Introduction

Seasonal reproduction of teleost fish depends on the hormonal regulations of gonadal maturation in a specific time of the year in relation to the specific environmental conditions. Thus, coordination is essential between brain, pituitary and gonads to activate hypothalamus-hypophysis-gonads axis. Environmental stimuli such as day-length and temperature are presumably received by the brain, which releases a decapeptide hormone, gonadotropin-releasing hormone (GnRH), the key regulator of reproductive events in all vertebrates. Four different forms of GnRH, so far has been isolated from various piscine sources and they vary from mammalian GnRH (Millar *et al.,* 1997). GnRH stimulate pituitary to release gonadotropic hormone (GTH), the primary mediator of germ cell growth and maturation. GTH in turn regulates gonadal functions by occupying specific receptors in the cell membrane of theca and granulosa cells of ovary and leydig cells of testis. GTH induces the synthesis and release of 17β-estradiol from ovarian follicular cells or 11-ketotestosterone from testicular leydig cells at the initial stage. At the latter stage, that is during maturation of germ cells, GTH induces the synthesis of 17α,20β-dihydroxy-4-pregnen-3-one, the maturation inducing hormone (MIH) of most piscine species, from ovarian follicular cells and testicular somatic and germ cells.

There is growing evidence that pituitary GTH is not the sole regulator of gonadal physiology. Oocyte growth and maturation is a complex process, which is regulated by different kinds of hormones and growth factors. Like all vertebrates, fish gonadotropins (FSH and LH) which are release from anterior pituitary, plays the key regulatory role for oocyte growth and maturation. One kinds of gonadotropin is GTH I which is known as follicle stimulating hormone (FSH) regulates folliculogenesis, vitellogenesis and growth of oocyte etc. Other kind is GTH II or leutinizing hormone (LH) which regulates oocyte maturation process. During oocyte growth, FSH induces granulosa cell to synthesis 17β-estradiol (E_2) and it regulates vitellogenesis in liver to synthesis of vitellogenin which successively involved in growth of oocyte. LH regulates synthesis of 17α,20β dihydroxy 3 pregnone 2 one (17,20β-P) in granulosa and it induces oocyte maturation by activation of maturation promoting factor (MPF). 17,20β-P acts as Maturation inducing hormone (MIH), which down regulates the concentration of cAMP that activates IP3 kinase and sequentially other signaling cascade and ultimately leads to the activation of MPF.

Gonadotropin hormone (GTH) and its role in fish reproduction

Endocrine mechanism of fish, particularly the function of pituitary is always linked with the external factors by a certain network of the central nervous system. GnRH activity in the hypothalamic region indicates that it is the key regulator of hormonal cascade during reproduction of teleost fish (Ball, 1981; Peter, 1983). Fish GnRH is a decapeptide hormone. This 10 amino acid structure may varies considerably from other vertebrates, but conserved peptide length and in amino terminus and carboxy terminus, suggesting terminal part to be functionally critically important. GnRH receptor is a G-protein coupled receptor with extracellular amino-terminal, transmembrane and intracellular carboxy-terminal domain. Binding of GnRH receptors on fish pituitary gonadotroph cell membrane by GnRH follows several intracellular signal cascades; ultimately leading to release of GTH.

Until 1980s it was believed that a single GTH regulate steroidogenesis, vitellogenesis and oocyte maturation in female and spermatogenesis and spermeation in male fish (Burzawa-Gerard, 1982). But late 1980s this concept was changed that two kinds of gonadotropin invented in salmon fish (Suzuki *et al*., 1988a,b,c; Kawauchi *et al*., 1989; Swanson, 1991). Both gonadotropins are composed with common α and specific β subunits. The similarity between GTH Iβ and FSHβ, GTH IIβ and LHβ were based on sequence homology (Querat, 1994, 1995; Yaron *et al*., 2003).

In vertebrates, synthesis and release of gonadotropins (FSH and LH) is a complex signaling process, which is regulated by the hypothalamus-pituitary-gonad axis. It was previously established that GTH I and GTH II are both same as FSH and LH (Suzuki *et al*., 1988a). FSH bind with specific receptor FSHR on granulosa cell layer and stimulate oocyte growth and synthesis of estradiol 17β and LH stimulate synthesis of 17, 20β-P in granulosa cell layer of teleost fishes (Suzuki *et al*, 1988c; Nagahama, 1994; Zhang *et al*., 2015). FSH regulates early developmental stages of oocytes such as previtellogenesis and vitellogenesis phase but LH induces late developmental stages of oocytes such as ovulation (Kim *et al*., 2011). Several reports reveled that FSH and LH secretion from pituitary gland is regulated by the GnRH and Dopamine (Chang *et al*., 2009; Zohar *et al*., 2010; Kim *et al*., 2011).

In salmon, plasma FSH levels are high during the vitellogenic and spermatogenic stage but LH reaches its maximum level during spawning stage (Suzuki *et al*., 1988c; Swanson *et al*., 1991). Plasma FSH level were low in immature stage but increased in vitellogenic stage and

level were decreased during maturation and spawning in female coho salmon (Kawauchi *et al*., 1989; Swanson *et al*., 1991). So, different studies showed that FSH has an important role in ovarian development and spermatogenesis, while LH play major role in oocyte maturation and spawning (Planas and Swanson, 1995).

Different studies showed that GnRH stimulates FSH production from pituitary in early vitellogenic stage of oocytes, whereas FSH stimulates the aromatase activity which actually responsible for the conversion of testosterone to estradiol-17β in granulosa follicular cell of oocytes (Fig. 1). Then estradiol 17β enhanced vitellogenesis process in liver and this vitellogenin accumulated into oocyte. GnRH also stimulates the synthesis of LH in post vitellogenic and pre-spawning stage for oocytes maturation. Actually LH helps to production of 17,20β-P (maturation inducing hormone) in granulosa cells which stimulates oocyte maturation (Kagawa *et al.*, 1981; Sundararaj and Nath, 1981; Nagahama, 1994; Heidari, 2010).

Receptors for FSH and LH, are single polypeptide chains that are members of the rhodopsin-β_2-adrenergic receptor subfamily of G-protein coupled receptors having N-terminal extracellular domain, a serpentine region containing seven transmembrane segments and an intracellular C-terminal (Ascoli *et al.,* 2002; Smits *et al.,* 2003). One of the more unique features of the glycoprotein hormone receptors is the extracellular domain which consists of three regions; a N-terminal cysteine-rich region, followed by a series of nine leucine rich repeats and a C-terminal cysteine-rich region known as the hinge region, adjacent to the transmembrane segments (reviewed by Swanson *et al.,* 2003).

