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Original Research Paper

# Oogenesis Control in Multi-Spawning Blue Gourami (*Trichogaster Trichopterus*) as a Model For the Anabantidae Family

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KEYWORDS :

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## ABSTRACT

*This review discusses a model of environmental, behavioral and hormonal involvement, and its influence on oogenesis in female blue gourami, Trichogaster trichopterus, which belongs to the Anabantidae family, based on more than 15 years of studies. The model presents the effect of environmental factors and pheromones (steroid glucoronides) on oogenesis and brain-pituitary hormones gene expression in blue gourami females. Environmental factors and pheromones affect gonadotropin-releasing hormone (GnRH1 and 3), pituitary adenylate cyclase-activating polypeptide (PACAP) and its related peptide (PRP), which act on follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH) and prolactin (PRL). 17β-estradiol (E2) and testosterone (T) increased during vitellogenesis (VTL) at a relatively high level in the plasma, and 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) increased during maturation and ovulation in blue gourami. In summary, this review proposes a quality model of the Anabantidae family combining important parameters, including water (temperature and salinity), the sexual behavior of males, pheromones and hormones involved in the brain-pituitary-gonad (BPG) axis, and the effect on oogenesis and reproduction of females.*

**Introduction**

The blue gourami (*T. trichopterus*) belongs to the Anabantidae family, which contains 16 genera and about 50 species distributed throughout most of southern Asia, India and Central Africa. This family belongs to the Labyrinthici suborder (Forselius 1973), which is characterized by the presence of an air-filled breathing cavity (labyrinth) located above the gills under the operculum. The blue gourami is multi-spawning and male-dependent, having an asynchronic ovary development (Degani 1993a, Degani and Schreibman 1993, Miller 1964). Reproduction and growth in vertebrates is controlled by a hierarchically organized endocrine system that is conserved throughout all vertebrates' species. GnRH plays (Sower et al. 2009) a central role in the control of vertebrate reproduction by affecting various hormones, e.g., FSH, LH (Degani and Boker 1992b, Nagahama 1994) and GH family, which in turn regulate gametogenesis and steroidogenesis (Degani 2001, Gomez et al. 1999, Montero et al. 2000).

In natural habitats, blue gourami reproduction occurs year-round but more rapidly during the heavy rain season (Degani 2001).

The hormone involved in reproduction control by the BPG (Yaron 2011, Zohar et al. 2010) (Yaron et al. 2001, Yaron 2011, Zhou et al. 2011) (Yaron 2011) axis has been studied extensively in various species of fish, many of them important in aquaculture or marine culture, and previously described. Some fish grown in aquaculture are synchronic whereby all the oocytes change uniformly in oogenesis; others are asynchronic whereby only part of oocytes undergo oogenesis (Yaron 2011, Zohar et al. 2010) (Yaron et al. 2001, Yaron 2011, Zhou et al. 2011). However, the BPG is not described in the asynchronic ovary of male-dependent and multi-spawning fish belonging to the Anabantidae family (see for review Degani 2001(Degani 2001)). Its oogenesis is controlled by male behavior, which affects female hormones, as is discussed in this review(Degani 2001).

The GnRH family includes 28 isoforms, 15 and 13 from representative vertebrate and invertebrate species, respectively (Sower et al. 2009). Genome study with phylogenetic analyses and functional proposed that there are four paralogous groups of GnRH in the brains that are considered to have originated from gene or genome duplications followed by a gene duplication event (Roch et al. 2011, Tostivint 2011). In the fish are fond in the present time only three GnRH paralogous groups, namely GnRH1 , GnRH2 and GnRH3 are present, whereas the GnRH4 group was probably lost after the divergence of gnathostomes (Roch et al. 2011, Tostivint 2011). From a functional point of view, the involvement of GnRH1 in the regulation of pituitary functions was well established in fish (for review see (Yaron et al. 2003, Zohar et al. 2010)). The function of three GnRH are study in various fish (Zohar et al. 2010) and there are some time similar function and on the other hand different function in various modern fish species (Levy and Degani 2013). GnRH1 control gonadal maturation and related to sexual behavior in the bland porgy (An et al. 2008). GnRH2 is involved in sexual behavior and food intake in goldfish (Canosa et al. 2008, Matsuda K and Uchiyama M 2008). GnRH3 effect on LH release in one species was demonstrated in vivo and in vitro in the African catfish (Bosma et al. 2000).

