

# **The Germinal Epithelium: Its Dual Role in Establishing Male Reproductive Classes and Understanding the Basis for Indeterminate Egg Production in Female Fishes**

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## **ABSTRACT**

The germinal epithelium in fish gonads is defined herein. An active germinal epithelium in the ovaries of mature fish is the basis for indeterminate egg production—the ability to produce an unlimited number of eggs. In males, changes that occur in testicular germinal epithelia during annual reproductive cycles can be used to define five reproductive classes: regressed, early maturation, mid maturation, late maturation, and regression. It is proposed that gonadal function changes during annual reproductive cycles in male fish. Mitosis is the mode of germ cell division during the regressed class, and the mode by which diploid germ cells, the spermatogonia, repopulate testis lobules. Meiosis never occurs. When reproductive, meiosis is the predominate mode by which germ cells divide, and sperm are produced. Mitotic divisions of spermatogonia may occur. In the ovary of the group-synchronous fish studied, common snook, meiosis occurs throughout the year. Oocytes, arrested in the diplotene stage of meiosis, and follicles are continuously produced by the germinal epithelium. Vitellogenesis occurs only during the reproductive season. In contrast to male fish, changes in the stages of oocytes that are present within the ovarian lamellae, not changes in the germinal epithelium, are used to define reproductive classes in females.

**KEY WORDS:** Germinal epithelium, spermatogenesis, vitellogenesis

## **INTRODUCTION**

In mammals and birds, there is a fixed number of oocytes present at the time of birth (Tokarz 1978). This is determinate egg production. In contrast, teleosts have indeterminate egg production, i. e., females can produce an unlimited number of eggs during their reproductive lifetimes (Tyler and Sumpter 1996) because they possess an active ovarian germinal epithelium (Grier 2000).

Herein, the concept of a germinal epithelium in male and female teleosts is reviewed, particularly as it can be used to understand indeterminate egg production in females and to define reproductive classes in males. An ovarian germinal epithelium may be present in almost all fish. However, given the reproductive diversity of fish, an exception occurs in the pipefish, *Sygnathus scovelli* (Begovac and Wallace 1987, 1988) and the seahorse, *Hippocampus erectus* (Selman et al.

1991). In both of these syngnathids, germinal ridges, one in the pipefish and two in the seahorse, occur along the ovary length. Oogonia are located in the germinal ridge where meiosis and folliculogenesis commence. The process of folliculogenesis, whether oocytes originate from a germinal ridge or a germinal epithelium, are similar. However, all of the criteria that define an epithelium (see below) do not apply to a germinal ridge.

#### GERMINAL EPITHELIUM CONCEPT

The ovarian germinal epithelium produces the germ cells in the fish ovary, and the testicular germinal epithelium produces those in the fish testis (Grier 2000, Grier and Lo Nostro 2000). The concept that a germinal epithelium continuously producing oocytes and follicles in the ovaries of fish (indeterminate egg production) has hardly been explored. In males, annual changes in the testicular germinal epithelium have been used to define annual reproductive classes, as in common snook, *Centropomus undecimalis* (Taylor et al. 1998, Grier and Taylor 1998), spotted sea trout, *Cynoscion nebulosus* (Brown-Peterson et al. 2001), and cobia, *Rachycentron canadum* (Brown-Peterson et al. in press). These changes in the germinal epithelium are based on definitive histological criteria.

The definition of the germinal epithelium is based upon histological definitions of an epithelium (Grier 2000, Grier and LoNostro 2000). The morphology and functions of epithelia depend on their location in the body--form and function coincide. Although morphologically diverse, all epithelia share the same characteristics:

- i) All epithelia border a body surface, lumen, or duct.
- ii) All epithelial cells are joined laterally (desmosomes, tight junctions, gap junctions, etc.).
- iii) All epithelia are supported by a basement membrane.
- iv) All epithelia are avascular.

If any of these characteristics are absent, then a tissue is not an epithelium. The germinal epithelium is unique among the different kinds of epithelia because it is the only epithelium that produces haploid germ cells that are associated with a somatic cell component (Grier and LoNostro 2000). Both ovarian and testicular germinal epithelia are supported by basement membranes and border a body lumen. In evolutionarily advanced female fish, the lumen is the ovarian lumen into which the ovigerous lamellae project from beneath the outer tunica. In more primitive fish, such as sturgeon and salmonids, the ovarian lamellae are suspended into the body cavity. In males, the body cavity that is bordered by the germinal epithelium is the lumen of the lobule (higher fish) or anastomosing tubule (lower fish) (Grier 1993). As a unifying concept, the definition of a germinal epithelium applies to both males and females and between chordate taxa (Grier and Lo Nostro 2000).

