INFLUENCE OF GAMMA IRRADIATION ON THE ULTRASTRUCTURE OF THE OVARIES OF THE OASES DATE MOTH, *EPHESTIA CALIDELLA* (GUEN.) (LEPIDOPTERA: PYRALIDAE)

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INTRODUCTION

Palm is one of the most important fruit crops in Egypt. It is successfully grown in different ecosystems. More than 7 million palm trees are grown in the newly reclaimed area and delta region, (Bekheit, 1999).

The oases date moth, *Ephestia calidella* (Guen.) is considered a serious pest infesting date both in the field and in stores. The pest cause huge losses and qualitative damage of date. Demianyk and Simha (1981), reported that, many insect pests cause qualitative damage of food grains and many other food stuff in storage within a short period of time because of feeding inside grains, encouragement of high moisture and development of micro-organisms.

In recent years genetic control in Lepidoptera is used as inherited sterility, which is especially pronounced in the first filial (F_1) generation following the exposure of the parents to substerilizing doses of ionizing radiation Mikhaiel (2003).

In the present investigation an attempt was made to study the effect of gamma irradiation on ovaries structure of sterile females and partial sterile females of F_1 and F_2 generations using electron microscope.

MATERIAL AND METHODS

The main stock cultures of the oases date moth, *Ephestia calidell* (Guen.) was obtained from infested dates brought from Siwa oases reared in laboratory media (according to Waites and Gothilf, 1969). The colony was maintained for

several generations in the laboratory of the Biological Applications Department, Atomic Energy Authority.

The insect stock culture was maintained at 30±2° C and 70±5 R.H. To obtain large number of pupae, the last instars larvae ready to pupate were collected, sexed and isolated in glass jars provided with fine tissue paper.

The fullgrown female pupae were irradiated at 350 Gy, sterile dose, Mikhail (2003). A group of 25 full grown pupae were kept as control.

To study the effects of gamma irradiation on progeny, full grown male pupae were irradiated at 150 Gy. Immediately after adults emergence Mikhail (2003), irradiated males paired with normal virgin females. Newly hatched larvae (F_1 progeny) resulting from the cross I♂ x N♀ (irradiated males X normal females) were reared in groups (250 larvae) and provided with rearing media until the full grown larvae, then collected sexed and kept separately in glass jars to pupate. Twenty five F_1 female adults after emergence were kept for dissection. F_1 male adults were paired with normal virgin female; three pairs were placed in plastic vials for mating and oviposition in order to obtain F_2 generation. Twenty five female adults were isolated for dissection.

Mature pupae (10 day-old) used for experimental purposes were exposed to irradiation dose in a Cobalt-60 unit installed at the laboratory of Middle Eastern Regional Radioisotopes Center for the Arab Countries, Cairo, Egypt. The radiation, dose rate was 0.05 Gray/sec.

The ovaries were dissected and fixed in 2.5% cold glutaraldehyde in 0.1M phosphate buffer at pH 7.2, for 2-3 hrs at 4°C then washed twice in buffer and left overnight in buffer solution. Ovaries were post-fixed in 1% osmium tetroxide in 0.1M phosphate and ovaries were post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer for 30 min at room temperature. After the post fixation, the gonads were washed then dehydrated in successive 50%, 70%, 90%, 95% and 100% ethanol (10 min. each), followed by propylene oxide for two times (15 min. each). The dehydration process was followed by infiltration of the tissue in a mixture of 2:1 propylene open polymer overnight. The specimens were transferred with a dissecting needle into oven dry embedding forms filled with open and left to polymerize for 1 day at 60° C as described by Smagghe *et al.*, (1996). Tissue blocks were then cut into thin section in the LKB ultramicrotome. Section were cut with glass knives and collected on 200-mesh copper grids. Stained with uranyl acetate and lead citrate and were examined under Joel electron microscope.

RESULTS AND DISCUSSION

Normal non-irradiated female *E. calidella,* has meriostic polytrophic ovarioles which are typical in all lepidopterous insects. In lepidoptera oogenesis and vitellogensis are usually completed during late larval and pupal stage.

Figure (1, A) show ovarian follicular sheath and different stages of oocyte formation during oogenesis. Trophocytes are well developed and differentiated, contain rough endoplasmic reticulum, sparsely dispersed mitochondria and dividing nuclei (Fig. B, C).

Figure (C) show well developed microvillar border which has developed at the interface of the oocyte. The oocyte is charged with protein and lipid spheres (Fig. D).

