

Occurrence of an Accessory Cavity in the Testis of the Topmouth Gudgeon, *Pseudorasbora parva*, and Its Relation to Juvenile Intersexuality

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Abstract The structure of the testis-sperm duct system of the topmouth gudgeon, *Pseudorasbora parva*, was examined histologically. In almost all of the young and adult males examined, a cavity was found to exist between the dorsomedian side of the testis and the opposed peritoneal wall. The testicular cavity was generally flat in shape and ran along the entire length of the testis, with a blind end at the level of the common sperm duct. It appeared that the testicular cavity was not implicated in the transport and storage of spermatozoa in the fish. By studying the process of differentiation and development of gonads in juvenile fish, it was confirmed that some germ cells in differentiating testes underwent oogenesis in many cases, and that the cavity was formed by fusion of the distal edge of the testes with the peritoneal wall in quite the same manner as the ovarian cavity. Thus, the testicular cavity of the topmouth gudgeon is homologous with the ovarian cavity and is apparently a preserved vestige of juvenile intersexuality occurring in this species of cyprinid teleosts.

Juvenile intersexuality appearing in some cyprinid fishes includes, in addition to the occurrence of oogenesis in differentiating testes of males, the modification of stromal somatic tissues of the testes to construct a cavity homologous with the ovarian cavity of females. The cavity appearing in the testis is only vestigial in the goldfish, *Carassius auratus* (Takahashi and Takano, 1971), and is often undetected in adult testes of the rose bitterling, *Rhodeus ocellatus ocellatus* (Shimizu, 1979) and the Sumatra barb, *Barbus tetrazona tetrazona* (Takahashi and Shimizu, 1983). In comparison, the cavity in zebrafish, *Brachydanio rerio*, may be implicated in the sperm-transport system of the testis in adult males (Takahashi, 1977). Thus the phenomenon of a somatic intersexuality is an important consideration regarding the histo-architecture of the testis in some teleost fishes.

The topmouth gudgeon, *Pseudorasbora parva*, is a cyprinid fish commonly found in ponds and lakes of central and western Japan, and also in some places in Hokkaido (Hikita, 1964). The gudgeon is apparently gonochoristic in sexuality, though the occurrence of previtellogenic oocytes in maturing testes has been noted in fish introduced to Rumania (Ionescu-Varo and Grigoriu, 1963). In the course of studies on the reproductive cycle of the gudgeon, the appearance of a

peculiar cavity adjacent to the testis-sperm duct system was noticed in adult males. The testicular cavity, which turned out to be an ovarian cavity homologue originating from juvenile intersexuality in males of the topmouth gudgeon, will be dealt with in the present paper.

Material and methods

Young and adult topmouth gudgeons, *Pseudorasbora parva*, were collected from an earthen pond in a fish-culture farm in Higashi-Onuma, near Hakodate, in 1983 and 1984. A total of more than 100 males were chosen from monthly samplings of the fish for the present study. Maturing and mature males captured in April and June ranged in body length from 60.3 to 74.0 mm (mean 67.4 mm) and in weight from 3.6 to 8.1 g (mean 5.5 g). Young immature fish caught in January measured 43.9 mm in mean body length and weighed less than 2.5 g. The fish were transported to the laboratory, measured under light anesthesia, and preserved *in toto* in Bouin's fluid after having been subjected to a midventral incision which was held open. For histological preparations, the entire coelomic area with the testes in position was excised from juvenile fish, whereas the anterior, middle and posterior portions of testes and adjoining peritoneal wall were removed from adult fish. Serial paraffin sections

of these specimens were cut frontally at 6–8 μm in thickness and were stained with Delafield's hematoxylin and eosin for histological observations.

