Ovary, oogenesis

Oogenesis in its broadest sense is the process by which primordial germ cells become ova that are ready for fertilization. Oogenesis is fundamentally similar in vertebrates and differences between the taxa are superficial. Oogenesis can be broadly divided into six major steps: (1) formation of primordial germ cells (germline segregation), (2) transformation of primordial germ cells into oogonia (sex differentiation), (3) transformation of oogonia into oocytes (onset of meiosis), (4) growth of oocyte while under meiotic arrest (vitellogenesis), (5) resumption of meiosis (maturation), and (6) expulsion of ovum from its follicle (ovulation; Patino and Sullivan, 2002).

In spite of great diversity with an equally diverse array of reproductive strategies, teleost ovaries show a general structure (Nagahama, 1983). Ovaries of adult fish are generally paired structure surrounded by conjunctiva tunica and mesovarium attached to the body cavity on either side of dorsal mesentery. The posterior part of each ovary is prolonged by an oviduct connected to genital papilla. Ovaries are compartmentalized by numerous septa formed by folds of the germinal epithelium, called ovigerous lamellae, projecting into the ovarian lumen. These lamellae contain nest of oogonia, oocytes at early stages of entry into the meiotic prophase-I and follicles at various stages of oocyte and follicle growth and differentiation. In contrast to mammals, teleost oogonia keep on proliferating in adult females thus renewing the stocks of oocytes and follicles (Tokarz, 1978). Apart from this general scheme, there are some exceptions. Viviparous poecilids have only one ovary (Dodd, 1986) which presents specialized structures devoted to long
term preservation of spermatozoa (Jalabert, 2005). On the contrary, the ovaries of salmonids such as trout and salmon are not completely surrounded by the mesovarium and ovigerous lamellae that are open to the body cavity where mature oocytes are directly released during ovulation. At times, they remain for a while before being laid down through genital papilla (Jalabert, 2005).

The functional unit within the ovary is ovarian follicle, which has remarkably similar architecture in most teleosts. The developing oocyte is located in the centre of follicle and is surrounded by follicular cell layers. The granulosa and thecal layers are separated by a basement membrane. Between the surface of oocyte and granulosa layer there is an acellular layer, zona radiata the future egg chorion in which the microvilli originating from both oocyte and granulosa layer forms pore canals. Cytoplasmic gap junctions between granulosa cells and oocyte have been shown to form essential conduits between them for the transfer of nutrients and chemical messengers (Nagahama, 1983; Kessel et al., 1985; York et al., 1993). Granulosa cells form a regular monolayer, but the morphology and functions of these cells vary between animal and vegetal poles (Iwamatsu et al., 1994). Highly specialized cells at animal pole, the micropylar cell, closes up the micropyle and appears as a kind of funnel with a small opening on the oocyte side which will be just enough to allow a sperm to get through (Riehl, 1974). In comparison with a pre-ovulatory mammalian follicle, fish post-vitellogenic follicle has no antrum and most of its volume is occupied by oocyte with enormous amount of yolk and a large germinal vesicle (Jalabert, 2005).
**Oocyte growth - Vitellogenesis**

Following the onset of meiosis, significant growth of the oocyte begins after formation of ovarian follicle (folliculogenesis) and the structure of follicle remains essentially unchanged throughout follicular growth (Wallace and Selman, 1990; Patino and Takashima, 1995; Patino and Sullivan, 2002). Follicular growth generally occurs in two stages, pre-vitellogenic and vitellogenic. At the onset of pre-vitellogenic growth, oocytes arrest their meiosis at the diplotene stage of prophase-I. Large amounts of ribosomal RNA and much of the mRNA present in full-grown oocytes are produced during pre-vitellogenic growth. The content of certain mRNAs, such as vitellogenin receptor (VgR) and vitellogenin (Vg) processing enzyme mRNAs thought to peak during pre-vitellogenic growth (Wallace and Selman, 1990). Large amounts of glycoproteins are also synthesized and incorporated into alveoli (cortical alveoli) at the oocyte’s periphery. Deposition of lipids derived from circulating very low density lipoprotein generally begins during pre-vitellogenic growth (Prat et al., 1998).

