INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
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www.vmsindia.org

HISTOMORPHOLOGY, HISTOLOGY AND ULTRASTRUCTURE OF THE MALE GENITAL TRACT OF DAMSELFLY *ISCHNURA AURORA* BRAUER (ODONATA: ZYGOPTERA)

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Abstract: Histomorphology of male reproductive organs of *Ischnura aurora* Brauer consist pair of testis, vasa differentia and sperm sac. Testes are cylindrical, translucent body located ventrolaterally from seventh and eighth segment. Each testis opens into thin vas deference which joins in the center forming sperm sac. Sperm sac opens ventrally through slit like male gonopore on ninth segment. Testis shows adipose tissue, peritoneum, lobule and lateral duct. The lobules are filled with germ cells representing only one stage of spermatogenesis. The lumen of the duct contains spermatozoa in a group. An ultramicroscopic structural study of the internal male reproductive system reveals movement of mature cyst towards lateral canal. Early spermatids arecylindrical with conical nucleus and accumulation of mitochondrion at the base of nucleus forming neberkern. A mature spermatozoon is long filamentous divisible in to elongated head consisting nucleus and long tail axoneme mitochondrial derivative and secretary vesicle. Vas deference shows two types of secretary granules below microvilli.

Keywords: Dragonfly, Vas deferens, Sperm sac, Neberkern, Axoneme

Introduction:

Tillyard (1917) gave a salient account of the male reproductive organs in dragonfly while Whedon (1918) described the morphology and development of genitalia in the Zygoptera *Agrion*. Asahina reported presence of pair of accessory glands in Anisozygoptera, *Epiophlebiasuperstes*. Studies on the structure and various physiological aspect of the male reproductive system in Odonata were later undrer taken by various worker Midttunn(1974), Prasad and Shrivastava (1960 and 64), Tembhare and Thakare,1982, Andrew and Tembhare, 1993. Formation of sperm bundle and/or spermatophore has proved advantageous in the evolution of the insect since they facilitate safe transfer of spermatozoa in to female reproductive tract avoiding the risk of desiccation and predation during copulation. Omura (1957) reported formation of sperm bundles by the union of head region of spermatozoa to form a compact mass, while tail remains free. Bakare and Andrew (2008, 2010), Abro (2004) described male reproductive system, ultrastructure and biochemistry of genital tract of *Anaxguttatus*. The present work has been undertaken to study the histomorphology, histology and ultrastructure of male reproductive organs of *Ischnura aurora* Brauer.

Material and Method

The male reproductive organs were fixed in Bouin's fixative for histological preparation. The Bouin's tissues were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax at 60-62 °C. The sections of 4 – 6 µm thick were cut on the Cambridge Rocking Microtome and proceeded for histological staining techniques as described in Table 1.

Table- 1 Histological Techniques

Technique	Abbreviation	Fixatives	Substances	References
Ehrlich's Haematoxylin-Eosin	HE	Aqueous Bouin's	Nuclei cytoplasm	Humason (1962)
Heidenhain's Iron-HaematoxylinOrange-G	IH	Aqueous Bouin's	Nuclei cytoplasm	Humason (1962)

Transmission Electron Microscopic studies (TEM)

The internal male reproductive organs (testis, vasa deference, seminal vesicle and sperm sac) were dissected in cacodylate buffer and was fixed in 3% glutaraldehyde in cacodylate buffer (pH 7.3) at 4°C for two to three hours. The material was then washed in cacodylate buffer for 24 hour, post fixed in 1% osmium tetraoxide for 1 hour, dehydrated through graded series of

ethanol and passed through 1,2-epoxypropane before embedding in emscope CY212 resin. 80 nm sections were cut on a Reichert OmU3 ultra microtome and mounted on Athene 400 EM grids. After staining with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963) they were viewed and photographed with Phillips EM400T at 80KV.

