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Cytology studies of spermatogenesis of *Bilorchis mehrai*

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Abstract
The developing primordial germ cells and spermatogonia are located in the peripheral region of the testis while the other stages are more central in position in *Bilorchis mehrai* species studied. 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.
The 2-celled, 4-celled, 8-celled, 16-celled and 32-celled clusters show clear rosette formation having conical cells with nice tapering towards the centre of the rosette. The author concludes that the nature of the rosette formation in the whole Digenea depends upon the duration of spermatogonial and spermatocyte stages.

Keywords: Cytology, Spermatogenesis, *Bilorchis mehrai*

1. Introduction
During the present century the cytology of spermatogenesis, oogenesis and fertilization in the digenetic trematodes have been extensively studied. The earlier observations were based on the limited methods of the histological and the cytological techniques and were primarily concerned with general features of the gametogenesis and chromosome number. Hanumantha *et al.* (1966) have reported certain cytomorphological variations in the cytoplasm of the Oocytes in certain group of digenetic trematodes.
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 germinalline 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]
Tripathi (1993) [12] has given extensive review on the systematics of digenetic trematodes of Madhya Pradesh and discussed the cytology of germ cells and the evolutionary significance of chromosome numbers, phylogeny and ecology in Digenea.
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 *Bilorchis mehrai* Dwivedi, 1963 of Rewa region.

2. Methodology
The parasites were removed from the organs of the host very gently and carefully in normal saline solution. Their colour, movement, collar spine and cuticular spines and excretory organs were studied in living condition. For morphological studies and identification, some of the parasites were fixed in aqueous or alcoholic Bouin's fluid, 90% alcholol, or 5% to 10% formaline. Formaline and alcoholic Bouin's fluid gave better results. While fixing the parasites every precaution was taken to avoid over or under pressure. Some parasites were flattened on a slide under slight pressure of a cover slip gently, where some bigger and muscular ones were flattened by using two over glasses giving more pressure.
The materials after washing were stained. A variety of stains were used such as, Haemalum, Borax carmine, Gower's Carmines, Ehrlich's Haematoxylin 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 *Bilorchis mehrai* Dwivedi, 1963
Reproductive system
i) Male genital organs
Testes are symmertical, parallel, unequal lobed (2-3 lobes) and a little below the middle of body length. Testicular zone measuring 0.27-0.43 x 0.120-0.180 mm. vesicula seminalis present in the proximal part of cirrus sac, pars prostatica tubular surrounded by prostatic gland cells. Cirrus short, unarmed, measuring 0.114 mm. Genital opening submedian. dextral. just below the intestinal bifurcation.

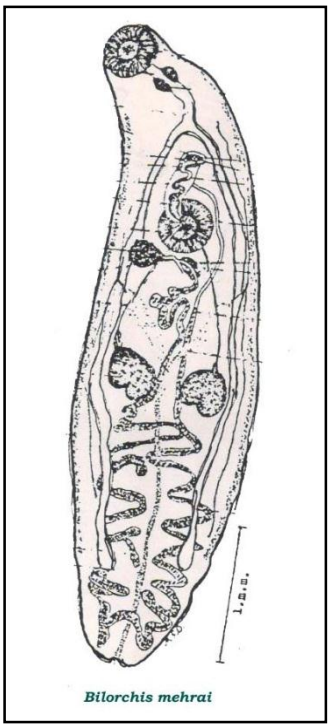

Fig 1: *Bilorchis mehrai*

ii) Spermatogenesis

The testis is bounded by a thin sheath of a fibrous tissue, in which the nuclei of the sperm mother cells were apparent. There were very clear patches of the primordial germ cells in the testis, forming small groups. Some primordial germ cells also reached upto the peripheral region, but their outline were not clear. The growing spermatogonia, primary spermatocytes and the secondary spermatocytes alongwith spermatids, all were seen clearly scattered in the testis.

iii) Spermatogonia

The mitotic division of the primordial germ cells, gave rise to more primary speramatogonia of which few tended to divide further giving rise to secondary spermatogonia. The primordial germ cells ordinarily separated from each other after division, while the primary spermatogonium formed a rosette of two secondary spermatogonia.

The two secondary spermatogonia then divided simultaneously to form four tertiary spermatogonia which were interconnected to each other though fine cytoplasmic stands. These tertiary spermatogonia divided further, giving rise to eight primary spermatocytes in a rosette form.

iv) Spermatocytes

The eight primary spermatocytes emerging from the tertiary spermatogonia divided further to form a cluster of sixteen cells. The cytoplasmic boundaries of these secondary spermatocytes, remained clearly defined and intact. The nuclei of these were more prominent and the rosette formed showed a clear compactness then the interphase stage.

The sixteen secondary spermatocytes entered into the next maturation division leading to the formation of thirty two spermatids in cluster form. The complete cluster of the thirty two spermatids (rosette form) measureed 10-20 microns in diameter.

v) Spermiogenesis

The thirty two spermatids rosette stage remained for a vary short duration and very quick change took place. The cyteoplasmic boundaries started vanishing but the spermatids remained attached to the central mass of the cytoplasm. The spermatids took a deeper stain, making the nuclei more prominent each of them.

The nuclei gradually got elongated in shape as the head formation of the sperms started from this time. The cytoplasmic extension gave the shape to the tails and the heads got elongated further. Ultimately, each rosette formed showed clearly, thirty two elongated sperms. The cytoplams thus got absorbed in the formation of the tails. The remaining mass of the cytoplasm was left behind as residual mass and the sperms liberated from the bundles and were released free for individual movement.

The individual mature sperm measured 138-246 microns in length. The fully matured (motile) sperms showed three clear regions, i.e. head, middle piece and tail.

4. Discussion and Conclusion

The author has attempted here todiscuss the various views regarding the gonadial complex of male genital system, cytology of spermatogenesis and spermiogenesis in Indian species of Digenetic trematodes available in Rewa region of Madhya Pradesh.

The average size of the Digenea ranges between 2-15 mm except for *Fasciola gigantica* and *Fasciolopsis buski* which may even to up to the length of 80 mm. The majority of trematodes are parasitic in chordate hosts living in different parts of the body. The digeneans represent a somewhat heterogeneous group of parasites. Body is generally dorsoventrally flattened. The absence of rigid skeleton and the presence of an extensive musculature allow continued changes of the body shape to occur. On the tegument are found hooks and spines of various shapes and types.

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.

Willey *et al.* (1950) [14], John (1953) [8] and Dhingra (1954) [4] had given the brief account of the sperm development without mentioning the origin of various parts. Chen (1937) [2], Rees (1939) [10], Yamaguti (1958) [16], Markell (1943) [9], Aeppli (1951) [1], Willmott (1950) [15], Guilford (1961) [6], Tripathi *et al.* (1996) [13] and Govaert (1960) 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.

Singh (1972) [11] reported the distribution of mitochondria in *Lissemysia sinhai and Lissemysia jagtai* as a mass of small granules forming a cup like covering enclosing the half of the proximal portion of the nuclei. He also observed that the number of mitochondrial granules in each nuclei gradually decreased in number in the later stages of spermatogenesis.

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