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DEVELOPMENT OF THE GONADS AND GAMETES IN THE SOFT-SHELL CLAM (MYA ARENARIA)

WESLEY R. COE AND HARRY J. TURNER, JR.
Osborn Zoological Laboratory, Yale University

TWENTY-THREE FIGURES

AUTHORS' ABSTRACT

The clam **Mya** differs from other pelecypods which have been investigated in this respect in having most of the reserve nutritive materials formed within the vacuolated follicle cells of the gonads instead of within the very limited amount of mesenchymatous connective tissue of the visceral mass. The profusely branching tubular gonads originate from two groups of primordial germ cells situated in the position of the future genital apertures. The germinal primordia soon become differentiated into two types of nuclei, one of which becomes associated with the large, vacuolated follicle cells, which form the principal volume of the gonadal tissue, while the other type proliferates to form the primary gonia which become widely scattered along the walls of each alveolus.

There is much degeneration and cytolysis during gametogenesis in both sexes, with the accumulation of characteristic inclusions within the follicle cells. Atypical spermatogenesis followed by cytolysis occurs throughout the year but the normal method only in preparation for spawning. No evidence of protandry or change of sex was obtained; only three hermaphrodites were found in the examination of more than 1000 individuals.

During the past few years it has been found that in several species of pelecypods all or nearly all individuals are protandric, with a strong tendency toward alternating female and male phases in later life. In the viviparous oysters (Coe, '34; Orton, '36) and in at least one species of Teredo (Coe, '36) there may be two or more of these functional phases within a single year. In the oviparous oysters the dominance of protandry is less complete, the proportion of individuals functioning as females during their first breeding season depending upon the locality and the environmental conditions. In older individuals the sexual phase may change in the interval between two breeding seasons or even during a single breeding season in the case of young individuals under certain conditions (Coe, '36 a). In the quohog, on the other hand, an initial male phase leads to either of the unisexual phases, with no evidence of a second change of sex (Loosanoff, '36, '37).

The question as to the extent of protandry among the pelecypods requires further investigation, and it was with the object of answering this question for the soft-shell clam that this study was undertaken. Although it was soon determined that in this species there is no evidence of protandry, the histological structure and developmental history of the gonads were found to be of such aberrant types that it seems advisable to describe in some detail the processes observed.

DEVELOPMENT OF PRIMARY GONAD

The primary gonads develop from a pair of germinal primordia situated near the posterior end of the body and just ventral to the pericardium. These positions are retained in the adult as the later openings of the genital ducts (figs. 1, 2).

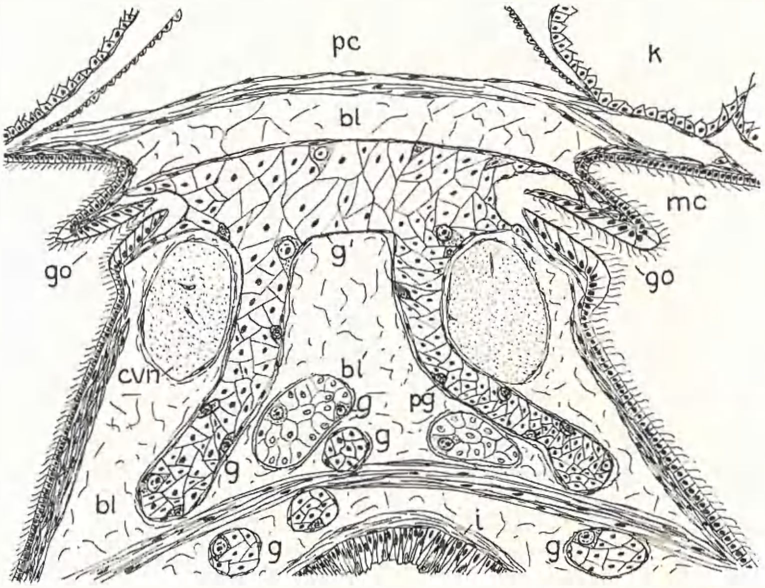
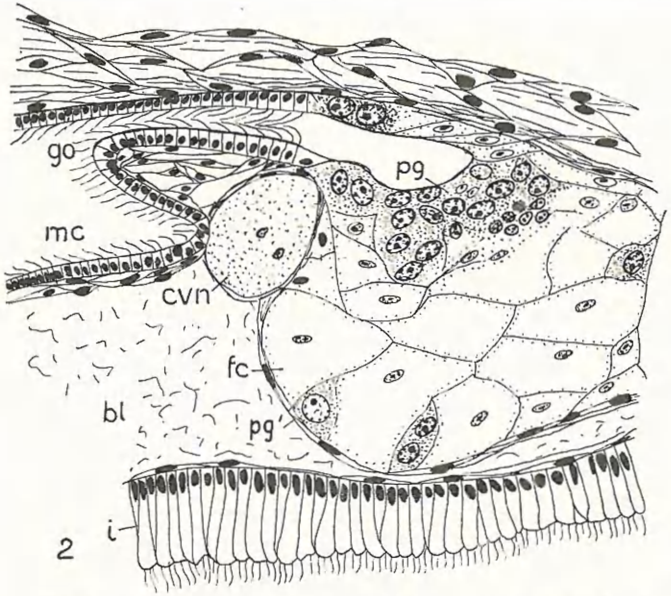


Fig. 1 Portion of section through body of young individual 17 mm. in length, showing position of the pair of primary gonads (g) with transverse connective (g') and transverse sections of several branches; bl, vascular connective tissue; cvn, cerebro-visceral nerve cords; go, genital openings; k, kidney; i, intestine; mc, epibranchial chamber; pc, pericardial cavity; pg, primary germ cells separated by more numerous follicle cells.