Results

The internal reproductive organ of *Ischnura aurora*Brauer consists of pair of testes, vas deferens, median sperm sac and ejaculatory duct seminal vesicle is absent (Fig. - 2).i. Testes The testes are whitish colour cylindrical structure above gut extend from half of seventh and whole eighth segment (Fig.-1-2). Each testis anteriorly prolonged as suspensory ligament which is part of adipose tissue enveloping testis (Fig.-3). Each testis measures about 2.237 mm and 0.163 mm in diameter.The testes shows following structures beginning from the outermost side: a)adipose tissue , b) peritoneum, c) lobules and d) common lateral duct. The adipose tissue consists of spongy mass of highly vacuolated cells. The peritoneum (Fig.-3&4) comprising single row of small, starched cells, encircles numerous lobules and a lateral duct. Each lobule is nearly spherical, of varying size, and each is bounded by thin limiting membrane; the germinal epithelium being absent. Each lobule is filled with germ cells representing only one stage of spermatogenesis. The lateral duct (Fig.-5)runs throughout, on one side of the testes but terminate little before the anterior end. The wall of lateral duct composed of epithelium, basement membrane and a muscle layer. The epithelium composed of cuboidal epithelium measuring 17 ± 4 µm and 14± 3µm arranged in 2-3 rows. The cell possesses granular cytoplasm, and a large spherical nucleus bearing central nucleolus and many deeply staining granules. The lumen of the duct contains spermatozoa in groups.

Ultra structural studies of the cyst wall reveals thickness measuring 200nm in damselfly *Ischnura aurora*Brauer and does not contain any specialized organelles but for the movement of mature cyst towards the lateral canal, a thin pair of fibril (50 nm thick) develops in the core of cyst wall containing mature spermatozoa (Fig.-13). During early spermatid stage the spermatozoa is cyllendrical shaped with conical nucleus. The accumulation of mitochondrion at the base of nucleus forming neberkern (Fig.-14) is also observed. At maturation spermatid elongates and forms two distinct areas, the head and the tail (Fig.-12). A fully grown mature spermatozoa is long filamentous 47± 5.03 µm in length divided into two regions elongated head composed of acrosome and nucleus. A long tail composed of axoneme mitochondrial derivative and secretary vesicle (SV) (Fig.- 14). The apical tip of spermatozoa contains rod shaped acrosome 120 nm in diameter. The nucleus is long rod like and electron dense containing highly packed inert chromatin. The nucleus is 18.7± 0.95 µm long and 190 nm in diameter

Vas deferens Each vas deferens (VD) emerges out of the testis, confined within ninth segment. It measures about 1.53 ± 0.12 mm in length and about 0.176 ± 0.03 mm in diameter. It changes its position from dorsolateral of gut, in the beginning to ventral side along its course up to middle of ninth segment.The vas deferens is divisible into proximal and distal regions. Histologicaly the proximal region resembles with lateral duct of testis but differ with the distal region. In the latter, the peritoneum and adipose tissue surround the musculature(Fig.- 6). The epithelium is composed of single row of small cuboidal cell. The lumen contains spermatozoa and secretary material arranged in two zones. In the peripheral zone secretary material dense and granular central zone shows homogenous secretion and spermatozoa found in segregated condition.

Electron microscopic observation reveals that the epithelial cells present in the wall of vas deferens are with two type of secretary granule large and small (Fig. - 8). The peripheral region around the nucleus filled with secretary granules of various size(Fig.- 9). The secretary vesicles are also found below microvilli. The microvilli measures about 250-300 nm in length and 30 nm in width. The network of fibril and

giant mitochondria are also visible below the epithelial layer.