The presence of multiple gonadotropin receptors in fish was initially suggested by binding studies of salmon FSH and LH to the membrane of isolated theca interstitial layer and granulosa cells of the ovary (Miwa *et al.,* 1994). A two- receptor model was proposed where by one receptor (GTHRI) did not discriminate between FSH and LH, while the second type of receptor (GTHRII) was highly selective for LH. Oba *et al.,* (1999 a,b) first cloned cDNAs for two fish gonadotropin receptors. This was followed by cloning of FSH and LH receptors in African (Bogerd *et al.,* 2001; Vischer and Bogerd 2003) and channel catfish (Kumar *et al.,* 2001 a,b), and an FSH receptor in zebrafish (Laan *et al.,* 2002). Although FSH is more potent than LH as a ligand, recent studies suggested that FSH receptors are not selective. LH receptors on the other hand are highly selective for LH. These data are consistent with the proposed two-receptor model and the observed overlap in activities of FSH and LH in several fish species. Gene

expression studies in channel catfish females suggest that FSH receptor transcript increase during recruitment of oocytes into secondary growth just after the spawning season, and LH receptor transcript increases at the time of spawning (Kumar *et al.,* 2001 a,b).

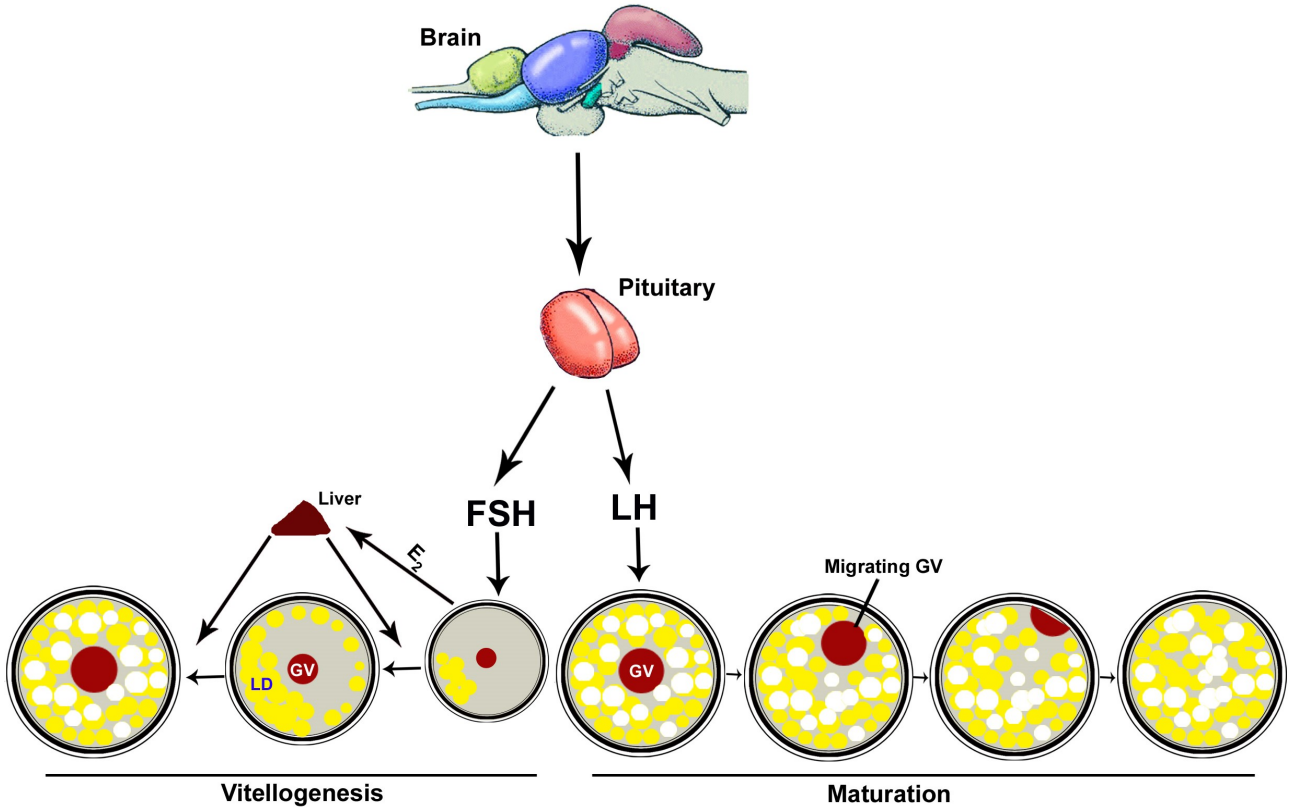

Fig. 1 Mechanism of gonadotropin regulated oocyte growth and maturation.

Gonadotropins and oocyte growth:

Oocyte growth and development is very complex process in teleost. This process is regulated by the gonadotropins, steroids and other factors. Most of the histological study shows that oocyte growth occurred in different developmental stages such as primary growth, cortlcal alveolar stage, vitellogenic and postvitellogenic etc in teleosts (Tyler and Sumpter, 1996; Correiro *et al*., 2003). Major event in oocyte growth is vitellogenesis, where vitellogenin are accumulated into oocyte and sequentially size also increased. Several studies showed that FSH was plays very crucial role in oocyte growth (Nagahama, 1994; Devlin and Nagahama, 2002; Yaron *et al*., 2003). E_2 is one of the major steroids for oocyte growth and its production and secretion regulated by the FSH (Wallace, 1985; Kawauchi *et al*., 1989; Suzuki *et al.,* 1988c; Pakdel *et al.,* 1989, 1991; Swanson *et al.*, 1991; Nagahama, 1994; Devlin and Nagahama, 2002).