Vitellogenic growth is characterized by accumulation of exogenously synthesized yolk proteins within the oocyte (Wallace, 1985). Vg is a large glycosphospholipoprotein (300-600 kDa) synthesized by liver, transported via the blood stream to ovary and taken up by growing oocytes (Patino and Sullivan, 2002). Using tracer technique, it has been demonstrated that Vg enters the ovarian follicle through capillaries of thecal layer. Then it passes through the basement membrane, the intercellular spaces of granulosa cells, finally through the pore canals of vitelline envelope and makes contact with oocyte

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*General introduction*
surface (Fig. 1). Vg binds to a specific VgR on the oocyte surface and internalization of Vg-VgR complex occurs at specialized areas of the oocyte membrane called coated pits (Stifani et al., 1990). Proteolytic cleavage of Vg into yolk proteins, lipovitellin and phosvitin occurs in the internalized vesicular bodies and lysosomal cathepsin D acts as yolk protein generating enzyme (Hiramatsu, 2002). Yolk proteins are stored as yolk globules or platelets throughout the ooplasm (Blazer, 2002). Natural induction of Vg synthesis in liver ultimately ensues from activation of the hypothalamic-hypophyseal-gonadal axis in response to endogenous as well as environmental cues. Reports in teleosts indicate that increased levels of plasma follicle stimulating hormone (FSH) induce follicular estradiol-17β (E₂) production, which in turn, stimulates hepatic Vg synthesis (Fig. 1; Specker and Sullivan, 1994). In some species, ovarian E₂ production may be regulated by luteinizing hormone (LH; Okuzawa, 2002). Estrone may contribute to induction of Vg by priming the liver to respond more strongly to E₂ (van Bohemen et al., 1982). Besides gonadotropins, several other hormones such as melatonin, thyroxine, insulin and growth hormones have been implicated in regulating vitellogenesis (Wallace, 1985; Senthilkumaran and Joy, 1995; Patino and Sullivan, 2002).
Fig. 1. Schematic representation of the hormonal regulation of vitellogenesis (adopted from Senthilkumaran et al., 2004).
Final oocyte maturation (FOM)

In many teleosts, plasma LH levels begin to rise (well known as pre-ovulatory LH surge) after the completion of vitellogenesis (Joy et al., 1998; Khan and Thomas, 1999). LH binds to its receptor on granulosa cells and stimulates a sequence of events including acquisition of maturational competence, production of maturation-inducing hormone (MIH), MIH-dependent resumption of oocyte meiosis and cytoplasmic maturation. Upon completion of the first meiotic division and release of first polar body, meiosis in the matured oocyte is arrested again at metaphase-II that is resumed during fertilization (Nagahama, 1994).

In general, the most obvious morphological change of FOM is migration of germinal vesicle towards animal pole and is often used as an indicator (Upadhyaya and Haider, 1986; Senthilkumaran and Joy, 2001). This process involves changes in cytoskeletal networks such as microtubule distribution (Lessmann et al., 1988). Following migration, several other processes occur in the nucleus and cytoplasm including germinal vesicle breakdown (GVBD) that indicates the completion of prophase-I, condensation of chromosomes, formation of spindle and expulsion of first polar body indicating the completion of meiosis-I (Nagahama, 1994). Cytoplasmic changes observed during GVBD include coalescence of lipid droplets and yolk globules with increased oocyte translucency (Goetz, 1983). In some teleosts, especially marine species, GVBD is accompanied by marked hydration involving Na⁺/K⁺-ATPase
dependent ion regulation, resulting in the swelling of oocyte (LaFleur and Thomas, 1991).