Sperm sac The sperm sac (SS) is distensible organ, formed by joining of distal regions of vasa deferentia below the gut in the posterior two-third part of ninth segment. When fully distended its caudal end project into the tenth segment. In tenerals the sperm-sac is faint red, flattened wrinkled and heart shaped. The sperm-sac resembles with distal region of vas deferens in histological structure, except that in it a) adipose tissue is lacking b) peritoneum (Fig.- 7) and musculature are well developed and c) the epithelium is thin and nuclei are large, oval and without nucleoli. The lumen is specious, filled with granular secretion (G) in which group of spermatozoa are observed (SP). Sperm-sac lacks the chitinous intima. The ultra structural observation reveals that the wall is highly muscular and epithelium contains dense granule in large number. The epithelial cells of sperm sac shows large nucleus with rich amount of chromatin material. The mitochondria and secretary vesicle found abundant in the cytoplasm of the cell (Fig.10-11).

Ejaculatory duct (ED) It is small translucent, ventral prolongation of the sperm-sac wall which communicates to the exterior through gonopore. The ejaculatory duct is the ventral prolongation of the sperm-sac wall which communicates to the exterior as gonopore. Its muscular coat consists of an outer layer of circular fibre and inner layer of longitudinal fibres. The epithelium is lined over by a thin chitinous intima. The transmission electron microscopic observation reveals the thick cuticle on the inner side along with the thick muscle layer.

Fig. 1 Anatomical organization of the internal male reproductive organs in the damselfly *Ichnura aurora aurora*.

Fig. 2 Diagrammatic representation of Fig. 29 (SS- sperm sac; T- testis; VD- vas deferens; VII-IX- abdominal segments)

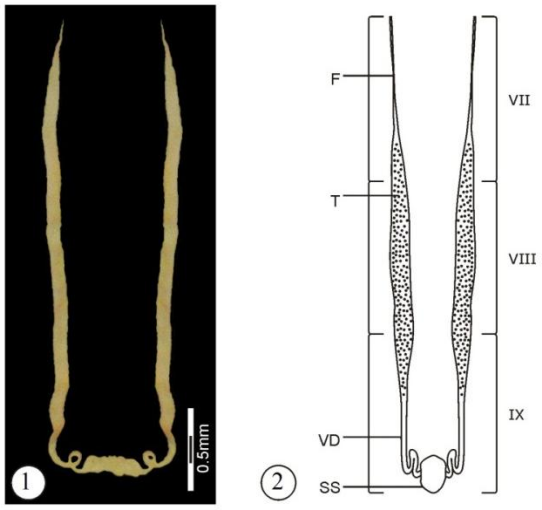


Fig. 3 Transverse section of testis of *Ichnura aurora* showing adipose tissue (AT), lateral duct (LD), lumen of lateral duct (LU), cyst (CY) & peritoneal membrane (PM) (HE X 200)

Fig.4 Magnified view of the cyst showing adipose tissue (AT), darkly stained nucleus (NU) of the spermatozoa peritoneal membrane (PM).(HE X 400)

Fig. 5 Transverse section passing through lateral canal (LC)showing epithelial cell (EC) & lumen (LU) filled with secretion (Asterix)(HE X200)

