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Oogenesis in teleosts: How fish eggs are formed

Esther Lubzens [a,*], Graham Young [b,c], Julien Bobe [d], Joan Cerdà [e]

[a] Department of Marine Biology, Israel Oceanographic and Limnological Research, 81080 Haifa, Israel
[b] School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA
[c] Center for Reproductive Biology, Washington State University, Pullman, WA 99164, USA
[d] INRA, UR1037, IFR140, Ouest Genopole, Campus de Beaulieu, F-35000 Rennes, France
[e] Laboratory of Institut de Recerca i Tecnologia Agroalimentàries (IRTA)-Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), 08003 Barcelona, Spain

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ABSTRACT

One of the major objectives of the aquaculture industry is the production of a large number of viable eggs with high survival. Major achievements have been made in recent years in improving protocols for higher efficiency of egg production and viability of progeny. Main gaps remain, however, in understanding the dynamic processes associated with oogenesis, the formation of an egg, from the time that germ cells turn into oogonia, until the release of ova during spawning in teleosts. Recent studies on primordial germ-cells, yolk protein precursors and their processing within the developing oocyte, the deposition of vitamins in eggs, structure and function of egg envelopes and oocyte maturation processes, further reveal the complexity of oogenesis. Moreover, numerous circulating endocrine and locally-acting paracrine and autocrine factors regulate the various stages of oocyte development and maturation. Though it is clear that the major regulators during vitellogenesis and oocyte maturation are the pituitary gonadotropins (LH and FSH) and sex steroids, the picture emerging from recent studies is of complex hormonal cross-talk at all stages between the developing oocyte and its surrounding follicle layers to ensure coordination of the various processes that are involved in the production of a fertilizable egg.

In this review we aim at highlighting recent advances on teleost fish oocyte differentiation, maturation and ovulation, including those involved in the degeneration and reabsorption of ovarian follicles (atresia). The role of blood-borne and local ovarian factors in the regulation of the key steps of development reveal new aspects associated with egg formation.

1. Introduction

Studies on teleost fish reproduction assist the aquaculture industry in meeting the ever increasing demand for fish, by improving protocols for higher efficiency of egg production and enhanced viability of progeny. In recent years, there is a pressing need for new cultured species due to the dwindling of natural resources by overfishing and an increasing demand for diversification of marketable products. One of the main concerns of the aquaculture industry is the production of large numbers of viable eggs with high survival. Most of the research efforts until recently were focused on endocrine regulation of spawning and optimizing

Abbreviations: 11-KT, 11 ketotestosterone; 17,20βP, 17,20β-dihydroxy-4-pregnen-3-one; 20β-HSD, 20β-hydroxysteroid dehydrogenase; ADAMs, a disintegrin and metalloprotease; AMH, anti-Müllerian hormone; *amh/amhrII*, anti-Müllerian hormone/anti-Müllerian hormone receptor II; AQP1, aquaporin-1; Aqp1b, aquaporin-1b; AQPs, aquaporins; BMP15, bone morphogenetic factor 15; *cxcr4*, chemokine receptor 4; *dnd*, *dead end* gene; E2, estradiol 17β; EGF, epidermal growth factor; FAA, free amino acids; FSH, follicle stimulating hormone; GDF9, growth and differentiation factor 9; GSDF, gonadal soma-derived growth factor; GSI, gonadosomatic index; GTH, gonadotropin; GVBD, germinal vesicle breakdown; HDL, high density lipoproteins; Hsp60, heat-shock protein 60; Hsp90, heat-shock protein 90; IGF-BP, IGF binding protein; IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; LDL, low density lipoproteins; LDLR, LDL receptor; LDLR, low density lipoprotein receptor; LH, luteinizing hormone; LLTP, Large Lipid Transfer Protein; LRAT, lecithin:retinol transferase; LvH, heavy chain of lipovitellin; LvL, light chain lipovitellin; MBV, multivesicular bodies; MIP, major intrinsic proteins; MIS, maturation inducing steroids; MPF, maturation promoting factor; *nanos-1*, nanos homolog 1 (*Drosophila*) gene; PGCs, primordial germ-cells; PGs, prostaglandins; Piwi, P-element induced wimpy testis (*Drosophila*), regulates the proliferation and maintenance of germ-line stem cells in diverse organisms; RAR, retinoic acid receptors; RBP, retinol-binding protein; RE, retinyl-ester; RXR, retinoid X receptors; SAGE, Serial Analysis of Gene Expression; *sdf-1*, stromal cell-derived factor-1 gene; SDF-1, Stromal cell-derived factor-1 protein; SSH, suppression subtractive hybridization; StAR, steriodogenic acute regulatory protein; TGF-β, transforming growth factor β; TNF, tumor necrosis factor; *vasa*, The *vasa* gene encodes an ATP-dependent RNA helicase belonging to the DEAD-box family; VEP, vitelline envelope proteins; VLDL, very low density lipoproteins; *vldlr*, a transcript encoding the VLDL receptor; *vldlro*, a transcript encoding VLDL receptor containing an O-linked sugar domain; Vtg, vitellogenin; VtgR, Vtg receptor; WGD, whole genome duplication; Ziwi, the zebrafish homolog of the *Drosophila piwi*; ZP, zona pellucida; ZRP, zona radiata proteins.

* Corresponding author. Fax: +972 4 8511911.
E-mail address: esther@ocean.org.il (E. Lubzens).

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rearing protocols. Major gaps in our knowledge, however, remain in the molecular, biochemical and physiological mechanisms that lead to the production of so-called "high quality eggs".

An egg is the final product of oocyte growth and differentiation. We are still far from understanding the dynamic processes associated with the formation of an egg from the time germ cells turn into oogonia, until the release of ova during spawning. While studies on endocrine, cellular and ultra-structural aspects of oogenesis and oocyte growth (Wallace and Selman, 1981, 1990; Le Menn et al., 2007) have been fortified in recent years with molecular aspects, including large scale transcriptome and proteome analyses (Cerdà et al., 2008a,b), this information is far from complete. New information on primordial germ-cells (PGCs), yolk protein precursors and their processing within the developing oocyte, the deposition of vitamins in eggs, the structure and function of the egg envelope and oocyte maturation processes, further reveal the complexity of the process of oogenesis. Moreover, numerous circulating endocrine and locally-acting paracrine and autocrine factors regulate the oocyte developmental and maturation stages. Though it is clear that the major regulators during vitellogenesis and oocyte maturation are the pituitary gonadotropins (luteinzing hormone, LH, and follicle-stimulating hormone, FSH) and sex steroids, the picture emerging from recent studies is of complex hormonal cross-talk at all stages between the developing oocyte and its surrounding follicle layers to ensure coordination of the various processes that are involved in the production of a fertilizable egg. Thus, the notion that the oocyte is a passive passenger subject to control by the follicle cells is no longer tenable, and emerging though often indirect evidence indicates that oocyte-derived growth factors profoundly affect the activity of the follicle cells which in turn influences development of the ovary.

This chapter aims at highlighting some recent advances on the processes of teleost fish oocyte differentiation, maturation and ovulation, including those involved in the degeneration and reabsorption of the ovarian follicles (atresia). It also provides a brief and selective summary of the involvement of blood-borne and local ovarian factors in the regulation of the key steps of development needed to produce a viable egg.

2. Reproductive strategies

Most teleost fish species are oviparous, producing yolk containing eggs, although viviparity, with embryos developing within the female reproductive system, is also known (see Jalabert, 2005 for an overview of the specificities of reproduction and oogenesis in teleost fish). One of the striking features in teleost fish is the plasticity of sex determination with a range of gonochoristic species, where individuals are either males or females, or species displaying a change in gender during their lifetime. For example, protogynous fish will develop first as females and after one or more spawning seasons may change to males, while protoandrous fish will develop first as males and change later to females. Simultaneous hermaphroditism is also displayed in some species with gonads showing testicular and ovarian components at the same time (Devlin and Nagahama, 2002; Yaron and Levavi-Sivan, 2006).

Three main types of ovarian development have been described for fish (Wallace and Selman, 1981): (a) synchronous, where all oocytes develop and ovulate at the same time, (b) group-synchronous, where at least two populations of oocytes can be recognized in the ovary throughout the reproductive season (i.e. vitellogenic and maturing), and (c) asynchronous, where oocytes of all stage of development are present without a dominant population. Spawning pattern will depend on the rhythm of oocyte ovulation with "synchronous ovulators" shedding the full population of eggs in a single episode or over a short period of time. Alternatively, in "asynchronous ovulators" or batch spawners, eggs are recruited into maturation and ovulation from the population of yolked oocytes in several batches during the spawning season. The high investment in reproduction is reflected in the gonadosomatic index (GSI) of females, which can reach 15–40% during the spawning season (Tyler and Sumpter, 1996). Most cultured fish exhibit external fertilization without parental care and release a large number of benthic (benthophil species) or pelagic (pelagophil species) eggs (Fig. 1).

3. From primordial germ-cells to fully-grown ovarian follicles

While strategies for egg formation and spawning may differ between fish species, most studies show several common features in the steps leading to the formation of a mature egg. During the course of differentiation of the primary ovarian follicle into an egg, the oocyte developing within the ovarian follicle, acquires the capability of forming a viable embryo after fertilization. This process is accompanied by massive structural and functional changes and their wide, complex and intertwining scope are only starting to be revealed through genomic and proteomic studies (Cerdà et al., 2008a,b). Observations based on light and electron micrograph sections, reveal the structural changes occurring from primary oocytes residing in oogonial nests, up to the structure of the mature egg (Wallace and Selman, 1981; Selman et al., 1993; Le Menn et al., 2007). Major stages during egg development (Patiño and Sullivan, 2002) include formation of primordial germ-cells (PGCs), the transformation of PGCs into oogonia and subsequently their transformation into primary oocytes, with the onset of meiosis. This is followed by the massive growth of the oocyte during vitellogenesis, whereby the oocyte accumulates nutritional reserves needed for the development of the embryo. At this period, the oocyte also accumulates RNA (known as maternal RNA) and completes the differentiation of its cellular and non-cellular envelopes. During this time the oocyte remains in meiotic arrest, at the end of prophase and in the diplotene stage. Maturation processes are characterized by reduced or stopping of endocytosis, resumption of meiosis, germinal vesicle breakdown (GVBD), the formation of a monolayer of cortical alveoli under the oolemma, and yolk platelet dissolution and pelagophil oocytes undergo hydration. The first meiotic division gives rise to two cells differing in size, the small cell with first polar body degenerates and the large secondary oocyte is formed, and finally ovulation takes place at the end of the maturation process. The secondary oocyte is then extruded from its surrounding follicular cell layers and moves into the ovarian lumen or abdominal cavity (depending on the species). At this stage the female gamete is known as an ovum. It is haploid as a result of the occurrence of the second meiotic division and the formation of the second polar body that also degenerates. During fertilization, the haploid ovum nucleus fuses with the haploid nucleus of the spermatozoon and forms the diploid egg.

The usual division into different stages or steps is rather artificial as oocyte development is a dynamic process and it is difficult to identify the beginning or the end of each event. Fig. 2 shows some visual distinguishable stages (with a light microscope) of isolated basophil (*Danio rerio*) and pelagophil (*Sparus aurata*) ovarian follicles. Stages of oocyte development were extensively reviewed (Wallace and Selman 1990; Selman et al., 1993; Tyler and Sumpter, 1996; Devlin and Nagahama, 2002; Patiño and Sullivan, 2002; Le Menn et al., 2007; Cerdà et al., 2008a) and therefore the following summary aims at highlighting some recent advances on specific topics and does describe the full events associated with each stage or step of ovarian follicle development that were described before (Selman et al., 1993; Le Menn et al., 2007). The topics that will be discussed include the formation of PGCs, oocyte maturation and ovulation, hormonal regulation of oocyte development and follicular atresia.

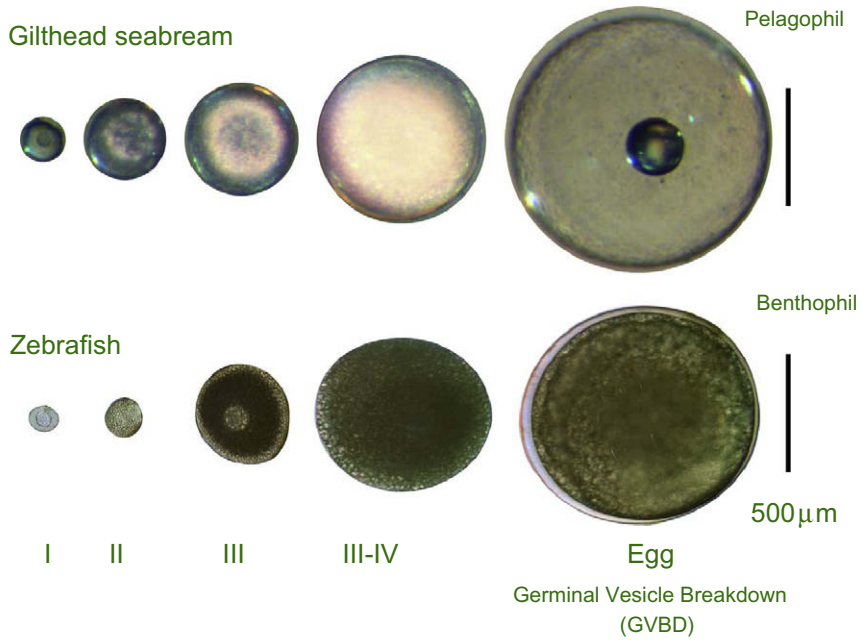

Fig. 1. Microscopic view of oocytes isolated from ovaries at different stages of development. Pelagophil oocyte from the gilthead seabream (*Sparus aurata*) and benthophil oocytes from the zebrafish (*Danio rerio*) are shown on top and bottom panels, respectively.

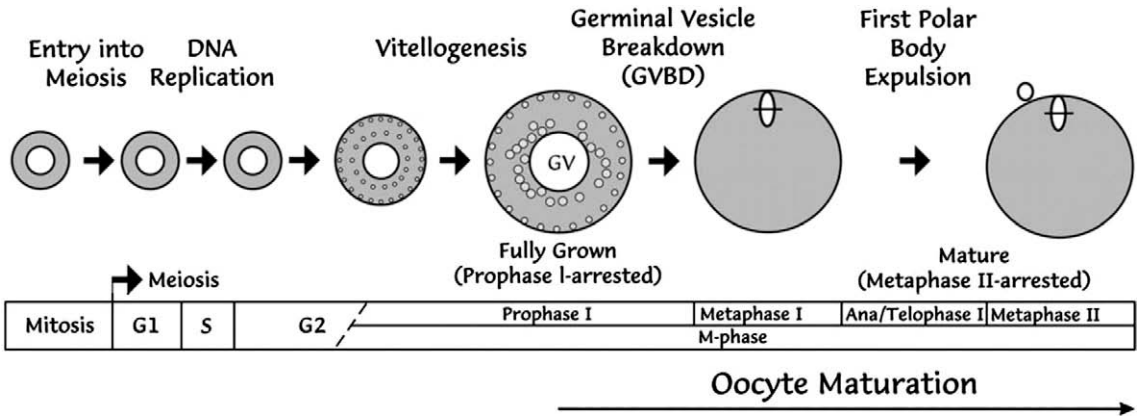

Fig. 2. A schematic description of oocyte developmental stages in relation to meiosis in teleost fish, adapted from Suwa and Yamashita (2007). From left to right: from primary oocytes, to vitellogenic and mature oocytes. See text for more detailed description.

