Hormonal and Testicular Ultrastructural Changes at Puberty in Rat Offspring from Diabetic Mothers

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Abstract: Diabetes mellitus has multiple consequences which affect the fertility of the patients and their offspring. Male rats born to diabetic mothers usually show testicular changes at the age of puberty which affect the function of the testis. This investigation was conducted to evaluate the ultrastructural changes of testes in adult male albino rats born to diabetic mothers in attempt to understand the relation between these changes with the changes in the serum levels of testosterone, FSH and LH. Forty adult female albino rats were used in this investigation. The rats were divided into two groups. Diabetes Mellitus was induced in one group by STZ injection. Both groups became pregnant by natural mating. Blood was collected from 60-day-old male offspring from both groups and the level of testosterone, FSH and LH were measured in their sera. At the same time, the testes were prepared for light and electron microscope study. Results showed significant decrease in LH, FSH and testosterone in sera of offspring from diabetic mothers compared with the control group. Light microscope examination of the testes of experimental group revealed loss of the normal arrangement of seminiferous epithelium, multiple intercellular spaces, significant reduction in the thickness of seminiferous epithelium and widening of interstitial spaces while ultrastructural examination of the testes of experimental rats showed folding of the basement membrane of seminiferous tubules, seminiferous epithelium with wide intercellular spaces, defective acrosome formation,few sER and absent lipid droplets in Leydig's cells.

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1. Introduction

Diabetes mellitus presents a significant public health burden due to its associated morbidity, mortality and economic costs (Stratton *et al.*, 2000). The prevalence of diabetes in Saudi Arabia is about 24%, one of the highest in the world (Al-Nozha *et al.*, 2004). The world health organization estimates that the Middle East region will have the highest increase in the prevalence of diabetes mounting at 163% by the year 2030 (Wild *et al.*, 2004).

Diabetes mellitus has many complications which affect most of the body systems. These complications are not restricted to the patients only but extend to their offsprings (Jelodar *et al.*, 2009). They added that, the consequences of diabetes during pregnancy are not only confined to foetal and neonatal life, but also extend to adult life and even to the next generation through the maternal line. Chung and Myrianthopoulos (1975) reported that pre-gestational maternal diabetes is associated with strong teratogenic effects on the heart, central nervous system and urinary tract. Drazancic *et al.* (1993) reported that major congenital anomalies are two to four times more common in diabetic pregnancies than in normal pregnancies. Knowler *et al.* (1985) stated that the risk for diabetes is significantly higher in the offspring of mothers who have non-insulin-dependent diabetes.

One of the mammalian systems that is clearly impaired in diabetes is the male reproductive system. Decreases in the serum levels of follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin and growth hormone in diabetes were reported by Hutson *et al.* (1983). Moreover; Benítez and Pérez Díaz (1985) stated that diabetes induced alterations of Leydig cell functions as decrease in androgen synthesis and in the total number of these cells leading to disturbances in sexual function, including a decrease in libido, impotence and infertility. Cameron *et al.* (1990) reported that these changes could be due to testicular dysfunction associated with sustained hyperglycaemia in diabetic rats. Furthermore Hassan *et al.* (1993) stated that spontaneously diabetic BB (biobreeding/Worcester) rats showed decrease in testicular testosterone production and altered spermatogenesis indicating that this process is inherent to this disease. Moreover, Jelodar *et al.* (2009) described the effect of maternal diabetes on the hormonal profile and testicular histomorphometry of rat offsprings. They said that testicular function is primarily controlled by pituitary hormones; FSH regulates spermatogenesis, whereas LH controls Leydig cell function. Zhao *et al.* (2011) demonstrated that oxidative stress could be a major cause for diabetic testicular damage.

To our knowledge testicular ultrastructure in adult rat born to diabetic mothers was not studied yet. So, this investigation is designed to study the testicular ultrastructural changes at puberty in rat offspring from

diabetic mothers and to correlate these changes to the changes in testicular hormonal axis.

