

## *Results*

## RESULTS

### A) Testis:

In sections of the control testis of mice, eight stages could be recognized during the seminiferous apithelial cycle according to Roosen Runge (1952) and Morsi, et al., (1985).

These stages are described as follows:-

#### Stage I:

(Fig. 1) This stages starts from the release of matur spermatozoa into the leumen of the seminiferous tubules and end with the start of nuclear elongation of spermatids. On the basement membrane spermatogonia type A & B are predominant.

The Sertoli cells with their characteristic large triangular nuclei are located near the basement membrane. There are two generations of primary spermatocytes, the leptotene and the pachytene phases. Near the lumen there is is one genration of spermatids with rounded nuclei. The frequency of this stage was found to be 13.7% (table 3).

#### Stage II:

(Fig. 2) This is the stage of elongation of spermatid nuclei. It starts with elongation of spermatid nuclei and with the formation of bundles of spermatids in the Sertoli cell cytoplasm. On the basement membrane type A spermatogonia are still present beside the sertoli cells. There are two generations of primary spermatocytes, the zygotene and the

pachytene. The shape of elongated spermatids varies from ovoid to eleptical with centrally located nuclei at the end of the elongation phase. The frequency of this stage is 16.7% (tuble 3).

#### **Stage III:**

(Fig. 3) This stage starts from the formation of bundles of elongated spermatids in the sertolian cytoplasm up to the appearance of the metaphase plates of the first maturation division. On the basement membrane type A spermatogonia are still present beside the sertoli cells. The zygotene phase of the primary spermatocytes is still present. This stage is characterized by the appearance of the diplotene phase of primary spermatocytes, which is characterized by large nuclei. Afew diakinesis were seen. The elongated spermatids are arranged in bundles. The frequency of this stage is 14.8% (table 3).

#### **Stage IV:**

(Fig. 4) This stage is characterized by the appearance of intermediate and type A spermatogonia on the basement membrane.

The zygotene phase of primary spermatocytes is still present near the basement membrane.

A bove this zygotene generation the configuration of metaphase plate will be seen with the appearance of a

secondary spermatocyte generation. The nuclei of the secondary spermatocytes are smaller than those of primary spermatocytes and larger than those of spermatids. It is characterized by having a thin network of chromatin to which are attached fine chromatin granules and each nucleus contains about six chromatin particles. The elongated spermatids are in bundles. The frequency of this stage is 2.2% (table. 3).

#### **Stage V:**

(Fig. 5) It starts from the end of last maturation division up to appearance of dusty chromatin in the nuclei of young spermatids. This stage is characterized by the presence of type A spermatogonia.

The zygotene phase of the primary spermatocytes is still present. There are two generations of spermatids, young spermatids which arise from the secondary spermatocytes of the previous stage and the bundles of elongated spermatids. The frequency of this stage is 9.1% (table. 3).

#### **Stage VI:**

(Fig. 6) This stage starts from the appearance of dusty chromatin in young spermatids up to the migration of the bundles of elongated spermatids toward the lumen of the seminiferous tubules. On the basement membrane, type B spermatogonia are predominating beside type A spermatogonia.

Pachytene spermatocytes, spermatids with rounded nuclei and the bundles of elongated spermatids which start to migrate into the lumen are observed in this stage. The frequency of this stage is 10.7% (table. 3).

#### **Stage VII:**

(Fig. 7) It starts and ends with the centripetal migration of the elongated spermatids towards the lumen of the seminiferous tubules. On the basement membrane type A and B spermatogonia are present. This stage is characterized by the appearance of leptotene spermatocytes, having a nucleus characterized by individualised thin filaments within the chromatin.

In a later stage, it assumes more diffuse enchromatin. The pachytene spermatocytes are still persisting. The rounded as well as elongated spermatids are centripetally arranged around the circumference of the seminiferous tubules. The frequency of this stage is 14.2% (table. 3).

#### **Stage VIII:**

(Fig.8) It is the stage of release of mature (type d) spermatids from the sertoli cytoplasm into the lumen as spermatozoa leaving the residual bodies behind. On the basement membran individual number of type A spermatogonia is present together with a few number of type B.

The majority of the basement membrane is occupied by leptotene spermatocytes while more centrally the pachytene spermatocytes then the rounded spermatids are arranged along the whole circumference of the seminiferous tubules. The frequency of this stage is 18.6% (table. 3). The mean diameter of the seminiferous tubules is  $175.6 \pm 2.7$  U (table 1).

The average number of cells of the seminiferous epithelial cycle is shown in table (1) and the sertoli cell ratio is shown in table (2).

In animals of the second group (animals which were injected with vitamin A for 8 days) the diameter of the seminiferous tubules showed a slight decrease ( $172.7 \pm 2.8$  U) as compared with the control ones. All the eight stages of the seminiferous epithelial cycle were identified. Generally there is no appreciable changes in the cellular associations of the different stages of the seminiferous epithelial cycle compared with control ones, but the germ cells appeared more defined and better stained than in control ones.

