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Morphogenesis of Accessory Reproductive Organs in Male Goldfish, *Carassius auratus*

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Abstract

In goldfish, *Carassius auratus*, the sperm duct lying along the testicular hilus is not a simple duct but a cephalo-caudal succession of complicatedly anastomosing cavities which are homologous to the seminal lobules of the testis. During the initial period of spermatogenesis, some seminal lobules existing in the testicular hilus become almost entirely devoid of germ cells and joined to one another, taking an appearance of a convoluted duct containing spermatozoa. No prominent accumulation of stroma cells is observed in the testicular hilus in any stage of the testicular development. A different process in the construction of cavities, which is the same as that of the ovarian lumen, also occurs during the course of the testicular development, but the resulting cavities are vestigial in structure and function. Epithelial cells lining the vestigial cavities can react to exogenous estrogen by displaying columnar hypertrophy and ciliation just like those of the ovarian lumen affected by steroid hormones.

The posterior part of goldfish testes forms an inconspicuous structure composed of branching lumina on the dorsal side of the intracoelomic common sperm duct. The structure as well as the common sperm duct arises from solid genital ridges near the point of their fusion, and is evidently lacking in germ cells from the beginning of its morphogenesis. Morphological characteristics of the structure fairly correspond to those of the seminal vesicles of other fishes, though the organ of the goldfish seems to serve essentially as a sperm reservoir but not as a glandular organ.

Whereas comparatively many studies have been carried out on the sexual differentiation of germinal elements of the gonad in teleost fishes1)2), quite a few work has so far been concerned with the morphogenesis of male accessory reproductive organs such as the sperm duct and the seminal vesicle. Although the organs are rather simple in anatomical and embryological senses as compared with those of higher vertebrates, they seem to display an interesting variety in their structure and function as briefly reviewed by Forbes3) and Hoar4). This has made us recognize the need of further studies to ascertain the view offered by Egami and Arai5) that the variety of the organs has a relation to the reproductive habits of the fishes to be examined. Furthermore, it is considered that the work on an embryological basis may facilitate to elucidate the nature of the variety of the accessory reproductive organs in teleost fishes.

In a previous report6), we described the process of the formation of the

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ovarian lumen in the goldfish, *Carassius auratus*, and explained the behaviour of epithelial cells lining the lumen of adult ovaries by embryological and experimental approaches. In this paper, the process of the formation of the sperm duct, together with the occurrence of a seminal vesicle-like structure, in the testis of the goldfish will be described.

Before going further, we wish to express our sincere thanks to Professor Kiichiro Yamamoto, Hokkaido University, for his kind advice and critical reading of the manuscript.

Material and Methods

Each of the observations made in the present study was done employing fry and juvenile goldfish, *Carassius auratus*, of separate spawns obtained in the laboratory. Several broods of the fish were raised in indoor glass aquaria with filtered and aerated water of 20–23°C, while others were cultured in outdoor plastic tanks under natural water temperature, different spawns being kept in their respective aquaria or tanks under a natural condition of light in both cases. They were mainly fed on powdered diet of a commercial assorted feed for fish culture (Nihon Haigō Jiryō Co.), sometimes being supplemented with small aquatic animals such as branchiopods especially in the cases of outdoor rearing. The rearing water was refreshed whenever it showed signs of injurious pollution. Quite similar results were obtained from the two different methods of rearing at least in regard to the essential process of differentiation and development of the organs to be studied.

The fish totalling about 180, usually 6 to 10 fish at a time, were fixed with Bouin's fluid or Heidenhain's Susa at varying intervals during the period from the 9th to the 130th day after hatching. The desired part of the body with the urogenital system *in situ* was taken out, embedded in paraffin, sectioned serially at 8–10 μ in thickness, and examined after the staining of the sections with Delafield's hematoxylin and eosin or with Mallory's triple stain.