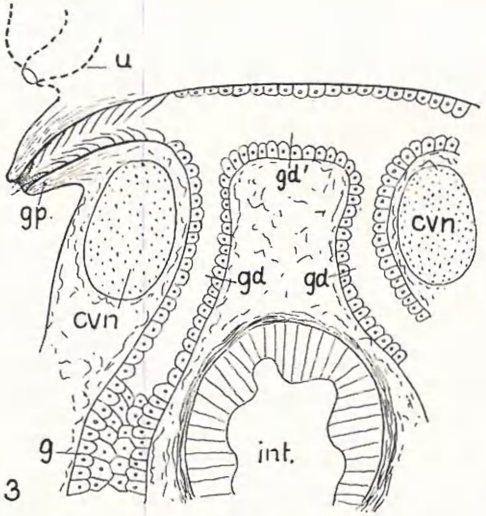


Fig. 2 Portion of section through 6 mm. individual, showing primordium of gonad with group of proliferating nuclei of germ cells (pg) and follicle cells, followed by large vacuolated cells (fc) separating the germ cells (pg'); go, genital opening; other letters as in figure 1.

Fig. 3 Outline of terminal portion of primary gonads of 16-mm. individual, showing genital ducts (gd) and transverse connective (gd'), leading from solid gonad (g) to genital papilla (gp); u, ureter; other letters as in figure 1.

From the germinal primordia a pair of slender processes grow out ventrally and anteriorly through the loose vascular connective tissue adjacent to the intestine to form the basis of the profusely branched follicles of the adult gonads. The gonadal systems of the two sides of the body are connected by a transverse process at the posterior end (fig. 1). These terminal portions of the gonad become the genital ducts of the adult by the formation of large central lumens (figs. 2, 3).

The young gonads consist of cylindrical, branching root-like masses of vacuolated follicle cells, with scattered germinal cells along the periphery (fig. 4). From these few germinal cells all the future gametes will be derived; the follicle cells are strictly nutritive.

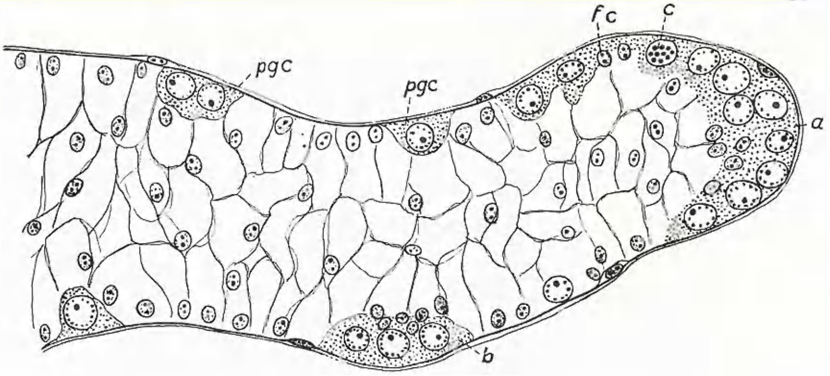
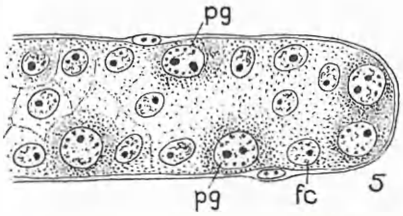


Fig. 4 Terminal portion of primary gonad of young individual, 6 mm. in length, showing syncytial group of germ cell nuclei at tip of growing point (a) and nuclei of follicle cells (fc) beneath. At one point (b) at the side of the gonad a similar, but smaller, group of germ cells indicates the primordium of a lateral branch of the gonad, while other primary gonia (pgc), scattered along the wall, represent the antecedents of the functional gametes; c, nucleus in prophase of mitosis.

The apical growing point of each branch is composed of a syncytium containing two quite different types of nuclei. The larger of these will later be incorporated into the primary germ cells, while the smaller ones will become the nuclei of follicle cells (figs. 4, 5, 6). No transitional stages between the two types of nuclei are found and it is evident that each produces only its own kind, although both have had a common origin in the gonadal primordium.

A short distance behind the apex each of the follicle cell nuclei becomes enclosed in a thin layer of cytoplasm surrounding a large central vacuole and incompletely separated from its neighbors by a delicate cell membrane.

As the root-like growth proceeds an occasional germ cell is left behind along the wall of the cylinder formed by the much more numerous follicle cells (fig. 4). These scattered germ cells become themselves the centers for the outgrowth of lateral branches similar in all respects to the primary portion (figs. 5, 6). In this manner a complex system of branching root-like processes eventually ramifies ventrally and anteriorly among the other viscera and even into the foot. These will later form the follicles of the functional gonads with the genital ducts opening into the suprabranchial cavity at the sites of the original primordia (fig. 1). Small groups of proliferating germ cells and follicle cell nuclei remain for several weeks at the sites of the gonadal primordia as sources of supplementary gonia and follicle cells for the rapidly growing gonad (fig. 2). As the body increases in size the proportional growth of the gonads is accelerated, but even with a body length of only 6 mm. the principal parts of the system are already established.



