CHAPTER - 6

SUMMARY AND CONCLUSIONS

6.1 CHROMOSOMAL STUDIES -

The previous authors have reported that there is a tendency of chromosomal condensation, shortening, breakage, fragmentation, swelling, stickiness, clumping, and fusion etc., when the male germinal cells are irradiated by different doses of X-rays.

It may be mentioned here that in the present investigation a detailed observation has been made about the changes taking place in the chromosomes of the different types of cells formed during the process of spermatogenesis at the radiation of 1500r, 3000r and 4000r of x-rays.

6.1.1 Chromosomal breakage, fragmentation, condensation and fusion -

When irradiated by different doses of x-rays, the chromosomal breakage, fragmentation, condensation and fusion has been observed in the different developing cells found during the process of spermatogenesis.

The spermatogonial cells when exposed to 1500r and 3000r the chromosomes break into small fragments and become condensed at prophase and metaphase in all the four insect species. These chromosomes during anaphase and telophase stage at the radiation of 4000r become much fragmented and extremely condensed. Some of these fragments even fuse with each other and form clumped masses at their poles.
The chromosomes of the primary spermatocyte cells also become fragmented when irradiated by x-rays. At the radiation of 1500r and 3000r the chromosomes of leptotene, zygotene, and pachytene stages become fragmented and little condensed but at the high exposure of 4000r at diplotene, diakinesis, metaphase, anaphase and telophase stage the chromosomes become very much fragmented and condensed. Some of the fragments even fuse with each other and appear as clumped masses in the cells.

The secondary spermatocyte cells of the four insect species when irradiated by X-ray at 1500r and 3000r, the chromosomes of prophase stage become fragmented. Normally the chromosomes at metaphase become much condensed. However at the exposure of 4000r at anaphase and telophase stages, the chromosomes become very much fragmented and highly condensed. Some of the chromosomes fuse with one another at their poles and form clumped masses.

6.1.2 Unequal distribution of chromosomes at anaphase and telophase-

In the present study, the spermatogonial, primary and secondary spermatocyte cells, when exposed to 3000r and 4000r of X-rays, the chromosomes of anaphase and telophase stages become unequally distributed for both the future daughter cells. It is difficult to give any explanation for such mis-saggregation of chromosomes at these stages. It can be simply speculated that due to the high radiation of X-ray the chromosomes somehow become disorganized and give rise to this condition which may be responsible for many abnormalities, producing mutations and genetic disbalance in the future generation.

6.1.3 Stickiness of the chromosomes -

It has been observed that the spermatogonial, primary and secondary spermatocyte cells when irradiated at 1500r, 3000r and 4000r the
chromosomes of some of the stages become sticky. This condition is not seen uniformly in all the cells but is visible in some of the stages in some of the insects. However, the stickiness of chromosomes is more frequent at the radiation of 3000r and 4000r during metaphase, anaphase and telophase stage of spermatogonial and secondary spermatocyte cells. In the primary spermatocyte cells it has been observed during leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase, anaphase and telophase stages.

One of the reasons for the stickiness may be that, the X-ray radiations probably produce some chemical changes in the chromosomes, cytoplasm or in the nucleoplasm. As reported earlier the unequal distribution of the chromosomes at anaphase and telophase stage, the stickiness of the chromosomes may also be a reason for their mis-aggregation.

6.2 **NUCLEOLUS**

When irradiated by different doses of x-rays, the behaviour of the nucleolus in quite interesting in the various cells of spermatogenesis of the insects under study.

At the radiation of 1500r the nucleolus of the spermatogonial cells at prophase is enlarged in all the four species. It also enlarges in the primary spermatocyte cells in all the four species at their Leptotene, Zygotene and Pachytene stages.

At the radiation of 3000r in *A.foveicollis*, the nucleolus of the spermatogonial cells shrink and become smaller in size. In the primary spermatocyte cells of *A. foveicollis* and *C. peregrinus* it also becomes small at their leptotene,
zygotene and pachytene stages. Similarly in *A. intermedia* and *D. cyngulatus* also the nucleolus shrinks only at the pachytene stage.

It is remarkable to note here that at the same radiation of 3000r the nucleolus of the spermatogonial cell enlarges and increases in its size in *D. cyngulatus*. Similarly in the primary spermatocyte cells of *A. intermedia* and *D. cyngulatus* also the nucleolus enlarges at leptotene and in *D. cyngulatus* at the zygotene stages.

It is interesting to note here that at the radiation of 3000r the nucleolus of certain cells become smaller in size in some species but become larger in other as described above. It is difficult to give any explanation why the nucleolus shrinks / enlarges in its size at one radiation only.

At the exposure of 4000r the nucleolus shrinks in *A. foveicollis* and *C. peregrinus* at their leptotene, zygotene and pachytene stages. Remarkably at the diplotene stage, the nucleolus disintegrates and is lost at the radiation of 1500r, 3000r and 4000r in all the four species.