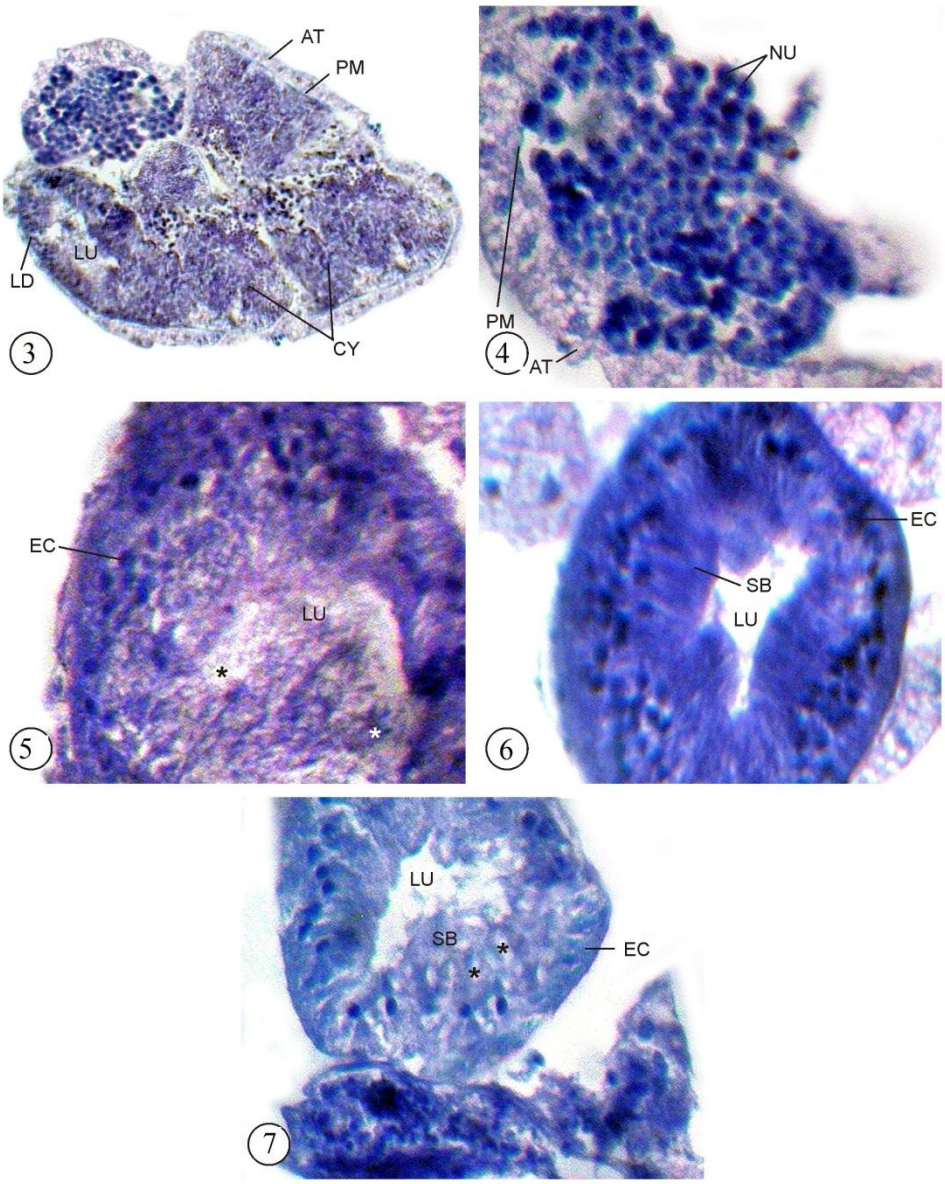
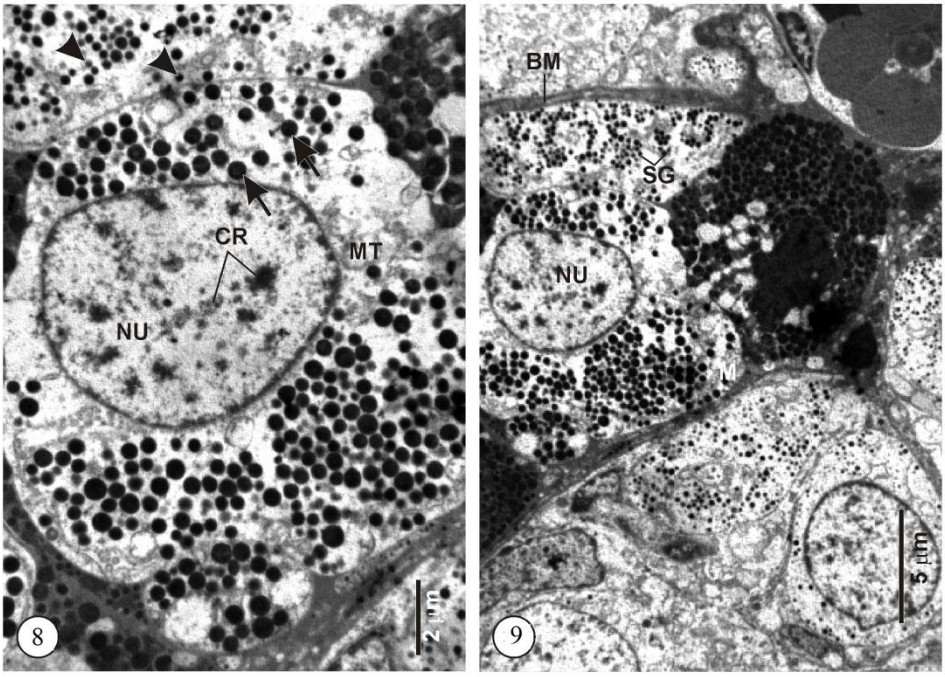