The major event takes place during vitellogenesis is sequestration and packaging of vitellogenin, a hepatically derived protein into yolk protein.17β-estradiol, in most cases provided by the ovary under the influence of gonadotropin, moves to the vascular system and stimulate the synthesis and secretion of vitellogenin. Nagahama and his associates proposed a two-cell-type model in the production of follicular 17β-estradiol in amago salmon (Kagawa *et al.,* 1985; Nagahama 1983, 87). In this model, the thecal cell layer, under the influence of gonadotropin, synthesize aromatizable androgen, predominantly testosterone, which then diffuses into the granulosa cell layer where the aromatase is exclusively restricted (Kagawa *et al.,* 1985; Nagahama, 1987a; Adachi *et al.,* 1990). Regarding gonadotropin influence of increase in aromatase activity in fish granulosa cells it is now known that in salmonids FSH is elevated during vitellogenesis, whereas LH appears during final oocyte maturation in females (Gomez *et al.,* 1999; Prat *et al.,* 1996). Moreover, in salmonids, P450arom enzyme activity (Kagawa *et al.,* 1983) and its mRNA level (Tanaka *et al.,* 1992) also increases during the vitellogenic oocyte growth. These results suggest that FSH regulate 17β-estradiol production through stimulating P450arom gene expression and enzyme activity during vitellogenic period. In a recent study Kagawa *et al*., (2003) demonstrated that LH from red sea bream (*Pagrus major*) pituitary, but not FSH stimulated both aromatase activity and p450 arom gene expression. *In vitro* experiments have shown that GTH induces P450 arom activity in ovarian follicles of gold fish (Kagawa *et al.,* 1984) and medaka (Nagahama *et al.,* 1991) but not in amago salmon (Young *et al.,* 1983). However, it remains unclear whether FSH or LH can stimulate the expression and activation of P450arom in teleosts because most *in vitro* studies have been performed with either partially purified chinook salmon GTH (SGG 100) (Young *et al.,* 1983) or heterologous GTH such as human chorionic gonadotropin (Kagawa *et al.,* 1984) and pregnant mare serum gonadotropin (Nagahama *et al.,* 1991).

Different studies reported that pre-spawning stage; FSH levels were significantly high for oocyte development (Kawauchi *et al*., 1989; Swanson *et al*., 1991). FSH stimulates follicular cells to secrete E_2. During different ovarian development of fish, E_2 play the major role to synthesis of vitellogenin (Vtg) in liver (Flouriot *et al*., 1997; Miura *et al*., 2007; Nagahama and Yamashita, 2008). Egg yolk lipoprotein, vitellogenin is transported to oocyte through blood and accumulated into oocytes via receptor mediated endocytosis (Campbell and Idler, 1976; Campbell, 1978; Crim and Idler, 1978; Ng and Idler, 1978; Lubzens *et al*., 2010; Kagawa, 2013).

It was reported that teleost have three kinds of vitellogenin (Vtg A, Vtg B and Vtg C) (Sawaguchi *et al*., 2006). The volume of oocytes is increased hundred times during primary oocyte growth. Nucleus and cytoplasm ratio also decreased at that time (Wallace and Selman, 1981). It was also reported that during primary oocyte growth, gonadotropin has no role but in transition into secondary oocyte growth regulated by the gonadotropin (Khoo, 1979).

In red seabream, LH is considered to be more important than FSH and hence, LH might regulate these processes (Kagawa *et al*., 1998; Gen *et al*., 2000; Kagawa *et al*., 2003). The GTHs might also employ E_2 to differentially regulate both vitellogenin and estrogen receptors during vitellogenesis (Flouriot *et al*., 1997). Thus, the regulation of E_2 production by GTHs is considered to be important during vitellogenesis. In this regard, the primary target of FSH/LH is the ovarian cytochrome P450 aromatase as it is considered to be one of the rate-limiting enzymes for E_2 biosynthesis (Tanaka *et al*., 1992; Gen *et al*., 2001; Kagawa *et al*., 2003).

Gonadotropins and oocyte maturation

Oocyte maturation is a crucial sequential multistep process where chromosomal condensation, assembly of meiotic spindle, germinal vesicle breakdown occurred. After completion of oocyte growth, different factors such as gonadotropins, maturation inducing hormone (MIH) and maturation promoting factor (MPF) induce oocyte maturation process (Nagahama, 1987c; Redding and Patino, 1993). Oocyte maturation means reinitiation and completion of meiosis I and other sequential changes occur in oocytes. During oocyte maturation, germinal vesicle (GV) shifted towards animal pole of oocyte and finally released from oocytes. This is known as germinal vesicle breakdown (GVBD) which is the indicator of final oocyte maturation (FMO). During this time first polar body is migrated and finally disappears from oocytes (Masui and Clarke, 1979; Yoshikuni and Nagahama, 1991; Nagahamam 1994; Yaron, 1995; Nagaham and Yamashita, 2008).

After completion of vitellogenic stage, oocyte enters into postvitellogenic stage where oocyte maturation occurs with the control of gonadotropin i.e., LH (Khan and Thomas, 1999; Nagahama, 1997; Senthilkumaran *et al.,* 2004*;* Planas and Swanson, 1995; Pramanick *et al*., 2013). It was previously reported that during postvitellogenic stage, LH levels become high in teleosts (Planas *et al*., 2000; Swanson *et al*., 2003). LH is more potent than FSH during oocyte maturation in red seabream (Kagawa *et al*., 1998; Gen *et al*., 2000; Kagawa *et al*., 2003; Senthilkumaran *et al*., 2004). After binding of LH with its receptor on granulosa cell, stimulates

series of events such as synthesis of maturation inducing hormone (MIH), resumption of meiosis and maturation of cytoplasm (Nagahama, 1997; Planas *et al*., 2000; Patiño *et al*. 2001; Patino and Sullivan, 2002). In most of teleost, 17α, 20β-dihydroxy-4-pregnane-3-one (17, 20β-P) acts as MIH (Nagahama, 1997) but 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S) acts as MIH in sciaenid fish (Thomas, 1994). LH stimulates the synthesis of 17, 20β-P (MIH) in granulose cell of follicle. MIH activates maturation promoting factor (MPF) and ultimately final oocyte maturation (FMO) occur.

