Preliminary study on the early development of embryos and ovarian diverticula in the viviparous scorpion, *Liocheles australasiae* (Fabricius) (Scorpiones, Ischnuridae)

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Introduction

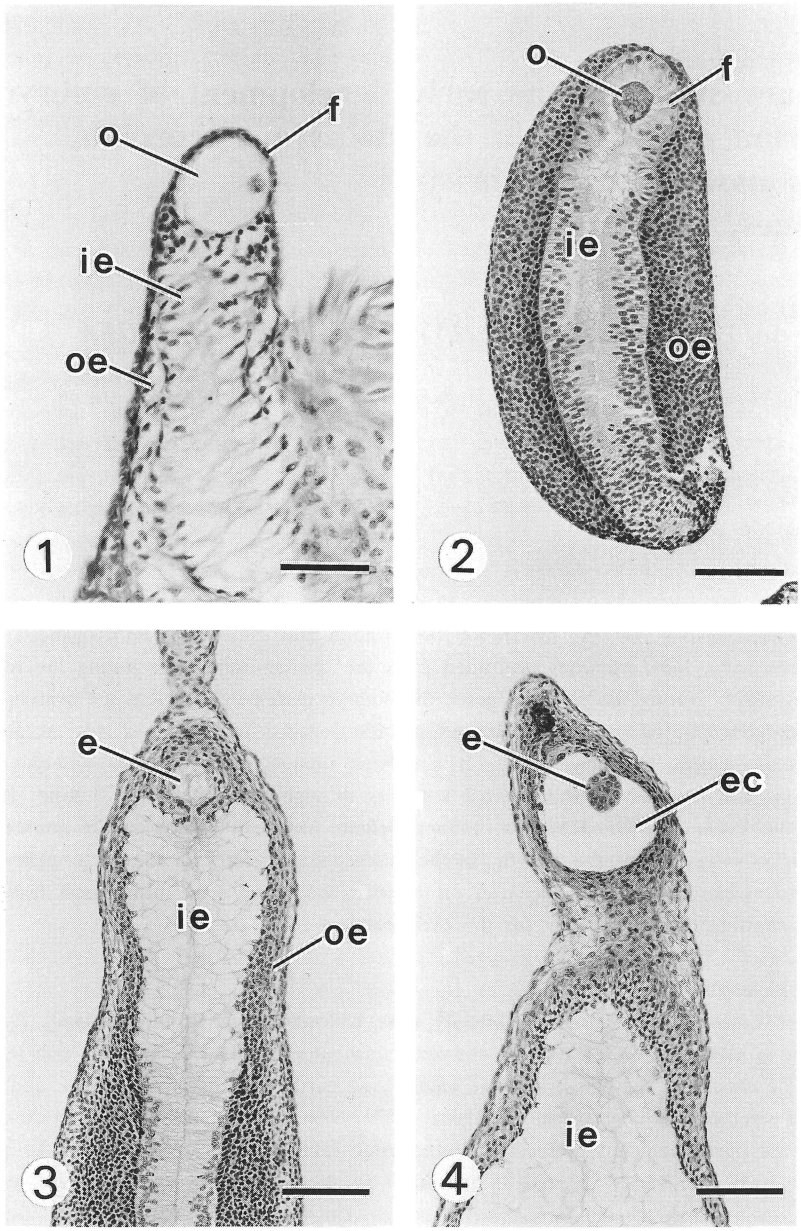
In viviparous scorpions, the egg maturation, fertilization and subsequent embryogenesis take place in the ovarian diverticula, blind blanches protruded from the ovarian tubes constituting the reticular-shaped ovary (Franke, 1982). During the embryogenesis, the embryo extremely grows in the ovarian diverticulum, which begins to grow and to form a cord-shaped vascular process, "an appendix", on its distal end prior to the embryonic growth (Mathew, 1956). It has been demonstrated that the embryos at the morphogenetic stages are nourished with maternal nutrients through the appendices (Laurie, 1891; Mathew, 1948, 1959, 1968; Vachon, 1953). However, nothing definite has been known on the nourishment for the embryos at earlist stages before the growth. In the course of our study on the early embryogenesis of a thelytokous and viviparous scorpion *Liocheles australasiae*, we found some histological features of diverticula possibly showing the nourishment for the early embryos.