Several pairs of male and female fish were separately reared in laboratory aquaria with rippled plastic plates as a spawning bed, under natural light and temperature conditions in June and July. Two broods of eggs thus spawned were then transferred to other aquaria with well aerated water at 20°C. After hatching, larvae from the two broods were simultaneously reared in their respective aquaria under natural light, and were fed daily with copepod larvae supplemented by powdered commercial feed for carp. Aquarium water was changed with fresh temperature-controlled water approximately once a week. From the two broods, groups of about 10 fish were sampled at 5 day intervals from day 1 (the day following hatching) to day 53, preserved *in toto* in Bouin's fluid, and processed for histological observations of the gonads in the same manner as described above.

Results

The testis of mature males of the topmouth gudgeon, *Pseudorasbora parva*, was a rather long, bilateral band adhering closely to the dorsolateral wall of the peritoneal cavity. It was a triangular mass in cross sections with the two longer sides reaching the peritoneal wall beneath the ventrolateral side of the swim bladder. Histologically, the testis was mainly made up of numerous seminal lobules and was of the "unrestricted" spermatogonial testis-type (Grier, 1981). A tubular, main sperm duct was absent along the entire length of the testis. Instead, complicated anastomosing lobules, devoid of germ cell elements on their walls, were arranged anteroposteriorly along the dorsal edges of the testis and apparently functioned in collecting and transporting spermatozoa. These sterile lobules may be called the "sperm sinuses" (Fig. 1A). The bilateral testes fused with each other at their caudal extremities to form a single tubular structure in which numerous anastomosing compartments of irregular sizes and shapes were formed by complicated villous projections on the wall (Fig. 1B). The sperm sinuses of the bilateral testes were confluent with these compartments which acted as a sperm reservoir, and led caudally to the common sperm duct which pene-

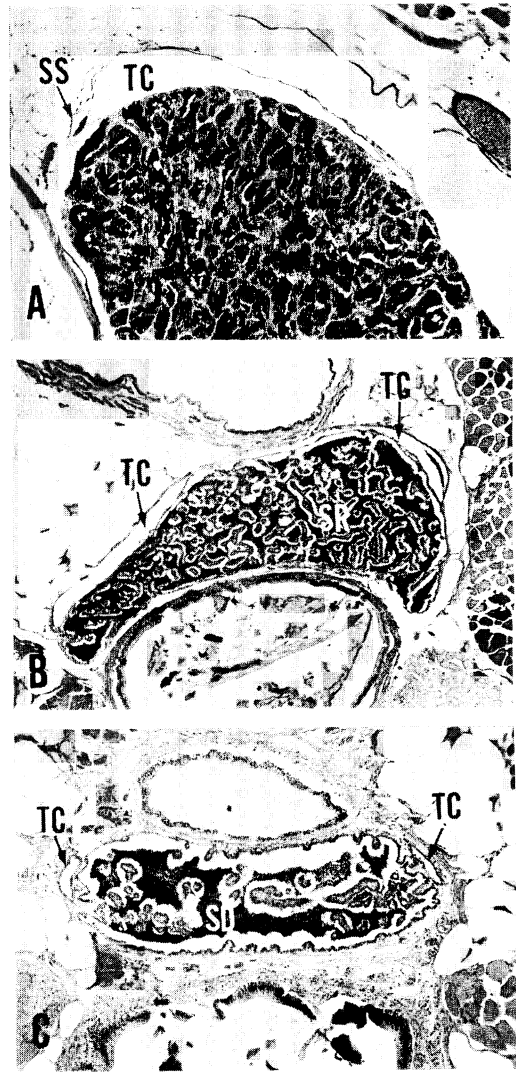


Fig. 1. Cross sections through testes of adult topmouth gudgeons, showing a topographic relationship between the testicular cavity (TC) and the testis-sperm duct system. SS, sperm sinus. A, at the level of the middle region of testis in April, $\times 55$; B, at the level of the sperm reservoir (SR) in July, $\times 30$; C, at the level of the common sperm duct (SD) in July, $\times 55$.

trated the body wall and finally joined the urinary duct in the vicinity of the urogenital pore.