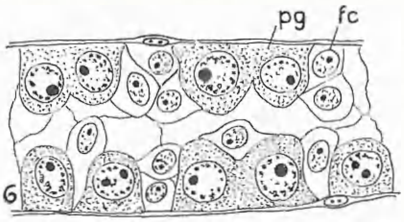
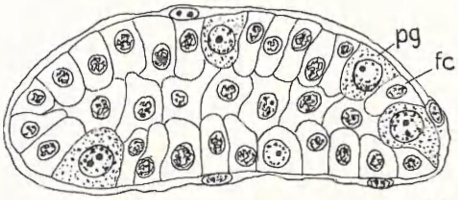


Fig. 5 Terminal portion of branch of primary gonad with syncytial group of nuclei of germ cells (pg) and follicle cells (fc). Length of body 9 mm.

Fig. 6 Portion of branch in which the cell membranes of germ (pg) and follicle (fc) cells have become established.

A few weeks later both the germ cells and follicle cells have been carried forward by the elongation of the terminal portions of the gonads. In their progress the germ cells have come to lie separately or in small groups between the follicle cells along the gonadal walls. The follicle cells separate to form a large central lumen in each gonad from the genital opening forward, thus producing a slender canal which becomes the future genital duct. The transverse connective is also included, thereby allowing the genital products of both gonads to pass from the body through either genital opening (fig. 3).



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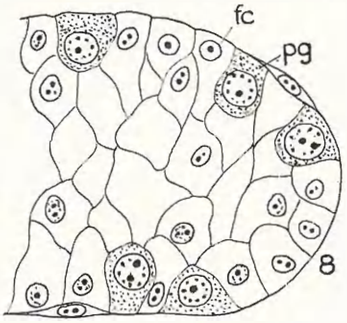


Fig. 7 Section of branch in which the primary gonia and follicle cells are sharply differentiated.

Fig. 8 Follicle cells (fc) have increased in size and separated the primary gonia (pg) more widely. Length of body 20 mm.

The young gonads of this species of clam show little resemblance to those described for other pelecypods because of the vastly greater development of the follicle cells and the widely scattered gonia. The difference is accentuated by the extremely small amount of mesenchyme in Mya, so that the young alveoli are packed closely together (fig. 9) instead of being separated by the great abundance of mesenchyme found in many other bivalves. The follicle cells of Mya, however, resemble superficially the mesenchyme of other forms, particularly when they contain granular inclusions, and their nutritive functions are similar but their origin is quite different.

At the age of about 3 months, when the body length of well-nourished individuals has reached about 20 to 30 mm., the profusely branching gonads fill almost the entire visceral mass posterior to the digestive gland. Each of the larger branches has from thirty to sixty follicle cells in a transverse section (figs. 7 to 11), with only a thin sheath of loose connective tissue separating adjacent branches (fig. 9).

In the young gonad the follicle cells are quite transparent, with extremely thin and delicate walls bordered internally by a thin layer of cytoplasm surrounding a large central vacuole. But with increasing age the cytoplasm shows an accumulation of rounded granules or globules of various sizes. The lipoid globules are visible in life because of their higher index of refraction and in the prepared sections the proteins become conspicuous because of their differential staining. At the time of sexual differentiation and later the follicle cells accumulate inclusions which are almost as characteristic of the two sexes as are the gametes themselves (figs.

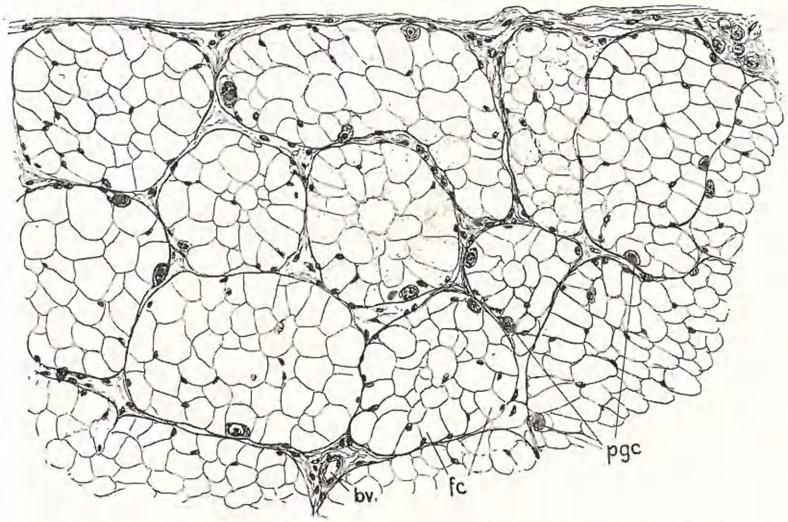
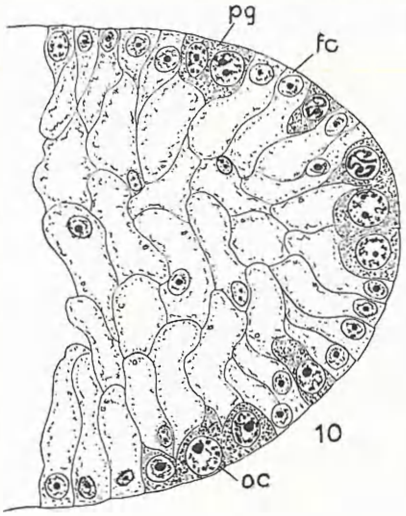


Fig. 9 Small portion of gonad, showing the closely crowded branches separated by thin sheaths of vascular connective tissue and small blood vessels (bv); widely scattered germ cells (pgc) are shown along the walls, with numerous follicle cells (fc) filling the rest of the gonad. Length of body 20 mm.