When most, but not all of the criteria that define an epithelium apply to a tissue, then the term "epithelioid" applies (Ross et al. 1995). An epithelioid tissue is the layer of follicle cells surrounding an oocyte. They rest upon a basement membrane, are laterally connected, and are avascular. However, they do not border a free body

space but are associated with the developing or mature oocyte. In cobia (Brown-Peterson et al., in press), solid cords of spermatogonia and Sertoli cells grow from the distal ends of the lobules during the regression and regressed classes that are also epithelioid tissue, until a lumen develops.

Germinal epithelia are composed of two cell types: somatic and germ cells. In the testis, the somatic cells are the Sertoli cells (Figure 1, Grier 1993). In the ovary, the somatic cells are the epithelial cells that give rise to prefollicle cells and eventually become follicle cells (Figure 2, Grier, 2000). Within the germinal epithelium, the diploid germ cells are either oogonia (ovary) or spermatogonia (testis). At the initiation of meiosis, an oogonium becomes an oocyte, and a spermatogonium becomes a spermatocyte. These germ cells undergo two successive meiotic divisions; the first one is the reduction division, i. e., the chromosome number is halved. The first meiotic prophase is extended and is subdivided into leptotene, zygotene, pachytene, diplotene, and diakinesis. In pachytene, the paired, homologous chromosomes are condensed and joined by a synaptonemal complex that is distinctive. The presence of synaptonemal complexes, seen by using electron microscopy, in the ovaries of regressed common snook was used to discern and define the ovarian germinal epithelium and document that meiosis and folliculogenesis occur throughout the annual reproductive cycle (Grier 2000).

A major difference exists between meiosis in cells that form sperm and those that form eggs. This is true, not only of fish, but also other vertebrates (Tokarz 1978), including mammals (Pelliniemi et al. 1993). In males, meiosis is a continuous process involving two successive cell divisions resulting in the production of four haploid spermatids. These, in turn, begin a morphological transformation called "spermiogenesis" and become sperm. In contrast, during oogenesis, meiosis becomes arrested during diplotene of the first meiotic division. Fish oocytes remain in diplotene through three growth stages: primary growth; secondary growth (vitellogenesis), and final oocyte maturation, after which the oocyte is preovulatory (Figure 3). Primary growth oocytes are marked by a synthesis of heterogenous RNA and ribosomal RNA, the latter produced by multiple nucleoli within the nucleus or germinal vesicle (Selman and Wallace 1989). Oocyte diameters increase from approximately 8-10  $\mu\text{m}$  to approximately 250  $\mu\text{m}$ . During secondary oocyte growth, vitellogenin is synthesized within the liver. Circulating vitellogenin is sequestered from the circulation and incorporated in protein yolk globules, increasing oocyte diameter to about 450  $\mu\text{m}$  in saltwater fish that lay pelagic eggs. Pelagic eggs contain an oil globule for buoyancy and a clear cytoplasm. The process by which the single oil globule and cleared cytoplasm forms from a mature oocyte is called final oocyte maturation (Wallace and Selman 1981).