Maturation inducing hormone (MIH)

Maturation inducing hormone (MIH) has an important role in oocyte maturation (Bhattacharyya *et al*. 2002; Schmitt and Nebreda 2002). Several studies showed that *in vitro* effects of various steroids on oocyte maturation (Jalabert, 1976; Iwamatsu, 1978; Fostier *et al., 1983;* Nagahama *et al.,* 1983; Greeley *et al.,* 1986; Canario and Scott, 1988; Trant and Thomas, 1988; Nagahama, 1994). It was reported that C21 steroid has the potential inducing effect on oocyte maturation than other two steroid groups (C18 and C19). C21 steroid are 20β dihydroprogesterone, 17, 20β-P, 17α, 20β, 21- trihydroxy-4-pregnen-3-one (20b-S) and 11-deoxycorticosterone (DOC). All this steroids have effective role in oocyte maturation (Jalabert 1976; Sundararaj & Goswami 1977; Young *et al.* 1982; Goetz 1983; Nagahama *et al.* 1983; Nagaham and Yamashita, 2008) but among these steroids 17, 20β-P has most effective in oocyte maturation (Nagahama *et al.* 1983; Nagaham and Yamashita, 2008). In Amago Salmon, 17, 20β-P level was high in plasma during sexual maturity but very low level in plasma during vitellogenic female (Young *et al.,* 1983c; Nagaham and Yamashita, 2008). Previous studies showed that 17, 20β-P is a naturally occurring MIH in many other teleost (Nagahama *et al.,*1994) but other kinds of naturally occurring MIH (20β-S) also identified later in Atlantic croaker and spotted sea trout (Trant & Thomas, 1989; Thomas, 1994; Nagaham and Yamashita, 2008).

Steroidogenesis

In teleosts as well as other vertebrates, gonadotropins (FSH and LH) regulate gonadal development and oocyte maturation by synthesis of steroids (Suzuki *et al*., 1988a; Planas *et al*., 1993; Li and Ford, 1998; Yoshiura *et al*., 1999; Swanson *et al*., 2003) from the follicular cells surrounding the oocyte (Nagahama, 1994; Heidari, 2010). Steroids synthesis is a very complex sequential process, which is occurred in theca (outer follicular cell) and granulosa (inner follicular cell) of oocytes (Senthilkumaran *et al.,* 2004; Nagaham and Yamashita, 2008). It is

reported that oocyte growth regulated by E_2 (Nagahama, 1994; Devlin and Nagahama, 2002) and the oocyte maturation by 17, 20β-P in teleosts (Nagahama, 1997).

Follicle stimulation hormone (FSH) stimulates E_2 production in follicular cell of oocytes via different intermediate steroid synthesis and gene activation (Senthilkumaran *et al.,* 2004; Nagaham and Yamashita, 2008). In theca follicular cell testosterone is synthesized from the cholesterol by the action of different gene product. After that testosterone is diffused into granulose cell where gonadotropin (FSH) has vital role to increase aromatase activity for the production of 17β-estradiol (E_2) (Kagawa *et al,* 1982; Nagahama, 1987a; Nagahama *et al.,* 1994; Senthilkumaran *et al.,* 2004; Nagaham and Yamashita, 2008). In teleost, it was documented that plasma E_2 level increase in vitellogenic stage and decrease in maturational stage (Bromage *et al.*, 1982; Kagawa *et al.*, 1983; Shimizu *et al.*, 1985; Sakai *et al.*, 1988, Heidari, 2010; Pramanick *et al*., 2013).

Luteinizing hormone (LH) regulates 17,20β-P production in granulosa cell prior to oocyte maturation. 17,20β-P biosynthesis occurred by series of process from cholesterol. Cholesterol side chains were cleaved by P450scc gene product and produce precursor of steroid, prognenolone. 17α hydroxypregnenolone or progesterone is converted from prognenolone by the action of 17α hydroxylase and 3β HSD respectively. Ultimately, progesterone and 17α hydroxypregnenolone are transformed into 17α hydroxyprogenterone in granulose cell which produced 17,20β-P through the action of 20β HSD (20β hydroxysteroid dehydrogenase) (Senthilkumaran *et al.,* 2004; Nagahama and Yamashita, 2008). In several fish species it was showed that plasma MIH start to increase in late vitellogenic stage and a sharp increased peak in postvitellogenic stage (Scott *et al*., 1980; Pankhurst and Thomas, 1998; Sen *et al*., 2002; King and Pankhurst, 2003; Pramanick *et al*., 2013).

Steroidogenic shift from E_2 to 17,20β-P

It is well known that in teleosts, E_2 and 17,20β-P are regulates oocyte growth and maturation respectively (Fig. 2). After completion of vitellogenic stage, a drastic change occurred in steroid level (i.e., a shift from E_2 to 17,20β-P) during postvitellogenic stage of teleosts. This drastic change is pre-required for oocyte maturation. This shift performed by two steps, firstly production of precursor steroid in theca cells and ultimately final steroid mediator synthesis in granulosa cells (Senthilkumaran *et al*., 2004; Nagahama and Yamashita, 2008).

For precursor steroid production, 17α hydroxylase and 17,20 lyase have an important role in teleosts. This is encoded by single gene, P450c17 (Chung *et al*., 1987; Picado-Leonard and Miller, 1987). This gene products activity was regulated by cAMP dependent protein kinase phosphorylation and dephosphorylation in teleosts (Zhang *et al*., 1995; Pandey *et al*., 2003; Senthilkumaran *et al*., 2004).

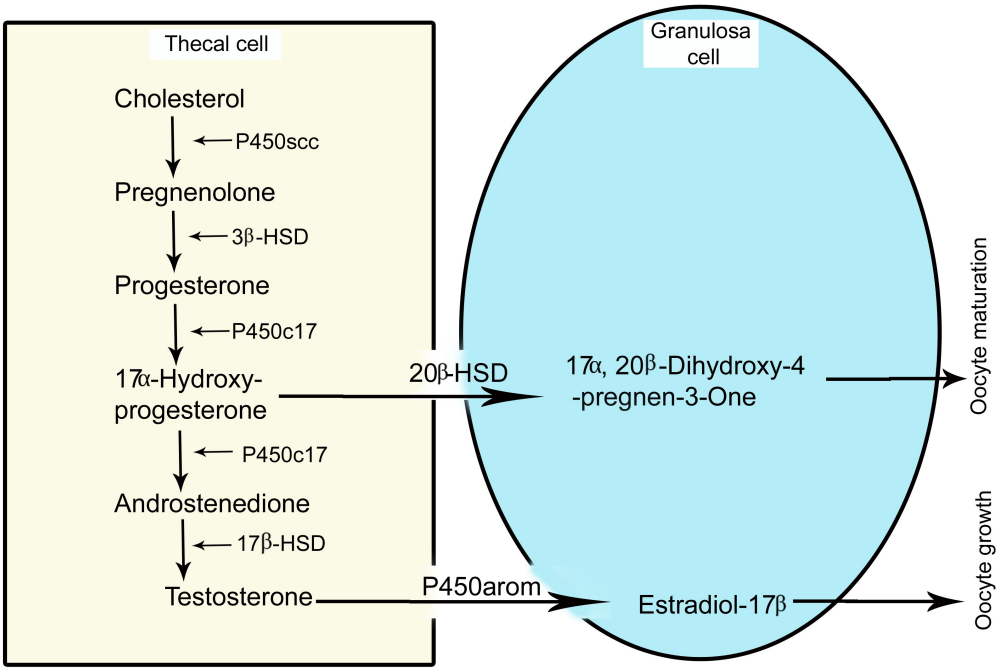

Fig. 2 Two-cell type model: schematic representation of shift in steroidogenesis in ovarian follicles.

