

Germinal Epithelium, Folliculogenesis, and Postovulatory Follicles in Ovaries of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Teleostei, Protacanthopterygii, Salmoniformes)

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ABSTRACT The rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is a salmoniform fish that spawns once per year. Ripe females that had ovulated naturally, and those induced to ovulate using salmon gonadotropin-releasing hormone, were studied to determine whether follicles were forming at the time of spawning and to describe the process of folliculogenesis. After ovulation, the ovaries of postspawned rainbow trout were examined histologically, using the periodic acid-Schiff procedure, to stain basement membranes that subtend the germinal epithelium and to interpret and define the activity of the germinal epithelium. After spawning, the ovary contained a few ripe oocytes that did not ovulate, numerous primary growth oocytes including oocytes with cortical alveoli, and postovulatory follicles. The germinal epithelium was active in postspawned rainbow trout, as determined by the presence of numerous cell nests, composed of oogonia, mitotic oogonia, early diplotene oocytes, and prefollicle cells. Cell nests were separated from the stroma by a basement membrane continuous with that subtending the germinal epithelium. Furthermore, follicles containing primary growth oocytes were connected to the germinal epithelium; the basement membrane surrounding the follicle joined that of the germinal epithelium. After ovulation, the basement membrane of the postovulatory follicle was continuous with that of the germinal epithelium. We observed consistent separation of the follicle, composed of an oocyte and surrounding follicle cells, from the ovarian stroma by a basement membrane. The follicle is derived from the germinal epithelium. As with the germinal epithelium, follicle cells derived from it never contact those of the connective tissue stroma. As with epithelia, they are always separated from connective tissue by a basement membrane. *J. Morphol.* 268:293–310, 2007.

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The Salmonidae is a teleost fish family of global economic value and, thus, is also of primary interest to the aquaculture of coldwater fish species. Salmonids include many of the most economically

valuable fishes, such as Pacific and Atlantic salmon, trout, charrs, and whitefish (Tyler, 1991; Tyler and Sumpter, 1996). The rainbow trout is likely the most important aquaculture salmonid species. The breadth of research devoted to rainbow trout husbandry provides extraordinary insight with respect to vitellogenesis, the formation of protein yolk (Sumpter et al., 1984; Copeland et al., 1986; Bromage and Cumarantunga, 1988; Tyler et al., 1990a,b, 1993; Blandine et al., 1998; Celius and Walther, 1998; Davail et al., 1998), refinement of the science of aquaculture (Bromage et al., 1992), development of strains that spawn at different times of the year (Scott and Sumpter, 1983; Billard, 1992), photoperiodic control of reproduction (Bromage et al., 1992; Davies et al., 1999), steroidogenesis (van den Hurk and Peute, 1979), fecundity (Allan and Sancher, 1960; Tyler et al., 1990b), and sex differentiation, gametogenesis, and gamete preservation (Billard, 1983, 1992).

Rainbow trout spawn once a year (Scott, 1987; Tyler et al., 1990b), having an “annual determined fecundity” as defined by Hunter et al. (1985). Prior to spawning, a clutch of oocytes matures and subsequently ovulates, and this maturation process is repeated during the next annual spawning event. To provide eggs over a prolonged season, hatchery strains of rainbow trout have been developed that spawn in the northern hemisphere during autumn and spring. Hormonal profiles for testosterone, 17 β -estradiol, 17 α ,20 β -dihydroxyprogesterone, gonadotropin, and vitellogenin, have been determined

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and compared for two such strains (Scott and Sumpter, 1983). Peak concentrations of these hormones have shifted and occur earlier in the year (September and October) in the early-spawning strain and later (November through January) in the winter spawning strain. Oocytes in the vesicle stage (possessing cortical alveoli) occur throughout the annual reproductive cycle in mature fish (Bromage and Cumaranatunga, 1988), leaving open to question the timing of follicle formation (folliculogenesis): is the number of eggs produced annually for a single spawning event determined throughout the year or only seasonally?

An emerging concept in vertebrate reproduction is the existence of a germinal epithelium (Parenti and Grier, 2004), the source of germ cells in both male and female gonads. The germinal epithelium is described by the same criteria that define an epithelium, as reported in numerous histology texts (Grier, 2000, 2002). The germinal epithelium differs from all other epithelia in that, in males and females, it possesses germ cells, which produce sperm or eggs, and somatic cells, involved in gamete maturation. In males, the somatic cells are Sertoli cells. In females, the somatic cells are epithelial cells that become prefollicle cells when associated with a meiotic oocyte in the germinal epithelium. Together, the prefollicle cells and oocytes initiate folliculogenesis, formation of a follicle. Upon completion of folliculogenesis, the follicle is surrounded completely by a basement membrane, and the prefollicle cells become the follicle (granulosa) cells. A germinal epithelium has been mentioned (van den Hurk and Peute, 1979), but not described in rainbow trout or any other salmonid. Here, we focus on description of an active germinal epithelium in postspawned rainbow trout, folliculogenesis, and postovulatory follicles.

MATERIALS AND METHODS

In the central plateau of Mexico, the peak spawning of hatchery-reared rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is during November and December. The spawning season was almost over in January 2002 and 2003, when two collections of fish used in this study were made. During the first collection on January 4, 2002, three adult rainbow trout were obtained from Truchon, a public park. They originated from nearby El Zarco, a state trout hatchery. One female was preovulatory, one was ovulating, and the third was postovulatory. During the second collection, between December 27, 2002 and January 4, 2003, broodstock rainbow trout were obtained directly from El Zarco and from the trout farm Tepehuaje, both northwest of Mexico City. The trout ranged in weight between 0.99 and 3.3 kg and length between 400 and 706 mm TL.

To obtain ovaries from rainbow trout after ovulation at Tepehuaje, 12 females were implanted with a time-release, salmon gonadotropin-releasing hormone pellet (Ovaplant, Syndel International). These females were checked twice daily for ovulation by applying gentle abdominal pressure. They ovulated 3 or 4 days, after being implanted.

Trout were pithed, and the ovaries quickly excised. In the Truchon collection, Bouin's fixative was used exclusively. At El Zarco

and Tepehuaje, both Bouin's fixative and Trump's glutaraldehyde and formaldehyde fixative (McDowell and Trump, 1976) were used. Because the ovaries were quite large and the tissue was not fixed until the fixative penetrated, the jars containing the gonads in fixative were placed on ice to maintain low temperature and to slow postmortem changes. After 24 h in iced fixative, the ovaries were sliced, and smaller pieces were returned to fresh fixative. The picric acid (yellow) component of Bouin's fixative had penetrated the ovaries only to an approximate depth of 5–10 mm during 24 h. After 48 h in Bouin's fixative, picric acid was washed from the samples with multiple water changes before storage in 70% ethanol, until further processing. Gonads in Trump's fixative were sliced into smaller pieces after 24 h and placed in fresh fixative, where they were stored until further processing, ~2 weeks later. According to McDowell and Trump (1976), samples can be stored in this fixative.

For histological processing, pieces of rainbow trout ovaries were cut to appropriate sizes for embedding and were passed through a series of increasing concentrations of ethanol, ending with 95%. These samples were then embedded in glycol methacrylate (JB-4 plastic, Polysciences). Sections were cut at 4 and 6 μm on a retracting microtome and stained with hematoxylin-eosin and the periodic acid-Schiff reaction for glycoproteins, metanil yellow, and hematoxylin (Quintero-Hunter et al., 1991). As a modification, the latter stain was also used, but hematoxylin was omitted, resulting in brilliant periodic acid-Schiff-staining of glycoproteins that was contrasted against the metanil yellow staining of the surrounding tissues that were rendered in low contrast. This simple modification visually enhanced the staining of periodic acid-Schiff-positive basement membranes beneath the germinal epithelium, especially after Bouin's fixation compared to Trump's fixation. The bracketed letters in the figure legends designate Bouin's fixative [B], Trump's fixative [T], Periodic acid-Schiff and metanil yellow stain [PAS/MY], and the same stain minus hematoxylin [PAS/MY-H]. Distinctive staining differences due to fixation are noted. Histological slides have been deposited in the Division of Fishes, National Museum of Natural History, Smithsonian Institution, Washington, DC. Further information on this material is available from the third author.

RESULTS

Ovarian Morphology

The morphology of the rainbow trout ovary is best viewed in spawned fish when large follicles with preovulatory oocytes do not obscure tissue relationships. In gross section, the walls of each ovarian lobe formed a "U-shaped" structure that is not enclosed dorsally (Fig. 1A), i.e., the ovarian lumen is continuous with the coelom dorsally. Ovarian lamellae extend between the ovarian walls, bridging the ovarian lumen. Even after spawning, some preovulatory oocytes, ~4.5 mm in diameter, may be observed in the lamellae along with numerous small follicles, containing primary growth oocytes with basophilic cytoplasm and numerous larger oocytes with cortical alveoli (Fig. 1B,C). The different sizes of follicles indicate some level of oocyte growth. Because the mature oocytes are large, the postovulatory follicles are clearly observed in both fresh and fixed tissue as saccular structures (Fig. 1A). Histologically (Fig. 1B,C), postovulatory follicles are a prominent component within the ovarian lamellae of postspawned rainbow trout.

In histological section (Fig. 1B,C), the ovarian lumen between the lamellae appears discontinuous

as juxtaposed lamellae contact each other (Fig. 1B). There are also distinctive, large spaces, the lacunae, within the lamellae. Some contain amorphous material. Some lacunae partially surround follicles with developing oocytes (Figs. 1B, 3B, 6A,B, 7A). A simple squamous layer of cells lines the lacunae (Figs. 3B, 6A). These cells sometimes appeared to contribute to the thecal layer surrounding follicles (Fig. 3B).