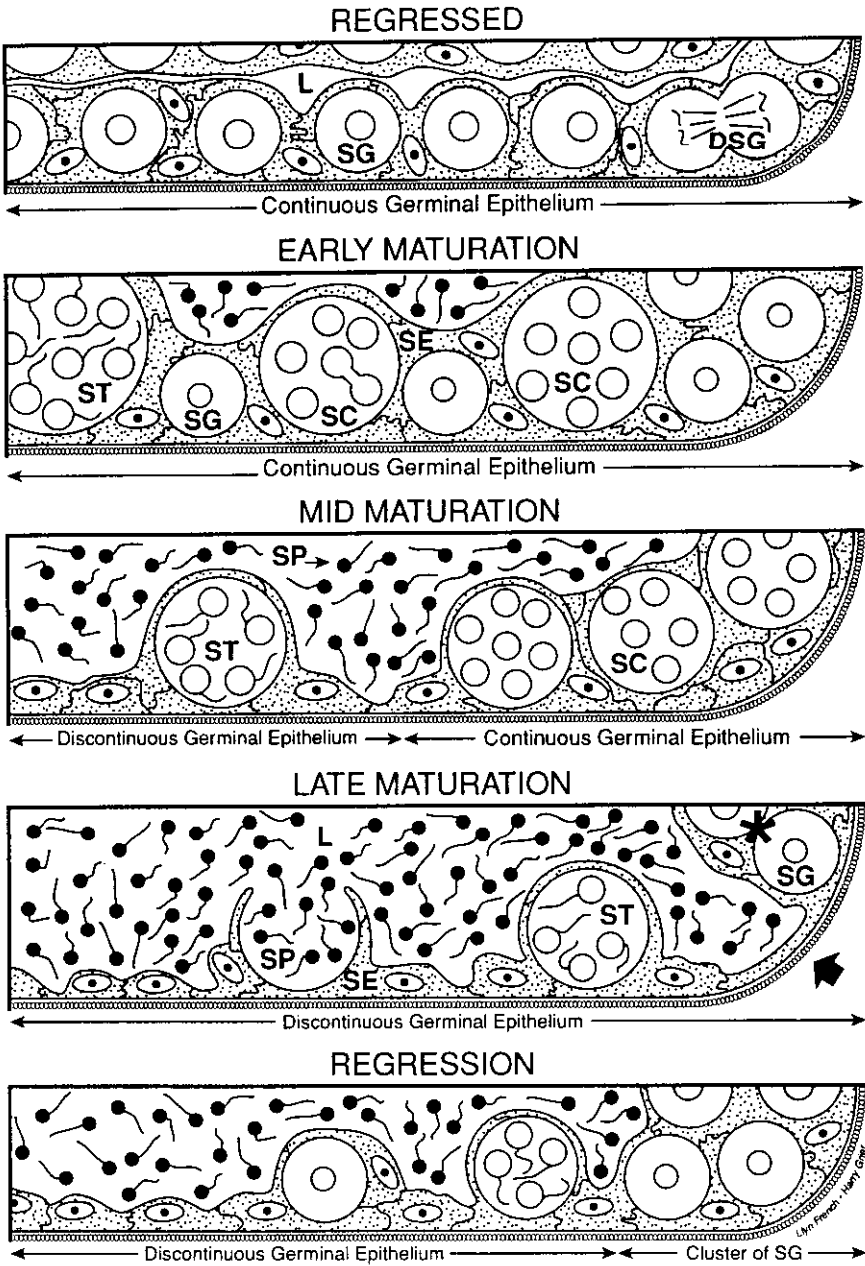


Figure 1. See legend at end of manuscript

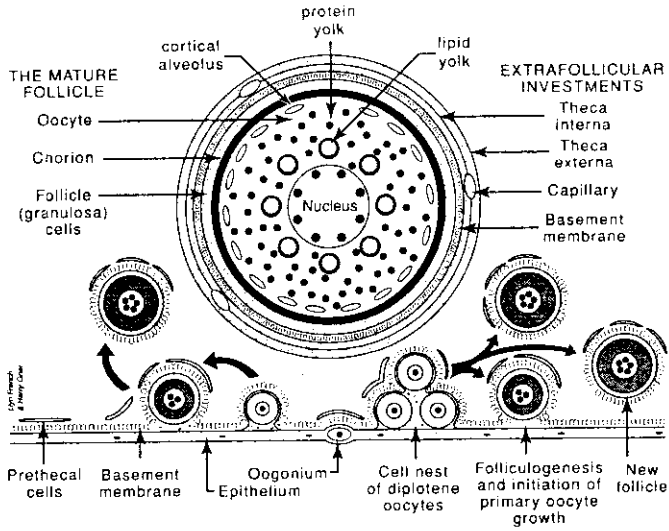


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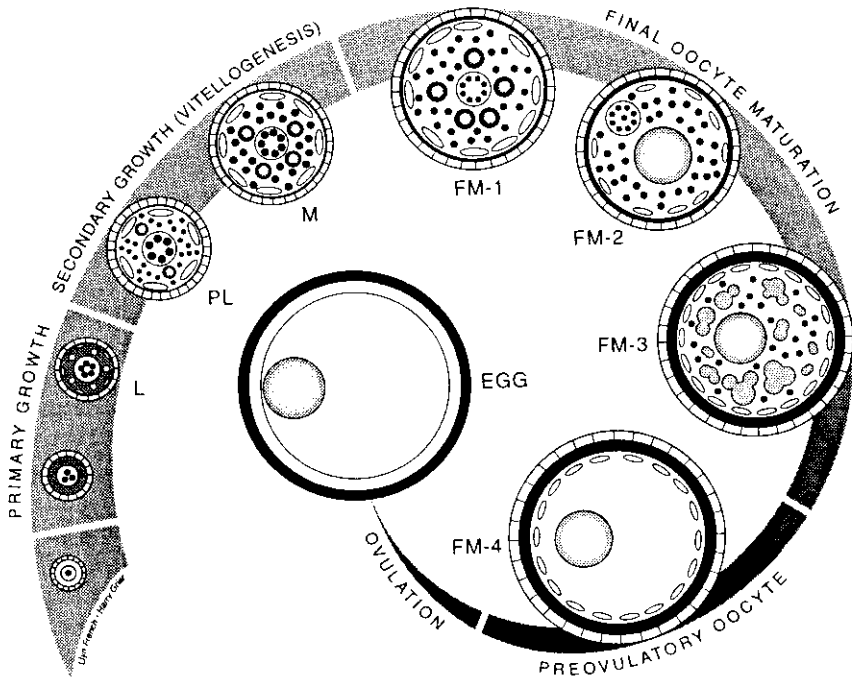