The regulation of reproduction, several observations have shown t PACAP work cooperatively toward the induction of the expression in both β subunits of βLH and βFSH genes ( Purwana IN 2011, Purwana IN and H 2010 ). PACAP is brain peptide that PACAP synergistically upregulate GH and βFSH gene expression in fish. In fish demonstrated a cross-talk between GnRH and PACAP intracellular signaling pathways (Yaron et al. 2001, Yaron 2011). The GH expression during the reproductive cycle could contribute to understanding the interactions between somatotropic and gonadotropic axes at the pituitary level and elucidating the effects of GH on fish reproduction. GH mRNA and protein are expressed soon after hatching (Funkenstein and Cohen 1996, Yang et al. 1999) and persist during the growth and reproductive stages [23]. The GH/IGF-1 axis is involved in eliciting aromatase activity, E2 production and inducing GVBD to promote oocyte maturation (Reinecke 2010). Regarding reproduction and behavior, PRL is essential in the physiology of lactation and plays an important role in parental care behavior (de Ruiter et al. 1986).

The rate of oocyte development has been found to be inversely proportional to temperature, to $E_2$ and vitellogenin synthesis (Watts 2004). The important steroids among others involve in ovarian development and oogenesis in are $E_2$ and 17,20P. E2, which acts directly on oogonial proliferation, and 17,20P which acts directly on the initiation of the first meiotic division of oogenesis (Miura C 2007) .

**Over the past 1**5 years, the internal and external effects of various parameters on reproduction were studied. The key hormones involved in regulating oogenesis in the brain

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that are affected by temperature are GnRH1 and 3 (Levy et al. 2011), PACAP and PRP (Levy et al. 2009), pituitary follicle-stimulating hormone β FSH, β LH (Degani 1990) (Jackson et al. 1999a, b), GH (Goldberg et al. 2004), prolactin (PRL) (Degani et al. 2010), and ovary steroids (Degani 1990).

The proposed model (Fig. 4A and B) examined the effect of different factors on oocytes development in female blue gourami.

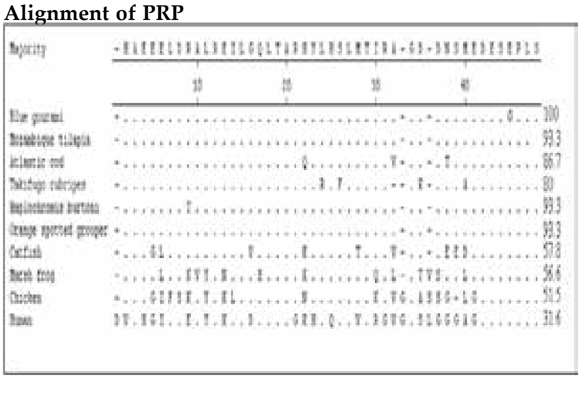
The proposed model (Fig. 4A and B) examined the effect of different factors on oocytes development in female blue gourami. Sequence phylogenetic tree analysis of GnRH1, 2 and 3), PACAP and its PRP, β FSH, β LH, GH and PRL sequences

To examine the evolutionary relationship between different pre-pro-GnRHs, we carried out a phylogenetic analysis using the ClustalW method. The deduced sequences of GnRH1, GnRH2 and GnRH3 in blue gourami were compared to homologous sequences from a number of other fish species, which are presented in Figure 1. The phylogenetic tree comparing three isoforms of GnRH pre-pro-hormones presented in Figure 1 shows a close relationship between GnRH1 and GnRH3(Levy and Degani 2012). The cDNA sequences of the full-length blue gourami brain PACAP and that of its related peptide (PRP) were acquired. PACAP cDNA had two variants obtainable by alternative splicing: a long form encoding for both PRP and PACAP; and a short form encoding only for PACAP (Levy et al. 2010).

