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Innervation of Steroid-Producing Cells in the Ovary of Tilapia *Oreochromis niloticus*

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ABSTRACT—The innervation of the tilapia ovary was examined histochemically and ultrastructurally. Thick nerve bundles were localized in the area near the ovarian artery and vein in the ovarian wall on the side facing the mesentery. Groups of a few axons ramified from the thick nerves and were terminated in the proximity of clusters of steroid-producing cells which are distributed in the interstitial area among yolky oocytes. The axon terminals were in intimate relation with the steroid-producing cells. The terminals contained many clear vesicles, a few dense-cored vesicles and some mitochondria. Moreover, a few terminals were observed on the surface of steroid-producing theca cells surrounding yolky oocytes. Histochemical results using a nerve-specific stain were in agreement with the ultrastructural observations. Our observations of direct innervation of steroid-producing cells bring to light a possible new avenue for regulation of steroid production in the tilapia ovary.

INTRODUCTION

Innervation of the ovary of teleost fish has been described in several species (Young, 1931; Nilsson, 1970; Uematsu, 1986; Uematsu *et al.*, 1989). The focus of these and other reports (e.g. Uematsu, 1985, 1990) on the function of this innervation has been on the neural regulation of ovarian muscular contraction for expelling oocytes at oviposition. Innervation of the testis of teleost fishes has also been described (Young, 1931): however, an additional observation and area of inquiry has clarified the significance of the innervation of the Leydig cells (Follénius, 1964; Gresik, 1973; Hurk *et al.*, 1974, 1982; Nakamura and Nagahama, 1995). To our knowledge, similar findings of innervation of steroid-producing cells (SPCs) in the ovary of fishes have not been reported.

We now report our observations on the distribution of nerves directly innervating the SPCs in the ovary of tilapia.

MATERIALS AND METHODS

Tilapia (*Oreochromis niloticus*) were reared in aerated water at 25±2°C until use. Twenty females, ranging from 75-170 mm in total length and 70-150 days after hatching, were used for ultrastructural and histochemical analyses.

After anesthetization (MS 222), the middle part of the ovaries were fixed in Karnovsky's solution at room temperature for 2 hr. After rinsing with 0.1 M cacodylate buffer, they were postfixed with 1% OsO_4 in the same buffer at room temperature. Ovaries were immersed in saturated uranyl acetate for 2-4 hr to block staining. They were dehydrated in a graded ethanol series and embedded in epoxy resin. One micron sections were cut on an ultramicrotome and stained with toluidine blue for observation by light microscopy. Thin sections (50-80 nm) were stained with lead citrate for observation by electron microscopy (Hitachi-7000).

For histochemical staining of nerves, mature ovaries were fixed in neutral formalin, glacial acetic acid, and 80% ethanol (1:1:18) at 4°C for 2 days. Ovaries were sectioned at 12 micrometer and then stained with Bodian's silver impregnation method as modified by Otsuka (1962).

RESULTS

Light and electron microscopies

In the immature ovary of fish at 70-100 days after hatching, a few thick nerves were localized in the ovarian wall along the ovarian artery and vein on the medial side facing the mesentery (Fig. 1). These consisted of more than one thousand axons of various sizes ranging from 100 to 1000 nm in diameter (Figs. 2 and 3). Bundles of axons branching off from the thick nerves invaded the central part of the ovigerous lamellae. Capillaries branching from the ovarian vein were also distributed in the ovarian interstitium of the ovigerous lamellae. Clusters of SPCs, with ultrastructural characteristics including well-developed endoplasmic reticulum and mitochondria with tubular cristae were located in the interstitium near the capillaries (Figs. 4 -6). Bundles of nerve axons were observed

