Japanese Journal of Ichthyology
Vol. 28, No. 4 1982

魚類学雑誌
28巻4号 1982年

Gonadal Sex Differentiation in Whitespotted Char, *Salvelinus leucomaenis*

Masaru Nakamura

(Received July 3, 1981)

Abstract The process of gonadal sex differentiation in the whitespotted char, *Salvelinus leucomaenis*, was investigated histologically. Sexually indifferent gonads consisted of a few large germ cells mounted in a mass of somatic cells. Morphological differentiation of the gonad either into ovary or into testis became distinguishable 131 days after hatching. Characteristic features of initial ovarian differentiation are the transition from oogonia to premeiotic oocytes in the anterior region of the gonad and the appearance of a mass stromal tissue of the gonad along the side facing the mesentery. A residual ovarian cavity was formed but restricted to the cranial part of the ovaries. On the other hand, initial testicular differentiation was characterized by the formation of a few narrow lumina in the proximal region of the gonads, though germ cells still remained at the gonial stage in these gonads. Germinal and stromal cells in the testis increased in number gradually, and the occurrence of spermatogenesis was not observable even by 250 days after hatching. The process of gonadal sex differentiation in the whitespotted char was essentially similar to that in other salmonid fishes.

Histological observations on gonadal sex differentiation in salmonid fishes have hitherto been reported by several investigators (Robertson, 1953; Ashby, 1957; Okada, 1973; Nakamura et al., 1974; Takashima et al., 1980). However, no study has been concerned with gonadal sex differentiation of fishes belonging to the genus *Salvelinus*.

In the present study, the normal course of gonadal sex differentiation of the whitespotted char, *Salvelinus leucomaenis* (Pallas), was examined histologically with special reference to the behaviour of somatic elements of the gonad during that process, and compared with that of other salmonid fishes.

Material and methods

Juvenile fish of the whitespotted char used in the present study were obtained from the Nanae Fish-Culture Experimental Station, Hokkaido University. They were kept under natural temperatures of river water ranging from 1 to 6°C during a period from January to May in 1974. Seventy-one fish in total were sacrificed at intervals of 1～2 weeks from 40 to about 250 days after hatching, and their gonads were examined histologically.

For histological observations of the gonad, fish were fixed in toto in Bouin's solution. Serial paraffin sections of the body including gonadal regions were cut frontally at 8 μm in thickness and stained with Delafield's hematoxylin and eosin.

Observations

Sexually indifferent period. A pair of gonadal anlages of fish at 40 days of age was located on both sides of the dorsal root of the mesentery (Fig. 1A). They ran caudalwards from the level of the proximal region of the pectoral fins, and ended in the peritoneum near the genital pore. Germ cells were seen to be distributed solitarily and evenly throughout the whole length of the gonadal anlages. The germ cells were provided each with a large nucleus of roundish or oval form and a clear cytoplasm, and were surrounded by a small number of somatic cells (Fig. 1A). Although germ cells undergoing mitotic division were not detectable, mitosis of somatic cells was frequently found in the gonads at this age.

Dividing germ cells were often observed in the anterior region of gonads of the fish at 47 days of age (Fig. 1B). From 61 to 75 days of age, no remarkable development was noticeable either in germinal or somatic elements of the gonad.

At 89 and 103 days of age, paired gonads were seen to be hung down each by a thin and short mesogonium from the dorsal peritoneal wall into the coelomic cavity. Germ cells and somatic cells of these gonads increased slightly in number, and some clusters of germ cells smaller than those of the previous age were present frequently in the anterior region of the gonad (Fig. 1C).

In 6 out of 12 specimens examined at 115 days of age, the gonads had many cysts of germ cells together with larger solitary ones in their anterior region (Fig. 1D). In the remaining 6 fish, gonads were furnished exclusively with large solitary germ cells scattered in the gonadal stroma, though the germ cells were sometimes observed to be in mitosis in the anterior region (Fig. 1E). No apparent differences in histological aspects of stromal cells were detected between the two different types of gonads at least at that age.

