RESEARCH ARTICLE

Histological study on the ovaries of *Haemonchus contortus* (Nematoda)

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ABSTRACT

The didelphic female reproductive system in *Haemonchus contortus* possesses telogonic ovaries, each divisible into a germinal zone and a growth zone and are enveloped by a thin layer of squamous epithelium. Many cytoplasmic strands congregate in the formation of rachis in the germinative zone. The rachis acts as a supporting structure only and keeps the young gametocytes in position till these are mature for fertilization. In the growth zone, the ovaries are tightly coiled around the intestine suggesting the trans-membrane flow of nutrients from the later to the former.

Keywords: Ovary, rachis, histology, *Haemonchus contortus*, nematoda.

INTRODUCTION

The nature of the ovarian epithelium differ in different species of nematodes. In *Syngamus trachea*, the wall of the ovarian germinal zone resembled a thin syncytium. However, towards the end of germinal zone and in the growth zone, it becomes thick consisting of epithelial cells (Brunanska, 1991, 1992). The ovarian wall of *Toxocara canis* was found to be composed of epithelial cells having large number of mitochondria in the germinal zone and abundant rough endoplasmic reticulum, ribosomes and bundles of microfilaments in the growth zone (Brunanska, 1994). In *Trichinella spiralis,* the ovarian wall was found to be composed of a mono-layered epithelium covered by a basal lamina and at the basal surface of epithelium a moderately developed basal labyrinth was present. The epithelial cells possessed minute nuclei and very thin cytoplasm, but were devoid of any secretory granules (Takahashi *et al*., 1995). Zograf *et al*. (2008) found variations in the gonad morphology among six species of nematodes from genus *Steinernema*.

The gastrointestinal nematode *Haemonchus contortus* (Rudolphi, 1803) is a major pathogen of small ruminants throughout the temperate and tropical regions of the world and is a significant cause of production losses. Over years attempts have been made to study various aspects of *Haemonchus* spp. from different parts of the world (Ashad *et al*., 2011; Sood, 2006).

Previously, the microscopic structure of various organ systems of *Haemonchus contortus* has been studied by Singh and Johal (1997), Singh (2000), Singh and Johal (2001a, 2001b and 2001c) and Singh and Johal (2004), Singh (2015a; 2015b; 2015c; 2015d; 2015e) and Singh (2016a; 2016b) The present paper describes the histological structure of the rachis and ovarian wall of *Haemonchus contortus* to substantiate the hitherto existing information in this aspect in nematode parasites.

MATERIALS AND METHODS

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 7µm 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

Female reproductive system of *Haemonchus contortus* is diorchic i.e. the presence of two sets of reproductive organs, which open externally through a common vulva at a distance of about 930-960 µm from the tip of the tail. The reproductive system 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 (Fig. 1).

Each set of reproductive organs includes a long tubular highly coiled ovary, a narrow oviduct followed by a uterus and an ovijector leading into a common short vagina which opens to outside through a common vulva covered by a vulvar flap (Fig. 1).

Both ovaries project anteriad, one slightly behind the other (Fig. 2). These are highly coiled, thin walled tubes of simple squamous epithelium limited by a basal lamina and accommodate diverse stages of developing female germ cells in them. Each ovary is telogonic and is divisible into a small germinal zone, followed by a large growth zone (Fig. 1).

