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THE DISTRIBUTION OF CARBOHYDRATES, PROTEINS AND LIPIDS IN THE FEMALE GENITAL TRACT OF *HAEMONCHUS CONTORTUS* (NEMATODA)

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ABSTRACT

In *Haemonchus contortus*, the concentration of various metabolites differs in different regions of female genital system. An adequate quantity of carbohydrates is found in the ovarian epithelium. Though a high quantity of glycogen is evidenced in ovarian epithelium and rachis but the protoplasmic processes connecting the oogonia to the rachis are completely devoid of the same. Both structural as well as cytoplasmic proteins are found in abundance in the female reproductive organs. The ovarian epithelium of the growth zone in particular depicts a high concentration of general as well as – NH2 bound proteins. The concentration of proteins gradually decreases in the epithelium of growth zone of ovaries, seminal receptaculum and uterus. The genital epithelium is basically lipoidal in nature and lipids seem to be a major constituent of the outer fibrous layer of vagina.

Key words: Histochemistry, Female genital tract, Nematoda, Haemonchus contortus.

INTRODUCTION

Haemonchus contortus is the trichostrongylid nematode occurring in the mucosa and contents of abomasum portion of stomach of sheep and other ruminants. It has been ranked as the most important parasite of small ruminants in all tropical and temperate areas of the world (Sood, 1981; Meeusen, 1996). Haemonchosis, the disease caused by this nematode is responsible for considerable disease, poor milk yield and wool production. Medium infection causes sheep to lose condition and heavy infection may prove fatal. The death of the host may occur within one week without significant signs (Fraser, 1991). Previously, the histomorphological studies on the various organ- systems of *Haemonchus contortus* were performed by Singh and Johal (1997), Singh (2000), Singh and Johal (2001 a, b and c), Singh and Johal (2004) and Singh (2015 a, b and c). The present research paper describes the distribution of carbohydrates, proteins and lipids in the female genital tract of this pathogenic nematode. This histochemical study will fill the hitherto existing gaps in information about this aspect. Moreover, it can form the basis for the development of effective chemotherapeutic measures against this endoparasitic nematode.

MATERIALS AND METHODS

The adult females of nematode *Haemonchus contortus* were extracted from the abomasum portion of stomach of sheep (*Ovis aries*). In order to remove debris, the nematode worms were washed in 0.85% NaCl solution. For histochemical studies, the worms were fixed in alcoholic Bouin's fixative and Carnoy's fixative, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 8µm in transverse and longitudinal planes by using rotary microtome. The serial sections arranged on albuminised slides were stained with following staining methods. General carbohydrates were studied by Periodic acid Schiff's staining technique (McManus, 1948). Glycogen was detected histochemically by Best's carmine staining (Best, 1906) and acid mucopolysaccharides by Alcian blue (Steedman, 1950). For the localization of proteins, Mercuric bromophenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa, 1953) were used. The histochemical presence of lipids was detected by Sudan black B staining (McManus, 1946) and Oil red O in isopropanol (Lillie and Ashburn, 1943). The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

An adequate quantity of carbohydrates is found in the ovarian epithelium of *Haemonchus contortus* as evidenced by Periodic acid Schiff's staining (Fig. 1). Glycogen is seen in the wall of rachis whereas the protoplasmic processes connecting it with oogonia are devoid of it (Fig. 2). Both structural as well as cytoplasmic proteins are found in abundance in the female reproductive system of *Haemonchus contortus*. The bounding epithelium of germinal region near the tip of the ovary is not well separated off and shows a profuse and rachis a moderate concentration of proteins (Figs. 3 and 4). Little further down, the ovarian epithelium is well demarcated and depicts a high

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concentration of protein along with the basal lamina which also is strongly positive for – NH_2 bound proteins (Figs. 5, 6 and 7). By making a comparative observation it is found that in the genital tract of *Haemonchus contortus* the concentration of protein gradually decreases in growth zone of the ovary, seminal receptacle and the uterus (Fig. 5, 6 and 8). In the vaginal region, general as well as – NH_2 bound proteins are observed in the luminal border as well as basal region of fibrous layer, whereas the outer peripheral portion has a poor supply of the same (Figs. 7 and 9). Both the ovarian as well as the uterine wall are lipoidal in nature (Fig. 10). In the vaginal region, some lipid is observed in the epithelial layer lining the lumen. Some septa observed in the middle layer are also lipoidal and a high concentration of lipid is seen in the outer fibrous layer of the vagina (Fig. 11).