3.1. Primordial germ cells and formation of oogonia

The development of germ-line cells is associated with the transmission of specific maternal RNAs accumulated within the oocyte during its development. The germ-cells are unique as they generate haploid reproductive cells or gametes. They transmit the genetic information from one generation to the next, by forming sperm cells or eggs. Shortly after fertilization of the egg and during the early stage of embryogenesis, a small number of non-dividing PGCs are produced. PGCs contain components known as germ-plasm that is characterized by the presence of polar granules or electron dense structural organelles associated with mitochondria, RNA and proteins also identified as "nuage" or "ciments" (reviewed in Braat et al., 1999a; Lyman-Gingerich and Pelegri, 2007; Le Menn et al., 2007). Nuage has been found not only in PGCs but also in oogonia, oocytes, spermatogonia, spermatocytes and spermatids. PGCs can be distinguished from somatic cells (with a light microscope) by their relatively larger size and larger nuclei with a distinct nuclear membrane and one or two prominent nucleoli. For several decades it has been suspected that germ line-specific electron dense structures representing storage of RNA and proteins were essential for differentiation and or determination of PGCs in various teleost species. The identification of the zebrafish ortholog of the *vasa* gene assisted in revealing the site of germ plasm in the mature oocyte and its incorporation into PGCs during embryogenesis (Olsen et al., 1997; Yoon et al., 1997; Braat et al., 1999a,b; Yoshizaki et al., 2002; Raz, 2003). The *vasa* encodes an ATP-dependent RNA helicase of the DEAD box family, originally identified in *Drosophila* and whose homologs are present as RNA or protein in the germ cell lineages of all organisms studied so far. Transcripts of *vasa* have been shown to become cortically localized during early stages of oocyte development and homologs were isolated from several fish species (Yoshizaki et al., 2002; Saito et al., 2004). In zebrafish, *vasa* transcripts were detected in the cytoplasm by in situ hybridization of oogonia or early developing oocytes and in the oocyte cortex of vitellogenic oocytes (Braat et al., 1999a,b). Distribution of *vasa* transcripts and other germ-plasm or maternal transcripts or proteins may differ between fish species (Yoshizaki et al., 2002;

Lyman-Gingerich and Pelegri, 2007). During embryonic cell cleavage in the zebrafish, the *vasa* transcripts are limited to a small number of cells and were found in four cells at the 32-cell stage and only 4–12 cells at the 4000-cell dome stage. This number increases to 16–25 cells at the shield stage and these cells are localized in four evenly spaced clusters near the blastoderm margin. During epiboly the four clusters concentrate on the dorsal side of the embryo, forming two groups, with each group placed laterally to the midline and dorsal to the gut. The total number of *vasa* positive PGCs in zebrafish will reach 25–30, representing 2–3 mitotic divisions, before their migration and arrival at the genital ridges. As in other organisms, zebrafish PGCs are formed before gastrulation in the embryo, at a location distinct from the gonadal site. Several other genes were found to be associated with germ-plasm in zebrafish such as *nanos-1*, *dazl*, *dead end*, *cxcr4b* and *sdf-1a* receptor (Raz, 2003; Knaut and Schier, 2008).

Recent data in zebrafish unveils the processes associated with PGC migration towards the gonadal site, where they become associated with somatic cells and differentiate into gametes. During this migration, the PGCs are guided by somatic cells expressing a chemokine *sdf-1a* along their migratory route. It is assumed that PGCs expressing the SDF-1 receptor CXCR4b are attracted and guided by a source of chemokine SDF-1a expressed in somatic cells. As the location of SDF-1a shifts during development, germ cells follow the source until they reach their final destination (Raz and Reichman-Fried, 2006; Knaut and Schier, 2008). PGC migration also requires a second SDF1a-receptor (CXCR7) that sequesters SDF1a, highlighting the importance of ligand clearing during guided cell migration (Boldajipour et al., 2008). The maintenance of germ cells depends on Piwi (or Ziwi) proteins (members of Argonaute protein family, associated with RNA silencing) that are components of the "nuage" germ-line structure (Houwing et al., 2007). After reaching the gonadal ridge, the relatively small number of germ cells colonizes the gonad and within the gonad they embark on proliferation (Sawatari et al., 2007; see also Section 3.2). The PGCs colonizing the gonad stroma are relatively large, irregular in shape, have a relatively large nucleus and may exhibit pseudopodial structures indicating their migratory status. Several ultra-structural features were shown to characterize the cytoplasm of these cells such as electron dense "ciment" or "nuages" mentioned earlier. A reciprocal cross-talk between germ cells and gonadal somatic cells involving the expression of *amh/amhrII* (anti-Müllerian hormone/anti-Müllerian hormone receptor II) and unknown signal molecule(s) from the somatic cells was found important for sexual differentiation in medaka gonads (Tanaka et al., 2008).

Although PGCs are sexually bipotential during their migratory stage, sexual differentiation is initiated after colonization of the gonads by the timing of the onset of meiosis. During this process in mammals (or more specifically in the mouse), the female germ cells enter into meiosis and proceed to the diplotene stage of the meiotic prophase I, whereas the male germ cells arrest at G1/G0 and undergo genome-wide DNA methylation and parental imprinting (see Suzuki and Saga, 2008). It was shown recently, that retinoic acid (RA) is probably involved in inducing meiosis in germ cells in mammals (Swain, 2006). RA induces *Stra8*, an RA responsive gene in the developing female germ cells, leading to meiotic initiation in the embryonic ovary. However, RA signaling was inhibited by the gene *Cyp26b1*, which encodes an enzyme metabolizing RA that is expresses exclusively in somatic cells of the male (XY) gonad (Swain, 2006). The source of RA was attributed to the mesonephros and enters the gonad through the mesonephritic tubules that are connected to the gonad at the anterior end (Bowles et al., 2006; Bowles and Koopman, 2007). More recently it was shown that *Nanos2*, a male PGC specific gene involved in the maintenance of male germ cells, maintains the suppression of meiosis by preventing *Stra8* expression, after the decrease of Cyp26B1. The testis may use RA signaling to induce meiosis postnatally (Suzuki and Saga, 2008).

While the occurrence of similar pathways have yet to be shown for fish, it has been demonstrated for medaka and zebrafish, that the germ line plays a critical role in female sex determination (Kurokawa et al., 2007; Siegfried and Nüsslein-Volhard, 2008, respectively). In zebrafish, the germ line is essential for female sex determination by maintenance of ovary-specific gene expression (e.g. *foxl2*, *cyp19a1a*) and down-regulation of testis gene expression in the surrounding somatic cells. Zebrafish without germ cells develop a testis and the somatic tissue of the germ-line deficient testes show normal structure, cell types and somatic expression compared with wild type-testis. Germ deficient zebrafish have male coloration and male mating behavior, demonstrating that steroids produced by the testis are sufficient for masculinization of the animal. The number of germ cells and timing at which they are required for determining the fate of ovary development is not yet clear for zebrafish but transplantation of a single PGC into a germ line deficient zebrafish yields a male fish (Saito et al., 2008). Medaka has an XY sex determination system where a dominant gene, the *DMY* or *DmrtbY*, on the Y chromosome determines the male gonad. The expression of *DMY* in the pre-Sertoli cells of the male gonad is the first sexually dimorphic character demonstrating the important role of the somatic supporting cells of the gonad in sex determination. However, germ cells also play a critical role in medaka, as germ cell deficient XX medaka morphants manifest female-to-male sex reversal of their secondary sex characteristics. The gonadal somatic cells in medaka are predisposed to male development (Kurokawa et al., 2007). In summary, in both zebrafish and medaka, two distantly related fish, the germ line plays a critical role in female sex determination as germ line loss results in masculinization, supporting the contention of a conserved role of the germ line in female sex determination. Successful in vitro proliferation of cultured zebrafish PGCs achieved recently will assist in future investigations on germ cell differentiation and embryonic germ cell pluripotency (Fan et al., 2008).

The formation of gametes from primordial germ cells (PGCs) has gained specific attention in recent years with the availability of specific molecular markers and the striking potential as a valuable resource for genetic preservation and production of individuals from gametes of germ-line chimeras (Ciruna et al., 2002; Yoshizaki et al., 2002; Saito et al., 2008). The totipotency of PGCs to proliferate into spermatozoa or eggs was used for developing a surrogate broodstock technology for genetic resource preservation for fish. Moreover, by transplantation of spermatogonia into xenogeneic recipients, it was possible to obtain female or male progeny, depending on the sex of the recipient fish (Okutsu et al., 2006a,b, 2007). Complete replacement of the gonad of a host fish by one xenogeneic PGC from distantly related species was also demonstrated recently (Saito et al., 2008). The application to cultured fish species was successfully demonstrated recently by germ cell transplantation in tilapia (Lacerda et al., 2008). These studies aspire to provide an important contribution to germ-plasm preservation and offer an opportunity for preservation of specific traits of cultured species, side by side with prospects of replenishing the genetic diversity of dwindling natural fish populations.

The transformation of PGCs into oogonia involves structural changes within the PGC and each oogonium multiplies by mitotic divisions forming oogonial nests in association with pre-granulosa cells. At this stage, each oogonium becomes surrounded by a monolayer of somatic granulosa cells that secrete a basement lamina, separating it from the ovarian stroma cells. Somatic cells forming a monolayer outside the basement lamina, constitute the theca that becomes associated with blood vessels. The oocyte with its

surrounding granulosa cells, basement lamina and theca somatic layer constitutes the ovarian follicle and forms the primary oocyte. The transition from oogonium to a primary oocyte is also characterized by the initiation of the first meiotic division, before leaving the oogonial nest (Selman et al., 1993).

3.2. Endocrine aspects of oogonia proliferation and transition into meiosis

The hormonal mechanisms controlling oogonial proliferation and oocyte recruitment are incompletely understood for any vertebrate. Early evidence for the hormonal mediation of oogonial division in teleosts mainly came from the analysis of ovaries of fish after hypophysectomy, which reduced number and divisions of oogonia (Tokarz, 1978). Injection of pituitary extracts restored oogonial proliferation in hypophysectomized goldfish (Yamazaki, 1965), and unilateral ovariectomy of tilapia increased the number of dividing oogonia in the remaining ovary; the increase was blocked by the gonadotropin (GTH) antagonist, methallibure (Dadzie and Hyder, 1976), suggesting that GTHs, either directly, or indirectly via stimulation of ovarian mediators, increase oogonial proliferation. Recent studies have implicated two sex steroids in controlling oogonial proliferation and transition of oogonia into meiosis. Data from experiments using a long term explant culture system for Japanese huchen (*Hucho perryi*) and common carp (*Cyprinus carpio*) ovarian fragments suggest that progression of germ cells through early oogenesis involves estradiol-17β (E2), which acts directly on oogonial proliferation, and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP), which in addition to promoting oogonial proliferation initiates the first meiotic division, leading to the development of the first stage in primary growth, chromatin nucleolar oocytes. Plasma 17,20βP levels doubled at the time of the initiation of the first meiotic division in huchen. Plasma E2 levels were about 0.4 ng/ml during mitotic proliferation of oogonia (Miura et al., 2007). The factors and mechanisms regulating steroid production at this time are unknown. Interestingly, an early study reported that the mitotic activity of oogonial nests increased after administration of estrone in minnows (*Phoxinus laevis*; Bullough, 1942). The onset of meiosis has also been linked to the expression of insulin-like growth factor-I (IGF-I) in somatic cells and oocytes of tilapia (Berishivili et al., 2006).

Gene expression profiling has revealed that the genes encoding the β subunits of the GTHs follicle-stimulating (FSH) and luteinizing (LH) hormones are overexpressed in the developing trout ovary when the first meiotic oocytes were observed. The GTHs have been suggested to function as anti-apoptotic agents at this time (Baron et al., 2005). However, although FSHβ, LHβ and the common α-subunit have been identified as products in the adult sea bream oocyte (Wong and Zohar, 2004), the gene encoding the α-subunit was not expressed in the developing trout ovary (Baron et al., 2005).

Gonadal soma-derived growth factor (GSDF) is a novel transforming growth factor belonging to the transforming growth factor-β (TGFβ) superfamily that has recently been cloned from rainbow trout (Sawatari et al., 2007). Homologs of GSDF have been identified in the expressed sequence tag databases of Atlantic salmon (*Salmo salar*), stickleback (*Gasterosteus aculeatus*), fathead minnow (*Pimephales promelas*), fugu (*Fugu rubripes*), and zebrafish (*Danio rerio*). GSDF appears to be a teleost-specific growth factor expressed in the genital ridge somatic cells in direct contact with primordial germ cells of rainbow trout embryos and in the gonadal tissue of adults. Although no data are available for ovary, GSDF promoted spermatogonial proliferation in a dose-dependent manner in the rainbow trout testis and GSDF antisera reduced spermatogonial proliferation (Sawatari et al., 2007). Therefore, GSDF could potentially play a similar role in controlling oogonial proliferation.

3.3. Primary growth and folliculogenesis

Primary growth encompasses the period of oocyte development from meiotic chromatin-nucleolus stage to early cortical alveoli stage and is intimately linked with the development of the follicle layers surrounding the oocyte. During this stage, the organelles and molecules used at later stage are synthesized. Cortical alveoli are synthesized prior or concurrent with the commencement of lipid and vitellogenin endocytosis. Studies aimed at revealing stage specific gene expression were reported recently for the developing ovary of rainbow trout (*Oncorhynchus mykiss;* von Schalburg et al., 2005, 2006) and coho salmon (*Oncorhynchus kisutch*; Luckenbach et al., 2008a,b). Differentially expressed ovarian genes during early stages of oogenesis were revealed in the ovaries removed from the coho salmon (Luckenbach et al., 2008a). This semelparous species spawns only once in a lifetime, exhibits synchronous follicle development and is therefore a good model for studying stage specific gene expression in the ovary, especially of early developmental stages (Luckenbach et al., 2008a). The more striking results include the intriguing temporal expression patterns of several genes with important roles in oogenesis that are expressed during primary oocyte growth, but with a functional role during later stages of oogenesis or during embryogenesis. This includes gene transcripts of proteins associated with lipid uptake although lipid droplets were not yet observed by microscopic examination. Evidently, it is difficult to determine the localization of gene transcripts in the ovary as they may arise from follicle cells (granulosa or theca cells), interstitial cells or the oocyte (Luckenbach et al., 2008a). Morphogenic gene families were found to be expressed in the adult rainbow trout and some of them are probably involved in regulating division and germ line stem cells in the ovary (von Schalburg et al., 2006). However, proteome profiling of developing ovarian follicles of zebrafish and the gilthead seabream (*Sparus aurata*) did not reveal stage specific proteins, although more than 600 proteins were identified (Ziv et al., 2008). This maybe associated with group-synchronous development of ovarian follicles displayed by these species.