Interestingly the nucleolus of the secondary spermatocyte cells at their prophase stages enlarges at the radiation of 1500r, 3000r and 4000r in all the four species.

6.3 **NUCLEOLAR VACUOLE**

An unstained small clear space, the nucleolar vacuole within the nucleolus has been found in different cells during spermatogenesis. The only account available is of Kulmami, Hema, Ghosh, N. and Sharma, Sarita (2006). They described the process of spermatogenesis in *Aulacophora foveicollis* (coleopteran) with special
reference to nucleolar vacuole and perinucleolar ring. The authors have not given any findings on the effect of X-rays during this process.

The spermatogonial cells at prophase stage, when irradiated by the x-rays at 1500r and 3000r the nucleolar vacuoles are visible in *A.foveicollis* and at 3000r and 4000r it is present only in *D. cyngulatus*. It is totally lost in *A.intermedia* when irradiated at 3000r and 4000r.

Remarkably, in the spermatogonial cells of *C.peregrinus* during prophase stage the nucleolar vacuole is totally lost at all the three radiations.

In the primary spermatocyte cells the nucleolar vacuoles are also not traceable in all the four insect species at all the three radiations. (i.e. 1500r, 3000r and 4000r).

In the secondary spermatocyte cells the nucleolar vacuole is totally absent in *A.foveicollis, A.intermedia* and *C.peregrinus* at all the three radiations but present only in *D.cyngulatus* at 1500r and 3000r but not in 4000r.

6.4 **PERINUCLEOLAR RING** -

In the literature only one reference is available in which the account of the perinucleolar ring has been given. Kulmarni, Hema, Ghosh, N., Sharma, Sarita (2006) have described the process of spermatogenesis in *A.foveicollis* (a coleopteran) with special reference to nucleolar vacuole and perinucleolar ring. However, they did not observed the effect of X-ray in their study.

The perinucleolar ring is a clear 'unstained ring' like structure seen all around the nucleolus the various cells formed during spermatogenesis in some insects. It may be mentioned here that the presence and persistance of the perinucleolar
ring varies in different cells of different species at the different do seses of X-rays.

In the spermatogonial cells of *A. foveicollis*, *C. peregrinus* and *D. cyngulatus* the perinucleolar ring is present at the prophase stage at the radiation of 1500r and 3000r except in *A. intermedia* where it is totally absent (i.e. absent even in the resting stage of the prophase). At the exposure of 4000r the perinucleolar ring is totally lost due to high irradiation in all the species. Nucleolus is not found during metaphase, anaphase and telophase stages therefore the perinucleolar ring also doesn't exist in these stages.

In the primary spermatocyte cells the perinucleolar ring is present in leptotene, zygotene and pachytene stages in all the species at all the three radiations. During leptotene at the radiation of 1500r, 3000r and 4000r it is present in *A. foveicollis*, *C. peregrinus* and *D. cyngulatus* but in *A. intermedia* it is visible up to 1500r and 3000r and not in 4000r as it is lost. However at the radiation of 4000r it persists only in *A. foveicollis* and is lost in other three species. On the basis of the results obtained it can be concluded that the primary spermatocyte cells of *A. foveicollis* are less sensitive to X-rays at their leptotene, zygotene and pachytene stages as the perinucleolar rings persists at the exposure of 1500r, 3000r and even at the high radiation of 4000r.

The secondary spermatocyte cells when exposed to 1500r and 3000r, the perinucleolar ring in the prophase stage is visible in *A. foveicollis*, *C. peregrinus* and *D. cyngulatus* but not in *A. intermedia*. Remarkably at the exposure of 4000r the ring is present only in *A. foveicollis* and is totally lost in other three species.

It may be concluded that the secondary spermatocyte cells of *A. foveicollis* also show less sensitivity as the perinucleolar ring persists at the radiation
of 1500r, 3000r and even at the high exposure of 4000r. This characteristic feature
is similar to the primary spermatocyte cells of this species i.e. *A.foveicolis* as
mentioned above.

6.5 **CYTOPLASMIC VARIATIONS** -

In the present investigation it has been noticed that during the
process of spermatogenesis of insects the cytoplasm of the spermatogonial, Primary
and secondary spermatocyte cells become thicker and their stickiness increases
gradually with the increasing doses of X-ray radiations.

In *C.peregrinus* at the radiation of 4000r the cytoplasm becomes very
thick and sticky; therefore, for the anaphase stage the two sets of chromosomes fail
to separate from are another from the equatorial plate of the metaphase stage with the
result they lie very close to each other.

The primary spermatocyte cells of *A.foveicolis* and *C.peregrinus* when
exposed to 3000r and 4000r, at their anaphase stages, some of the chromosomes
fail to move to their respective poles through the sticky and thick cytoplasm in which
they lie. As a result of this obstruction the chromosomes are unequally distributed.
This abnormality may be responsible for the genetic disbalance and mutagenic
expressions in the newly formed individuals.