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At first, diplotene oocytes have a spherical nucleus and a single, prominent nucleolus, which is surrounded by a clear cytoplasm. These diplotene oocytes have frequently been misinterpreted as oogonia (see Grier 2000). Once they enter the primary growth phase of development, increase in diameter, and their cytoplasm becomes basophilic due to the accumulation of ribonucleic acid, there is no mistaking the correct staging of these cells as meiotic oocytes. Oocytes remain in arrested meiosis until the four phases of final oocyte maturation (Figure 3, Neidig et al. 2000) are completed, and they are preovulatory (Wallace and Selman, 1981; Selman and Wallace 1989). At the end of final oocyte maturation, the nucleus has migrated to the periphery of the oocyte, the nuclear membrane has dissociated, and the chromosomes have become free in the nucleoplasm. The first meiotic division is completed and the first polar body is extruded. The remaining chromosomes then proceed to the second meiotic metaphase and again arrest in division (Selman and Wallace 1989).

#### DETERMINATION OF REPRODUCTIVE CLASSES IN MALES

In the annual reproductive cycle of male fish, descriptive classes can be recognized because of distinct changes in the germinal epithelium (Figure 1). These changes are easily perceived histologically. They are based on whether there is a continuous or a discontinuous germinal epithelium and the germ cell stages that are present (Grier and Taylor 1998). In a continuous germinal epithelium, a continuous population of germ cells and Sertoli cells exists along the lobule basement membrane. The germ cells may be spermatogonia, meiotic spermatocytes, maturing spermatids during spermiogenesis, or sperm. These germ cells develop synchronously within spermatocysts whose borders are formed by Sertoli cells. In a discontinuous germinal epithelium, sperm mature and undergo spermiation (release into the lumen of the lobule). Because the germ cells are not replaced as the spermatogonia all transform into meiotic cells, there is a gradual loss of the germ cell component from the germinal epithelium. Spermatocysts, and generally some spermatogonia, become widely spaced, and an epithelium results that eventually is almost entirely composed of a monocellular layer of Sertoli cells along the lobule basement membrane. The alternation of continuous and discontinuous germinal epithelia, and the stages of the germ cells that are associated with Sertoli cells in the germinal epithelium, are the criteria that are used to determine reproductive classes in male fish (Figure 1).

*The regressed class* — is defined by the presence of only spermatogonia in the testis (Figure 1). A continuous germinal epithelium extends from the ducts to the distal termini of the lobules. It is significant that the spermatogonia, and their associated Sertoli cells, divide mitotically (Lo Nostro et al. in review) during the regressed class. It is not generally recognized that this is a period when the population of spermatogonia is renewed within the germinal compartments and important testicular growth processes occur. In regressed red drum, mitotic divisions of

spermatogonia produce lobule elongation, which is represented by epithelioid outgrowths, called cords, containing spermatogonia and Sertoli cells just prior to the breeding season (Grier et al. 1987). Cords of spermatogonia and Sertoli cells are particularly evident in cobia (Brown-Peterson et al. in press) during the regression and regressed classes. However, even before the initiation of spermatogenesis, a lumen begins to develop within the epithelioid cords; they then fulfill the criteria for an epithelium and are a germinal epithelium.

*The early maturation class* — is also defined by the existence of a continuous germinal epithelium between the ducts and the termini of the lobules, but in this class spermatogenesis has been initiated (Figure 1). Because spermatogenesis involves the formation of spermatocysts, that contain a clone of synchronously developing germ cells, the width of lobules increases, and the lobular lumen is generally partially restricted or closed because opposing sides of the lobules press against each other. It becomes continuous when sperm are voided from the spermatocysts (spermiation). There is considerable elongation of the lobules, and the entire testis becomes larger. Early maturation marks the switch from a mitosis-dominated to meiosis-dominated cell division among germ cells (Figure 4).

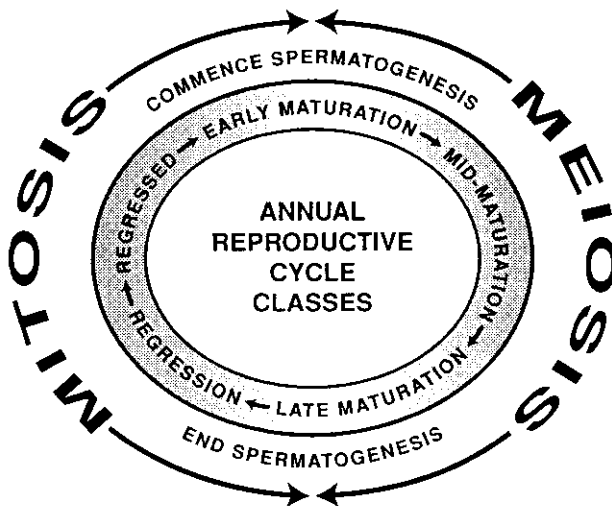


Figure 4. See legend at end of manuscript

*The mid maturation class* — is characterized by the presence of a discontinuous germinal epithelium in the region of the testis ducts and a continuous germinal epithelium at the lobule termini (Figure 1). As maturation progresses, the lobules can grow in length so long as there are distal spermatogonia and Sertoli cells at their termini. Upon spermiation, or sperm release into the ducts, the germ cell component of the germinal epithelium is lost. Spermatogonia and spermatocysts containing germ cells become scattered along the lobule basement membrane and are separated by Sertoli cells, producing a discontinuous germinal epithelium (Grier 1993).