14, 20 to 22). In hermaphrodites which have the ovogenic and spermatogenic cells in separate alveoli, each alveolus has only the inclusions peculiar to its own type of sexuality (fig. 23).

SEXUAL DIFFERENTIATION

The primary germ cells of the two sexes appear to be indistinguishable but as early as September, when well-nourished individuals of the earlier broods have reached a length of 20 mm. or more, a stage is reached where the male gonad is characterized by the rapid multiplication of the primary gonia and their differentiation into spermatogonia. In an equal proportion of individuals the primary gonia are differentiated into ovocytes after passing through the spireme and synaptic phases.



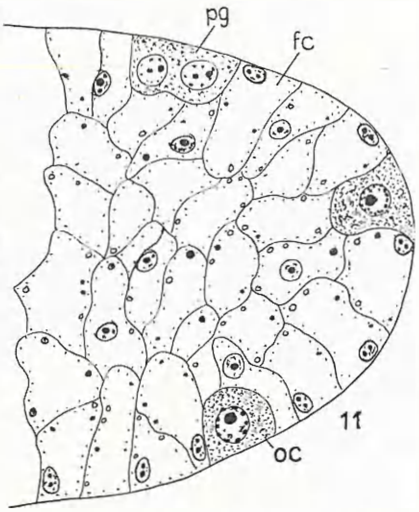


Fig. 10 Initial phase of sexual differentiation; female. Follicle cells (fc) have increased in size and a few gonia have transformed to ovocytes (oc); follicle cells have small inclusion granules. Same magnification as in figures 7 and 8. Length of body 26 mm.

Fig. 11 Differentiation of ovocytes (oc); follicle cells are beginning to show spherical inclusions.

Young which are hatched in mid-summer or later, as well as those with retarded growth, do not reveal their type of sexuality until early winter or in some cases until early spring.

Although the differentiated young gonad shows some indications of bisexuality, as is the case with so many other pelecypods, it is only rarely that functional hermaphroditism occurs. There seems to be a direct transformation of most of the primary gonia into one type of sexual cell or the other, although a few typical ovocytes are sometimes present in the spermaries of young individuals (fig. 17). Differentiation into the two sexes is remarkably uniform, however, for only three functional hermaphrodites were found in more than a thousand adult individuals. One of these was an example of bilateral hermaphroditism, while the others were of the mixed type.

Ovogenesis

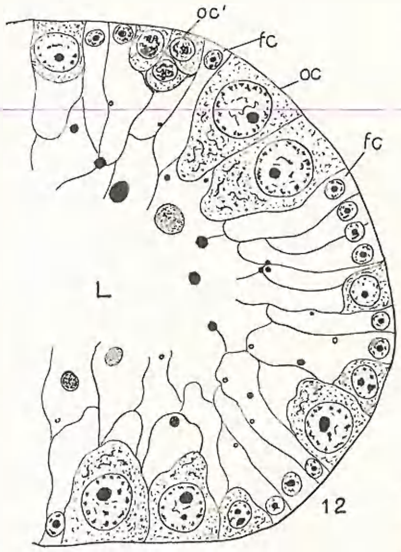
The primary gonad is transformed into an ovary by the growth of the ovocytes and the formation of a central lumen by the separation of the vacuolated follicular cells (figs. 12, 13). At first only a few of the many young ovocytes continue their growth as definitive ova (fig. 14), the number evidently depending on the abundance of nutrition, for there is a direct correlation between the size of the young female and the number of ova produced.

On reaching full size (0.069 to 0.073 mm.) in April or May the ova remain for a time attached by slender stalks to the walls of the greatly enlarged follicles and then drop off into the lumens.

The first spawning usually occurs in early June in the vicinity of New Haven, but may take place earlier in seasons or situations where the water reaches a suitable temperature in May (Mead, '00; Mead and Barnes, '03). It seems probable that some females may spawn several times during the summer, for many large ova may be found in the gonads after partial spawning. Many small ovocytes as well as residual gonia remain after spawning as antecedents of the gametes to be formed preparatory to the next breeding season. Unspawned ripe or nearly ripe ova undergo cytolysis (fig. 15).

SPERMATOGENESIS

This species differs from most other pelecypods in having two distinct types of spermatogenic transformations. One of these is similar to that described for other forms (Coe, '32), while the other method is highly atypical and reminiscent of the aberrant processes which occur in some of the gastropods, lepidoptera and other groups.



