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HISTOMORPHOLOGY OF FEMALE ORGAN SYSTEM OF A NEMATODE *OESOPHAGOSTOMUM COLUMBIANUM*

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The telogonic ovary is enveloped by a thin epithelial layer and contains various stages of developing oocytes attached to a central cytoplasm rachis. Oviduct is muscular and acts just as a passageway. The oviduct-uterine junction is consisted of large oval cells acting as a sphinctor. Anterior region of the uterus is modified to act as a fertilization chamber and is lined by a delicate membrane under the epithelium. Uterine epithelium of the mid region is secretory in nature and is responsible for the formation of the outerwall of the egg sheli.

INTRODUCTION

Different aspects of the reproductive system of nematodes are discussed by a host of workers, Anya (1964), Wharton (1979), Wu & Foor (1983), Weber (1987) and Mackinnon (1987), but the histomorphology of the complete genital tract is studied only in a limited number of species, there too, the observations of the earlier workers appear contradictory. Keeping in view the histological variations occuring in the nematode reproductive organ systems, *Oesophagostomum columbianum* was selected for the present endeavour. This study would be helpful in clearing some of the hitherto persisting gaps in information.

MATERIAL AND METHODS

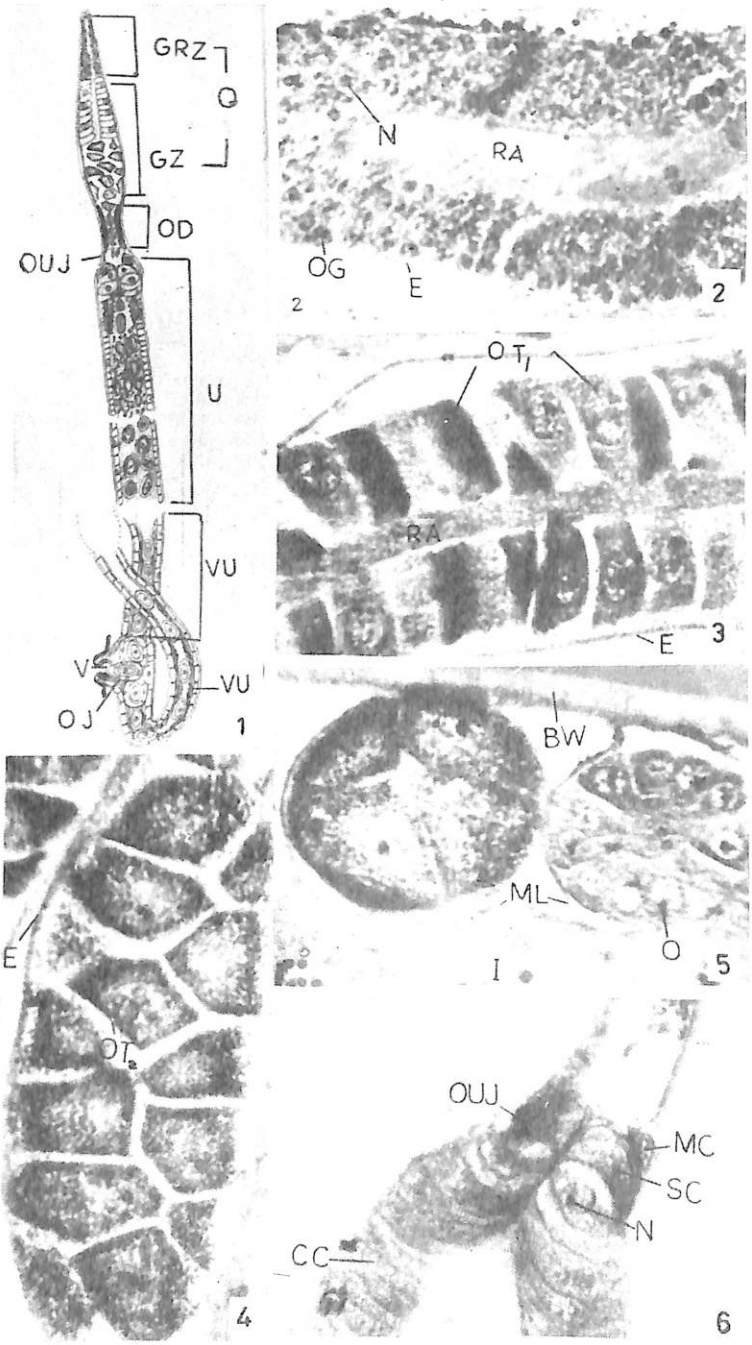
The parasites were recovered from the large intestine of sheep slaughtered in local abattoirs. The female worms were separated, washed in 0.85% saline, stretched in iced water and fixed in Bouin's fluid. The material was processed for sectioning and for histological studies, the slides were stained in Heidenhain's hematoxylin. To study the topography of the female reproductive system, some of the specimens were stained in 0.1% Methylene blue at 60°C and cleared in lactophenol.