Hypophysectomy in teleosts does not appear to inhibit the primary growth of oocytes until arrest at the late perinucleolar or very early cortical alveoli stage (Pickford and Atz, 1957; Khoo, 1979). Thus, prior to the cortical alveoli stage, follicles are able to proceed through development in the absence of pituitary GTHs and primary oocyte growth has been termed GTH-independent (Billard, 1992). However, it may be more accurate to regard primary oocyte development in teleosts as being pituitary-independent, because both FSHβ and LHβ transcripts and proteins were detected in the primary and secondary oocytes of gilthead seabream (*Sparus aurata,* Wong and Zohar, 2004). By analogy with mice where FSH knockout studies indicate that FSH has a facilitatory role in the development of pre-antral follicles which is largely under the control of local regulators (Britt and Findlay, 2002), results from the relatively few hypophysectomy studies in teleosts do not necessarily imply that pituitary hormones are not involved in regulating primary growth.

Several growth factors have been implicated in the regulation of primary oocyte growth in correlative studies, but no experimental evidence so far exists on their specific roles. Two members of the TGF-β family that are expressed in oocytes, growth and differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), both of which play important roles in early ovarian follicle growth in mammals (Gilchrist et al., 2004; Juengel et al., 2004; Moore and Shimasaki, 2005), have been implicated in primary ovarian follicle development in teleosts. Baron et al. (2005) reported that GDF9 transcripts were highly expressed in trout ovary between 60 and 110 days after first feeding, coincident with the period of very early development of primary ovarian follicles when granulosa cells

proliferate. Androgen treatment down-regulated GDF9 expression. Subsequently, in zebrafish (Liu and Ge, 2007) and European sea bass (Halm et al., 2008), elevated levels of GDF9 and BMP15 mRNA transcripts in the ovary during primary ovarian growth and a subsequent reduction during secondary growth were reported, suggesting an important, but currently ill-defined role in development of primary ovarian follicles. However, Clelland et al. (2006) reported that BMP15 was expressed in zebrafish follicles at all stages of development with no significant changes over the course of folliculogenesis. In mammals, in which early ovarian follicle development appears to be GTH-independent, GDF9 and BMP15 are of critical importance, particularly in granulosa cell proliferation and differentiation (Dong et al., 1996; Findlay et al., 2002; McNatty et al., 2005). It appears that the onset of GTH-receptor expression coincides with decreased GDF9 and BMP15 expression (Ge, 2005; Halm et al., 2008).

3.4. Transition into secondary growth

An early event associated with the enlargement of the oocyte is the appearance of cortical alveoli that fill the periphery of the oocyte and this stage was also termed as "primary vitellogenesis" (Selman et al., 1993). Cortical alveoli are membrane-limited vesicles of variable size that stain with dyes for protein and carbohydrates. They appear in proximity to Golgi complexes that were shown to participate in the synthesis of their contents. As the oocyte grows, cortical alveoli increase in number and size, filling the oocyte cytoplasm. Cortical alveoli are eventually displaced to the oocyte periphery during the late stages of oocyte development, due to the centripetal accumulation of yolk proteins. The content of the cortical alveoli is released to the egg surface after as part of the "cortical reaction" at fertilization. This release leads to the restructuring of egg envelop proteins forming the chorion (Selman et al., 1993). Transcripts associated with cortical alveoli components include the serum lectin isoform 2 (*lcal*), rhamnose binding lectin STL3 (*lrham*) and alveolin (*alv*) (Luckenbach et al., 2008a).

Early studies on hypophysectomized fish led to the idea that the transition of primary oocytes into secondary growth (initially the accumulation of cortical alveoli) was dependent on the presence of the pituitary (Yamazaki, 1965; Tokarz, 1978; Khoo, 1979). Detailed studies on the endocrine changes associated with the primary–secondary oocyte transition are largely lacking, as are experimental approaches to determining the role of pituitary and ovarian factors in regulating this transition. Plasma levels of FSH in salmonids increase during the transition from primary to secondary follicular growth, with elevated levels maintained as follicles are recruited into vitellogenesis (Swanson, 1991; Breton et al., 1998; Santos et al., 2001; Swanson et al., 2003). Perinucleolar salmon ovarian follicles respond to stimulation with GTHs by increasing E2 production (Swanson et al., 1989). In the most comprehensive study currently available, changes in circulating hormones and expression of endocrine-related genes were traced during the transition of oocytes of coho salmon from perinucleolar to lipid droplet stage (Campbell et al., 2006). Body growth during the fall-spring months (1 year to 6 months before spawning) was strongly related to the degree of oocyte development, with larger fish possessing more advanced oocytes than smaller, slower growing fish. The synthesis of cortical alveoli was associated with increases in plasma and pituitary FSH, plasma E2, and expression of transcripts encoding ovarian steroidogenic acute regulatory protein (StAR), which serves to move cholesterol across the inner mitochondrial membrane, the rate limiting step in steroidogenesis. Ovarian transcripts for growth hormone receptor and somatolactin receptor decreased during this time. Subsequent accumulation of lipid droplets was associated with increased plasma IGF-I levels and components of the FSH-ovary axis, including plasma FSH, E2, and ovarian mRNAs for GTH receptors, StAR, IGF-1 and IGF-II. The positive relationship between plasma IGF-1, E2, and pituitary FSH during growth in the spring suggest that they are part of the mechanism through which body growth influences the rate of oocyte development (Campbell et al., 2006).

A subsequent study has identified genes that were differentially expressed between follicles at late perinucleolar and cortical alveoli stage of coho salmon. Highly significant increases in expression of the FSH receptor, and in the expression of anti-Müllerian hormone (AMH) and GSDF, both members of the TGF-β family, were identified (Luckenbach et al., 2008a). AMH is expressed in granulosa cells and expression peaks at the cortical alveoli stage of zebrafish follicles and then progressively declines during vitellogenesis (Rodríguez-Marí et al., 2005). Similarly, GSDF is expressed in granulosa cells (Sawatari et al., 2007) and may play a role in granulosa cell proliferation (Luckenbach et al., 2008a). Increased FSH signaling, together with increased potential for steroid production are characteristic of this transition, but the role of FSH in regulating expression of genes associated with the accumulation of cortical alveoli is currently unknown.

Both steroids and IGF-I have been experimentally implicated in the transition of oocytes into secondary growth. In the shortfinned eel (*Anguilla australis*), in vivo and in vitro experiments have demonstrated that 11-ketotestosterone (11-KT), a non-aromatizable androgen that is found at high levels in female eels when secondary growth begins, increased the diameter of late perinucleolar oocytes (Rohr et al., 2001; Lokman et al., 2007) and, in the presence of the triglyceride triolein, 11-KT significantly increased lipid accumulation. IGF-I treatment in vitro also increased oocyte diameter but E2 was without effect (Lokman et al., 2007). In cod, androgens have also been reported to stimulate growth of primary oocytes, but the specific initial oocyte stage and final stage achieved were not reported (Kortner et al., 2008, 2009). However, E2 treatment induced cortical alveoli formation in oocytes from hypophysectomized goldfish (*Carassius auratus*; Khoo, 1979). More recently, the accumulation of cortical alveoli has been associated with increased aromatase mRNA expression and E2 production in zebrafish (Kwok et al., 2005).

3.5. Vitellogenesis

The term vitellogenesis generally describes the incorporation of vitellogenin proteins by the oocyte and their processing into yolk proteins (Le Menn et al., 2007), but needs to be extended to include also the incorporation of other molecules such as lipids and vitamins. At the end of this process, the oocyte becomes competent to undergo fertilization, and contains maternal mRNAs, proteins, lipids, carbohydrate, vitamins and hormones that are important for the proper development of the embryo. The massive ovarian follicle enlargement occurs during the spectacular uptake of lipids and yolk protein from the plasma by the oocyte. In parallel, the formation of the egg envelope surrounding the oocyte takes place (Wallace and Selman, 1981, 1990; Brooks et al., 1997; Le Menn et al., 2007).

3.5.1. Lipid accumulation

The lipids accumulating within the oocyte ooplasm originate from plasma very low density lipoproteins (VLDL) and from vitellogenins (Vtgs). Two VLDL receptors involved in the uptake of lipids were described in rainbow trout. One receptor containing an O-linked sugar domain (encoded by *vldlro*) is expressed in the ovary and somatic tissues of some fish species, and a Vtg receptor (encoded by *vldlr*) that is expressed specifically in ovaries (Davail et al., 1998; Prat et al., 1998). It was suggested (Le Menn et al., 2007) that lipids are sequestered from the plasma VLDL by the binding of apolipoprotein B in VLDL with O-linked sugar specific

VLDL/Vtg receptors anchored in the plasma membrane of the oocyte or/and the action of an endothelial-attached lipoprotein lipase. Lipolysis of VLDL triacylglycerols and esterification of the fatty acids released through lipolysis may result in neutral lipids that are stored in the lipid globules. Phospholipids required for embryonic development probably originate from lipids carried by Vtg. Lipid globules appear in the ooplasm at about the same time as the cortical alveoli and their abundance predominates in early stages of vitellogenesis over the yolk globules. The expression of several genes associated with lipoprotein uptake was found to take place during early stages of vitellogenesis (Luckenbach et al., 2008a).

3.5.2. Vitellogenins

The Vtgs are phospholipoglycoproteins that are found in the blood of females of all oviparous vertebrate species females during vitellogenesis. They are synthesized mainly in the liver, under the regulation of E2 but Vtg synthesis can also be induced by several other hormones (see summary in Babin et al., 2007; see also Section 3.6). In addition, expression of *vtg* genes in extra-hepatic tissues, including intestine and ovary, has been shown for zebrafish (Wang et al., 2005), indicating that the liver may not be the only source of Vtgs in plasma.

The Vtgs belong to the Large Lipid Transfer Protein (LLTP) superfamily (Babin et al., 1999). A number of studies indicate that teleosts have at least three different Vtgs, VtgA, VtgB and VtgC, and all of them are incorporated in the oocyte. Their amino acid sequence can be divided into several domains located in linear fashion: NH_2-heavy chain of lipovitellin (LvH)-phosvitin (polyserine domain)-light chain of lipovitellin (LvL)-β′ component (β′-C)-COOH. Each of these domains corresponds to the different yolk proteins, lipovitellins (lipoproteins), phosvitins and phosvettes (highly phosphorylated proteins), and β′-C, which are stored in granules or globules distributed in the oocyte cytoplasm. VtgC is similar to VtgA and VtgB, but lacks the phosvitin domain. The Vtgs are posttranslationally glycosylated and phosphorylated and additional prosthetic groups such as retinal are added before being excreted into the circulating blood as a homodimeric lipoprotein complex (Finn, 2007). The Vtg receptor binding site is presumed to be located on the Lv domain of Vtg (Stifani et al., 1990).

The Vtgs reach the oocyte by passing from the theca capillaries to the granulosa layer, arriving at the oocyte surface through the pore canals of the zona radiata and are sequestered by receptor mediated endocytosis, involving specific receptors in the endocytotic clathrin-coated pits of vesicles. The coated vesicles move into the peripheral ooplasm and fuse with lysosomes forming multivesicular bodies (MVB) (Wallace and Selman, 1990). The specific Vtg receptors (VtgR) belong to the supergene family of low density lipoprotein receptor (LDLR) related proteins (LRPs; Le Menn et al., 2007). The complete molecular cloning and characterization of a fish VtgR was obtained for the rainbow trout oocyte showing a ligand-binding domain with 8 LA (type A binding) repeats and lacking an O-linked sugar domain showing the endocytotic active domain FDNPVY (Davail et al., 1998). VtgR transcription predominates during previtellogenesis, suggesting that VtgR is recycled to the oocyte surface during the vitellogenic stage (Perazzolo et al., 1999). The molecular structure and function of two VtgRs from the white perch and tilapia were also characterized (Tao et al. 1996; Li et al., 2003; Hiramatsu et al., 2004). Within the MVBs, the Vtgs are probably cleaved by lysosomal enzymes such as cathepsin D, into the smaller yolk proteins (Sire et al., 1994; Carnevali et al., 1999a,b; see detailed description below).

3.5.3. Uptake of vitamins by developing oocytes

Vitamins are compounds required in trace amounts for growth, health and reproduction and must be supplied in the diet. The vitamin content of eggs is related to egg quality in teleosts and depends on the nutrition provided to the broodstock (Izquierdo et al., 2001; reviewed in Palace and Werner, 2006). Dietary intake concentrations of ascorbic acid, vitamin A and vitamin E were determined for several fish species but there is very little information on other vitamins and most importantly, there is almost no information on the mode of transport and uptake of vitamins by the developing oocytes. Studies on lipid soluble vitamins such as vitamin A and vitamin E gained more attention in recent years and it was proposed, in general, that they are delivered to the ovary in the same pathways as lipids. The primary physiological function of vitamin E is to serve as an antioxidant. While vitamin A may also serve as an antioxidant, its main role in developing embryos lies in regulating gene transcription (Balmer and Blomhoff, 2002).

During oogenesis, vitamin E and vitamin A are recruited from peripheral tissues and muscles in the adult female and their movement within the body is mediated by lipoprotein particles via the endogenous transport pathway (Palace and Werner, 2006). In general, lipid transport takes place in two forms; exogenous transport whereby lipids move from the gut to the liver and extra-hepatic tissues (Paik et al., 2004) by chylomicrons and by fatty acids bound to protein carriers, and the endogenous transport involving export of lipids from the liver or other organs to peripheral tissues. Lipids are packaged in VLDL, LDL and high density lipoproteins (HDL) and in adult females there is also a very high density lipoprotein (VHDL) fraction consisting mainly of Vtg. Lipid soluble vitamins are transported by passive inclusion in these hepatically derived lipoprotein fractions in parallel to protein mediated transfer. Vtg was found to transport vitamin E (as α-tocopherol; Lie et al., 1994; Tokuda et al., 2000) and vitamin A (in the form of retinal; Irie and Seki, 2002) during vitellogenesis but other protein carriers may also be involved, such as retinol-binding protein (RBP) for retinol transport and a tocopherol binding protein with a higher affinity for α-tocopherol (Hamre et al., 1998). In salmonids, vitamin E and vitamin A accumulate in all tissues and prior to spawning a large portion is redistributed to the ovary. Only 20% of the serum plasma tocopherol was associated with Vtg, and most of the tocopherol was transported by the HDL fraction but also by the VLDL and LDL fractions (Palace and Werner, 2006).

