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SPERMATOGENESIS IN HAEMONCHUS CONTORTUS (NEMATODA: TRICHOSTRONGYLIDAE)

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Abstract. Basic structure and development of spermatogonia, spermatocytes (primary and secondary), spermatids and spermatozoa have been studied in *H. contortus* (Rud., 1803) with the aid of light microscopy.

Male gametes in nematodes have been studied both with light and electron microscopy (Chitwood and Chitwood 1950, Nath 1965, Bird 1971, Anya (1976). Such studies, though mostly on large forms parasitic in animals include those carried out on divergent forms from widely different environments (Bird 1971). Although the economic importance and wide distribution of *Haemonchus contortus* (Rud., 1803) have prompted the publication of much information relating to its ecology, immunology and biology, there is no information on the spermatozoa and spermatogenesis in this nematode. The present communication deals with the light microscopy study of the basic structure and development of spermatogonia, spermatocytes, spermatids and spermatozoa in *H. contortus*.

MATERIAL AND METHODS

The mature worms of *H. contortus* were collected from the abomasum of goats (*Capra hircus*) procured from the local slaughter houses. All goats had been pastured continuously, and thus became naturally infected. Small, cut pieces of worms were immediately fixed in Bouin's liquid with alcohol modification for subsequent histological treatment. Paraffin sections 6—7 μm thick were stained with haematoxylin and eosin. The stained sections were then studied with the aid of light microscopy.