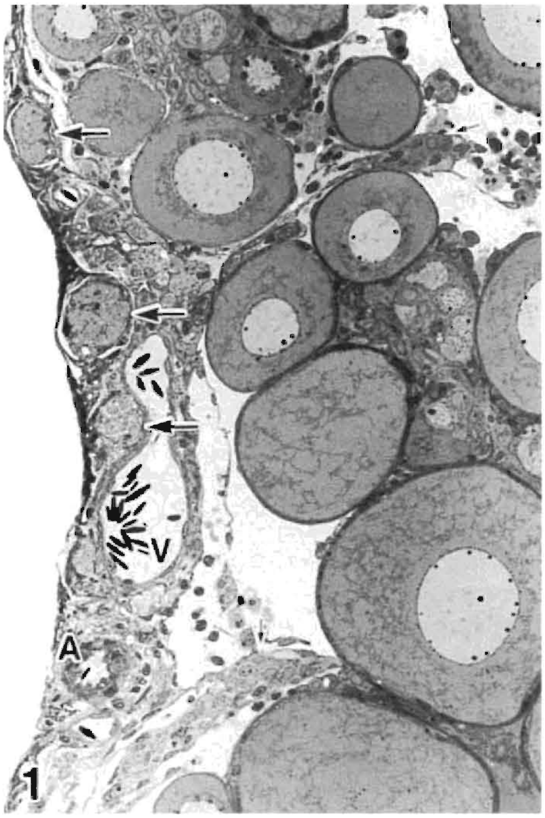


Fig. 1. Light micrograph of a part of the ovary of tilapia *Oreochromis niloticus*. Some thick nerve bundles (large arrows) are distributed near the artery (A) and the veins (V). Clusters of steroid-producing cells (small arrows) are seen in the interstitial area. × 360.

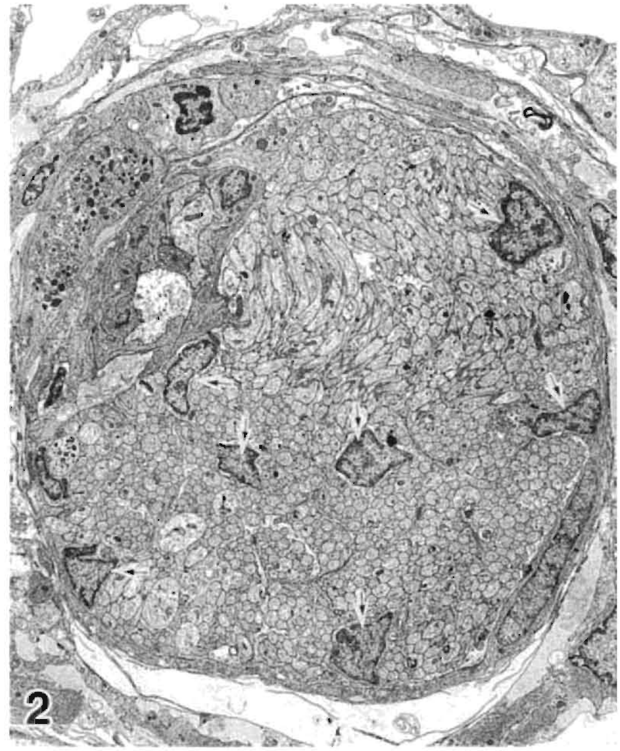


Fig. 2. Electron micrograph of a thick nerve bundle in the ovary of tilapia *Oreochromis niloticus*. Nerve bundle consists of more than one thousand nerve axons and some Schwann cells (arrows). × 3,800.

beside the cluster of SPCs (Fig. 4) and penetrated into the narrow space among the SPCs (Fig. 6). The terminals of these nerve axons swelled slightly and were in intimate relation with the SPCs (Figs. 5 and 6). Frequently, membrane of nerve terminals was in close proximity to the membrane of SPCs (5-10 nm). These endings contained many synaptic vesicles (30-50 nm in diameter), a few dense-cored vesicles (30-80 nm in diameter), and some mitochondria (Figs. 5 and 6). However, we did not observe synaptic structures such as the presence of synaptic cleft with electron dense material or pre- and post-synaptic membrane specializations.

The follicle surrounding yolky oocytes consisted of an inner granulosa layer and an outer theca layer which included SPCs (Fig. 7). There were junctions with gaps between nerve terminals and the thecal SPCs (Figs. 7 and 8). However, the numbers of the junctional structures seen on the steroid-producing thecal cells were very few, in comparison with those on the interstitial SPCs. No innervation of the granulosa cells was observed.