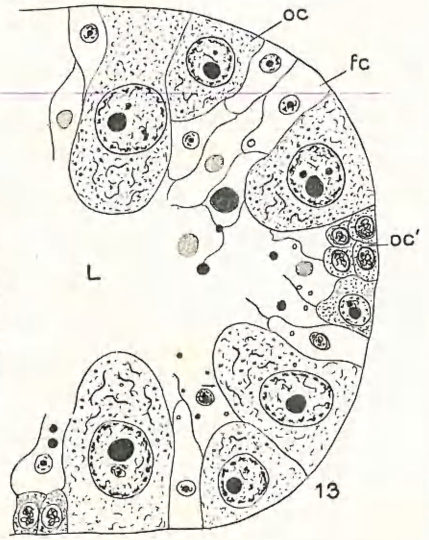
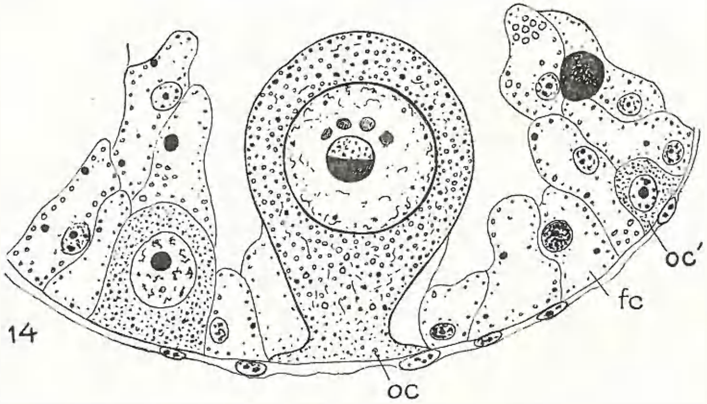


Fig. 12 Portion of ovary of young clam 28 mm. in length, showing growth of ovocytes (oc) and smaller ovocytes (oc') in synaptic phase; a lumen (L) is forming in the central axis between the follicle cells; inclusions larger. January.

Fig. 13 Portion of ovary with disintegrating follicle cells and large central lumen. Same magnification and lettering as in figures 7, 8, 10 to 12. March.

In the typical method the spermatogonia along the walls of the solid gonad begin proliferation as soon as sexual differentiation becomes apparent in young animals and in early autumn in older individuals. These proliferating groups of spermatogonia force their way between the follicle cells and begin migration toward the center of the alveolus. As the spermatocytes and spermatids approach the center a central lumen is formed and in this space spermatogenesis is completed (fig. 16).



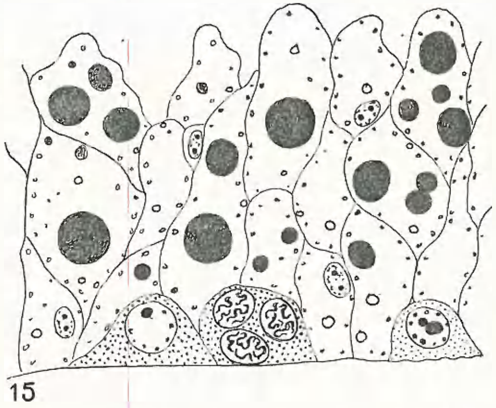


Fig. 14 Portion of ovary with large lumen, showing one half-grown ovum (oc) and two smaller ovocytes (oc′); disintegrating follicle cells have large inclusions. Same magnification as preceding figures. April.

Fig. 15 Portion of ovary soon after spawning; residual gonia and young ovocytes in spireme stage on wall; follicle cells with large inclusions. Same magnification as in preceding figures. September.

In well-nourished individuals nearly all the follicle cells are eventually eliminated and the multitudes of spermatogenic cells fill the entire alveolus (fig. 17). The mature spermatozoa are usually retained until all the active cells of the entire spermary have completed their transformations. Each alveolus is then filled with irregular radial columns of spermatozoa with their tails occupying the interradial spaces and filling the central lumen. Before the sperm are discharged a complete layer of follicle cells is built up along the periphery, with scattered residual gonia along the connective tissue wall (fig. 18).

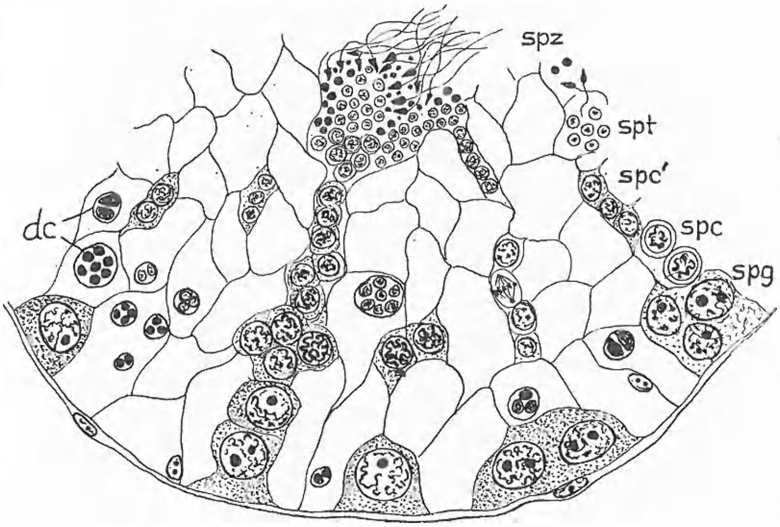


Fig. 16 Portion of spermary of poorly nourished individual with a shell length of only 10 mm. Slender radial columns of spermatogenic cells extend between the vacuolated follicle cells from periphery to the central lumen which contains a small number of spermatozoa (spz); follicle cells enclose several atypical cells (dc); spt, spermatids; spc, spc', primary and secondary spermatocytes; spg, spermatogonia. May.