**Oocyte maturational competence**

Maturational competence refers to the ability of folliculated oocyte to resume meiosis when stimulated with MIH (Patino *et al.*, 2001). LH-dependent acquisition of maturational competence requires activation of protein kinase A pathway (Chang *et al.*, 1999), *de novo* protein synthesis and is also associated with increased mRNA levels of gap junction proteins, oocyte membrane MIH receptor (Thomas *et al.*, 2001 & 2004) and formation of homologous and heterologous gap junctions. Insulin-like growth factor (IGF) and activins may modulate or mediate LH-dependent maturational competence in teleost ovarian follicles. In red seabream, IGF-I is a strong inducer of maturational competence (Kagawa *et al.*, 1994) as well as gap junction contacts (Patino and Kagawa, 1999). IGF-I also induces membrane MIH receptor activity (Thomas *et al.*, 2001). Activins A and B induce maturational competence in ovarian follicles of zebrafish and co-treatment with follistatin suppresses it (Pang and Ge, 2000a).

**Three stage concept of oocyte maturation**

Several studies both in teleosts and amphibians, demonstrated that oocyte maturation is regulated by three major mediators, namely, gonadotropin (GTH), MIH and metaphase promoting factor (MPF; Nagahama, 1994 & 1997; Planas *et al.*, 2000; Senthilkumaran
et al., 2004; Nagahama and Yamashita, 2008; Fig. 2). Gonadotropic control of oocyte maturation has been best studied in fishes. Pituitary GTH induces the production of MIH by ovarian follicle cells in a two-step process (Nagahama, 1994). First, GTH induces the production of 17α-hydroxyprogesterone (17α-OHP) in thecal cells. Secondly, this precursor steroid is converted to 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP) by the granulosa cells.

Fig. 2. Schematic representation of hormonal regulation and three stage concept of oocyte maturation (adopted from Senthilkumaran et al., 2004).
Identification of MIH

Numerous studies have been conducted to test the effects of various steroids on the induction of FOM \textit{in vitro}. Among the C18, C19 and C21 steroids tested so far, the C21 steroids have been shown to induce FOM more potently than other two groups of steroids. $17\alpha$, $20\beta$-DP has been first identified as amago salmon MIH (Nagahama and Adachi, 1985). Subsequent reports designated it as MIH for several teleosts. Trant and Thomas (1989) identified a related MIH, $17\alpha$, $20\beta$, 21-trihydroxy-4-pregnen-3-one (20$\beta$-S), as a potent inducer of GVBD in a marine perciform, the Atlantic croaker. There is strong evidence that MIH is a steroid, specifically progesterone or its derivatives in fish species studied to date (Jalabert \textit{et al}., 1991). However, androgen has been shown to be produced by \textit{Xenopus} oocytes and induce FOM potently (Lutz \textit{et al}., 2001). Apart from these, corisol and catecholestrogens were also reported to induce FOM in Indian catfishes (Goswami and Sundararaj, 1974; Senthilkumaran and Joy, 2001). Although MIH is generally considered as mediator of LH-induced meiotic resumption, a number of other factors are also known to mediate or modulate this process. IGF-I (Kagawa \textit{et al}., 1994; Weber and Sullivan, 2000 & 2001), activins (Ge, 2000; Wu \textit{et al}., 2000), epidermal growth factor, transforming growth factor (Pang and Ge, 2002b) and others have been shown to induce FOM. Serotonin seems to negatively modulate MIH-dependent meiotic maturation in ovarian follicles of \textit{Fundulus heteroclitus} (Cereda \textit{et al}., 1998). Arachidonic acid (AA) and its metabolic products appear to regulate an unidentified step in the biochemical pathway leading to meiotic
maturation in ovarian follicles of the European seabass (Sorbera et al., 2001). However, neither AA nor its metabolites seem to influence MIH-dependent oocyte maturation in ovarian follicles of the Atlantic croaker (Patino et al., 2003).