A complicated but more detailed picture is available for transport of vitamin A. Vitamin A, as retinol or retinyl-ester and provitamin A carotenoids are acquired from the food as these compounds are not synthesized in vivo by vertebrates. Their metabolites, including retinoic acid, are crucial for proper embryonic development in vertebrates. Retinoic acid is required as it is a ligand of two classes of nuclear receptors (retinoic acid receptors, RARs and retinoid X receptors, RXRs), regulating the transcription of ~500 genes involved in a large array of biological processing, including ovarian follicle and embryonic development in vertebrates (Blomhoff and Blomhoff, 2006). Moreover, retinoic acid was found to be associated, in mammalian species, with the entry into meisosis and the differentiation of germ cells as egg or sperm (Swain, 2006; Bowles and Koopman, 2007). The eggs of oviparous vertebrates contain carotenoids (e.g. astaxanthins, canthaxanthins, zeaxanthins, and others, reviewed in Scheidt, 1998), retinals, retinols and retinyl-esters (RE), and their relative abundance changes between the different taxonomic groups (Lubzens et al., 2003). Carotenoids within fish eggs are located in oil droplets (chylomicra particles) or in association with lipoproteins (Ando and Hatano, 1991). Retinals are the main stored form of retinoids in fish eggs and ovaries (Plack, 1964) and may constitute almost 100% of all retinoids in eggs of marine fish (Irie and Seki, 2002).

Transport of carotenoids and retinoids for deposition in oocytes was suggested to take place through four plasma carriers: (a) retinol-binding protein (Rbp4), (b) chylomicrons, (c) lipoproteins and (d) serum albumins (Levi et al., 2008). As mentioned before,

retinal carried by Vtg is deposited in fish eggs and constitutes a major component of the stored retinoids. A model explaining the metabolism of retinol, retinyl-ester and carotenoids was offered recently for the ovary of rainbow trout (Levi et al., 2008). This model is based on transcripts of genes known to participate in vitamin A metabolism and on immuno-histochemical studies of Rbp4 in the ovarian cells. In this model, retinol transported in the plasma bound to Rbp4 enters the ovary via Stra6, the receptor for Rbp4. Inside the ovarian cells (stromal or granulosa cells), cellular retinol-binding protein (Rbp1) regulates the cellular metabolism of retinol and metabolizes retinol to retinyl-ester by lecitin:retinol acyltransferase (LRAT) or oxidizes retinol to retinal. Intracellular retinol could also bind to Rbp4 for subsequent secretion from the stromal or granulosa cells and taken up possibly by the oocyte. While such a pathway was not demonstrated for fish ovarian follicles, this was suggested to take place in bovine ovaries (Brown et al., 2003). Ovarian cells may also take up retinyl-esters from plasma chylomicrons and either store them or convert them to retinol when needed. Beta-carotene or other carotenoids carried by either lipoproteins or serum albumin in the plasma may enter the ovarian cells, where they could be cleaved to retinal and subsequently converted into retinol and retinoic acid. It remains to be shown whether abundant plasma carotenoids such as xanthophylls can be cleaved by ovarian cells or ovarian follicles and can serve as a source of retinol and retinoic acid. Differences were found in the relative abundance of gene transcripts associated with vitamin A metabolism, in the theca and granulosa cells surrounding the oocyte. Moreover, temporal changes in gene expression levels were found during different oocyte developmental stages. These results indicate specific pathways in ovarian follicle cells with different roles during vitellogenesis, and suggest novel pathways for providing retinoids and carotenoids to developing oocytes in parallel to Vtg or lipoproteins.

3.6. Endocrine control of oocyte growth

Early vitellogenesis is characterized by further increases in plasma FSH and E2, and increased expression of ovarian FSH receptor (e.g. Swanson, 1991; Tyler et al., 1997; Breton et al., 1998; Oba et al., 2001; Santos et al., 2001; Swanson et al., 2003; Kwok et al., 2005; kobayashi et al., 2008, 2009). In vitellogenic salmonids, steroidogenic thecal cells supply androgen substrate to ovarian granulosa cells that express P450 aromatase and produce E2 (Fig. 3; Nagahama, 1994; Senthilkumaran et al., 2004; Young et al., 2005). E2 promotes hepatic Vtg synthesis, and FSH has been demonstrated experimentally to increase Vtg uptake by rainbow trout ovarian follicles in vitro (Tyler et al., 1991). Growth hormone (GH) potentiates the effects of E2 in stimulating Vtg synthesis by eel primary hepatocyte cultures (Peyon et al., 1996). GH also stimulates E2 synthesis by ovarian tissue of killifish (*Fundulus heteroclitus*; Singh et al., 1988) and spotted seatrout (*Cynoscion nebulosus*; Singh and Thomas, 1993).

Numerous studies have reported that FSH, LH, and partially purified and heterologous GTHs stimulate E2 production by vitellogenic teleost ovarian follicles in vitro (Fig. 4; Young et al., 2005). Several recent reports show that the GTH stimulation of E2 production in short term culture is accompanied by increased expression of one or more of the genes encoding ovarian steroidogenic proteins, including StAR, 3β-hydroxysteroid dehydrogenase, and P450 aromatase (Young et al., 2002; Kagawa et al., 2003;

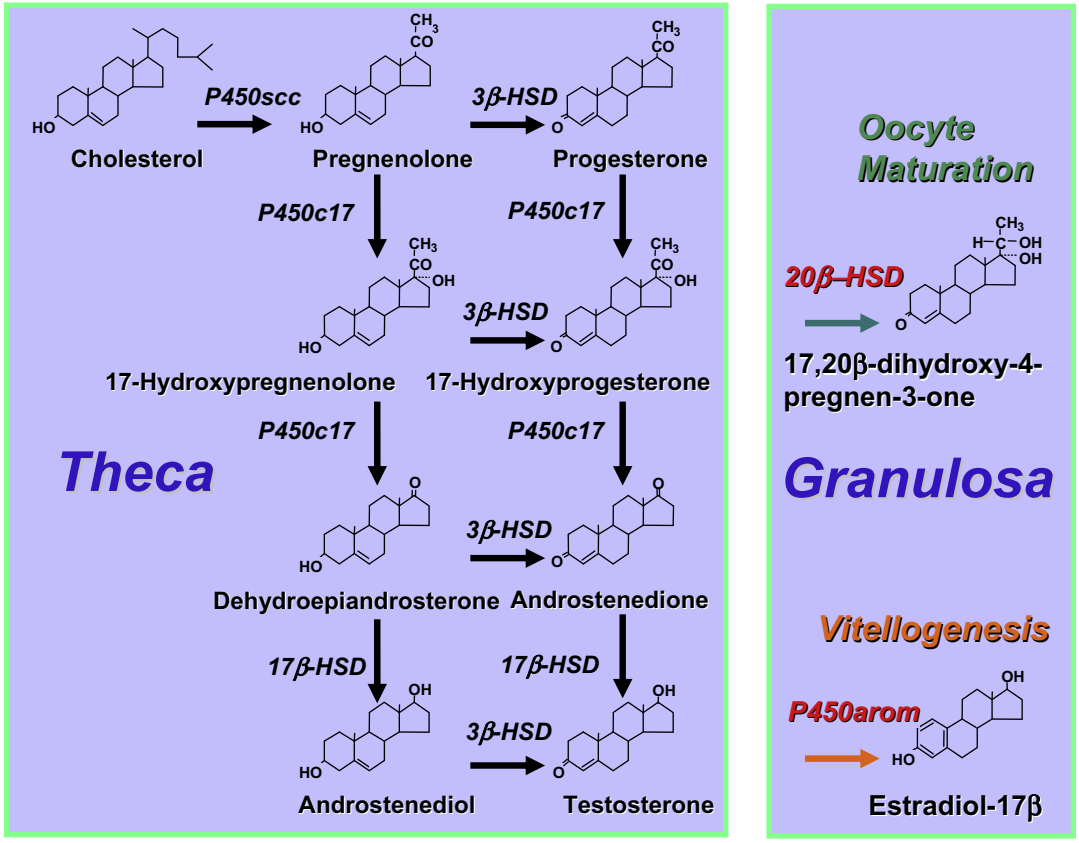

Fig. 3. Interaction of the salmonid thecal and granulosa layers of the ovarian follicle in the production of estradiol-17β during vitellogenesis and 17,20β-dihydroxy-4-pregnen-3-one during oocyte maturation. Enzymes: P450scc, P450 side-chain cleavage; P450c17, 17-hydroxylase/C17-C20-lyase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 20β-HSD, 20β-hydroxysteroid dehydrogenase; P450arom, P450 aromatase.

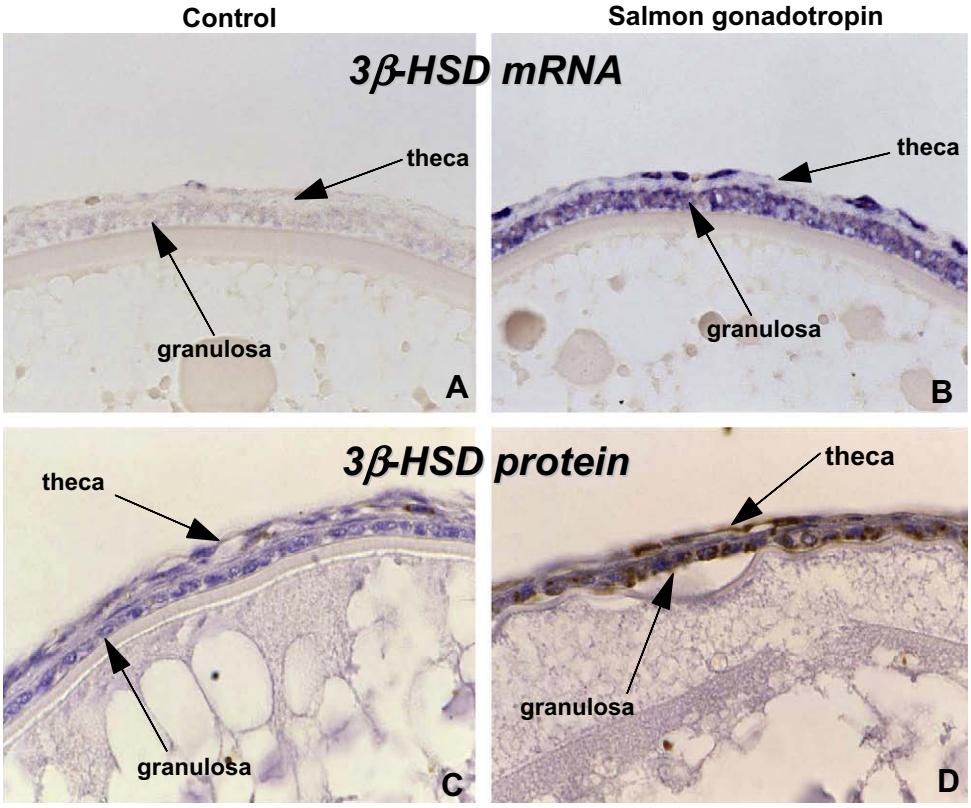

Fig. 4. Enhanced expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) after incubation of early vitellogenic follicles of rainbow trout *Oncorhynchus mykiss*) with partially-purified salmon gonadotropin. (A and B) 3β-HSD mRNA transcripts, in situ hybridization, 18 h incubation. (C and D) 3β-HSD protein, immuno-histochemistry, 36 h incubation. Only faint signals for 3β-HSD transcripts (A) and protein (C) are present in a few thecal cells in controls. After gonadotropin treatment, signals for both 3β-HSD transcripts (B) and protein are greatly enhanced in numerous thecal cells and moderate signals are induced in granulosa cells (Young et al., unpublished).

Nakamura et al., 2003; Montserrat et al., 2004; Ings and Van Der Kraak, 2006). IGF-1, whether systemic or produced by follicle granulosa cells (e.g. Kagawa et al., 1995; Perrot et al., 2000; Schmid et al., 1999; Berishivili et al., 2006), has been implicated in regulating ovarian steroidogenesis, though the underlying mechanism(s) are unclear. This growth factor inhibited steroid production by thecal layers in coho salmon ovarian follicles, yet stimulated steroid production by granulosa layers (Maestro et al., 1997b). Furthermore, IGF-I increased P450 aromatase expression in red sea bream (Kagawa et al., 2003) and trout ovarian follicles (Nakamura et al., 2003). IGF-I receptors are expressed in both layers of carp follicles (Maestro et al., 1997a). Expression of both IGF-I and IGF-I receptor in the ovaries of sterlet (*Acipenser ruthenus*) became elevated at the onset of vitellogenesis (Wuertz et al., 2007). Together, these studies indicate that the IGF-I signaling system is important in regulating the steroidogenic activity of the ovarian follicles during vitellogenesis. In addition to affecting the steroidogenic machinery, mitogenic effects of IGF-I on granulosa cells at the onset of vitellogenesis have been suggested (Kagawa et al., 1995).

The increase in both FSH and LH receptor expression as vitellogenesis starts and progresses is associated with a decline in the expression of GDF9 and BMP15 in European sea bass (Halm et al., 2008), and the same has been reported for GDF9 expression in zebrafish follicles entering vitellogenesis (Ge, 2005). Halm et al. (2008) suggest that these correlative observations may indicate that GTH receptor expression is regulated by these growth factors, and/or vice versa, since human chorionic gonadotropin reduced GDF9 expression in fully-grown zebrafish oocytes in vitro (Liu and Ge, 2007).

In zebrafish follicles, the temporal expression of activin βA (TGF-β superfamily) was low during primary follicle growth, but expression progressively increased with further ovarian development to peak at mid-vitellogenesis. During the daily ovulatory cycle of zebrafish, expression of activin βA gradually increased after 1800 h and peaked at 0400 h, coincident with the migration of the germinal vesicle to the periphery of full-grown oocytes (Wang and Ge, 2004a). By contrast, activin βB only increased at 0700 h when mature oocytes started to appear. Therefore, activin βA appears to be involved in promoting ovarian follicle growth, and activin βB appears to be important in the processes of oocyte maturation and/or ovulation. Epidermal growth factor (EGF), produced in zebrafish oocytes, stimulated expression of both activin βA and βB, and suppressed basal and human chorionic gonadotropin-induced expression of follistatin (a protein which binds and neutralizes activins) in cultured follicle cells. Since the EGF receptor was expressed in the follicle cells, EGF appears to act in a paracrine fashion to regulate the function of the follicle cells (Wang and Ge, 2004b). Recombinant goldfish activin B and recombinant TGF-β suppressed testosterone production by vitellogenic goldfish follicles (Calp et al., 2003).