OBSERVATIONS

Female reproductive system of *O. columbianum* is didelphic *i.e.* having two sets of reproductive organs opening externally through a common vulva situated at the mid-ventral line about 1200-1250 μ anterior to the tip of the tail. In the vulvar region the reproductive system is amphidelph with uteri opposed, the anterior uterus extends anterior and posterior, after leaving the ovejector immediately makes a u-turn and comes to lie parallel to the anterior uterus. Each set of the reproductive organs includes a long tubular and reflexed ovary, a narrow oviduct followed by a uterus and a vagina opening into the common ovejector which communicates externally through vulva (Fig. 1).

Ovary : Each ovary is telogonic, *i.e.* the germ cells proliferate at the anterior end, the germinal zone, which is followed by a long growth zone. In the germinal zone, rapid division of relatively smaller cells takes place. It is enveloped by a thin epithelial layer. The proliferating germ cells are arranged around a central axis the rachis. The rachis is a non-nucleated protoplasmic cord extending in 3/4 of the ovary (Figs. 1 & 2).

In the proximal region of the growth zone, oocytes are attached to the rachis at their bases (Fig. 3), as they increase in size, their connection with the rachis weakens and ultimatley disrupts, thus



Figs. 1-6. 1. Female reproductive system of *Oesophostomum columbianum*; **2.** L.S. of germinal zone; **3.** L.S. of growth zone; **4.** L.S. through last portion of ovary; **5.** Section showing mesenterial layer; **6.** L.S. showing oviduct-uterine junction (x 200).
(BW = Body wall; CC = Columnar cells; E = Ovarian epithelium; GRZ = Germinal zone; GZ = Growth zone; I = Intestine; MC = Sphincter muscle cells; ML = Mesenterial layer; O= Ovary; OD = Oviduct; OG = Oogonia; OJ = Ovejector; OT_1 = Primary oocytes; OT_2 = Secondary oocytes; OUJ = Oviduct-uterine-junction; RA= Rachis; SC = Sphincter cells; U= Uterus; V = Valva; VU = Vagina uterina)

leaving them free in the lumen (Fig. 4). The diameter of the proximal region of the growth zone measures 125 μ and the size of the oocyte is 35 × 30 μ. But, there is a progressive increase in the diameter of ovary (180 μ) and the size of oocytes reaching 90 × 83 μ accompanied by the thickness of epithelial wall, in the region prior to the beginning of the oviduct. In the growth zone the bounding epithelial layer becomes comparatively thicker. The nuclei of the cells are very prominent (Figs. 3 & 4). The ovary is held in position by the mesenterial membrane of the same nature, that envelops the intestine (Fig. 5).

Oviduct : The oviduct is a narrow tube and seems to act just as a passageway for the mature oocytes (Fig. 8). The oocytes are arranged in a single file to squeeze their way to the uterus (Fig. 7). The wall of the oviduct is muscular and comparatively thicker than the ovary. The diameter of the oviduct is 26 μ, it opens into the uterus through the oviduct-uterine junction (Fig. 6).