During oocyte maturation 20β HSD is a key enzyme to synthesis 17,20β-P (Senthilkumaran *et al*., 2002) while P450 arom decreased E_2 production after vitellogensis(Yoshiura *et al*., 2003). It was reported that Ad4BP/SF1 and CERB are transcription factors that binds on promoter region of P450 arom and 20β HSD gene, regulates the production of E_2 and 17,20β-P respectively. Several authors demonstrated that amounts of P450 aromatase and Ad4BP/SF1 increased parallely during vitellogenic but decreased in late vitellogenic stage (Fukada *et al*., 1996; Watanabe *et al*., 1999; Yoshiura *et al*., 2003). However, during spawning stage 20β HSD gene expression was high (Senthilkumaran *et al*., 2002).

***Role of cyp19a1* and SF-1 in ovarian function**

Cytochrome P450 aromatase (*cyp* 19 family) has an important role in vertebrates reproduction. It was documented that aromatase is found in different type of tissues such as gonad, brain, liver, skin, kidney, bone etc (Harada *et al*., 1999; Simpson *et al*., 2002; Callard *et al*., 1993; Chiang *et al*., 2001; Kishida and Callard, 2001; Chang *et al*., 2005; Choi *et al*., 2005; Fenske and Segner, 2004; Goto-Kazeto *et al*., 2004; Kitano *et al*., 1999; Sawyer *et al*., 2006; Strobl-Mazzulla *et al*., 2003; Tchoudakova and Callard, 1998; Tong *et al*., 2001; Wong *et al*., 2006; Jeng *et al*., 2012). In fish two kinds of aromatase (*cyp19a1* and *cyp19b1*) were found. *cyp19a1* mRNA is predominantly expressed on gonads and *cyp19b1* mRNA is predominantly on brain in many teleosts (Barney *et al*., 2008; Kishida and Callard, 2001; Kobayashi *et al*., 2010; Rasheeda *et al*., 2010; Uno *et al*., 2012).

During steroidogenesis, *cyp19a1* has significant role for 17β-estradiol biosynthesis from androgen in granulosa cell (Guiguen *et al*., 1999). The expression of *cyp19a1* mRNA significantly high during vitellogenic stages of oocyte (Goto-Kazeto *et al*., 2004; Cheshenko *et al*., 2008; Rodriguez-Mari *et al*., 2005; Nakamura *et al*., 2005; Nunez and Applebaum, 2006). Several studies showed that a significant correlation of increased ovarian E_2 and *cyp19a1* expression occurred during vitellogenic stage in most of the teleosts (Kagawa *et al*., 1984; Kobayashi *et al*., 1988; Tan *et al*., 1986; Tanaka *et al*., 1992; Fukada *et al*., 1996; Chang *et al*., 1997b; Jensen *et al*., 2001; Panter *et al*., 2004; Gen *et al*., 2001; Goto-Kazeto *et al*., 2004; Jeng *et al*., 2005; Choi *et al*., 2005).

Steroidogenic factor 1 (SF-1) is an orphan receptor present in nucleus. It has an important role in gonadal development via. aromatase expression in fish (Dalla Valle *et al*., 2002; Kazeto *et al*., 2001; Das *et al*., 2013). SF-1 acts as a transcription factor, which regulates expression of StAR protein and gonadal aromatase ((Lynch *et al*., 1993; Sugawara *et al*., 1996; Leers-Sucheta *et al*., 1997; Hu *et al*., 2001; Senthilkumaran *et al., 2004*). It has been documented that SF-1 binds to the promoter region of *cyp19a1*in zebra fish and gold fish (Tchoudakova *et al*., 2001; Tong and Chung, 2003). Fitzpatrick *et al*. reported that decreased of sf1 mRNA leads to reduction of *cyp19a1* transcript (Fitzpatrick *et al*., 1997). In *Labeo rohita*, SF1 transcript and protein regulates expression of *cyp19a1*and aromatase activity in ovary with the stimulation of HCG (Roy Moulik *et al*., 2016).

Maturation promoting factor (MPF)

Maturation promoting factor (MPF) activation occurred during postvitellogenic oocyte developmental stage. The indicator of MPF activation is shifting germinal vesicle (GV) position towards periphery of oocyte. In fish, MIH binds to the oocyte surface receptor (mPRα) which activates inhibitor G protein (G_i). This G_i decreased intracellular cAMP level as well as protein kinase A (PKA) activity and finally MPF activation occurred. In fish MPF is consists of two components, cdc2 (34 kDa) and regulatory protein cyclin B (46-48 kDa), which is universal for most of the species (Yamashita *et al.,* 1992; Balamurugan and Haider, 1998; Basu *et al.,* 2004). In fish two types of cdc2 are present, 35kDa (inactive) and 34kDa (active). Phosphorylation and dephosphorylation on Thr14, Tyr15 and Thr161 of cdc2 regulates MPF activity .cdc2 is activated by Phosphorylation on T161 (T) of cdc2, after binds with cyclin B with the help of cdk activating kinase (CAK) and ultimately MPF activation occurred (Fig. 3). It was reported that in goldfish, cyclin B protein is absent in immature oocytes while in MIS induced mature oocytes, cyclin B protein was synthesised from stored mRNA of its. This cyclin B binds with pre-existing cdc2 protein and MPF activation occurred prior to cyclin B bound cdc2 phosphorylation (Nagahama and Yamashita, 2008).