During vitellogenesis the numbers and/or size of oocytes developing are adjusted to match available energy resources (see Luckenbach et al., 2008a). Follicle loss may also occur because of environmental stressors (see Section 6). FSH has anti-apoptotic effects on trout ovary (Janz and Van Der Kraak, 1997; Wood and Van Der Kraak, 2002). Furthermore, unilateral ovariectomy of vitellogenic coho salmon reduced loss of oocytes by atresia (Luckenbach et al., 2008a), and elevated levels of FSH have been reported after unilateral ovariectomy of rainbow trout (Tyler et al., 1997) and complete ovariectomy of coho salmon (Larsen and Swanson, 1997). Pharmacological inhibition of GTH action blocked the compensatory response in the remaining ovary to unilateral

ovariectomy in tilapia (Dadzie and Hyder, 1976). EGF and E2 suppressed DNA fragmentation associated with apoptosis in rainbow trout ovarian follicles in vitro (Janz and Van Der Kraak, 1997; Wood and Van Der Kraak, 2002). Ovarian-derived GnRH also appears to be involved in regulating apoptotic processes. GnRH has anti-apoptotic action in mid-vitellogenic goldfish follicles, but may have pro-apoptotic action in fully-grown follicles (for review, see Habibi and Andreu-Vieyra, 2007).

3.7. Egg envelope proteins

In all vertebrate species the egg is surrounded by an acellular vitelline envelope, known by several synonyms as the egg envelope, vitelline membrane, egg capsule or radiate membrane (Modig et al., 2006, 2007). In mammals, this envelope is transparent and called zona pellucida (ZP) and consists of 2–4 major proteins, the zona (pellucida) proteins (ZPs), also called zona radiata proteins (ZRP), vitelline envelope proteins (VEP) or choriogenins.

The egg envelope is formed by three main layers consisting of a cellular plasma membrane and two acellular layers that are deposited by the oocyte. Preceding the entry of yolk precursors, the oocyte is surrounded by cellular and acellular layers consisting of the theca cells, the basement lamina and the granulosa cells closely surrounding the plasma membrane or oolemma (Selman et al., 1993; Le Menn et al., 2007). The space between the oolemma and the granulosa cells fills with an extra-oocyte matrix within which oocyte microvilli protrude from the oocyte surface towards the granulosa cells. At the same time, the mesenchymal cells of the ovary differentiate into thecal cells forming a thick outer cell layer separated from the granulosa cells by a basal lamina. At the onset of oocyte growth, the oocyte extends microvilli that will make contact with the plasma membrane of granulosa cells. The synthesis of the egg envelopes in the oocyte starts from the base of these microvilli (Selman et al., 1993; Le Menn et al., 2007). The ZP precursor proteins are synthesized, modified with oligosaccharides, secreted and associated into cross-linked filaments that exhibit a structural repeat. The nascent ZP glycoproteins are incorporated into secretory vesicles that fuse with the oocyte plasma membrane, and the proteins are deposited into the innermost layer of the thickening ZP. In tangential histological sections this layer appears to be perforated by channels, through which the oocyte microvilli pass. In some species this external envelope seems to be composed of two structurally distinct layers. At a later stage of oocyte growth, the third layer of the ZP known as zona radiata interna is secreted from the oocyte, displacing the zona radiata externa toward the granulosa cells. The zona radiata interna shows a fibrillar structure, in contrast to the amorphous structure of the zona radiata externa. The layered vitelline envelope is perforated by pore canals which contain long microvilli directed from the oocyte, extending deep into the spaces between adjacent follicle cells, and generally shorter microvilli from the follicle cells, although some may reach the oocyte surface. The microvilli from the two directions may reside in the same pore canal and junctional contacts and gap junctions between them (Cerdà et al., 1999). The acellular vitelline envelope continues to differentiate throughout the growth of the oocyte and becomes highly ordered and architecturally complex (Le Menn et al., 2007; Modig et al., 2007).

Four groups of ZPs containing a similar ZP domain have been identified in vertebrates, namely ZPA, ZPB, ZPC and ZPX (Modig et al., 2006, 2007). The ZPA, ZPB and ZPC were found in mammals, while ZPX was identified in frogs, chicken and fish. The ZPA has not been found so far in fish. The thick egg envelope of teleost fishes consist of 3–4 proteins with a mass ranging from 47 to 129 kDa and all share a conserved ZP domain that can be found in many components of the extracellular matrix and membrane proteins. These proteins show proline and glutamine rich repetitive domains that maybe involved in the egg envelope hardening after fertilization forming the chorion or egg shell.

The synthesis of ZP proteins in fish occurs in the liver or in the ovary (e.g. carp *Cyprinus carpio* or zebrafish, Chang et al., 1996, 1997; Mold et al., 2001, respectively) or both (Hyllner et al., 2001; Modig et al., 2006). In teleost species where ZP proteins synthesis takes place in the liver, it is regulated by E2. The ZP precursor proteins are transported to the ovary, where they are incorporated into the oocyte and reshuffled via vesicles formed by the Golgi apparatus to the oocyte plasma membrane and deposited by the oolemma in the innermost layer of the zona radiata interna as described before. While estrogen regulation of ZP is widespread among teleosts, it has been shown that the estrogenic response could be altered by cortisol in the Arctic char (Berg et al., 2004). Estrogenic control of ZP synthesis is not a universal model for fish and other hormones such as cortisol and androgens have been shown to alter estrogen dependent regulation of ZP proteins in some species (Modig et al., 2007). The site of ZP synthesis varies among species and between different ZPs in the same fish. The dual expression of rainbow trout ZPC and gilthead sea bream ZPBa and ZPX in both liver and ovary (Modig et al., 2006) adds to the complexity. While liver expression appears to be regulated by estrogen, ovarian expression can either be under estrogenic control or independent of it as in the case of zebrafish (Liu et al., 2006; see Table 1 in Modig et al., 2007).

In most vertebrates, the eggshell is involved in fertilization through sperm binding and sperm guidance. In contrast to mammals, fertilization in fish takes place only through a funnel like micropyle that is located at the animal pole. In teleosts, a single sperm travels through the micropyle and reaches the egg cell. After the egg is activated by the sperm, the micropyle closes and prevents polyspermy. The vitelline envelope also possesses antimicrobial and bactericidal functions, protecting the egg from bacterial pathogens (Modig et al., 2007). After fertilization, the egg envelope, also named chorion at this stage, will protect the embryo in the aquatic environment.

4. Oocyte maturation and hydration

4.1. Hormonal control of oocyte maturation

Numerous studies, both in vivo and in vitro, have shown that the major endocrine events associated with termination of vitellogenesis and progression to meiosis resumption (oocyte maturation) are an acute increase in plasma LH levels, increased expression of the LH receptor, and an LH-driven switch in the ovarian follicle steroidogenic pathway from the production of predominantly E2 during vitellogenesis, to the production of maturation-inducing steroids (MIS) (Nagahama and Yamashita, 2008). These compounds are species-specific derivatives of progesterone which bind to oocyte membrane-specific receptors to activate the maturation promoting factor (MPF) in the ooplasm that triggers the dissolution of the germinal vesicle and reinitiates meiosis.

The MIS of salmonids and many other species is 17,20βP while 17,20β,21-trihydroxy-4-pregnen-3-one is found in some, but not all, perciforms and some other species (Young et al., 2005; Nagahama and Yamashita, 2008). In a few species, both steroids appear to participate in regulating oocyte maturation (e.g. red sea bream, Ohta et al., 2002; kyusen wrasse, Matsuyama et al., 2002). The switch in the steroidogenic pathway is associated with decreased expression of P450 aromatase (e.g. Nakamura et al., 2005; Bobe et al., 2006) and increased 20β-hydroxysteroid dehydrogenase (20β-HSD) activity in granulosa cells, accompanied by increased 20β-HSD transcripts in some (Tanaka et al., 2002; Senthilkumaran et al., 2002, 2004; Nakamura et al., 2005) but not all studies (Wang and Ge, 2002; Bobe et al., 2003, 2004; Von Schalburg et al., 2005).

Activity of 17α-hydroxylase increased and C17, C20 lyase decreases in thecal cells, such that the predominant thecal product switches from androgens to 17α-hydroxyprogesterone, which is converted to 17,20βP via 20β-HSD in granulosa cells (see Young et al., 2005). There is some evidence to suggest that the decrease in P450 aromatase activity is due to the actions of LH (Maestro et al., 1997a; Montserrat et al., 2004; Nakamura et al., 2005). Since 17α-hydroxylase and C17, C20 lyase activities were assumed to be contained within a single enzyme, P450c17, the mechanism through which 17α-hydroxylase activity became dominant was thought to be essential to this switch in the steroidogenic pathway. However, recent work in tilapia and medaka has identified two forms of P450c17 that are encoded by two different genes. P450c17-II exhibits only 17α-hydroxylase activity while P450c17-I possesses both 17α-hydroxylase and C17, C20 lyase activities. Analysis of expression of P450c17-I and -II in the ovary of tilapia during secondary growth strongly suggests that P450c17-I is responsible for the synthesis of E2 during vitellogenesis. However, P450c17-II is expressed during the steroidogenic shift to 17,20βP once vitellogenesis is completed (Zhou et al., 2007a,b). Distinct *p450c17-I* and *-II* genes have also been identified in the genomes of fugu (*Fugu rubripes*) and tetraodon (*Tetraodon nigroviridis*) (Zhou et al., 2007a,b) suggesting that the existence of these two forms may be widespread in fish. This would explain the paradoxical observations that *p450c17* transcripts decline in rainbow trout follicles at a time of enhanced MIS production during maturation and ovulation (Bobe et al., 2004; Nakamura et al., 2005). Several studies have also reported impressive increases in expression of the ovarian StAR gene at the time of oocyte maturation, suggesting that the overall capacity of the follicle to produce steroid hormones may be enhanced during this time (Kusakabe et al., 2002; Bobe et al., 2004; Nakamura et al., 2005).

The ability to synthesize MIS by the follicle cells must also be accompanied by the acquisition of sensitivity of the oocyte to respond to the MIS. This process is known as maturational competence, and includes an increase in MIS receptors on the oocyte cell membrane and an increase in communication among the granulosa cells, and between the granulosa cells and the oocyte, through gap junctions. The role of gap junctions during this process is however not known. Substantial evidence is available to show that maturational competence is induced by GTHs (see Patiño and Sullivan, 2002; Patiño et al., 2003; Weber et al., 2007; Yamamoto and Yoshizaki, 2008). Large scale gene expression profiling of rainbow trout ovarian follicles during acquisition of maturational competence has implicated a number of hormones and growth factors in this process, but their precise roles have yet to be determined. Transcripts for FSH receptor (Bobe et al., 2003) and IGF-II (Bobe et al., 2003, 2004) were significantly higher in ovarian follicles displaying high maturational competence. In addition, certain IGF binding proteins (IGF-BPs) have been implicated in the acquisition of maturational competence (IGF-BP3 and 5); transcripts for all six IGF-BPs were regulated by partially purified GTH, and IGF-BP2–5 were regulated by 17,20βP and E2 (Kamangar et al., 2006). The involvement of IGFs in both acquisition of maturational competence and in oocyte maturation is supported by a number of other studies. IGF-I, and in some cases IGF-II, stimulate acquisition of maturational competence and/or induce oocyte maturation, depending on the species (Kagawa et al., 1994, 1995; Kagawa and Moriyama, 1995; Negatu et al., 1998; Patiño and Kagawa, 1999; Weber and Sullivan, 2000, 2005; Mukherjee et al., 2006; Weber et al., 2007). In some cases, this has been linked to increased MIS production, while in others the effects appear to be independent of alterations in steroid output. In addition, expression of IGF-BP6 and IGF-BP related protein 1 were up-regulated at the time of oocyte maturation in rainbow trout (Kamangar et al., 2006).

A number of other ovarian factors have been implicated in the processes leading to maturation. The system has been shown to be involved in transducing the surge in LH associated with maturation of zebrafish follicles: activin and follistatin synthesis were up-regulated by GTH, goldfish activin β enhanced both GTH and MIS effects on oocyte maturation, and human activin A and goldfish activin B enhanced maturational competence (Pang and Ge, 1999, 2002a; Ge, 2000; Wang and Ge, 2003a,b). Further, GTH up-regulated activin βA and activin type II receptor expression (Pang and Ge, 2002c). Recombinant human EGF and TGF-β significantly enhanced GTH-induced maturation of zebrafish ovarian follicles, their effects were blocked by follistatin, and both growth factors increased the expression of activin βA and the activin type II receptor (Pang and Ge, 2002b). In killifish ovarian follicles, porcine inhibin inhibited GTH-stimulated oocyte maturation, but not via effects on synthesis of MIS, and also blocked MIS induction of maturation. Conversely, human activin A enhanced MIS-induced maturation rates (Petrino et al., 2007).

Evidence has recently emerged suggesting that BMP-15 is a key factor controlling oocyte maturation, at least in zebrafish ovarian follicles. Although reporting no change in expression during the course of oocyte development, Clelland et al. (2006, 2007) have demonstrated that incubation of ovarian follicles with recombinant human BMP-15 suppressed human chorionic GTH-induced oocyte maturation. Conversely, treatment of follicles in vitro with antiserum against zebrafish BMP15 increased oocyte maturation. Furthermore, suppression of expression of BMP15 using antisense morpholino oligonucleotides increased the rate of oocyte maturation, while enhanced expression via microinjection of a BMP-15 cDNA construct reduced GTH- and MIS-stimulated oocyte maturation. Together these results indicate that BMP15 functions to prevent premature oocyte maturation, partly through suppressing sensitivity to MIS.

4.2. Transcriptome and proteome studies

It should be stressed that apart from the thoroughly studied LH/MIS/MPF cascade and related endocrine and paracrine events, several concomitant processes are likely to participate in the differentiation of the ovarian follicles in order to prepare ovulatory and post-ovulatory functions as well as the release of a developmentally competent oocyte. High throughput transcriptome and proteome analyses have proven to be powerful tools to uncover genes and proteins that may be involved in these mechanisms (Cerdà et al., 2008a; Bobe et al., 2008a, 2009). At the follicular level, a transcriptome analysis carried out in rainbow trout showed that a drop in the expression levels of genes involved in estrogen synthesis was one of the earliest molecular events prior to oocyte maturation (Bobe et al., 2006). A concomitant drop of the mRNA levels of a novel sex hormone-binding globulin, called shbgb, that could participate in the modulation of estrogen and androgen action at the time of oocyte maturation, was also uncovered using this approach (Bobe et al., 2008b). In zebrafish, a serial analysis of gene expression (SAGE) has led to the identification of a repertoire of genes expressed in fully-grown ovarian follicles prior to oocyte maturation (Knoll-Gellida et al., 2006). According to the *in silico* analysis carried out by the authors, the three most expressed transcripts were rhamnose-binding lectin, beta actin 2, and a transcribed locus similar to the H2B histone family. A parallel analysis carried out using two-dimensional electrophoresis led to the identification of several proteins expressed in fully-grown follicles. Comparison of transcriptome and proteome data revealed that most proteins identified by proteomic analysis had at least one transcript counterpart. In goldfish (*Carassius auratus*), a proteomic analysis of 26S proteasome was carried out during oocyte maturation (Horiguchi et al., 2006), which identified eight proteins differentially

expressed and showed that modifications of proteasomeal subunits occurred during oocyte maturation. Moreover, most of the proteins of maturing ovarian follicles were also identified in early developing oocytes (Ziv et al., 2008). Several of these proteins (e.g. Serpin A1, importin and ZP3) show higher abundance at late stages of oocyte differentiation, while others (e.g. Hsp90, Hsp60, calreticulin and piwi) were observed at significantly higher levels at early stages. The relatively enormous amount of Vtgs in maturing oocytes is one of the main obstacles in protein profiling of oocytes containing yolk and reduces the capacity of identifying stage specific proteins.

