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# Cytology studies of spermatogenesis of *Paryphostomum bubulcusi*

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**Abstract**
Cytology of spermatogenesis in the digenea has attracted much attention of various investigators, but most accounts are restricted to the routine features of processes. The mitochondria and other cytoplasmic components of the male and the female germ cells have received less attention. However, recently, the utilization of the electron microscope and other advanced techniques have led the scientists to investigate the ultra-structure and biology of various components involving gametogenesis.
A number of previous workers have observed a great similarly in the general pattern of spermatogenesis in the digenetic trematodes. In this process the three spermatogenesis division and two subsequent mitotic divisions result in the formation of the clusters of 2, 4, 8, 16 and 32 spermatids. These spermatids along with centrosomal granules and flagella finally form the sperms. After the liberation of fully mature sperms, the residual mass of cytoplasm is left behind. In the membrane of residual cytoplasm many holes are seen through which the nuclei of the sperms come out. The general pattern of the dividing male germ cells during gametogenesis are diagramatically.

**Keywords:** Cytology, Spermatogenesis, *Paryphostomum bulbulcusi*

## 1. Introduction
The present investigation of the author is concerned with the studies of cytology of spermatogenesis, of certain species of digenetic trematodes. Critical survey of the available literature in this field reveals that there has been a constant accumulation of the literature in the taxonomic studies of trematodes. Many new species are being added every day from different corners of the world and it is seen that there is an overlap of different species showing only marginal differences between them. There are difficulties to understand the limits of interspecific variations. Therefore, a need is felt to supplement the morphological studies of the species with their cytological data. The karyotype has been recognized as a definite species character. The morphology of the chromosomes among the individual of the same species is generally reasonably constant.
The germinal cells undergo no reduction division. They remain separate from the somatic cells during the development of germinal sacs and they are never localized in reproductive glands. Therefore, the multiplication of these germinal cells in the body cavity of the germinal sacs is really by a polyembryony of the original zygote. According to the germinal line age hypothesis the only cells in all the stages of lifecycle of the digenetic trematodes that have the haploid number of chromosomes are the spermatozoa and mature ova which have gone through reduction division is the reproductive organs of the adult (Cort *et al.* 1950) [3].
There are several difficulties during the study of spermiogenesis. This active dividing stages are rarely available, staining capacities of germinal cells of different species are variable, the temporary squash preparations get heavy granulations and the germ cells in this group are very small and they do not stain easily.
Keeping in view of the above facts I have attempted to add cytological data of certain available Indian species of digenetic trematodes of *Paryphostomum bubulcusi*, Pidiha, 1997 of Rewa region.

## 2. Methodology
The materials after fixation were washed in or 70% alcohol. When Bouin's fluid was used as a fixative, the worms were removed after 12-18 hours, washed thoroughly to remove the fixative, bleached in chlorinated water or chlorinated alcohol.
The materials after washing were stained. A variety of stains were used such as, Haemalum, Borax carmine, Gower's Carmines, Ehrlich's Haematoxyl in and Acetocarmine. Gower's carmine gave better results, when fixative used were formaline or aqueous Bouin's fluid. The worms were dehydrated and cleared in clove oil or xylol, mounted in Canada balsom and dried at 40 °C to 45 °C in the oven.

## 3. Observation
**Spermatogeneis of *Paryphostomum bubulcusi* Agarwal, 1958**
**Reproductive system**
**i) Male genital organs**

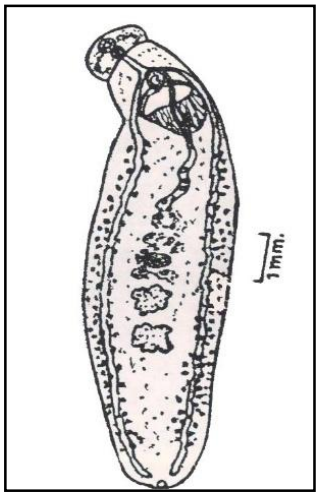


**Fig 1:** *Paryphostomum bubulcusi*

The anterior testis lies at a distance of 0.52 mm. from the anterior end and is slightly smaller than the posterior testis. It measures 0.08 mm. in length and 1.2 m.m. in breadth. Two testes are 0.06 mm. apart from each other. The vas efferenta of both the testes join each other just above the anterior testis to form a looped seminal vesicle which opens into thin walled pars musculosa which finally opens into the genital pore. The genital pore has week musculature and is 0.784 mm. away from the anterior end. It measures 0.08 mm x 0.09 in diameter.

