GAMMA IRRADIATION-INDUCED SPERMATOZOA ANOMALIES IN THE GROUND BEETLE, BLAPS POLYCRESTA

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Abstract: Exposure of the gonads to radiation known to lead to sperm abnormalities. The effect of Gamma rays from Cs137 source on the ultrastructure of the sperm of the ground beetle Blaps polycresta were studied. Two groups of 10 beetles each were irradiated with a dose rate of 32 Gy and 64Gy. Electron microscopic examination of spermatids and sperms of Blaps polycresta in the group which were exposed to 32 Gy exhibited many alterations of the general architecture and were more pronounced in the group exposed to 64 Gy. The results showed that the increase in morphological aberration of spermatids and sperms induced by gamma radiation were found to be positively correlated with the dose level. A major finding of this review was the paucity of data regarding the effects of Gamma radiation on spermiogenesis in adult insects.

Key words: Gamma radiation, Beetles, spermatozoa

INTRODUCTION

Radiological protection has focused on humans. Recently the ecological impact of ionizing radiation has emerged as an important research field. Exposures to natural radiation sources in particular, natural radionuclides are released to the environment in mineral processing and uses, such as phosphate fertilizer (production and use) and fossil fuel combustion (large amounts of fly ash and bottom ash result from coal combustion), causing enhanced natural radiation exposures [1]. Many persons are exposed to enhanced levels of natural radiation at their places of work such as underground miners, processing workers of minerals, and aircraft flight crew. Some exposures are caused by medical radiation procedures, through mishandling of radiation sources allowing radionuclides to be released to the environment. Also natural radiation include metal ore processing, uranium mining, zircon sands, titanium pigment production, oil and gas extraction, building materials (in the cement industry and clay in the ceramics industry), thorium compounds, scrap metal industry and emissions [2]. The highest dose rate for a critical group in nature (about 250 µSv) came from the use of fly ash in building materials [3]. Reproduction is considered to be one of the most sensitive to radiation (UNSCEAR, 1996), and not only determines the fate of the single organism but also may influence the population dynamics and the balance of higher ecological units. In most contamination situations, the majority of the radionuclide inventory in terrestrial ecosystems is found within soil; thus soil invertebrates can receive significant external and internal doses [4]. The effects of mutagenic radiation on ecosystems is the induction of germ cell mutations which cause directly affect the reproductive potential of populations [5]. The most radiosensitive cells are those with a high
mitotic rate and with a long mitotic future under normal circumstances. In this contemplates, germ cells are the most radiosensitive, and show different killing and sterilization susceptibility according to their developmental stage [6].

Many tissues show negligible damage in mature insects but the reproductive organs are sensitive to gamma radiation because the germinal cells are usually in active division [7]. In some cases it appears that innate genetic factors determine the time and mode of post-radiation mortality [8]. Age and developmental stage are important parameters to be taken into consideration when deciding on radiation process. In general, adults are more radio-resistant than pupae, which in turn are more resistant than larvae [9-12]. Early studies suggested that species with large adults would tend to be more radiosensitive than those with small adults, in this regard experiments have shown that Periplaneta americana is killed or sterilized by radiation doses to which smaller insects in genera such as Drosophila, Habrobracon and Tribolium are resistant [13,14].

**Fig. 1a:** Semithin section showing testicular follicles surrounded by epithelial sheath (arrow). Note the follicles are wrapped together by connective tissue (CT) and trachea (T). Note also: spermatids (St), spermatozoa (S), Sertoli cells (SC). **b:** Magnified part of Fig. (1a) showing testicular follicles surrounded by epithelial sheath (arrow). Note: connective tissue (CT), spermatids (St), spermatozoa (S), Sertoli cells (SC), cyst wall (arrow head). **c:** Semithin section showing early spermatids (St) in pairs with nucleus (N), Nebenkern (NK), Sertoli cells (SC), cyst wall (arrow head). **d:** Semithin section showing epithelial wall (arrow), spermatozoa (S), Sertoli cells (SC). a: X 10, b: X40, c: X100, d: X 100 [Specimen fixed in 4F1G and stained with toluidine blue].
Female arthropods are usually more radiosensitive than males [15-17], but there are numerous exceptions, for example, males were found to be more radiosensitive than females in the hemipteran families Pyrrhocoidae, Piesmidae, and Pentatomidae [18], the American cockroach *Periplaneta americana* (L.) [13], certain Coleoptera [19] and ixodid ticks [20]. Pupal or adult irradiation has similar effects and accordingly, histological damage is expected to be similar [21].