Germinal Epithelium and Folliculogenesis

In the ovary of ovulated rainbow trout, there is an active germinal epithelium producing follicles. The luminal surface of ovarian lamellae is covered by the germinal epithelium, in which epithelial cells could be squamous, cuboidal, or columnar (Figs. 2B, 6A, 7B), and among which are scattered oogonia (Figs. 2A–C, 3A, 7B, 8). Meiotic oocytes in diplotene may be within the germinal epithelium (Fig. 3A) or, in appropriate plane of section, observed within cell nests that are extensions of the germinal epithelium (Figs. 3B, 4A–C, 6C), protruding into the stroma. Cell nests are always separated from the stroma by a basement membrane (Figs. 3B, 4A–C, 6C). The germinal epithelium rests upon a basement membrane, which always separates it from the stroma. The basement membrane of the germinal epithelium is usually well-stained after Bouin's fixation (Figs. 2B,D, 3A), but is not as distinctive after Trump's fixation (Fig. 2A,C). Concurrently, Bouin's fixation also resulted in the loss of nuclear basophilia compared with Trump's fixation.

Oogonia divide mitotically producing cell nests, composed of clusters of germ cells (oogonia and early oocytes) and prefollicle cells. Dividing oogonia and oocytes may be juxtaposed (Figs. 3B, 5A,B) within a nest. Both mitosis of oogonia (Figs. 3B, 5A,B) and the initiation of meiosis (Fig. 3A) occur within the germinal epithelium and within cell nests.

The extent to which basement membranes are a part of the structure of ovarian lamellae in rainbow trout, compartmentalizing and separating stroma from tissue that is the germinal epithelium or the follicle which is derived from it, is particularly well demonstrated by omitting hematoxylin from the periodic acid-Schiff trichrome stain (Figs. 4A–C, 6C), although nuclear staining and basophilia are lost. This simple modification is an adequate method for demonstrating the basement membranes (Fig. 4A–C) that separate tissue compartments, i.e., germinal epithelium and cell nests from the stroma.

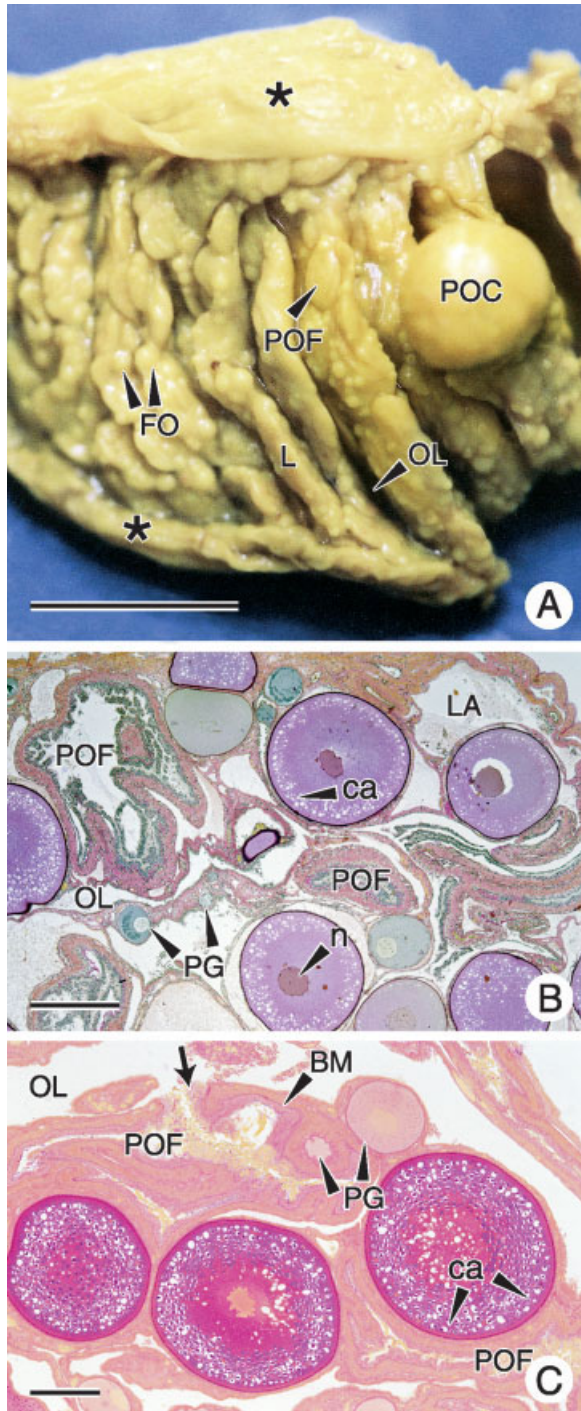
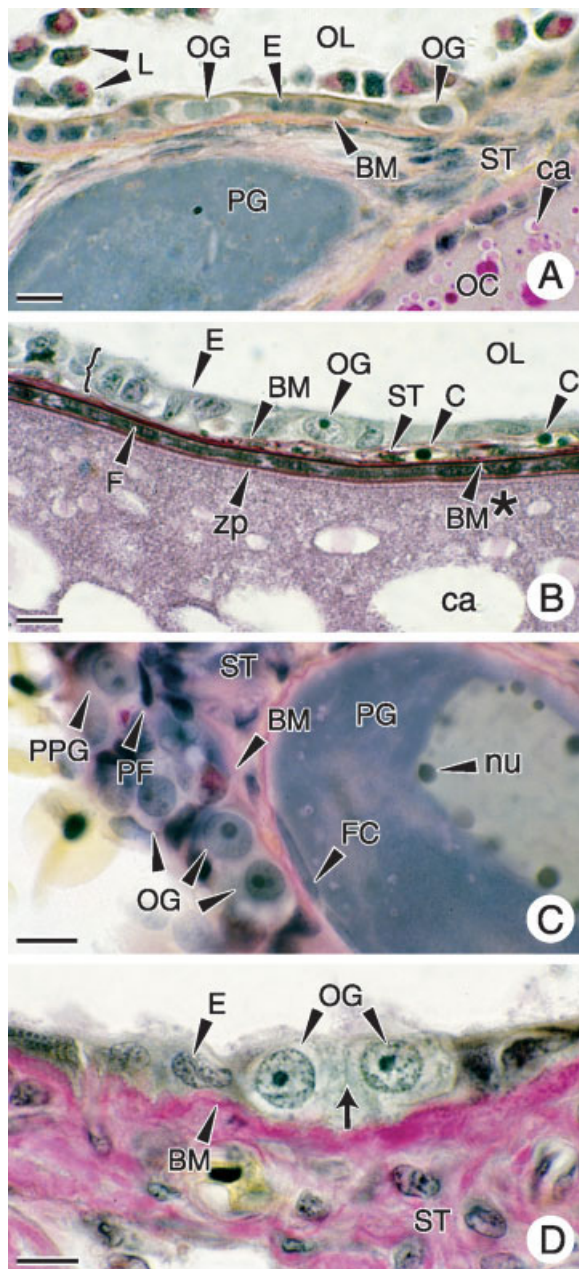


Fig. 1. *Oncorhynchus mykiss*. Ovaries from spawned rainbow trout. **A:** A dissection. Lamellae extend between the walls. Primary growth oocytes in follicles, a mature oocyte, and postovulatory follicles are present. **B:** Primary growth oocytes, and those having cortical alveoli, and lumina of the ovary, of postovulatory follicles, and of lacunae are present. [T, PAS/MY-H]. **C:** Three oocytes with PAS-positive cortical alveoli and smaller primary growth oocytes are present along with postovulatory follicles. The lumen of one postovulatory follicle is continuous (arrow) with that of the ovarian lumen. A prominent basement membrane delineates the postovulatory follicle. [B, PAS/MY-H]. BM, basement membrane; ca, cortical alveoli; L, lamella; LA, lacunae; FO, follicle; n, oocyte nucleus; OL, ovarian lumen; ovary walls, (*); PG, primary growth oocyte; POC, preovulatory oocyte; POF, postovulatory follicle. Scale bar in A = 5 mm; in B = 500 μ m; in C = 400 μ m.

Following the pachytene stage of the first meiotic division, when the homologous chromosomes are paired and condensed, the oocytes enter the diplotene stage. The nucleus is spherical and the chromosomes stain distinctly (Figs. 3A,B, 6A,B). Initially, the cytoplasm of these early diplotene or preprimary growth oocytes is not basophilic (demonstrated by staining with hematoxylin) as in primary growth oocytes, where there is an accumulation of RNA (Figs. 2A,C, 3A,B, 5A, 6A–C). Early diplotene oocytes have a lucent cytoplasm (Figs. 3B, 5A,B) prior to the beginning of primary growth.

Primary growth of oocytes (Figs. 3A,B, 5A, 6A,B) begins prior to the completion of folliculogenesis.

This may occur when prefollicle cells are still encompassing primary growth oocytes, and the cell nest in which they reside is surrounded by a basement membrane (Fig. 5A). By the beginning of primary growth, the appearance of basophilia in the cytoplasm of oocytes and the form of the follicle have been established, but it is not complete. As a scale of size, two primary growth oocytes, still within a forming follicle, can be compared to a cell nest with a dividing oogonium in metaphase (Fig. 5A,B). The spindle fibers of the dividing oogonium are evident (Fig. 5B). The cell nest contains oogonia, a metaphase oogonium, and early diplotene oocytes.



Attachment of the Follicle to the Germinal Epithelium

During folliculogenesis, the connection between the forming follicle, or cell nest, and its origin, the germinal epithelium, is illustrated clearly (Figs. 3B, 4A–C, 6C). The basement membrane of the germinal epithelium is continuous with that of the forming cell nest, and there is a continuity of cells, both germ cells and prefollicle cells, between the nest and the germinal epithelium. Upon completion of folliculogenesis, a single basement membrane is interposed between the germinal epithelium and the follicle (Fig. 6A–C). At the periphery of the attachment, the basement membrane supporting the germinal epithelium forms a distinctive branch. One branch extends between the germinal epithelium and the follicle. The other branch extends around the follicle.