*The late maturation class* — commences when at least one lobule within a histological section is observed to possess a discontinuous germinal epithelium at its distal terminus (Figure 1, arrow). In common snook, clusters of spermatogonia (Figure 1, asterisk) also become reestablished in late maturation (Grier and Taylor et al. 1998), but this does not happen until the regression class in cobia (Brown-Peterson et al., in press). At first, only a single lobule within a histological section may have a discontinuous germinal epithelium at its terminus. As maturation progresses, additional lobules lose a continuous germinal epithelium at their termini (Grier and Taylor 1998, Brown-Peterson et al. in press).

Spermiation, or release of sperm from the spermatocyst, is illustrated during the late maturation class of Figure 1. Spermiation, coupled with exhaustion of primary spermatogonia, is the basis for changes in the germinal epithelium through an annual reproductive cycle. At a time when the testis is producing sperm for reproduction, parts of it are ceasing to produce sperm and are becoming involved in sperm storage. The testis undergoes transformation from sperm production to sperm storage, beginning in the duct area of the testis. Most fish must produce large volumes of sperm during a spawning event, particularly if they are group-synchronous spawners and broadcast gametes into the environment. Lacking separate sperm-storage organs, the testis serves this purpose.

As maturation progresses, the process of germ cell exhaustion within the lobules extends towards the testis periphery. However, upon close histological examination, there are generally a few inactive spermatogonia along the lobule lengths. Therefore, the germinal epithelium becomes truly “discontinuous,” where there is only a Sertoli cell presence along the lobule walls and very few scattered spermatogonia or spermatocysts. Recognition of these two states of the germinal epithelium, continuous and discontinuous, and the observation that germ cells persist within lobules throughout annual reproductive cycles, led to the conclusion that the lowest taxon in which a permanent germinal epithelium evolved is the fishes (Grier 1993, Grier and Lo Nostro 2000).

*The regression class* — is characterized by widely scattered spermatocysts along lobule walls, generally lobules and ducts are filled with sperm, spermatogonia are few in number (Figure 1), and there is a decreasing gonadosomatic index. Sperm may be phagocytized by Sertoli cells, as has been observed in snook, or possibly voided from the testis at the cloaca (Grier and Taylor et al. 1998), or they may



remain accumulated in the lobules and ducts as in cobia (Brown-Peterson et al. in press). Species differences regarding the accumulation of sperm within the testis lobules, both during the regression and regressed classes, underscore the reason for not including sperm in the assignment or reproductive classes (Brown-Peterson et al. in press).

#### COMPARISONS BETWEEN SPECIES (MALES)

The five reproductive classes (regressed, early maturation, mid maturation, late maturation, regression), that are based upon changes in the male germinal epithelium and the types of germ cells that are present, have been applied to other teleosts. Annual changes in the testicular germinal epithelia of common snook, *Centropomus undecimalis* (Grier and Taylor 1998), spotted seatrout, *Cynosion nebulosus* (Brown-Peterson and Warren 2001), cobia, *Rachycentron canadum* (Brown-Peterson, et al. in press), and the synbranchid eel, *Synbranchus marmoratus* (Lo Nostro et al. in review) all cycle by a similar mechanism. The latter species is in the order Synbranchiformes while the former three species are in different families in the order Perciformes. Comparative information on annual changes in the male germinal epithelium is species-limited at present as there are only a few studies. However, the potential application of germinal epithelium criteria for the determination of annual reproductive classes in fish may become common as more fisheries biologists interpret reproductive cycles in light of these new criteria.

The testicular annual reproductive cycle can also be divided into two periods, dominated by either meiosis or mitosis (Figure 4). Sperm production is dominated by meiosis (early maturation, mid maturation, and late maturation classes). The sperm production phase is followed by mitosis-dominated renewal of stem germ cells, the spermatogonia. Meiosis does not occur. The discontinuous germinal epithelium observed in late maturation and regression is replaced by a continuous germinal epithelium, but the only germ cells present are spermatogonia. Reestablishment of a spermatogonial population in the testis may begin as early as late maturation, as in common snook (Taylor et al. 1998), and it continues into the regression and regressed classes. These two phases in male fish, dominated by either meiosis or mitosis, are obviously under distinctively different physiological controls. When fish are in the regressed class, the primary activity is mitotic division of spermatogonia and their associated Sertoli cells to restore a stem germ cell population within the germinal compartment. Herein, it is recognized that there is continual germ cell activity within the testis throughout an annual reproductive cycle, the periods of activity are either mitosis or meiosis dominated. Learning how these mechanisms—mitosis, meiosis, and lobule elongation—temporally interact and differ in fish species with different reproductive strategies, is an arena for future fish research.