ii) Spermatogenesis

The testis sac shows fibrous tissue inside the testis, clear crypts are visible. The developing germ cells lie in group attached to the fibrous wall. In the crypts the spermatogonia, primary spermatocytes and secondary spermatocytes, spermatids, along with developing sperms and interstitial cells are clearly seen. The author has been able to see the various developmental and growth period stages, some of which have been photomicrograped.

iii) Spermatogonia

The primordial germ cells and the spermatogonial cells exist in the form of irregular patches and sometimes it became difficult to make differentiation. However, the spermatogonia became clear when some dividing stages were located forming primary spermatocytes.

The spermtogonia measured 6-9 microns in diameter. The dividing stages show the spermatogonia preparing to divide mitotically and form more spermatogonial cells.

The subsequently formed spermatogonia so called secondary spermatogonia which normally have not separated were found as separating in this species. They divided mitotically further and formed four spermatogonial cells which remained connected together by their cytoplasmic strands and mass. Thus formed tertiary spermatogonia now dividing simultaneously and formed eight primary spermatocytes in a rosette fashion which also remain stranded by their cytoplasmic mass. The 2 and 4 celled stages showed syncytial condition.

iv) Spermatocyte

The above primary spermatocytes which formed a rosette. started, loosening a bit from the cytoplasmic mass at the time of subsequent division which underwent the reduction division. The division produced sixteen secondary spermatocytes in cluster.

The rosette of 16 celled syncytial secondary spermatocytes now starts expanding in size, and prepared for further division. The cytoplasmic mass got further loosened and the stages enlarged since 32 secondary spermatocytes were formed. They took deeper stain and the nuclei gradually elongated.

v) Spermiogenesis

The nuclei of the secondary spermatocytes after getting elongated. Started becoming cosized and the chromation material got condensed. The nuclei started to distribute towards periphery of the cytoplasmic mass to be recognized as spermatides. These then got elongated further and took the shape of spermatozoa, with fusiform head a tail.

In the previous stages of the development, the nuclei showed less affinity towards the basic dyes in staining but after the head and tail formation the affinity increased and they took deeper stain. The spermatozoa then got irregularly arranged in the central mass and their movement started. Then they became grouped and thinner for an active movement, Further, the residual mass got dissolved. The residual mass of cytoplasm measured 16-21 microns in diameter. The sperms left the residual mass, flowing in one direction showing the single sperm leaving the residual mass. It measured 145-263 micron in length.

The fully formed sperm showed clear demarcation of Head. Middle region and tail.

## 4. Discussion and Conclusion

The male system of Digenea follow the typical Platyhelminthes model and present no unusual features. The testes lie embedded in the general parenchyma in the species region of the body. During the histological and cytological studies the fact became obvious that the certain variation in the germinal cell structure and their arrangements in gonadial tissues do exist in different Digenean groups.

Number of workers have observed a similar pattern of cell divisions during spermatogenesis in digenetic trematodes. This process involves the three spermatogonial divisions and the two maturation divisions. They form clusters of 2, 4, 8, 16 and 32 cells. These sperms form a bundle and come out of the cytoplasm of the spermatid rosette leaving behind a residual mass of cytoplasm. The dividing stages of primary, secondary and tertiary spermatogonia are not commonly seen and also these stages are less in number. This indicates that these stages of mitotic divisions are followed in quick succession and the interphase stages between them are of a short duration.

Willey *et al.* (1950) [11], John (1953) [7] and Dhingra (1954) [4] had given the brief account of the sperm development without mentioning the origin of various parts. Cable (1931) [1], Chen (1937) [2], Rees (1939) [9], Markell (1943) [8], Willmott (1950) [12], Guilford (1961) [6], Tripathi *et al.* (1996) [10] and Govaert (1960) [5] had regarded the sperm to be purely nuclear product. It is likely that their observations had been influenced by the presence of a large quantities of the residual mass of cytoplasm and the absence of apparent regions differentiation of the thread like sperm.

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