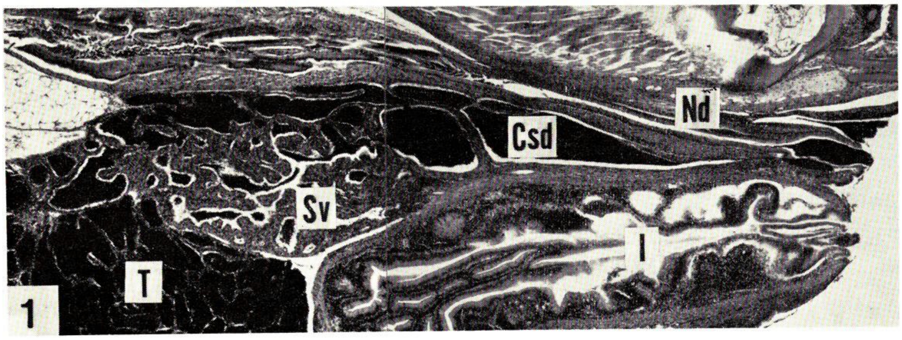
In parallel with the above observations, adult goldfish of various maturational stages were examined to get precise knowledge concerning the anatomical and histological characteristics of the genital system. Moreover, an additional result of the observations was secured by estrogen administration to 12 young males of 56.4 mm and 7.2 g in average body length and body weight at the start. Being kept in an aquarium under water temperature of 20–23°C and natural light, they were fed on pelleted diet containing ethinylestradiol (50 μg/g diet) daily for 16 and 30 days. On the day after the last feeding of the hormonal diet, they were sacrificed by decapitation, their testes which remained attached to the coelomic wall were preserved in Bouin's fluid, and examined microscopically by similar histological procedures to those described above.

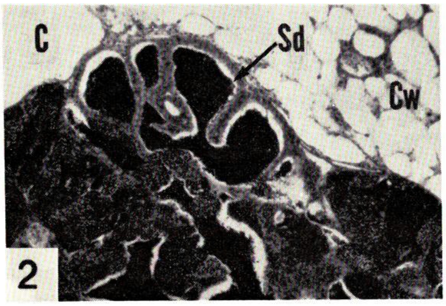
Observations

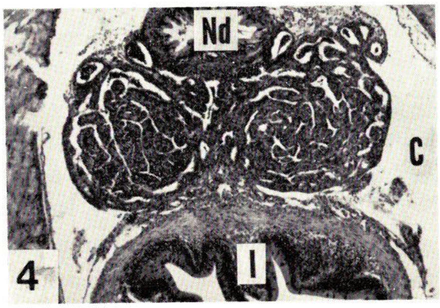
In the testis of adult goldfish, mature spermatozoa are found filling up the lumina of seminal lobules which appear to display a slight gradient of spermatogenetic activity in the direction from the periphery to the center or from the distal region to the proximal one of the testis. The lobule lumina, which show anastomosis with one another, are eventually confluent with the sperm duct lying along the proximal edge of the testis (Fig. 1). The sperm duct is not a simple duct but rather a cephalo-caudal succession of cavities which are joined complicatedly to one another as long as it runs within the testis, and opens into a single common sperm duct, or vas deferens, at the caudal end of the testicular region. The epithelial lining of the sperm duct consists of a layer of flat cells without any peculiar histological features. Not rarely, however, there can be detected several germ cell elements, which are mostly in the resting spermatogonial stage but are sometimes in the form of spermatocyte cyst, on the wall of the duct (Fig. 2).

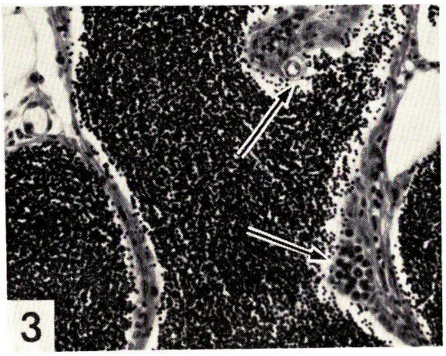
In mature testes, the lumina of the sperm duct, and of the seminal lobules as well, are generally packed with a large amount of mature spermatozoa and appear to serve not merely as a sperm-transporting system but also as a storehouse of the sperm that has accumulated prior to its efficient shedding. Moreover, the testes come to unite to each other at their caudal end to form a single dorso-median structure which is characterized by a network appearance of narrow lumina (Figs. 3 and 4). The lumina are connected to one another and proximally to the sperm duct, containing a variable amount of spermatozoa according to the degree of testicular maturation. As shown in Fig. 5, the epithelial layer of the lumen is flat or at most of low cuboidal form being 11–12 μ in height, and is provided with no germinal elements except in the anterior region of the structure where the sterile epithelium is gradually replaced by the germinal epithelium of the seminal lobules chiefly in the periphery. The structure bears a morphological resemblance to the so-called seminal vesicles of other teleosts, but it is quite small in size, being about 2–2.5 mm in length and about 0.6–0.8 mm in height in maturing males of about 45 mm in mean body length. While no evident sign of secretory function is observable in the epithelial cells in any time of the reproductive cycle, the structure may play a supplemental role in maintaining mature spermatozoa until the time of their discharge.

