

Reinvestigation of epithelial lining of the genital coelomic sinus in asteroids. An ultrastructural study

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ABSTRACT

Ultrastructural study of gonadal muscles in sea star, *Asterina pectinifera*, showed that myoepithelial cells were located only in the epithelial lining of the genital coelomic sinus. No myoepithelial cells were found in the visceral peritoneal epithelium or within connective tissue layer of the outer sac. Morphology of the myoepithelial cells in gonads of *A. pectinifera* varies during the reproductive cycle. During the gametogenic phase of the reproductive cycle, the myoepithelial cells get an elongated, spindle-like shape having a length of 20–30 μm . In prespawning gonads, many of the myoepithelial cells form cytoplasmic extensions of 3–5 μm in length, filled with myofilaments and penetrating into the underlying connective tissue of the outer sac or haemal sinus. Besides, myoepithelial cells, simultaneously anchored in the inner and outer sacs, were also observed. These changes result in development of more elaborated musculature and increase in contractility of the gonadal wall in prespawning gonads as compared to that during other stages of the reproductive cycle.

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1. Introduction

The histology and ultrastructure of gonadal wall in asteroids was studied for a number of species (for review, see Walker, 1982; Chia and Walker, 1991; Chia and Koss, 1994). These studies showed that the gonadal wall consists of the outer and inner sacs, separated from one another by the genital coelomic sinus (Fig. 1). This sinus is an outgrowth of the genital coelom (Ezhova et al., 2013), also referred to as aboral or genital sinus by Hyman (1955, p. 275) or as aboral coelomic sinus by Chia and Koss (1994, p. 237), a coelomic cavity which unites all gonads in sea star. The outer sac contains a visceral peritoneal epithelium, a discontinuous layer of myoepithelial cells, and a connective tissue layer, which is located between the two epithelia (Fig. 1). The inner sac includes a layer of myoepithelial cells, the haemal sinus, and germinative epithelium (Fig. 1), where developing germ cells are located (Chia and Koss, 1994).

Despite the general organization of asteroids' gonads has been commonly recognized, data on the structure of different parts of gonadal wall, provided by various researchers, are often inconsistent. This is especially true for gonadal wall musculature. For example, according to Walker (1974, 1982) peritoneal epithelium

of the gonadal wall in *Asterias rubens* (as *Asterias vulgaris*) contains myoepithelial cells, while no myoepithelial cells were observed in the peritoneal epithelium of the gonadal wall in this species by other researchers (Shoenmakers et al., 1981; Beijnkink et al., 1984). No myoepithelial cells were also found in the peritoneal epithelium in *Ctenodiscus crispatus*, *Hippasteria phrygiana*, *Cryptasterina hystera*, and *Echinaster (Echinaster) sepositus* (Walker, 1979; Byrne, 2005; Riesgo et al., 2011). However, Riesgo et al. (2011) reported that in the gonadal wall of *E. sepositus* some myoepithelial cells were completely enclosed within the connective tissue layer of the outer sac. Similar results were reported for *A. rubens* (Shoenmakers et al., 1981). It should be emphasized, that neither Riesgo et al. (2011), nor Shoenmakers et al. (1981) provided information whether myoepithelial cells, located within the connective tissue of the outer sac, belong to the myoepithelial layer of the outer sac, which lines the genital coelomic sinus. This indicates the necessity to refine data on gonadal musculature in asteroids. The aim of the present study is to describe organization of musculature in the gonads of sea star, *Asterina pectinifera*, at different stages of reproductive cycle.

2. Materials and methods

Adult individuals of *A. pectinifera* were collected in Emar Bay (in the vicinity of Vladivostok, Sea of Japan, Russia) from May to

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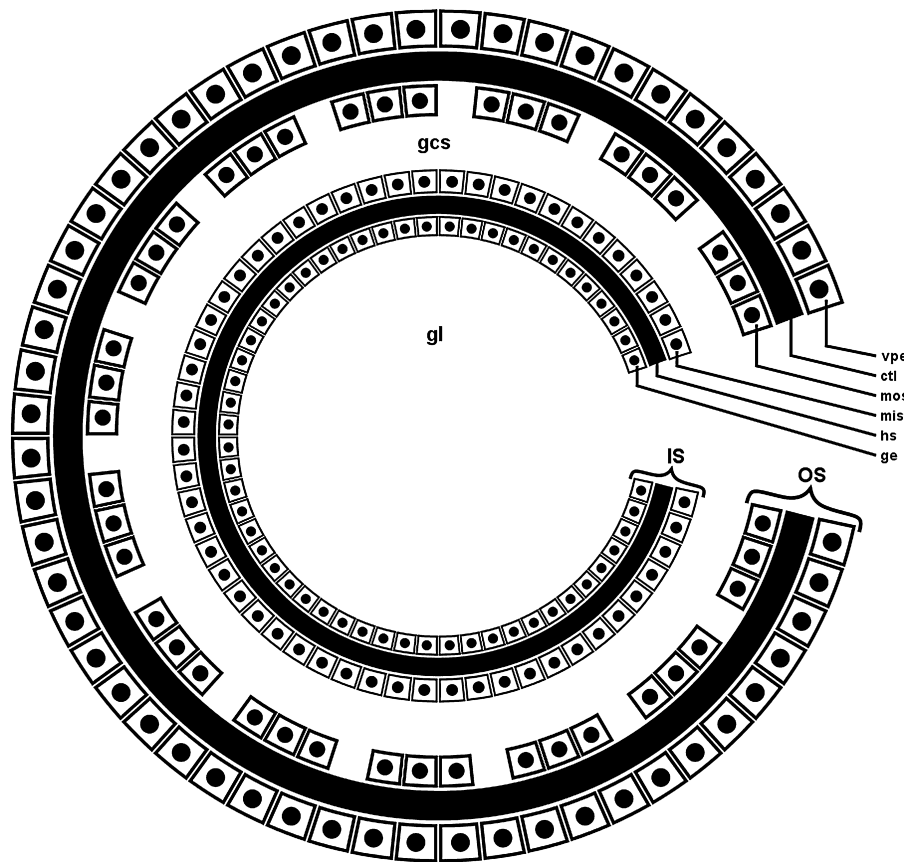