A fair amount of glycogen has been reported by Lee (1960) in the epithelial cells of the germinal zone of ovary followed by a decrease in its concentration towards the growth zone, till it becomes totally lost. The oviduct is described to be free from the glycogen but small amount of the same appears in the uterine epithelium and the ovijector. However, a complete absence of glycogen in the epithelial cells of ovary of *Aspiculuris tetraptera, Loa loa* and *Tanqua anomala* has been reported by Anya (1964 a), Weber (1987) and Kankal (1989) respectively. Anya (1964 b) has described large deposits of glycogen in the uterine wall and muscular layer of ovijector in three species of Oxyuroids, *Aspiculuris tetraptera, Enterobius vermiculuris* and *Syphacia obvelata*. In *Paranisakis kherai*, Gupta and Garg (1976) described the presence of polysaccharides with 1:2 glycol groups from the wall of both oviduct and uterus.

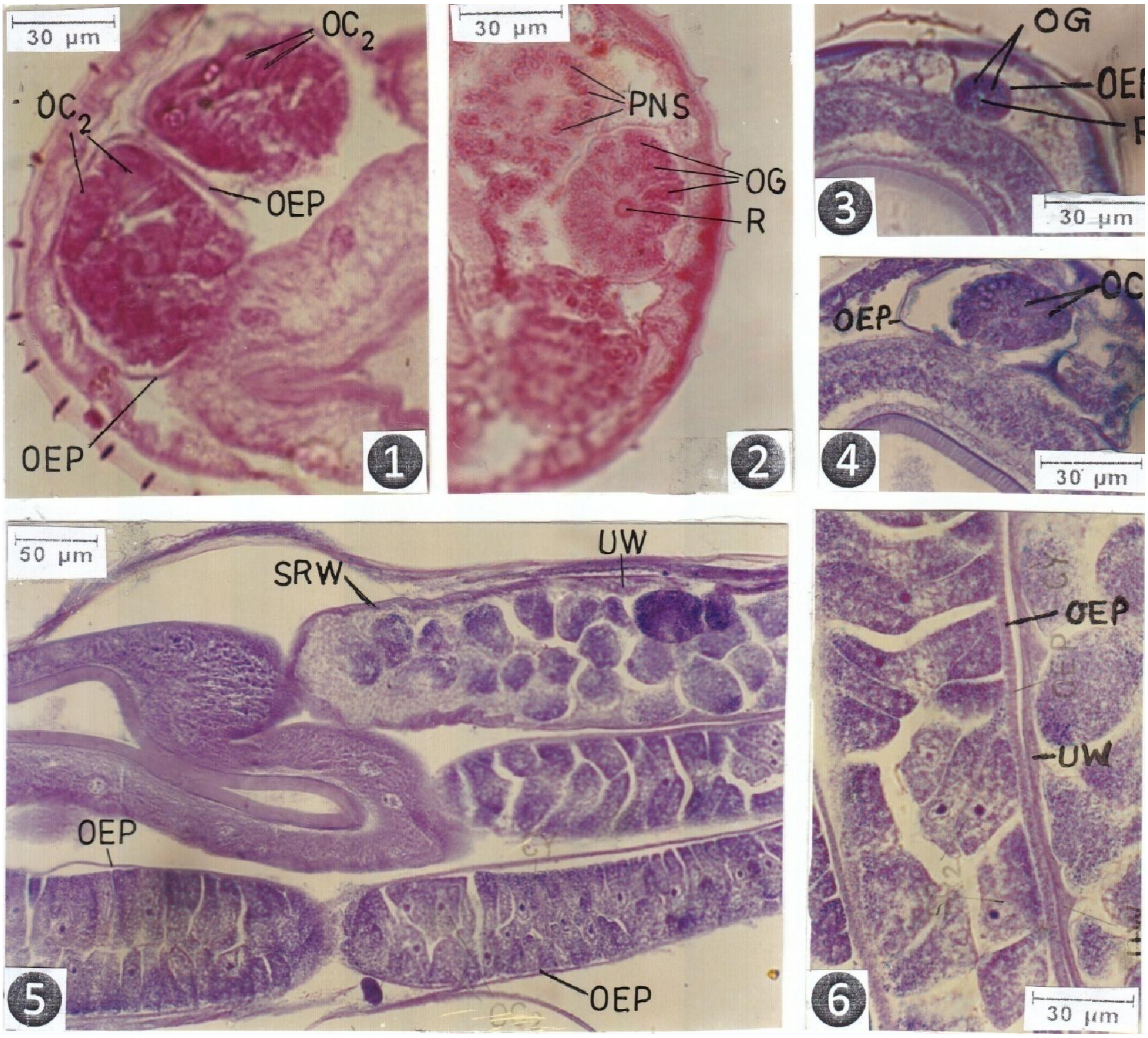

Fig. 1-6. *Haemonchus contortus*

Fig. 1. T. S. of female showing concentration of carbohydrates in the ovarian epithelium (OEP) and secondary oocytes (OC_2) (Periodic acid Schiff's staining);
Fig. 2. T. S. of female showing concentration of glycogen in the perinuclear spaces (PNS) of oogonia (OG) and rachis (R) (Best's carmine staining);
Fig. 3 and 4. T. S. of female showing concentration of proteins in the ovarian epithelium (OEP), oogonia (OG), primary oocytes (OC_1) and rachis (R) (Mercuric bromophenol blue staining)
Fig. 5. A Portion of longitudinal section (L.S.) of female showing distribution of proteins in the ovarian epithelium (OEP) of growth zone, wall of seminal receptaculum (SRW) and uterine wall (UW) (Mercuric bromophenol blue staining);
Fig. 6. A Portion of L.S. of female showing concentration of proteins in the ovarian epithelium (OEP) near the end of growth zone and uterine wall (UW) (Mercuric bromophenol blue staining).