6.6 **CYTOPLASMIC BLOCKS** -

The different cells of spermatogenesis when irradiated by different
doses of X-ray, the cytoplasm of some of the cell becomes condensed and get
converted into thick "block" like structures which are seen arranged just below the
cell membrane. The formations of such *cytoplasmic block* have not been reported
by earlier workers. These cytoplasmic blocks are not formed always by the X-ray exposure but it varies in different cells at different doses. It is just possible that the physico-chemical properties of the cytoplasm below the cell membrane is changed due to the effect of the radiation with the result such cytoplasmic blocks are formed. However, the exact reason for their formation is not known.

Further, it is remarkable that the cytoplasmic blocks are not at all formed at the metaphase, anaphase and telophase stages of spermatogonial, primary and secondary spermatocyte cells and also at the pachytene stage at all the X-ray exposure (i.e. 1500r, 3000r and 4000r) in all the four insect species under study.

On the basis of the frequency of formation of these cytoplasmic blocks during spermatogenesis it may be concluded that *A.foveicollis* and *C.peregrinus* are more X-ray radiation resistant whereas the *A.intermedia* and *D.cyngulatous* are more radio sensitive.

6.7 SPERMIOHISTOGENESIS -

The literature reveals that some of the previous authors have observed the X-ray effects on the cells of spermatogenesis. They reported that the sperms are sensitive to X-ray and their fertility is depressed, ionization of sperms and spermatids cause several times more genetic mutations, nuclear disorganization, ultra structure variations, abnormal variations in spermiogenesis cells and presence of two lateral grooves in the sperm nucleus etc.

During the present investigation the effect of the X-ray exposures of 1500r, 3000r and 4000r on the cells of spermiogenesis have been recorded in all the four insect species.
There are some remarkable and interesting features which have not been noticed by the previous workers and are being reported probably for the first time.

When the spermatids are irradiated by the x-ray, the size of the nucleus is increased due to its swelling at all the three radiations and in all the four insect species. The chromatin material of the nucleus becomes condensed and form granules, these even fuse together to form 2-3 large granules as found in *D. cyngulatus* but in the case of *A. foveicollis* it is different. Here chromatin material becomes very much condensed and aggregates at the lateral margins of the elongating spermatid nucleus at the high radiation of 3000r and 4000r.

In *C. peregrinus*, at the high radiation of 3000r and 4000r the elongating spermatid nucleus becomes much condensed and appear like a spirally coiled thread like structure.

6.7.1 Formation of tubular vacuoles -

The literature reveals that there is only one report published by Khatoon, Shahida, Ghosh, N. and Sharma, Sarita (2006) on the formation of longitudinal tubular vacuoles during insect spermateliosis.

In the process of spermateliosis *A.intermedia* and *C. peregrinus* the elongating spermatid nucleus contains 3-4 or more vacuoles within it. Later these vacuoles fuse with each other and form a longitudinal tubular vacuole.

On further condensation of the chromatin material and the radiation effect this vacuole is ultimately lost and a condensed filamentous sperm nucleus is formed.
In the case of *A. foveicollis* and *D. cyngulatous* the formation of the longitudinal tubular vacuole is different from one which has been mentioned above. Here the elongating spermatid nucleus becomes flattened appearing as a 'trypanosome' or a 'leaf' like structure. Later the lateral margins of the flattened spermatid nucleus roll up and fuse with each other longitudinally at the midline. As a result of this a longitudinal vacuole is enclosed within the nucleus. On further elongation and condensation and also the radiation effect the sperm nucleus becomes condensed and the vacuole is totally lost.
CONCLUSIONS

The present work is related to the X-ray effects produced at the radiation of 1500r, 3000r and 4000r on the male germinal cells of four insects may be concluded in short on the points mentioned below:-

1. As already reported by some authors previously, in the present investigation also the chromosomal condensation, breakage, shortening, swelling, stickiness, clumping, union and fusion etc. has been observed when the male germinal cells of the four insect species were exposed to different doses i.e. 1500r, 3000r and 4000r of X-ray.

   It has been noticed that cells during spermatogenesis are not equally sensitive to irradiation's in all the phases of its existance. The extent of radiation damage to the cell depends on the doses as the number of chromosomal abnormalities increase with increasing dose.

   It may be mentioned here that there are certain features which have not been observed by any of the previous authors and probably this is being reported for the first time now.

2. Nucleolus: It is interesting to note here that at the high radiation's of X-ray the nucleolus enlarges and also shrinks in its size at one particular radiation. It is difficult to find any explanation for this behaviour. It appears that this behaviour may be specific to the particular species.