Fig. 2. *O. mykiss*. Germinal epithelium in spawned rainbow trout. **A:** The germinal epithelium is composed of individual oogonia and epithelial cells and is separated from the stroma by a basement membrane. Leukocytes are in the ovarian lumen. [T, PAS/MY]. **B:** The germinal epithelium (bracket), composed of oogonia and a monolayer of squamous or cuboidal epithelial cells, borders the ovarian lumen and is subtended by a basement membrane. The stroma, containing capillaries, encompasses the basement membrane surrounding a follicle composed of follicle cells, a zona pellucida, and an oocyte. The oocyte peripheral cytoplasm contains cortical alveoli. [B, PAS/MY]. **C:** Multiple oogonia of the germinal epithelium are associated with epithelial cells that have associated with preprimary growth oocytes and become prefollicle cells. An indistinct basement membrane separates the germinal epithelium from the stroma and a primary growth oocyte. [T, PAS/MY]. **D:** A pair of oogonia, flanked by epithelial cells, is observed in the germinal epithelium; a cleavage furrow (arrow) is barely resolved between them. A basement membrane separates the germinal epithelium from the stroma. [B, PAS/MY]. BM, basement membrane; BM*, basement membrane surrounding follicle; C, capillaries; ca, cortical alveoli; E, epithelial cells; F, follicle cells; FC, follicle cell nucleus; L, leukocytes; nu, oocyte nucleoli; OG, oogonia; OL, ovarian lumen; PG, primary growth oocyte; PPG, preprimary growth or early diplotene oocytes; PF, prefollicle cells; ST, stroma; zp, zona pellucida. Scale bar in A = 10 μ m; in B = 10 μ m; in C = 10 μ m; in D = 10 μ m.

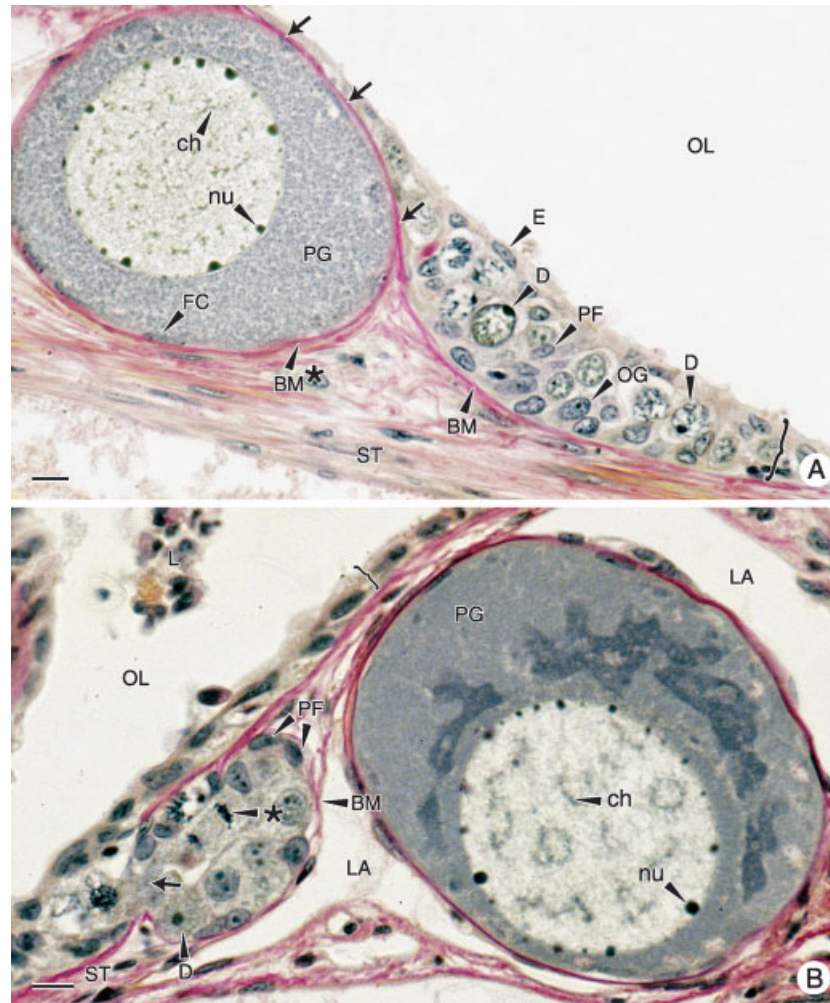


Fig. 3. *O. mykiss*. Cell nests. **A:** A forming cell nest within the germinal epithelium contains oogonia, early diplotene oocytes, and prefollicle cells. The germinal epithelium is separated from the stroma by a basement membrane and borders the ovarian lumen. The basement membrane beneath the germinal epithelium joins with that over the follicle surface (arrows) and becomes continuous with that surrounding the follicle (BM*), in which follicle cells and a primary growth oocyte are observed. [B, PAS/MY]. **B:** A cell nest is continuous (arrow) with the germinal epithelium. Prefollicle cells and an early diplotene oocyte, that has not begun primary growth, are present. A basement membrane separates the cell nest from the stroma within which a lacuna encompasses most of a follicle containing a primary growth oocyte. In the nucleus of the latter, lampbrush chromosomes and perinucleolar nucleoli are present. Leukocytes are in ovarian lumen. [B, PAS/MY]. BM, basement membrane; BM*, follicle basement membrane; ch, chromosomes; D, early diplotene oocyte; E, epithelial cells; FC, follicle cell; (I), germinal epithelium; L, leukocytes; LA, lacuna; nu, perinucleolar nucleoli; OG, oogonium; OL, ovarian lumen; PF, prefollicle cells; PG, primary growth oocyte; ST, stroma; dividing cell, (*). Scale bar in A = 10 μ m; in B = 10 μ m.

Oocytes

Three stages of oocytes were observed in the post-ovulatory ovary of rainbow trout. The largest oocytes were preovulatory (Fig. 1A) and measured ~ 4.5 mm in diameter. They were scattered along the lamellae, but were visually the predominant oocytes. A much more numerous population of oocytes was in primary growth, ranging in size up to 300 μ m in diameter. Most of these oocytes had a basophilic cytoplasm. Some diplotene oocytes had a spherical nucleus, ~ 8 μ m in diameter, a single nucleolus, and lucent cytoplasm. These were classified as early diplotene or preprimary growth

oocytes (Figs. 3B, 5A,B). At the time of ovulation, primary growth had commenced in oocytes. Some of these, even with perinuclear nucleoli, had not completed folliculogenesis (Fig. 5A). Excluding preovulatory oocytes, the largest oocytes in the spawned rainbow trout ovary possessed numerous cortical alveoli (Figs. 1B,C, 2B, 4A).

Postovulatory Follicles

The postovulatory follicles in spawned rainbow trout are large, 1.5-mm long as observed in gross section (Fig. 1A). Histologically (Fig. 1B,C), they