Ovarian differentiation. Histological differentiation of the above-mentioned two types of gonads became increasingly evident toward 131 days of age. At that age, gonads of one type had many cysts of premeiotic germ cells (oocytes) in their anterior region along with cysts of germ cells (oogonia) which had increased in number, indicating their ovarian nature. Stromal cells in these gonads were found to be crowded especially along the side facing the mesentery (Fig. 1F). Gonads of the other type were utterly devoid of premeiotic germ cell cysts and still retained solitary gonial germ cells (spermatogonia) dispersed in increased stromal tissue (Fig. 1G).

The presence of cysts of premeiotic oocytes were evident in ovaries at 146 days of age (Fig. 1H). At 159 days of age, cysts of premeiotic oocytes showed an increase in number, and stromal cells along the median side of ovaries also increased in amount gradually, in which a few blood vessels began to appear. Stromal tissue of the lateral side of ovaries were very thin and formed a layer covering the germ cells (Fig. 1I).

In ovaries examined at 174 days of age, premeiotic oocytes further increased in number in the anterior region (Fig. 2A), and some of them reached the auxocytes stage of oogenesis. Owing to such a development of germ cells, the anterior part of ovaries showed intense swelling.

Later, by 250 days of age, many auxocytes were found to develop and to occupy the anterior region of the ovaries. By that time the distal part of the ovary started to fold toward the dorsolateral side to make a groove intruding deeply into the ovarian tissue (Fig. 2B). The groove seemed to become closed at its edges, since an entovarian cavity was observed to be shaped in the anterior region of the ovary (Fig. 3C). However, the middle and posterior regions of the ovary were evidently lacking such a cavity, still consisting of a small amount of stromal tissue and a few auxocytes and static oogonia. A few thick blood vessels appeared on the median side of the proximal region of the ovary.

Testicular differentiation. Some of the gonads observed at 131 days of age could be regarded as testes by having large germ cells (spermatogonia) which were located dispersedly and solitarily in the stromal tissue (Fig. 1G). A few narrow lumina were present in the stromal tissue of the anterior region of the testes at that time (Fig. 1G). These lumina, or anlages of blood vessels, occurred only in the proximal region of the testes and on the side facing the mesentery. The posterior region of the testes consisted of a few spermatogonia surrounded by scanty stromal cells.

In testes examined at 146 and 159 days of age, stromal cells having increased in number in the anterior region though spermatogonia were still quite quiescent and existed quite dispersedly (Fig. 2D).

In testes at 174 days of age, narrow lumina in the stromal tissue of their proximal region differentiated into an artery and a vein. Other lumina, which seemed to develop into the seprm duct, were found to exist near the blood vessels (Fig. 2E). Spermatogonia were still large in size (12 μm in diameter) and distributed singly in the stromal tissue of the anterior region (Fig. 2E). In testes at 250 days of age, blood vessels were more expanded than in the preceding period. Moreover, the lumina existing around blood vessels increased in size and number. Germ cells also increased in number slightly but were distributed still singly in the stromal tissue (Fig. 2F).