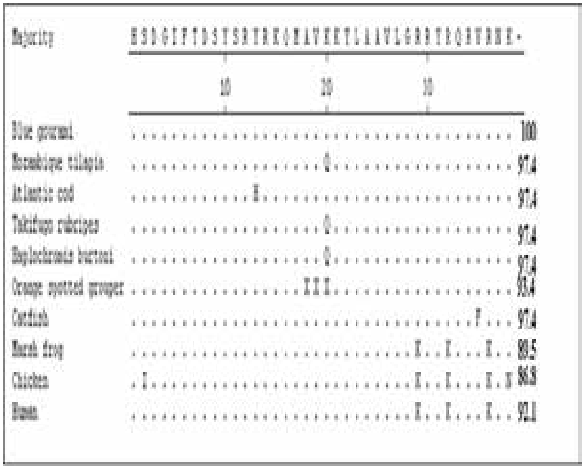
A sequence comparison of amino acids was made of the PRP (A) and PACAP (B) peptides. A sequence alignment was conducted using DNAStar WI Megalign ClustalW in the analysis of PRP and PACAP mature peptide sequences. PRP and PACAP of blue gourami were aligned with corresponding sequences of other vertebrates. Identical amino acids are represented by dots, whereas differing residues are shown with the single letter code for amino acids. PACAP is highly conserved in contrast to PRP peptides (Table 1).

Table 1: Sequence comparison of amino acids making up the PRP (A) and PACAP (B) peptides. Sequence alignment was conducted using DNAStar WI Megalign ClustalW in the analysis of PRP and PACAP mature peptide sequences. PRP and PACAP of blue gourami were aligned with corresponding sequences of other vertebrates. Identical amino acids are represented by dots, whereas differing residues are shown in the single letter code for amino acids. PACAP is highly conserved in contrast to PRP peptides.

**Alignment of PRP**



**B. Alignment of PACAP**



The phylogenetic tree comparing three isoforms of blue gourami β FSH and β LH are presented in Figure 1. The blue gourami β FSH is most similar to its striped bass (*Morone saxatilis*) counterpart, with the two polypeptides sharing 73% of their residues. The lowest similarity was found between blue gourami β FSH and goldfish β FSH (Fig. 1), with only 44% of their residues identical. A dendrogram, which graphically represents the relationships among the various β subunits of β FSH belonging to the different fish species, is presented in Figure 1A. Similarly, when the amino acid sequence of blue gourami β LH was compared with β LH polypeptides from the same group of fish as in the former comparison, the highest similarity was found with striped bass β LH, 84% of their residues being identical. The lowest similarity was found with baikal omul β LH (only 65% identical residues). The phylogenetic tree, which represents the relationships among β LH polypeptides, is presented in Figure 1B.