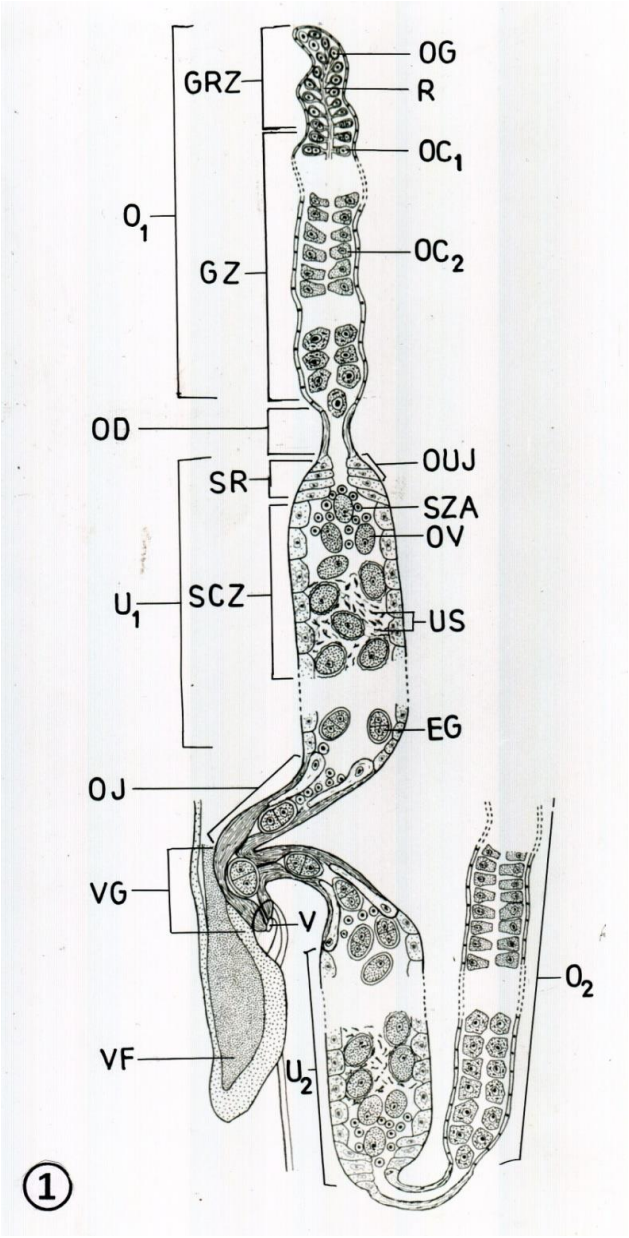

Fig. 1 : A diagrammatic representation (reconstructed with the help of transverse and longitudinal sections) of the female reproductive system of *Haemonchus contortus* showing germinal zone (GRZ) and growth zone (GZ) of anterior ovary (O_1), posterior ovary (O_2), oviduct (OD), ovo-uterine junction (OUJ), Seminal receptaculum (SR), secretory zone of uterus (SZ), anterior uterus (U_1), posterior uterus (U_2), ovijector (OJ), vagina (VG), vulva (V), vulvar flap (VF), oogonia (OG), rachis (R), primary oocyte (OC_1), secondary oocyte (OC_2), ovum (OV), spermatozoa (SZA), uterine secretion (US) and fertilized egg (EG).