Fig. 6 Transverse section passing through vas deference showing epithelial cells (EC) and lumen (LU) filled with sperm bundle (HE-X200).

Fig. 7 Transverse section passing through sperm sac (SS) showing lumen filled with sperm bundle (SB) and secretion(Asterix) (HE-X200).

Fig. 8 TEM of the epithelial cells of the vas deferens showing two types of secretary granules large (arrow) and small (arrow head) in the perikaryons and nucleus (NU) with defined chromatin material (CR) and Mitochondria (MT) near nuclear membrane.

Fig. 9 TEM of the epithelial cells of vas deferens in active secretary phase. Note centrally located nucleus (NU), peripheral region filled with secretary granules of various size.



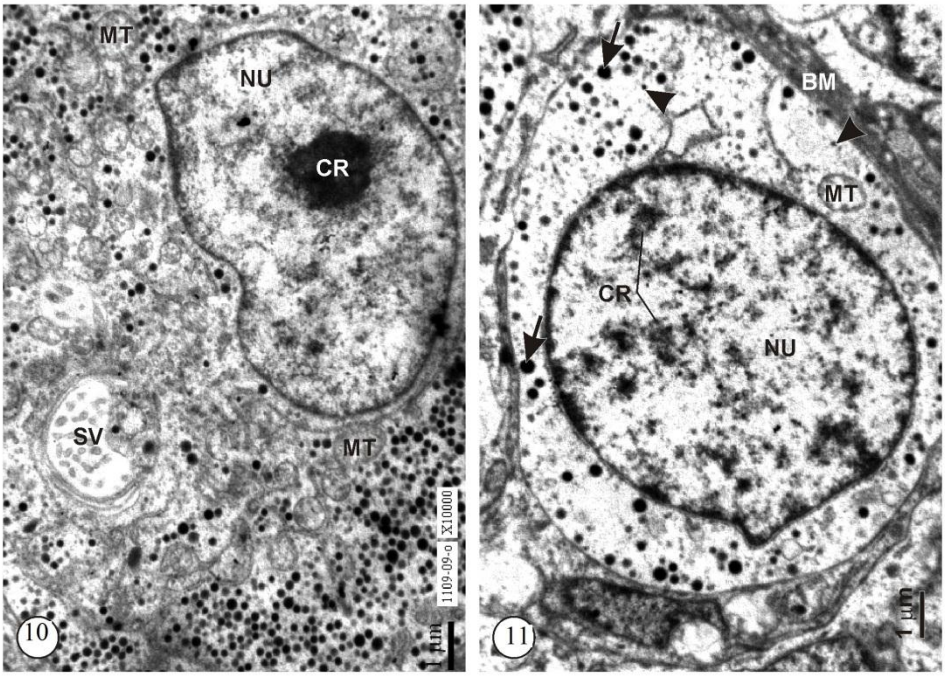


Fig. 10 TEM of the epithelial cell of sperm sac showing large nucleus with centrally located chromatin granules(CR) and towards the periphery mitochondria (MT) and secretary vesicle (SV) in the cytoplasm.