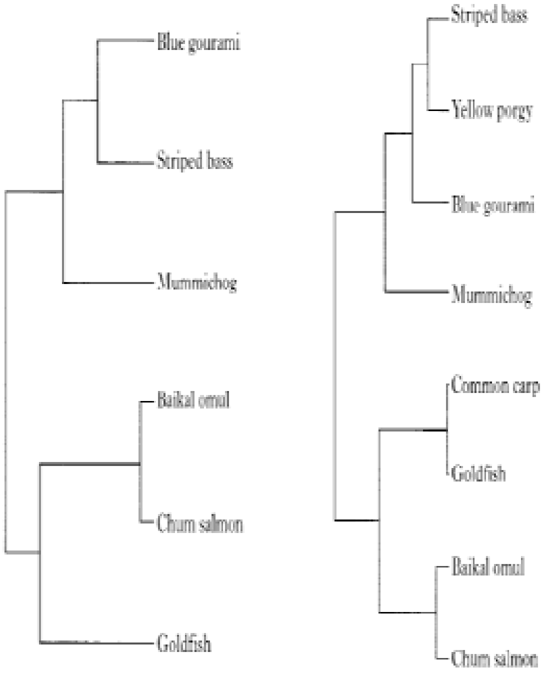


**Fig.1: Dendrograms graphically showing the relationship between various fish GtH-I (A) or GtH-II (B). The dendrograms were created according to the UPGMA method from similarity matrices produced from corre-**

**sponding sequence alignments. The scale bar is a measure of the estimated number of amino acid substitutions per 100 residues that are found when the sequences are compared in a pairwise fashion (Jackson et al. 1999b).**

A cDNA coding for the complete growth hormone of blue gourami (bg GH) was cloned by RACE PCR using several sets of degenerate oligonucleotides. The full length of the cDNA sequence was determined from a clone 863 bp long that included a 43 bp poly-A tail. The bgGH shows an open reading frame (ORF) starting at nucleotid (nt) position 46 and ending at position 660 (Goldberg et al. 2004) A comparison of the deduced amino acid sequence of bgGH to nine similar fish hormones found in the GenBank database showed that bgGH was most similar to S. aurata GH (86% identical residues) and least similar to *Anguilla japonica* GH (43% identical residues). A dendrogram showing the relationship between these fish GH is presented in Figure 2 (Goldberg et al. 2004)

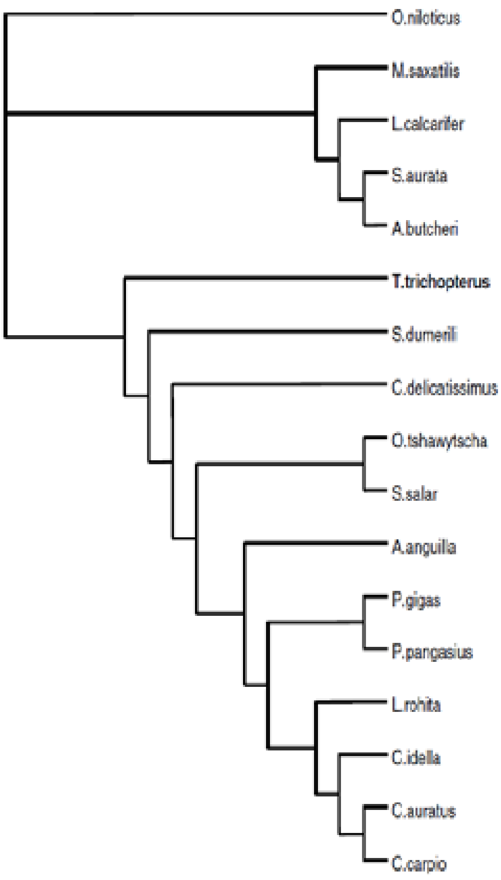


Fig. 2: Dendrogram graphically showing the relationship between various fish GH. The dendrogram was created according to the method of neighbor joining for cluster analysis (Goldberg et al. 2004) Blue gourami prolactin (bgPRL) was cloned by extracting total RNA from freshly excised pituitaries of gourami fish, followed by cDNA synthesis, rapid amplification of cDNA ends (RACE)-PCR, and finally sequencing. When bgPRL was compared to PRLs of other fish, it had the greatest homology with PRL of perciformes and the least homology with those of Anguilliformes (Table 2) (Degani et al. 2010).