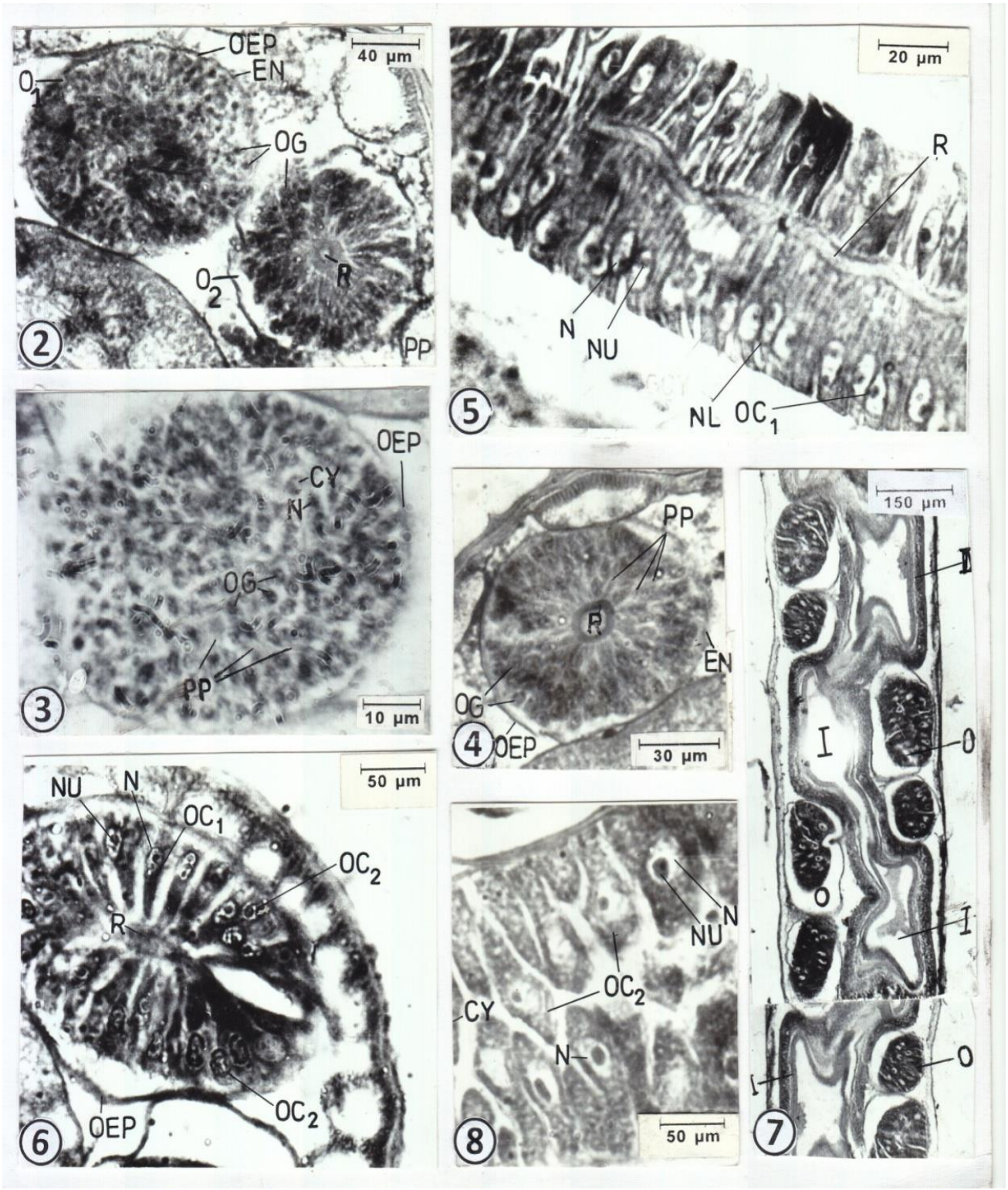

Fig.2 : A portion of T. S. of female showing ovary of the first set (O_1), ovary of the second set (O_2), ovarian epithelium (OEP), epithelial cell nuclei (EN), oogonia (OG),protoplasmic processes (PP) connected with the rachis (R); **Fig. 3 :** T.S. through the germinal zone of ovary showing ovarian epithelium (OEP), oogonia (OG) in dividing stage, nucleus (N) and protoplasmic processes (PP) lying in interoogonial spaces; **Fig. 4 :** A portion of T.S. of female showing ovarian epithelium (OEP), Epithelial cell nuclei (EN), oogonia (OG), protoplasmic processes (PP) and rachis (R); **Fig. 5 :** L.S. of ovary showing primary oocytes (OC_1), rachis (R), nucleus (N), nucleolus (NU) and nucleolemma (NL); **Fig. 6 :** T.S. of ovary showing primary oocytes (OC_1), rachis (R), formation of secondary oocytes (OC_2) nucleus (N), nucleolus (NU), ovarian epithelium (OEP); **Fig. 7 :** L.S. of female showing tight coiling of ovary (O) around the intestine (I); **Fig. 8 :** A portion of ovary showing secondary oocytes (OC_2),nucleus (N), nucleolus (NU), cytoplasm (CY) and ovarian epithelium. (All slides stained with Haematoxylin- Eosin staining).