Fig. 11 TEM of the epithelial cell of sperm sac showing basement membrane (BM), large nucleus (N) with chromatin material (CR), secretary granules of two type (arrow and arrow head)and mitochondria (MT)

Fig. 12 TEM of spermatozoa showing acrosome cap (AC), nucleus (N), mitochondrial derivative (MT) and axial filament.

Fig. 13 TEM of sperm bundle showing randomly placed different stages of development. Note sperm head (SH)and sperm tail (ST).

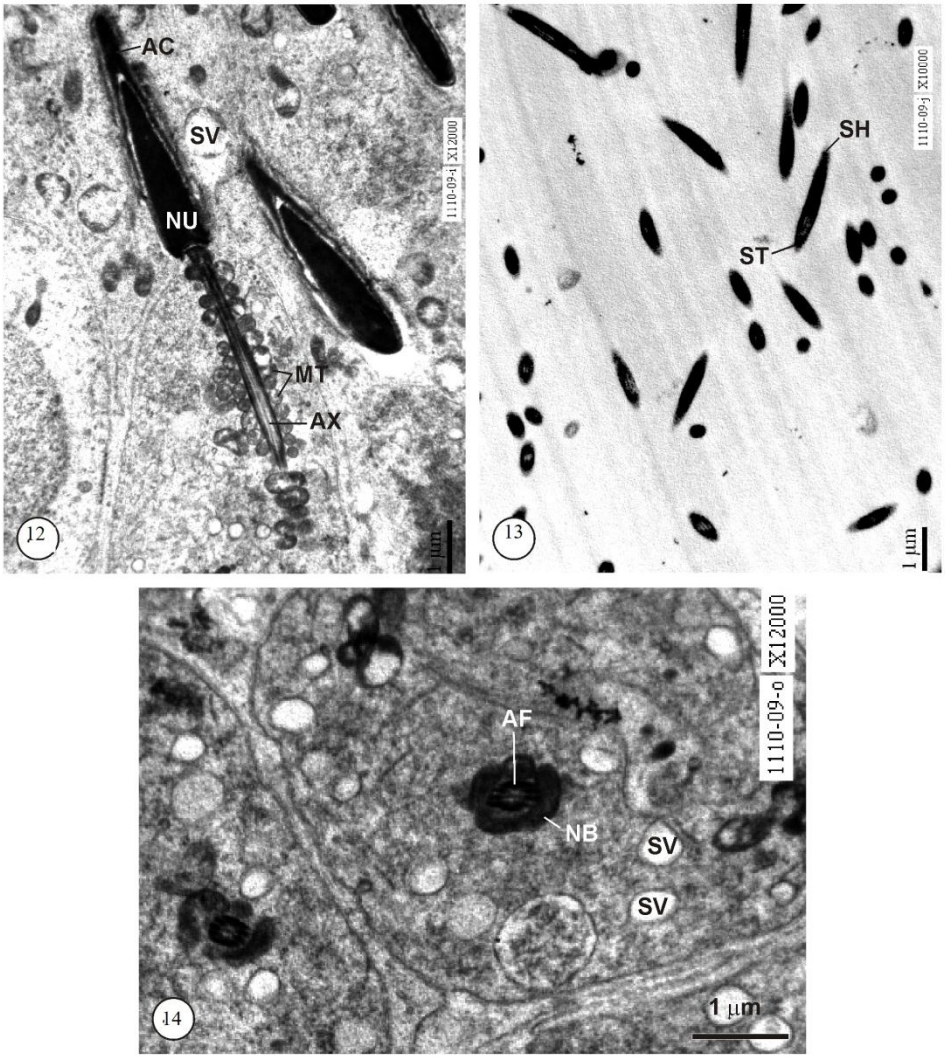


Fig. 14 TEM of early spermatid stage showing fused mitochondria forming neberkern (NB). Note centrally located axial filament (AF) and secretory vesicle (SV)