**Mechanism of synthesis of MIH: Two-cell type model**

Using incubation of isolated follicular preparations, a two-cell type model has been proposed, for the first time in any vertebrate, for the production of MIH (Nagahama, 1994 & 1997). In this model, the thecal cell layers produce 17α-OHP from progesterone by the action of the enzyme 17α-hydroxylase. 17α-OHP traverses the basal lamina and is converted to 17α, 20β-DP in the granulosa cells where GTH acts to enhance the activity of 20β-hydroxysteroid dehydrogenase (20β-HSD), the key enzyme required for the conversion of 17α-OHP to 17α, 20β-DP (Fig. 3; Young et al., 1986; Nagahama, 1987). The GTH stimulation of thecal cells to produce 17α-OHP involves receptor-mediated activation of adenylate cyclase and formation of cAMP. The GTH action on 20β-HSD enhancement in granulosa cells has been found to be mimicked by forskolin, dbcAMP and by two phosphodiesterase inhibitors (Nagahama et al., 1985a; Kanamori and Nagahama, 1988). Subsequently, *in vitro* experiments using both transcriptional and translational inhibitors have demonstrated that GTH and cAMP induction of 20β-HSD activity involves new RNA and protein synthesis (Nagahama et al., 1985b).
Fig. 3. Schematic representation of two-cell type model in the shift in steroidogenesis occurring in fish ovarian follicles prior to oocyte maturation (adopted from Senthilkumaran et al., 2004).
Shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation

The ability of ovarian follicles to produce E$_2$ in response to GTH increases during oocyte growth, but rapidly declines with the onset of FOM in response to GTH (Kagawa et al., 1983; Joy et al., 1998; Kumar et al., 2000). It is also reported that testosterone production by thecal layer preparations during oocyte growth and maturation gradually increases in response to GTH and peaks during post-vitellogenic period (Kanamori et al., 1988). This capacity of thecal layers is maintained during the period of oocyte maturation and ovulation. Ovarian aromatase (oP450arom), enzyme that produces E$_2$, activity in granulosa cells increases during vitellogenesis and decreases rapidly with the onset of FOM in response to GTH (Yoshiura et al., 2003). This decrease in oP450arom activity seems to be coincident with decreased ability of follicles to produce E$_2$. On the other hand, immediately prior to oocyte maturation, ovarian follicles acquire increased capability to produce 17α, 20β-DP. Granulosa cells acquire the ability to convert 17α-OHP to 17α, 20β-DP well before the onset of maturation and an increase in 20β-HSD activity was noticed during FOM (Senthilkumaran et al., 2002). However, thecal cells do not develop the capacity to produce 17α-OHP until they receive a signal from GTH during oocyte maturation (Kanamori et al., 1988). Therefore, a decrease in C17-20 lyase activity and an increase in 20β-HSD activity appear to be the major factors responsible for massive production of 17α, 20β-DP during FOM (Nagahama, 1994 & 1997). Though it cannot be
generalized, the two-cell type model seems to hold good for many teleost species (Fig. 3).

**Mechanism of action of MIH**

Following synthesis by follicle cells, MIH travels across the zona radiata to reach the oocyte and induce FOM. Granulosa cells and oocyte are physically coupled through their microvillar-gap junctions and this coupling is known to be regulated developmentally and hormonally. Using chemical inhibitors of gap junctions, it is now established that the gap junctions are essential conduits for intra-follicular transport of MIH to oocyte in vertebrates (Patino and Purkiss, 1993; York *et al.*, 1993).

In fishes, microinjection of MIH into full-grown immature oocytes was ineffective in inducing GVBD while external application was effective suggesting the presence of MIH receptors on oocyte membrane. Progesterone, a MIH in amphibians apparently acted via membrane receptors (Fig. 2; Nagahama, 1997). Further, membrane receptors for 17α, 20β-DP and 20β-S have been found in different fish species (Patino and Thomas, 1990; Yoshikuni *et al.*, 1993; Thomas *et al.*, 2004) and subsequently cDNA clones were obtained in fishes as well as in other vertebrates (Zhu *et al.*, 2003a & b). A significant increase in 17α, 20β-DP and 20β-S receptor concentrations was noticed in ovaries of flounder and seatrout undergoing FOM. Consistently, *in vitro* treatment of ovarian follicle with GTH also raised the concentration of MIH membrane receptors.
This GTH induced elevation in membrane MIH receptor coincides with development of oocyte maturational competence (Patino and Thomas, 1990). The mechanism by which the binding of MIH to its receptor induces FOM was controversial. Based on the studies from fishes as well as from other vertebrates, utilizing phosphodiesterase inhibitors such as 3-isobutyl-1-methylxanthine and theophylline and adenylate cyclase activators such as forskolin and choletera toxin, it has been found that the 17α, 20β-DP induced FOM involves a transient decrease in oocyte cAMP level and subsequently cAMP signaling cascade involving protein kinase A (Jalabret and Finet, 1986; Haider, 2003). Further, using pertusis toxin, inhibitory G protein has been shown to involve in mediating the action of 17α, 20β-DP (Yoshikuni and Nagahama, 1994; Nagahama, 1997). On the other hand, a decrease in membrane diacyl glycerol and protein kinase C signaling is also thought to be involved in 17α, 20β-DP action (Smith, 1989).