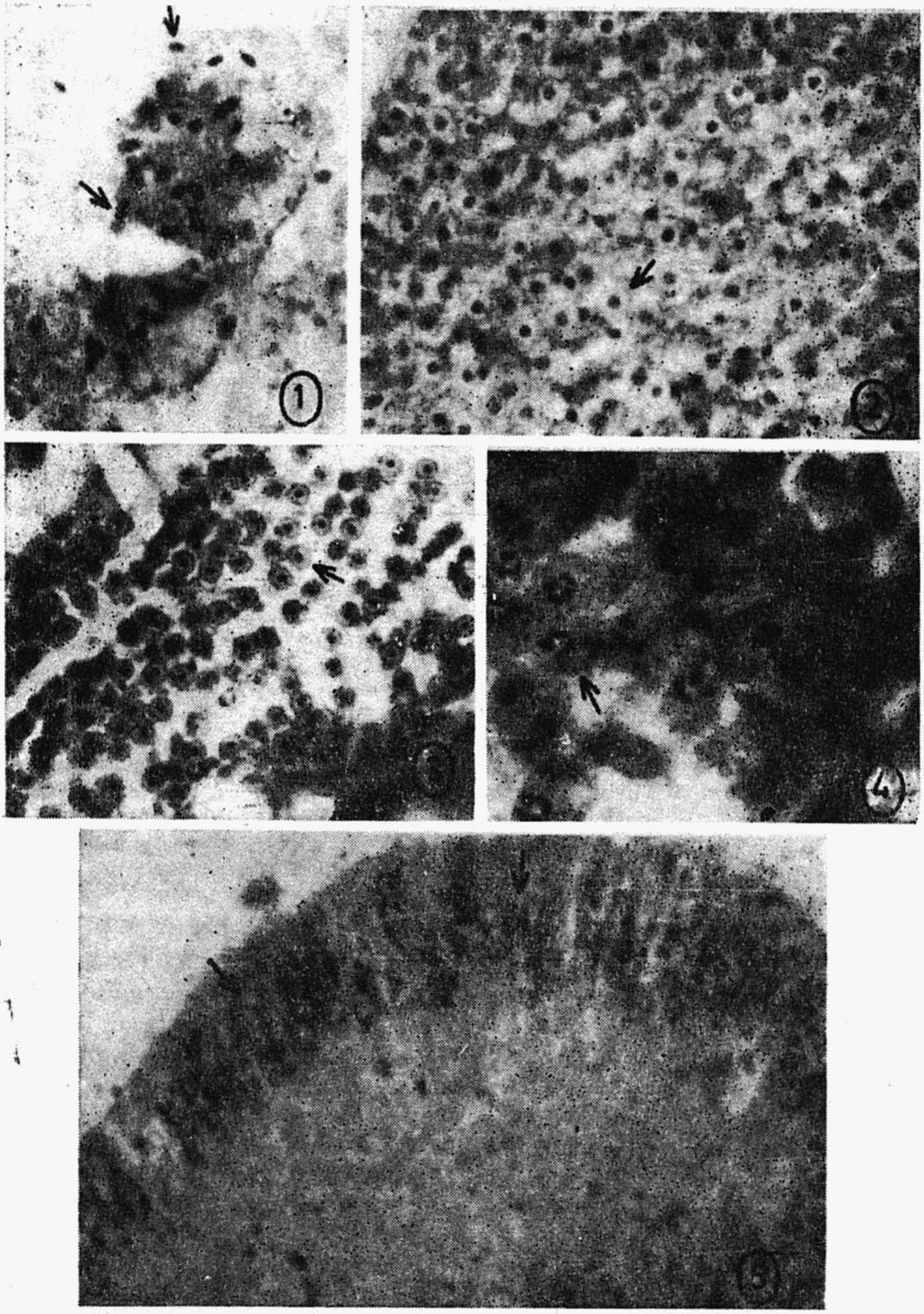
RESULTS

The male reproductive system of *H. contortus* consists of a single anterior, uncoiled tubular testis. The testis in *H. contortus* is telogonic i.e., the spermatogenesis starts at the tip of the testis and the spermatozoa are formed at the other end. This condition is in contrast to that in other hologonic nematodes where the spermatogenesis starts at the periphery of the testis and the spermatozoa are formed in the lumen. The whole process of spermatogenesis in *H. contortus* can be divided into the following stages:

Spermatogonial cells: The testis of *H. contortus* being telogonic, the spermatogenesis is initiated in the germinal zone at the tip of the testis, with the formation of spermatogonia. The spermatogonia lie as closely packed cells with large nuclei and indistinct cell boundaries (Fig. 1), and are present in the centre of the testis. The nuclei measuring up to 1 μm are rounded to oval and stain darkly with haematoxylin. The number of nuclei increases gradually towards the posterior end, showing thereby that this region is a multiplication zone.

Spermatocytes: At the end of the multiplication zone, cytoplasmic mass aggregates around each nucleus. The spermatogonia change shape, becoming polygonal, enlarged and thus transform into spermatocytes.

a) **Primary spermatocytes:** The compact crowded condition of the cells without any apparent intercellular spaces is very prominent at this stage. This results in the formation of the polygonal spermatocytes presenting a pseudoappearance, analogous to the sclerenchymatous tissue of the plants. Each spermatocyte possesses a distinct rounded



Figs. 1—5. Microphotographs showing different stages of germ-cell development during spermatogenesis in *H. contortus*. **Fig. 1.** Section through anterior region of testis showing spermatogonial cells. **Fig. 2.** Primary spermatocytes. **Fig. 3.** Secondary spermatocytes. **Fig. 4.** Spermatids. **Fig. 5.** Mature spermatozoa (1—5 × 1000).

to oval nucleus (Fig. 2). The cell boundaries are clearly demarcated. The primary spermatocytes measure 1.5 to 2 μm in diameter and the nuclei are 1 μm in size. Due to a comparatively large size of the nuclei as compared to their cell size, the nucleo- cytoplasm ratio is smaller in primary spermatocytes. The chromosomes are not well visualised at this stage.

b) **Secondary spermatocytes:** Secondary spermatocytes are rounded cells with clearly demarcated cell boundaries (Fig. 3). The cells measure 1.5 μm in diameter. The nuclei are rounded and measure less than 1 μm in diameter. Unlike that in primary spermatocytes, nucleo-cytoplasm ratio is greater in these cells. However, chromosomes as in primary spermatocytes, are indistinct.

Spermatids: The spermatids, formed from secondary spermatocytes, have no distinct nucleus. However, chromosomes are distinct and separate, and frequently up to 5 can be counted (Fig. 4). However, in some cells even 6 groups of chromosomes have been observed. The spermatids range from 3—4 μm in size. The cytoplasm is basophilic and stains light blue.