### THE GERMINAL EPITHELIUM IN FEMALE FISH

The first description of the ovarian germinal epithelium, in the common snook, *Centropomus undecimalis*, which is a group-synchronous spawning species (Grier 2000), established the origin of oocytes in the fish ovary and that meiosis and folliculogenesis occur throughout the annual reproductive cycle. Meiotic oocytes were observed in the ovarian germinal epithelium at the nadir of the annual reproductive cycle, during the regressed class. Unlike the testis maturation stages in male common snook, which occur once through the course of the annual reproductive cycle, there is a cycling between mid maturation and late maturation in common snook ovaries with every spawning event (Taylor et al. 1998).

It is not possible to define reproductive classes, via changes in the germinal epithelium, in the fish ovary. This is due to the marked differences in gamete development between the sexes in fish and the prolonged diplotene stage of the first meiotic division in oocytes. The paper that introduced the germinal epithelium as a potential new interpretative tool for fisheries biologists (Taylor et al. 1998) subtly makes this developmental difference clear. Reproductive classes in male snook were defined based upon distinctive, annual changes in the testicular germinal epithelium that are easily discerned histologically (Figure 1). Reproductive classes in female common snook were defined based upon changes in oocyte developmental stages and oocyte cycling through individual reproductive events in common snook (Figure 3). A single, hormone-induced spawn averaged 280,000 eggs (Neidig et al. 2000), and the natural, estimated time between spawns is 1.6 days (Taylor et al. 1998). In order to support continuous egg production in fish, there needs to be indeterminate egg production: folliculogenesis, or formation of follicles (Figure 2), needs to be continuous. Within the germinal epithelium, diploid oogonia must continuously enter into meiosis, and oocytes may be continuously recruited from the ovarian germinal epithelium into the ovarian lamellae where maturation (primary oocyte growth, vitellogenesis, and final oocyte maturation prior to ovulation, Figure 2).

In order to define folliculogenesis in the fish ovary, it was first necessary to define what a follicle is, because the fish literature has been inconsistent in its definition (Grier 2000). The fish ovarian follicle, by the definition taken from the mammalian literature (Grier 2000, Grier and Lo Nostro, 2000), is composed of the oocyte and its encompassing layer of follicle (granulosa) cells (Figure 2). This definition also reflects on the origin of the follicle from oogonia and epithelial cells (which become the follicle cells (Grier 2000) that are originally in the germinal epithelium (Figure 3). As oocyte maturation progresses, the follicle adds an intercellular layer, the chorion. The follicle is encompassed by an acellular basement membrane and the cellular theca interna and externa (Figure 3). Together, the follicle and its encompassing extrafollicular investments are termed the follicle complex (Grier 2000, Grier and Lo Nostro 2000). In mammals, the formation of an antrum in the developing follicle signifies the secondary stage in development (Fawcett and Jensch 1997). An antrum never forms in teleost ovarian follicles, and the term "secondary follicle" is inappropriate as a means of comparison between taxa.

The ovarian germinal epithelium is the thin epithelium that forms the surface of an ovarian lamella and borders the ovarian lumen (Figure 2); it is composed of two cell types: somatic epithelial cells and germ cells that may be either oogonia or meiotic oocytes. The epithelial cells are attached via desmosomes and tight junctions and rest upon a basement membrane (Grier 2000). They are avascular. Scattered among the epithelial cells are oogonia that may divide mitotically to maintain their population or enter into meiosis to produce single oocytes. The ovarian germinal epithelium is always a discontinuous germinal epithelium by the definition developed for determining male reproductive classes. After repeated mitotic divisions, oogonia may produce a cell nest composed of multiple oogonia and then, at the initiation of meiosis, multiple oocytes become arrested in the diplotene stage of the first meiotic prophase. Observations made on common snook indicate that diplotene oocytes in a cell nest are, at some point, still attached to and part of the germinal epithelium as there is no separating basement membrane (Grier, 2000). Primary oocyte growth begins when oocytes are still attached to the germinal epithelium (Figure 2, Grier 2000).

Within the germinal epithelium, oocyte meiosis proceeds through leptotene, zygotene, pachytene (when synaptonemal complexes are visible), and into diplotene of the first meiotic prophase. During diplotene, lampbrush chromosomes appear (Selman and Wallace 1989); primary oocyte growth, denoted by the accumulation of RNA within the cytoplasm, commences shortly thereafter.