The occurrence of a "testicular cavity" was characteristic of the testis structure in the topmouth gudgeon. The testicular cavity was generally located along the dorsomedian side of

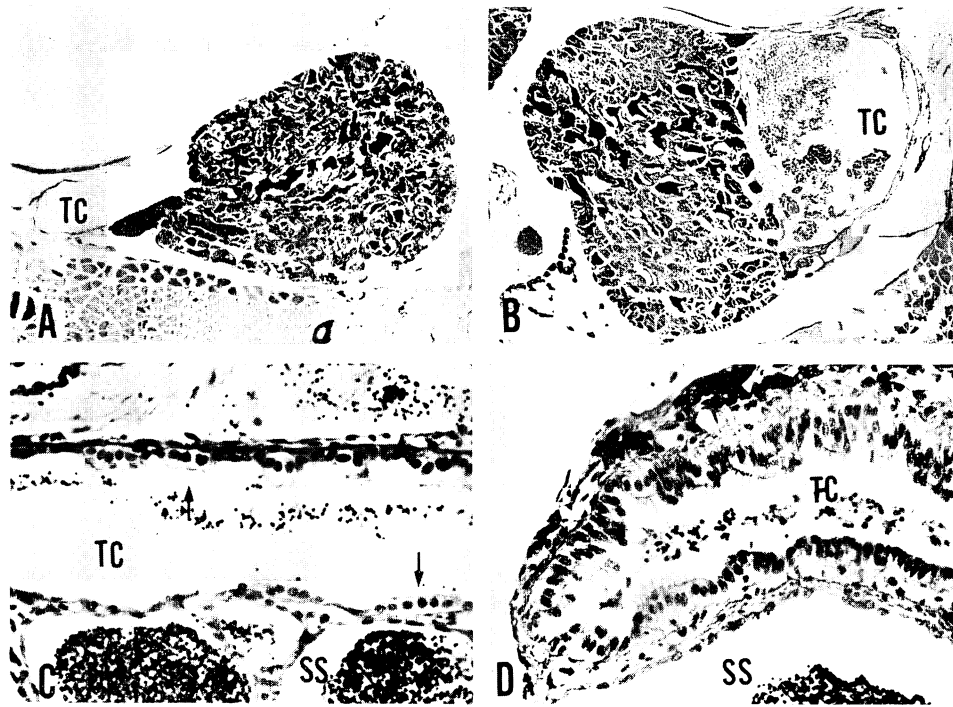


Fig. 2. Cross sections through testes of adult topmouth gudgeons, revealing histological characteristics of the testicular cavity (TC). SS, sperm sinus. A, the cavity filled with liquid material in April, $\times 20$; B, the cavity accumulating some amorphous and cellular material in June, $\times 25$; C, occurrence of patches of ciliated cells (arrows) in the epithelial layer of the cavity in August, $\times 290$; D, development of ciliated columnar epithelium lining the cavity in September, $\times 290$.

the testis in such a manner that a portion of the peritoneal cavity was confined in a space between the testis capsule and the confronted peritoneal wall (Fig. 1A). It was very narrow in many cases and its occurrence was recognizable only by careful observations on properly prepared sections of the testis attached *in situ* to the peritoneal wall. The cavity extended from the anterior end of testis in the caudal direction along the whole length of testes on both sides, and the two cavities joined at their caudal ends with a single median cavity which closely bordered the dorsal wall of the sperm reservoir (Fig. 1B). The cavity bifurcated caudally again to traverse from the dorsal to the lateral side of the sperm duct, becoming progressively narrower, and had blind ends at the region where the sperm duct enters the body wall (Fig. 1C). In some cases, the caudal parts of the cavities were shifted, anterior to their blind end, from the lateral to the ventral side of the sperm duct.