Spermatozoa: Each spermatid develops into an elongated, narrow, smooth-walled spermatozoan, bluntly pointed at both ends (Fig. 5). The most notable feature is the presence of distinct chromosomes showing a linear distribution, mostly confined to the periphery. The mature spermatozoa are 5—7 μm long. They are non-flagellate and lack a typical acrosome. The cytoplasm of the spermatozoa in *H. contortus* is not capable of forming any pseudopods.

During the whole process of spermatogenesis, the chromosomes are distinct only in two stages of development, i.e. spermatids and spermatozoa, while in all other stages, the chromosomes are not well visualised.

DISCUSSION

Spermatogonia in *H. contortus* are closely packed cells with large nuclei and indistinct cell boundaries resembling those in prophase of *Anguina triticii* described by Triantophyllou and Hirschmann (1966). In contrast to the large size of spermatogonia (8×2 μm) in *Dipetalonema viteae* (McLaren 1973), the nuclei of *H. contortus* spermatogonia measure up to 1 μm in size. Neill and Wright (1973), McLaren (1973) and Beams and Sekhon (1972) have shown the presence of one or more than one nucleoli in the spermatogonia of *Capillaria hepatica*, *D. viteae* and *Rhabditis pellio* respectively. However, no such observations have been presently made in the case of *H. contortus*. Unlike the five stages of spermatocyte development, found in *Onchocerca volvulus* (Miller 1966), only two types of spermatocyte stages have been observed in *H. contortus*.

Primarily spermatocytes in *H. contortus* are present a little behind the anterior end of the long thread-like telogonic testis, while the secondary spermatocytes are present behind the primary spermatocytes. This condition is in contrast to that in *Nippostrongylus brasiliensis* where the immature spermatocytes are found as cluster of cells at the anterior end and the developing spermatocytes are arranged in a single row posteriorly (Jamuar 1966).

In *H. contortus*, the spermatids are rounded to oval cells and the chromosomes are distinct and separate, a condition resembling the spermatids of *O. volvulus* and *W. bancrofti* as described by Miller (1966). In *H. contortus*, frequently five chromosomes were counted in a single cell but in some cells even six chromosomes were seen, a condition quite similar to that found in *O. volvulus*. However, the cytological examination of ovine and bovine strains of *H. contortus* has revealed the chromosome number for both to be 2n = 11 in the male and 2n = 12 in the female (Bremner 1954, 1955).

Chromosomes in *D. viteae* line up in the long axis of the spermatid and are either 5 to 6 in number (McLaren 1973). The chromosomes in the spermatids of *H. contortus* do not lie along the long axis but are dispersed in the centre of the cell.

Spermatozoa of *H. contortus* are elongate cells bluntly pointed at both ends. The sperms thus resemble in shape to those of *D. viteae* and *W. bancrofti*, as described by Terry et al. (1961) and Miller (1966) respectively. Spermatozoa in *O. volvulus* described by Miller (1966) resemble the spermatozoa of *H. contortus* in being elongate, narrow smoothsurfaced structures but with the difference that spermatozoa in *O. volvulus* are pointed at both ends.

H. contortus spermatozoa lack a well-defined nucleus. The chromatin material is present in a linear fashion along the long axis of the cell. This condition resembles the chromosome distribution in *D. viteae* (Terry et al. 1961), and *W. bancrofti* and *O. volvulus* (Miller 1966).

In *H. contortus*, the spermatozoa are 5—7 µm long. However, in nematode sperms there is a great variability in size ranging from 2 µm in length as in *A. duodenale* (Looss 1905) to 148—170 µm in *O. volvulus* (Miller 1966).

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СПЕРМАТОГЕНЕЗ У *HAEMONCHUS CONTORTUS* (NEMATODA: TRICHOSTRONGYLIDAE)

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Резюме. В световом микроскопе изучена основная структура и развитие сперматогоний, сперматоцитов (первичных и второстепенных), сперматид и сперматозоидов у *H. contortus* (Rud., 1803).

REFERENCES

ANYA A. O., Physiological aspects of reproduction in nematodes. In: B. Dawes (Ed.), Advances in Parasitology, Academic Press, London, 14: 267—351, 1976.