Materials and Methods

Females of *Liocheles australasiae* (Fabricius) were collected from Iriomote Island, Ryukyu, Japan, separately kept in glass vials in a dark and moistened incubator of 28±1℃, and fed with termites once a week. Under the condition, they grew, became mature and parthenogenetically pregnant, and gave birth to juveniles about eight months later (Makioka, 1992a, 1993). Next embryogenesis started in the females about 20 days after the parturition and progressed at the same rate as the former one. In the present study, the females of 1, 18, 21, 24, 27, 29 and 31 days after the last parturition were dissected in a physiological saline, and the ovaries were removed from the bodies. The ovaries dissected out were fixed with Bouin's solution, dehydrated in a graded ethanol-*n*-butanol series, and embedded in paraffin. Serial sections of 3 μm thick were stained with Mayer's haematoxylin and eosin.

Results

The ovary one day after the parturition had 20-30 empty ovarian diverticula as remnants of the last pregnancy and a number of young ovarian diverticula preserved for the following pregnancies. The wall of the ovarian diverticula consisted of a layer of the inner thick columnar epithelium and several layers of the outer thin stratified epithelium (Fig. 1), both of which continued with the inner and the outer epithelia of the ovarian tube, respectively, at the base of the diverticulum. A fully grown alecithal oocyte, about 50 μm in diameter, with a distinct nucleolus in a large spherical nucleus was located at the apical part of young ovarian diverticulum of about 225 μm in length and 75 μm in width. The oocytes were surrounded by a



Figs. 1-4 Sagittal sections of growing ovarian diverticula including a fully grown oocyte (Figs. 1 and 2) or an early embryo (Figs. 3 and 4). 1. Ovarian diverticulum one day after the last parturition. Scale bar = 50 μm. 2. Rapidly growing ovarian diverticulum 18 days after the last parturition. An oocyte in maturation is surrounded by a thickening follicle epithelium. Scale bar = 100 μm. 3. Distal half of ovarian diverticulum 24 days after the last parturition. The inner diverticular epithelium starts to vacuolize at the distal part of the diverticulum. Scale bar = 100 μm. 4. Distal end of ovarian diverticulum 31 days after the last parturition. Embryo develops in an expanded embryonic cavity. Scale bar = 100 μm. e: embryo, ec: embryonic cavity, f: follicle epithelium, ie: inner diverticular epithelium, o: oocyte, oe: outer diverticular epithelium.

thin follicle epithelium, about 2.5 μm in thickness (Fig. 1). At the base of the oocyte, the follicle layer continued with the distal end of the inner diverticular epithelium, and at the apical part, the folliculated oocyte was covered by the outer diverticular epithelium.

In the ovary 18 days after the last parturition, a meiotic figure of the first maturation division was found in the apical periphery of the primary oocyte of about 50 μm in diameter, which was located in the distal part of growing ovarian diverticulum of about 550 μm in length and 260 μm in width (Fig. 2). The inner and the outer diverticular epithelia were thickened into about 50 μm mainly by cell growth and about 75 μm by cell division, respectively. The follicle layer was also extremely thickened up to about 30 μm in thickness (Fig. 2).

In the ovary 21 days after the last parturition, a mitotic figure of the first cleavage was found in the center of the folliculated egg in the growing ovarian diverticulum of about 850 μm in length and about 300 μm in width. At the apex of such a pregnant ovarian diverticulum with an embryo, a cluster of large spherical cells appeared in the outer diverticular epithelium distal to the follicle. Thereafter, the distal end of outer diverticular epithelium of the pregnant diverticulum rapidly elongated to form a cord-shaped appendix, which lacked the inner diverticular epithelium.