Ionizing radiation acts as a direct mutagen with minimal latency time of action, since it does not require absorption, distribution or metabolism; furthermore, the time of effective activity is practically zero [22]. Direct damage to the cell nucleus is believed to be responsible for mutations. Gamma rays are considered to be an ionizing form of radiation that traverses deeper into tissues and this penetration leads to a more even distribution of energy [23]. High doses of gamma radiation can inactivate sperm and lower doses have significant effects on sperm production, if the timing of the treatment has affected the developing sperm cells [24]. The frequency of abnormal sperm gradually increased by increasing the dose-rate of gamma radiation [25]. Apoptosis during spermatogenesis has been reported in Triatoma infestans a blood sucking insect and vector of Chagas disease after exposure to gamma-ray irradiation [26]. Cells in the process of spermatogenesis are very radiosensitive and apparently are easily killed [8]. The aim of this work was to evaluate the ultra-structural anomalies of sperms due to the effect of Gamma irradiation. This study could be an important tool in evaluating the retardation in spermiogenesis.

**MATERIALS AND METHODS**

Specimens: Live specimens were collected from the garden of Faculty of Science Moharram Bey, Alexandria University, Alexandria, Egypt and considered as a non-polluted site [27] in July 2015. After transport to laboratory specimens were sexed, males were chosen then maintained alive in native soil and plants in glass containers until processing and held under a day/night and temperature regime that approximated their place of origin.

Radiation treatments (gamma radiation): Thirty adult male insects divided into three groups; namely group A, group B, and group C. Animals of group A (10 insects) were used as control group, didn’t receive any treatment with radiation and housed at normal environmental conditions (the temperature inside the lab varied between 20° to 25°C, lighting condition were natural light from large windows during the day and complete darkness during the night). Insects of group B and C were irradiated at Gamma cell-40 Candian in the Egyptian Atomic Energy Authority (EAEA). Group B (10 insects) were housed inside a glass jar in front of Cs137 source to gamma rays at a dose rate 32 Gy and group C (10 insects) at a dose rate 64 Gy. At the end of the experiment insects were anesthetized, and dissected. Testes were removed carefully for experimental investigation.

Dissection procedures: Beetles were dissected under dissecting microscope in a drop of Ringer’s physiological solution on a wax-fixed petridish. A pair of dissecting forceps was used to open the abdominal cavity and then the testes were taken out. The surrounding tracheoles and fat body were removed.

Preparation of specimens for electron microscopy: Testis were fixed immediately in 4% formalin and 1% glutaraldehyde ([F.sub.4]G) fixative mixture in 0.1M phosphate buffer (pH 7.4) for 24 hrs. at 4°C. Specimens were then post fixed in 2% OsO4 in the same buffer for 2 hrs. at 4°C. Samples were washed in the buffer and dehydrated at 4°C through a graded series of ethanol. For scanning electron microscopy, dehydrated specimens were dried by the critical point method, mounted on an Al-stub and coated with gold in a sputter-coating. Observations of sperm morphology were performed by a JSEM-5300 operated at 20 Kv. Semithin sections (1 µm thick) stained with Toluidine blue were examined by LM to identify suitable area for ultrastructural evaluation. For transmission electron microscopy, dehydrated specimens of testes were embedded in Epon-araldite resin mixture. Ultrathin sections (60 nm thick) were double stained with uranyl acetate for 1/2 hr. and lead citrate for 20-30 min. and were examined in JTEM [28].