Histochemistry

Silver-staining was used to confirm the presence of nerve fibers in the mature ovary (Fig. 9). Nerve fibers reached the surface of follicle tissues enclosing yolky oocytes. Some nerve

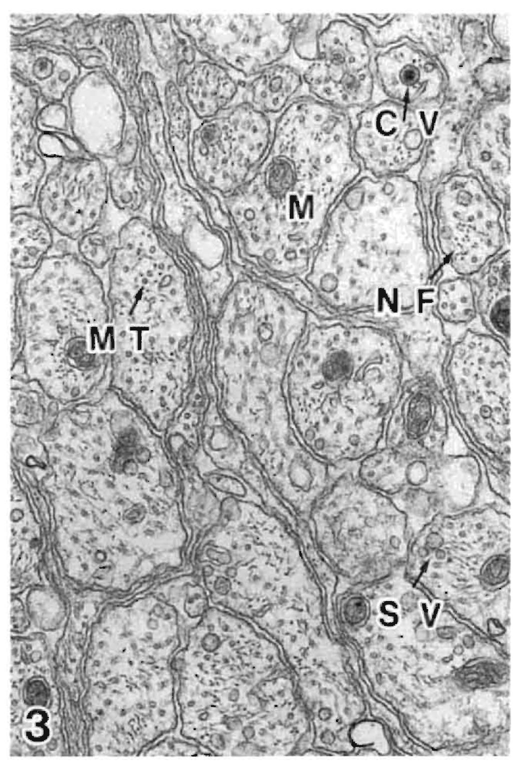
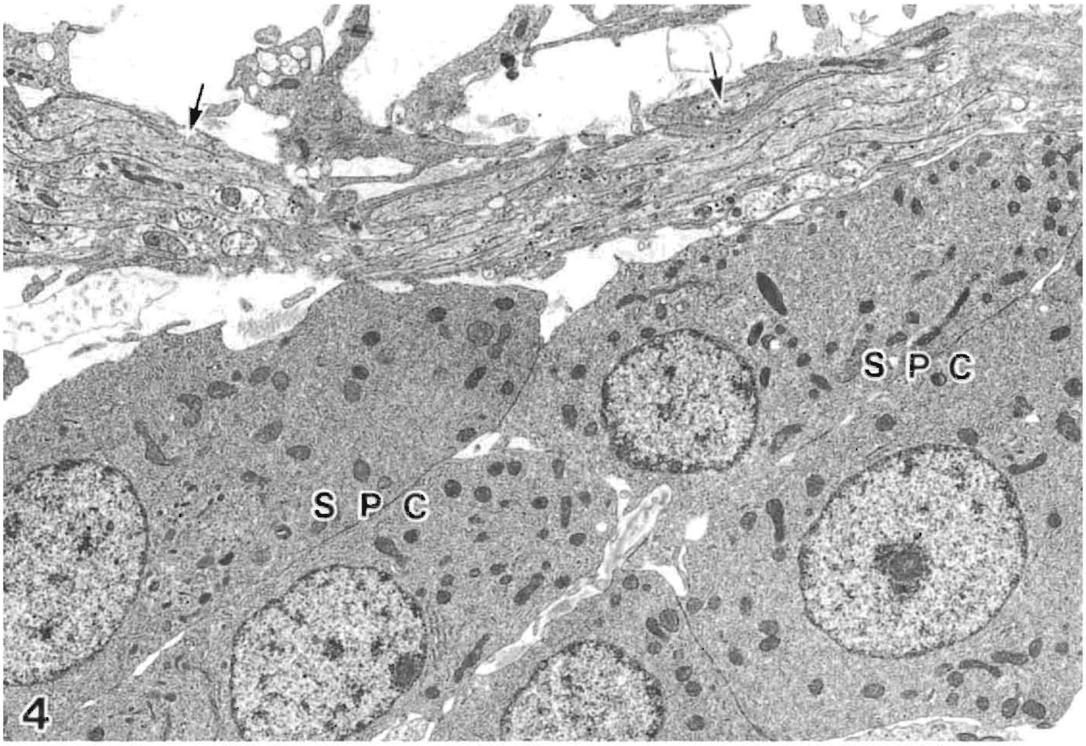


Fig. 3. High magnification of nerve axons in a nerve bundle. Neurofilaments (NF), microtubules (MT), synaptic vesicles (SV), dense-cored vesicles (CV) and mitochondria (M) are seen. × 33,200.