As reported previously$^{6)}$, a differentiation of ovaries in the goldfish becomes recognizable by 25 to 30 days after hatching. Thenceforth the ovaries continue their development to make many auxocytes appear by 50 days of age. On the other hand, the gonad of genetic males remains rather quiescent in its development during that period of ovarian differentiation, though a spermatogonial multiplication appears to proceed through the dilatory continuation of mitoses. There is a striking variation in the time course of farther testicular development in









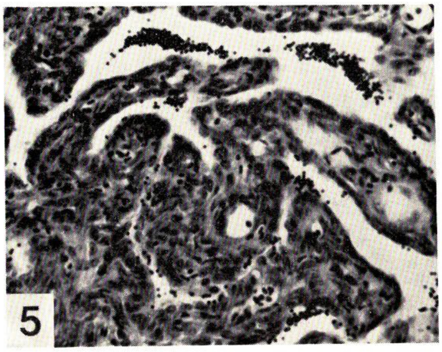


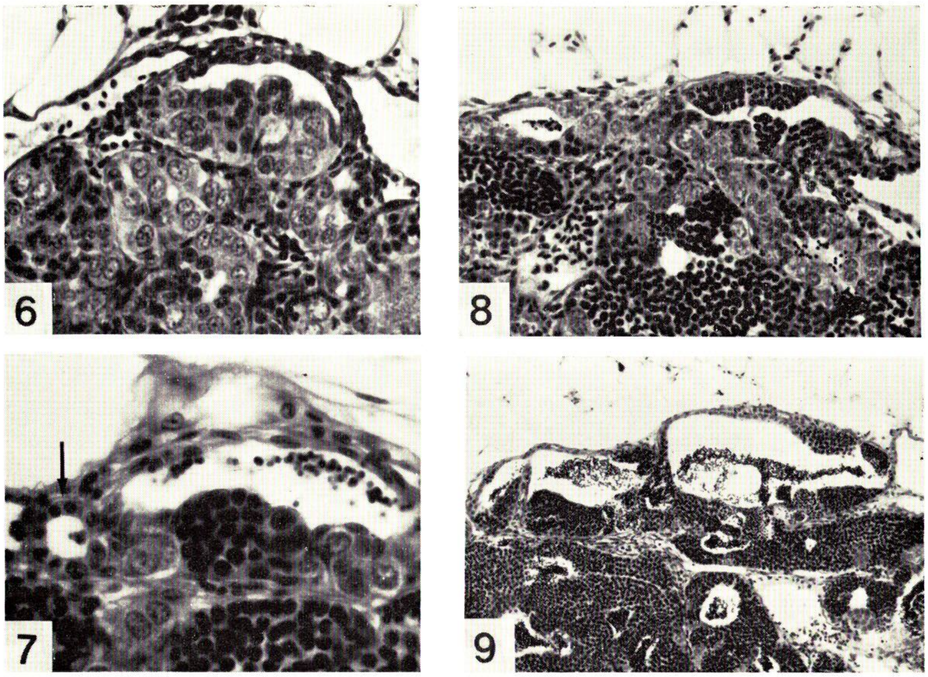
Fig. 1. Median sagittal section through posterior region of the trunk of a male goldfish, showing the arrangement of genital organs and their associated structures. *Csd*, common sperm duct; *I*, intestine; *Nd*, nephric duct; *Sv*, seminal vesicle homologue; *T*, germinal portion of testis. ×35.

Fig. 2. Frontal section through hilar region of maturing testis, indicating the presence of anastomosing lumina, the sperm duct (*Sd*), packed with sperm. *C*, coelom; *Cw*, dorsal coelomic wall. ×65.

Fig. 3. A part of the intratesticular sperm duct. Note germinal elements (arrows) surviving on the wall lining the duct. ×245.

Fig. 4. Frontal section through the seminal vesicle homologue posterior to the germinal portion of testis. *C*, coelom; *I*, intestine; *Nd*, nephric duct. ×50.

Fig. 5. A part of the seminal vesicle homologue, revealing the epithelium of lumina of the organ. ×245.