3. Nucleolar vacuole: The presence or absence of the nucleolar vacuole within the nucleolus has been observed in the male germinal cells of the insets. Its persistence during X-ray irradiation's is a remarkable feature.
4. **Perinucleolar ring**: The presence of an unstained perinucleolar ring around the nucleolus of the germinal cells and their persistence is also interesting to observe in different cells at different X-ray exposures.

5. **Nuclear membrane**: The swelling and the disintegration of the nuclear membrane has been observed at different doses of X-rays.

6. **Unequal distribution chromosomes**: An interesting phenomenon of unequal distribution of chromosomes has been observed at anaphase and telophase stages of the dividing cells when irradiated by high X-ray exposures in some species of insects. This feature has also been reported only by Verma, R.C., Reddy, B.M. and shevade, A. (1996) *P. dermondii*.

   The unequal distribution of chromosomes for the two newly formed daughter cells may be responsible for the chromosomal disorders in the future generations.

7. **Cytoplasmic and nucleoplasmic changes**: The cytoplasm as well as the nucleoplasm become thicker and more sticky when irradiated at the high doses of X-ray. It is interesting to observe that a few chromosomal fragments are unable to reach their respective poles as their movement is obstructed due to the stickiness of the cytoplasm during anaphase stage of the dividing cells. This is responsible for the mis-segregation of the chromosome for the newly formed cells. This may be a very important event for the genetic disbalance in the future generation.

   Similarly, during cytokinesis it has been observed that when the cell is irradiated by high doses of X-rays, their cytoplasm is also unequally divided with the result one of the daughter cell becomes larger and another smaller in size. This also may be responsible for the difference in the formation of two new individuals. It is difficult to give any explanation for this behaviour.
8. **Formation of cytoplasmic blocks:** When irradiated by different doses of X-rays the cytoplasm of some of the cells become condensed and form 'small cytoplasmic blocks'. These are found in a single row below the cell membrane. This feature has also not been reported by any author so far. These blocks are not formed in all the cells when exposed to the X-rays. It is difficult to give any reason for their formation. However, it is just possible that the physico-chemical properties of the cytoplasm below the cell membranes is changed due to the effect of the radiation.

On the basic of the formation of cytoplasmic blocks it has been concluded that *A. foveicollis* and *C. pergrinus* are more radio-resistant where as *A. intermedia* and *D. cyngulatus* are more radio-sensitive.

9. **Spermiohistogenesis:** During spermiohistogenesis the spermatids swell up due to the radiation effect of X-rays. The different doses of X-rays produce condensation of the chromatin material and form large granules.

The vacuoles within the elongating spermatids enlarge and at the high radiation they fuse with each other and form a tubular vacuole. This vacuole is lost in due course of time on account of condensation and elongation of the spermatid at high exposures.

In the case of *A. foveicollis* and *D. cyngulatus* the formation of the tubular vacuole is entirely different from one which has been described above. In these species the elongating spermatid nucleus becomes flattened and appears like a 'leaf' or a 'trypanosome'. The lateral margins of the flattened nucleus roll up and fuse at the mid-line and as a result of this an elongated tubular space is enclosed within it.
At the high radiation of $3000r$ and $4000r$ the sperm nucleus becomes condensed and the tubular vacuole is lost due to the X-ray effect. At the high radiation of $3000r$ and $4000r$ the sperm nucleus becomes spirally coiled like a 'spring' and later on it is broken into pieces.

The present study is very useful as the X-ray radiation's induce chromosomal aberrations and mutations in the germinal cells of insects. Which are useful for the pest control as well as for their irradiation. This type of study has its own importance in cytogenetical and also in the cytotaxonomical studies.
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STUDIES ON THE SPERMATOGENESIS OF AULACOPHORA FEMORALIS (A COLEPTERN) WITH SPECIAL REFERENCE TO NUCLEOLAR VACUOLE AND PERINUCLEOLAR RING

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ABSTRACT: The present work on the spermatogenesis of Aulacophora femoralis reveals certain remarkable features, such as the presence of two nucleolar vacuoles inside the nucleolus and a perinucleolar ring surrounding it in spermatogonial cells, primary spermatocyte and secondary spermatocyte cells, which however do not persist throughout all the stages. Further the elongating spermatids contain vacuoles which later on fuse together and form an unstained tube like structure. This tube is also lost due to elongation and condensation of the sperm nucleus.

Key words: Spermatogenesis, Perinucleolar, Spermatocyte.

INTRODUCTION

The cytological studies of male germ cycle of Coleopterans has been done by authors such as Bowen (1924), Virkki (1965), Phillips (1970), George and Klemetson (1955), Yadav and Vyas (1996), Venu et al. (2000) and many other workers. There have been varying reports on the presence of cytoplasmic connections between germinal cells, synchronous division within the cyst, number and behavior of the nucleolus, shape of the elongating spermatid, the formation of acrosome, number of centrioles etc. The present paper is an addition to the studies made on insect spermatogenesis.

MATERIAL AND METHODS

Insects were dissected under normal saline solution in living condition and their single testes were taken out. Its squash and smear preparations were made and stained in aceticarmine stain for observations.