As meiosis advances, epithelial cells become associated with oocytes, these complexes of epithelial cells and oocytes "sink" below the surface of the germinal epithelium and epithelial cells become prefollicle cells. During folliculogenesis, a basement membrane is synthesized around the forming follicle (Figure 3). The follicle is completely separated from the germinal epithelium by the basement membrane (Figure 3). Undifferentiated cells within the ovarian stroma, actually within the extravascular space (Grier 2000), become associated with the follicle as it is forming. These cells differentiate to form the theca interna and externa.

Prior to the completion of folliculogenesis, cortical granule formation, occurrence of lipid yolk, and advancement of the oocyte to the perinucleolar stage of development have not been observed, at least not in common snook (Grier 2000). Presumably, these events in oocyte maturation occur after folliculogenesis is completed, possibly in all fishes. The oocyte within a mature follicle (Figure 3) possesses cytoplasmic lipid and protein yolk globules and peripheral cortical granules. A chorion is not initially present in a newly formed follicle, but it develops during primary oocyte growth.

The events leading to ovulation begin and end with the diplotene oocyte. All of the processes of maturation, until the oocyte is preovulatory (final oocyte maturation stage FM-4, Figure 3), when the nuclear membrane disassociates and chromosomes become free in the cytoplasm and the first meiotic division, occur in the arrested diplotene stage of the first meiotic prophase. When first formed, diplotene oocytes do not possess a basophilic cytoplasm but are large cells with a distinct nucleolus. Primary oocyte growth is initiated within the germinal epithelium

with accumulation of basophilic, cytoplasmic RNA.

Simple techniques, developed for hatchery application (Neidig et al. 2000), divided final oocyte maturation of common snook into four distinct stages (Figure 3) that can be used, if ovarian biopsies can be taken, to track maturation until broodstock are preovulatory. These stages probably apply to a number of other perciform fish and have been applied to red drum production (unreported). During stage 1 of final oocyte maturation (FM-1), lipids begin to coalesce and are much more distinctive than is nuclear migration to define both FM-1 and FM-2. During FM-1, using a wet mount technique called the coverslip method, it is possible to view the fusion of lipid globules within the oocyte cytoplasm until FM-2 is reached. At FM-2, there is but a single lipid globule, but protein yolk dissolution is not observed. Frequently, protein yolk globules begin to "clear" or become fluid before there is a single oil droplet. The oocyte can proceed from FM-1 directly to FM-3, the stage characterized by oocyte clearing. In the last final oocyte maturation stage, the oocyte possesses a completely clear cytoplasm, a single oil globule, and is preovulatory. At ovulation, the oocyte becomes an egg, voided into the ovarian lumen, and loses its encompassing layer of follicle and thecal cells that become a post-ovulatory follicle.

#### DISCUSSION

The changes that take place in the regressed testes and ovaries are subtle. Until recently, it has been rare to witness cell divisions or derive growth processes. The regressed testis appears to contain only spermatogonia and Sertoli cells while the ovary has numerous primary growth oocytes. In the ovary of the wahoo, *Acanthocybium solandri* (Scombridae), Brown-Peterson et al. (2000) recognized that multiple spawning species (group synchronous) have "continual oocyte recruitment." However, the underlying cellular basis for this recruitment became known only when the ovarian germinal epithelium and process of folliculogenesis were described (Grier 2000).

New criteria for the determination of reproductive classes in male fish are based upon naturally occurring changes that take place in the germinal epithelium. The reproductive classes--regressed, early maturation, mid maturation, late maturation, and regression--have been applied to male and female common snook (Grier and Taylor 1998, Taylor et al. 1998). Researchers are finding that these classes may be used to describe the reproductive classes in other fishes such as male sea trout, *Cynoscion nebulosus* (Brown-Peterson and Warren 2001), cobia, *Rachycentron canadum* (Brown-Peterson et al. in review), and swamp eels, *Synbranchus marmoratus* (Lo Nostro et al. in review). Nine gonadal reproductive stages were initially described in sea trout, including an immature stage (Brown-Peterson et al. 1988). By using changes in the germinal epithelium to describe the annual reproductive cycle, eight of the reproductive stages were replaced by five classes (Brown-Peterson and Warren 2001). The immature stage should become an immature class following the definitions of stage and class (Taylor et al. 1998).

“Stage” refers to a stage in germ cell development. “Class” refers to changes in gonad development.

The ability of fish to produce continuous numbers of eggs throughout their reproductive lifetime is because of an active ovarian germinal epithelium in adults. The ovarian germinal epithelium has only recently been described (Grier 2000), but its activity undoubtedly underlies fish egg production. The germinal epithelium is active at the nadir of the annual reproductive cycle in common snook, in both males and females. Testicular germinal epithelia produce spermatogonial renewal during the regressed class in cobia and swamp eels. I propose that male fish cycle between gamete production and the coincident exhaustion of diploid spermatogonia (meiosis-dominated part of the annual reproductive cycle) and renewal of the spermatogonia population in the testis (mitosis-dominated part of the annual reproductive cycle). These two processes are temporally separated on an annual basis and occur within respective reproductive classes (Figure 4). The implication is not made that during the meiosis-dominated part of the reproductive cycle there are no mitotic divisions of spermatogonia, only that one type of cell division is predominate. However, meiosis does not occur during the regressed class when only spermatogonia are present within the testis.