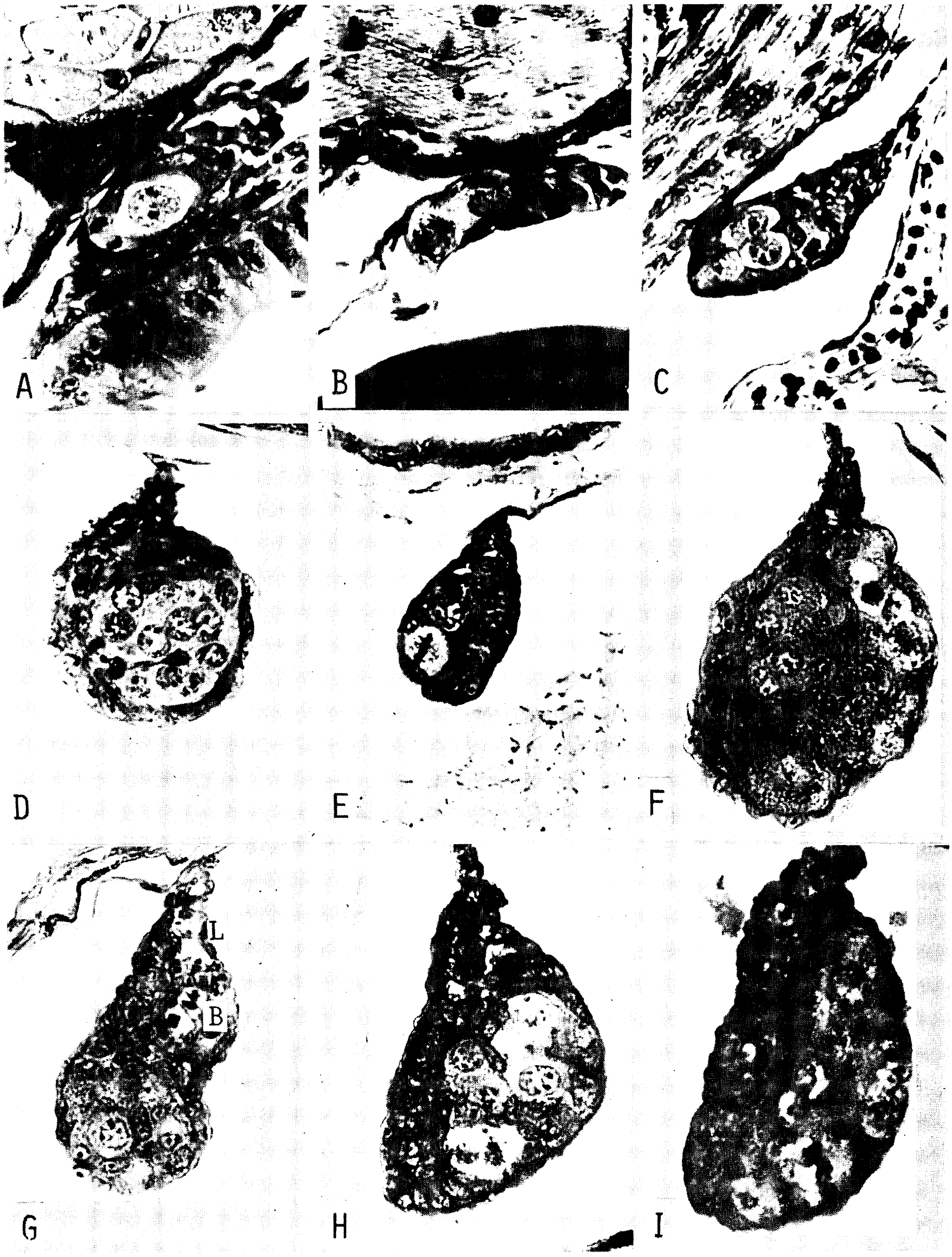

Fig. 1. Cross sections through developing gonads of the whitespotted char, *Salvelinus leucomaenis*. A: Sexually indifferent gonad of a fish 40 days after hatching, revealing mitotic division of a follicle cell. ×540. B: Sexually indifferent gonad of a fish 47 days after hatching, showing mitosis of a germ cell. ×540. C: Sexually indifferent gonad of a fish 89 days after hatching. ×540. D and E: Gonads of fish 115 days after hatching. The gonads are furnished with many cysts of germ cells or with large solitary germ cells. ×540. F: Ovary of a fish 131 days after hatching. Premeiotic oocytes together with germ cells are seen. ×540. G: Testis of fish 131 days after hatching. Spermatogonia are found dispersedly in the stroma. Lumina (L) and blood capillaries (B) exist in the stroma. ×540. H and I: Ovaries of fish at 146 (H) and 159 (I). ×540.