Discussion:

The internal male reproductive organs in *Ischnura aurora*resemble those of other species of odonates in their anatomical organization (Prasad and Srivastava, 1960, 64; Tembhare and Thakare, 1982; Srivastava and Srivastava, 1986, 87a; Andrew and Tembhare , 1993a). In insect the testis is mostly a multifollicular structure consisting of variable number of long finger-like follicles containing gametes undergoing successive stages of spermatogenesis mostly in the antero-posterior direction (Wigglesworth, 1972). In *Ischnura aurora* testis is cyllendrical multilobular while in *Anaxguttatus* the testis is a unifollicular structure consisting of numerous cysts each with gametes

representing only a single stage of spermatogenesis and it seems to be a characteristic feature of odonatan testis (Tillyard, 1917; and Richards and Davies, 1977). Midttun (1974) reported the presence of inner epithelium and outer longitudinal muscle layer in the vasa efferentia of *Somatochloraarctica*, whereas in *Pantalaflavescens* (Prasad and Srivastava, 1960), *Bradinopyga geminate* (Prasad and Srivastava, 1964), *Ictinogompusrapax* (Tembhare and Thakare, 1982); *Enallagmaparvum* (Srivastava and Srivastava, 1986c) and *Trameavirginia*(Andrew and Tembhare, 1993a) the structure of the wall of vasa efferentia is merely a noncellular membrane similar to that of lobule wall.

Mature spermatozoa produced by lobules are transferred to common duct via fine ductules. In *Ischnura aurora* the central canal runs along the lateral margin of the testis and it is termed as the 'lateral duct' as in Zygoptera, (Srivastava and Srivastava, 1986c), whereas in anisopterans it is located at the central region of testis and is termed as the 'central duct' or central canal (Prasad and Srivastava, 1960; Midttun, 1974; Tembhare and Thakare, 1982; Andrew, 1988). In zygoptera the vas deferens is convoluted and confined to ninth abdominal segment (Shrivastava, 1979). The central canal runs throughout the length of testis but in *Agrion* (George, 1928) it extends only upto the middle of the testis. In *Enallagmaparvum* (Srivastava and Srivastava, 1986) the central canal terminates a little before the anterior end of testis. In *Anaxguttatus* the central canal runs along the whole length of testis in a zig-zag manner as found in *Trameavirginia* (Andrew and Tembhare, 1993a). In *Trameavirginia* (Andrew and Tembhare, 1993b) the central canal is quite passive but the vasa deferentia is actively secreting seminal fluid.

In *Enallagmaparvum* and *Trameavirginia* the central duct opens into the vas deferens (Srivastava and Srivastava, 1986; Andrew and Tembhare, 1993b). Similar continuity of the central duct is found in *Anaxguttatus*& lateral duct in *Ischnura aurora*. In *Trameavirginia* (Andrew and Tembhare, 1993a) a valve is present at the anterior tip of vasa deferentia. Such structural modification has not been noticed *Ischnura aurora*. Histologically, the vasa deferentia and seminal vesicle of *Anaxguttatus*&*Ischnura aurora* are similar to that of other dragonflies & damselflies (Tillyard, 1917; Prasad and Srivastava, 1960, 64; Srivastava and Srivastava, 1986c). The columnar epithelium of vas deferens is provided with microvilli in *Aeshnajuncea* (Abro, 2004), these microvilli are not observed in light microscopy. Although Midttun (1974) using light microscopy stated that the epithelium of vas deference is ciliated (i.e. motile Kinocilia), but according to Abro (2004) this seems to be a misconception due to the use of obtrusive, strongly acid fixative. In *Aeshnajuncea,* two types of cells have been reported in the vas deference one containing numerous tall, non-motile microvilli and other with short irregular microvilli (Abro, 2004). The absence of cells containing numerous tall, non-motile microvilli containing cell, is probably due to the fact that these help to move the sperm bundles through a narrow lumen as found in *Aeshnajuncea,*. Abro (2004) postulated that the exposure of epithelial cells of the vas deferens to the sperms may help in their further maturation. In *Ischnura aurora*Large number of electron dense granules and cell-coat particle are release in the lumen of vas deferens. Thus the spermatozoa as such do not change in the vas deferens but change occurs in the covering of the material attached to or associated with covering and in composition of seminal plasma (Riemann and Giebultowich, 1992). In the dragonfly *Aeshnajuncea,* the sperm bundles are carried in the viscous jel-like substances that stain heavily with toludine blue which also tends towards metachromasia. In the vas deferens, a dense layer of globules mostly mucoporteins accumulate around the hyaline cap of the sperm bundles (Abro, 2004).