Fig. 1. Schematic representation of the tissues which comprise the gonadal wall in asteroids. IS – inner sac, OS – outer sac, ctl – connective tissue layer, gcs – genital coelomic sinus, ge – germinative epithelium, gl – gonadal lumen, hs – haemal sinus, mis – myoepithelium of the inner sac, mos – myoepithelium of the outer sac, vpe – visceral peritoneal epithelium. Not to scale.

September 2009. For electron microscopic studies, small pieces of gonads were taken out and immediately prefixed with a primary fixative solution (containing 2.5% (v/v) glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) with addition of NaCl (21 mg/ml) to make the fixative solution isotonic to seawater) for 2 h at 4 °C. After the prefixation, the specimens were rinsed in the same buffer and postfixed with 2% (w/v) solution (20 mg/ml) of osmium tetroxide (OsO₄) in 0.2 M cacodylate buffer (pH 7.4) for 1 h at room temperature. Fixed tissues were dehydrated in a series of ethanol and acetone solutions and embedded in Epon-Araldite. Ultrathin sections (ca. 75 nm) were made using a Leica UC 6 ultramicrotome. The specimens were stained with 1% (w/v) alcoholic uranyl acetate (10 mg/ml in 10% ethanol) and Reynold's lead citrate (Reynolds, 1963), and then examined under a Zeiss Libra 120 transmission electron microscope (Far East Centre of Electron Microscopy, Institute of Marine Biology FEB RAS) operated at 120 kV.

3. Results

The gonadal wall in *A. pectinifera* had the same pattern as it had been previously described for other asteroids. It consisted of the outer and inner sacs, separated from one another by the genital coelomic sinus (GCS). The outer sac included the visceral peritoneal epithelium, a layer of connective tissue, and a discontinuous layer of epithelial and myoepithelial cells (Fig. 2A and B). Morula-like cells and nerve cells processes were observed among the peritoneocytes; no myoepithelial cells were observed in the visceral peritoneal epithelium at any stage of the reproductive cycle (Fig. 2A). Similarly, no myoepithelial cells were observed completely enclosed within the connective tissue layer of the outer sac;

occasionally, some fibroblast-like cells were found in the connective tissue of that layer (Fig. 2B). The myoepithelial layer of the outer sac included myoepithelial and epithelial cells connected to each other by desmosome-like junctions. As well, nerve cells processes were found among the cells of this layer. The inner sac contained the myoepithelial layer, the haemal sinus and the germinative epithelium (Fig. 2B). The myoepithelial layer of the inner sac contained epithelial and myoepithelial cells. This layer was similar to that of the outer sac with two exceptions. First, it was a continuous myoepithelial layer without any gaps between the cells. Second, the number of the processes of nerve cells was reduced as compared to their number in the myoepithelial layer of the outer sac. Numerous amoebocytes were found in the haemal sinus throughout the reproductive cycle (Figs. 2B, 3A and 3C).

The epithelial cells, observed at the opposite sides of the GCS, were similar in their morphology. They were flattened flagellated cells with irregularly shaped nuclei (Fig. 2B and C). They were anchored on the basal lamina by hemidesmosomes. These cells often extended thin cytoplasmic processes that penetrated into folds of the underlying basal lamina (Fig. 2B). Electron-dense cytoplasm of the epithelial cells contained numerous mitochondria, Golgi complex, electron-opaque globules, and electron-lucent vesicles (Fig. 2C). These cells do not changed significantly in their morphology during the reproductive cycle.

In contrast, the morphology of myoepithelial cells varied significantly throughout the reproductive cycle. During the active gametogenic phase of the cycle (May–June), the myoepithelial cells were elongated, spindle-shaped, reaching a length of 20–30 μm (Fig. 2B and D). These cells were connected to their neighbors by desmosome-like junctions. On the basal lamina, myoepithelial cells

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