In the pregnant diverticulum 24 days after the last parturition, about 900 μm long and about 300 μm wide, the embryo of 8 cell-stage was found in the follicular capsule (Fig. 3). The follicle epithelium was flattened into about 10 μm in thickness. In the distal end of the inner diverticular epithelium connecting with the follicle epithelium, the epithelial cells began to be extremely distended up to about 90 μm in height, storing a number of vacuoles in their cytoplasm. Thereafter, the distension and vacuolization of the inner diverticular epithelium (Fig. 3) successively progressed toward proximally.

In the pregnant diverticulum 27 days after the last parturition, about 1000 μm long and about 350 μm wide, the embryo developed into the early blastula without blastocoel. The distal half of the inner diverticular epithelium was vacuolated and distended, though the proximal half not yet. In the distal end, the distended inner diverticular epithelial cells reduced in volume successively from the distal, possibly by releasing the contents of vacuoles, and the inner diverticular epithelium became thinner up to about 50 μm in thickness. Consequently, a gourd-shaped embryonic cavity appeared around the embryo. It consisted distally of wide cavity surrounded by the extended follicle epithelium and proximally of narrow cavity surrounded by the degenerate inner diverticular epithelium. The embryo floated in the distal wide cavity, about 80 μm in diameter.

In the pregnant diverticulum 29 days after the last parturition, about 1000 μm long and about 350 μm wide, the embryo in the embryonic cavity developed into a spheroidal stereoblastula, about 50 μm in major length. The constriction of the embryonic cavity was gradually weakened, and the proximal part was gradually widened.

In the pregnant diverticulum 31 days after the last parturition, about 1100 μm long and about 360 μm wide, the embryo developed into a spherical gastrula of about 50 μm in diameter. The constriction of the embryonic cavity was completely disappeared, and the cavity was widened into about 120 μm in major length (Fig. 4). The vacuolization and distension of the inner diverticular epithelium nearly reached the proximal end of the diverticulum, while the release of the vacuolar contents and reduction of the vacuolated inner epithelial cells had proceeded only up to the distal quarter of inner diverticular epithelium.

The oocytes observed which were largest in the ovaries just after the last parturition and the gastrulae 31 days later were almost the same in size. The development of the cord-shaped appendices was left to be unfinished, and there were not found any signs supportive of the nourishment through the appendices.

Discussion

In *Liocheles australasiae*, Laurie (1896) observed the ovarian diverticula including embryos in an early morphogenetic stage and the developing appendices, and Pflugfelder (1930) described the embryos and ovarian diverticula in various embryonic stages including early cleavage ones. These and other authors such as Vachon (1953) and Mathew (1956) who studied the development of the ovarian diverticula in viviparous

scorpions, however, paid little attention to the nourishment in the early embryonic stage before the morphogenesis. In the present study, we found a series of structural changes suggesting a secretory process in the inner epithelium of the growing ovarian diverticulum containing an early embryo before morphogenesis. It is noteworthy that a liquid material released from the inner epithelial cells of the ovarian diverticulum flew into the embryonic cavity.

In the viviparous scorpions, it has been known that the embryo in morphogenesis extremely grows to fill the ovarian diverticulum (Laurie, 1896; Pflugfelder, 1930; Mathew, 1956; Makioka, 1992a, b). Therefore, it may be supported that the embryos are nourished with maternal blood including nutrients essential for their morphogenesis through the appendix of ovarian diverticulum. In the present study, however, the early embryos were not found to grow in the embryonic cavities. We may presume that the early embryos gain nutrients for the early embryogenesis from the secretory fluid of the inner epithelial cells, and that the late embryos are supplied with other nutrients for the morphogenesis through the appendices. Further studies should be necessary in particular on the substances included in the fluid secreted from the inner diverticular epithelial cells in the early embryogenetic stages.

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