2. Materials and Methods

Forty adult female albino rats (weighing 200 – 230 g and 4 – 5 months old) were housed at room temperature and supplied with standard pellet food with tap water ad libitum. Rats were divided into two groups, group I (thirty rats) and group II (ten rats). Diabetes was induced in the rats of group I by single intraperitoneal injection of streptozotocin (STZ) 150 mg/kg (Sigma, St. Louis, MO, USA) (Abo Abeeleh *et al.*, 2009). The rats were fasted 12 hours before and after injection. Diabetes was diagnosed by blood glucose level above 200 mg/dl one week after STZ injection. Rats from both groups were caged with male rat for mating. Mating was confirmed by the observation of vaginal plug. Nine female rats from group I died at different periods of pregnancy and 12 rats aborted spontaneously. The remaining rats completed their pregnancy. Offspring from both groups were reared in standard conditions in an animal house for 60 days. At the end of the experiment there were 28 living male offspring (experimental rats) from mothers of group I. Similar number was taken from the male offspring of group II and considered as control rats. Male offspring were anaesthetized with diethyl ether and killed by whole blood collection through a heart puncture. Blood serum was isolated and used for hormonal assay. Testosterone, FSH and LH levels were measured by radioimmunoassay (RIA) technique, Commercial kit (Immunotech - Radiova, Prague, Czech Republic).

The testes were removed surgically. Specimens from the testes were removed rapidly, cut into small pieces and processed for light and electron microscopic examination. Specimens for light microscope were fixed in Bouin's solution and processed to prepare 5 µm thick paraffin sections for Hematoxylin and Eosin (H&E) stain (Bancroft *et al.*, 1996). Specimens for electron microscope were immediately fixed in 2.5% glutaraldehyde solution buffered with 0.1 phosphate buffer at pH 7.4 for 2 hours at 4 °C. Specimens were dehydrated with ascending grades of ethanol and then put in propylene oxide for 30 minutes at room temperature followed by impregnation in a mixture of propylene oxide and resin (1:1) for 1 hour, then at 48°C for 1 hour. The specimens were embedded in EMbed-812 resin in BEEM capsules at 60 °C for 24 hours (Glauret *et al.*, 1998). By using Leica ultra cut UCT semi-thin sections will be obtained and stained with toluidine blue for light microscope examination. Ultra thin sections were cut and stained with uranyl acetate and lead citrate and examined using Jeol 100 S transmission electron microscope at Tanta university, Egypt.

For Stereological Procedures, A digitizing set consisted of Digitizer KD 3040 B connected to integer IBM compatible personal computer, was used with a specially prepared program to measure lengths. The thickness of seminiferous epithelium was calculated by measuring the total thickness of seminiferous epithelium from the diagrams in cm. Then, the true magnification was estimated. The actual thickness in microns was calculated as follow: The thickness in cm X 10000 / the true magnification.

The mean value and the standard deviation were calculated for the studied parameter. Unpaired student t-test was used to compare between the mean values of different groups. The values were considered significant when $P < 0.05$.

3. Results

Hormonal assay

The offspring of the diabetic mothers showed decreased serum levels of FSH, LH and testosterone (chart 1). The hormonal values obtained from the diabetic and control groups are presented in table 1. A high significant difference was observed for all measured hormones between the two groups ($P < 0.01$).

Table 1: Comparison of the mean values ± SD of hormones levels in offspring of control and experimental groups.

	Control group (No. = 28)	Experimental group (No. = 28)	*P* value
FSH (IU/ml)	4.38± 0.31	1.63± 0.27	P < 0.01**
LH (IU/ml)	0,54± 0.04	0.25± 0.03	P < 0.01**
Testosterone (ng/ml)	3.19± 0.26	1.3± 0.24	P < 0.01**

** = high significant difference

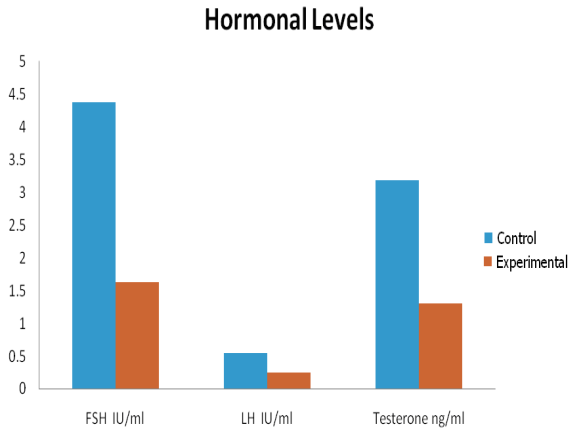

Chart 1: Comparison of hormonal levels in offspring of control and experimental animal groups

Light microscopic examination

Light microscopic examination of the testes of the experimental rats in comparison to control rats revealed loss of the normal arrangement of seminiferous epithelium together with multiple wide intercellular spaces and reduction in the thickness of seminiferous epithelium in the experimental rats. There is also severe exfoliation of the cells of seminiferous epithelium forming cellular embolus in the lumen of seminiferous tubules. The interstitial spaces are apparently widened (Figs. 1-A&B and 2-A&B).