4.3. Oocyte hydration

In marine pelagophil fish, 67–75% of the final volume of the egg is acquired during oocyte maturation due to water uptake (reviewed by Cerdà et al., 2007). This process of oocyte hydration is unique among vertebrates and is thought to contribute with a water reservoir for embryos to survive in the hyperosmotic seawater as well as to the buoyancy of eggs, thereby increasing their survival and dispersal in the ocean. Thus, the water content of mature oocytes from pelagophil species that produce highly hydrated, floating eggs in seawater, can account for up to 90–95% of the egg weight, whereas in benthophil species that produce minimally hydrated, demersal eggs, showing no buoyancy in seawater, water content can reach 85% in weight (Fig. 5A).

4.3.1. The osmotic effectors: free amino acids and inorganic ions

In the killifish (*Fundulus heteroclitus*), Wallace and collaborators described for the first time the process of yolk protein hydrolysis that occurs specifically during oocyte hydration and maturation (Wallace and Selman, 1985; Wallace and Begovac, 1985). This unique process in oviparous vertebrates was observed to be more pronounced in pelagophil teleosts, and it was suggested that this hydrolysis could be the source of amino acids that increased the osmotic pressure of the oocyte thereby allowing water influx (Greeley et al., 1986). In further studies, it was reported that the abundance profile of amino acids between the free amino acid (FAA) pool of mature oocytes and some yolk proteins is very similar, indicating that the FAA pool most likely originates from the hydrolysis of these proteins (Thorsen and Fyhn, 1996; Thorsen et al., 1996). The role of FAA as osmotic effectors during oocyte hydration has been later confirmed by a number of different studies that have shown a positive correlation between water and FAA content in the egg (Fig. 5B–D) (Cerdà et al., 2007).

The egg content in FAAs, however, accounts for only half of the osmolality of the maturing oocytes, and therefore the accumulation of other low molecular weight compounds may also be osmotically active (Finn et al., 2002b). This is the case for benthophil species, such as the killifish, where the hydration of the oocyte depends on the accumulation of K^+ during the maturation process (Greeley et al., 1991; Wallace et al., 1992). In pelagophil species, K^+ ions have also been related to the increase of the oocyte osmolality during maturation, although during this period an increase in Mg^{2+}, Ca^{2+}, Cl^-, total ammonium (NH_4^+) and/or inorganic phosphorus (Pi) (LaFleur and Thomas, 1991; Selman et al., 2001; Finn et al., 2002b; Fabra et al., 2006), has also been found. Thus, in Atlantic halibut (*Hippoglossus hippoglossus*) it has been estimated that FAAs derived from yolk hydrolysis contribute roughly to 50% of the oocyte osmolality, whereas ions, such as K^+, Cl^-, Pi and NH_4^+, make up the balance (Finn et al., 2002b).

The mechanisms of transport and accumulation of ions in the fish oocyte are however largely unknown. In some species, experiments in vitro using specific inhibitors suggest a function for Na^+, K^+, ATPases, although the cellular localization of these pumps or their hormonal regulation are unknown (LaFleur and Thomas, 1991; Chen et al., 2003). In *F. heteroclitus*, however, no evidence has been obtained for the role of typical ouabain-sensitive Na^+, K^+, ATPases during oocyte hydration (Wallace et al., 1992). In this species, gap junctions are essential for oocyte hydration, suggesting

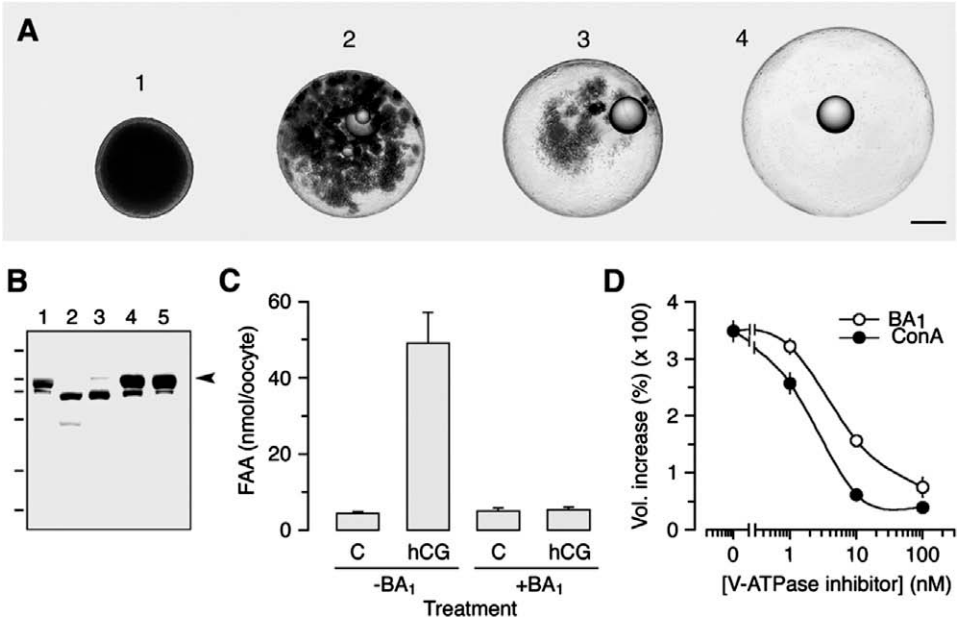

Fig. 5. Oocyte hydration in marine fish and role of free amino acids (FAA). (A) Follicle-enclosed oocytes undergoing oocyte maturation and hydration in vitro in a pelagophil teleost, the gilthead sea bream (*Sparus aurata*). 1, pre-maturation oocytes; 2 and 3, oocytes undergoing maturation; 4, mature oocytes prior to ovulation. Bar, 200 μm. (B) Western blot of sea bream yolk proteins of control (lane 1) and 17,20βP-stimulated ovarian follicles in the absence (lane 2) or the presence of 1, 10 and 100 nM bafilomycin A1 (BA1), an inhibitor of yolk hydrolysis (lanes 3, 4 and 5, respectively). Note that the degradation of a yolk protein of approximately 100 kDa, possibly LvH, is inhibited by treatment with BA1. The position (bars) of molecular weight markers is indicated on the left (from top to bottom: 200, 116, 97, 66, 45 and 29 kDa). (C) Effect of BA1 on the generation of FAAs during hCG-induced oocyte maturation in vitro in the black sea bass (*Centropristis striata*). (D) Treatment of follicles with yolk proteolysis inhibitors prevents oocyte hydration in sea bream. Data are from Selman et al. (2001) and Fabra et al. (2006).

that these intercellular channels are employed to transport K^+ into the oocyte (Wallace et al., 1992; Cerdà et al., 1993).

4.3.2. Processing of yolk proteins during oocyte maturation

During oocyte maturation, especially in pelagophil species, yolk proteins are further cleaved, generating FAAs and small peptides used for oocyte hydration. During this process, the yolk globules merge with one another, eventually forming a central mass of liquid yolk, and the internal crystalline structures disassemble conferring to the mature oocytes their characteristic transparency (Fig. 5A) (Cerdà et al., 2007). The hydrolysis of VtgA- and VtgB-derived yolk proteins occurs concomitantly by the action of cysteine proteases, such as cathepsin L and/or cathepsin B, that are specifically activated during maturation (Carnevali et al., 2006; Cerdà et al., 2007). However, studies in several pelagophils have shown that yolk proteins are differentially processed (Matsubara et al., 1999; Reith et al., 2001; Finn et al., 2002a,b; Finn, 2007; Kolarevic et al., 2008). In most species, LvHb (from VtgB) is dissociated into two monomers, LvHa (from VtgA), phosvitins and β′-C are extensively degraded to produce FAA, and LvLs are partially hydrolyzed. The degradation of the LvHa thus contributes fundamentally to the pool of FAAs for oocyte hydration, whereas the LvHb and LvL remain stored in the oocyte as a source of nutrients for further embryonic development. VtgC-derived yolk proteins are only slightly hydrolyzed during oocyte maturation, and therefore they do not seem to be major contributors to the hydration of the oocyte (Amano et al., 2008). In benthophil species, however, only the LvHa is partially cleaved, while phosvitins are fully degraded, and altogether results in a more modest increase of the FAA pool (Finn et al., 2002b; LaFleur et al., 2005).

Finn and Kristoffersen (2007) have recently examined the evolution of vertebrate *vtg* genes in relation to the "3R hypothesis" of whole genome duplication (WGD). Based on Bayesian and fossil record analyses, these authors suggest that lineage-specific duplication of *vtg* and further neofunctionalization of duplicated genes, which allowed one paralog (*vtga*) to be proteolyzed into FAAs driving hydration of the maturing oocytes, was a key event in the evolution and success of the teleosts in the oceanic environment. The evolution of *vtg* genes was possibly accompanied by the appearance of cellular mechanisms in the oocyte allowing the differential hydrolysis of VtgA- and VtgB-derived LvHs during maturation. The nature and regulation of these mechanisms are however largely unknown, although it may be hypothesized that they may be related to differences in the amino acid sequence of Vtg paralogs, in their secondary structure, or in their different compartmentalization inside of yolk globules, which would allow the cathepsins distinguish between the two LvHs. The elucidation of these biological processes will be essential to understand the metabolism and function of yolk proteins during fish oogenesis and embryogenesis.

4.3.3. Role of aquaporins during oocyte hydration

For a long time, it was assumed that water flux into the fish oocyte occurred by simple diffusion through lipid membranes following the osmotic gradient created by the accumulation of ions and FAAs. However, the discovery of the molecular water channels, aquaporins (AQPs), in practically all organisms, from prokariota to eukariota (King et al., 2004), has prompted detailed investigations into the molecular mechanisms involved in the hydration of the oocyte of marine teleosts.

The AQPs are integral membrane proteins that form pores for the transport of water along an osmotic gradient, where the direction of water flow is determined by the orientation of the gradient. These channels consist of six transmembrane domains, which are connected by five loops (A–E) and have their N- and C-termini located intracellularly. One molecule consist of two repeats, each containing the highly conserved asparagine-proline-alanine (NPA) motif (in loops B and E), which is the hallmark of the major intrinsic proteins (MIP) family (King et al., 2004). Currently, thirteen different AQPs are known in mammals that can be divided in water-selective AQPs and aquaglyceroporins, which are permeable to water and to small solutes, such as glycerol and urea. AQPs are differentially expressed in many types of cells and tissues where they regulate water transport and glycerol content, although the specific physiological roles for most AQPs is yet unknown (Takata et al., 2004; Rojek et al., 2008).

The role of AQPs during oocyte hydration in pelagophil teleosts has recently been investigated using the gilthead sea bream (*Sparus aurata*) as experimental model. These studies have identified a novel water-selective AQP highly expressed in the ovary, related to mammalian AQP1, that has been named aquaporin-1b (Aqp1b, formerly AQP1o) (Fabra et al., 2005). Investigations into the physiological role of Aqp1b provided immunological and functional evidence for its involvement mediating water uptake into the oocyte during hydration (Fig. 6) (Fabra et al., 2005). In sea bream, Aqp1b is synthesized at early vitellogenesis and transported towards the oocyte cortex throughout oocyte growth (Fabra et al., 2006). During oocyte maturation, shortly after germinal vesicle breakdown and before complete hydrolysis of yolk proteins is reached, Aqp1b is translocated transiently into the oocyte plasma membrane. Thus, in sea bream yolk hydrolysis seem to play a major role to create the osmotic driving force, while Aqp1b possibly facilitates water influx into the oocyte. These results support a novel model for fish oocyte hydration, whereby the accumulation of osmotic effectors and Aqp1b intracellular trafficking are two highly regulated mechanisms that allow water uptake into the oocyte (Fabra et al., 2006).

Phylogenetic analysis of vertebrate AQP1-related channels suggest that Aqp1b originated during evolution early in the teleost lineage possibly by local duplication of an ancestral AQP1-like gene and further structural divergence of the C terminus (Fig. 7A) (Tingaud-Sequeira et al., 2008). In extant marine and migrating (catadromous) pelagophil teleosts that spawn highly hydrated eggs, *aqp1b* mRNA is predominantly expressed in the ovary, where it encodes a functional water channel that possibly mediates oocyte hydration (Fig. 7B). In contrast, in strict freshwater species that spawn non-hydrated eggs, such as zebrafish (*Danio rerio*) or medaka (*Oryzias latipes*), *aqp1b* has a completely different expression pattern or is not found in the genome, respectively. In addition, functional expression analyses have revealed that the sea bream Aqp1b C-terminus, unlike that of Aqp1a (the teleost ortholog more similar to mammalian AQP1), contains specific residues involved in the control of Aqp1b intracellular trafficking through phosphorylation-independent and -dependent mechanisms (Tingaud-Sequeira et al., 2008). These data thus suggest that Aqp1b is encoded by a gene unique to teleosts that represents a neofunctionalized water channel adapted to oocytes of marine and catadromous teleosts.