**Table 2: Degree of homology between blue gourami (bg) PRL and PRL of other classes. Multiple sequence alignments were converted into distance matrices by the DISTANCES software using the correction method for superimposed substitutions. Sequence alignment was conducted using DNAStar WI Megalign ClustalW in the analysis.**

| *Species* | Class/Order | Accession No. | bgPRL (%) |
|---|---|---|---|
| *Perca flavescens* | Perciformes | AY332491 | 79 |
| *Dientrarchus labrax* | Perciformes | X78723 | 79 |
| *Spaurus aurata* | Perciformes | AF060541 | 77 |
| *Paralichthys olivaceus* | Perciformes | AF047616 | 75 |
| *Onchorhynchus mykiss* | Salmoniformes | M24738 | 66 |
| *Coregonus autummalis* | Salmoniformes | Z23114 | 66 |
| *S. salar* | Salmoniformes | X84787 | 66 |
| *Heteropneustes fossilis* | Siluriformes | AF372653 | 62 |
| *I. punctatus* | Siluriformes | AF267990 | 62 |
| *Hypoththalmichtys molitrix* | Cypriniformes | X61052 | 62 |
| *Danio rerio* | Cypriniformes | AY135149 | 61 |
| *Cyprinus carpio* | Cypriniformes | X12541 | 61 |
| *A. japonica* | Anguiliformes | AY158009 | 59 |
| *A. anguilla* | Anguiliformes | X69149 | 59 |

Effect of environmental factors (water temperature and salinity) on oocyte maturation (OMT), ovulation (OVL) and brain-pituitary hormones gene expression in blue gourami females

Environmental factors affect the VTL and OMT of fish, e.g., water temperature (Degani and Levy 2013, Levy et al. 2011) and quality (salinity) (Levy et al. 2011). The effect of temperature on the expression of genes encoding for growth and reproduction hormones in adult females under non-reproductive conditions (NRC) (in several females maintained under non-reproductive conditions with non-reproductively active few males) and under reproduction conditions (RC) (one female with one male that exhibited sexual behavior and nest-building)(Degani 1993a, Degani 1993b) in blue gourami females in temperatures ranging between **23 °C to 31°C** (the optimum reproduction temperature being 27°C (Degani 1989)) was studied (Levy et al. 2011). Three separate aquariums, each containing underground water (0%, 50%, 100%) with different salinity (0.2, 0.56 and 1.11 ppm NaCl) and conductivity (346, 955 and 1908 uS) (Degani and Levy 2013, Levy et al. 2011) levels were populated with 10 non-reproductively active adult blue gourami males (with an average weight of 7.61 ± 0.28 g). After an acclimation period of four days, the experiment was carried out for four days.

In females maintained under non-reproductive conditions (NRC), several fish were maintained in one aquarium with non-reproductively active males (Jackson et al. 1999b) and a percentage of females were in the advanced VTL oocyte stage, mRNA levels of pituitary PACAP and PRP-PACAP, gonadotropins and GH were affected by temperature changes (Jackson et al. 1999b). The trigger to advanced stages of VTL and oocytes maturation was affected by both the pheromones and male reproductive behavior.

In females maintained under reproductive conditions, only females and males with no other fishes, the reproductively active males, the nest-building males, GnRH3 and the insulin-like growth factor 1 (IGF-1)(Degani 2014) were affected by temperature changes. In blue gourami, as in other species of fish, pheromones are the chemical signal between genders (Becker et al. 1992, Degani et al. 1994). In fish, pheromones (steroid glucoronides) dissolved in

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water, as described in fish and blue gourami and detected by gas chromatography-mass spectrometry (GCMS), the radioimmunoassay method (RIA) and thin layer chromatography (TLC), are found in gonads and in the water in which the fish were maintained (Becker et al. 1992, Degani et al. 1994). The function of pheromones is to attract males and females to water, affecting behavior and gametogenesis. The pheromones of blue gourami males affect females' brain hormones and gonadotropins (Degani and Schreibman 1993), which control VTL and OMT. Pheromones affect female BPG and oogenesis (Degani et al. 1994, Degani and Schreibman 1993).(Degani 1994a, Degani et al. 1994, Degani 1994b).