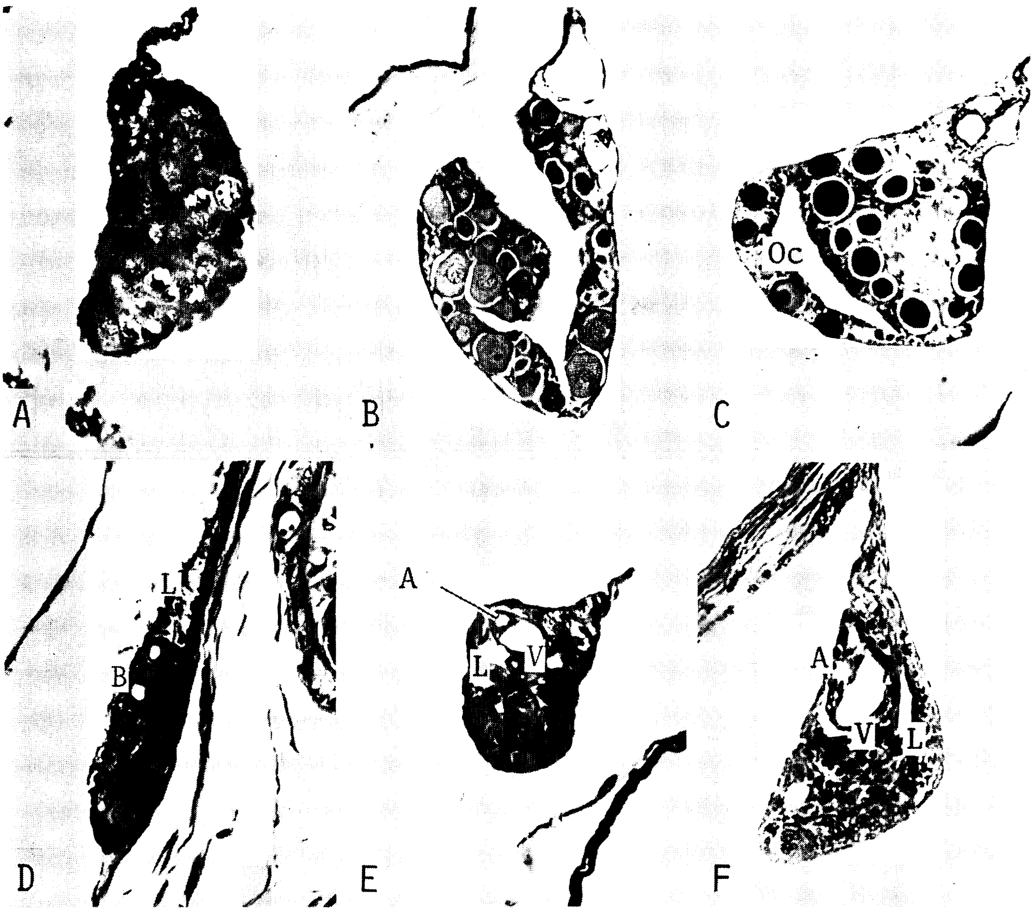

Fig. 2. Cross sections through developing gonads of the whitespotted char, *Salvelinus leucomaenis*. A～C: Ovaries of fish at 174 (A) and about 250 days of age (B and C). Oocytes increase in number and size gradually. In B and C, formation of ovarian cavity (Oc) is seen. A, ×270; B and C, ×160. D～F: Testes of fish at 159 (D), 174 (E) and about 250 days of age (F). A, artery; B, blood capillaries; L, lumina; V, vein. ×330.