The present ultrastructure study revealed clearly the tropholytes and oocytes during active normal oogensis. This study is in agreement with the findings of many authors on other insects. Abdalla (1995) worked on *Sitotroga cerealella* and cleared the same fine ultrastrudutre of normal ovary. Also, Abd El-Meguid and Haiba (1996) worked on the potato tuber moth. Salam *et al.*, (1996) reported that the growth of a trophocyte is accompanied by an increase in the rate of RNA synthesis (M. Tochondria, Ribosomes). Also, microvilli play a role in the vitellogenic process. The synthesized proteins are transported to the oocyte via the microvilli.

The oogonia are found in the apical part of the germarium. Each oogenium has a large spherical nucleus containing dense concentrated chromatin. The germarium of ovariole consists of a central mass containing comparatively large nuclei surrounded by a layer in which rough endoplasmic reticulum is abundant microvilli found between trophocyte and oocyte. (Hafez and Hamed, 2004). Worked on *Spodoptera littoralis.*

The irradiation caused extreme deterioration in ovarian development, especially the sterile dose (350 Gy). Figure (2 A&B). The microvilli surface between follicle cells lost its uniformity and ruptured. The most prominent damage appeared in the cytoplasmic organelles, which were vesiculated. Most mitochondria become disorganized. Fig.(B) show clearly extreme lysis of egg yolk. Protein yolk bodies were severely disorganized, disintegrated, fragmented and lost their crystalline appearance. Some morbid organells came together in the cytoplasm of the follicular cells.