The testicular cavity in maturing males generally

appeared to be vacant and was lined by flat epithelial cells. However, in males during the breeding period extending from April to August, there frequently emerged some amorphous or liquid material often with dispersed cells of undetermined origin in expanded testicular cavities (Fig. 2A, B). Spermatozoa were present only sporadically in the cavity. In addition, the wall of the cavity of mature males was provided, especially on its dorsal and lateroventral sides, with many specks of cuboidal or columnar epithelial cells which showed ciliation on their free borders (Fig. 2C). The development of epithelial cells was quite prominent in some males examined in September, shortly after the breeding period: the dorsal epithelial layer lining the testicular cavity was extensively composed of columnar cells of 10–13 μm in height with numerous cilia of 6–7 μm in length on their free borders (Fig. 2D). During the successive period of testicular quiescence, epithelial cells of the cavity were mostly flat in shape and no longer showed

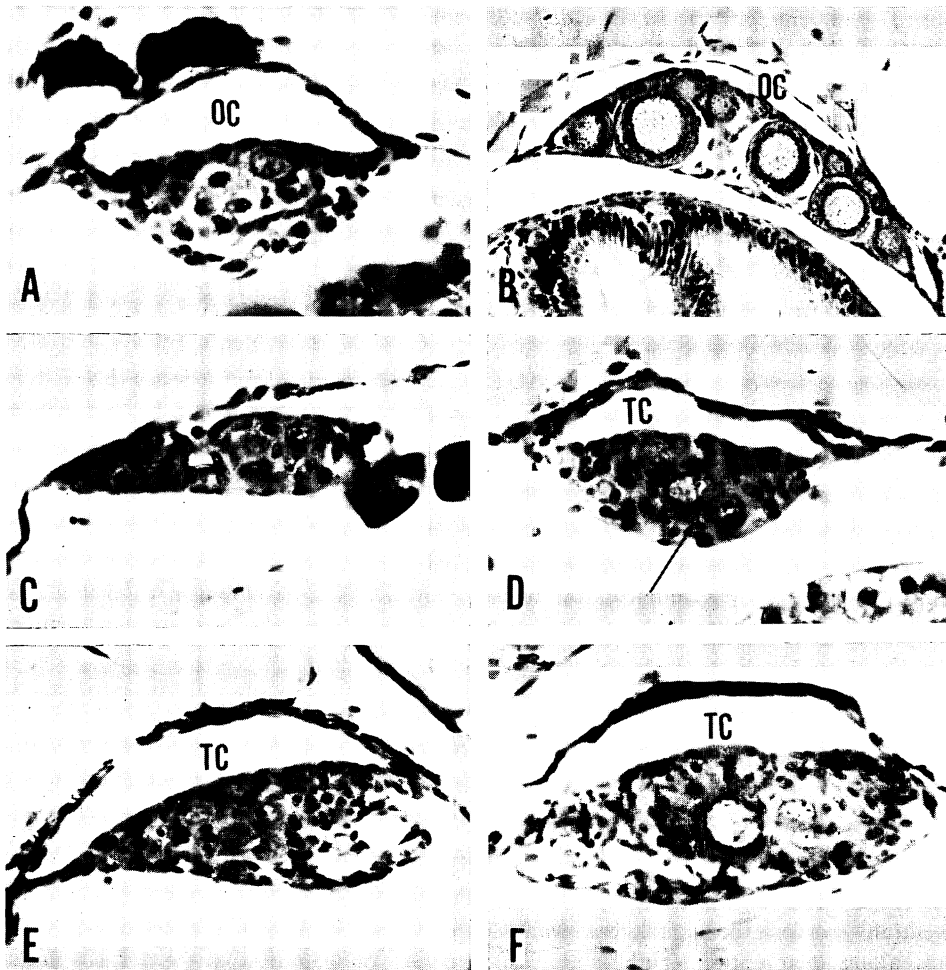


Fig. 3. Cross sections through developing gonads of juvenile topmouth gudgeons. A, a differentiating ovary with a distinct ovarian cavity (OC) on day 27, $\times 620$; B, a defined ovary on day 37, $\times 260$; C, a testicular gonad on day 37, $\times 620$; D, a testicular gonad with the testicular cavity (TC) and degenerative oviform germ cells (arrow) on day 37, $\times 520$; E, a differentiating testis on day 48, $\times 400$; F, a differentiating testis of the same specimens as E, with an oviform germ cell, $\times 440$.

noticeable ciliation.