**RESULTS**

Histological and ultrastructural patterns observed in spermiogenesis of *Blaps polycresta*, group A: Each follicle was found to be surrounded by epithelial sheath (Fig.1a) and partitioned into a number of
progressive reduction of spermatid head during SEM preparations revealed that there was a As spermiogenesis proceeded (at late spermatids), where it became the acrosome (Fig. 2a). The basal part of the cells and migrated to the apex form the proacrosome since it will be differentiated through-out the remainder of the growth period to and the developing Nebenkern. Golgi bodies fused apparatus move into the area between the nucleus juxtanuclear position (Fig. 2a). At this stage the Golgi large body commonly known the Nebenkern, in a aggregate and fuse into a single mass to form a in mitochondria, adjacent mitochondria began to of chromatin (Fig. 2a). There was a little change sometimes with an intensely stained peripheral rim of the nucleus which was pale staining centrally, and were connected by intercellular bridges (Fig. 2a). spermatids and sperms appeared filling the follicles, these features indicating that spermatogenesis was at its peak (Figs.1a,b,d).

In the electron micrographs, initial spermatids of group A (the control group) were hemispherical in shape and were arranged in pairs with their nuclei abutting closely, apparently denoting their origin from a single spermatocyte (Fig. 2a). Spermatids were connected by intercellular bridges (Fig. 2a). The nucleus which was pale staining centrally, and with an intensely stained peripheral rim of microtubules, MD: mitochondrial derivatives, accessory bodies (double head arrow). Note: transverse sections in sperm head (H), middle piece (arrow), and flagellum (FL). Note: mature sperm (S) with diminished head.

As spermiogenesis proceeded (at late spermatids), our SEM preparations revealed that there was a progressive reduction of spermatid head during their development ranging from 2.92 µm in initial spermatids to 0.66 µm in late spermatids (compare Fig. 2b with Fig. 2e). The axoneme was elongated from the centriole and the Nebenkern undergo a structural differentiation, then divided into two separate mitochondrial derivatives toward the posterior end to form the flagellum which is approximately 7µm in length (Figs. 2b,c).

Mature spermatozoa had condensed nuclei. During chromatin condensation, the nucleus diminished in size and became small between thread-like acrosome and a long flagellum ranged about 0.094µm in size (Figs. 2 c,d,e). Mitochondria transformed into two mitochondrial derivatives and two longitudinal rods that flank the axoneme which are called accessory bodies (Fig. 2f). Transverse sections through the flagellum showed a presence of 9 accessory tubules in circle around the doublets, the bulk of the intertubular material was connected to the doublets. The axoneme was described by having the familiar 9 + 9 + 2 array (Fig. 2f).

Electron microscope preparations illustrated Sertoli cells this cell can be easily identified by its characteristic nucleus. The central large nucleus of the Sertoli cell had a polymorphous shape and was highly irregular being lobulated with intended nuclear envelope. The irregular nucleus revealed extensive nuclear envelope infoldings. The chromatin were evenly distributed. Few patch-es of heterochromatin and some clumps were associated with the nuclear membrane (Fig. 2g). Electron dense nucleoli were located peripherally in the nucleus. The cytoplasm extend and actually forms the wall of the cysts and contained numerous mitochondria, smooth endoplasmic reticulum and rough endoplasmic reticulum. Free ribosomes were