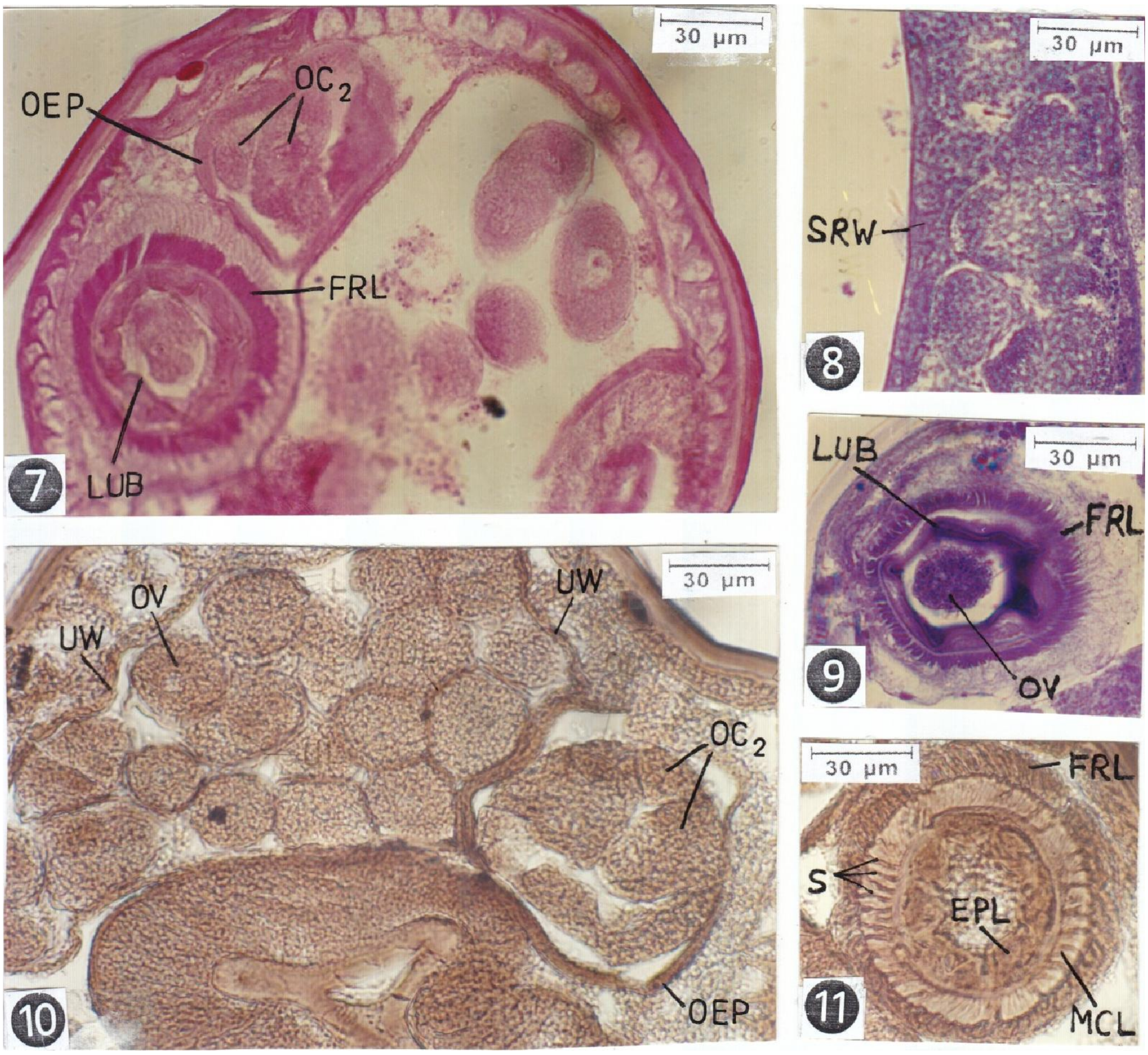

Fig.7-11. ***Haemonchus contortus***

Fig. 7. T. S. of female showing concentration of $-NH_2$ bound proteins in the ovarian epithelium (OEP), secondary oocytes (OC_2) and luminal border (LUB) & fibrous layer (FRL) of vaginal wall (Ninhydrin Schiff's staining);
Fig. 8. A portion of L.S. of female revealing distribution of proteins in the seminal receptaculum wall (SRW) (Mercuric bromophenol blue staining);
Fig. 9. T. S. of vagina of female revealing distribution of proteins in the luminal border (LUB), Fibrous layer of vaginal wall (FRL) and ovum (OV) (Mercuric bromophenol blue staining);
Fig. 10. T. S. of female showing distribution of lipids in the ovarian epithelium (OEP), secondary oocytes (OC_2), uterine wall (UW) and fertilized ova (OV) (Oil red O in isopropanol);
Fig. 11. A Portion of T. S. through vagina of female showing distribution of lipid in the epithelial layer (EPL), septa (S) of the middle muscular layer (MCL) and outer fibrous layer (FRL)of the vaginal wall (Oil red O in isopropanol)

In addition, mucopolysaccharides have been reported in the outer uterine wall of *Setaria cervi* by Gupta and Kalia (1978). Polysaccharides form the main structural constituent of the uterine wall and the epithelial lobes forming the uterovaginal junction of *Trichuris ovis* (Johal and Joshi, 1993). Johal (1995) while working on *Oesophagostomum columbianum* has observed that the genital epithelium of germinal zone of ovary is devoid of carbohydrates whereas an adequate amount of polysaccharides is evidenced in the growth zone which subsequently diminishes further down the ovary. In the present study on *Haemonchus contortus*, a rich quantity of carbohydrates is found in the ovarian epithelium and glycogen too, is detected in the wall of the cylindrical rachis, whereas the uterine wall has a poor supply of it. Like carbohydrates the distribution of protein differs in the various organs included in the genital tract of different nematodes. Complete absence of protein in the ovarian epithelium of many nematode species has been reported by Fairbairn (1957), Anya (1964 a), Wharton (1979) and Adamson (1983). However, the presence of $-NH_2$ and – SS – bound proteins is reported by Gupta and Garg (1976) in the female genital epithelium of *Paranisakis kherai*. A rich quantity of protein forms the main structural