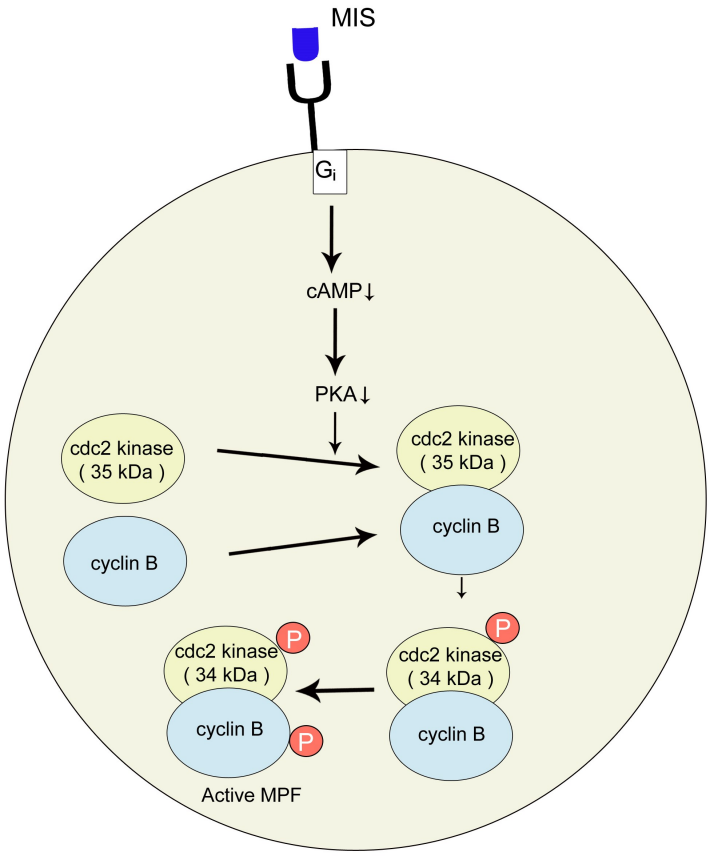

Fig. 3 Activation of maturation promoting factor (MPF).

Signaling events upon hormonal stimulation of oocyte maturation

Signal transduction cascade of oocyte maturation is a complex process in all vertebrates including fish. After completion of vitellogenic stage, oocyte under goes in maturation process by the induction of MIH. Oocyte maturation leads to condensation of chromosome, metaphase spindle formation, polar body extrusion and germinal vesicle breakdown (GVBD), finally oocyte become ready to fertilize (Masui and Clarke, 1979; Masui, 2001; Contreras *et al*., 2003).

One common signaling event initiated upon hormonal induction of oocytes maturation in lower vertebrates and starfish is a rapid decrease in oocytes 3'-5' cAMP levels by modulation of heterologous gap junction coupling between the follicle cells and the oocytes, or by both methods (Sadler and Maller, 1982; Meijer *et al.,* 1987; Ferrel, 1999; Yoshizaki *et al.,* 2001; Webb *et al.,* 2002a,b). It has been hypothesized that decreasing cAMP concentrations in the oocytes is sufficient to promote maturation in *Xenopus* and mouse oocytes (Andersen *et al.*,

1998; Conti *et al.,* 2002), presumably through inhibition of cAMP- dependent protein kinase (PrKa) activity, which leads to MPF activation (Duckworth *et al.,* 2002).

Meiotic arrest is maintained by constitutive signals that elevate cAMP and prevent maturation. These signals are endogenous to the oocyte, and include Gαs and Gβγ, which may be signaling independent of or in response to a G proteincoupled receptor (GPCR) (perhaps a member of the mPR family). Steroids overcome this inhibition, allowing meiosis to progress. Steroid-bound mPR might rapidly activate Gαi, resulting in a decrease in intracellular cAMP. mPR-mediated activation of the PI3K or MAPK pathways might also occur. Steroid binding to the constitutively activated mPR could shut off signaling, which would also lower intracellular cAMP. These inactivated receptors could even "switch classes" from Gαs to Gαi signaling, resulting in even greater reductions in intracellular cAMP. Thus secondary signals such as Mos, MAPK, and cdc2 are activated. These signals then promote each other in a powerful positive-feedback loop, resulting in germinal vesicle breakdown (GVBD) and maturation.

Activation of Akt is necessary and sufficient to induce oocyte maturation in star fish, *Xenopus* and mouse (Andersen *et al.,* 1998; Okumura *et al*., 2002; Hoshino *et al.,* 2004). One potential downstream of PI3K / Akt is the activation of phosphodiesterase (Pdes), the enzyme that degrades and inactivates cyclic AMP. Activation of oocyte-specific PDE3 by PI3K / Akt was found to mediate insulin like growth factor-induced, but not progesterone-induced oocyte maturation.

Activation of mitogen activated protein kinase is universal during oocytes maturation, although its requirement for GVBD is uncertain (Maller, 1998; Yamashita, 1998; Ferrel, 1999; Nebreda and Ferby, 2000). In *Xenopus* oocyte over expression of Mos and MAP kinase kinase or constitutively active MAP kinase 1 / 3 induces GVBD in presence of various MAP kinase inhibitors (Gross *et al.,* 2000). Activation of MAP kinase in follicle cells but not in oocytes is necessary for oocyte maturation in mouse (Su *et al.,* 2003) activation is neither necessary nor sufficient for inducing oocyte maturation in gold fish (Yamashita, 1998; Kajiura-Kobayashi *et al.,* 2000). Pace and Thomas, (2005), reported that in Atlantic croaker inhibition of MAP kinase 1/3 activity using PD98051 or U0216 had no effect on GVBD.

Several investigators reported that phosphatidylinositol 3 kinase (PI3 kinase) has important role in signal transduction cascade of oocyte maturation in most of the vertebrates. PI3 kinase activation is essential for steroid and growth factors induced oocyte maturation (OM)

(Anderson *et al*., 1998; Sadler and Ruderman, 1998; Weber and Sullivan, 2001; Ju *et al*., 2002; Pace and Thomas, 2005; Paul *et al*., 2009; Pramanick *et al*., 2014) while in xenopus PI3 activation is not essential for progesterone induced oocyte maturation (OM) (Liu *et al*., 1995; Mood *et al*., 2004). Pramanick el at., (2014) reported that blocking PI3 kinase activity by inhibitors (Wortmannin and LY294002) failed to induce GVBD in 17,20β-P stimulated oocyte maturation (OM) of *T. ilisha*.