5. Oocyte ovulation from the ovarian follicle

After meiosis resumption, the metaphase II oocyte is released from the follicle as a result of the ovulatory process. Ovulation is defined as the release of a mature oocyte from its follicle into the ovarian cavity or into the abdominal cavity depending on the species. Arachidonic acid and its metabolites, including prostaglandins (PGs), have been shown to be involved in ovulation in fish (Jalabert and Szöllösi, 1975; Stacey and Pandey, 1975; Goetz, 1983; Goetz et al., 1991; Bradley and Goetz, 1994; Goetz and Garczynski, 1997; Patiño et al., 2003), and in some species, stimulation by MIS leads to both oocyte maturation and ovulation in vitro. In yellow perch, 17,20βP induces both oocyte maturation and ovulation in vitro, whereas ovulation is inhibited by PG synthesis inhibitors, indicating that one or more PGs are probably the

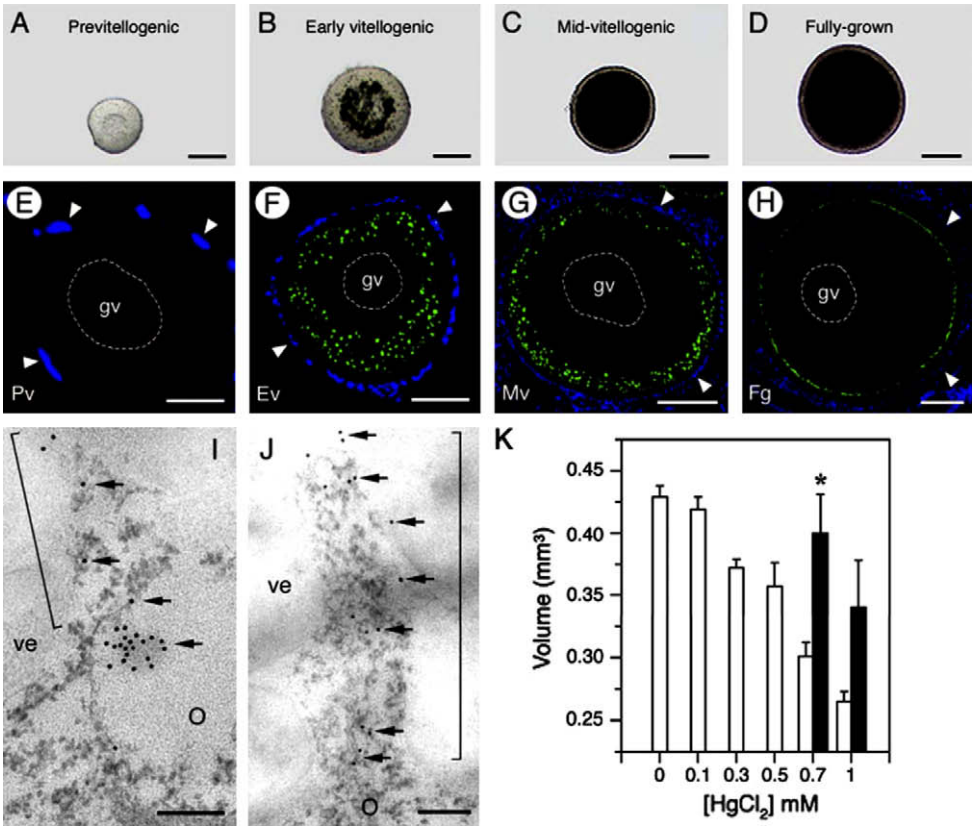

Fig. 6. Aquaporin-1b subcellular localization and function in sea bream oocytes. (A–D) Photomicrographs of follicle-enclosed oocytes at previtellogenesis (Pv; A), early vitellogenesis (Ev; B), mid-vitellogenesis (Mv; C), and fully-grown (Fg; D). Bars 100 μm (A and B), 200 μm (C and D). (E–H) Immunofluorescence microscopy from the ovary showing ovarian follicles at the same stages than above after reaction with an anti-Aqp1b antisera. The sections were counterstained with Hoechst (blue color) for visualization of cell nucleus from somatic cells. Bars, 20 μm (E), 50 μm (F), 100 μm (G and H). (I and J) Immunoelectron microscopy micrographs of fully-grown (I) and maturing (J) oocytes showing subcellular localization of Aqp1b predominantly within vesicles and in the microvilli extending from the oocyte, respectively. The immunogold particles are indicated by black arrows, and the microvilli are indicated by brackets. (K) Mercury inhibition of Aqp1b-mediated oocyte hydration in vitro (mean ± SEMs) and recovery by β-mercaptoethanol (closed bars). $^{*}p < 0.05$. Data are from Fabra et al. (2005, 2006).

final hormonal inducers of ovulation. Furthermore, PG levels increase in yellow perch follicles stimulated to ovulate with 17,20βP (Goetz and Garczynski, 1997), and 17,20βP-stimulated ovulation and follicular PG synthesis requires the close interaction of extrafollicular tissue and other follicle wall layers (Goetz, 1997). However, it appears that the pathway activated from MIS stimulation (via receptors on the oocyte plasma membrane) to oocyte maturation is separated but linked to that which leads to ovulation. For the latter, a MIS-induced increase in oocyte nuclear progestin receptors has been postulated to be a key step in the ovulatory process (Nagahama and Yamashita, 2008). A number of studies suggested that in addition to prostaglandins, ovulation in fish probably involves the cooperation of other ovarian factors such as proteases and protease inhibitors, progestins, other eicosanoids, catecholamines, and vasoactive peptides (reviewed by Goetz and Garczynski, 1997).

In general, meiotic maturation of follicle-enclosed oocytes is rapidly followed by ovulation. Indeed, it is now considered that during the pre-ovulatory period the ovarian follicle undergoes a progressive differentiation during which oocyte maturation and ovulation successively occur (Patiño et al., 2003; Bobe et al., 2008a). At the follicular level, oocyte maturation and ovulation are thus closely integrated and partly overlapping events. This process requires the separation of the oocyte from the granulosa layer and the localized rupture of follicle layers (Fig. 8). This process requires a proper retraction of the oocyte microvilli tightly connected to the granulosa cells during vitellogenesis, which disrupts cell–cell adhesion structures and gap junctions between the oocyte and the granulosa cells (York et al., 1993; Cerdà et al., 1999).

5.1. Mechanical and ultra-structural aspects

As already indicated, during ovulation the oocyte is expelled from the follicle through a localized rupture of follicular layers. Observations made in vitro have shown that the ovulating oocyte stretches through this localized "hole" in the follicular envelopes (Fig. 8) (Jalabert, 1978). This suggests not only a localized rupture of follicular layers, possibly through the action of specific proteolytic enzymes and/or directed cell death, but also a mechanical action of the oocyte within the follicle onto surrounding follicular layers. These mechanical properties have been used to study the action of several molecules on in vitro ovulation using follicle contraction assays of punctured brook trout (*Salvelinus fontinalis*) follicles (Hsu and Goetz, 1992; Hajnik et al., 1998). In rainbow rout, a 25% increase in oocyte hydration is observed during the pre-ovulatory period (Milla et al., 2006). It is therefore likely that the associated increase of oocyte volume would mechanically participate in the ovulatory process through an increase of intrafollicular pressure applied by the oocyte onto surrounding envelopes as shown in Fig. 8. This hypothesis would be totally consistent with the dramatic increase of *aqp4* (aquaporin 4) and *slc26a4* (also known as pendrin, a sodium-independent chloride/iodide transporter) mRNA expression reported in the rainbow trout ovary prior to ovulation (Bobe et al., 2006). It is also noteworthy that the theca is composed

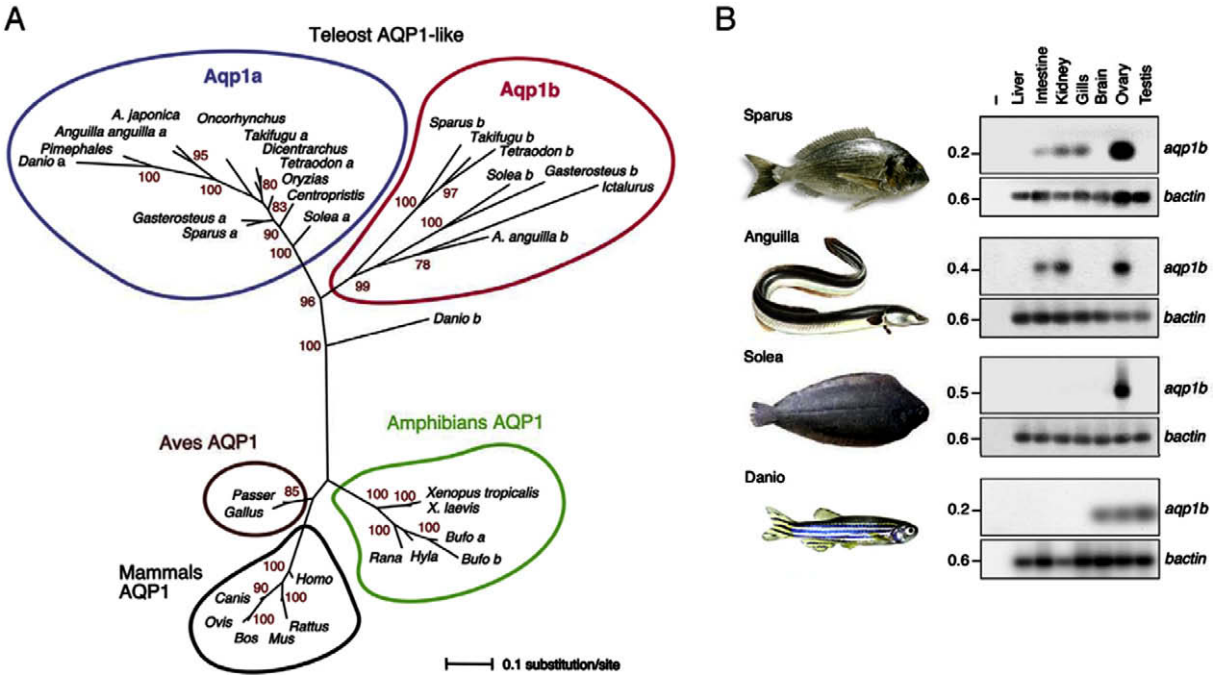

Fig. 7. Phylogenetic relationships of AQP1-like proteins in vertebrates and expression pattern of Aqp1b in teleosts. (A) Bayesian majority rule consensus tree for the amino acid alignment of teleost and tetrapod AQP1-like sequences. Nodes with ⩾70% Bayesian posterior probabilities are shown. (B) Representative RT-PCR analysis of *aqp1b* (upper panels) and *bactin* (lower panels) transcripts in different pelagophil and benthophil teleosts. PCR products were detected by Southern blot. Minus indicates absence of RT during cDNA synthesis. The size (kb) of PCR products and molecular markers are indicated on the left.

of several cell types including smooth muscle-like cells and collagen fibers (Szöllösi and Jalabert, 1974; Jalabert and Szöllösi, 1975; Szöllösi et al., 1978). It is thus possible that follicular contractions participate also in the ovulatory process has it has been hypothesized in many vertebrate species (Schroeder and Talbot, 1985). In favor of this hypothesis is the observed inhibition of in vitro ovulation by cytochalasin B in rainbow trout (Jalabert, 1976) and medaka (*Oryzias latipes*) (Schroeder and Pendergrass, 1976).

5.2. Molecular mechanisms during ovulation

In the past decade, several molecular studies have been used to decipher the molecular mechanisms of the ovulatory process. These studies have been carried out using successively stage-specific subtractive cDNA cloning, differential display PCR (DDPPCR), suppression subtractive hybridization (SSH), and cDNA microarrays (Jalabert, 1976; Garczynski and Goetz, 1997; Hajnik et al., 1998; Langenau et al., 1999; Bobe and Goetz, 2000a,b, 2001a; Bobe et al., 2006). Several of these studies have shown that a wide variety of proteases and antiproteases were expressed in the peri-ovulatory ovary (Garczynski and Goetz, 1997; Hajnik et al., 1998; Bobe and Goetz, 2001b,c; Bobe et al., 2006). Among the identified proteases, serine proteases seem to play an important role in the ovulatory process. In rainbow trout, *prss23* (serine protease 23) is progressively up-regulated in the pre-ovulatory ovary (Bobe et al., 2006), and in brook trout, serine proteases have been hypothesized to participate in follicular contraction (Hajnik et al., 1998). In mammals, ovulation is accompanied by broad-spectrum proteolysis and the implication of several classes of proteases is well documented (Ohnishi et al., 2005). In addition, serine proteases have been implicated in both ovulatory and inflammatory reactions (Espey, 1980). Similarly, *adam22* metalloprotease-disintegrin gene was shown to be strongly up-regulated prior to ovulation (Bobe et al., 2006). In mammals, several members of the metalloprotease-disintegrin protein family (also known as ADAMs: A Disintegrin And Metalloproteinases) participate in the ovulatory process and in brook trout, metalloprotease activity increases in the ovary prior to ovulation (Berndtson and Goetz, 1988, 1990). Together, these observations suggest that several proteases, including serine proteases and members of the ADAMs family participate in the ovulatory process.

In addition, to proteases and antiproteases, molecular screens also showed that several members of the tumor necrosis factor (TNF) family and the TNF receptor family were differentially expressed in the peri-ovulatory ovary (Bobe and Goetz, 2001b; Bobe and Goetz, 2001d). These observations suggested that TNF ligands and receptors are involved in the ovarian physiological processes around the time of ovulation possibly through the induction of cell death in specific cell populations of the follicle. In addition, suppression subtractive hybridization (SSH) led to the identification of osteopontin expression in the brook tout ovary at the time of ovulation (Bobe and Goetz, 2001a). This protein is expressed in granulosa cells and could participate in macrophage invasion, and T-cell activation or recruitment in agreement with its role in mammals (Patarca et al., 1989; Singh et al., 1990; O'Regan et al., 1999).

In rainbow trout, it was also shown that several inflammation-related genes such as *cxcl14, f5* (coagulation factor V), *fgf2* (fibroblast growth factor 2) and *ace2* (angiotensin-converting enzyme 2) were dramatically up-regulated in the ovary prior to ovulation (Bobe et al., 2006). In mammals, CXC chemokines are involved in chemotaxis of neutrophils, monocytes and lymphocytes (Walz et al., 1987;Yoshimura et al., 1987). *Cxcl14* expression was induced by inflammation in a murine model used to study Crohn's disease (Abad et al., 2005). Together, these observations suggest that *cxcl14* gene expression induction contributes to an inflammatory-like reaction occurring in the trout ovary during ovulation. The nature and expression profile of *f5* would also be consistent with a participation in an inflammatory-like process. Similarly, a strong increase of *ace2* and *fgf2* mRNA expression was observed in the ovary during the pre-ovulatory period (Bobe et al., 2004, 2006).