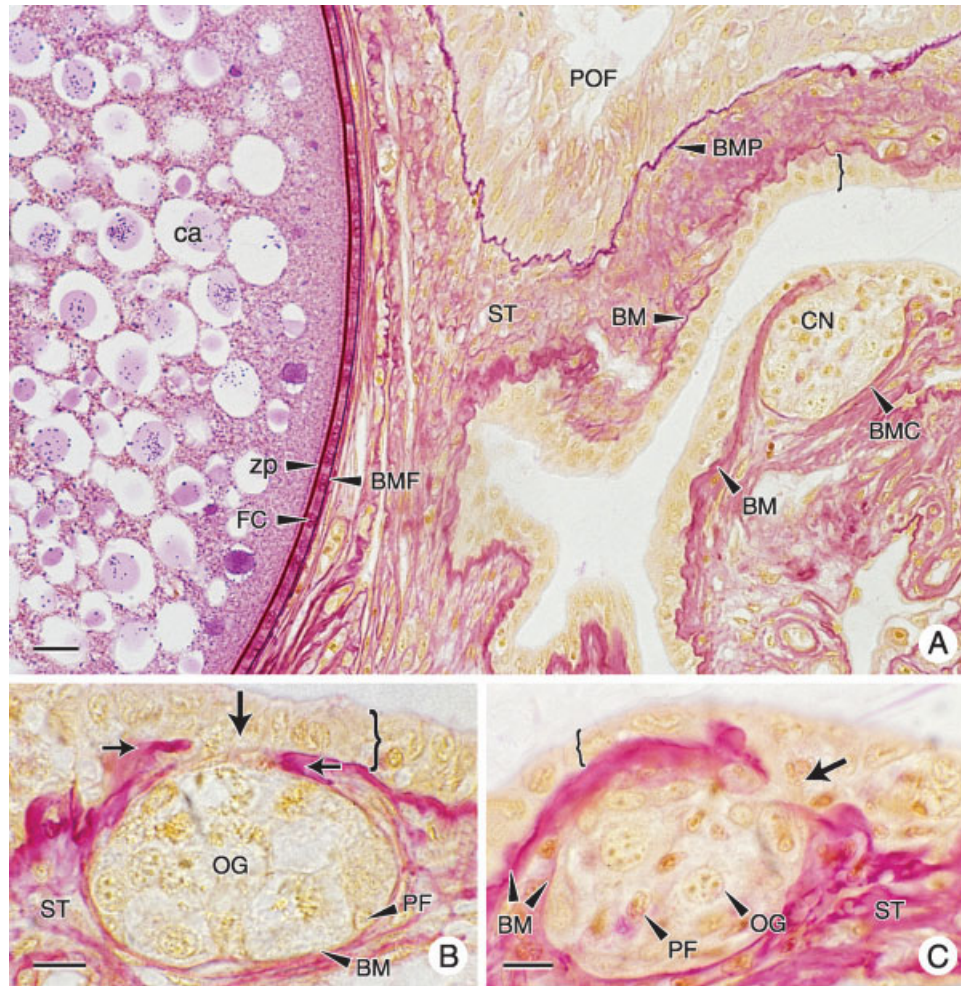


Fig. 4. *O. mykiss*. Basement membranes compartmentalize the ovary. **A:** The germinal epithelium is subtended by a continuous basement membrane, separating it from the stroma containing PAS+ fibrils (purple). A forming cell nest, containing germ cells and prefollicle cells, is encompassed by a basement membrane that is continuous with that of the germinal epithelium. A follicle, containing an oocyte with numerous cortical alveoli, is surrounded by a zona pellucida and follicle cells, and is also separated from the stroma by a basement membrane. Finally, the follicle cell component of a postovulatory follicle is separated from the stroma by a basement membrane. [B, PAS/MY-H]. **B:** The basement membrane around a forming cell nest is continuous with that subtending the germinal epithelium (small arrows). Cells within the cell nest form a continuum with those in the germinal epithelium (larger arrow), while the basement membrane appears to be isolating cells in the nest by expanding dorsally (apposed arrows). In the cell nest, oogonia and prefollicle cells are observed, but are indistinct without a hematoxylin stain. [B, PAS/MY-H]. Bar = 10 μ m. **C:** A basement membrane subtends the germinal epithelium (bracket) and is continuous around a forming cell nest, separating it from the stroma. Oogonia and prefollicle cells are within the cell nest and form a continuum with cells of the germinal epithelium. Metanil yellow renders chromosomes of a pachytene oocyte barely visible. [B, PASM-Y-H]. BM, basement membrane subtending germinal epithelium; BMC, basement membrane of cell nest; BMF, basement membrane of follicle; BMP, basement membrane of postovulatory follicle; ca, cortical alveoli; CN, cell nest; FC, follicle cell; (}), germinal epithelium; ST, stroma; OG, oogonia; PF, prefollicle cell; POF, postovulatory follicle; zp, zona pellucida. Scale bar in A = 50 μ m, in B = 10 μ m; in C = 10 μ m.

were polymorphic structures resulting from the collapse of the follicle at ovulation. Postovulatory follicles possess a central lumen that is continuous with that of the ovarian lumen (Figs. 1C, 7A,B, 8), being established at the time of ovulation. Most frequently, the lumina of postovulatory follicles contained numerous red blood cells and leukocytes, the cytoplasm of which was partially periodic acid-Schiff-positive (Fig. 8). Both red blood cells and leukocytes were abundant in the ovarian lumen (Fig. 8), possibly due to dissection. Because the postovu-

latory follicles are so huge in the spawned rainbow trout ovary, and their basement membranes stain intensely with periodic acid-Schiff, especially when hematoxylin is omitted from the periodic acid-Schiff stain, the continuity between the basement membranes of the postovulatory follicle and that subtending the germinal epithelium was easily demonstrated (Figs. 7A,B, 8). The basement membrane of the postovulatory follicle was thickened, where it joined that membrane subtending the germinal epithelium (Figs. 7A, 8).

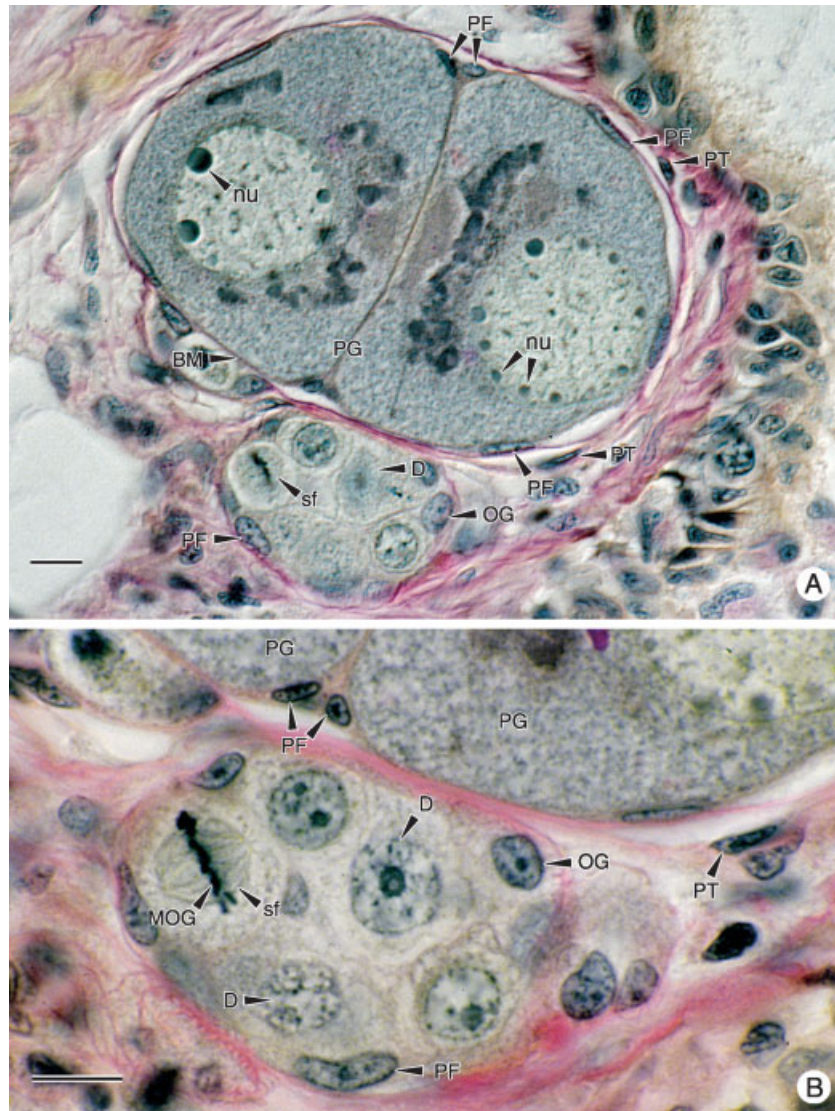


Fig. 5. *O. mykiss*. **A:** A pair of primary growth oocytes, with perinuclear nucleoli, in the process of being encompassed by prefollicle cells. Next to the oocytes is a cell nest containing one oogonium in metaphase with discernable spindle fibers. Oogonia, oocytes in early diplotene, and prefollicle cells are in the cell nest. [T, PAS/MY]. **B:** An enlargement and different plane of focus of the cell nest in Figure 8 illustrating oogonia, clearly showing the spindle fibers in the metaphase oogonium, an early diplotene oocyte, and prefollicle cells. Above the cell nest, two prefollicle cells extend processes between the primary growth oocytes. [T, PAS/MY]. D, early diplotene oocyte; MOG, metaphase chromosomes; nu, nucleoli; OG, oogonium; PF, prefollicle cell; PG, primary growth oocyte; PT, prethecal cell; sf, spindle fibers. Scale bar in A = 10 μm ; in B = 10 μm .

The postovulatory follicle is a compound structure that includes both the former follicle cell layer and the theca. These layers are separated from one another by a basement membrane. Where the oocyte emerged at ovulation, the follicle cells in the postovulatory follicles form a continuum with the epithelial cells of the germinal epithelium (Figs. 7A,B, 8). Identification of the germinal epithelium rests with the observation of oogonia (Figs. 7B, 8) among its somatic epithelial cells. The latter lie a short distance from where they join with the follicle cells at the opening of the postovulatory follicles into the ovarian lumen. It seems

that the stratified follicle cell layer, observed in newly formed postovulatory follicles (Figs. 7A,B, 8), could result from detachment of the follicle cells from the basement membrane. Those cells furthest from the basement membrane appear vacuolated (Fig. 7B).

Basement Membrane

A basement membrane was consistently (Fig. 4A) observed to separate the germinal epithelium and its derivatives (forming cell nests, the follicle, and the postovulatory follicle) from the stroma.