There is a basic difference between male and female fish regarding meiosis. In males, only mitosis and division of spermatogonia occur during the regressed class. In females, however, meiosis also occurs during the regressed class, at least in common snook as of this writing (Grier 2000), and follicles are produced in which oocytes enter the primary growth phase. However, they do not become vitellogenic until the early maturation class during the annual reproductive cycle.

#### FIGURE LEGENDS

**Figure 1.** Reproduction classes through the year as interpreted by histological changes in the germinal epithelium. Dividing Spermatogonium (DSG); Lumen (L); Sperm (SP); Spermatoctye (SC); Spermatogonia (SG); Spermatisd (ST); Sertoli cell (SE).

**Regressed class:** only spermatogonia are present which are associated with Sertoli cells. The lobule lumen is frequently discontinuous, and spermatogonia may divide mitotically.

**Early maturation class:** There is a continuous germinal epithelium extending the entire length of the lobule, but spermatogenesis has been initiated.

**Mid maturation class:** a discontinuous germinal epithelium is present proximally, near the testis ducts. A continuous germinal epithelium is observed at the lobule periphery.

**Late maturation class:** a discontinuous germinal epithelium is observed at the termini of at least one lobule in a histological section (arrow) and increasingly more lobules as maturation progresses. Spermiation, the release of sperm from a spermatoctyst, is depicted.

**Regression Class:** Spermatogonia begin to reestablish their population along the basement membrane of the lobule in association with Sertoli cells. Spermatogonia are also present at the distal termini of lobules, sometimes being organized as epithelioid cords of cells. There may be a few spermatoctysts present, but the

germinal epithelium is primarily made up of Sertoli cell epithelium; the GSI is declining and the testis is becoming smaller.

**Figure 2.** The ovarian germinal epithelium is composed of oogonia and epithelial cells that rest upon a basement membrane. At the initiation of meiosis the oogonium becomes an oocyte, extending into the ovarian lamella. If oogonia divide mitotically prior to entering meiosis, a cell nest is produced that is attached to the basement membrane. All oocytes within a cell nest eventually become follicles composed of the oocyte and encompassing layer of follicle cells that are derived from the original epithelial cells of the germinal epithelium. As the oocyte matures, an acellular chorion forms between the oocyte and the follicle cells. Primary oocytes possess a RNA-rich cytoplasm (gray area), whereas mature oocyte cytoplasm has cortical alveoli, lipid yolk, and protein yolk. The follicle is surrounded by the basement membrane, derived from that supporting the germinal epithelium, and a vascularized theca that is divided into a theca interna and theca externa. Undifferentiated cells within the extravascular space, the prethecal cells, form the theca interna and externa.

**Figure 3.** The process of oocyte maturation begins with a diplotene oocyte that enters the primary growth stage. The cytoplasm becomes basophilic due to the accumulation of RNA. Nucleoli become oriented around the periphery of the nucleus (perinucleolar stage), lipid yolk (L Stage), and cortical alveoli form during primary growth. During secondary growth or vitellogenesis, lipid yolk and protein globules (PL Stage) accumulate in the oocyte cytoplasm until the oocyte is mature (M Stage) and will respond to a gonadotropin surge from the pituitary gland, whereupon final oocyte maturation commences. Based upon easily discernable characteristics, revealed by using the coverslip method, final oocyte maturation has been subdivided into four substages (Neidig et al., 2000). These are: (1) Final Maturation-1 (FM-1), where lipids fuse into larger, distinct globules; Final Maturation-2 (FM-2), where there is a single oil globule, but no apparent "clearing" of the oocyte cytoplasm; Final Maturation-3 (FM-3), where protein yolk begins to liquify and the cytoplasm becomes clear; and Final Maturation-4 (FM-4), where the cytoplasm is completely clear and the oocyte is preovulatory. Prior to ovulation, the first meiotic division is completed; the oocyte progresses to metaphase of the second meiotic division and again rests in development. The oocyte ovulates and becomes an egg, losing its encompassing layer of follicle cells in the process. These remain in the ovarian lamellae as a post-ovulatory follicle.

**Figure 4.** Diagrammatic representation of annual testis cycling in fish. The testis cycles between mitosis-dominated (late maturation, regression and regressed classes) or meiosis-dominated (early maturation, mid maturation, late maturation classes) cell division. Mitosis-domination represents renewal of diploid spermatogonia, whereas meiosis domination cell division represents spermatogenesis and haploid sperm production.

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