MPF
A key event during 17α, 20β-DP induced oocyte maturation is formation and/activation of MPF in the cytoplasm of oocyte. Identification and purification of MPF in unfertilized amphibian eggs was a land mark in cell biology research (Masui and Clarke, 1979; Lokha et al., 1988). In contrast to GTH and MIH, MPF displays no species-specificity and acts not only in inducing meiotic maturation but also as a universal regulator of G2/M phase transition in the cell cycle of various organisms.
ranging from yeast to mammals (Nagahama and Yamashita, 2008). In all animals examined to date, including fishes, MPF is composed of two protein subunits: a protein having MPF kinase activity that is homologous to cdc2\(^+\) gene product of fission yeast referred to as p\(^{34\text{cdc2}}\) (cdc2) and a regulatory protein cyclin B (Yamashita, 1998). In general, MPF activity is controlled by phosphorylation and dephosphorylation of cdc2 on Thr14, Tyr15 and Thr161 after a complex formation with cyclin B. The cdc2 activity requires Thr161 phosphorylation, which is catalyzed by cyclin dependent kinase (CAK) consisting of cdk7 and cyclin H. phosphorylation of Thr14 and Tyr15 by Weel/Myt1 inactivates cdc2 even if Thr161 is phosphorylated. Dephosphorylation of Thr15/Tyr15 is catalyzed by cdc25 (Coleman and Dunphy, 1994; Morgan, 1995; Nurse, 2002).

Two models concerning activation of MPF have been proposed and investigated extensively in several vertebrate species. First, a constant level of cdc2 is maintained during oocyte maturation and cyclin B protein is newly synthesized from its stored mRNA in oocyte after a signal from MIH (Yamashita et al., 2000). The newly synthesized cyclin B immediately forms a complex with pre-existing cdc2, rendering the CAK to phosphorylate cdc2 on Thr161 thus leading to the activation of MPF. Since CAK can not phosphorylate monomeric cdc2, complex formation with cyclin B seems to be compulsory. Thus, synthesis of cyclin B protein in response to MIH is necessary and sufficient to induce oocyte maturation in goldfish (Katsu et al., 1993). This proposition was confirmed by the finding that MIH-induced GVBD is blocked by the

Secondly, immature oocytes of *Xenopus* and starfish consists inactive MPF called pre-MPF (Yamashita *et al.*, 2000). Here in this case, in pre-MPF, Thr161 and Thr14/Tyr15 of cdc2 are in phosphorylated state and since dephosphorylation of Thr14/Tyr15 is necessary to make MPF active. Hence, the action of MIH (progesterone in *Xenopus*) is to activate cdc25 that impairs the ability of Myt1 to phosphorylate Thr14/Tyr15 of cdc2 which leads to the activation of MPF (Smith and Nebreda, 2002; Haccard and Jessus, 2006).

The model proposed for goldfish is known to operate in several other fish species too including carp, catfish, zebrafish and also in lamprey giving the impression that absence of pre-MPF is common to fish (Yamashita *et al.*, 2000). But later on, in fresh water perch (Basu *et al.*, 2004) and trout (Qiu *et al.*, 2007) pre-MPF has been found in immature oocytes and the activation of MPF as in *Xenopus* seems to be the key event to induce oocyte maturation in these species although this contention needs further clarification. In between these goldfish and *Xenopus* models, the presence of low levels of pre-MPF has been identified in the axolotl (Vaur *et al.*, 2004) immature oocytes. Slow and progressive dephosphorylation of Thr14/Tyr15 of cdc2 is the turning point in this species. However, in spite of differences in the formation and activation of MPF from species to species, the molecular structure and functions are common in all eukaryotes (Yamashita *et al.*, 2000; Nurse, 2002).
Testis, spermatogenesis