Electron microscopic examination

Ultrastructural examination of the testes of control rats revealed Sertoli cells having triangular nuclei with apical invaginations resting on basement membrane. In contrast experimental rats showed folding of the basement membrane of seminiferous tubules together with wide intercellular spaces. They also showed Sertoli cells with nuclear basilateral invaginations (Figs. 3-A&B).

In control rats, spermatogonia have large nuclei and scanty granular cytoplasm which has vesicular mitochondria. Spermatocytes have spherical nuclei with fine granular nucleoplasm and chromatin accumulation. The cytoplasm is scanty with rounded mitochondria aggregating in groups (Fig. 4 A). Compared with the control animal group, the spermatogonia of the experimental rats have no morphological alterations except vacuolated cytoplasm. The experimental spermatocytes show reduction in the number of the mitochondria (Fig. 4 B).

In control rats, early spermatids are rounded cells with large spherical nuclei. Their cytoplasm contains vesicular mitochondria (Fig. 5 A). Oval nuclei with defected acrosome formation and swollen mitochondria are revealed in early spermatids of the experimental animal group (Fig.5 B).

Study of control tubular interstitium using electron microscope shows Leydig's cells with vesicular smooth endoplasmic reticulum together with lipid droplets (used for testosterone synthesis) distributed throughout the cytoplasm (Fig. 6 A). Contrary to the control animal group, few endoplasmic reticulum were seen with no apparent lipid droplets in the experimental animal group (Fig. 6 B).

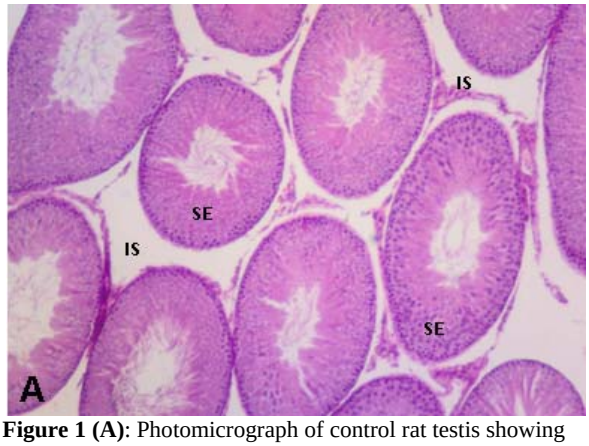

Figure 1 (A): Photomicrograph of control rat testis showing the seminiferous epithelium (SE) of seminiferous tubules and interstitial space (IS). (H&E X 100)

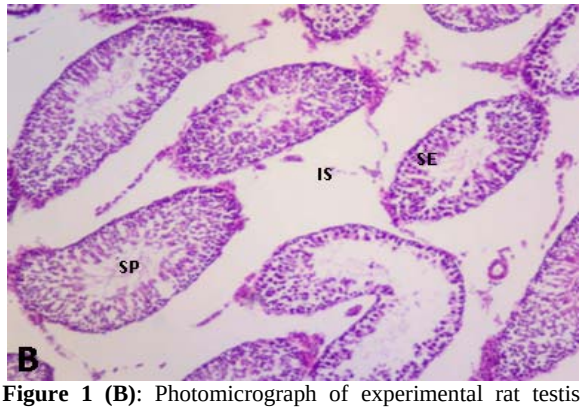

Figure 1 (B): Photomicrograph of experimental rat testis showing seminiferous epithelium (SE), sperms in the lumen of seminiferous tubules (SP) and widened interstitial space (IS). (H&E X 100)

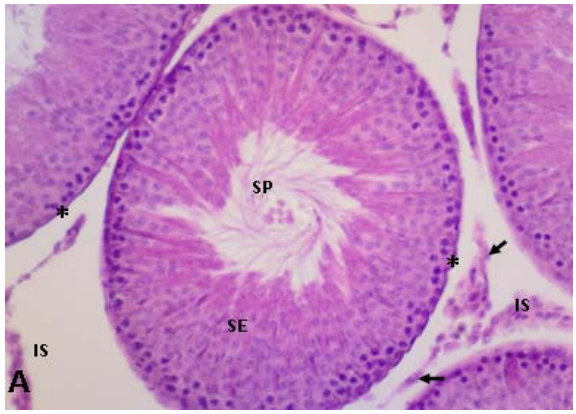

Figure 2 (A): Photomicrograph of control rat testis showing the seminiferous epithelium (SE) resting on a basement membrane (asterisks), sperms in the lumen of the tubule (SP) and Interstitial space (IS) containing Leydig's cell (arrow). (H&E X 400)