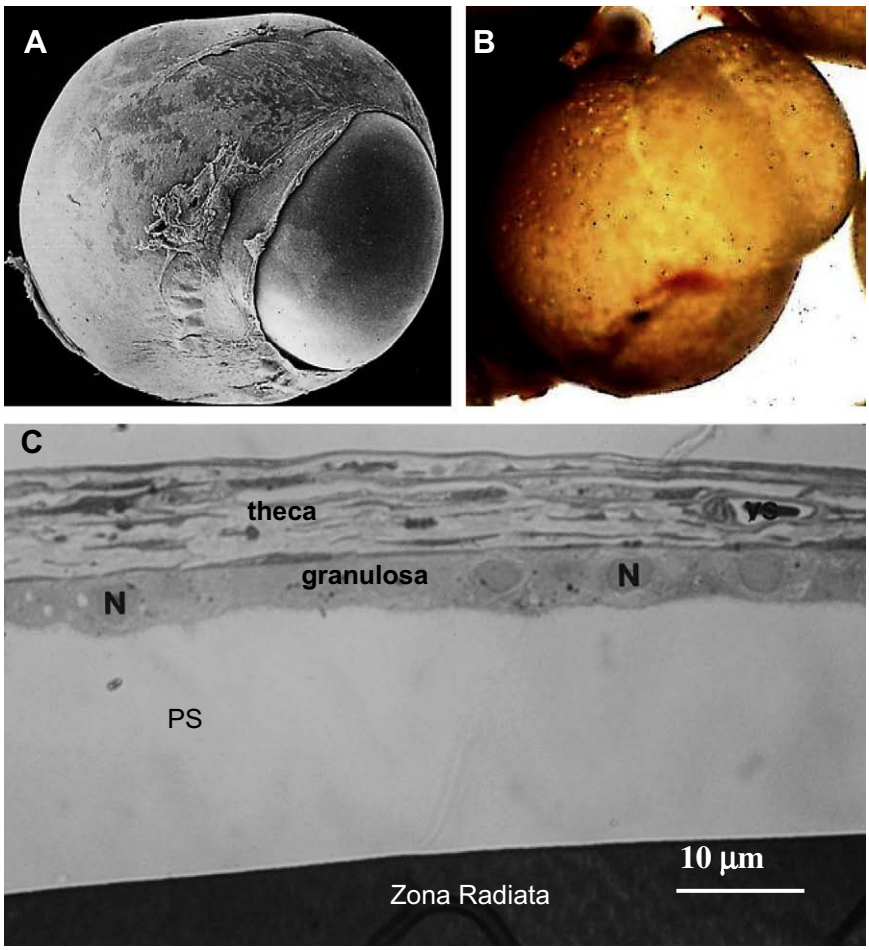

Fig. 8. (A) Scanning electron microscopic view of the localized rupture of the ovarian follicle (Foll) at the time of ovulation in rainbow trout (*Oncorhynchus mykiss*) ultimately leading to the release of the oocyte (Oo) into the body cavity. Copyright INRA Cauty-Jalabert. (B) Light macroscopic view of a rainbow trout follicle during the ovulatory process. The oocyte (Oo) stretching through a localized rupture of follicular layers (Foll) is shown. (C) Separation of the oocyte from the granulosa layer occurring prior to ovulation in rainbow trout. Copyright INRA Cauty-Jalabert.

While very little is known on the role of ACE2 in the vertebrate ovary, it is known in mammals that ACE2 can function as an Ang II degrading enzyme, forming the vasodilatator peptide Ang(1–7) (Vickers et al., 2002; Zisman et al., 2003). In the bovine, *FGF2* is expressed in follicles during final growth to pre-ovulatory follicle (Berisha et al., 2000) where it could be involved in the proliferation of capillaries. Interestingly, local vasodilation and changes in vascular dynamics are usually associated with inflammatory response and are also observed during the mammalian ovulatory process (Espey, 1980).

In mammals, it is now well accepted that ovulation is an inflammatory-like reaction (Espey, 1980, 1994). In fish, a number of existing studies also suggest that ovulation resembles an inflammatory-like reaction given the large number of inflammatory-related factors involved in the process (Goetz, 1997). This hypothesis is supported by molecular studies including recent transcriptome analyses suggesting that genes with pro-inflammatory, vasodilatory, vasoproliferative, proteolytic and coagulatory functions are expressed during ovulation (Bobe et al., 2006, 2009).

6. Follicular atresia

Ovarian atresia is a common phenomenon in vertebrate ovaries under both natural and experimental conditions during which a number of ovarian follicles recruited into the vitellogenesis pool fail to complete maturation and ovulation (Saidapur, 1978). This mechanism is in fact a highly regulated process that it is believed to be essential for the maintenance of ovarian homeostasis, and in fish it is usually seen at the end of each reproductively cycle (Krysko et al., 2008). However, a number of factors have been described in teleost fish as causing increased follicular atresia, such as hypophysectomy, starvation, temperature changes, stress, and inadequate hormone treatments (Guraya, 1986). In captivity, atresia is more frequent in vitellogenic oocytes, although it can also occur in previtellogenic oocytes (Guraya, 1986; Rizzo and Bazzoli, 1995; Miranda et al., 1999).

The process of atresia and resorption of ovarian follicles in fish is preceded by marked morphological changes in both the oocyte and follicle cells. The first morphological signs of atresia are the disintegration of the oocyte nucleus and of other cytoplasmic organelles (mitochondria, cortical alveoli, annulate lamellae), followed by the fragmentation of the zona pellucida and hypertrophy of the follicle cells (Saidapur, 1978; Miranda et al., 1999). The follicle cells become phagocytic with digestive vacuoles and incorporate and digest the oocyte yolk as well as other oocyte components as mitochondria and other organelles, and they may also secrete enzymes which digest the yolk. Several studies have also reported the presence in atretic follicles of blood cells derived from the ovarian stroma and/or the theca, such as eosinophilic granulocytes and macrophages, which invade the degenerating oocyte releasing their granules containing lytic enzymes (Besseau and Faliex, 1994; Miranda et al., 1999). By the end of the atretic process, both

follicle and immune cells degenerate leading to the formation of a deposit of pigments characterized as lipofuscins. At the same time, the number of theca cells decreases and the richly vascularized connective tissue surrounds the remnants of the follicle (Miranda et al., 1999).

The mechanisms that initiate and regulate oocyte atresia in teleost fish are poorly known, especially at the molecular level. In many organisms, atresia is considered as an apoptotic process, often initiated within the granulosa cells, and therefore granulosa cell apoptosis has been proposed as a key feature of atresia in the vertebrate ovary (Krysko et al., 2008). Gonadotropin-releasing hormone (GnRH) is one of the few hormones that have been proposed to participate in follicular atresia in mammals, since treatment with GnRHa agonists has been shown to induce DNA fragmentation (Hong et al., 2008). In contrast, current studies available in teleosts have been unable to demonstrate a direct link between granulosa cell apoptosis and the initiation of follicular atresia, although apoptosis has been shown to occur in the theca cell layer. (Drummond et al., 2000; Wood and Van Der Kraak, 2001, 2002). Accordingly, expression of GnRH and its receptors have been demonstrated in the fish ovary, which might be involved in the induction of apoptosis during gonadal regression resulting from lack of the appropriate ovulatory surge of LH (Habibi and Andreu-Vieyra, 2007).

In contrast to the apoptotic hypothesis in granulosa cells, the proteolytic degradation of the oocyte yolk proteins, mediated by the differential activation of lysosomal cathepsins, has been proposed as the initial event leading to oocyte cell death (Wood and Van Der Kraak, 2003). This hypothesis is supported by histological observations in a wide variety of teleosts where breakdown and resorption of the oocyte generally precedes somatic cell disappearance (Saidapur, 1978), and by the observation that a massive transfer of some yolk proteins combined with high density lipoproteins to the bloodstream occurs during the course of follicular atresia (Babin, 1987). The activation of this mechanism for yolk resorption in atretic follicles agrees with the elevated expression of fatty acid-binding protein 11 mRNA that has been detected in hypertrophic follicle cells surrounding atretic oocytes (Agulleiro et al., 2007). Therefore, the processes of yolk hydrolysis and resorption during ovarian atresia in fish have possibly evolved to facilitate the redistribution of energy-rich yolk materials from oocytes that fail to develop properly (Wood and Van Der Kraak, 2003). However, the specific oocyte mechanisms involved in the regulation of protease activity during atresia, as well as the origin of the signals that activate this system, are yet unknown and warrant further investigation.

7. The mature egg

The mature egg is a metaphase II oocyte released from the ovary after the completion of the ovulatory process. At this stage, the egg is fully formed and contains all the molecules and nutritive reserves needed for embryonic development as previously reviewed (Brooks et al., 1997). However, several investigations have suggested that the oocyte released at ovulation might not have acquired full developmental competence. This is mostly true for salmonids that can hold their eggs for a long time after ovulation. In rainbow trout for instance, several studies have indicated than the best timing for fertilization is 4–5 days after ovulation (Springate et al., 1984; Aegerter et al., 2005). In these two studies, fertilization of eggs sampled at the time of ovulation resulted in lower embryonic survival, whereas eggs sampled a few days after ovulation show significantly higher developmental capacities.

The unfertilized egg of vertebrates contains DNA and RNA polymerases, histone and non-histone chromatin proteins, transcription and translation factors, rRNAs, tRNAs, and maternal mRNAs (Tata, 1986). After fertilization, maternal factors support early embryonic development until activation of zygotic transcription. The initiation of zygotic transcription occurs during the "mid-blastula transition" (MBT) (Kane and Kimmel, 1993). In zebrafish, MBT occurs at cycle 10 and is characterized, in addition to the initiation of zygotic transcription, by the increase of cell cycle length, the loss of cell synchrony, and the appearance of motility (Kane and Kimmel, 1993). In parallel to the activation of zygotic transcription, differential degradation of maternally inherited mRNAs takes place (Mathavan et al., 2005; Giraldez et al., 2006). Maternal mRNAs are therefore extremely important for the functionality of the female gamete. For instance, mutation screens carried out in zebrafish have shown that the function of maternal factors (i.e. mRNAs) in the developing embryo depends on a precise plan of storage and localization that is initiated during oogenesis (Pelegri, 2003). These studies have also shed light on the function of some maternally inherited mRNAs during early embryonic development (Dosch et al., 2004). Data on maternal mRNAs have also shown that the abundance of some mRNAs varied significantly with egg quality. For instance, the mRNA levels of nucleoplasmin (*npm2*) in the unfertilized eggs dropped dramatically throughout the post-ovulatory period. This oocyte-specific gene encodes for a chaperone protein involved in the decondensation of sperm chromatin after fertilization in *Xenopus laevis* (Philpott et al., 1991). In addition, Npm2 knockout female mice have fertility defects owing to failed preimplantation embryo development (Burns et al., 2003).

Several protein families were identified apart from the Vtgs and ZPs, in the fish egg. These families include proteins associated with metabolic functions, chaperones, peroxiredoxins and translation regulatory proteins. Interestingly, discrepancies were found between the transcriptome (revealed by SAGE analysis) and proteome profiles, indicating that proteins were either deposited in the oocytes from extra-oocytic sources (most notably example are the Vtgs or ZPs) or that some proteins were synthesized at earlier stages (Ziv et al., 2008). In addition, the mature eggs contain vitamins and growth factors with specific functions during embryonic development. Mechanical protection of the embryo from the surrounding environment is provided by the eggshell proteins that also posses antimicrobial factors.

8. Conclusions

The formation of an egg is a complex process. Upon ovulation, the egg is self sufficient to protect and sustain the developing embryo until hatching, with almost no contribution from its surroundings. Therefore, all the contents of an egg, whether in the form of mRNA, nutrients or hormones, must be incorporated into an egg while it is developing as an oocyte within the ovary.

Understanding the mechanism underlying the processes of oocyte growth and development and how these processes are coordinated, is essential for perceiving the factors affecting egg quality and fertilization. Research efforts were directed to identify environmental influences on egg quality, including the difference in quality as a consequence of diet, especially lipids, protein and vitamin content, photoperiod and physiochemical properties of the water, and husbandary practices (reviewed in Brooks et al., 1997; Bobe and Labbé, 2009). Great advances were also made in revealing endocrine pathways regulating egg formation and functional aspects that contribute the proper development of the future embryo.

Several gaps still remain in our knowledge on the molecular mechanisms leading to the formation of a viable egg. One of these is the pathways involved in accumulation of vitamins in eggs that may contribute to their "quality". While some initial progress was

made in studies of vitamin A and vitamin E, these are far from providing a clear view on the state of events. Furthermore, there is almost no information on the transport and accumulation of vitamin D or others. Moreover, the mode of regulation of these compounds by carrier proteins is virtually unknown. Another important aspect not yet uncovered is the transductional pathways that are triggered in the oocyte just after the activation of the MIS receptor and that ultimately drive the activation of the MPF and the reinitiation of meiosis. These intracellular mechanisms are possibly involved in the regulation of cathepsin-mediated yolk proteolysis and AQP intracellular trafficking, but they still remain completely unknown.

In recent years, increasing efforts are made to provide ovarian or egg specific transcriptome profiles in model fish species such as zebrafish (Zeng and Gong, 2002; Li et al., 2004; Wen et al., 2005; Knoll-Gellida et al., 2006; Santos et al., 2007; Sreenivasan et al., 2008) and *Takifugu rubripes* (Shen et al., 2008) and in parallel of several cultured fish species such as rainbow trout (Bobe et al., 2004,2006; von Schalburg et al., 2005, 2008), coho salmon (Luckenbach et al., 2008a,b), bluefin tuna (*Thunnus thynnus*; Chini et al., 2008), Senegalese sole (*Solea senegalensis*; Cerdà et al., 2008b) and sea bream (Ferraresso et al., 2008). While recent molecular and proteome tools provide deeper insight into the composite events associated with oocyte development, we are still far from resolving the question raised by Brooks et al., 1997: What makes a good egg? Several markers were proposed in the past as quick indicators, including markers of apoptosis (e.g. P53, IL1, IL8 or FAS) and the level of MPF and cathepsin enzymatic activity (Carnevali et al., 2003, 2007; Suwa and Yamashita, 2007) that can serve for identifying eggs with potentially low viability. Early studies on relating egg quality to yolk lipoprotein nutrients, vitamins or hormonal factors were extended recently to identifying maternal mRNA transcripts as markers for egg quality (Aegerter et al., 2004, 2005; Bonnet et al., 2007; Ziv et al., 2008). For example, in the rainbow trout, post-ovulatory aging-induced egg quality defects were associated with relatively low mRNA levels of nucleoplasmin (*npm2*), *igf1* and beta-tubulin and higher abundance of keratins 8 and 18, cathepsinZ and prostaglandin synthase 2. Moreover, the abundance of *igf1* and *igf2* transcripts in ovulated oocytes was correlated with oocyte developmental competence after post-ovulatory aging. Also, the abundance of *igfr1b* or cyclin b transcripts was correlated with the incidence of post-ovulatory aging-induced morphological abnormalities (Aegerter et al., 2004, 2005). Similarly, abundance of mRNA of prohibitin 2 (*phb2*) was negatively correlated with the developmental potential of trout eggs (Bonnet et al., 2007). One of the obstacles in developing specific markers is the inherent diversity in egg viability, not only between different individual females but even between eggs obtained at different times after spawning from the same female (Aegerter et al., 2004, 2005). Identification of maternal mRNAs whose abundance in the oocyte is correlated with developmental competence has pointed out specific molecular mechanisms that could be participating in the control of egg quality. It remains to be shown whether these molecular mechanisms are also relevant for other fish species. An expectation for a protein marker or markers has not been fulfilled so far, especially as most proteins were identified already at early stages of oocyte development (Ziv et al., 2008).

Future advances in transcriptome and proteome profiling for deeper understanding of fish oogenesis highly depends on more genome sequencing especially of cultured fish species. Mining the data is one of the great challenges in high-throughput studies and this depends on improving our understanding of specific functions. These include pathways regulating the synthesis and response to endocrine and growth factors, regulation of hydration during oocyte maturation and the cascade of events leading to ovulation and readiness of the oocyte for fertilization and embryonic development. Post-genomic analyses are additionally required to functionally characterize the role of candidate genes and maternally-inherited mRNAs identified from genomic screens, during the development of the oocytes and early stages of embryos.

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