A great diversity do exists in the morphology of testis. In agnathans, testicular tissue is present in a single cord and in hagfish it is common for this cord to contain ovarian tissue too (Gorbman, 1983). Germ cells develop in follicles which eventually rupture to release sperm into body cavity. In elasmobranches, testes are paired structures within which germ cells and their supportive Sertoli cells divide and develop together in discrete spherical units called spermatocysts (ampullae; Dodd, 1986). Teleost testis is typically paired and has a lobular or tubular organization within which germ cells develop. During this process they were partially embedded in Sertoli cells (Nagahama, 1983). The mitotic spermatogonia are organized in lobules along entire length of tubular segment and the mature sperm are released into central lumen which eventually leads to efferent ducts followed by urogenital opening. Sperm production in some species is a single synchronous event, while in others it is cyclic or even continuous.

Despite great diversity in testis types, it contains germ cells in synchronous or variable stages of development and a complement of somatic cells specialized for physical support and regulation of spermatogenesis, including Sertoli and Leydig cells. Sertoli cells are found in direct association with germ cells which they support physically and nurture by modifying the chemical microenvironment. Leydig cells are typically interspersed in the connective tissue and their primary function is to produce steroids needed for spermatogenesis (Nagahama, 1983).
Fish spermatogenesis is fundamentally similar to that of vertebrates (Nagahama, 1983; Callard, 1991). Differences between taxa are superficial. Characteristic cytological changes during spermatogenesis include mitotic proliferation of committed spermatogonial stem cells and the subsequent differentiation of some, but not all, into primary spermatocytes. The first meiotic division marks the conversion of primary spermatocytes into secondary spermatocytes which in turn undergo a second meiotic division to form spermatids. During spermiogenesis the spermatids mature, develop a flagellum and separate from their supportive Sertoli cells to become spermatozoa (Schulz and Miura, 2002).

Culture of testicular fragments with GTH or other steroids induced the entire process of spermatogenesis within 24 days (Miura et al., 1991). The human chorionic gonadotropin (hCG)-induced spermatogenesis in vitro activates Sertoli and Leydig cells markedly in terms of ultrastructural changes and active production of steroids. Addition of hCG to culture media containing testicular fragments induced a rapid increase in 11-ketotestosterone (11-KT) which is known to be a potent androgen in most of the fishes, whereas in Fundulus, only testosterone promoted the spermatogenesis in vitro (Cochran, 1992). However, it is believed that the action of 11-KT is mediated by other factors produced by Sertoli cells, where the presence of androgen receptor is detected (Ikeuchi et al., 2001). It is possible that some of these factors are growth factors such as IGF-I and activin B. Further, up-regulation of activin B cDNA has been demonstrated within the first 24 hours of hCG-treatment in vivo in eels (Miura and Miura, 2003). All
these studies indicate that the hormonal regulatory mechanisms for spermatogenesis include GTH induced spermatogonial proliferation and testicular 11-KT production which in turn activates the Sertoli cells to produce activin B. Activin B then acts on spermatogonia to induce mitosis leading to the formation of spermatocytes (Fig. 4; Miura and Miura, 2003).

Fig. 4. Schematic representation of hormonal regulation of spermatogenesis in fish.
Sperm maturation