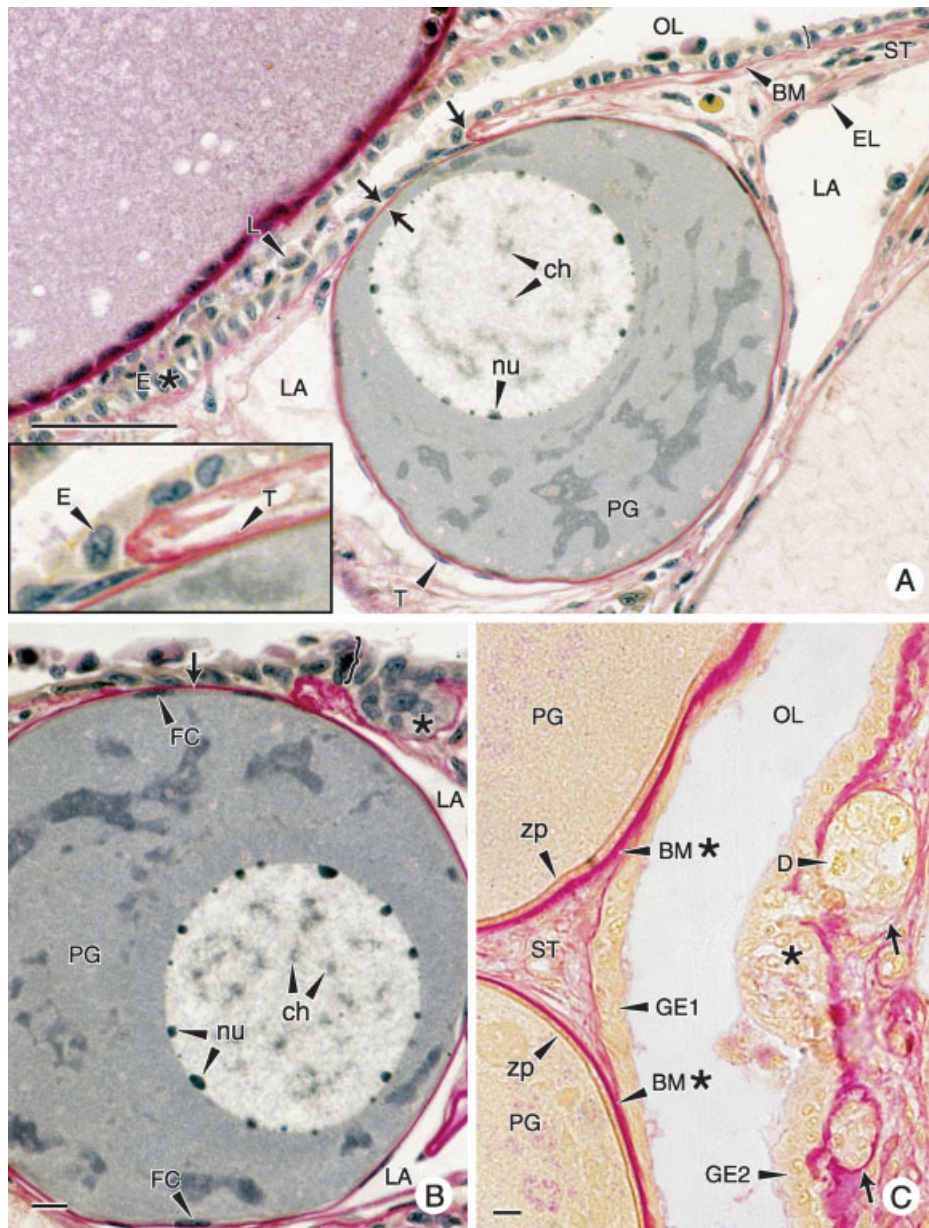


Fig. 6. *O. mykiss*. Follicle attachment to the germinal epithelium. **A:** The basement membrane subtending the germinal epithelium involutes (arrow) and forms a continuum over the surface of the follicle and between the follicle and the germinal epithelium (apposed arrows). The follicle contains a primary growth oocyte whose nucleus contains both lampbrush chromosomes and perinucleolar nucleoli. A lacuna partially surrounds the follicle. In this micrograph, two lamellae are closely apposed. In the space between them, part of the ovarian lumen, leukocytes are present. [B, PASM-Y-H]. Bar = 50 μ m. Inset: an enlargement of the infolded germinal epithelium basement membrane is continuous over the follicle surface and singular between the germinal epithelium and follicle. [B, PAS/MY]. **B:** A single basement membrane (arrow) is between the germinal epithelium and a follicle composed of a primary growth oocyte, the nucleus of which contains lampbrush chromosomes and perinucleolar nucleoli. Follicle cells surround the oocyte. A lacuna partially surrounds the follicle and a cell nest is forming (*) within the germinal epithelium. [T, PAS/MY]. **C:** The ovarian lumen separates two lamellae, each with a simple germinal epithelium. Where cell nests are forming (arrows), the germinal epithelium becomes multilayered, and its basement membrane is continuous with that of each cell nest. A stratified germinal epithelium designates a forming cell nest, but cells are poorly discerned with this stain. An early diplotene oocyte lies within a cell nest. Two primary growth oocytes are surrounded by zona pellucidae and are separated from the stroma by a basement membrane between them, but their basement membranes fuse with that of the germinal epithelium, and there is a common basement membrane between them. [B, PAS/MY-H]. BM, basement membrane; BM*, basement membranes fuse between the follicle and germinal epithelium; ch, lampbrush chromosomes; D, early diplotene oocyte; (l), germinal epithelium; E*, columnar epithelial cells of the germinal epithelium; EL, lacunar epithelium; FC, follicle cell; GE1 and GE2, germinal epithelia; L, lymphocyte; LA, lacuna; nu, nucleolus; (*), stratified germinal epithelium forming cell nest; OL, ovarian lumen; PG, primary growth oocyte; ST, stroma; T, putative theca; zp, zona pellucida. Scale bar in A = 10 μ m; in B = 50 μ m; in C = 50 μ m.

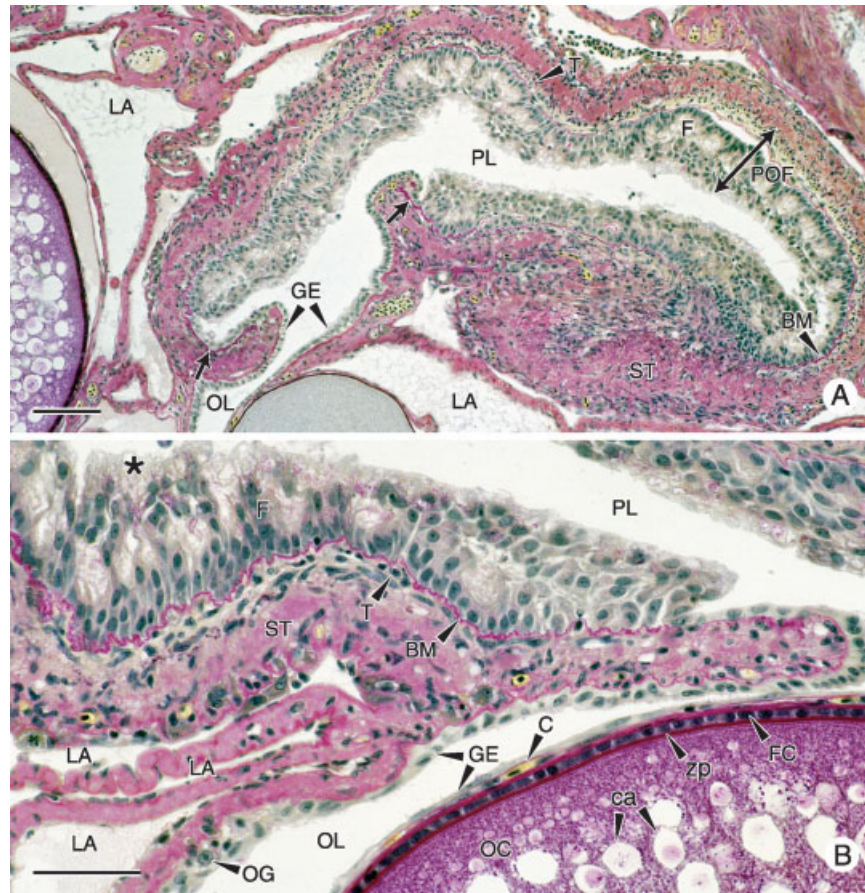


Fig. 7. *O. mykiss*. Postovulatory follicles. **A:** After ovulation, a postovulatory follicle is composed of the follicle and thecal cell layers of the former follicle. Its lumen, previously occupied by an oocyte, is continuous with that of the ovarian lumen. The follicle cell layer of the postovulatory follicle is stratified and separated from an indistinct theca and stroma by a basement membrane that joins the basement membrane subtending the germinal epithelium. Extensive lacunae are also present. [B, PAS/MY]. **B:** A stratified follicle cell layer characterizes the newly formed postovulatory follicle. Near the postovulatory follicle lumen, these cells lose their staining affinity and become vacuolated. A continuous basement membrane separates follicle cells from those of the theca and stroma. The basement membrane of the postovulatory follicle is continuous with that of the germinal epithelium in which an oogonium is observed. A follicle, containing an oocyte surrounded by a PAS-positive zona pellucida and follicle cells is beneath the germinal epithelium and is separated from it by an indistinct theca containing a capillary. Epithelial cells in the germinal epithelium are either squamous or cuboidal. Lacunae are also present. [B, PAS/MY]. BM, basement membrane; C, capillary; ca, cortical alveoli; F, follicle cell layer of post ovulatory follicle, FC, follicle cell of follicle; GE, germinal epithelium; LA, lacuna; OC, oocyte; OG, oogonium; OL, ovarian lumen; PL, lumen of post ovulatory follicle; breadth of post ovulatory follicle, POF and two-headed arrow; ST, stroma; T, thecal cell layer of postovulatory follicle; vacuolated follicle cells (*); zp, zona pellucida. Scale bar in A = 100 μ m; in B = 50 μ m.

The germinal epithelium basement membrane subtends both the germinal epithelium and forming cell nests. Follicles are always separated from the theca, which is part of the stromal compartment of the ovary, by a basement membrane.

DISCUSSION

Synchronous Spawning

Numerous species of fish spawn multiple times within a limited reproductive season. Their spawning mode has been called asynchronous, partial, serial, or heterochronal. Other fish are synchronous, isochronal, or total spawners (Hunter and Mace-

wicz, 1985; Hunter et al., 1985; Tyler and Sumpter, 1996), having determinate annual fecundity such as the striped mullet (*Mugil cephalus*, Mugilidae) (Render et al., 1995; McDonough et al., 2003), and the salmonids, the brown trout (*Salmo trutta*) (Bagenal, 1969, 1978; Billard, 1987), and rainbow trout (*O. mykiss*, Salmonidae) (Scott, 1987; Tyler et al., 1990b). Some salmonids have lifetime batch or determinate fecundity and die after spawning, as does the anadromous coho salmon (*O. kisutch*), sockeye salmon (*O. nerka*), and Pacific pink salmon (*O. gorbuscha*) (Zelennikov, 2003). In the last and rainbow trout, two mechanisms for determinate annual or lifetime spawning may exist. Once the pool of oocytes is established in the 0+ age group of