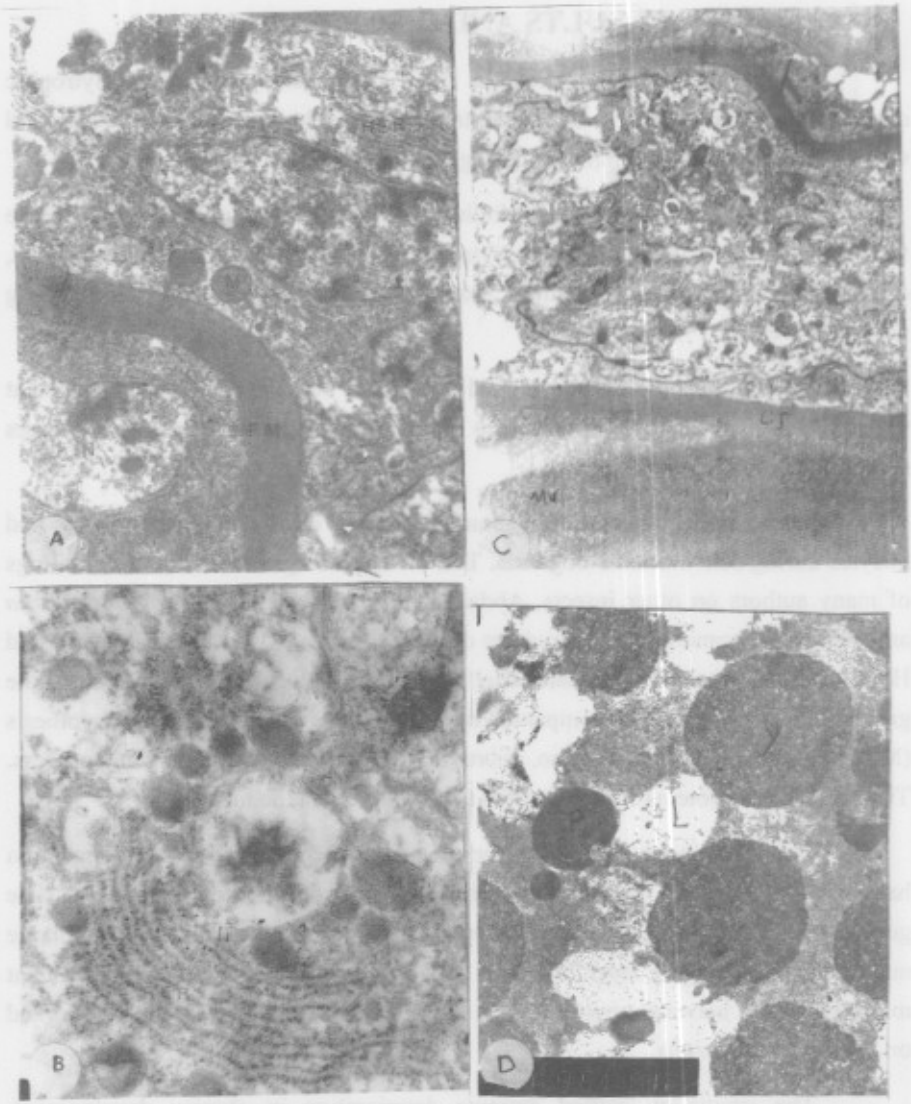

Fig.(1-A,B,C&D): Electron micrograph in ovariole of normal adult *E. calidella:* (A) Showing trophocyte cells at priphery of follicle, nuclei (N) appear ready to divide mitochondria (M), rough endoplasmic reticulum (RE R), ribosomes (R). 10000X. (B) Showing trophocyte cell, mitochondria (M) and rough endoplasmic reticulum (RER). 20000X. (C) Showing developing trophocyte cells, mitochondria (M), cell functions (CF), compact microvillar border and follicle sheath (FS). 4000X. (D) Showing protein (P) and yolk (Y) spherules in developing oocyte. 20000X.

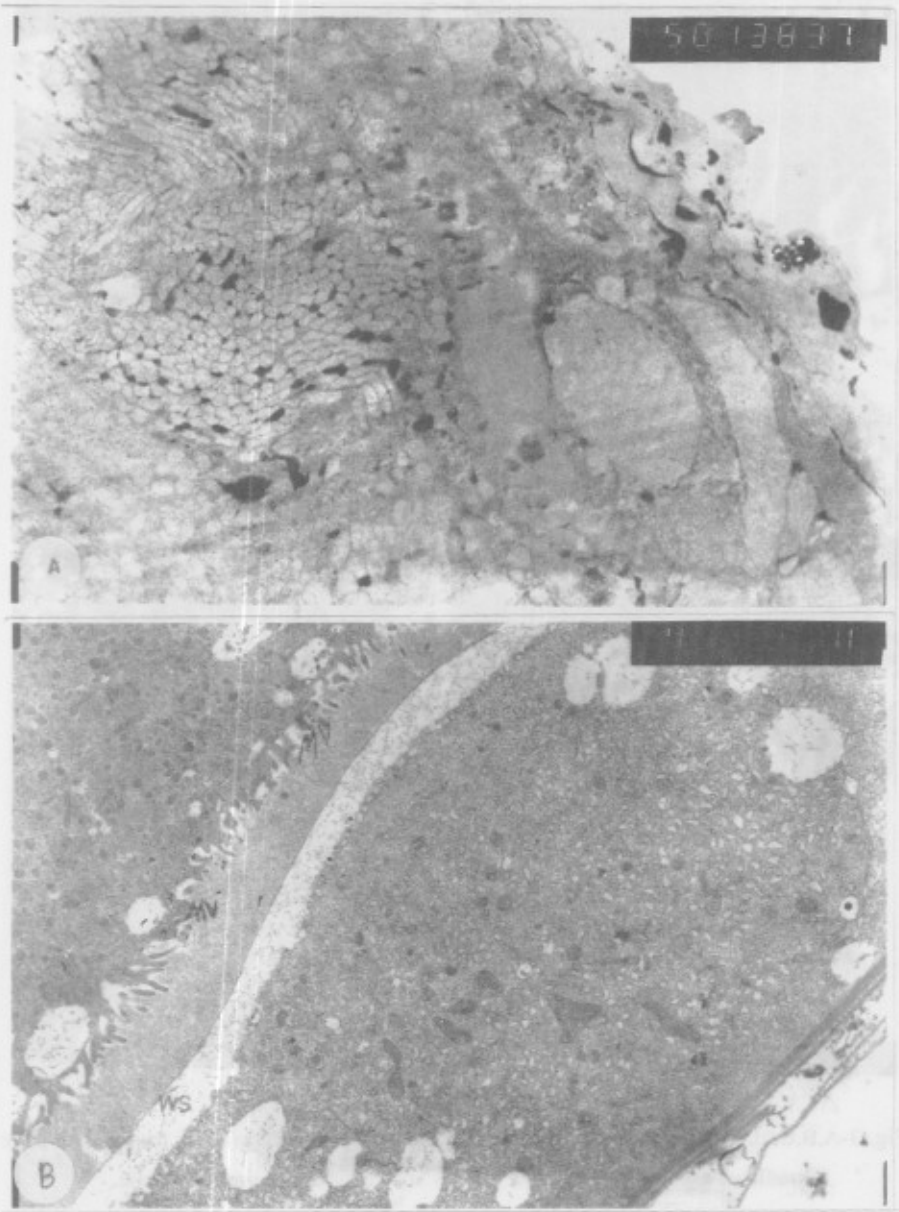

Fig.(2-A&B): Electron micrograph in ovariole of adult *E. calidella* irradiated as mature pupae with 350 Gy. (A) Showing degenerating cells and other organelles are unidentifiable 5000X.

