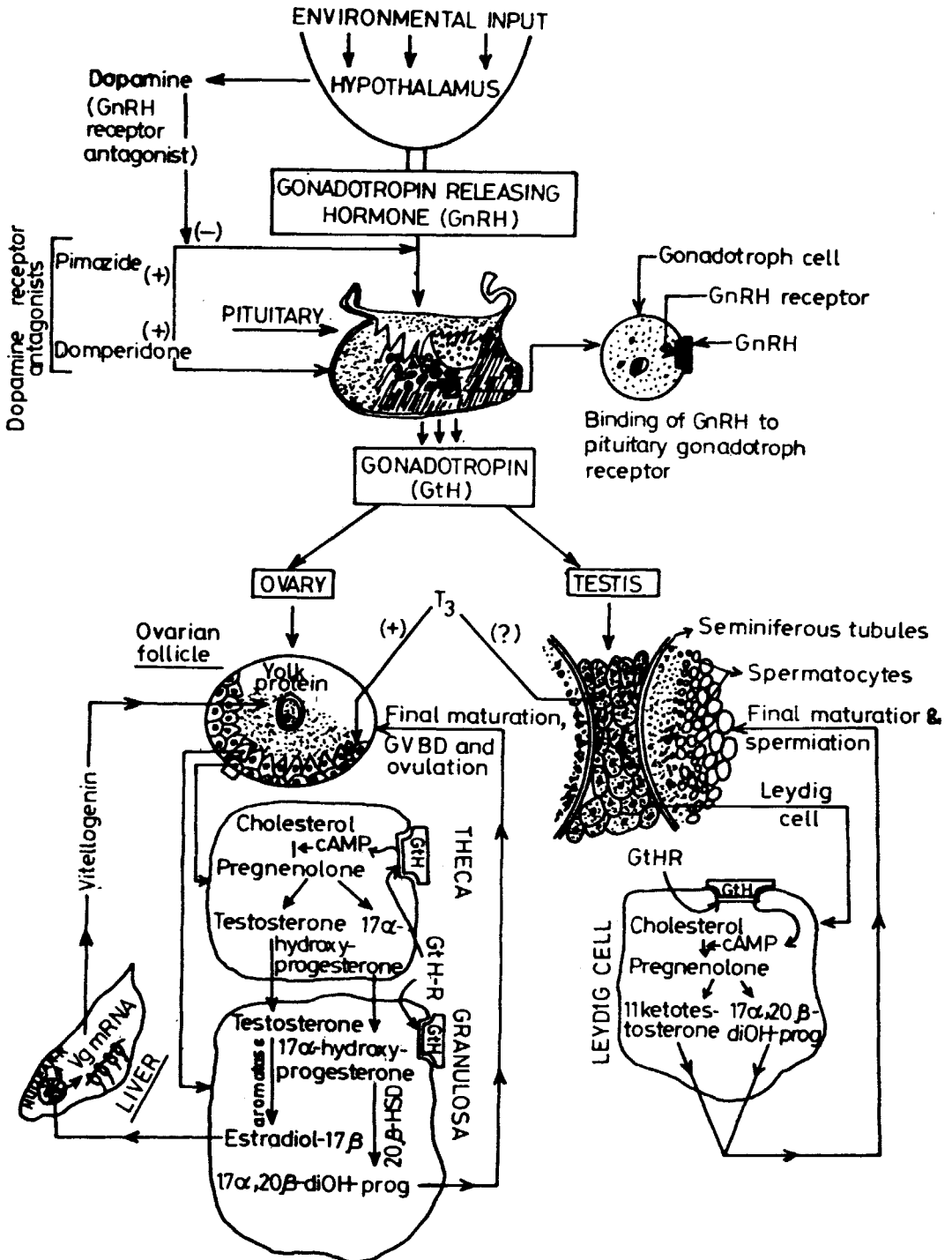


Figure 4 A model on the hormonal control of fish reproduction prepared on the basis of the present information

GtH in male increases the circulatory level of testosterone, 11-ketotestosterone with glucuronide ester and also  $17\alpha$ ,  $20\beta$ -diOH prog (Kime 1979, Ueda et al. 1983). In salmonid,  $17\alpha$ ,  $20\beta$ -diOH prog appears to be the most important steroid involved in spermiation and GtH surge during spermiation is accompanied by dramatic increase in circulatory level of  $17\alpha$ ,  $20\beta$ -diOH prog (Nagahama 1987c). Apart from all these regulations, thyroid hormone has long been implicated in the reproduction of vertebrates including fish. But how it influences the reproductive process is not known. It has been shown that thyroid hormone (Triiodothyronine,  $T_3$ ) has a specific high affinity receptor in the ovarian nuclei of the perch, *Anabas testudineus*, and  $T_3$  increases ovarian protein synthesis significantly (Chakraborti et al. 1986). The quantity of ovarian nuclear  $T_3$  receptor clearly coincided with the annual reproductive cycle of perch, highest was detected during prespawning and spawning stage while it dropped dramatically in the postspawning stage oocyte indicating functional relevance of  $T_3$  receptor in the

reproduction (Maitra & Bhattacharya 1989). This has been further elucidated by *in vitro* experiments. Addition of  $T_3$  to perch oocyte culture resulted a dose dependent increase in progesterone release. Cycloheximide, an inhibitor of protein synthesis, blocked the  $T_3$ -stimulated progesterone release suggesting a protein mediator of  $T_3$  effect and this protein was found to be soluble in nature (Guin et al. 1993). In the case of male perch, binding of  $T_3$  to testicular Leydig cell triggers the synthesis of a protein which in turn stimulates androgen release (Jana & Bhattacharya 1993). All these findings are indeed surprising as till date release of steroid hormone from the piscine gonad is known to be regulated only by GtH. How thyroid hormone does this? Further investigations will be required to answer this question but there is no doubt about the tangible existence of this new dimension in the field of endocrine regulation of fish reproduction. Current status of knowledge discussed so far is briefly summarised in a diagrammatic model (figure 4).

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