Figs. 6–9. Frontal sections through testes of juvenile goldfish, revealing sperm ducts in the process of differentiation in the testicular hilus. In Fig. 7, the occurrence of an atypical, vestigial cavity is shown by arrow. Age after hatching and body length of the fish examined: Fig. 6, 100 days, 40 mm; Fig. 7, 81 days, 39 mm; Fig. 8, 83 days, 41 mm; Fig. 9, 81 days, 42 mm. Fig. 6, ×320; Fig. 7, ×470; Fig. 8, ×235; Fig. 9, ×110.

different specimens, depending partly on environmental influences such as water temperature and population density, and partly on the differences of spawns to which the examined fish belong. Generally speaking, however, after the period of 50 to 60 days after hatching the testes become gradually enlarged in size with the development of spermatogonial cysts following an active mitosis.

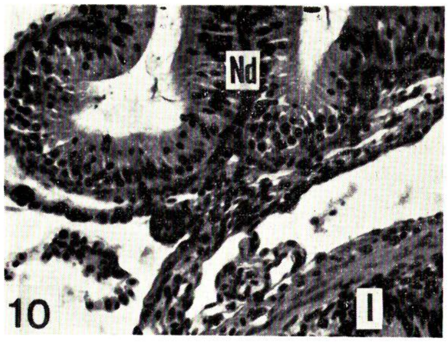
By that time testicular stroma cells are found permeating among germ cell cysts and through the peripheral region of the testis without forming special clusters or arrangement of the cells even in the testicular hilus. By 80 to 85 days after hatching, and in the fish of about 30 mm in body length, testes start spermatogenesis resulting in the occurrence of spermatocyte cysts along the wall of seminal lobule anlages which are now provided with narrow cavities in the center. A few of the lobule anlages come to be located in the testicular hilus in contact with the adipose tissue of the coelomic wall (Fig. 6). They include spermatogonia and spermatogenetic cysts enclosing small central cavities as in ordinary seminal lobules, though on some occasions the germ cells tend to lie prevailingly on the ventral wall of the hilar lobules (Fig. 7).

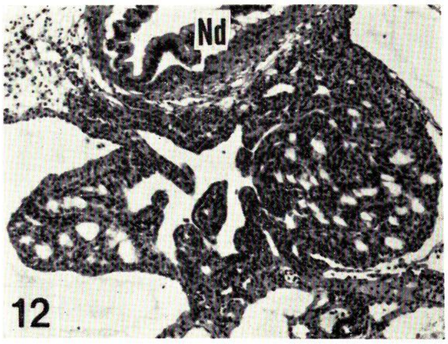
As spermatogenesis proceeds, the hilar lobules are expanded progressively to have larger lumina (Fig. 8), and become to exhibit complicated anastomosis with neighbouring lobules of cranial, caudal and ventral sides, thus having the appearance of the sperm duct of adult testes. Concurrently with these changes, germ cells of the hilar lobules are rapidly developed into mature spermatozoa which come to be aggregated in the lumina. Most of the germ cells eventually disappear from the hilar lobule leaving only a flat epithelial layer behind on the wall (Fig. 9), whereas those of the ordinary seminal lobules are seen to remain as single or clustered spermatogonia and as spermatogenetic cysts of various stages on the lobule wall throughout the breeding cycle. These facts may therefore prove evidence that the sperm duct of goldfish testis is homologous to seminal lobules of the testis.

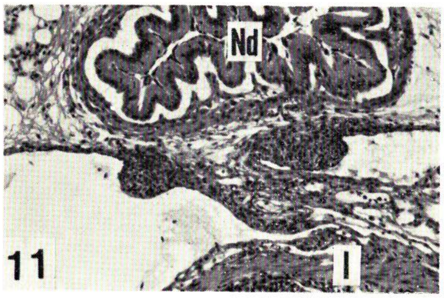
The development of the gonad of the goldfish generally begins at the anterior region and proceeds posteriorly in succession. During an initial period of testicular development, the posterior end of paired testicular ridges, which run bilaterally on the dorsal coelomic wall at some distance from the mesentery, does not reach the caudal limit of the body cavity. By 60 to 70 days after hatching, when the fish become to be about 20 mm in body length and their testes are still small in size with a small number of spermatogonia, the posterior ends of the testicular ridges arrive at the caudal end of the body cavity without having any germinal elements in them. As the ridges gradually converge in the caudal direction to the dorsal mesentery, their posterior ends are found to exist on both sides of the dorsal terminal of the mesentery near the point of union of the bilateral mesonephric ducts (Fig. 10).