In order to know the histo-architectural significance of the testicular cavity of the topmouth gudgeon, the gonads of two broods of fish spawned and reared in laboratory aquaria were histologically examined at various stages of gonadal morphogenesis. Gonads remained sexually indifferent until day 17 (7.6 mm mean BL), having only a small number of gonial germ cells surrounded by a thin somatic cell sheet in the genital ridge. In gonads of 7 out of 21 fish examined on day 22 (9.8 mm mean BL) and 27 (10.5 mm mean BL), germ cells were still few in number

and remained quiescent. In those of the remaining 14 fish, however, there appeared some cysts of germ cells in meiotic prophase together with solitary gonial germ cells. Moreover, gonads of 9 out of these 14 fish had completed the formation of ovarian cavities which resulted from a fusion of the distal edge of the genital ridge with the dorsolateral peritoneal wall, clearly indicating ovarian differentiation of the gonads (Fig. 3A). Growth of oocytes was conspicuous in such gonads on and after day 32, giving rise to a developing ovary with defined aspects (Fig. 3B).

Among the 42 fish examined during the period

from day 37 (14.0 mm mean BL) to day 53 (17.4 mm mean BL), 19 had developing ovaries, whereas 8 had small gonads with only a small number of quiescent gonial germ cells mounted in somatic cell stroma which had moderately increased in amount (Fig. 3C). The histological attributes apparently pointed to a testicular nature of these gonads. Gonads of the remaining 15 were similar in general aspects to the testicular gonads, but were characterized by having a few cysts of germ cells showing meiotic nuclear changes together with sporadic oviform germ cells which were mostly degenerative in appearance (Fig. 3D). Most of these gonads were provided each with a cavity on their dorsal side. It was confirmed by serial observations that the testicular cavity was formed through quite the same process as the ovarian cavity (Fig. 3E, F).

In the present study, a total of 100 adult male topmouth gudgeons were examined for their testicular histology. The presence of testis-ova was observed in testes of only 4 of these fish, while occurrence of the testicular cavity was evident in 98 fish.

Discussion

The present study indicates that the testicular cavity appears to be a permanent and normal accessory of the testis of adult male topmouth gudgeon, *Pseudorasbora parva*. The testicular cavity ventrally borders the dorsolateral wall of the testes along their entire length, and exists quite independently of the sperm-transport system. The dorsal wall lining the cavity is originally made up of peritoneal epithelium, indicating that the cavity is a compartment of the peritoneal cavity just as the ovarian cavity of the fish. The testicular cavity is formed in males during an early phase of testicular differentiation in an identical manner as that seen in the formation of ovarian cavity in females, which substantiates the homology of these two structures.

The occurrence of a similar cavity in differentiating testes has been shown in other cyprinid fishes such as zebrafish *Brachydanio rerio* (Takahashi, 1977), rose bitterling *Rhodeus ocellatus ocellatus* (Shimizu, 1979) and Sumatra barb *Barbus tetrazona tetrazona* (Takahashi and Shimizu, 1983), which display juvenile intersexuality during their testicular differentiation. No information has been given, however, about the

formation of such an accessory cavity in the testis associated with juvenile intersexuality in common carp *Cyprinus carpio* (Davies and Takashima, 1980). In the rose bitterling and the Sumatra barb, the cavity is often undetected as the testis grows in young males, while in the zebrafish the cavity is retained in adult testes and may function at least as part of the sperm-transport system. In contrast, the testicular cavity of the topmouth gudgeon does not appear to act as a route for sperm transport in adult testes, and seems to be nothing but the product of an innate potential of future testes to develop ovarian characteristics at an initial phase of their differentiation, as in other cyprinid fishes cited above.