### Expression of brain-pituitary hormones genes during oogenesis in blue gourami females

Based on the cDNA sequences of the full-length blue gourami brain GnRH1, 2, 3 (Levy et al. 2009) PACAP, PRP (Levy and Degani 2012) pituitary βFSH, βLH(Jackson et al. 1999b) GH (Goldberg et al. 2004), PRL (Degani et al. 2010), these genes' mRNA levels were measured by real-time PCR in females at pre-vitellogenic (PVTL), VTL and OMT stages of blue gourami.

Females under non-reproductive conditions (maintained in high density), the percentage of oocytes found in the VIT stage, and mRNA levels of genes encoding to GnRH3, βLH and GH were affected by temperature changes (Levy et al. 2011). The effect of all these hormones was studied *in vitro* and *in vivo* during oogenesis. The results support the idea that all these hormones are involved in oogenesis (as proposed in the model described in Fig. 3B). In females maintained in water where the males built nests, a higher percentages of oocytes was found in the ovary at advanced vitellogenesis, and the sexual behavior of the male affects oocytes in changing to OMT stages compared to the control group (water in which only females were maintained) (Degani 1993a, Degani and Schreibman 1993).

In the brain of fish, three forms of GnRH1,2 and 3 were found to have different sequences of amino acids, however, only two, GnRH 1 and 3, had an effect on hormone involvement in reproduction.

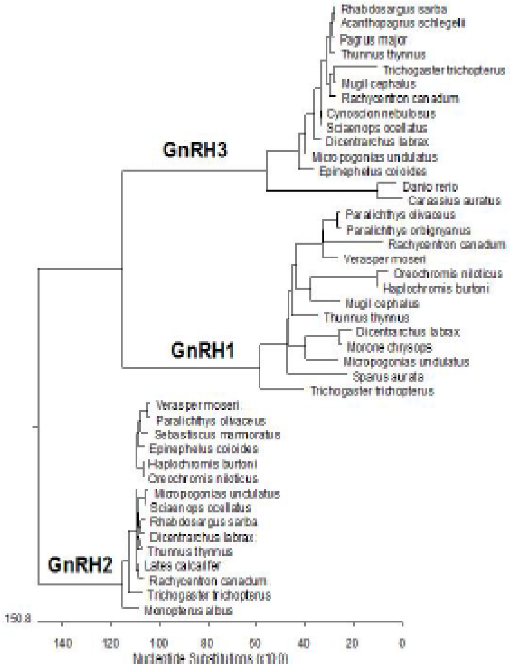


**Fig. 3: Phylogenetic tree showing the relationship between vertebrate pre-pro-GnRH amino acid sequences. The tree was generated by maximum ClustalW using DNAStar WI Megalign software. All sequences were obtained from NCBI Genebank according to the accession numbers. The accession numbers of GnRH3 sequences were as follows: Carassius auratus (CAU30301); Danio rerio (NM_182887); Epinephelus coioides (GU143807); Micropogonias undulates (AY324670); Dicentrarchus labrax (AF224280); Sciaenops ocellatus (AY677170); Rachycentron canadum (AY677173); M. cephalus (AY373449); T. trichopterus (EU107388); Thunnus thynnus (EU239501); Pagrus major (D26108); Acanthopagrus schlegelii (EU117212); Rhabdosargus sarba (EF433772); Cynoscion nebulosus (AY796310)(Levy and Degani 2012).**