In fish, spermatozoa in the sperm duct are immotile and acquire motility when confronted with water suggesting that after completing spermiogenesis, spermatozoa attains the ability of motility. Sperm maturation is the phase during which non-functional gametes develop into mature spermatozoa, which are fully capable of motility and fertilization. Sperm maturation seems to involve only physiological changes but not morphological changes. In salmonids, sperm maturation has been induced by increasing the seminal plasma pH to 8.0 (Miura et al., 1992) in the sperm duct. Later on, it has been found in eel also (Miura et al., 1995; Ohta et al., 1997). Sperm maturation is also under endocrine regulation and in some teleosts 17α, 2β-DP is known to induce sperm maturation (Miura et al., 1992). However, the action of 17α, 2β-DP is not direct on sperm, rather it increases seminal plasma pH, which in turn increases the sperm cAMP content thereby allowing the acquisition of sperm motility (Miura, 1995). Using subtractive cDNA library approach, a cDNA named as eel spermatogenesis related substance 22 has been cloned that is homologous to carbonic anhydrase (CA; Miura et al., 2002). CA catalyzes the reversible hydration of carbon and is involved in ion and acid-base regulation in various tissues and fluids. Subsequently, a progesterone receptor on spermatozoon membrane was identified and activation of CA by 17α, 2β-DP through membrane progesterone receptor is thought to increase the pH of sperm duct (Fig. 5; Nagahama, 1994; Todo et al., 2000; Miura and Miura, 2003).
Fig. 5. Schematic representation of sperm maturation in teleosts.

Molecular mechanism of steroidogenic shift-Scope and objectives

Shift in steroidogenesis can happen at two stages, first being the shift in synthesis of precursor steroid from testosterone to 17α-OHP while the second is shift in final steroid products from E₂ to MIH (Nagahama and Yamashita, 2008). The switch in production of these steroids is likely to be regulated by changes in abundance of steroidogenic enzymes that produce them. Hence, characterization in terms of form and function, expression of steroidogenic enzyme genes gained momentum soon after the initial identification of shift in plasma steroid levels.
**Shift in precursor steroid synthesis**

Since MIH is synthesized from the 17α-OHP, there should be a shift in synthesis of androstenedione from 17α-OHP to maintain the levels of 17α-OHP. This can occur with the down regulation of C17-20 lyase activity and up-regulation or maintenance of hydroxylase activity. Regulation of P450c17 is complex because it is a single enzyme catalyzing both the reactions. A similar kind of shift in steroid synthesis i.e. from E₂ to progesterone also occurs in mammalian ovary during the transition from follicular phase to luteal/maturation phase. But the production of progesterone takes an alternative pathway (Δ⁴) utilizing 3β-hydroxysteroid dehydrogenase (3β-HSD) only and P450c17 is not required in this case. However, the differential regulation of P450c17 is seen in adrenal steroidogenesis during aging and the selective production of corticosteroids and sex steroids are brought about by the differential regulation of C17-20 lyase and hydroxylase activities. Ser/Thr phosphorylation through a cAMP dependent kinase is known to selectively elevate C17-20 lyase activity while dephosphorylation by protein phosphatase 2A keeps hydroxylase activity high. Moreover, redox partners such as cytochrome b5 and P450 oxidoreductase are known to modulate lyase activity. In fish there are no studies to explain the differential actions of P450c17 during steroidogenic shift and it has been thought that similar mechanism that operates in mammalian adrenals also occurs in fish ovary. But recently, a second novel form of P450c17 was identified in tilapia and medaka and has been shown that the differential expression patterns of these enzymes are important for shift in steroidogenesis (Zhou et al., 2007a
& b). However, none of the studies in fish are comprehensive to relate the mRNA expression to enzyme activity.

Another possibility for the production of high amount of 17α-OHP during oocyte maturation is increase in the abundance of steroidogenic acute regulatory protein (StAR) and steroidogenic enzymes such as cytochrome P450 side chain cleavage enzyme (P450scc) and 3β-HSD. Possibility of involvement of StAR and above mentioned enzymes is reasonable because (i) many of the steroidogenic enzyme genes and StAR are influenced by the stimulatory effect of tropic hormones, in most cases with an increase in intracellular cAMP levels (ii) stimulation of adenylate cyclase activity and production of cAMP in follicle layers during GTH induced oocyte maturation and finally (iii) in vitro experiments with both RNA and protein synthesis inhibitors have suggested the involvement of de novo RNA and protein synthesis. Though cDNAs encoding StAR, P450scc and 3β-HSDs have been cloned from different fish species, little is known about role in shift in steroidogenesis. In addition, there are no precise studies utilizing in vitro and in vivo models to implicate a role for these proteins in steroidogenic shift.
Shift in final steroid production

Expression of $oP450arom$ gradually increased throughout vitellogenesis and became undetectable in post-vitellogenic ovarian follicles/during meiotic maturation (Watanabe et al., 1999). Further, *in vitro* incubation of tilapia post-vitellogenic follicles with hCG-purged $oP450arom$ transcripts (Yoshiura et al., 2003). Further studies on this line confirmed that AD4BP/SF-1 and FOXL-2 regulates the expression $oP450arom$ (Yoshiura et al., 2003; Wang et al., 2007).