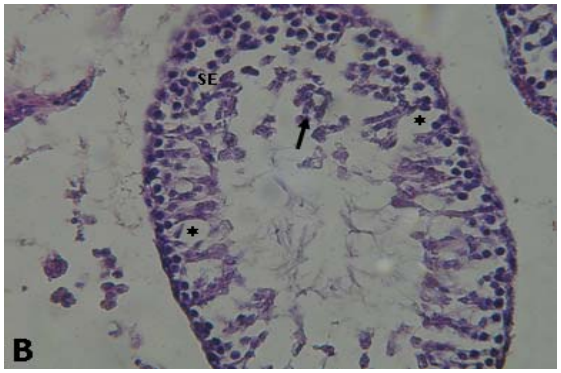

Figure 2 (B): Photomicrograph of experimental rat testis showing loss of the normal arrangement of the seminiferous epithelium (SE) with multiple intercellular spaces (asterisk) and exfoliated seminiferous epithelium (arrow). (H&E X 400)

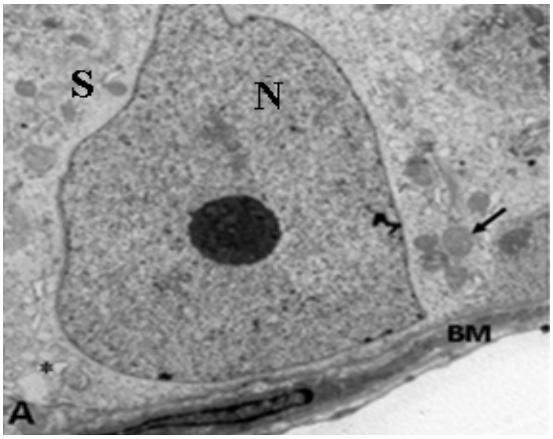

Figure 3 (A): Electron micrograph of control rat testis showing basement membrane (BM) of seminiferous tubule and Sertoli cell (S) having irregular nucleus (N) with apical invagination, mitochondria (arrow) and smooth endoplasmic reticulum (asterisk). (X 11700)

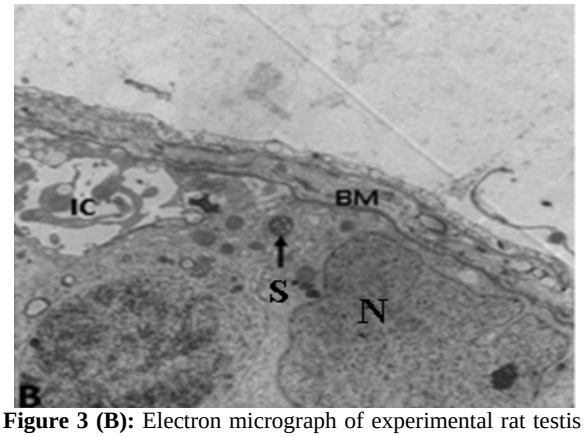

Figure 3 (B): Electron micrograph of experimental rat testis showing folded basement membrane (BM) of seminiferous tubule, wide intercellular spaces (IC) and Sertoli cell (S) having nucleus (N) with basilateral invagination and mitochondria (arrow). (X 8780)

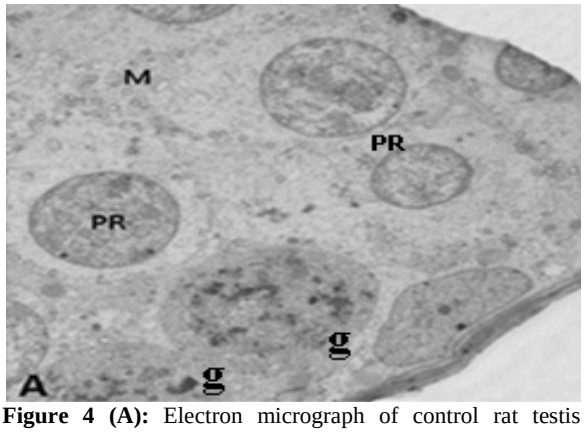

Figure 4 (A): Electron micrograph of control rat testis showing spermatogonia (g) and spermatocytes (PR) having peripheral rounded mitochondria (M). (X 5850)