In animals that are less well nourished, particularly in young and dwarfed animals, relatively few spermatogonia are formed and these remain separated by numerous follicle cells. The radial groups of spermatogenic cells are much more widely separated than those shown in figure 16 and only a few spermatozoa are produced. The alveoli are of about normal size, however, but are few in number. In other cases only a few of the alveoli participate in spermatogenesis in the early part of the season, the larger portion of the gonad remaining more or less inactive until after the first spawning. More extensive activation and a second spawning presumably follows if nutritive conditions later become favorable.

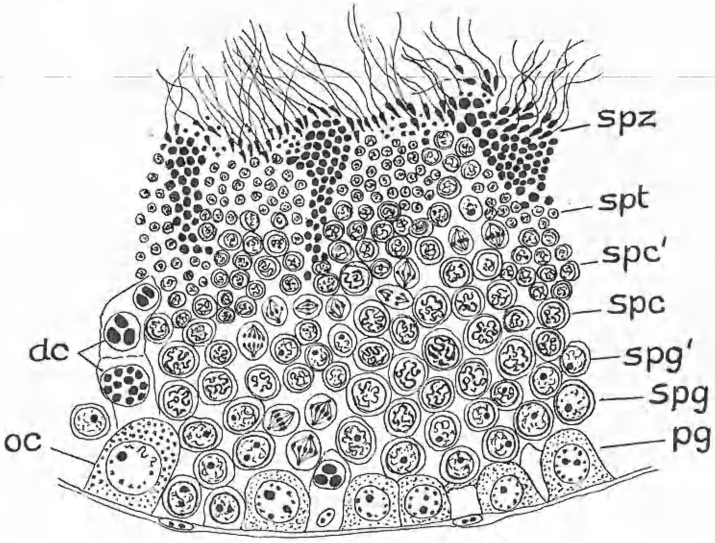
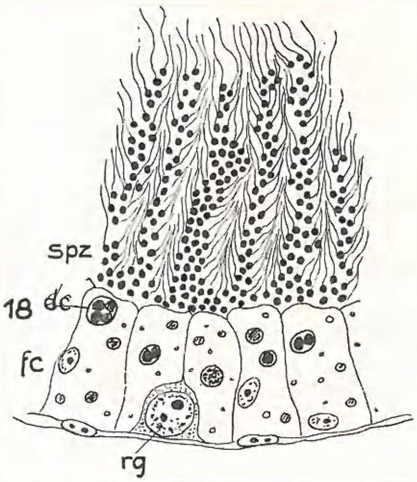


Fig. 17 Portion of spermary of well-nourished young individual, showing the crowded spermatogenic cells, with the tails of spermatozoa projecting into the central lumen of the gonad; pg, undifferentiated gonia; spg, spg', primary and secondary spermatogonia; dc, degenerating atypical cells; oc, ovocyte-like cell. Other letters as in figure 16. May.

The initial phases of spermatogenesis usually begin in the autumn or early winter, although young hatched in late summer will not commence the process until the following spring. A small proportion of the spermatozoa will be mature as early as February in the vicinity of New Haven but these will be retained in the gonads until all the active spermatogonia have completed their transformation.

Because of the differences in the time of spawning of its various members the breeding season of the population as a whole extends over the entire summer. These differences are presumably due not only to individual variations in the initial spawning period of the season but also to second spawnings by some of the males that have produced only a small amount of sperm earlier in the season. Under extremely unfavorable conditions some individuals fail to produce any normal spermatozoa at the usual time; all the active cells undergo atypical and degenerative transformations.



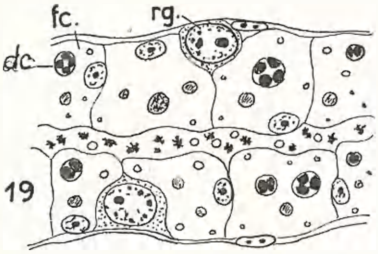


Fig. 18 Portion of spermary after completion of spermatogenesis, showing the slender radial columns of spermatozoa (spz) with tails between the columns and projecting into the lumen. A single residual gonium (rg) lies on the periphery between the basal follicle cells (fc); dc, atypical cells. May.

Fig. 19 A similar portion of the collapsed alveolus after the discharge of the spermatozoa; debris is scattered in the narrow lumen and two residual gonia (rg) are shown; other letters as in figure 18.

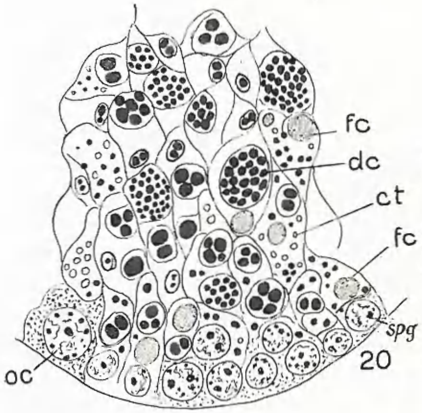
Immediately after spawning the alveoli of the gonads of some of the well-nourished males will have only a narrow lumen with a single layer of follicle cells and scattered residual gonia (fig. 19). Other individuals will have retained more numerous follicle cells with more or less extensive accumulations of degenerative products from atypical or abnormal meiosis (fig. 22).

Atypical spermatogenesis. This aberrant mode of meiosis usually begins before the onset of typical spermatogenesis in young males and shortly after spawning in older individuals (fig. 22). Vast numbers of these abnormal cells are often produced before the first normal spermatocytes appear. They are evidently derived from the same spermatogonia as the others, but their behavior is quite different. In the atypical spermatocyte the cytoplasm surrounding the nucleus involved withdraws from the loosely syncytial mass and becomes rounded into a sharply outlined cell. The nucleus later divides but the contour of the cell remains smooth and the homogeneous cytoplasm stains intensely with acid dyes.