The phenomenon of juvenile intersexuality appearing in the development of germ cells in male gonads is rather obscure in the gudgeon as compared with that in the zebrafish (Takahashi, 1977). However, the development of meiotic germ cell cysts and sporadic auxocytes in differentiating testes of the gudgeon is always accompanied by the formation of the testicular cavity. It is also characteristic of the gudgeon that false ovarian differentiation of male gonads takes place at varying times among different individuals, generally occurring later than the differentiation of definite ovaries in females. The development of oocytes in male gonads seems to cease in the early stages of growth: maturing and mature male gudgeons are observed to have testes with no traces of heterosexual germ cell development except for only a few with sporadic testis-ova. On the contrary, the testicular cavity is retained in the testis of almost all male gudgeons examined. Thus, the juvenile intersexuality of the gudgeon may cause a permanent modification of testicular structure through heterosexual differentiation of the somatic constituent of the gonad. Ionescu-Varo and Grigoriu (1963) also stressed intersexuality of *Pseudorasbora parva* introduced to Rumania, on the basis of the existence of oocytes in the testis of some immature males, but they did not mention the presence of accessory cavities in the testis of the fish. The structure of the testis of the gudgeon seems to resemble that of the testis of serranid fishes of the genera *Paralabrax* and *Liopropoma* which show secondary gonochorism possibly derived from ancestral hermaphroditic conditions (Smith and Young, 1966; Smith, 1971).

It is interesting to note that epithelial cells lining the dorsal wall of the testicular cavity of the gudgeon became thickened and ciliated especially at a later phase of the breeding period. The dorsal wall of the ovarian cavity in mature ovaries of the gudgeon is similarly lined with ciliated cells of tall columnar shape. Accordingly, the hypertrophy and ciliation of epithelial cells of the testicular cavity may simply reflect the fact that the testicular cavity is homologous with the ovarian cavity. It has also been shown that, in female goldfish *Carassius auratus*, similar changes of the epithelium of the ovarian cavity may be caused by sex hormones (Takahashi and Takano, 1971). Such is also the case for the epithelium of the ovarian cavity in the gudgeon (unpublished observation). Thus, there is a possibility that, in the gudgeon, the alterations of the epithelial cells of the testicular cavity may result from changes in endogenous levels of sex hormones, though it seems unlikely that the alteration has any significance in testicular function in the fish.

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モツゴの精巣の付属腔の出現とその幼時間性現象との関連

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モツゴの成魚の精巣には、その背側から正中側にかけて、体腔壁との間に扁平な腔所が付属するのがみられる。この腔所 (testicular cavity) は精巣の全長にわたって存在し、後方では主輸精管合一部の背側で左右が同様に合一し、さらに後方の総輸精管に接して盲端をもつ。この testicular cavity 内には液性ないし細胞性の内容物がみられることがあり、また時には少数の精子の混入もあるが、モツゴの testicular cavity は輸精管系とは全く別個の存在である。精巣成熟時には、testicular cavity の背側の上皮細胞が顕著な肥厚と線毛形成をみせるが、この変化は、成熟雌魚の卵巣腔上皮にみられる形態変化と一致する。

モツゴ稚魚の将来の精巣では、卵巣分化の開始にかなり遅れて、ふ化後 37-53 日に一部の生殖細胞が卵子形成過程に入り、やや不明確な幼時間性現象を示す。またそれと同時に生殖腺腹縁と体腔側壁との接着によって、体腔の一部をとりこんだ形式の腔所、すなわち testicular cavity が形成される。この形成様式は卵巣腔形成の様式と全く同様である。

すなわち、モツゴ成魚の精巣に付属する testicular cavity は幼時間性現象の所産が精巣発達の過程で痕跡化ないし消失することなく残存するに至ったもので、おそらく精巣活動に関する機能を有しないものと思われる。

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