Endocrine disrupting chemicals (EDC)

According to the U.S. Environmental Protection Agency (EPA), endocrine-disrupting chemicals (EDCs) defined as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process. (Diamanti-Kandarakis *et al*., 2009)". As such, understanding the biological impact of EDCs, for last two decades, investigators have grown interest to explore the mechanism of exerting the effect of such exogenous agents. Different types of endocrine disrupting chemicals are natural hormones, heavy metals, pesticides, herbicides, alkylphenol, organochlorines, phthalates, bisphenol A etc. in our environment (Huang *et al*., 2014). Numerous heavy metals such as mercury, cadmium, copper, lead, zinc and arsenic causes reproductive dysfunction in diverse fishes (Kirubagaran and Joy, 1988; Aditya *et al*., 2002; Pundir and Saxena, 1992; Jadhao *et al*., 1994; Munkittrick and Dixon, 1989; Ruby *et al*., 2000; Ebrahimi, 2007; Helena *et al*., 2008; Shukla and Pandey, 1984). EDCs exposures from numerous sources are reported which varying in different ways (Scholz and Mayer, 2008; Huang *et al*., 2014). EDCs functions through different receptors *viz.* nuclear, non-steroid, orphan, nonnuclear steroid hormone receptors and many more primarily thought to be prominent receptors for exerting actions (Diamanti-Kandarakis *et al*., 2009). EDCs salient role are also involved in various pathways of biological systems such as reproductive, endocrine, metabolic and neurological system (Fig. 4). It is noted that endocrine glands secret several types of hormones which helps in communicating cell-cell interactions via modulation of signaling pathways (Pramanick *et al.,* 2014). Very small alterations in the function of endocrine glands due to exposure of EDCs during developmental periods of any organisms may results in a number of unfavourable changes in growth, reproduction and behaviour. EDCs can mimic or antagonize the role of endogenous hormone which leads to detrimental physiological effects to an organism (Colborn *et al*., 1993; Huang *et*

al., 2014). Few EDCs have reported to exhibits inducible or inhibitory effects on sex steroid biosynthesis which directly associated with reproductive abnormalities in wildlife (Guillette *et al*., 1994; Jobling *et al*., 1998; Reeder *et al*., 2005).

In this review, we emphasize the role of mercury as EDC on reproductive biology of aquatic organism mainly fish species.

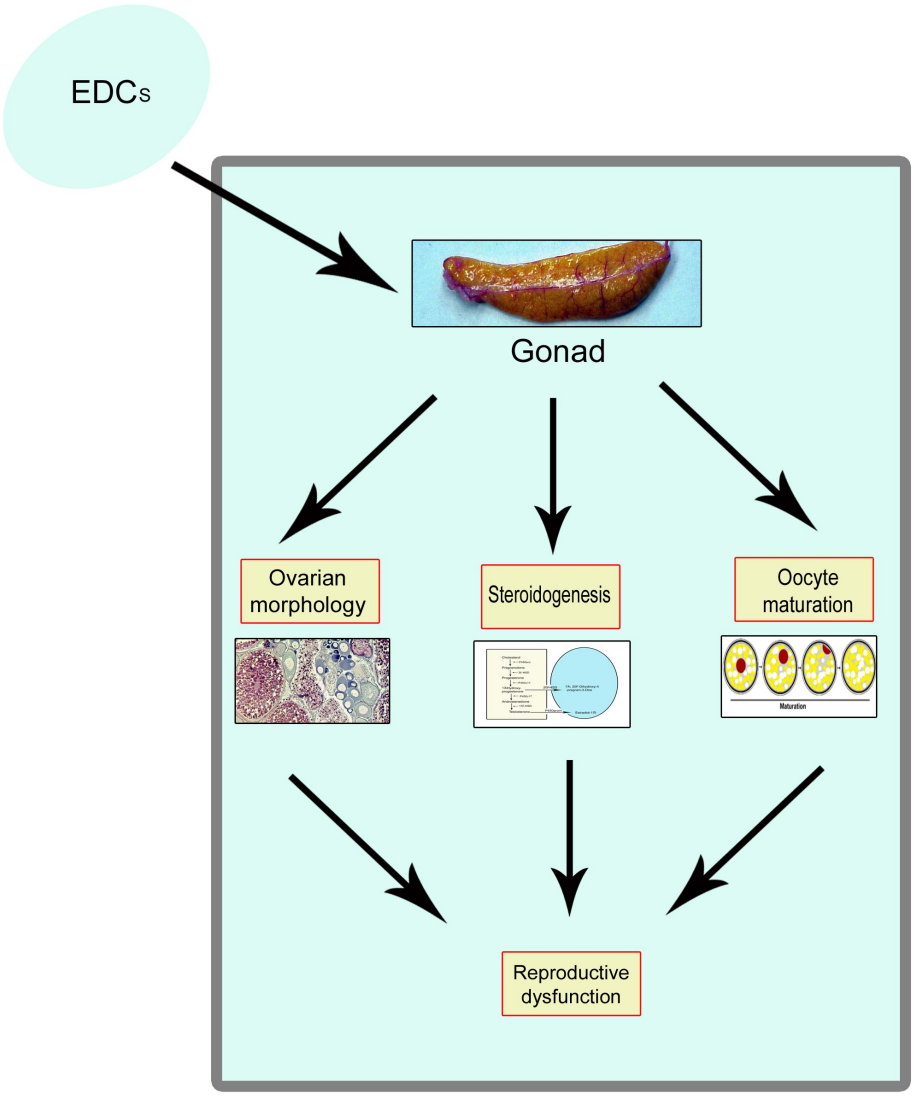

Fig. 4 Schematic representation of effect of EDC on reproductive dysfunction in fish.

Effect of EDC on teleost ovarian morphology

Several authors reported that EDC has an inhibitory effects on ovarian morphology and physiology (Ram and Sathyanesan, 1987a; Ram and Sathyanesan, 1987b; Dey and Bhattacharya,

1989; Crump and Trudeau, 2009). GSI value was significantly decreased in murrel (Ram and Joy, 1988; Ram and Sathyanesan, 1987a; Ram and Sathyanesan, 1987b), walking catfish (Kirubagaran and Joy, 1988; 1991), fathead minnows (Drevnick and Sandheinrich, 2003; Hammerschmidt *et al*, 2002) after exposed to inorganic mercury or mercurial fungicide at 10-50µg/l concentration while in nile tilapia, no significant changes occurred after exposed to methylmercury treatment (Arnold, 2000). Proportion of vitellogenic oocytes were decreased and increased atretic follicles number after EDCs exposure (Halden *et al*., 2011; Daouk *et al*., 2011). Several studies revealed that follicular cell degeneration (atresia) occurred after mercury treatment (Victor *et al*., 1986; Kirubagaran and Joy, 1988; Adams *et al*., 1999; Crump and Trudeau, 2009; Zhang *et al*., 2016) and other EDCs exposure (Sridevi *et al*., 2013; Chen *et al*., 2015) in ovary of fish.