$20\beta$-$HSD$ cDNA was first cloned from pig testis (Tanaka et al., 1991), since purified protein was available only in pig (Nakajin et al., 1988). Surprisingly, Tanaka et al. (1991) found that the pig testicular $20\beta$-$HSD$ cDNA has striking homology (about 85%) to that of human carbonyl reductase1 (CBR1) and shown to be structurally and functionally similar to mammalian CBR1. Since $20\beta$-$HSD$ has broad substrate specificity and low Km for endogenous compounds, involvement of this enzyme in very specific events such as FOM is questionable. Nevertheless, several reports demonstrated the involvement of $20\beta$-$HSD$ and CBR1 in oocyte maturation and ovulation respectively (Espey et al., 2000; Tanaka et al., 2002). Further, increase in $20\beta$-$HSD$ mRNA is known in ayu and trout (Guan et al., 1999; Tanaka et al., 2002) while sudden appearance was observed in the Nile tilapia during FOM (Senthilkumaran et al., 2002). In contrast, a stable expression pattern of $20\beta$-$HSD$ was noticed in hCG-induced maturation of zebrafish ovarian follicles (Wang and Ge, 2002). Unlike $oP450arom$, transcriptional regulation of $20\beta$-$HSD$ is least studied (Senthilkumaran et al., 2001 & 2004). Since,
expression of both $20\beta$-HSD and $oP450arom$ in granulosa cells is modulated by GTH via cAMP, understanding how cAMP regulates the up-regulation of $20\beta$-HSD and down regulation of $oP450arom$ at a time in the same cell is interesting.

In light of the above reviewed literature, present thesis work is an effort to understand the molecular mechanism underlying the shift in steroidogenesis more explicitly. Following set of objectives (see below) were designed using snake head murrel, *Channa striatus* and air-breathing catfish *Clarias gariepinus* as experimental models. Murrel is a delicious fresh water teleost species of India and is being used in alternate medical practice for the treatment of asthma in Hyderabad (India). Air-breathing catfish is domesticated species in southern parts of India and has high growth rate. The synchronous development of ovary, possibility of collection of matured eggs after inducing FOM with hCG, *in vivo* without sacrificing animals and availability of data on plasma steroid levels during ovarian development (Joy *et al*., 1998; Kumar *et al*., 2000) are some of the characteristic features of catfish model.
Objectives

1. Molecular cloning, characterization, localization and expression of $20\beta$-HSD during oocyte maturation.
2. Isolation of promoter region of $20\beta$-HSD and understating the transcriptional regulation.
3. Precursor steroid production for shift in steroidogenesis: Role of $P450c17$.
4. Precursor steroid production for shift in steroidogenesis: Role of StAR.
References


General introduction


General introduction


Nagahama, Y., 1997. $17\alpha$, $20\beta$-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanism of synthesis and action. Steroids 62, 190-196.


Nagahama, Y., Young, G., Adachi, S., 1985b. Effect of actinomycin D and cycloheximide on gonadotropin induced $17\alpha$, $20\beta$-dihydroxy-4-pregnen-3-one by intact follicles and granulosa cells of the amago salmon, *Oncorhynchus rhodurus*. Dev. Growth Diff. 27, 213-221.


Pang, Y., Ge, W., 2002b. Epidermal growth factor and TGFα promote zebrafish oocyte maturation in vitro: Potential role of the ovarian activin regulatory system. Endocrinology 143, 47-54.


Yoshikuni, M., Nagahama, Y., 1994. Involvement of inhibitory G-protein in the signal transduction pathway of maturation-inducing hormone (17α, 20β-Dihydroxy-4-pregnen-3-one) action in rainbow trout (*Oncorhynchus mykiss*) oocytes. Dev. Biol. 166, 615-622.


