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RESEARCH ARTICLE

LIGHT MICROSCOPIC STUDY ON THE OVIDUCT AND UTERUS OF *HAEMONCHUS CONTORTUS* (NEMATODA)

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ABSTRACT

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materials necessary for the formation of outer uterine layer of the egg shell.

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INTRODUCTION

The structural variations in the epithelial lining of female genital tract of nematodes have been studied by a host of workers in different species of nematodes. Lalchhandama (2010) found that in Ascaridia galli, the wall of the uterus is comparatively thicker than that of the ovary and oviduct. Brunanska (1997) studied the fine structure of oviduct of Toxocara canis and described columnar epithelial cells with many electron dense bodies and also the presence of numerous microvilli at the apical membrane bordering the oviduct lumen. Anya (1964) described that the wall of anterior portion of the uterus has cuboidal epithelial lining with rounded apical portions. The succeeding area of uterus was considered to be responsible for the secretion of uterine layers of egg shell in a microscopic study conducted by Wharton (1979). On the contrary, Takahashi et al. (1995) described that the cells lining the uterus of Trichinella spiralis are very thin and devoid of any secretory granules. Haemonchus contortus (Rudolphi, 1803) is a highly pathogenic blood-feeder helminth that causes anaemia and reduced productivity and can lead to death of infected animals (Burke et al., 2007). Over years attempts have been made to study various aspects of Haemonchus spp. from different parts of the world (Sood, 2006). Previously, the histomorphology and histochemistry of various organ-systems

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Department of Zoology, Baring Union Christian College, Batala-143505, Punjab, India. of *Haemonchus contortus* has been studied by Singh and Johal (1997), Singh (2000), Singh and Johal (2001a; 2001b; 2001c and 2004). In the present investigation, attention is directed to the microscopic structure of the oviduct and uterus of this pathogenic nematode.

MATERIALS AND METHODS

In Haemonchus contortus, the wall of the oviduct is thick and muscular, acting as a mere passageway

for the oocytes. The seminal receptaculum is not a distinct structural entity but a continuity of the

distal portion of the uterus. The epithelial lining near the ovo-uterine junction is of oblique columnar

type followed by cuboidal type of uterine epithelium which becomes secretory in the mid region and muscular near the vaginal end. The secretory epithelium of middle portion of the uterus secretes

The adult female *Haemonchus contortus* extracted from the abomasum of sheep (*Ovis aries*) were washed in 0.85% NaCl solution to remove debris. For whole mount preparation, after fixation in 70% alcohol at 60 0 C, the nematode worms were cleared and mounted in lectophenol. For histomorphological studies, each worm was fixed in alcoholic Bouin's fixative for 12-24 hours, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 7 μ m in transverse and longitudinal planes. The serial sections arranged on slides were stained with haematoxylin and eosin. The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

Reproductive system of female *Haemonchus contortus* is diorchic i.e. the presence of two sets of reproductive organs. It is amphidelphic with uteri opposed, the anterior uterus extends anteriad and the posterior uterus makes a U-turn at some distance from the vulva and then runs anteriad. The oviduct is a narrow tube (46.6 μ m in diameter) extending between the

ovary and anterior part of uterus, the seminal receptaculum. The oocytes align themselves in a single file inside its lumen. Its wall is thick and muscular as compared to that of the ovary (Figs. 1 and 2). The distal portion of uterus is modified into a seminal receptaculum. It is distinguished by the presence of a thick wall composed of oblique high columnar epithelium and some muscle fibers. The ovo-uterine junction is provided with a sort of sphincter which can be opened by the pressure of ova lying in the oviduct and closes immediately to ensure the fertilization (Figs. 2 and 3). A large number of ova and spermatozoa are seen lying in its lumen (Fig. 4). In fact whole of the uterine tract shows the presence of migrating spermatozoa, only the number varies being maximum in the seminal receptaculum (Fig. 4) or near the vulva just after copulation. The succeeding portion of uterine wall is composed of flat cuboidal epithelium (Fig. 5) changing into a thin secretory one (Fig. 6) From the surface view, transversely elongated uterine cells with large nuclei full of chromatin granules and cytoplasm full of secretory granules are visible (Fig. 8).