(B) Showing the follicle cells separated by a wide space (WS) and microvillar border (MV) lost its uniformity. 4000X.

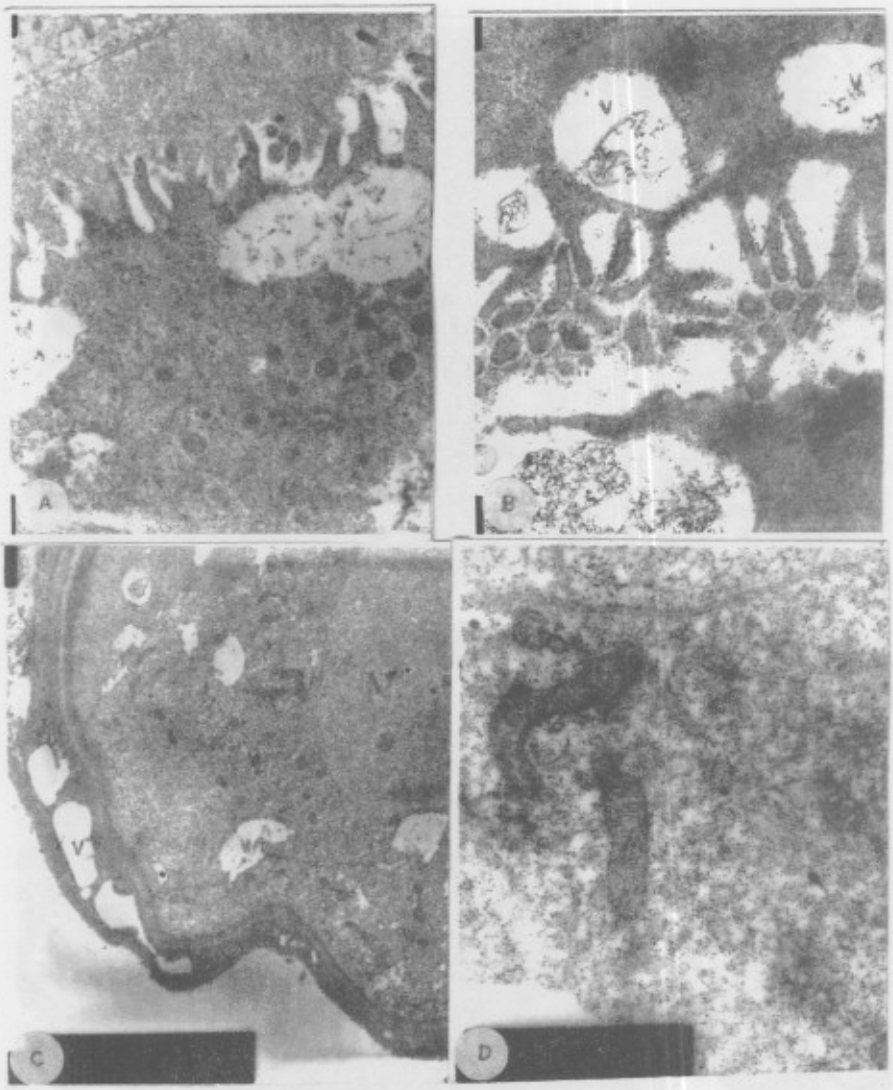

Fig.(3-A,B,C&D): Electron micrograph in ovariole of F_1 adult *E. calidella* from parent male irradiated as mature pupae with 150 Gy and crossed with normal female.

(A&B): Showing degenerate mitochondria (M) and vacuolation of cytoplasm (V). Microvillar border (MV) appear torn and irregular. 10000X & 20000X.

(C&D): Showing trophocyte cells deteriorated, mitochondria (M) reduced in size, cytoplasm vacuolated (V). 10000X & 20000X.