RESULTS AND DISCUSSION

The process of spermatogenesis in A. femoralis also follows the general trend as seen by most of the authors in insects. The spermatogonial cells at their resting stage has only one nucleolus containing one or two unstained clear vacuoles in it. A distinct unstained perinucleolar ring surrounds the nucleolus. At prophase the chromosomes appear in the nucleoplasm and at metaphase they arrange themselves on the equatorial plate. Further at anaphase they move to their respective poles and at telophase they appear as deeply stained clumped masses at both the poles.

The primary spermatocytes at their resting stage have one or two unstained nucleolar vacuoles, within the nucleolus and a clear perinucleolar ring surrounds it. At leptotene and zygote stages one to two nucleolar vacuoles are seen and a clear unstained perinucleolar ring encircles the nucleolus. At pachytene the chromosomal loops are formed and the nucleolus shifts at the margin of the cell, close to the nuclear membrane. During
Abbreviations:

Anaphase - A, Acroblast - Ab, Acrosome - Ac, Anterior - Ant, Nuclear vesicle - Nvs
Prophase - Pro, Perinucleolar ring - PNR, Posterior - Post, Chromatin material - Cm, Sperm tail - SpT
Centriole - C, Sperm nucleus - SpN, Diakinesis - D, Telophase - T, Diploïene - Dip, Vacuole - V
Metaphase - M, Zygotene - Z, Nucleus - N, Nucleolar vacuole - Nuv

Explanation of figures:

Aulacophora femoralis -
Spermatogonial cells - 1. resting stage with two nucleolar vacuoles and a perinucleolar ring
2. prophase showing perinucleolar ring, 3. metaphase, 4. anaphase, 5. telophase
Primary spermatocyte cells - 6. resting stage with one to two nucleolar vacuoles and a perinucleolar ring, 7. lepote, 8. zygotene, 9.diploïene, 10. diakinesis, 11. anaphase, 12. metaphase.
diplotene stage, the chromosomes form chiasmata and at diakinesis they appear as deeply stained dots, rods and rings due to extreme condensation. The nucleolar vacuoles and the perinucleolar ring could not been seen in pachytene, diplotene and diakinesis stages. Further, at metaphase the chromosomes are seen on the equatorial plate, at anaphase they move towards their respective poles and at telophase they form clumps at two poles connected by cytoplasmic strands. Later due to cytokinesis two secondary spermatocyte cells are formed.

The newly formed secondary spermatocyte cells do not posses nucleolar vacuole but an unstained perinucleolar ring is seen surrounding the nucleolus. At metaphase the chromosomes become short and stain deeply. Later these cells pass through anaphase and telophase stages and divide to form spermatids.

After a short while the chromatin material of the spermatids arrange itself at the inner surface of the nuclear membrane and appear like a horse-shoe shaped structure. As a result of this an unstained space, the nuclear vesicle is formed inside it. A deeply stained acrosomal granule appears within the acroblast situated at the margin of the nucleus. Further, the spermatids elongate gradually and the chromatin material also becomes condensed. The acrosomal granule occupies the apical position so as to from future acrosome.

Later the shape of the spermatid changes showing tapering anterior and broad posterior ends. In most of the cases the elongating spermatid contains one large and two small vacuoles inside it. During further elongation of the spermatid these vacuoles fuse together so that a single elongated vacuole is seen inside. The broad posterior end of the spermatid becomes rounded and here the cytoplasmic tail is seen connected. The elongated vacuole disappears on further elongation of the sperm nucleus. Now the sperm nucleus becomes filamentous with its faintly stained cytoplasmic tail attached to its posterior end.

Favard (1968) and Klag (1977) reported only one nucleolus in the germinal cells, but Noelle (1979) found two nucleoli in larval testis of Doriphora and Leptinotarsa decemlineata. Herbault (1972) reported the fragmentation of nucleolus in these cells. However, in the present investigation only one nucleolus is present in the cells it has also been reported by Favard (1968) and Klag (1977).

It is remarkable to note here that in the spermatogonial and primary spermatocyte cells of A. femoralis the nucleolus contains one or two vacuoles and a clear perinucleolar ring surrounds it. As the literature reveals both the vacuoles as well as the perinucleolar ring have not been reported by any of the previous authors. Further these vacuoles are also present in leptotene and zygotene stages of primary spermatocyte cells but are absent in pachytene and onward stages. It is remarkable that only perinucleolar ring is again seen in the prophase of the secondary spermatocyte cells. The peculiar variable behavior of their disappearnce and reappearance in spermatogenesis has not been reported earlier. It is difficult to understand and speculate the role of these vacuoles and the perinucleolar ring during spermatogenesis.