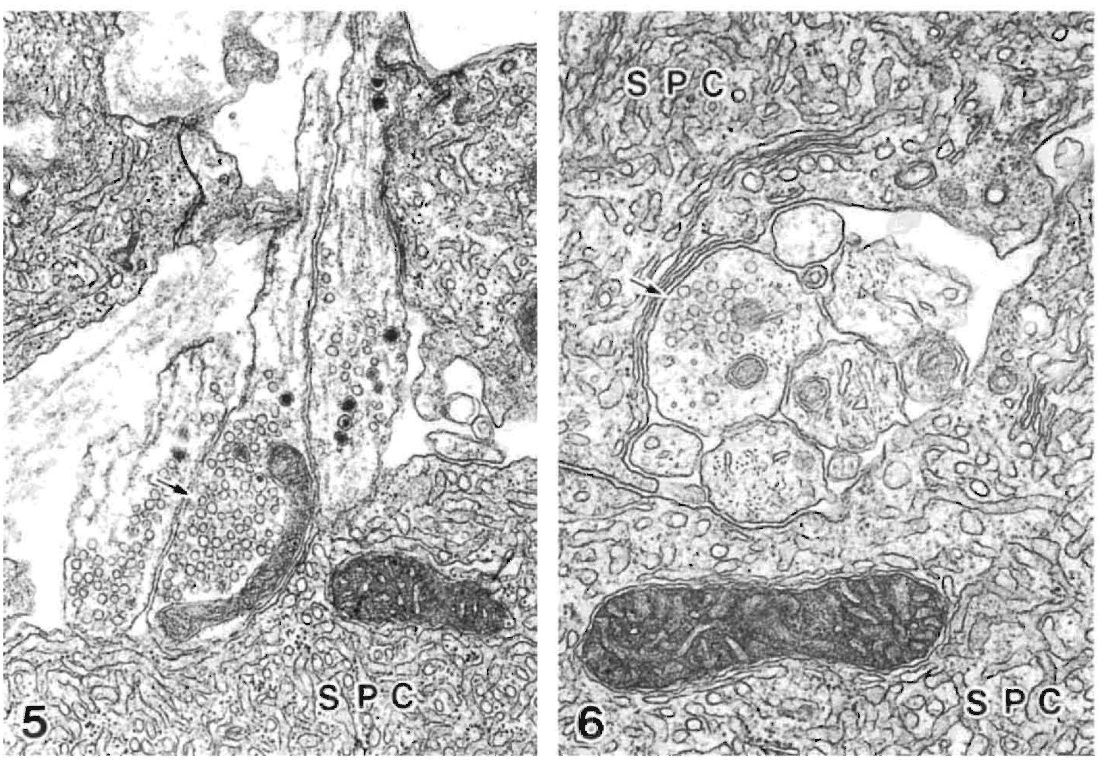
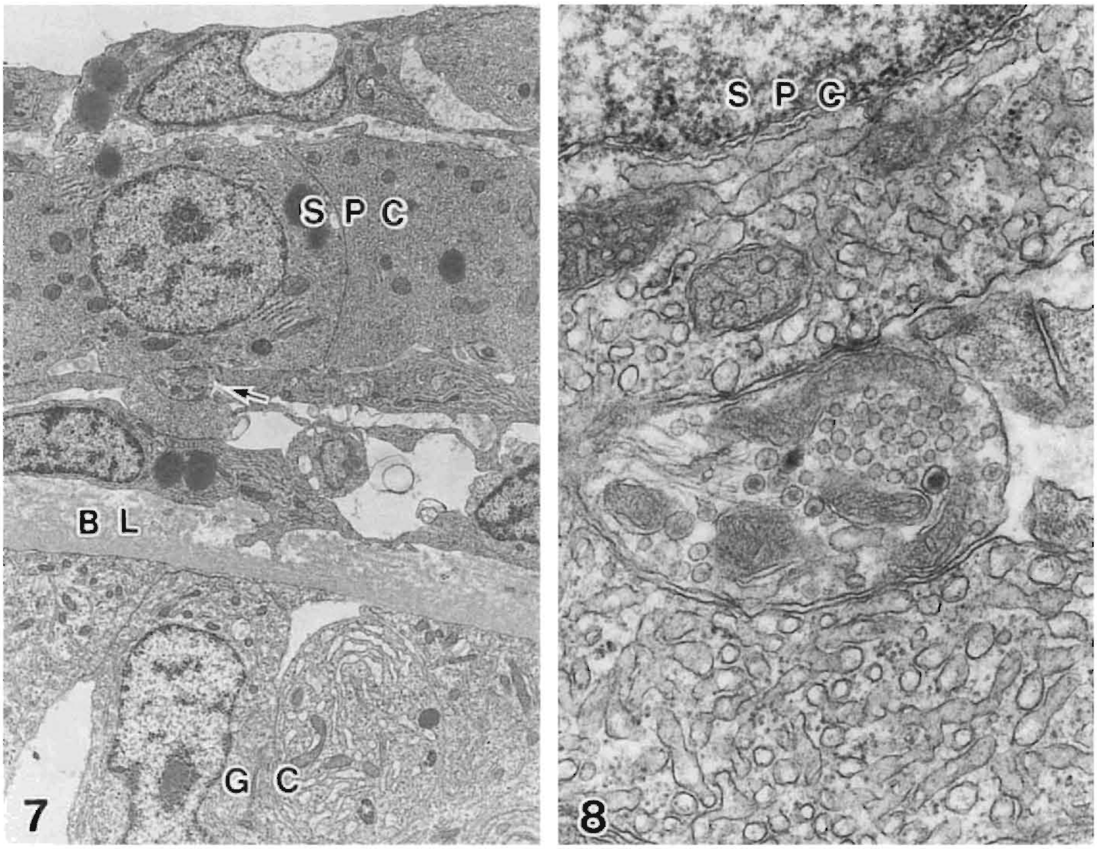