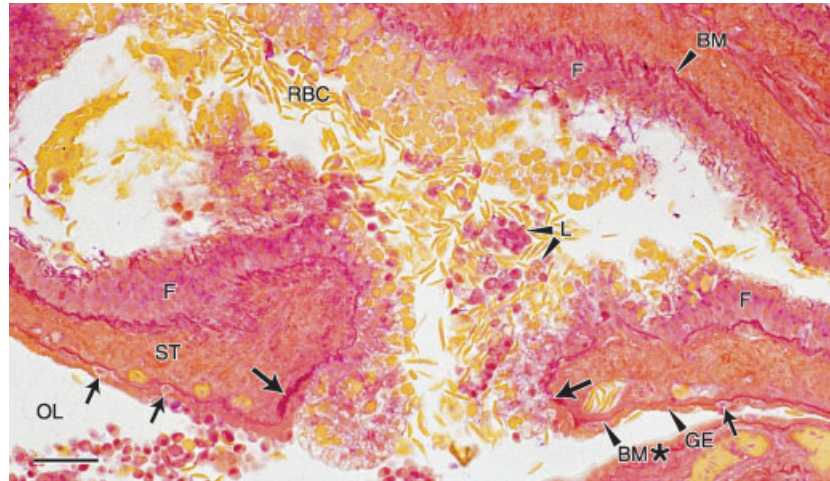


Fig. 8. *O. mykiss*. Basement membranes and the postovulatory follicle. A basement membrane separates the follicle cell layer from the theca (not distinguished with this stain) and the stroma. The basement membrane is thickened (large arrows) where the postovulatory follicle joins that of the germinal epithelium. Oogonia (small arrows) are scattered between epithelial cells of the germinal epithelium. The lumen of a postovulatory follicle contains numerous red blood cells and leukocytes. These cells are also present in the ovarian lumen. [B, PASM-Y-H]. BM, basement membrane of postovulatory follicle; BM*, basement membrane of germinal epithelium, GE, germinal epithelium; F, follicle cell layer of postovulatory follicle; L, leukocytes; OL, ovarian lumen; RBC, red blood cell; ST, stroma. Small arrows, oogonia; Large arrows, thickening of the basement membrane connection between the germinal epithelium and the postovulatory follicle. Scale bar = 20 μm .

pink salmon, *O. gorbuscha*, further divisions of oogonia are blocked. In other Pacific species of salmon (chum salmon [*O. keta*], salmon trout [*O. masou*], sockeye salmon [*O. nerka*], and coho salmon [*O. kisutch*]), another mechanism exists as lifetime determinate fecundity involves resorption, or atresia, of new germ cells resulting from divisions of oogonia (Zelennikov, 2003). In the brown trout, *S. trutta fario* (Billard, 1987, 1992), and rainbow trout (Bromage and Cumarantunga, 1987, 1988), there is an increase in oogonia following spawning. Herein, we demonstrate that in the rainbow trout there is an active germinal epithelium and ongoing folliculogenesis at the time of spawning, demonstrating one aspect of synchronous or annually determinate spawning.

Oocyte atresia is common throughout vitellogenesis of rainbow trout (Bromage and Cumarantunga, 1987) and was identified as an important determinant of fecundity. In a subsequent study (Tyler et al., 1990b), in contrast, atresia was not considered an important determinant of fecundity. There was no significant change in the number of developing oocytes once vitellogenesis had begun, indicating that annual determined fecundity might have been established by the time vitellogenesis began. Tyler et al. (1990b) did not measure oocytes less than 500 μm diameter. Therefore, their study may be biased because, as van den Hurk and Peute (1979) indicated, secondary oogonia (those beneath the germinal epithelium and representing ongoing folliculogenesis as determined herein) are present in the rainbow trout ovary from August through January. This is corroborated in the present study

in rainbow trout that were spawned in January. Cell nests represent an ongoing process of folliculogenesis. The largest previtellogenic rainbow trout oocytes have a diameter between 100 and 300 μm (those with yolk nuclei [cortical alveoli]; van den Hurk and Peute, 1979), less than the smallest oocytes measured by Tyler et al. (1990a,b), which indicates that significant production of follicles and atresia may have been overlooked. Oocytes with cortical alveoli (yolk vesicles) were observed in rainbow trout ovaries at the time of spawning (Sumpter et al., 1984), as reported also herein, and vitellogenin uptake by oocytes began in March. Processes leading to the formation of follicles and the growth of oocytes overlap. The studies conducted to date form a strong foundation upon which the added knowledge of germinal epithelium structure, the process of folliculogenesis, including the formation of cell nests and rates of atresia necessarily and simultaneously need to be integrated to elucidate when annual determinate fecundity is established in rainbow trout.

Oogonia and Follicles

In the rainbow trout ovary, numerous mitotic divisions of oogonia occur within the germinal epithelium and within cell nests that are attached to the germinal epithelium at the time of spawning. When the single, annual clutch of eggs is ovulated, the ovary is composed of a few follicles with mature oocytes that did not ovulate, an active germinal epithelium, including its oogonia entering meiosis to become oocytes, cell nests, primary growth oocytes,

those with cortical alveoli, and the stroma. We focus here on the activity of the germinal epithelium and the process of folliculogenesis at spawning. Primary emphasis is placed on revelation of basement membranes that separate tissue compartments within the ovary. These two compartments are the (1) epithelial compartment and the cells derived from it (oocytes and follicle cells), and (2) the stromal compartment. Throughout follicle development, basement membranes separate epithelium and its derivatives from the stroma, as is particularly well illustrated in Figure 4. The germinal epithelium, forming cell nests, follicles, and postovulatory follicles is separated from the stroma by a basement membrane, a configuration that also occurs in males where the germinal epithelium is separated from the stroma or interstitial tissue (Grier, 1993).

Description of folliculogenesis includes the definition of an epithelium, the separation between the follicle and the stroma by a basement membrane, and the ovarian follicle as a derivative of an epithelium, the germinal epithelium. Among follicles of different maturational stages in the mammalian ovary, the stage specifically analogous to the fish ovarian follicle is the primordial follicle; an oocyte surrounded by a layer of squamous follicular cells (Tokarz, 1978; Williams, 1995; Fawcett and Jensch, 2002). As a mammalian follicle develops, follicle cells become cuboidal and divide, producing typical multilayers of granulosa cells surrounding growing oocytes in secondary and tertiary (containing a fluid-filled space, the antrum) follicles. The terms follicle cells and granulosa cells are used interchangeably in the fish literature. We endorse the name "follicle cells," given their description in the primordial follicle and that follicle cells are called granulosa cells in secondary and tertiary mammalian follicles. Multilayers of stratified follicle cells have not been observed in fish ovaries.

The follicle cells surrounding an oocyte have been called an epithelium (Tokarz, 1978) or follicular epithelium (Dodd and Sumpter, 1984). While follicle cells form intercellular contacts with each other, are avascular, and rest upon a basement membrane, all criteria used to describe and define an epithelium, a fourth criterion for defining an epithelium is not met, as follicle cells do not border a lumen. Therefore, follicle cells surrounding an oocyte are an epithelioid layer of cells, not an epithelium (Grier, 2002). Cell layers that lack some of the defining criteria of an epithelium are termed "epithelioid," defined as "resembling epithelium" (Lawrence, 1995), a term first applied in fish to the interpretation of annual testicular changes in cobia, *Rachycentron canadum* (Brown-Peterson et al., 2002). During the regression class, Sertoli cells and spermatogonia initially grow as solid cords of cells, lacking a lumen. They become a germinal epithelium when a lumen develops.

The follicle complex is composed of the oocyte and its surrounding investments of mixed origins, the follicle cells, basement membrane, and theca (Grier, 2000). The outermost layer of thecal cells, but lacking a supporting basement membrane, has been interpreted as a "surface epithelium" in the ovary of the atherinomorph fundulid *Fundulus heteroclitus* (Selman and Wallace, 1989), but reinterpreted as an epithelioid layer of cells forming the theca externa in the follicle complex of *Centropomus undecimalis* (Grier, 2000). In this species, a basement membrane also was not observed beneath these thecal cells. The designation "epithelioid," instead of "epithelium," is based upon the accepted definition of an epithelium and the criteria required to satisfy that definition.

We adapted the traditional definition of an ovarian follicle in teleosts from *Gray's Anatomy* (Williams, 1995) and histological literature (Grier, 2000; Grier and Lo Nostro, 2000), wherein the follicle is defined precisely as an oocyte and surrounding follicle cells, as has also been used by Begovac and Wallace (1987) for pipefish, *Syngnathus scovelli*. The follicle is an epithelial derivative that is always encompassed and separated from the thecal layers by a basement membrane, as is illustrated herein in rainbow trout. Given its origin and the definition of an epithelium, the basement membrane and thecal cells are not to be included in the definition of a follicle. The follicle, its encompassing basement membrane, and theca form the follicle complex, a term introduced by Grier (2000). Begovac and Wallace (1988) referenced the complex of follicle cells and oocyte being surrounded by the basement membrane, but the follicle is composed of these cells. Within the follicle complex, the follicle is separated from the theca, originating from the stroma in fish (Grier, 2000) and in mammals (Williams, 1995). The theca is derived from prethecal cells underlying the germinal epithelium in the perciform fish *C. undecimalis* (Grier, 2000). These are actually stromal cells at a specific location within the ovarian lamellae. The theca is not derived from the peritoneal epithelium, as indicated by Dodd and Sumpter (1984). Follicle cells (pregranulosa cells) are also not derived from undifferentiated cells of the stroma, as indicated for mammals (Guraya, 2000). The origin of follicle cells from an epithelium has been acknowledged (Tokarz, 1978).

In fish, speculation on the origin of follicles in the ovary dates back at least to the work of BULLOUGH (1942) on the Eurasian minnow, *Phoxinus laevis* (= *phoxinus*), and MENDOZA (1943), on the livebearing atherinomorph goodeid *Skiffia* (= *Neotoca bilineata*), both indicating origin of oocytes from the epithelium lining the ovarian lumen. In a literature review, HOAR (1969) stated, "ovarian follicles develop from or in association with the germinal epithelium," but could not give a detailed

description of the germinal epithelium, because such a description did not exist at that time. Herein, a germinal epithelium and folliculogenesis are clearly depicted in rainbow trout, based on the specific fixation and staining for basement membranes and in consideration of the definition of an epithelium.