Discussion

In the present study, the origin of stromal cells of gonadal primordia in the whitespotted char, *Salvelinus leucomaenis,* could not be determined since the genital ridges had already been formed when the observation started. The dualistic structure of gonads, which is manifested particularly in the sexually indifferent stage in amphibians (Witschi, 1957), was never evident in gonads of the char prior to and throughout the stage of sex differentiation. As many investigators have reported in other teleost fishes, stromal cells in the gonads of the char seem to be of monistic origin.

Tuzuki et al. (1966) and Satoh and Egami (1972) reported in medaka, *Oryzias latipes*, that germ cells in possible ovaries were more in number than those in possible testes, and that the meiosis of germ cells occurred earlier in the ovaries than in the testes. As many investigators have noticed, the early appearance of premeiotic germ cells was one of the criteria for morphological judgement of initial differentiation of ovaries in teleost fishes including salmonids such as *Oncorhynchus keta* (Robertson, 1953), *Salmo trutta* (Ashby, 1957), *Salmo gairdnerii* (Okada, 1973; Takashima et al., 1980) and *Oncorhynchus masou* (Nakamura et al., 1974). In the whitespotted char, *Salvelinus* *leucomaenis*, too, the appearance of premeiotic germ cells in gonads of some of the fish examined at 131 days of age was a reliable character to indicate the differentiation of these gonads into ovaries.

Another histological character of ovarian differentiation in the char was the increment in amount of stromal cells of the gonad along the side facing the mesentery. In the stromal tissue, a few blood vessels made their appearance later. Nakamura and Takahashi (1973) revealed that initial differentiation of the ovary of *Tilapia mossambica* was characterized not only by early appearance of premeiotic germ cells but also by development of stromal tissue in the gonad to form the ovarian cavity. Although the aggregation of stromal cells in gonads of the char did not indicate their participation in the formation of the ovarian cavity, it could be considered as denoting the ovarian nature of those gonads. Such a characteristic development of gonadal stroma has not been described for ovarian differentiation in other salmonid fishes.

On the other hand, opinions are rather conflicting with regard to morphological characteristics indicating the initiation of testicular differentiation in teleost fishes, because germ cells in future testes usually remain quiescent for a long time. For example, the transition from spermatogonia to spermatocytes in testes of masu salmon, *Oncorhynchus masou*, does not occur until the time just before the fish begin their anadromous migration (Hiroi and Yamamoto, 1970). As mentioned before, however, behaviour of somatic cells of the gonad during the course of morphological sex differentiation is often sex-specific and successfully manifests the gonadal sex. Ashby (1957) reported that, in *Salmo trutta*, an increase in the amount of both vascular and connective tissues, which was regarded by Padoa (cited from Ashby, 1957) as a diagnostic masculine feature of juvenile salmon, was an obvious change in early testicular development. In rainbow trout, *Salmo gairdnerii*, the initial testicular differentiation was marked by the development of the seminiferous tubule (Okada, 1973) and by the differentiation of follicle cells and Sertori cells (Takashima et al., 1980). In *Oncorhynchus masou*, the development of blood vessels surrounding a cavity was observed in future testes in their hilar tissue facing the mesentery (Nakamura et al., 1974). In the char observed in the present study, primordia of efferent ducts and blood vessels occurred in stromal tissue of the proximal region of the gonad by 131 days after hatching, showing that morphological differentiation of testis started by that age.

It is interesting to note that a residual ovarian cavity is constructed restrictedly to the cranial part of ovaries of the char. Nakamura et al. (1974) revealed previously that in *Oncorhynchus masou*, an ovarian cavity of the parovarian type was formed only at the cranial region of ovaries during their early development. Robertson (1953) noted in the chum salmon, *Oncorhynchus keta*, that an endovarial canal was formed by the curving of the distal edges of ovaries toward the lateral sides 69 days after hatching. The present writer also confirmed that a residual ovarian cavity was formed in the anterior region of ovaries of the chum salmon (unpublished data). Similar phenomena have been noted to occur in Atlantic salmon, *Salmo salar*, and in rainbow trout, *Salmo gairdnerii* (cited from Robertson, 1957). Thus it is likely that the formation of such a residual ovarian cavity is common in the ovary of salmonid fishes.