Fig. 4. Distribution of a nerve bundle in the interstitial area among oocytes. Nerve bundle (arrows) is distributed near cluster of steroid-producing cells (SPC) with ultrastructural characteristics such as well-developed endoplasmic reticulum and some mitochondria with tubular cristae. × 5,600.

Figs. 5 and 6. Nerve terminals of nerve axons on the surface of the interstitial steroid-producing cells (SPC). Nerve endings contain many synaptic vesicles (arrow) (Fig. 5). Nerve axons invade the narrow space among steroid-producing cells and terminate (arrow) on the surface of steroid-producing cells (Fig. 6). Fig. 5, × 27,700; Fig. 6, × 41,600.



Figs. 7 and 8. Distribution of nerve terminals in the follicle tissues enclosing yolky oocyte. Terminal ending (arrow) is seen near steriod-producing cell (SPC) in the theca layer (Fig. 7). High magnification of terminal ending that appears in the center of Fig. 7 (Fig. 8). Many synaptic vesicles and dense-cored vesicles are seen. BL basal lamina, GC granulosa cells. Fig. 7, × 5,700; Fig. 8, × 42,700.

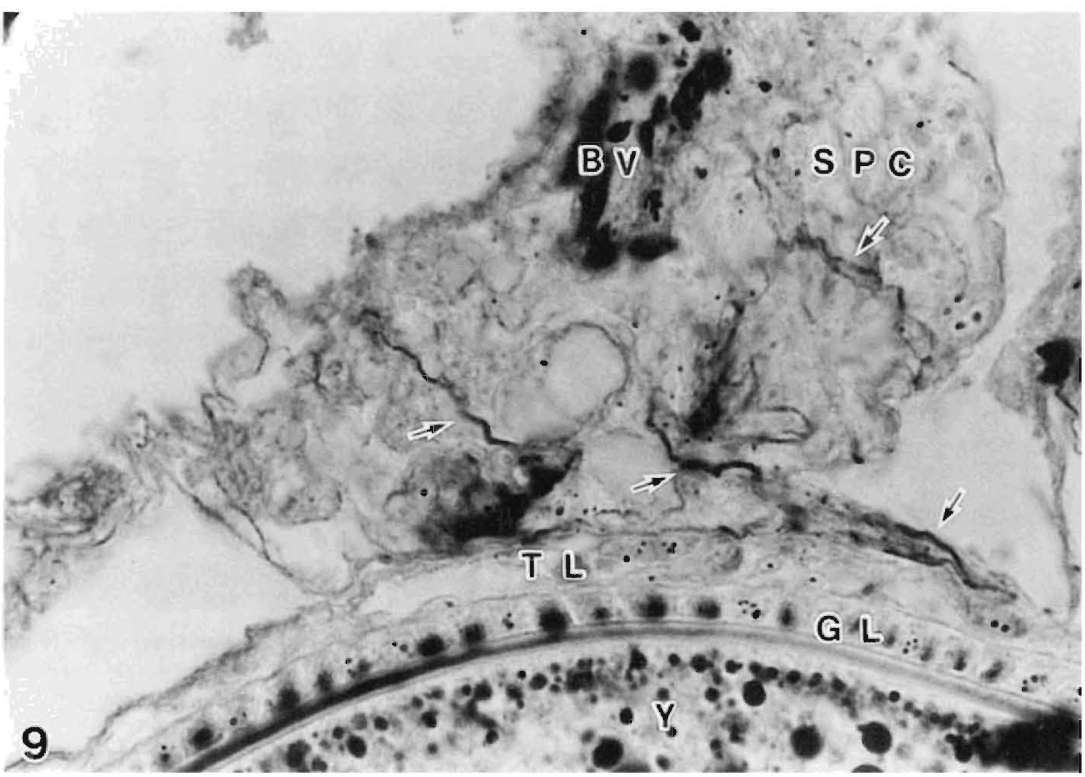


Fig. 9. Nerve axons (arrows) stained with silver are distributed in cluster of steroid-producing cells (SPCs) and are in contact with the surface of the theca layer (TL) enclosing yolky oocyte (Y). GL granulosa layer, BV blood vessel, × 1000.

fibers were distributed among clusters of SPCs. Silver also stained the cytoplasm of oocytes in the peri-nucleolus stage, yolky granules, the nucleus of granulosa cells, the nucleolus of SPCs, and capillaries.

DISCUSSION

This is the first report of the innervation of steroid-producing cells (SPCs) in the ovary of a fish. Bundles of nerve axons are interlaced among the SPCs located in the interstitium and make frequent intimate connections with the SPCs. These structures appear to be functional gap-junctions, since enlarged axon terminals contain secretory vesicles. However, they lack a synaptic cleft with electron dense vesicle, pre- and post-synaptic membrane specializations, which are characteristic of synaptic active zones (Thureson-Klein and Klein, 1990). This fact suggests that axon terminals on the surface of SPCs in tilapia ovary release its transmitter substances non-synaptically, as known in other animals (Buma and Roubos, 1986; Benedeczky and Halasy, 1988). These observations strongly suggest that the nervous system plays a part in regulation of the production and the secretion of steroid hormones in the tilapia ovary. Although there are no papers on this topic for the teleost gonads, a functional relationship between neurons and SPCs has been demonstrated in the rat ovaries, where adrenergic nerves play a role in maintaining preovulatory steroid secretion (Aguado and Ojeda, 1984). In addition, in rats it is also known that there is a direct link between the autonomic nervous system and the ovary for regulation of ovarian steroid synthesis (Weiss *et al.