**Fig. 2a:** TEM showing spermatids at early stage of development. Note: N: large nucleus, Ne: nuclear envelope, C: centriole, M: mitochondria, G: Golgi complex, intercellular bridge (arrow), SC: Sertoli cells, arrow head: cellular debris, rER: rough endoplasmic reticulum, sER: smooth endoplasmic reticulum, r: free ribosomes. **b:** SEM showing early spermatid with rounded head (H), middle piece (arrow), and flagellum (FL). c: SEM showing late spermatid with oval head (H), middle piece (arrow), and flagellum (FL).Note: mature sperm (S) with diminished head. d: TEM showing longitudinal sections through mature sperms (S). A: acrosome, N: nucleus, C: centriole. Note: transverse section of middle pieces (arrow), ax: axoneme, MD: mitochondrial derivatives. e: TEM showing longitudinal section of mature sperm (S).A: acrosome, N: nucleus, C: centriole; ax: axoneme, MD: mitochondrial derivatives. Note: transverse section of middle pieces (arrow), double head arrow points at accessory bodies. f: TEM showing transverse sections in middle piece of sperms (arrow). Note: axonemes (ax) with 9+1+2 arrangement of microtubules, MD: mitochondrial derivatives, accessory bodies (double head arrow). Note: transverse sections in sperm head (arrow head), N: nucleus. g: TEM showing Sertoli cell (SC). N: nucleus with nucleolus (Nu), indented nuclear envelope (*) and patches of heterochromatin (HC). Note: M: mitochondria, rER: rough endoplasmic reticulum, sER: smooth endoplasmic reticulum, r: free ribosomes, cellular debris (arrow). Scale bar=0.5µm in a; Scale bar=1µm in b, c; Scale bar=0.5µm in d, e, g; Scale bar=0.1µm in f. [Specimens fixed in 4F1G, post-fixed in OsO4 and stained with uranyl acetate-lead citrate].
Histological and ultrastructural patterns observed in spermiogenesis of Blaps polycresta, group B.: Histological examinations of the testes B. polycresta in group B displayed varying degrees of structural abnormalities relative to the testes of the control samples. Testicular abnormalities included fibrosis of follicular wall, partial occlusion of the lumen of some follicles (Fig. 3a), indistinct cyst boundaries and necrotic spermatids (Fig. 3b). Abnormal chromatin condensation, vacuolated cytoplasm, disintegrated Nebenkern, agglutinated early spermatids, necrotic spermatids (arrow). Note: nucleus (N) with abnormal chromatin condensation, vacuolated cytoplasm (V) and disintegrated Nebenkern (NK). Note also: rupture of cyst wall (arrow). 

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spermatozoa and hypertrophied Sertoli cells with dilated smooth endoplasmic reticulum were also observed in our histological preparations (Figs. 3c,d,e).

Electron microscope examination of spermatids and sperms of Blaps polycresta in group B exhibited many alterations of the general architecture. Initial spermatids had abnormal chromatin condensation in the nuclei and vacuolated cytoplasm as well as disintegration of Nebenkern and Golgi complex (Fig. 4a).

However, late spermatids exhibited an increase in peripheral dense chromatin (Fig. 4b). In the cytoplasm aggregation of mitochondria takes place to form the Nebenkern, Golgi complex and free ribosomes were observed. Changes in head morphology (Fig. 4b) deteriorated and vacuolated middle-pieces with signs of degeneration of mitochondrial derivatives (Figs. 4b,c) and degenerated axonemes (Fig. 3e). Higher incidences of spermatid agglutinations were observed (Figs. 4a,b,c). The spermatids mainly bound to each other by their heads (Figs. 4a,c) or by their tails (Figs. 4b,c) and occasionally the head was bound to tail of other spermatid (Fig. 4b). Double and tetra tail spermatids in some specimens were also observed (Figs. 4b,4c). Spermatids sometimes failed to separate its residual cytoplasm (Fig. 4c) i.e. the elimination of extensive cytoplasm was not occur. Thus in SEM preparations spermatid with biflagellate tail (Fig. 4d), spermatid agglutinations (Fig. 4e) and deep sutures in plasma membranes of spermatid heads (Figs. 4e,f) were noticed.