Successive nuclear divisions may follow, producing four, eight or sixteen smaller nuclei, all of which may sometimes have a normal appearance. More often, however, pycnotic changes appear in one or more of the constituent nuclei and frequently all are pycnotic (fig. 17). In other cases division is checked after two or three nuclei are formed. Each of these nuclei, surrounded by a layer of clear cytoplasm may then separate from the others and undergo further divisions or it may become pycnotic and later cytolyzed. These atypical cells and their degenerative derivatives pass through the broad openings into the follicle cells and occupy the central vacuoles. By the time that true spermatogenesis commences they may have become very numerous (figs. 20 to 22).

The definitive stages in this atypical method of meiosis are not usually reached until normal spermatogenesis has progressed as far as the formation of secondary spermatocytes or spermatids. At that time such multinucleate cells, or cysts, as have not become pycnotic or cytolyzed are found to contain from two to sixteen or more spermatids. Some of them later transform into spermatozoa with typical basal bodies but it is not certain whether any of them eventually become functional.

In some of the young males only a few of these atypical cells are formed before or during spermatogenesis but if the individual is kept for a few weeks under unfavorable conditions the ratio of atypical to normal cells is usually greatly



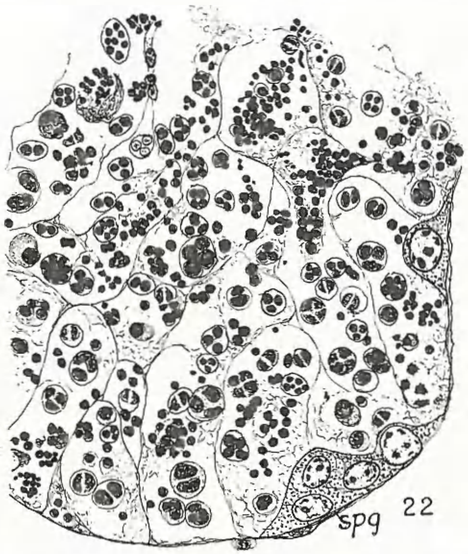


Fig. 20 Portion of gonad of adult male; winter condition with follicle cells (fc) crowded with atypical cell inclusions (ct, dc); spermatogonia (spg) have begun to multiply; oc, ovocyte-like cell.

Fig. 21 A more advanced stage in the disintegration of follicle cells and accumulation of atypical cell inclusions.

Fig. 22 Portion of spermary of clam a few weeks after spawning, showing follicle cells crowded with inclusions. September.

increased. They were found in great abundance in every one of the several hundred adult males from various localities along the coast when examined during the autumn and winter but most of them become cytolyzed during active spermatogenesis in early spring. They appear again as soon as normal spermatogenesis has been completed.

FOLLICLE CELL INCLUSIONS

It was noted above that the mesenchymatous tissue which in most species of bivalves serves for the accumulation and storage of reserve nutritive substances is practically lacking in Mya. As a compensation the follicle cells of the gonads function in this capacity, accumulating large amounts of material in the form of characteristic cell inclusions ready for utilization during the period of most rapid gametogenesis.

Female. In the female these inclusions consist of small globules of lipoid nature and larger globules of albuminous composition, the material of which is derived in part from the cytoplasmic activities of the follicle cells and in part from the cytolysis of degenerating ovocytes. They first become visible in the cells of the young clam at about the time of sexual differentiation and continue to increase in abundance during the growth of the young ova (figs. 11, 12). The plump and turgid aspect of the visceral mass during the autumn and winter in all sexually mature females is largely due to these inclusions.

The proportion of degenerative ova is largely dependent upon nutritive conditions but is always considerable (figs. 12 to 15, 23). As the ova approach maturity the follicle cells near the center of each alveolus undergo cytolysis and their inclusions become available for the nourishment of the rapidly growing ova. A continuous lumen is thus established throughout the entire alveolar system, including the terminal genital ducts. Eventually only a few of the follicle cells remain in well-nourished individuals which produce many ova, but in cases of malnutrition there are many remaining follicle cells and but few ova.

The alveolus collapses after spawning but the lumen is soon filled by the ingrowth of new follicle cells and the turgidity of the whole alveolar system is restored. Degenerative inclusions are always present (figs. 15, 23).

Male. The follicle cells of the males, as noted in a preceding section, always contain nutrient inclusions derived in part from the cytoplasmic activity of the cells themselves but more especially, except in very young males, from the cytolysis of the derivatives of atypical spermatogenesis (figs. 20 to 23). These reserve nutrients are utilized at the time of most rapid spermatogenesis.