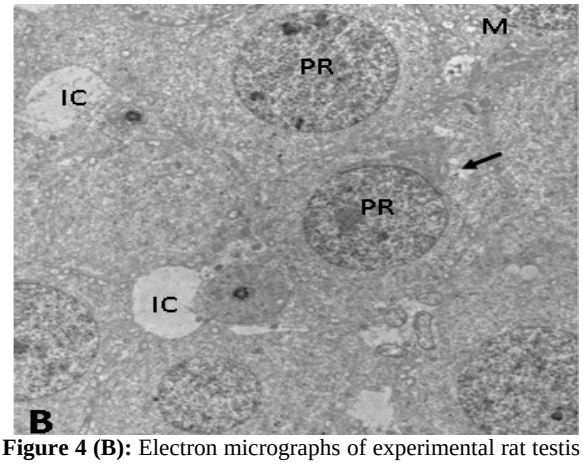

Figure 4 (B): Electron micrographs of experimental rat testis showing wide intercellular spaces (IC) and primary spermatocyte (PR) with vaculated cytoplasm (arrow) and reduced number of mitochondria (M). (X 5850)

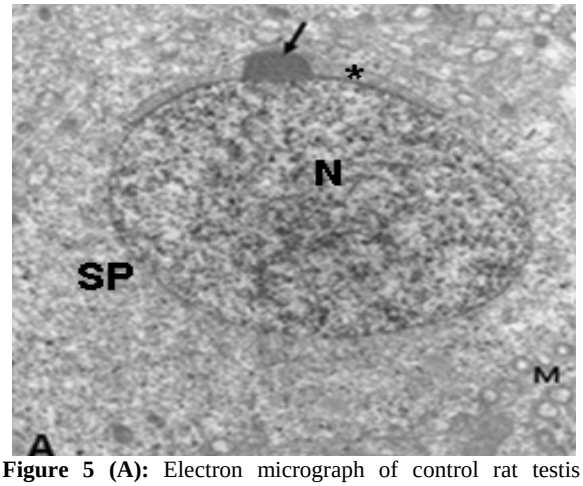

Figure 5 (A): Electron micrograph of control rat testis showing spermatid (SP) with spherical nucleus (N) and developing acrosomal cap (asterisk) containing acrosomal granule (arrow) and vesicular mitochondria (M). (X14600)

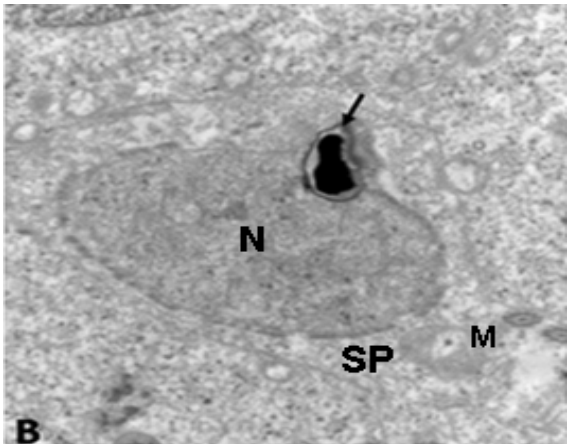

Figure 5 (B): Electron micrograph of experimental rat testis showing spermatid (SP) with oval nucleus (N), defected acrosomal cap (arrow) and swollen mitochondria (M). (X 14600)

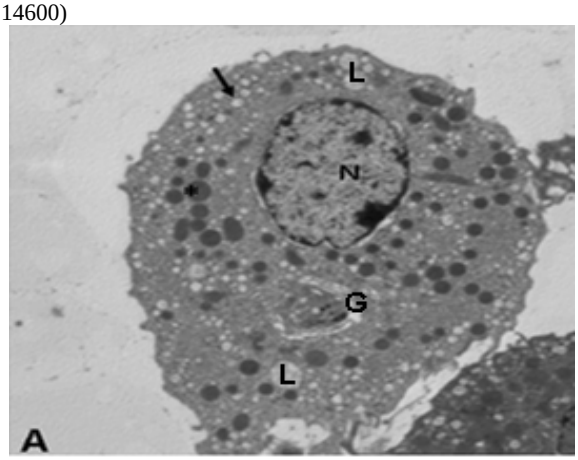

Figure 6 (A): Electron micrograph of control rat testis showing Leydig's cell with an oval euchromatic nucleus (N), numerous mitochondria (asterisk) vesicular sER (arrow), lipid droplets (L) and well developed Golgi apparatus. (X 11700)