Morphological changes in mature sperms revealed undulating plasma membrane in sperm head and rupture of the plasma membrane in the middle-pieces (Fig 4g). Our EM preparations revealed that Sertoli cells were hypertrophied and with high phagocytic activity to germ cells (Fig. 4h). The nucleus enlarged and contained granules of heterochromatin. In the cytoplasm, mitochondria with light matrices and short cisternae of rough endoplasmic reticulum (rER) were also noticed. Large amount of cellular debris and dilated smooth endoplasmic reticulum (sER) were found in the cytoplasm (Figs. 4h).

Histological and ultrastructural patterns observed in spermiogenesis of Blaps polycresta, group C.: In the present study, the influence of 64 Gy gamma irradiation on the histological structure of testes of B. polycresta revealed several abnormalities including shrinkage and massive necrosis of the follicle (Fig. 5a) (compare Fig. 5a with Fig. 1a), fibrosis and exfoliation of follicular wall (Fig. 5a), slight-to-marked derangement of the follicles (Fig. 5b), partial occlusion in the follicles (Fig. 5a,d) and disintegration or necrosis of spermatogenic elements (Figs. 5a,b,c). Furthermore, rupture of cyst walls was also noticed (Figs. 5a,b). Incomplete chromatin condensation in the nucleus of spermatids and hypertrophied Sertoli cells were also observed (Fig. 5d).

At the ultrastructural level, the follicles appeared with signs of various rates of injury. In few cases, germ cells were more or less normal, but in most cases, the germ cells undergo obvious changes and spermiogenesis was disturbed. A whole spectrum from healthy to necrotic cells were found. Spermatid differentiation or spermiohistogenesis was impaired variably. The initial large nuclear spermatids sometimes appeared with an oval nucleus, abnormal chromatin condensation, ill-defined nuclear membrane, vacuolated cytoplasm (Figs. 6a,b) and disintegrated Nebenkern (Fig. 6b). In addition agglutinated spermatids were commonly noticed (Figs. 6a,b).

In our electron micrographs changes in sperm morphology were observed. Morphologic assessment of sperms revealed winding of nuclear membrane and vacuolation of the nucleus (Figs. 6c,d). Sperms with double tail were observed (Figs. 6c,d,e), in addition to disintegration of plasma membrane in some middle pieces (Figs. 6c,d,e).

Sertoli cells were clearly affected, they appeared hypertrophied with expanded cytoplasm and displayed high phagocytic activity (Fig. 6f). The nucleus appeared with severe chromatolysis. Mitochondria with dense matrices, rER and dilated sER were also observed (Fig. 6f).

Our SEM preparations showed high incidence of spermatid and sperm agglutinations (Figs. 6g,h,i,j), biflagellate spermatid (Fig. 6g) and deep sutures in plasma membranes of spermatid heads were frequently noticed (Figs. 6g,h,i).

DISCUSSION

Ionizing radiation affects germ cells at all stages.
Each developmental stage responds with a different sensitivity [29]. In this study, a variation in the radiosensitivity among spermiogenic cells at the different stages of development was observed. Malformations were observed after irradiation with the dose rate 32 Gy and the maximum increase in these malformations were at 64 Gy. Bakri et al. [19] reported that the mean sterilization dose for Coleoptera ranged from 43 to 200 Gy. Curculionidae and Tenebrionidae, which represent the major groups of species that have been tested for radiation sterilization, both required a dose of about 76 Gy.

Our present study showed that the testes of B. polycresta consisted of testicular follicles partitioned into a number of germinal cysts and Sertoli cells which support the gamete producing cells. The follicles were covered by a layer of epithelial tissue and wrapped together by means of connective tissue and trachea. These results are in agreement with the results observed by kheirallah et al. [30].

Histologically, the irradiated testes of insects showed collapsed and shrinkage seminiferous follicles. These results similarly observed by Sallam et al. [31] who observed retardation in most of sperm bundles, less number of sperm bundles were found and sometimes few cysts were lost leaving large vacuoles instead in the testes of Spodoptera littoralis irradiated with the sub sterilizing gamma dose of 125 Gy.