During the successive period of testicular development, the caudalmost ridges of the testis are also increased in size but are still of solid structure without any germ cells (Fig. 11). Subsequently, in parallel with the initiation of spermatogenesis in the testis proper, they become to be furnished internally with many small cavities (Fig. 12). Concurrently they come to be merged into a single median structure, thus assuming the histological characteristics of the seminal vesicle homologue of adult goldfish testis. It is remarked that the organ is always lacking in any germinal elements as a structural component in any time during and after its morphogenesis.

Later on, the cavities in the sterile testicular ridge are developed to be connected to one another and antero-proximally to those of the seminal lobules in the testicular hilus which are differentiating into the sperm duct as described before. The sperm duct thus passes through the proximal region of the seminal vesicle homologue as a median luminal system and farther extends caudalwards as a single common sperm duct to open into the outside of the body.

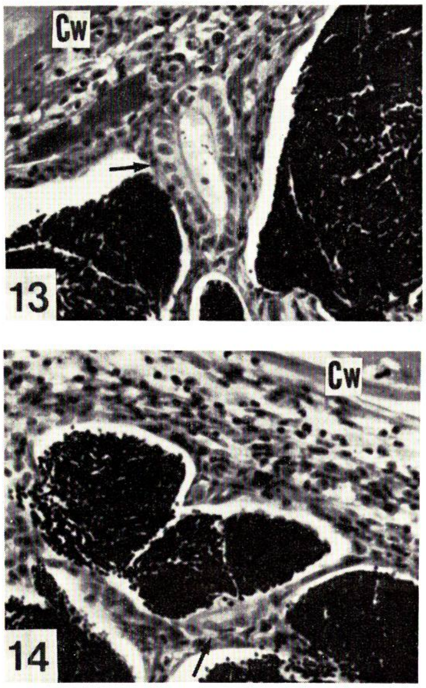


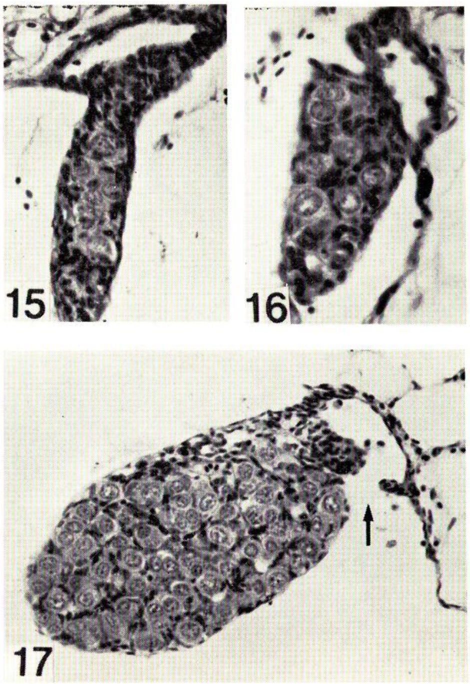




Figs. 10–12. Frontal sections through caudalmost portions of testicular ridges developing into the seminal vesicle homologue (Fig. 12) in juvenile goldfish. *I*, intestine; *Nd*, nephric duct. Age after hatching and body length of the fish examined: Fig. 10, 54 days, 20 mm; Fig. 11, 70 days, 24 mm; Fig. 12, 83 days, 41 mm. Fig. 10, ×245; Figs. 11 and 12, ×110.

On the other hand, careful examination of histological sections of maturing testes of the goldfish can detect in some specimens the presence of small discontinuous cavities, which are lined by a cell layer of exclusively somatic nature, in the testicular hilus (Fig. 7). Sometimes the lining cells of the atypical cavities are cuboidal to low columnar in feature. In young male goldfish administered with an estrogen, ethinylestradiol (50 μg/g diet), an occurrence of peculiar cavities was noticed in the hilar region of affected testes in most of the treated fish (Figs. 13 and 14). Judging from their location in the testicular hilus and their constituent cells of entirely somatic nature, the peculiar cavities surely correspond to those seen in normal testes mentioned above. Although being various in shape and size even in a single specimen, the cavities were commonly characterized by having a columnar epithelium with distinct cilia in a part or in the whole of the lining. Such a change of epithelial cells in response to the administered estrogen was in no case detectable on the wall of seminal lobules. The experimental results may denote the possibility that the epithelial lining of the peculiar cavities may originate at least in part in the coelomic wall, since the columnar hypertrophy with ciliation of the cells is a characteristic response to steroid hormones of the dorsal coelomic epithelium associated with the gonad in juvenile goldfish[6]. Other notable features of the peculiar cavities were that the cavities occurred rather intermittently, and even locally in some cases, along the length of the testis, and