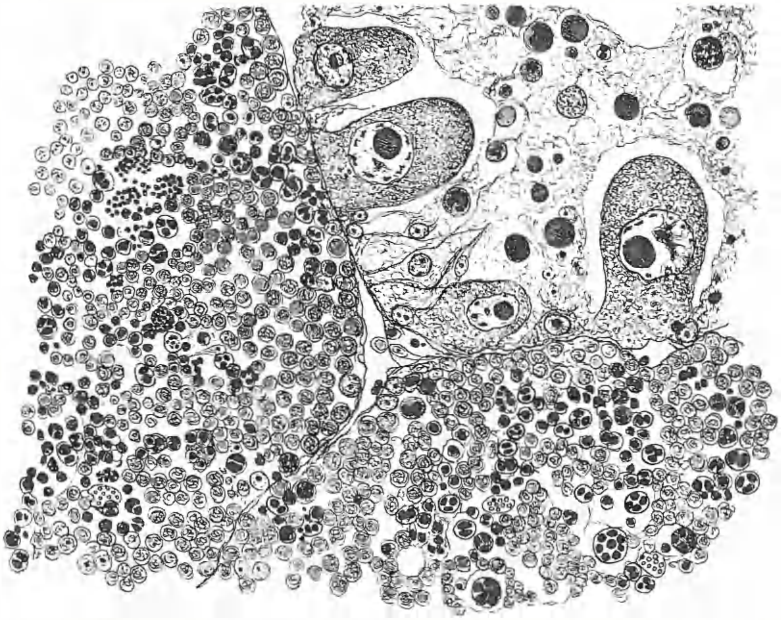


Fig. 23 Portion of gonads of bilateral hermaphrodite, indicating the characteristic inclusions in male and female (upper right in figure) alveoli. May.

SPAWNING

In the vicinity of New Haven most of the young clams are ready for their first spawning in the early summer, when they are about 1 year of age. It seems quite probable, however, that some individuals in favorable situations may spawn late in their first summer, when they are only about 4 months old. The gonads of small, thin-shelled individuals are sometimes fully ripe or recently spawned at that time, but proof of age has not been satisfactory. Many young clams, originating from the last set of the previous season, have remained over winter with a shell length of 10 mm. or less and these are not easily distinguished during the latter part of the following summer from individuals only 4 months of age. The evidence indicates that the clam resembles the oyster (Ostrea virginica) in respect to age at spawning, for the age of the latter at the first spawning period has been shown to vary from 4 months to 2 years, depending both on the individual and on the locality (Coe, '36 a).

The process of spawning in both sexes has been observed in the laboratory when the fully ripe individuals have been transferred from the refrigerator to water having a temperature of 20° to 25°C. The gametes are forced from the siphon in rhythmical jets. Mead and Barnes ('03) report success in rearing young clams from artificially inseminated ova but neither Belding ('16) nor ourselves were able to obtain normal development through the later stages except from those ova which had passed through the oviducts.

Rate of growth; dwarfs

The observations of Mead ('00) as well as of Mead and Barnes ('03) have demonstrated that growth may be so very rapid under favorable conditions that the shell of the young clam may reach a length of 10 to 15 mm. in July and 40 to 48 mm. by the end of September of its first year. The rate is retarded by the lower temperature of autumn and winter and by the formation of sexual products in the spring. Hence only relatively few individuals exceed 50 mm. in length during the spawning season when about 1 year of age. A length of 60 to 80 mm. may be reached when about 2 years old, but in later life the increment in size is relatively slow. An extreme length of 143 mm. has been reported for an old individual.

For most individuals, however, the growth rate is much less and some are only 6 to 8 mm. long at the end of their first year. In spite of this dwarfed condition a few gametes may be formed at the usual spawning season. The gonadal alveoli of these dwarfs are nearly as large as in well-nourished individuals but the gonia are so widely scattered that only a few of the sections contain even a single germ cell. Mead ('00) has shown that these dwarfs retain the capacity for rapid growth when placed under favorable conditions. On the more northern portions of the coast the rate of growth is much less, as Newcombe ('35) found in the Bay of Fundy, while the higher temperature and longer growing season to the southward favor a more rapid increase in size (Belding, '16).

SUMMARY

1. The primary gonads of the soft-shell clam (Mya arenaria) differ from those described for other pelecypods in having solid, cylindrical, profusely branching alveoli filled with large, vacuolated follicle cells and with widely scattered gonia on the periphery.

2. Each member of the pair of gonadal primordia consists of a cytoplasmic syncytium, in the position of the future genital opening, with nuclei differentiated into the two types which are characteristic of the germinal and the follicle cells. The smaller nuclei are incorporated into the large, vacuolated follicle cells, while the larger nuclei belong to the primary gonia.

3. Each gonad extends anteriorly and ventrally into a racemose system by root-like outgrowths, with syncytial caps of proliferating and differentiating cells, the primary gonia being scattered along the periphery of the cylindrical columns of follicle cells as growth proceeds.

4. Following sexual differentiation, characteristic inclusions are accumulated within the follicle cells and between them in both sexes. Those in the females consist of deeply staining globules of various sizes arising in part from cytoplasmic secretions and in part from the disintegration of ovocytes.

Those in the males may be followed through successive stages of division into 16 to 32 or more parts and evidently represent successive phases in the meiosis of atypical spermatocytes. Most of them usually become cytolyzed before or during normal gametogenesis, but others become differentiated into atypical spermatozoa.

5. A central lumen is formed in each alveolus during gametogenesis and most of the follicle cells are eliminated by the encroachment of gametes in well-nourished individuals but others are restored immediately after spawning. In poorly nourished individuals producing few gametes many of the follicle cells and their inclusions are retained.

6. The species is normally unisexual, with approximate equality in the sex ratios or with a slight excess of males during the first year. Functional hermaphrodites constitute less than ½ of 1% of the population in the vicinity of New Haven. No evidence of a change of sex has been observed.

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