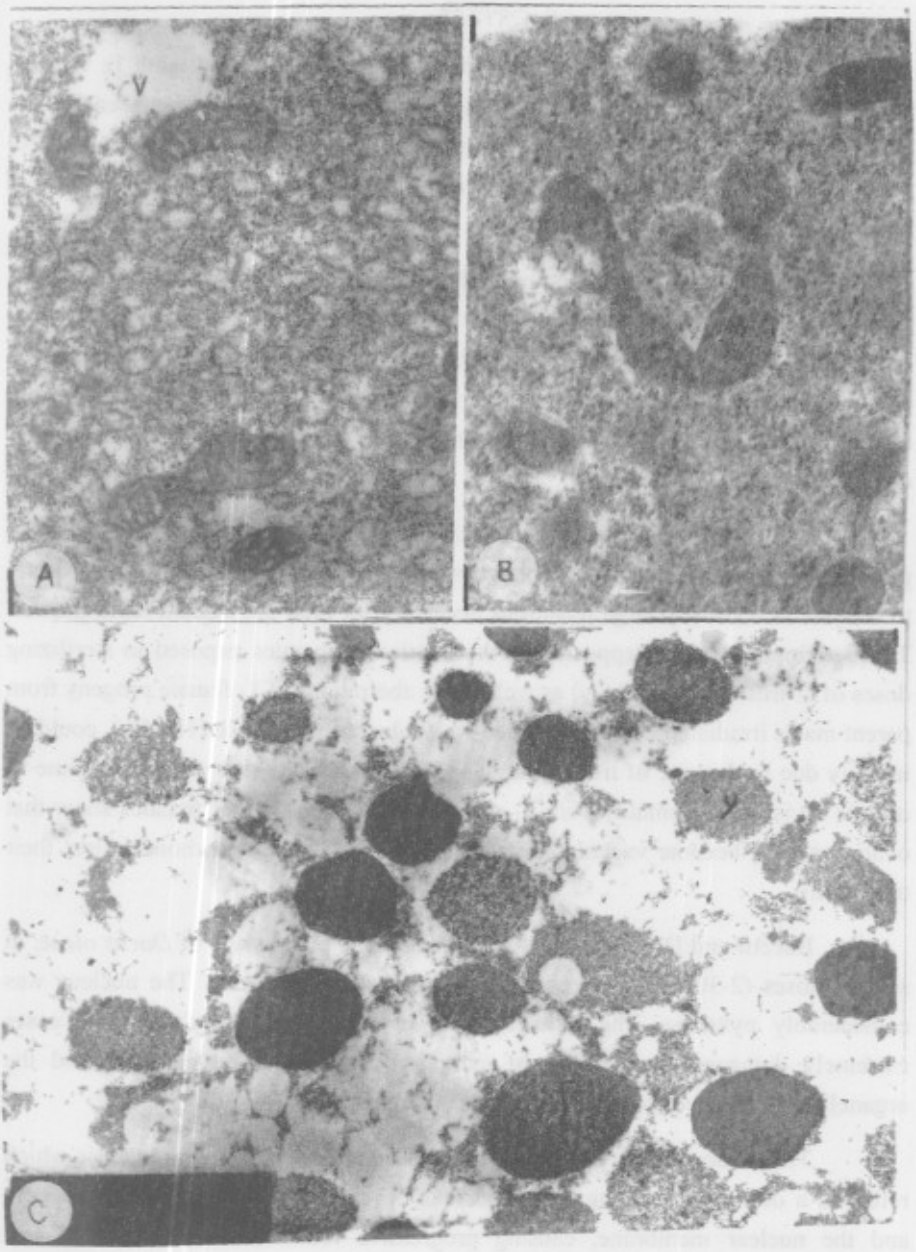

Fig.(4-A,B&C): Electron micrograph in ovariole of F_2 adult *E. calidella* , from F_1 male and crossed with normal female.

(A) Showing vacuolated cytoplasm of follicle cell (V). 14000 X.

(B) Showing degenerating mitochondria (M). 27000 X.

(C) Showing protein (P) and yolk (Y) spherules 27000 X.

Figure (3) shows the ultra structure of the ovarioles of 1-day-old adult F_1 females from P_1 adult males irradiated at 150 Gy, the microvillar border between follicle cells appear extremely distorted. Cytoplasmic degeneration could be observed. Disruption of mitochondria is very clear, showing swelling, fusion and disruption of cristae. Yolk bodies appeared shrunk.

Figure (4) the ultrastructure of the ovarioles of 1-day-old adult F_2 females from F_1 adult males; show clearly that ovarian sheath has become vacuolated. Cytoplasm of oocytes appears floccular, with the appearance of vacuoles. The cristae of some mitochondria lost their uniformity. Secondary lysosomes were observed. The chromatin in the nuclei seemed to be distorted apparently during their development.