A cell nest retains cellular contact with the germinal epithelium while bulging into the stroma; its basement membrane is a continuation and extension of the basement membrane beneath the germinal epithelium. Apparently, the basement membrane is synthesized around cell nests, as their volume increases. There is no information available on the synthesis of basement membranes during folliculogenesis. Herein, and with distinctive staining of basement membranes, the germ cells and prefollicle cells within cell nests were always connected to the germinal epithelium via a cellular continuity in the absence of an intervening basement membrane. The origin of the cell nest from the germinal epithelium is morphologically reflected by this observation. As prefollicle cells increasingly grow between oocytes within a cell nest, basement membranes also partition prefollicle cells and oocytes from one another as in the goodeid *Ilyodon whitei* (Grier et al., 2005) and pipefish (Begovac and Wallace, 1987). Precisely how the fully formed follicle remains attached to the germinal epithelium (*vide infra*) is unknown.

Primary oocyte growth begins prior to completion of folliculogenesis (Fig. 5A) as it does in a perciform fish, the common snook (Grier, 2000). In the pipefish, meiotic oocytes initiate some primary growth, while oocytes are within the germinal ridge (Begovac and Wallace, 1987), the equivalent of the germinal epithelium. Once an oocyte and its prefollicle cells are completely surrounded by a basement membrane, folliculogenesis is complete, and former prefollicle cells may be called follicle cells (Grier, 2000; Grier et al., 2005). The completion of folliculogenesis is quite specific and can be revealed by the periodic acid-Schiff procedure, as indicated herein. The timing of the completion of folliculogenesis seems to be slightly variable, however. In the pipefish, the definitive follicle seems to coincide with the appearance of multiple nucleoli (Begovac and Wallace, 1988). This is seen in rainbow trout (Figs. 3, 6), but not always (Fig. 5), as primary growth oocytes can possess multiple, perinuclear nucleoli and still be within forming follicles. At the completion of folliculogenesis, the oocyte and its encompassing follicle cells are surrounded by a basement membrane. The follicle remains attached to the germinal epithelium, however, where there is but one, shared basement membrane.

As revealed by periodic acid-Schiff-positive staining of the basement membrane in rainbow trout and by similar histological methodology in birds—*viz* chicken and Japanese quail (Callebaut, 1976)—

there is a permanent separation between cell nests (termed vase-shaped buds and ovigerous cords in these birds) and stroma and a common genesis of ovarian cell nests between a fish, the rainbow trout, and birds, the chicken and Japanese quail. As in the salmoniform rainbow trout, the perciforms *C. undecimalis* (Grier, 2000) and *Cichlasoma dimerus* (Meijide et al., 2005), the synbranchiform, *Synbranchus marmoratus* (Ravaglia and Maggese, 2003), and atherinomorphs (Parenti and Grier, 2004; Grier et al., 2005), the ovarian germ cells and the prefollicle cells are derived from a germinal epithelium. Likewise, when rabbit oogonia leave the germinal epithelium, they are accompanied by “large numbers of other cells from the germinal epithelium . . . which are the precursors of the future follicle cells” (Zamboni, 1972), *i.e.*, they are prefollicle cells. The composition of the germinal epithelium and the continuity of cell nests in rainbow trout, a basal euteleost (following the classification of Johnson and Patterson, 1996), is similar to that observed in the developing ovary of rabbits (Zamboni, 1972, Figs. 2.4–2.6). In sheep, follicle cells are also derived from the germinal epithelium (Sawyer et al., 2002), as in rainbow trout and the other osteichthyans, in which germinal epithelia have been described (*vide supra*). Tokarz (1978) (citing Okkelberg, 1921; Lewis and McMillan, 1965; Hardisty, 1971) concludes that, “the prefollicle cells of lampreys are derived from the coelomic epithelial cells,” as are those in a number of fishes, amphibians, reptiles, and birds. Dodd (1977) questioned whether the monolayer of follicle cells (granulosa) and the encompassing theca that surrounds the follicle in lampreys are homologous with those of mammals. As comparative information grows, however, there has been no evidence to contradict the hypothesis that follicle cells, oogonia, and oocytes are homologous among vertebrates.

In the present study, there is no supporting evidence that in rainbow trout “dictyate oogonia and meiotic oocytes become progressively isolated by epithelioid cells (prefollicular cells) and are included in the mesenchymal stroma that invades the multilayered epithelium” (Billard, 1992, and citing Upadhyay, 1977), which is the germinal epithelium. Oogonia are never “dictyate,” since this term refers to an oocyte in the first meiotic prophase. Prefollicle cells are not epithelioid (like an epithelium), as they are part of the germinal epithelium. A mesenchymal stroma never invades the germinal epithelium or its derivatives, but the germinal epithelium and its derivatives are always separated from the stroma by a basement membrane, as depicted in Figure 4A. The theca is derived from the stromal compartment of the ovary in fish (Grier, 2000), in mammals (van Blerkom and Motta, 1979), and other vertebrates (Tokarz, 1978). The definition of the germinal epithelium as a multilayered epithelium (Billard, 1992) requires

modification. As demonstrated herein, the germinal epithelium is primarily a simple epithelium composed of somatic epithelial cells, except in those regions where oogonia enter into meiosis and cell nests are being produced (compare Figs. 2A–D, 3A,B). Then, the structure of the germinal epithelium becomes more complex, being many cells thick. The epithelial cells, composing the germinal epithelium, are largely squamous, but are not always so; cuboidal and columnar epithelial cells have also been observed herein. The significance of this is unknown.

Primary and secondary oogonia in the ovary of rainbow trout have been described (van den Hurk and Peute, 1979). The staining techniques and microscopy used by these authors did not reveal basement membranes or the morphology of a cell nest. From the positional arrangement of oogonia, primary oogonia were within the germinal epithelium, whereas the secondary oogonia were clustered below the germinal epithelium. The designation of secondary oogonia has limitations regarding the actual stage of development of oogonia. Oogonia divide mitotically prior to entering into meiosis. Indications are that the number of mitotic divisions is species-specific (review by Patiño and Sullivan, 2002). Pachytene oocytes (Fig. 3A) may occur within the germinal epithelium, establishing that oogonia enter into meiosis both within the germinal epithelium and within a cell nest attached to the germinal epithelium. Secondary oogonia (those in cell nests beneath the germinal epithelium) were reported within the rainbow trout ovary in July through January, an observation corroborated herein for January when numerous cell nests were being formed in postspawned rainbow trout ovaries. In the common snook, primary growth begins while oocytes are still within the germinal epithelium or within a cell nest that is attached to the germinal epithelium (Grier, 2000). We demonstrate here that primary oocyte growth begins prior to the completion of folliculogenesis. Therefore, the designation of secondary oogonia might be used to refer to those that are within cell nests.

Early diplotene or preprimary growth oocytes have been observed within a cell nest (Fig. 3B). An unknown period of time is required prior to the onset of RNA production in early diplotene oocytes. RNA accumulates within the cytoplasm, rendering it basophilic. Cytoplasmic basophilia defines primary growth oocytes (Taylor et al., 1998). Preprimary growth or early diplotene oocytes were reported in the perciform gobioids *Cerdale floridana* and *Tridentiger bifasciatus* (Thacker and Grier, 2005) and in various species of livebearing fishes (Grier et al., 2005; Uribe et al., 2005). This stage of oocyte development is recognized herein to also occur in rainbow trout.

One finding of this study is the existence of the common basement membrane between follicles and

the germinal epithelium. In the fully formed follicle, the germinal epithelium basement membrane branches, one branch extends (in three dimensions) around the surface of the follicle, while the other branch extends between the follicle and the germinal epithelium, with a single basement membrane between them. The morphology is identical in the atherinomorph goodeid *I. whitei* (Grier et al., 2005) and was demonstrated via ultrastructure in the perciform cichlid *C. dimerus* (Meijide et al., 2005).

At ovulation, the single basement membrane breaks, the egg moves into the coelom (gynovarian ovary) or into the ovarian lumen (cystovarian ovary), and a postovulatory follicle is produced. According to Wallace and Selman (1981), an oocyte becomes an egg at the end of maturation when it is preovulatory and the first meiotic division has been completed, the first polar body has been formed and the oocyte proceeds to the second meiotic metaphase. In the pipefish, maturing oocytes are depicted within the ovary, eggs within the ovarian lumen (Begovac and Wallace, 1987).

Follicle Development

Follicle maturation in rainbow trout has been divided into three broad stages: previtellogenesis, exogenous vitellogenesis, and maturation (van den Hurk and Peute, 1979), a distinction maintained for ovarian follicle growth, maturation, and ovulation (Patiño and Sullivan, 2002). Within these three broad categories, seven oocyte stages have been described in rainbow trout, including a yolk vesicle (endogenous vitellogenesis) stage (Bromage and Cumaranatunga, 1987, 1988; Billard, 1992). As Billard (1992) pointed out, however, “yolk vesicles do not contain true yolk and these terms are not recommended.” Besides “yolk vesicles,” other terms have been applied to the structures that accumulate in oocyte cytoplasm prior to vitellogenesis: intravesicular yolk, endogenous yolk, cortical vesicles, and cortical alveoli (Selman and Wallace, 1989; Wallace and Selman, 1990). In rainbow trout and “in nonmammalian vertebrates, the principal events responsible for the enormous growth of the oocyte involve the sequestration and packaging of a hepatically derived plasma precursor, vitellogenin, into yolk protein” (Wallace and Selman, 1981, 1990; Selman et al., 1988, 1991). They suggested that the term “yolk vesicle” is a misnomer, since the vesicles “release their contents into the perivitelline space at fertilization” (Selman et al., 1988). The structures referred to by a variety of terms previously are herein called cortical alveoli. They are not yolk.