The secretory epithelium of middle portion of the uterus secretes materials necessary for the formation of outer uterine layer of the egg shell (Fig.6 and 7). The posterior part of the uterus is modified into a muscular ovijector (Fig. 5 and 7). Musso (1930) considers the oviduct as a narrow tube like structure consisting of high columnar epithelial cells and wanting of muscular layer except for the zone approaching the uterus. But in Aspiculuris tetraptera the oviduct consists of cuboidal epithelial cells whose length exceeds their height and their margins interdigitate, thus conferring a power of dilation to the tissue. The cells have prominent nuclei and are bounded by a basal membrane. It does not appear that the cells are secretory nor do they contain any appreciable stores of reserve material. Their main function is probably that of moulding the eggs into their usual shape and providing the right environment in the lumen for the initiation of certain processes prior to and after fertilization (Anya 1964). According to Adamson (1983), in *Gyrinicola batrachiensis*, the oviduct is bounded by simple cuboidal epithelium, encircled by a basal lamina.



Fig.1-8: *Haemonchus contortus* (All slides stained with Haematoxylin-Eosin staining). Fig.1. A portion of T.S. of female showing oviduct. Fig.2 & 3. L.S. of female showing narrow oviduct, ovo-uterine junction and seminal receptaculum. Fig.4. L.S. of female showing seminal receptaculum. Fig.5, 6 & 7. L.S. of female through uterus showing uterine epithelium. Fig.8. A portion of L.S. of female revealing surface view of uterine wall.

The cells are secretory since small electron-dense granules are present throughout the cytoplasm and also line the lumen. In Loa loa (Weber, 1987), the short oviduct has greatly thickened epithelial cells as compared to the ovary but the thickness of the basal lamina is the same as that of the ovary. The author has stated that although the structure of the oviduct seems to be derived from that of the ovary, yet it becomes so highly specialized that it is appropriate to consider it as a different structural entity rather than a mere functional part of the ovary. In Oesophagostomum columbianum, Johal (1988) too, has reported that the narrow oviduct tube is bounded by epithelial cells similar to those of the ovary but in addition some myofibrillar bundles are seen on its luminal border. According to Brunanska (1997) in Toxocara canis, the oviduct is bordered by columnar type epithelial cells with numerous microvilli at the apical membrane bordering the oviduct lumen.

In Haemonchus contortus, the wall of the oviduct is thick and muscular as compared to the ovarian wall. It is composed of cuboidal epithelium and a muscular layer. It is evident from the above mentioned findings that a number of diversifications exist in the bounding membranes of the oviduct in different species of nematodes which may be related to its functional aspects whether it acts as a mere passageway or helps in maintaining the shape of the eggs or functioning as a secretory structure. In many nematodes, a specialized area known as seminal receptaculum or fertilization chamber is present between the oviduct and the uterus (Rauther, 1918; Vogel, 1925; Chitwood and Chitwood, 1950 and Bird and Bird, 1991). Looss (1905) has stated that in the absence of a morphologically distinct seminal receptaculum, an area of the uterus adjacent to the oviduct may be modified to perform the function of receiving the sperms. Vogel (1925) suggested that the seminal receptaculum in Syphacia obvelata probably functions as a shell gland. According to Chitwood and Chitwood (1950), the nematode spermatheca is a specialized organ of the female reproductive tract wherein the fertilization is traditionally believed to occur and it is also the site of initiation of egg-shell synthesis. Pertaining to her studies on Aspiculuris tetraptera, Anya (1964) has described that the wall of the seminal receptaculum consisted of a single layer of cuboidal epithelial cells containing numerous mitochondria and microvilli on their inner surface. The cells are different from those of the oviduct. Their free surfaces are often rounded or hemispherical, the whole arrangement being such as to exclude much dilation of the lumen.