constituent of the outer contractile uterine wall and inner cuticular lining of vagina of *Setaria cervi* (Gupta and Kalia, 1978). In *Trichuris ovis*, the ovarian wall as well as the strands of tissue originating from it and connecting the oocytes is positive for proteins (Johal and Joshi, 1993). Johal (1995) in *Oesophagostomum columbianum* has observed that the ovarian epithelium of the germinal zone is the site where no protein is detected, whereas a rich quantity of the same is accumulated in the growth zone. On the contrary, the present study on *Haemonchus contortus* reveals that the germinal zone of the ovaries possesses a rich amount of general as well as $-NH_2$ bound proteins. Their concentration decreases further down the ovary till it becomes negligible in the growth zone. But the uterine epithelium again shows a small quantity of protein in it. Also, general as well as $-NH_2$ bound proteins are found in the luminal border and the basal region of the fibrous layer of vagina. von Kemnitz (1912) has been unable to detect even small traces of lipid in the ovarian epithelium of *Ascaris lumbricoides*. Lee (1960) and Anya (1964 b) have also reported a lipid free genital tract in *Thelastoma bulhoesi* and *Aspiculuris tetraptera*. In *Loa loa*, the ovary is totally free from lipids, however, some lipid droplets are found in the seminal receptaculum (Weber, 1987). Contrary to this, Flury (1912) and Fairbairn (1955) described significant amount of lipid in the female reproductive system of *Ascaris lumbricoides* and *Parascaris equorum* respectively. The significance of stored lipids which can be converted into glycogen whenever required has been discussed by Passey and Fairbairn (1957) and Barrett *et al*. (1970). The storage of lipid in the female reproductive system is accounted by a number of workers such as Beams (1964) in *Ascaris lumbricoides,* Foor (1967) in *Parascaris equorum,* Gupta and Kalia (1978) in *Setaria cervi*, Kankal (1989) in *Tanqua anomala*, Brunanska (1993 and 1994) in *Toxocara canis* and Johal (1995) in *Oesophagostomum columbianum*. Presently, in *Haemonchus contortus*, the ovarian wall, uterus and vagina reveal a rich concentration of lipid which is incorporated in all the stages of developing ova both as a cytoplasmic as well as a structural element.

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