Postovulatory Follicles

As stated earlier, ovulation occurs when the single basement membrane extending between the

germinal epithelium (Fig. 6A–C) and the follicle breaks and the egg moves into the ovarian lumen. The single basement membrane is the weak point around the follicle circumference where ovulation always occurs. Infolding of the epithelium lining the ovarian lumen in the viviparous goodeid *S. (= Neotoca) bilineata* was, according to Mendoza (1943), a weak point that was breached and allowed a fertilized egg to move into the ovarian lumen. Intrafollicular fertilization has been proposed to occur in *S. bilineata*. In rainbow trout, the tripartite branching of the basement membrane between the germinal epithelium, follicle, and between the follicle and germinal epithelium must break during ovulation, resulting in the continuum between the basement membrane of the germinal epithelium and the postovulatory follicle. The potential significance of this is the similar observation in a goodeid, *I. whitei* (Grier et al., 2005). The Goodeidae (atherinomorphs) and Salmonidae (basal euteleosts) represent distantly related teleost lineages. Yet, the morphological relationship between ovarian follicles and the germinal epithelium is identical between these taxa, supporting the proposal of homology or common origin and the germinal epithelium as an emerging concept in vertebrate reproduction (Parenti and Grier, 2004).

At ovulation, a new continuity is established between the formerly separated epithelial cells in the germinal epithelium and the follicle cells that were part of the former follicle (van den Hurk and Peute, 1979), and were derived from the epithelial cells of the germinal epithelium. A new structure, the postovulatory follicle, is formed, which is a follicle complex minus the oocyte. During this study, we observed postovulatory follicles in rainbow trout ovarian histology, 2 weeks after the fish had been spawned. However, they appeared degraded. It is doubtful if any structure similar to a corpus luteum forms. In fish, postovulatory follicles become atretic (Hunter and Macewicz, 1985), almost disappearing from the ovary within 24 h of spawning in marine fish that lay pelagic eggs. There is no information as to their assuming a role similar to the corpus luteum in higher vertebrates, although their persistence in rainbow trout ovaries after ovulation could indicate a possible model for future research.

In the postovulatory follicle, the former follicle cells of the follicle and the thecal cells in the stroma remain separated by the same basement membrane that separated them throughout oocyte development, beginning with early diplotene oocytes and extending until the oocyte is preovulatory. This phenomenon is clear in rainbow trout, because the follicles are extremely large, ~4.5 mm in diameter, and the basement membranes are demonstrated readily using the periodic acid-Schiff technique. Postovulatory follicles are not comparable to the follicles in which the oocytes developed, since they are also composed of the former thecal

cell layer, part of the stromal compartment of the ovary, and the basement membrane. As used in fishery literature (Hunter and Macewicz, 1985), these two cell layers compose the postovulatory follicle. The basement membrane that separates them is also inclusive, but has not been so described previously in teleosts.

The lumina of postovulatory follicles and ovarian lumen contain red blood cells and leukocytes. These were generally distributed in the stroma also, but a specific role for them has not been proposed in rainbow trout ovaries.

Gonoducts

In rainbow trout, the ovarian folds join ventrally to form a U-shaped trough across which the lamellae extend, as in Figure 1A, and so elegantly drawn in Kendall (1921) to illustrate the process by which eggs are shed into the coelom and then to the exterior via the genital papilla. Transit of the eggs to the exterior occurs via an additional structure that develops uniquely in the teleost ovary. Teleosts never develop Müllerian ducts, progenitors of the oviducts in other vertebrate taxa. Therefore to postulate homology and common origin, the term “oviducts” should be restricted to structures that are derived from the Müllerian ducts (Hoar, 1969; Wake, 1985; Uribe et al., 1988, 2005; Wourms et al., 1988). Without oviducts, communication to the exterior occurs near the caudal wall of the teleost ovary, by a gonoduct in cystovarian ovaries or retrieved by posterior funnels of the gonoduct, in salmonids and other noneuteleosts (Dodd, 1977; Wake, 1985; Lombardi, 1998). In these species, the eggs pass into a single median coelomic funnel at the posterior end of the ovary. This funnel opens directly to the genital pore (Lombardi, 1998). These structures have been termed “oviducts” (e.g., see Kendall, 1921; Dodd, 1977). The term gonoduct is more appropriate, recognizing that teleost gonoducts are not homologues of oviducts derived from the Müllerian ducts (Forbes, 1940; Wake, 1985; Wourms et al., 1988; Uribe et al., 2005).

Lumina

Various lumina dominate the histology of post-spawned rainbow trout, including the ovarian lumen, the lumina of postovulatory follicles, and lacunae. These are easily distinguished from one another: (1) the epithelium lining the ovarian lumen is the germinal epithelium composed of epithelial cells, among which are the oogonia or oocytes. The epithelial cells of the germinal epithelium rest upon a distinctive basement membrane, as revealed by selective staining. Basement membranes were not illustrated in earlier work that depicted the origin of ovarian germ cells from the

epithelium lining the ovarian lumen (Bullough, 1942; Mendoza, 1943). (2) The lumen of each postovulatory follicle is easily distinguished, because it is the only one that has a stratified epithelium composed of follicle cells that surround the oocyte prior to ovulation. (3) Lacunae are distinguished easily from the ovarian lumen as they generally possess a secretion, and their bounding cells always have extremely squamous nuclei. A basement membrane was not demonstrated to support these cells. Interestingly, cells forming lacunae boundaries also appeared to form part of the theca surrounding follicles.

Lacunae have been referred to as “fluid-filled spaces” in rainbow trout (van den Hurk and Peute, 1979), surrounding mature follicles. Here, lacunae were observed to have formed much earlier in oocyte maturation, around follicles with primary growth oocytes the cytoplasm of which contained yolk nuclei or Balbiani bodies (Stage 2 of van den Hurk and Peute, 1979). Lacunae have also been described in the ovaries of crocodylians (Forbes, 1940; Uribe and Guillette, 2000), turtles (Callebaut et al., 1997) and birds (quail [Callebaut, 1988; Callebaut et al., 1997], fowl [Guzsal, 1966], and hen [Kopp and Stahl, 1975; Nili and Kelly, 1996]). Lacunae were considered to be dilated lymphatic spaces (for review see Callebaut, 1988). In fish ovaries, these spaces were designated as lymphatic spaces (Begovac and Wallace, 1987) or as extravascular space (Grier, 2000). The homology and function of extravascular space and lymphatics between fish and other vertebrates has yet to be investigated.

Final Considerations

The rendering of basement membranes in stark contrast to surrounding cells is an important histological detail that brings clarity to the process of folliculogenesis in rainbow trout, and allows a better understanding of the mechanism of annual determinate fecundity in fishes, particularly salmonids. The set number of eggs to be spawned per female is established by two processes: folliculogenesis and atresia. This study, along with that of Zelennikov (2003), invites a new approach to study the mechanisms underlying two basic modes of reproduction in fishes, synchronous or annual determinate reproduction, and group synchronous or indeterminate reproduction. In salmonids, spawning may represent lifetime determinate reproduction. Female teleosts possess an active, or potentially active, germinal epithelium throughout life, and can produce an indeterminate number of eggs, the development and ovulation of which falls into these reproductive modes. Mammals, on the other hand, were believed to have lifetime determinate reproductive potential, with a given number of oocytes established in the ovary at birth (Williams, 1995). This axiom of vertebrate reproductive

biology was overthrown by the demonstration that the adult mouse ovary, like the fish ovary, possesses an active germinal epithelium (Johnson et al., 2004), and that follicles are produced in adults. Throughout female vertebrates “. . . the ultimate germ cell compartment is the follicle, with a diplotene oocyte and granulosa cells surrounded by a basement membrane” (Byskov et al., 1983). As in the male (Grier, 1993), a basement membrane in the female separates the germinal compartment from that of the interstitium, the stroma. This separation is a constant between sexes throughout the vertebrates.

A unifying concept, throughout the evolution of the vertebrate ovary, is the consistent origin of follicle cells, as indicated in Tokarz’s (1978) review on oogonial proliferation, oogenesis, and folliculogenesis in nonmammalian vertebrates. Follicle cells are derived from the coelomic epithelium, beginning as “prefollicular cells which surround and isolate each primary oocyte within its own follicle.” Tokarz (1978) described the process of folliculogenesis as we have seen it in rainbow trout, a basal euteleost, and also common snook (Grier, 2000), a perciform. This quote from Tokarz (1978), however, is of the description of folliculogenesis in lampreys. This process, as Tokarz (1978) points out, also occurs in the Atlantic hagfish, *Myxine glutinosa*, in which as “primary oocytes are formed and move into the mesenchyme, they are invested with prefollicular cells derived from the coelomic epithelium (Schreiner, 1955; Walvig, 1963).” Hagfishes and lampreys are among the oldest, most primitive, jawless vertebrates. Evidence from mammals indicates clearly that follicle cells are also derived from the coelomic epithelium (Gondos, 1969; Zamboni, 1972; Sawyer et al., 2002). As oogonia leave the surface of the ovary, they are “accompanied by large numbers of other cells from the germinal epithelium. These cells, which are the precursors of the future follicle cells, closely surround groups of oogonia and oocytes” (Zamboni, 1972). “These cells” are identified herein as the prefollicle cells, as described in all other vertebrate taxa (Tokarz, 1978).

The accumulating evidence corroborates homology of ovarian follicles among vertebrate taxa. Terminology applied to description of reproductive morphology should be consistent and reflect homology (Parenti and Grier, 2004). Our detailed examination of folliculogenesis in a basal euteleost, rainbow trout, when compared to existing reproductive biological literature, also corroborates the central role of the germinal epithelium in vertebrate reproduction and affirms the definition of an ovarian follicle as an oocyte and encompassing follicle cells. Elaboration of the form and function of the germinal epithelium has only begun to be investigated throughout vertebrates and is an exciting new arena of research in fish reproductive biology.

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