BEAMS H. W., SEKHON S. S., Cytodifferentiation during spermiogenesis in *Rhabditis pellio*. J. Ultrastructure Res. 38: 511 to 527, 1972.

BIRD A. F., Structure of nematodes. Academic Press, New York, 318 pp., 1971.

BREMNER K. C., Cytological polymorphism in the nematode *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898. Nature 174: 704—705, 1954.

—, Cytological studies on the specific distinctness of the ovine and bovine "strains" of the nematode *Haemonchus contortus* (Rudolphi) Cobb (Nematoda: Trichostrongylidae). Aust. J. Zool. 3: 312—323, 1955.

CHITWOOD B. G., CHITWOOD M. B., An introduction to Nematology. Monumental Printing Company, Baltimore, Maryland, 207 pp., 1950.

JAMUAR M. P., Studies of spermiogenesis in a nematode, *Nippostrongylus brasiliensis*. J. Cell Biology 31: 381—396, 1966.

LOOSS A., Rec. Egypt. Sch. Med. 3: 1—158, 1905. (Cited form Bird 1971.)

McLAREN D. J., The structure and development of spermatozoon of *Dipetalonema viteae* (Nematoda: Filarioidea). Parasitology 66: 447—463, 1973.

MILLER M. J., Observations on spermatogenesis in *Onchocerca volvulus* and *Wuchereria bancrofti*. Can. J. Zool. 44: 1003—1006, 1966.

NATH V., Animal gametes (male). Asia Publishing House, Bombay: 162—184, 1965.

NEILL B. W., WRIGHT K. A., Spermatogenesis in the hologonic testis of trichuroid nematode, *Capillaria hepatica* (Bancroft, 1893). J. Ultrastructure Res. 44: 210—234, 1973.

TERRY A., TERRY R. J., WORMS M. J., *Dipetalonema viteae*, filarial parasite of the jird, *Meriones libycus*. II. The reproductive system, gametogenesis and development of the microfilaria. J. Parasitol. 47: 703 to 711, 1961.

TRIANTOPHYLLOU A. C., HIRSCHMANN H., Gametogenesis and reproduction in the wheat nematode, *Anguina triticii*. Nematologica 12: 437—442, 1966.

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SEX RATIO IN THREE SPECIES OF THE GENUS ANTRICOLA

The members of the genus *Antricola* occur frequently in various hot caves in Cuba. Until now 7 species of this genus have been described from this territory (Cruz J., Poeyana 185: 1—5, 1978) and there exist some further undescribed species. During our investigations in March and April 1980 we had the opportunity to examine large quantities of bat guano in various cave systems and to collect the material of 3 species of this genus (see Table 1) from which the following conclusions can be made.

Table 1. Survey of examined material

Species	Cave	Collected		
A. habanensis Cruz, 1976	Cueva del Mudo Catalina de Güines, prov. Habana	578 ♂	322 ♀	1261 N
A. martelorum Cruz, 1978	Cueva de los Murciélagos Santa Cruz del Norte, prov. Habana	35 ♂	20 ♀	77 N
A. naomiae Cruz, 1978	Cueva de Santa Catalina Camarioca, prov. Matanzas	29 ♂	12 ♀	122 N

In all species the males prevail. The male: female ratio in *A. habanensis* was 1.8 : 1, in *A. martelorum* 1.75 : 1 and in *A. naomiae* 2.4 : 1. The nymphs were more numerous than the adults. The nymphs: adults ratio was in these 3 species 1.3 : 1, 1.4 : 1 and 3.0 : 1, respectively. The ticks were kept alive for one or several days under laboratory conditions and their behaviour was observed. It is interesting to note the copulation position in the members of this genus which is similar as in *Ixodes*. The male is situated on the lower body part of the female so that their venters are touching each other. The legs I and II of the male are directed anteriorly and sometimes one or both legs of the first pair enter the genital opening of the female. The legs III and IV embrace the body margin of the female behind its posterior legs. The female can walk without difficulties. Very frequently the pairs of pre-female nymphs and males are found. But in this case the male is always situated on the dorsal part of the nymph's body and never ventrally.

Our observations represent further contribution to the very poor knowledge on the bionomics of the *Antricola* species.

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