Effect of EDC on Steroid synthesis

Most of the studies revealed that mercury alter the function of hypothalamus-pituitary-gonadal axis in fish (Crump and Trudeau, 2009; Liu *et al*., 2013). It was reported that in Zebra fish, very low level of GnRH transcript were found after mercury exposure and sequentially decreased the production of FSH and LH from pituitary (Drevnick and Sandheinrich, 2003; Zhang *et al*., 2016). Plasma steroids (11-ketotestosterone, T and 17β-estradiol) concentration were decreased in both male and female fathead minnow after exposed to methoxychlor (Ankley *et al*, 2001). Biosynthesis of steroid hormones was required several enzymes action, which is sensitive to mercury exposure. It was well established that 17β estradiol is a key regulator of oocyte growth in teleosts (Devlin and Nagahama, 2002; Qiao *et al*., 2013). Several authors documented that plasma 17β estradiol level decreased due to EDCs exposure including mercury (Mondal *et al*., 1997; Fynn-Aikins *et al*., 1998; Hammerschmidt *et al.*, 2002; Drevnick and Sandheinrich, 2003). As we know aromatase (*cyp19a1a*) enzyme convert androgen to 17β estradiol in granulosa cell of oocyte (Fenske and Segner, 2004), this enzyme activity was minimized due to mercury exposure (Hinfray *et al*., 2006; Crump and Trudeau, 2009). So, mercury directly reduced the aromatase activity and consequently decreased plasma 17β-estradiol level in fish (Crump and Trudeau, 2009). Gonadal aromatase enzyme is encoded by *cyp19a1a* gene. It has a significant role in 17β-estradiol biosynthesis. Several investigations revealed that activity of *cyp*19a1a was upregulated or downregulated by diverse types of EDC. EDCs altered *cyp19a1a* expression at transcription level. *cyp19a1a* gene expression or activity

was modulated by the diverse EDCs through several mechanism. Diverse EDCs including xenoestrogens, phytoestrogens, pesticides, fungicides and organotin compounds, causes inhibition of aromatase activity in fish (Ankley *et al*., 2005; Monod *et al*., 1993; Monteiro *et al*., 2000; Noaksson *et al*., 2003; Shimazaki *et al*., 2003; Cheshenko *et al*., 2008) as well as mammals (Adlercreutz *et al*., 1993; Campbell and Kurzer, 1993; Ibrahim and Abul-Hajj, 1990; Kellis and Vickery, 1984).

Effect of EDC on oocyte Maturation:

Maturation of oocytes were inhibited or enhanced by the exposure of EDCs. Bake *et al*. (2007) noticed that Bisphenol A (BPA) and diethylstilbestrol (DES) *in vitro* exposure, induced oocyte maturation in long chin goby. While other investigators reported that diverse exposure of xenoestrogens including BPA and DES on fish increased or decreased vitellogenesis process and inhibited oocyte maturation (Thomas, 1999; Christiansen *et al*., 2000; Scholz and Gutzeit, 2000; Sohoni *et al*., 2001; Jobling *et al*., 2003).

Sources of Mercury (Hg) in the environment

Hg in the environment occurs through both natural and anthropogenic sources. Natural sources of Hg include volcanoes, geologic deposits of Hg, and volatilization from the ocean. Anthropogenic sources include release of Hg during alkali and metal processing, incineration of coal, and medical and other waste, and mining of gold and Hg (US Geological Survey, 2000). The majority of environmental Hg exposure occurs through atmospheric deposition of Hg released from both natural and anthropogenic sources.

Mercury as an EDC

Mercury is one of the known oldest toxicant present in three chemical forms: (1) organic mercury, used as fungicides, herbicides, and wood preservatives; (2) inorganic mercury, for antiseptic and dermatological preparations; and (3) elemental mercury, used in the production of batteries, thermometers, and fluorescent tubes. It is non biodegradable and biomagnifying heavy metal.It is evident that 5% methylmercury in the aqueous environment may increased to 15% in phytoplankton, 30% in zooplankton, and more than 90% in fish via biomagnifications (Morel *et al*., 1998; Farina *et al*., 2013). Many studies demonstrated that Hg accumulates in endocrine tissues at surprisingly high concentrations (Berlin and Ullberg, 1963; Falnoga *et al*., 2000; Hahn *et al*., 1989, 1990; Kosta *et al*., 1975). Thus, it is justified to find out the potential effect of mercury on endocrine-system function.

Mercury has not exhibit any beneficial effects on animal but it has numerous hazardous effects on animal (Pannetier *et al*., 2016). Most of the studies were conducted with methyl mercury; it has several harmful effects on organ including gonads (Hammerschmidt *et al*. 2002; Drevnick and Sandheinrich 2003). Different forms of mercury contaminated water and food exposure to fish shows reproductive impairments (Kirubagaran and Joy, 1992; Beckvar *et al*., 2005; Batchelar *et al*., 2013) including reduced GSI and oocyte diameter (Friedmann *et al*., 1996), increased atretic oocytes and ovarian follicular cell apoptosis (Drevnick *et al*., 2006), reduced spawning success, size of spawning eggs, hatching success, steroidogenesis and delayed development of embryo (Shrivastava *et al*., 1988; Bano and Hasan, 1990; Drevnick and Sandheirnrich, 2003; Latif *et al*., 2001; Hammerschmidt *et al*., 2002; Depew *et al*., 2012; Huang *et al*., 2011; Ismail and Shahrizad, 2011).

Concluding remarks

This review presents a current picture of the hormonal regulation of oogenesis in many teleosts, steroids and gonadotropins-regulated oocyte growth, oocyte maturation and signal transduction mechanism of oocyte maturation. Furthermore, this review also demonstrates the negative role of mercury in the normal physiochemical process of teleost fish. Scattered reports are available regarding the role of Hg as endocrine disruptor, but the mechanism by which Hg can alter the normal endocrine pathway is yet to be elucidated. Therefore, further research is needed to understand the possible mechanism of Hg-induced reproductive impairments in a freshwater teleost fish.