Figs. 13–14. Frontal sections through the hilar region of maturing testes of goldfish administered with ethinylestradiol for 30 days. Arrows exhibit atypical, vestigial cavities with hypertrophied and ciliated epithelial cells. *Cw*, dorsal coelomic wall. ×310.

Figs. 15–17. Frontal sections through testes of juvenile goldfish, indicating the occurrence of atypical cavities, which are homologous to ovarian lumen, in the proximal region. Fig. 17 shows incomplete connection of tissues between testicular and coelomic wall (arrow). Age after hatching and body length of the fish examined: Fig. 15, 105 days, 25 mm; Fig. 16, 75 days, 23 mm; Fig. 17, 100 days, 28 mm. Fig. 15, ×250; Fig. 16, ×350; Fig. 17, ×230.

that they were either empty in some cases or contained aggregated spermatozoa in others taking an aspect of a part of the sperm duct.

In the goldfish, the ovarian lumen is formed as a result of the union of the distal edge of the ovary with the lateral coelomic wall, enclosing a part of the body cavity along its dorsal border[6]. At some time during the early phase of testicular development, the testis also tends to display a partial connection of tissues between the dorsolateral edge of the testis and the dorsal coelomic wall at a little distance from the mesorchium (Figs. 15–17). This leads to the formation of a cavity, which is lined at least in part with the epithelial cells proper to the coelomic wall just as in the case of the ovarian lumen, in the testicular hilus.

In the present study, the formation of the cavity was distinctly noticed 75 to 90 days after hatching, a little later than the period of the appearance of the ovarian lumen in female fish, in some of the specimens of 23 to 28 mm in body length and with underdeveloped testes. These fish were considerably delayed in their body growth and associated testicular development in comparison with their age. The delayed testicular development seems to have made it easy to disclose the formation of the peculiar cavities. The cavity does not occur continuously but rather locally along the whole length of the testis, being wider in some places and quite narrower in others. On some occasions the formation of the cavity is incomplete, as indicated in Fig. 17. Along with further development of the testis the cavity becomes to be confined within the testicular hilus as a vestigial structure which is often hard to be detected. These characters of the atypical cavity appear to correspond to those seen in adult testes described before, suggesting that the cavity in question may survive as an atypical cavity or lobule existing in the hilus of adult testes of the goldfish.

Discussion

It is widely known that, unlike the sperm-transporting system of most vertebrate animals, that of teleost fishes has no anatomical relationship to the nephric duct, or Wolffian duct4). During the course of testicular morphogenesis, somatic cells deriving from the coelomic wall participate in the construction of the teleostean sperm duct. The duct posterior to the germinal part of the testis generally appears as a slit within the sterile gonadal ridge or fold. A corresponding pattern of the formation of the duct may be followed also by the sperm duct existing in the germinal portion of the testis. The sperm duct typical of the case can be noticed in the testis of cyprinodonts such as *Xiphophorus helleri*7) and *Lebistes reticulatus*8)9) in which the duct makes its first appearance as a narrow cavity within a prominent mass of stroma cells accumulated in the testicular hilus. Such is also the case in *Cottus bairdii*10) and possibly in *Tilapia mossambica* (unpublished observation).

In these teleost fishes mentioned above, the sperm duct is differentiated through the process clearly different from the formation of the ovarian lumen. However, the sperm duct, or testocoel according to Johnston11), of *Micropterus salmoides salmoides* is formed by the separation of the lateral epithelial layer from the germinal one of the gonad during the sexually indifferent stage, the same way as the ovarian lumen or ovocoel.