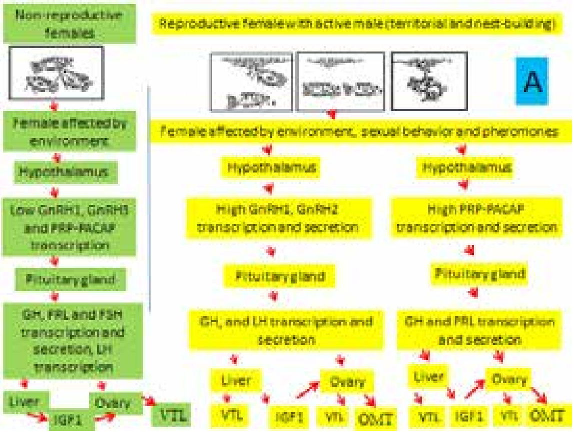
There are two pathways in which pituitary gonadotropins are regulated by the brain. The first is the GnRH1 and 3 systems controlling FSH, LH and GH in which the transcription of both GnRH1 and GnRH3 was high in VTL and the final OMT stage. The second is the BPG axis of PACAP and PRP-PACAP, GH and IGF1 that affect the gonads' oogenesis. In female blue gourami, PACAP is synthesized from a precursor, which includes the PACAP and PRP formerly known as growth hormone releasing hormone (GHRH)-like peptide (Levy and Degani 2011, 2012). PRP-PACAP mRNA levels were higher in females with oocytes in the final maturation stage than in VTL individuals. Stimulation of pituitary cells with blue gourami PRP caused an increase in βLH subunit transcription levels only in females, and an increase in GH mRNA levels and the PRP receptor in the pituitary cells (Levy and Degani 2011).

### Steroid levels profile during oogenesis in blue gourami females

Many steroids were found in the ovary during oogenesis, however, $E_2$ and T increased during VTL at a relatively high level in the plasma, and Vitellogenin (VTG) synthesis occurred in the liver and deposited in the oocytes(Jackson et al. 1994, Jackson et al. 2005). 17,20P increased during maturation and ovulation in blue gourami (Degani 1990, Degani 1993a, Degani 1993b, Degani and Boker 1992a, Degani et al. 1996, Degani et al. 1994).

### Conclusions

Oogenesis is a complex process in which many aspects are involved. In blue gourami females under NRC, VTL occurs through the environment, GnRH1→ FSH → $E_2$ and GH → IGF-1 pathways (Fig. 4A and B). Under reproductive conditions, VTL and FOM is promoted by hormones and sexual behavior, GnRH1 and 3, PRP → LH → 17,20P is promoted by the synergistic effect of PACAP, GnRH1 and 3 → FSH, and GH → $E_2$ and IGF-1 (Fig. 4A and B).



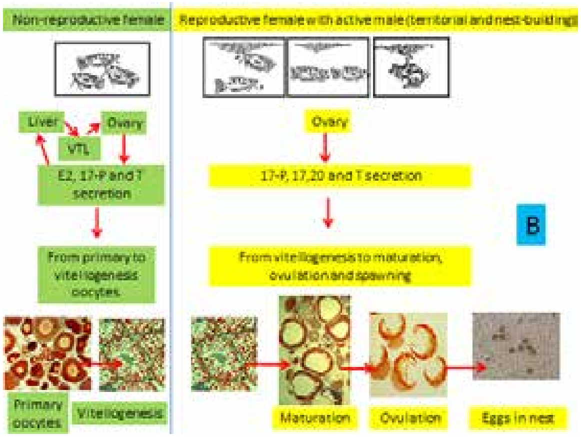


**Fig. 4: The proposed model examining the effect of different factors on oocytes development in female blue gourami. A - A flowchart depicting the hormonal and physiological events occurring along the BPG axis in non-reproductive and reproductive females. B - Histological presentation of the ovarian changes in non-reproductive and reproductive females. Shortening: gonadotropin-releasing hormone (GnRH1 and 3), pituitary adenylate cyclase-activating polypeptide (PACAP) and its related peptide (PRP), follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), prolactin (PRL), 17β-estradiol (E2), testosterone (T) , vitellogenesis (VTL) ,17 α-hydroxyprogesterone (17-P), 17α,20β-dihydroxy-4-pregnen-3-one (17,20P).**

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