Acknowledgments

I am very grateful to Prof. H. Takahashi, Faculty of Fisheries, Hokkaido University, for critically reading this manuscript. Thanks are also due to Associate Prof. T. Kubo, Nanae Fish-Culture Experimental Station, Faculty of Fisheries, Hokkaido University, for offering specimens used in the present study.

Literature cited

Ashby, K. R. 1957. The effect of steroid hormones on the brown trout (*Salmo trutta* L.) during the period of gonadal differentiation. J. Embryol. Exp. Morph., 5: 225～249.

Hiroi, O. and K. Yamamoto. 1970. Studies on the maturation of salmonid fishes-II. Changes in the testis of the masu salmon, *Oncorhynchus masou*, during anadromus migration. Bull. Fac. Fish. Hokkaido Univ., 20: 252～264.

Nakamura, M. and H. Takahashi. 1973. Gonadal sex differentiation in *Tilapia mossambica*, with special regard to the time of estrogen treatment effective in inducing complete feminization of genetic males. Bull. Fac. Fish. Hokkaido Univ., 24: 1～13.

Nakamura, M., H. Takahashi and O. Hiroi. 1974. Sex differentiation of the gonad in the masu salmon (*Oncorhynchus masou*). Sci. Rep. Hokkaido Salmon Hatchery, 28: 1～8. (In Japanese with English summary).

Okada, H. 1973. Studies on sex differentiation of Salmonidae-I. Effects of sex differentiation of the rainbow trout (*Salmo gairdnerii irideus* Gibbons). Sci. Rep. Hokkaido Fish Hatchery, 28: 11～21. (In Japanese with English summary).

Robertson, J. G. 1953. Sex differentiation in the Pacific salmon *Oncorhynchus keta* (Walbaum). Can. J. Zool., 31: 73～79.

Satoh, N. and N. Egami. 1972. Sex differentiation of germ cells in the teleost, *Oryzias latipes*, during normal embryonic development. J. Embryol. Exp. Morph., 28: 385～395.

Takashima, F., R. Patio and M. Nomura. 1980. Histological studies on the sex differentiation in rainbow trout. Bull. Japan. Soc. Sci. Fish., 46: 1317～1322.

Tuzuki, E., N. Egami and Y. Hyodo. 1966. Multiplication and sex-differentiation of germ cells during development in the medaka, *Oryzias latipes*. Japan. J. Ichtyol., 8: 176～182. (In Japanese with English summary).

Witschi, E. 1957. The inductor theory of sex differentiation J. Fac. Sci. Hokkaido Univ., ser. VI, Zool., 13: 428～439.

(Department of Zoology, Faculty of Medicine, Teikyo University, Hachioji, Tokyo 192-03, Japan)

アメマスの生殖腺の性分化

中村　將

アメマスの生殖腺の性分化過程を組織学的に調べた．性的未分化期の生殖腺は少数で大型の生殖細胞とそれを取り囲む体細胞より構成されている．卵巣及び精巣への分化はふ化後 131 日目に形態的に明らかになった．初期の卵巣の分化は，卵原細胞の減数分裂前期の卵母細胞への移行と多くの体細胞よりなる組織が腸間膜に面する側に出現することにより明らかとなった．痕跡的な卵巣腔が卵巣の先端部に限り形成された．他方，初期の精巣の分化は，生殖腺の基部に2・3の管腔が形成されることにより明らかとなった．その後，生殖細胞と体細胞は徐々に増加するものの，精子形成はふ化後 250 日においても認められなかった．アメマスの性分化過程は他のサケ科魚類のものと本質的に一致した．

(192-03　八王子市大塚 359　帝京大学医学部動物学教室)