Although a sexual differentiation of the gonad of goldfish was already reported by Stromsten12), he did not make any mention of the formation of the sperm duct during the course of testicular development. As the present study reveals, it undoubtedly admits that the intratesticular sperm duct of the goldfish is nothing but the seminal lobules which have become deprived of germ cells as a result of their promoted and complete development into spermatozoa. The seminal lobules destined for the future sperm duct are those lying along the testicular hilus and forming a series of cavities, to which open the fertile seminal lobules, by complicated anastomoses with one another. There is no conspicuous accumulation of stroma cells in the testicular hilus of the goldfish, in contrast to the cases of other fishes cited before. Perhaps this does not necessarily mean the fundamental difference of testicular morphogenesis but possibly reflects the difference in the distribution pattern of stroma cells of the testis between the two groups of fishes, since the testicular stroma tissue can be differentiated into a luminal structure regardless of the absence of germ cells as explained later.

It is interesting to note that the process similar to that of the formation of the ovarian lumen is observable at least locally in the testis during its early development, in the sense that this may imply the residue of female characteristics appearing in the course of testicular development. Actually the resulting cavities appear to be vestigial in structure and function in adult testis, though it remains uncertain whether or not they may take a supplemental but unavoidable part in the histogenesis of goldfish testes.

So far as we know, no report has hitherto made mention of the existence of a structure with complicatedly anastomosing lumina in the posterior sterile portion of goldfish testes. The structure corresponds evidently in its histological architecture to the so-called seminal vesicles of other teleost fishes. The seminal vesicle of gobies13)14), Indian catfishes15)16), and others is generally a large bilateral outgrowth of the common sperm duct, but that of the goldfish is merely an inconspicuous protrusion along the median ventral wall of the sperm duct. Moreover, the organ of gobiid fishes such as *Gillichthys mirabilis*13)17), *Tridentiger obscurus*14) and *Boleophthalmus chinensis*14), and catfishes such as *Heteropneustes fossilis*15) and *Clarias batrachus*16), and other fishes is regarded as an exocrine organ rather than a sperm storage, whereas that of the goldfish is no doubt serving as a sperm reservoir. A case comparable to that in the goldfish may be searched in a file fish, *Monacanthus cirrhifer*, in which the sperm duct itself has a structure resembling the seminal vesicle18). The "posterior testis" according to Sathyanesan19) of a catfish, *Mystus seenghala*, appears also to be similar in structure and function to the seminal vesicle homologue of the goldfish.

The bilateral sperm duct of a goby, *Pterogobius zonoleucus*, which is furnished with no distinct seminal vesicle, is of a structure characteristic of the seminal vesicle, but is clearly glandular in function14)20). The sperm duct of this species, as well as glandular seminal vesicles of other fishes, show a distention of the lumen as a result of an accumulation of secretory fluid. This situation is in no case observable in the seminal vesicle homologue of the goldfish. In *Gillichthys mirabilis* the main ingredients of secretory product of the seminal vesicle are known to be proteins17). Supposing that the structure in consideration of the goldfish can secret a certain substance for retaining the sperm in good condition, the secretion might be serous in nature. The possibility of the occurrence of such a secretion in the goldfish testis is being investigated in our laboratory.

The present observation on the morphogenetic process of the seminal vesicle homologue of the goldfish may provide an additional support for the homology of the seminal vesicle to the proper testis in teleost fishes. Sathyanesan19) described the "posterior testis" of *Mystus seenghala* as having abandoned its germinal potentiality, and considered that the cells lining the lumina of the organ might be degenerated germ cells. In the goldfish, however, the seminal vesicle homologue is entirely lacking in germ cells from the very beginning of its morphogenesis, except in the anteriormost region transitional to the germinal part of the testis. Such seems to be also the case in the seminal vesicle of *Gillichthys mirabilis*21). Accordingly it may be safe to conclude that the stroma tissue of the testis is capacitated for gaining luminal structure notwithstanding the presence or the absence of germ cells in the genital ridge.

The testis of the goldfish at the stage of functional maturation bears practically as a whole an aspect of a huge sperm reservoir, representing a condition similar to that of the testis of salmonid fishes at complete maturity22). From this point of view, the presence of a small sperm reservoir, the seminal vesicle homologue, in the testis might have no significance to accomplish the reproduction in the goldfish. Sundararaj15) affirmed, "if the testes are large, the seminal vesicles may be absent." Moreover, Egami and Arai5) suggested that the development of the male reproductive organs of teleost fishes might be related particularly to the reproductive habits of the fish. Further studies on various species of fishes are to be made to ascertain the propriety of these explanations on the significance of the male accessory reproductive organs of teleost fishes.

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