In the presence investigation the morphogenesis of spermatids and acrosome is almost the same as described by the previous authors such as George and Klemetson (1955) in Anthomonous grandis, Tiwari (1985) in Aspidomorpha miliaris, Nath (1965) and Phillip (1970). The presence of two centrioles have been reported by Friedlander and Wahrmann (1966) and Smith (1969). But in the present case it is only one as reported by Phillips (1970) in his review. In the present study it has been found that the elongating spermatids contain some vacuoles in it which fuse together during the course of nuclear elongation and form an unstained tube. This is ultimately lost on further condensation of the sperm nucleus. Similar to other insects in this case, also the sperm is filamentous and stains deeply with its cytoplasmic tail attached posteriorly.
VARIATIONS OBSERVED IN THE SPERMIOHISTOGENESIS OF AULACOPHORA FOVEICOLLIS AND LACCOPTERA QUADRIMECULATA, PHYTOPHAGUS BEETLES

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ABSTRACT : During the study of spermiohistogenesis in A. foveicollis and L. quadrimeculata, remarkable differences have been observed between them. The shape of the elongating spermatid nucleus in A. foveicollis changes into a "trypanosome" like flattened structure. On further elongation, the lateral margins of the flattened spermatid nucleus roll up and fuse together with the result an elongated tube like space is enclosed inside it. This space is gradually lost due to further condensation and elongation of the sperm nucleus. In the case of L. quadrimeculata the flattening of the spermatid nucleus and "trypanosome" like structure is not formed. Here the nucleus contains 2-3 vacuoles. During elongation of the nucleus these vacuoles increase in number but their size decreases. Later, all these vacuoles fuse together and form a single long vacuole. During further elongation this elongated vacuole is also lost as in the case of A. foveicollis.

Key words : Spermiohistogenesis, Spermatid nucleus.

INTRODUCTION

Bowen (1924) reported that in early spermatids the chromatin material becomes condensed at the inner surface of the spermatid's nuclear membrane and appear as a "horse-shoe" shaped structure so that a clear unstained nuclear vesicle appears within the nucleus. Phillips (1970) found the chromatin in a very diffused condition. Gassner et al. (1975) reported compact aggregation of certain strands and dispersed strands in early spermatids. Phillips (1970), Gassner et al. (1975), Breland (1968) and Smith (1969) gave a good account on the formation of nuclear vesicle, acroblast, acrosome and centriole during the process of spermiohistogenesis in insects.

MATERIAL AND METHODS

Insects were dissected under normal saline solution and their testis were taken out. Later their squash and smear preparations were made and stained in acetocarmine for the present study.

RESULTS AND DISCUSSION

At the end of the second meiotic division the spermatids are formed. These undergo most interesting cellular metamorphosis where the chromatin material concentrate on the inner surface of the nuclear membrane and appear as a "horse-shoe" shaped structure. As a result of this an unstained nuclear vesicle appears at its centre. This vesicle remain conspicuous for some period. A deeply stained acrosomal granule is seen within the acroblast, quite close to the nuclear membrane. As far as the centriole is concerned, in A. foveicollis it appears...
Abbreviations:

Acrosome - Ac, Acrosomal granule - Ag, Centriole - C, Chromatin material - Cm, Flagellum - Fl, Nucleolar vacuole - NuV.
Nuclear vesicle - NVs, Posterior - Post, Sperm nucleus - SpN, Sperm tail - Spt, Tubular space - TuS, Vacuole - V
SPERMIOHISTOGENESIS OF *A. foveicolis* AND *L. quadrimeculata*

as a granule at the nuclear margin connected to a small cytoplasmic projection, however, in the case of *L. quadrimeculata* this could not be traced. During condensation of chromatin material of the spermatid in *L. quadrimeculata* 2-3 vacuoles are seen inside the nucleus. On further elongation the vacuoles increase in number but their size decreases. Later on all these vacuoles fuse together and form a hollow tubular space within the nucleus.

Finally due to further condensation of the nuclear material the hollow space disappears with the result a mature sperm nucleus stains deeply having a faintly stained long cytoplasmic tail attached at its posterior end. However, in *A. foveicolis* the vacuoles are not seen in the chromatin material of the elongating spermatid. In this case the spermatid becomes fusiform and changes into a pear shaped structure with its anterior pointed acrosomal end and broad posterior end connected with its cytoplasmic tail. The middle portion of the nucleus becomes broad and flattened so the whole structure looks like a “trypanosome”. On further elongation the lateral margins of the flattened nucleus roll up and fuse on the midline. As a result of this a tube like hollow clear space is enclosed within the nucleus. On further elongation and condensation of the nucleus this tubular space is lost. Finally a mature sperm is formed consisting of a deeply stained filamentous nucleus and a long faintly stained tail attached to its posterior end.