*, 1982; Dyer and Erickson, 1985; Hernandez *et al.*, 1988). The SPCs in the interstitium of the avian ovary are also densely innervated by the autonomic nervous system, suggesting a possible functional role (see Unsicker *et al.*, 1983).

Our knowledge of the innervation of mammalian ovaries is based largely on the development of the rat ovary. Two concepts now emerging are (1) endocrine cells of the ovary produce neurotrophic factors which are critical to folliculogenesis, and (2) the density of innervation may contribute to the selection of follicles for further development (see Dissen *et al.*, 1993). The embryonic chick ovary contains interstitial steroidogenic cells with adrenergic receptors, that are thought to be important for normal development (Müller-Maschhausen *et al.*, 1988). We have already reported the folliculogenesis during early vitellogenesis in the tilapia ovary (Nakamura *et al.*, 1993). Clusters of SPCs that originate near blood vessels migrate into the interstices among oocytes and finally enclose early vitellogenic oocytes. While SPC clusters migrate, the bundles of nerve axons elongate into the interstices among oocytes (unpublished data). We add tilapia to the short list of vertebrates in which there is a structural association in the ovary between the SPCs and the autonomic nervous system. This supports the hypothesis that the ovarian development in all vertebrates is under at least partial neural control.

The observations reported here did not allow us to identify the kinds of nerves that innervate SPCs in the tilapia ovary. On the basis of histochemical and physiological studies, the nerves in the gonads of teleosts were identified as autonomic (Uematsu, 1986; Uematsu *et al.*, 1989). Thus, it is possible that the nerves in the tilapia ovary are also autonomic. Specific immunohistochemical identification of nerves in the fish gonads, as shown in mammals (Papka *et al.*, 1985; Kannisto *et al.*, 1986; Schultea *et al.*, 1992), is essential for understanding the role of innervation in teleost gonads. In addition, it is also still unknown on the development of the terminal ends on the surface of SPCs in the interstitium and in the theca layer accompanying maturation, and the differences in distribution pattern of the terminal ends in the different parts of the ovaries.

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