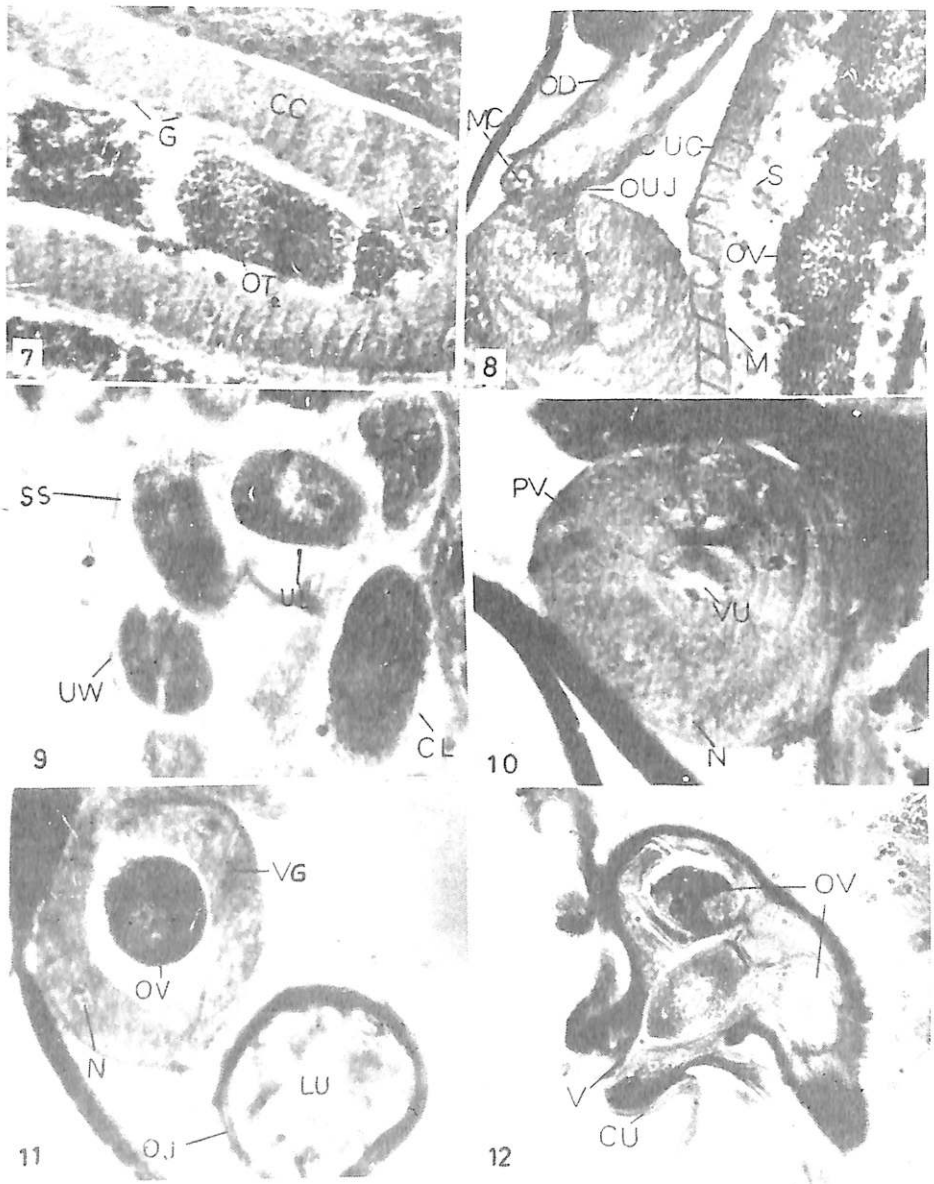
Oviduct-uterine junction : The epithelial cells of the oviduct-uterine junction are large, thick and oval in shape and possess prominent nuclei. In addition a pair of muscle cells is present at the terminal end of the oviduct (Figs. 6 & 8). The epithelial walls are quite distensible as to allow the passage of oocytes through them, otherwise, they lie in close approximation forming a sort of sphinctor. As the oocytes pass through the oviduct and oviduct-uterine junction, their hexagonal shape is transformed into an oblong one.

Uterus : The anterior part of the uterus is thick walled composed of columnar cells with rounded nuclei filled with chromatin granules. On the inner surface of these cells is present a delicate membrane with dark secretory granules adhering to it (Fig. 7). The next portion of the uterus acts as a seminal receptacle or fertilization chamber, this division of uterus into functional units is without any perceptible boundaries. The wall of seminal receptacle is composed of cuboidal epithelial cells (Fig. 8). It is also lined by a delicate membrane. The lumen of the uterus is full of eggs, spermatids and spermatozoa.

The last part of the uterus lodges the fertilized ova in it. Fertilization initiates the egg shell formation and maturation of the ova. The uterine wall in this region is secretory in nature and the secretion is mainly proteinaceous which forms the outer wall or uterine layer of the egg shell (Fig. 9).

Uterine tube shows some flexures and opens into the vagina uterina. The cells forming vagina uterina are basically of the same type as those forming the wall of the uterus but the lumen is very narrow (Fig. 11), in which the ova are arranged in a single file. This arrangement helps the eggs to orientate themselves for their expulsion through chambered ovejector and also gives them a final characteristic shape (Fig. 1). The length of vagina uterina is 458 μ and near its opening to the muscular ovejector its lumen is greatly reduced so as to form a slit within the valve like pulvillus. The pulvillus is made up of about four concentric rings of sausage shaped cells, each ring containing four cells and the total number being 16 (Fig. 10). This dilated bulb like structure represents the junction of vagina uterine with vagina vera.

Ovejector : Vagina is modified into an ovejector shaped like an inverted triangle with a broader dorsal region narrowing gradually towards the ventrally situated vulva (Fig. 12). Broader upper portion is 410 μ diameter with 45 μ thick walls. The ovejector has a crossed lumen (Fig. 11). Each arm of the cross dilates to form a knob-like compartment where the egg to be ejected out comes to lie. The lumen is surrounded by four epithelial cells which in turn are surrounded by a powerful muscular layer. About 16 cells are involved in its formation. The ova are ejected through the vulva by their own pressure coupled with the contraction of the vulvar muscles. The vulva is lined by an invagination of the external cuticle layer (Fig. 12).