Literature reveals that a lot of work has been done on the spermatogenesis of various insects including Coleopterans. However, there is a little difference of opinion regarding the process of spermiohistogenesis. Bowen (1924) in *Chelymorpha* (Cassidinae) found that in early spermatids the chromatin gradually get condensed on the inner surface of the nuclear membrane. Phillips (1970) concluded that the chromatin in early spermatid appear in a very diffused form. It gradually becomes condensed in elongating sperm nucleus. He reported that in *Spur-throated grasshopper* chromatin filaments appear thicker during spermiohistogenesis and seen uniform throughout the nucleus. But in tree-hopper *Ceresa*, chromatin condenses around the periphery of the nucleus then in the centre. Gassner et al. (1975) in boll weevil *Anthonomous grandis* observed compact aggregated chromatin strands or dispersed strands in spermatid nucleus. In early stage the compact chromatin get aggregated at the inner margin of the nuclear membrane with the result a clear nuclear vesicle is seen within the nucleus in almost all the species. Whereas in dispersed condition the chromatin strands are found distributed throughout the anterior part of the nucleus.

In a review Phillips (1970) concluded that acrosome is a product of golgi bodies and is spherical in shape. This granule changes its shape and reaches at the anterior end of the sperm nucleus Bowen (1924), Vishwanath (1951) reported that golgi appears as deeply stained granules of varying sizes which unite and fuse to form a large vesicle with a chromophilic rim and chromophobic interior. He termed this as “acroblast”. This loses its staining capacity and now is termed as acrosomal vesicle. Later a deeply stained acrosomal granule appears on the border of the acrosomal vesicle and moves infront of the nucleus and forms the acrosome. Gassner et al. (1975), in boll weevil *Anthonomous grandis* reported that in a mature sperm a spherical acroblast is derived from golgi bodies which ultimately forms a cone shaped acrosome.

Vishwanath (1951) in *Coccinella septumpunctata* and *Ploceadera obesus* reported a single centriole situated at the base of the sperm. Werner (1964) and Smith (1969) found two centrioles in the young spermatids. Phillips (1970) reported that the centriole disappears during spermiogenesis. During the present study single centriole is found attached to the cytoplasmic tail in *A. foveicolis* but in *L. quadrimeculata* the centriole could not be traced. Bowen (1924) in *Cicindela-sexguttata* reported that within the chromatin material of the elongating spermatid several vesicles are formed during spermiophlogiosis. These vesicles in advanced stages tend to collect and fuse together with the result a single vesicle is formed. During nuclear elongation an intermediate stage looks like a “trypanosome”. In the present study in *A. foveicolis* the middle portion of the nucleus
becomes flat and broad and appears like a leaf or "trypanosome". On further elongation the lateral margins of the flat nucleus roll up and fuse together, therefore, a tube like space is found enclosed within it. This tube gradually disappears as the sperm nucleus elongates and becomes condensed. However, in *L. quadrimaculata* during early elongation the spermatid nucleus contain two to three vacuoles but on further elongation the vacuoles increase in number but their size decreases. In more elongated forms a single long vacoule is formed due to fusion of the small vacuoles. This is lost due to further elongation and condensation of the sperm nucleus. Kulkarni (1999) has also observed 1-4 vacuoles in *A. femoralis* and 2-3 vacuoles in *A. indica* in the elongating spermatid nucleus. These vacuoles fuse together and form an elongated vacuole which on further condensation and elongation of the sperm nucleus, disappear.

**EXPLANATION OF FIGURES**

*Aulacophora foveicollis*: 1. Spermatid showing nuclear vesicle and the acrosomal granule within the acroblast, 2. Spermatid with the flagellum attached to the centriole, 3. An elongated spermatid with acrosome attached at its anterior end, 4. Elongation of sperm nucleus showing anterior pointed acrosomal end and broad posterior end to which the tail is attached. The lateral margins are seen expanded thus appear like a "trypanosome", 5-a. A tubular space is enclosed within the nucleus due to, 5-b. folding and union of the lateral margins of the flattened nucleus, 6. Sperm nucleus more elongated, 7. mature sperm released are elongated and condensed *Laccopera quadrimaculata*. 8. Spermatid showing aggregation of chromatin material at the inner surface of the nuclear membrane and appearance of nuclear vesicle within it and the acrosomal granule inside the acroblast, 9. Chromatin material of the spermatid nucleus getting condensed having unstained vacuole in it. The acrosomal granule seen close to the nuclear membrane, 10. Spermatid containing one or two vacuoles in it, 11. More elongated spermatids showing one end pointed, 12. Elongation of spermatid nucleus with pointed acrosomal end and broad posterior flagellar end. The chromatin material gets more condensed, the vacuoles increase in number and their size decreases, 13. Small vacuoles fuse together forming a hollow tubular space within the nucleus, 14. A mature sperm bundle in which the tubular vacuole is lost and showing well stained sperm nuclei with their faintly stained cytoplasmic tail attached to the posterior end.