Also, the present study revealed fibrosis of follicular walls, and rupture of cyst walls. McGee et al. [32] reported that changes in the cell surface morphology including irregularities of membrane contour and focal thickening often occur at an early stage in the course of cell injury, since the cell membrane is the first point of contact.

Necrosis and degeneration spermatogenic elements were also apparent in the irradiated testes of insects. Such decrease in the quality of gametes may in turn impair the reproductive success of adult organism. Similar results were obtained by Tallarico et al. [33] who observed that doses of 2.5, 10 and 20Gy gamma radiation had cell-killing effects in the germ cells of snail Biomphalaria glabrata.

The present ultrastructural observations of spermiogenesis in Blaps polycresta showed several features that are typical of insects. Conical acrosome covered the anterior of the nucleus which appeared compact with electron-dense material. The centriole appeared as a straight rod along the posterior nuclear membrane. This is in agreement with Kheirallah et al. [30] worked on the coleopterous insect Tachyderma hispida and Alzahrani et al. [34] worked on coleopterous insect Rhynchophorus ferrugineus.

In the present study, the development of the nucleus involves changes in shape and in degree of chromatin condensation: initially it is with a low electron density, and finally compacts with an electron-dense material. This is in agreement with

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**Explanation of figures**

**Fig. 4a:** TEM showing transverse section in early spermatids head (St). Note: nucleus (N) with abnormal chromatin condensation, disintegrated Nebenkern (NK) and vacuolated cytoplasm (V). Note also: sutures in the plasma membrane (arrow). Ne: nuclear envelope, G: Golgi complex, r: free ribosomes.

**b:** TEM showing transverse section in spermatids head (St) and middle pieces. Note: change of head morphology of spermatid (curved arrow), vacuolation in spermatid heads and middle pieces (V). Note also: middle pieces with degenerated or malformed mitochondrial derivatives (arrow), biflagellate spermatid (double head arrow). LSt: late spermatid, N: nucleus, NK: Nebenkern, G: Golgi complex, ax: axoneme, MD: mitochondrial derivatives. e: TEM showing transverse section in malformed middle pieces of spermatids. *: indicates agglutinated spermatids. Arrow indicates biflagellate spermatids with degenerated mitochondrial derivatives (MD). Note: tetra flagellated spermatozid (arrow head), middle piece with degenerated axoneme (double head arrow) and disintegrated mitochondrial derivatives. Ax: axoneme, V: vacuoles. d: SEM showing biflagellate early spermatid (double head arrow). Note: late spermatid (arrow). H: spermadid head, FL: flagellum. e: SEM showing biflagellate spermatozid (arrow head), agglutinated spermatid heads and flagella (*), suture in the plasma membrane (double head arrow). H: head, FL: flagellum, arrow middle piece. f: SEM showing agglutinated spermatid heads and flagella (*), suture in the plasma membrane (double head arrow). H: head, FL: flagellum, arrow middle piece. g: TEM showing mature spermatooza (S) with nucleus (N) and convoluted plasma membrane (arrow). Note: transverse section of middle pieces (double head arrow) with rupture plasma membrane. A: acrosome, C: centriole, ax: axoneme, MD: mitochondrial derivatives, arrow head: accessory bodies. b: TEM showing hypertrophied Sertoli cell with high phagocytic activity and expanded cytoplasm. Note: nucleus (N) displaying chromatolysis, large residual bodies (double head arrow). Note also: dilated smooth E/R (sER). S: spermatooza, arrow: middle piece. Scale bar = 0.5µm in a, b, e, c; Scale bar = 1µm in d, e; Scale bar = 5µm in f; Scale bar = 0.1µm in h. [Specimens fixed in 4F1G, post-fixed in OsO4 and stained with uranyl acetate-lead citrate].

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Swiderski and El Said [35], Fernandes and Bao [36], Alzahrani et al. [34] and Kheirallah et al. [30] worked on different hemipteran and coleopteran tribes. The acrosome is formed by Golgi complex [37]. The centriole in *B. polychresta* appeared as a straight rod and then divided into two equal parts this is similary observed by Alzahrani et al. [34] in the coleopterous insect *Rhyynchophorus ferrugineus*.