In the growth zone, numerous oogonia undergoing mitotic divisions are seen. The oogonia lying near the apex and its sides send some protoplasmic branches which pass through the interoogonial spaces and join to form thicker branches which subsequently merge into a centrally positioned anucleate cytoplasmic core, the rachis (Figs. 3 and 4). Oogonia lying near the rachis are directly attached to it. The rachis is a cylindrical structure with well defined walls and a clear lighter central axis measuring 13.3 µm in diameter (Fig. 4 and 5).

In the growth zone of the ovary, the primary oocytes still maintain their connections with the rachis, show a considerable increase in size and divide to form the secondary oocytes which ultimately detach and come to lie free in the lumen of the ovary (Fig. 6). This region of ovary is tightly coiled around the intestine suggesting the direct trans -membrane flow of nutrients from the gut to the gonads (Fig. 7). At the distal end of the growth zone the rachis is absent and the epithelium is slightly thicker (Fig. 8).

The nature of the bounding epithelium of ovarian region differs in different species of nematodes. In *Ascaris lumbricoides*, Musso (1930) has reported that the ovarian epithelium consists of a single layer of greatly elongated spindle shaped cells which may reach 1 metre in length. However, Adamson (1983) in *Gyrinicola batrachiensis* and Johal (1988) in *Oesophagostomum columbianum* describe it to be composed of a thin layer of squamous epithelium. Weber (1987) and Takahashi *et al*. (1995) in *Loa loa* and *Trichinella spiralis* respectively have reported a monolayered epithelium covered by a basal lamina enveloping the ovaries. In *Syngamus trachea*, Brunanska (1991 and 1992) accounts some drastic differences in the ovarian wall in the germinal zone which resembles a syncytium, whereas in the growth zone it is 3.5-8 µm thick and comprises of epithelial cells, basement membrane and an external electron dense layer.

The present studies on *Haemonchus contortus* reveal that the ovarian wall is composed of a single layer of very thin simple squamous epithelium limited by a basal lamina corresponding to the one that surrounds the intestine.

Despite the considerable information present regarding the histomorphology of the female genital system in the telogonic nematode parasites, the origin of the rachis is still a debatable feature. According to Prestage (1960) the rachis in *Ascaris suum* originates proximal to the germinal zone but according to Foor (1967 and 1972) it stems out from the germinal zone and extends to most of the length of growth zone in *Ascaris suum* and *Ascaris lumbricoides*. McLaren (1973) has depicted that the rachis originates as a central cytoplasmic strand in the germinal zone and changes to a much branched structure in the growth zone in *Dipetalonema viteae*. Whereas, the rachis is described as a single stranded structure present only in the germinal zone in *Gyrinicola batrachiensis* (Adamson, 1983). The rachis is reportedly absent in hologonic ovaries as in *Trichuris ovis* (Joshi, 1991) and *Trichinella spiralis* (Takahashi *et al.,* 1995).

Brunanska (1991) describes that in *Syngamus trachea,* the much vacuolated and densely granulated rachis appears at the end of the germinal zone and can be seen only in the initial portions of the growth zone to wane away gradually in the posterior region. The same author in 1994 accounts that in *Toxocara canis*, the rachis is just a branched cytoplasmic mass without any cell components in the germinal zone, whereas in the growth zone the cytoplasmic mass reassembles into a central axis cylinder containing small dense granules, lipid droplets and glycogen granules. A number of cytoplasmic inclusions like endoplasmic reticulum, numerous mitochondria, scattered electron-dense bodies and fewer ribosomes are also seen in the cytoplasm of the rachis of *Heligmosomoides polygyrus* (Mackinnon, 1987).

In *Haemonchus contortus*, many cytoplasmic strands congregate in the formation of rachis in the germinative zone. Subsequently, it stands out as a well defined cylindrical column of nonnucleated protoplasmic mass having a lighter core and well demarcated darkly staining walls. It diminishes and totally disappears at the termination of the secondary oocyte formation area. The rachis here seems to be just a supporting structure which keeps the young gametocytes in position. It is absent in most of the growth zone and consequently has no nutritive value for the oocytes..

Conflicts of interest: The author stated that no conflicts of interest.

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