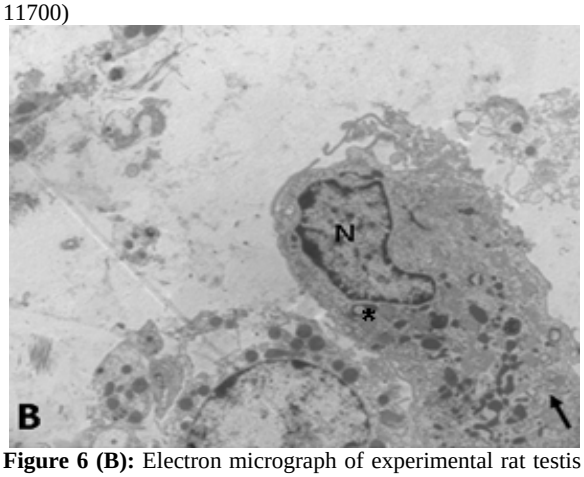

Figure 6 (B): Electron micrograph of experimental rat testis showing Leydig's cell with irregular nucleus (N), few endoplasmic reticulum (arrow) and bizarre-shape mitochondria (asterisk). There is no apparent lipid droplets. (X 11700)

Stereological examination

The thickness of the seminiferous epithelium shows highly significant lower values in the experimental group when compared to the control one.

Table 2: The mean values ± SD of epithelial thickness in offspring's seminiferous tubules of control and experimental groups.

Parameter	Control group (No. 28)	Experimental group (No. 28)	*P* value
Epithelial thickness (µ)	72.53± 6.04	61.26± 5.19	*P* < 0.01**

** = high significant difference

4. Discussion

The present study revealed a significant decrease in the levels of testosterone, LH and FSH in the offspring of diabetic mothers (experimental group) when compared with the controls. This is in agreement with Jelodar *et al.* (2009) who studied the hormonal profile and testicular morphology of the offspring of the diabetic mothers. Benitez and Parez Diaz (1985) explained the decrease in the testosterone level by reduction in the LH serum level caused by diabetes which in turn affects the normal Leydig's cells testosterone secretion, while Jelodar *et al.* (2009) explained this decrease in testosterone level by the reduction in the number of the Leydig's cells. In our study Leydig's cells showed decrease in the amount of sER together with absent lipid droplets which indicates decreased Leydig's cells function. Ballester *et al.* (2004) reported that the decrease in the number of the Leydig's cells observed in the offspring of diabetic mothers may be due to fetal hypoinsulinaemia. Fetal hypoinsulinaemia induces insulin-dependent decrease in FSH level, which in turn reduces LH level. This fetal hypoinsulinaemia was reported by Aerts and Van Assche (1977) and Devaskar *et al.* (1990) as a result of severe maternal hyperglycaemia.

Microscopic examination of the testis of the offspring of STZ-diabetic mothers revealed widening of interstitial spaces together with germinal epithelial changes in the form of cellular vacculation and presence of wide intercellular spaces between the seminiferous epithelium and folding of the basement membrane. There was a significant reduction of the thickness of seminiferous epithelium confirmed by stereological analysis. These results are more or less in consistence with previous studies on testes of STZ-diabetic rats. Asuquo *et al.* (2010) studied the effects of ethanolic extracts of Vernonia Amygdolina and Ocimum Gratissimum on the testes of STZ-diabetic male rates. They reported alterations, vaculations and distortion of both seminiferous epithelium and peritubular tissue. Ballester *et al.*, 2004 reported the same changes in the interstitial tissue in STZ-diabetic rats but the authors didn't find any significant differences in the size and density of the semineferous

tubules. These changes were partially attributed to the augmented oxidative stress in the interstitial tissue. (Tang *et al.*, 2008).

The tubular alterations shown in the present study could be explained by a decrease in the FSH level in the serum of the offspring of STZ-diabetic mothers. The decrease in FSH decreases the tubular FSH receptors which in turn diminishes significantly the response of the tubular epithelium to FSH stimulation. Besides, a decrease in the expression of the insulin receptors, could lead to loss of insulin mediated cell proliferation in seminiferous tubules. Both conditions may be responsible for the testicular histological changes reported in the present study.

In conclusion, maternal hyperglycaemia has a significant deleterious effects on the structure and hormonal function of the testes of their male offspring. These effects are likely to occur during fetal life and continue in the adult life.

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