Cytoplasmic bridges were observed in spermatids but not in spermatozoa in our study. Throughout spermiogenesis, sometimes broad cytoplasmic bridges...
are formed among spermatids making them share cytoplasm. The excess of cytoplasmic mass is eliminated at the end of spermiogenesis leading to spermatid individualization [38]. Similar criterion was found in the hemipteran insect *Sphaerodema urinatar* [39].

Mitochondria in early spermatids of many insects aggregate and form a round body, the Nebenkern. This body undergoes a structural differentiation which divides into separate equal-sized bodies, the mitochondrial derivatives [40-42]. In this body identity of the individual mitochondria was lost but their essential structure remained and acrosomal and flagellar organization had been initiated [43]. This comes in full agreement with the findings in *B. polycresta*. No separate mitochondria were found after Nebenkern formation. This observation was also reported in the testes of the coleopterous insects, *Rhynchophorus ferrugineus* [34] and *Tachyderma hispida* [30].

Mature spermatozoa possess diminished nucleus which appeared as a small rod between thread-like acrosome and a long flagellum and had an axonemal array 9+9+2 in microtubule pattern and two mitochondrial derivatives. This comes in full agreement with the findings of earlier workers [34,44-47].

In the present study, Sertoli cells had an irregularly shaped nucleus, elongated and spherical mitochondria, well-developed system of sER, strands of rER and free ribosomes. Cytoplasmic features of Sertoli cells are phagocytosis and elimination of residual cytoplasm by the intracellular digestive system [48]. Moreover, transportation, nutrition of spermatogenic cells and hormone production in invertebrates are important functions of Sertoli cells [49].

Ultrastructurally, the testes of insects of group B and C showed many pathological lesions. The magnitude of the pathological lesions increased from group B to group C. In the present investigation, ultrastructural diagnosis identified various spermatid and sperm morphological abnormalities in insects of group B and group C affected by gamma rays. TEM and SEM were used to make a detailed qualitative analysis and intercellular detailed feature of the structure of immature and mature spermatozoa of insects from group B and C.

The presence of vacuolated, lytic areas in some sections of spermatids are evidence of cytotoxicity. This injuries might be a consequence of the release of lysosomal hydrolases into the cytoplasm [50]. The appearance of abnormal chromatin condensation, vacuolation in the nucleus and irregular nuclear envelope resembled those reported by Alvares – Garcia [26] after gamma – ray radiation on the hemipteran Triatoma infestans.

In fact, sperm morphology assay is said to provide a quantitative method for locating genetic damage in male germ cell lines as reported previously by Soares et al. [51]. Incidences of structurally abnormal sperm shapes are reported to be genetically controlled by numerous autosomal and sex-linked genes [52]. Thus, the obvious relative decrease in the density of chromatin condensation seen in sperm of insects from group B and C might be associated with chromosomal abnormalities. Similar abnormalities in chromosomes in Lepidoptera *Ephestia kuchniella* due to the effect of gamma radiation were recorded by Koudelova and Cook [53].

In this study, a variation in the radiosensitivity among spermiogenic cells at different developmental stages were observed. Germ cells at later phases of spermatogenesis were more sensitive to the induction of dominant lethal mutations than those at earlier stages [33]. Ashrafi et al. [54] found that all spermatogenic materials of the fifth-stage male larvae and 1-day-old male adults in Indian meal moth, *Plodia interpunctella* were affected by gamma radiation and damage to all stages increased with increasing radiation dose. Examination of the irradiated testis in the red flour beetle *Tribolium castaneum* revealed that the organ was very sensitive to gamma radiation of 15 Gy and induced reduction in the size of the testes.