The EM micrographs showed that gonads exposed to □-irradiation in the pupal stage, were affected. The trophic tissue (trophocytes) is known to supply RNA to oocytes in relatively large quantities necessary for their subsequent rapid growth. The deterioration which appeared in the ovaries of females exposed to sterilizing doses of □-irradiation (350 Gy) as well as the aberrations of F_1 female progeny from parent males irradiated as mature pupae with sub sterilizing dose (150 Gy), could be initially due to damage of trophic tissue, which must have been affected at time of mitotic division. F_2 females from F_1 males crossed with normal females show that ovarian sheath become vacuolated and the cristae of some mitochondria lost their uniformity.

Bacetti and Dominicis (1963), irradiated the pupal stage of *Dacus oleae,* at several doses (2-30 K rad), reported some deterioration effects. The nucleus was considerably pyknotic. The mitochondria were few, small and in some cases extremely damaged, large areas of cytoplasm seemed to be necrotic, and the organelles, became condensed into compact clumps.

Theunissen (1976) said that a common symptom is hypochromatosis which refers to a decreased staining of the nucleus in onion fly, including the chromatin and the nuclear membrane, causing progressive reduction of the nuclear size terminating in pycnosis.

Bindu & Sehgal (1988) working on *C. quinquefasciatus* at a dose level of 18 Krad of gamma radiation found that the cell nuclei were small and showed a large variety of degeneration symptoms (e.g. chromatokinesis and chromatolysis). These symptoms cause inhibition of DNA synthesis in the nucleus of nurse and

follicle cells and attributed to the decrease of growth and development of the ovary which resulted from exposure to gamma irradiation.

According to Degheele *et al.* (1988), germinal cells of the female cabbage root fly decreased in number and vacuolization, cytolysis and cell debris were wide spread, are symptoms of radiation damage. Tamouh (1988) found that the ultrastructure of the ovarioles of cowpea weevil irradiated as late pupae with 10 K rad showed damaging effects which increased with time, after exposure. At emergence only the apical region of the germarium is damaged; the rest of the germarium and egg follicles show alterations a few days later. He found the follicle cells in a degenerate state and follicular epithelium vacuolated and containing deformed mitochondria.

Salem (1990) reported that irradiation of 4-8 day old pupae of *Callosobruchus maculatus* with 10 Krad resulted in germarium damage of 2-day-old adult. The nuclei lost their spherical shapes, nuclear membrane was irregular and karyosome or chromatin clumping occurred in some of the nuclei. All these degenerating effects inhibited egg chamber formation.

Also, Abd-Alla (1995), found that the sterilization of 1-day-old-adult of *Sitotroga cerealella* caused some histological damage. The F_1 female from parent males exposed to 300 Gy, were fully sterile. Ultrastructural examination showed that the interface between the oocyte and the follicle cells was undulated, microvilli disappeared and there was black depositions observed at the oocyte surface. The nuclei of the follicle cells were very poor in chromatin and their cytoplasm showed undeveloped mitochondria, endoplasmic reticulum few and ruptured.

Abd El-Meguid and Haiba (1996), cleared the effects of sub sterilizing (100 Gy) and sterilizing (150 Gy) doses of gamma irradiation on the ovaries of the potato tuber moth found that irradiation caused extreme deterioration in ovarian development. They claimed that the reasons for the inhibition of the oocytes development could have been affected at time of mitotic division.

Salem *et al.* (1996) said that gamma irradiation of pupae of *C. pipiens* produced several histopathological changes in the ultrastructure of the ovaries the germarium and follicle were sensitive to gamma rays doses, vaculization and degeneration of the cells were the most obvious sings of damage. The degree of atrophy increased by increasing the irradiation dose level. The cytoplasm losts its density, cells debris and remmants of cell components are observed. At the

sterilizing dose (80 Gy) the ovarian development stopped completely. A similar effect was found by Wakid *et al.* (1996) on *C. pipiens*.

The findings obtained during the course of this study coincide with the fundamental radiology knowledge confirming the higher radio-sensitivity of undifferentiated cells and cell gametes.

SUMMARY

Gamma irradiation of mature pupae had histopathological effects on the ovaries of the resulting females. The sterilizing dose (350 Gy) on female, showed by ultra structure the microvillar surface between follicles cells lost its uniformity. Trophocyte became vacuolated and most of the cell organelles were hard to identify. The ovaries of F_1 sterile females from P_1 males showed microvillar border between follicle cells appear extremely distorted and mitochondria deterioration. The ovaries of F_2 females from F_1 males. Show the cristae of some mitochondria lost their uniformity. Secondary lysosomes were observed.

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