#### **Stage I:**

(Fig.9) This stage showed no changes in the basement membrane. Type A&B spermatogonia, sertoli cells, the leptotene and pachytene phases of the primary spermatocytes and the spermatids with rounded nuclei showed well defined features. Compared with control ones. The frequency of this stage is 13.7% (table. 3).

**Stage II:**

(Fig.10) This stage is characterized by the same cellular association as in control ones, but the shape and nuclei of the germ cells of the seminiferous tubule are more defined (table.1). The frequency of this stage is 15.2% (table . 3).

**Stage III:**

(Fig. 11) In this stage there was an increase in the bundles of elongated spermatids with deeply stained nuclei. The diplotene phase of the primary spermatocytes showed an increase in the size and stainability of nuclei compared with control ones. The frequency of this stage is 15.8% (table 3).

**Stage IV:**

(Fig. 12) In this stage the seminiferous tubules is almost normal and the cells are deeply stained as compared with the control. The frequency of this stage is 0.3% (table.3).

**Stages V, VI, VII, & VIII:**

(Figs. 13,14,15,&16). There is no change in the shape and size of the cellular associations of these stages of the seminiferous epithelial cycle. It was noticed that the number of spermatids especially type d (spermatozoa) was increased than those of control ones (table 1).

The frequency of these stages are 13.7%, 9.2%, 11.6%, & 20.5% respectively as shown (table.3). The average number of cells

of seminiferous epithelial cycle and sertoli cell ratios of the treated animals are shown in tables 1&2.

In animals of third group (animals which were injected with vitamin A for 15 days) the diameter of the seminiferous tubules showed a slight increase as compared with control ones ( $179.9 \pm 2.20$ ). The eight stages of the seminiferous epithelial cycle were found and the cellular association was observed to be nearly the same as in control ones (Figs. 17-24). The frequency of these stages is shown in table (3).

In animals of the fourth group (animals which were injected with vitamin A for 22 days), the seminiferous tubules showed a complete degeneration and involution as show in figures (25 - 29).

A complete damage, dilatation and thickening of the wall of the blood vessels which appeared engorged with blood indicating the presence of congestion and hemorrhage was noticed (Figs. 27 & 28).

Many cellular debris were found in some seminiferous tubules (Fig. 29).

There are noticeable changes in the elastic fibres of the tunica albuginea of the testes of control and treated animals (Figs. 30-33), but there is a marked increase in the elastic fibres of the wall of the saminiferous tubules of the animals treated for 22 days with vitamin A, compared with control ones (Figs. 30 & 33).



Table (1): Average number of cells of seminiferous epithelial cycle and diameter of seminiferous tubules.

Experiments	Number of sertoli cells	Spermatogonia		Total spermatogonia	Primary spermatocytes				Secondary spermatocytes	Spermatids				Diameter of seminiferous tubules
		Type A	Type B		P-L	Z	P	D-K		Sa	Sb	Sc	Sd	
Control	15.7 ±0.2	17.6 ±1.3	12.4 ±0.5	30.6 ±1.0	2.2 ±0.2	18.8 ±0.8	28.3 ±1.6	6.4 ±0.7	5.1 ±0	75.2 ±3.8	11.7 ±0.6	18.9 ±0.9	20.7 ±0.8	175.6 ±2.7
8 days after infection with Vit.A	15.1 ±0.2	16.6 ±1.1	11.4 ±0.7	27.7 ±1.2	2.6 ±0.3	20.1 ±0.8	27.2 ±1.3	5.9 ±0.5	10.6 ±1.3	74.8 ±4.1	13.5 ±3.5	31.2 ±2.5	25.1 ±0.6	172.7 ±2.8
15 days after infection with Vit.A	15.0 ±0.3	13.1 ±0.7	13.6 ±1.0	26.8 ±0.6	3.1 ±0.3	22.5 ±1.6	31.8 ±0.7	7.5 ±0.5	5.9 ±0.4	90.8 ±1.8	15.3 ±0.8	2.9 ±1.4	27.1 ±1.8	179.9 ±2.2

Table ( 2): Sertoli cell ratio.

Experiments	Number of sertoli cells	Spermato- gonia		Total spermato- cytes	Primary spermatocy- tes				Secondary spermato- cytes	Spermatoids			
		Type A	Type B		P-L	Z	P	D-K		Sa	Sb	Sc	Sd
Control	15.7	1.1	0.8	1.9	0.1	1.2	1.8	0.4	0.3	4.8	0.7	1.2	1.3
8days after injection with Vit. A	15.1	1.1	0.7	1.8	0.2	1.3	1.8	0.4	0.7	4.9	0.9	2.1	1.7
15 days after injec- tion with Vit.A	15.0	0.9	0.9	1.8	0.2	1.5	2.1	0.5	0.4	6.1	1.0	1.9	1.8

Table (3): Frequency of the stages of seminiferous epithelial cycle.

Stages	Control	8 days after injection with Vitamin A.	15 days after injection with Vitamin A.
I	13.7	13.7	9.1
II	16.7	15.2	18.6
III	14.8	15.8	7.8
IV	2.2	0.3	1.7
V	9.1	13.7	16.0
VI	10.7	9.2	12.6
VII	14.2	11.6	12.1
VIII	18.6	20.5	22.1