Wharton (1979) in Aspiculuris tetraptera maintains that the seminal receptaculum appears to be an extension of the oviduct, as both are bounded by cuboidal cells containing numerous mitochondria, glycogen reserves and microvilli on their inner surface. According to Preston and Jenkins (1984), the spermathecal lining of Trichuris muris is composed of tall columnar epithelial cells. The apical portion of these cells are modified to form finger-like projections and large numbers of electron-dense lysosome like bodies and vesicles containing membranous whorls are connected to these. The basal portion of these cells are deeply inserted into a basement layer which also encompasses 2-3 external muscle layers. The presence of pseudopodial projections and lysosomal-like inclusions at the cell apices also indicate a potential phagocytic role for the spermathecal epithelium. Based on their studies on Ascaris suum, Wu and Foor (1983) have suggested that in the absence of a distinct seminal receptaculum the sperms may congregate in the region of oviduct-uterine junction awaiting the passing

mature oocytes, thus assuring fertilization. Cross-section of the oviduct-uterine junction reveals elongate epithelial cells distinctive in ultrastructure from the cells of adjacent areas and surrounded by a muscular layer. In *Loa loa*, Weber (1987) has depicted that the seminal receptaculum possesses a thinner basal lamina than the oviduct and its epithelium contains a weakly developed basal labyrinth in its outer zone.

The present author maintains that in *Haemonchus contortus* the distal portion of the uterus is modified to perform the function of a fertilization chamber. It is not a distinct structural entity but a continuity of the uterus although its epithelial lining near the oviduct-uterine junction is modified to form oblique columnar type cells rather than the cuboidal ones. The oblique cells form a sort of constriction through which the ova are passed singly into the uterus. In the fertilization chamber itself the cells are of simple columnar type. The appearance of the spermatozoa stored in the fertilization chamber is similar to that of the mature spermatozoa in the male genital tract. Looss (1905) in Ancylostoma duodenale has reported two cell rows forming the uterine epithelium. The uterine epithelium is described to be composed of five or six sided irregular or high cuboidal cells in Ascaris (Musso, 1930). A squamous epithelium is reported in the uterine wall of Aspiculuris tetraptera by Anya (1964). It is capable of great extension and there are usually four of these cells in a cross-section which are enclosed by strands of muscle fibres. However, in the upper uterus it is found to be secretory in nature. A secretory epithelium containing numerous mitochondria, large glycogen reserves and having numerous microvilli at its inner surface is reported by Wharton (1979) in Aspiculuris tetraptera. In Gyrinicola batrachiensis, Adamson (1983) has described a thin discontinuous layer of smooth muscle cells, in addition to a layer of simple epithelium and basal lamina enveloping the uterine wall. The microvilli are found to be longer and more numerous in the distal uterus (near oviduct) than in the proximal uterus (near vagina). In Trichinella spiralis, Takahashi et al. (1995) have reported that the thin uterine epithelial cells in this nematode are devoid of secretory granules and are circumscribed at regular intervals by thin layered contractor muscles which enable the uterus to move in a peristaltic fashion, thus propelling the embryos towards its anterior side.

In *Haemonchus contortus*, the uterine wall can be differentiated functionally as well as histologically into three separated regions. In the region of fertilization chamber it is lined by tall columnar cells which form a sort of sphincter to control the passage of eggs to ensure the fertilization. In the mid region the cuboidal epithelium secretes a large number of granular aggregations for the formation of outer uterine layer of the egg shell. The uterus becomes slightly muscular near the vaginal end for the propulsion of the eggs.

Abbreviations used:

CSR: Constriction between uterus and ovijector; COC: Oblique Columnar Cells; CUC: Cuboidal Cells; MW: Muscular Wall; N: Nucleus; NU: Nucleolus; OD: Oviduct; ODW: Oviduct Wall; OJ: Ovijector; OUJ: Ovo-Uterine Junction; OV: Ovum; PU: Primordium of Uterine Layer; RGR: Ribosomal Granules; SE: Secretory Epithelium; SEG: Segmented ova; SMC: Sphincter Muscle Cell; SR: Seminal Receptaculum; SZA: Spermatozoa; UL: Uterine Layer of egg shell; US: Uterine Secretion; UWC: Uterine Wall Cells; VMR: Vitelline Membrane.

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