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**REFERENCES**


STUDIES ON THE BEHAVIOUR OF CHROMOSOMES DURING SPERMATOGENESIS IN AULACOPHORA FOVEICOLLIS (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT: This paper deals with the chromosomal behaviour during the process of spermatogenesis in *A. foveicollis* along with the nucleolar persistence. The presence of the nucleolar vacuole inside the nucleus and an unstained perinucleolar ring are the remarkable features. During spermateliosis, the elongating sperm nucleus appears as a flattened leaf or trypanosome like structure. The flattened margins of the spermatized nucleus roll up to unite with the result, the nucleus encloses a tube like space within it. At further elongation and condensation of the sperm nucleus, the tubular space is lost.

Key words: Chromosomes, Behaviour, Spermatogenesis, *A. foveicollis*.

INTRODUCTION

A number of authors such as Bown (1924), Nath *et al.* (1957), Nath (1965), Phillips (1970), George *et al.* (1975), Neolle (1979), Khatoon *et al.* (2006), Kulkarni *et al.* (2006) and many other investigators worked on insect spermatogenesis. Despite of their contributions, considerable difference of opinion and gaps exists in our knowledge.

MATERIAL AND METHODS

Insects were dissected under normal saline solution and their testis were taken out. Later their squash and smear preparations were made in acetocarmine for the present investigation.

RESULTS AND DISCUSSION

In the present investigation, efforts have been made to observe the behavior of the chromosome during spermatogenesis with certain features like nucleolar vacuole inside the nucleus, perinucleolar ring and the trypanosome like flat spermatized nucleus which later fuses with its lateral margins enclosing a tubular space within it.

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REFERENCES

Spermatogonial cells:

Fig. 1 Interphase: Cell membrane and nuclear membrane distinct, cytoplasm is agranular but nucleoplasm is granular, nucleolus and perinucleolar ring are present.

Fig. 2 Prophase: In the nucleoplasm, chromosomal threads, nucleolus, the nucleolar vacuoles within the nucleolus and an unstained perinucleolar ring are visible.

Fig. 3 Metaphase: Deeply stained and condensed chromosomes aggregate at the equatorial plate.

Fig. 4 Anaphase: Chromosomes at their respective poles are seen connected with spindle fibers.

Fig. 5 Telophase: Deeply stained chromosomes aggregate at their respective poles. Cytokinesis has started.

Primary spermatocyte cells:

Fig. 6 Leptotene: Long threads of chromosomes appear within the nucleus, a perinucleolar ring around the nucleolus is distinct.

Fig. 7 Zygotene: Nucleus contains paired chromosomal threads, nucleolus and perinucleolar ring are distinct.

Fig. 8 Pachytene: Nucleolus shifts close to the cell membrane seen connected with chromosomal loops.

Fig. 9 Diplotene: Nuclear membrane is lost, chromosomes become condensed, stain deeply and chiasma are formed.

Fig. 10 Diakinesis: Extremely condensed chromosomes lie in the cytoplasm, nuclear membrane is lost.

Fig. 11 Metaphase: Deeply stained and condensed chromosomes lie at the equator.

Fig. 12 Anaphase: Chromosomes of the respective poles are seen connected with spindle fibers.

Fig. 13 Telophase: Condensed and clumped chromosomes are seen at their poles.

Secondary spermatocyte cells:

Fig. 14 Interphase: Cytoplasm is agranular but nucleoplasm is granular, nucleolus and unstained perinucleolar ring is visible.

Fig. 15 Prophase: Chromosomal threads appear within the nucleus, nucleolus and perinucleolar ring visible.
BEHAVIOUR OF CHROMOSOMES DURING SPERMATOGENESIS

Fig. 16 Metaphase: Short and condensed chromosomes aggregate at the equator.
Fig. 17 Anaphase: Chromosomes have reached at their respective poles.
Fig. 18 Telophase: Deeply stained chromosomes become clumped together, seen at both the poles. Spermatids, their elongations and the sperm.
Fig. 19 Spermatids: Chromosomes aggregate at the inner surface of the nuclear membrane. Nuclear vesicle appears inside the spermatid. Acrosomal granule is deposited on the nuclear membrane.
Fig. 20 Spermatid's 1st elongation: Spermatid is little elongated containing a small vacuole inside it. Faint and small cytoplasmic tail projects at one end.
Fig. 21-2nd Elongation: A little more elongated spermatid has uniformly distributed chromatin containing a vacuole within it.
Fig. 22-3rd Elongation: It's anterior end is pointed and the posterior end is broad, nucleus contains 1-2 vacuoles.
Fig. 23-4th Elongation: Chromatin material get uniformly distributed inside the nucleus. The flagellum is seen connected at the posterior broad end.
Fig. 24-5th Elongation: Middle portion of the nucleus is broad, its margins roll up, fuse together therefore a narrow tubular vacuole is enclosed within it.
Fig. 25 Sperm: Nucleus becomes condensed, the elongated vacuole is lost, Cytoplasmic tails are seen at their posterior end.