In *Ischnura aurora* the sperm sac is formed on the ninth segment. The sperm sac in *Aeschna* is ill-defined (Tillyard, 1917) while it is absent in *Somatochloraarctica*(Midttun, 1974) but in

most of the dragonflies studied so far, it is known to be present as well defined median structure (Matsuda, 1976). Asahina (1954) reported that in *Epiophlebiasuperstes*, the seminal vesicles and sperm sac are absent and the vasa deferentia open outside by a common opening but this is compensated by a pair of accessory glands which enter the vasa deferentia just before the opening. Matsuda (1976) proposed that since the terminal ampullae of vasa deferentia often gives rise to the accessory glands, in *Epiophlebiasuperstes* these glands can be considered as a form of sperm sac. The accessory glands are not found in the male *Anaxguttatus* similar to the report of other Odonatologists (Tillyard, 1917; Whedon, 1918; Tembhare and Thakare, 1982; Andrew and Tembhare, 1993a). The absence of sex glands in Odonata suggests that the accessory sex glands are relatively new structures as they are also lacking in Ephemeroptera, Plecoptera, and Dermaptera (Matsuda, 1976).It is found that Aeshnid, Cordullid and Anisozygoptera dragonflies have a very well developed ejaculatory duct compared to the libellulids (Asahina, 1954; Midttun, 1974; Andrew and Temhare, 1993a).

During the last three decades, extensive work on the structure, mechanism and functions of male accessory glands has been extensively studied (Chen, 1985; Happ, 1984; Gillott, 1988).

Conclusion:

In *Ischnura aurora* (Brauer) reproductive system consist testes, vasa deferentia and single median sperm sac opening into short ejaculatory duct. The seminal vesicle and accessory glands are lacking. Testis opens into thin vas deferens, running initially dorsolaterally and afterwards ventrally. The seminal vesicle is absent, vas deferens directly opening into sperm sac in ninth segment. The sperm sac when fully distended its caudal end project into tenth segment. It opens outside through ejaculatory duct in the ninth segment. Each testis shows on its one side lateral duct posteriarly continues as vas deferens. The lumen of lateral duct filled with secretary material, dense granular in peripheral region and homogenous in the central region. Ultra microscopic studies reveal that the epithelium cells present in the wall of vas deferens are with two type of secretary granule large and small. The epithelium of sperm sac contains dense granule in large number. The nucleus is large with rich amount of chromatin material. The mitochondria and secretary material found abundant in cytoplasm. Ultra structural studies reveals cyst wall 200 Aº thick and does not contain any specialized organelle except pair of fibril in the core of cyst wall for the movement of cyst towards lateral canal. Ultra structure of spermatozoa reveal presence of “nebenkern” at the base of nucleus. Apical head region composed of acrosome and nucleus. Acrosome is rod shaped. The long tail is composed of axoneme, mitochondrial derivative and secretary vesicle.

Acknowledgment- I am grateful to Dr. R. J. Andrew, Head P. G. Department of Zoology, Hislop College, Nagpur, for the laboratory facilities and guidance. I thanks UGC Pune for partial financial support under M.R.P. F- 47-169/07

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