Reduction in growth of the testis after irradiation may account for the decreases in or absence of spermatogenic activity as a possible consequence of radiation damage to the germ cells as reported by Banu et al. [21]. Anwar et al. [55] worked on the pupae and adults of the Mediterranean Fruit Fly *Ceratitis capitata*. The flies were irradiated with 10 kilorad (krad) of gamma radiation from a cobalt-60 source. The radiation dose of 10 krad completely stopped spermatogenesis. The spermatogonial cells, primary and secondary spermatocytes, and the
spermatids were completely aborted and produced dominant lethality in the fully differentiated sperm. Sallam et al. [31] studied the histological effects of gamma radiation on the testes of *Sodoptera littoralisir* radiated with the sub sterilizing dose of 125 GY and they found several morphological abnormalities included shrinkage of testes contents, vacuolations and dispersion of sperm bundles, spermatogonia and spermatocytes failed to develop to the next stages which lead to appearance of large vacuoles among the septum and retardation in sperm maturation.

In some affected specimens, the mid-piece showed convoluted plasma membrane, and mitochondrial abnormalities that were accompanied by axonemal degeneration and disruption of microtubles. This is in agreement with Joose et al. [56] who reported that gamma radiation cause damage to all stages of gametogenesis and caused a temporary sterile period in the snail *Lymmaea stagnalis*. Sperm axonemal alterations are known to be the primary cause of sperm immotility and it was found that a reduction of axonemal components caused lowered motility [57].

The present study demonstrated spermatid and sperm agglutinations. It seems likely that the sperm agglutination results from an influence of autologous IgM, which comes to penetrate the efferent duct as autoimmune responses in the testis progress [58,59].

Several investigations have been carried out on the effect of gamma irradiation on insect’s development and reproduction. Exposure of newly emerged adult of the codling moth, *Carpocapsa pomonella* (L.), to 40,000 rads of gamma radiation induced dominant lethality in at least 98% of the sperm without affecting adult emergence, mating behavior, or adult longevity and higher dosages decreased the frequency of mating as reported by Proverbs and Newton [60]. Bloem et al. [61] stated that male codling moths, *Cydia pomonella* (L.), which was treated with increasing doses of gamma radiation, and mated or outcrossed with fertile counterparts showed a slowly decline in fecundity of untreated females mated with treated males. As the dose of radiation increased fertility decreased and mortality increased. Fertility of treated males declined almost linearly to approach 0 near 400 Gy. Aye et al. [62] reported the inhibitory effects of gamma irradiation were demonstrated on the development and reproduction of *Plodia interpunctella*. Failure of all these events increased with increasing doses from 0.1 to 1.0 kGy. Same results were found by Salem et al. [63] worked on three substerilizing doses 50, 100 and 150 Gy of gamma radiation against full – grown male greasy cutworm, *Agrotis ipsilon*. The results showed that increase in sterility % induced by gamma radiation were found to be positively correlated with the dose level. Prabhakumary et al. [64] tested the effectiveness of different doses of gamma radiation ranging from 100 to 350 Gy for the control of *Triolium castaneum*. Higher doses caused mortality of *T. confusum*, while lowerdoses caused inhibition of developmentand sterility of the surviving insects. Dushmanrimana et al. [65] evaluated the mating performance of *Schistocerca*...
gregaria males irradiated with 4 Gray of Gamma radiation and found that irradiated males were able to mate but the resulting number of offspring was dramatically reduced compared to the average number of offspring observed during a regular mating. It is obvious that germ cells and Sertoli cells organelles are affected by gamma irradiation. Based on this data Blaps polychresta may be used as reference animals to environmental radiation protection.

CONCLUSION

The data obtained in this work by studying the effects of exposure to gamma radiation will be useful reference and helpful for the interpretation of data resulted from the analysis of environmental samples, which are usually complex mixtures of many chemicals. It is worthy to mention that, the micro morphological changes in spermatozoa present in this study should be included as a model for predicting gamma–rays effect and since exposures to natural radiation sources are more significant for the world’s population, then efforts should continue to broaden the database used for determining both representative values and extremes in exposures.

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