Figs. 7-12. 7. L.S. of Proximal part of uterus; **8.** L.S. of oviduct, its juction with uterus; **9.** L.S. of secretory portion of uterus; **10.** T.S. of pulvillus (PV) having concentric rings of cells; **11.** T.S. of vagina (V) having thick cells; **12.** T.S. of ovejector (x 200).
(CC = Columnar secretory cells; CL = Chitinous layer; Cu = Cuticle; CUC = Cuboidal cells; G = Grannule; OD = Oviduct; OJ = Ovejector; OT_2 = Oocytes; OUJ = Oviduct-uterine-junction; OV = Ova; Lu = Lumen; M = Membrane; MC = Muscle cells; N = Nuclei; PV = Pulvillus; S = Sperms; SS = Secretory strand; UL = Uterine layer; UW = Uterine wall; V = Vagina; VU = Vagina uterina)

DISCUSSION

In the ovary both oogonia and oocytes are attached to a central rachis by cytoplasmic bridges which is a common feature of all telogonic forms (Wu & Foor, 1983), but the size and form of rachis varies from species to species. In *O. columbianum* the rachis is a thick, non-nucleated protoplasmic cord originating at the germinal zone and extending to some length in the initial part of growth zone.

Functionally, the rachis appears to be just a supporting structure necessary to maintain the basic spatial arrangement of oocytes and preventing the migration of immature oocytes to the oviduct.

The oviduct is a narrow tube consisting of high columnar epithelium and no muscular layer except for the zone approaching the uterus (Musso, 1930). But, in *Aspiculuris tetraptera*, the oviduct is bounded by cuboidal epithelial cells whose length exceeds their height. It moulds the eggs into their proper shape (Anya, 1964). In *O. columbianum*, however, the oviduct is a narrow tube which acts just as a passageway for oocytes. It is bounded by epithelial layer similar to that of ovary but the myofibrillar bundles present in the luminal border of the oviduct make it capable of expansion. A pair of muscle cells present at the oviduct-uterine junction acts like a sphinctor and operates the uterine opening.

The function of the cells of oviduct-uterine junction is still under debate. Earlier, these cells were regarded as nurse cells (Leuckart, 1876), however, later, it was thought that they serve to engulf old unused spermatozoa (Romieu, 1911). In addition to more obvious morphological changes, according to Wu & Foor (1983), the antigenic determinants on the surface of the mature oocytes collected from the oviduct-uterine junction differ significantly from those present in the immature oocytes they suggest that this change is under the influence of the secretions of oviduct-uterine cells.

The epithelial cells of oviduct-uterine junction in *O. columbianum*, are quite distinct from the general uterine epithelium. These are thick, oval and oriented in such a way as to form a sphinctor which allows the passage of the oocytes in one direction only. At rest, the cells of the junction lie in close approximation but their contraction can make a passageway large enough for the oocytes to pass. This distensibility is regulated by the pressure of oocytes against the walls of oviduct-uterine junction and also by the action of a pair of muscle cells present in antero-lateral position of the junction. The cells of the anterior-most region of the uterine wall are elongated, columnar with densely granular cytoplasm. On their luminal border, densely staining secretory granules are present. The possible function of their secretion in *O. columbianum* is to revitalize the oocyte before fertilization.

The wall of the uterus becoms comparatively thinner and is composed of cuboidal epithelium in the region of the fertilization chamber. A very thin membrane lines the luminal border of epithelium which probably prevents the anchoring of spermatozoa to the epithelial cells and thus assures the fertilization of oocytes. A similar interpretation has also been given earlier by Wu & Foor (1983) to the presence of surface coating membrane present in the oviduct-uterine junction of *Ascaris suum*. Beyond the area where fertiiization takes place, the uterine wall becomes immensely active and secretes the outer protein coat of the egg shell. In *Ascaris* this area is rich in mitochondra, golgi complex, rough endoplasmic reticulum and glycogen & is referred as quadricollumella by Foor (1967). The cells of this area are considered responsible for secreting the uterine layer of egg shell (Eharton & Jenkins, 1978; Adamson, 1983). But the actual process of secretion of the uterine wall is not seen by any of these workers.

In *O. columbianum*, thick strands of colourless jelly-like material are actually seen emerging from the uterine wall. These strands envelop the eggs rather loosely but as the oocytes roll down the uterus, the shell becomes compact. Rest of the uterus acts as a storage space for ova before these are passed to vagina. The thick and muscular vaginal wall regulates the passage of eggs in a single file and also gives the characteristic oval form to them. The further moulding of egg shell is effected by the chambered ovejector.

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