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Original Article

Histological Impact of Long Term Varicocele-Induction on Right and Left Testes in Rat (Evidence for the Reduction of Sperm Quality and Mating Abilities)

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Abstract

This study was designed to clarify bilaterally effect of long-time varicocele on left and right testes and to identify the effect of varicocele on maturation divisions during spermatogenesis. Moreover, the percentage of seminiferous tubules (STs) with I-VIII and IX-XIV stages of spermatogenesis and sperm parameters such as sperm motility and abnormality types were evaluated. The left varicocele was induced in test group (n=18) and control-sham animals (n=6) were gone under simple laparatomy. The test group further sub-divided into three subgroups based on the duration of varicocele (4, 6 and 8 months after varicocele induction). The histological alterations, spermiogenesis index (SPI), Sertoli cells (SCs), cytoplasmic lipid and alkaline phosphatase (ALP), germinal cells first and second maturation divisions, spermatogenesis stages, natural mating and sperm characteristics (count, motility and abnormality) were evaluated. Histological results showed that the varicocele-induced damage affected both stages of I-VIII and IX-XIV and up to 20% of the STs exhibited negative SPI and SCs degeneration. After 8 months, majority of STs demonstrated a remarkable reduction in the first and second maturation division in both of the testes. Nevertheless, the right testicles were manifested with significantly ($P < 0.05$) lower detrimental features in comparison with the left ones. Following varicocele induction the sperm count, viability and motility reduced time-dependently. Our data suggested that monolateral varicocele-induction affected both right and left testicles simultaneously albeit with differences. The varicocele-induced injuries were manifested as negative SPI and SCs degeneration, which may have led to poor sperm quantity and quality.

Key words: Maturation division; Spermatogenesis; Sperm characteristics; Varicocele

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Introduction

According to epidemiological data and hospital reports the clinical varicocele is observed in 10-20% of the general male population, in 35-40% of men with primary infertility and in up to 80% of men with secondary infertilities.[1,2] The known and common etiology of varicocele is a retrograde blood flow down from the internal spermatic and the creamasteric veins into the pampiliform plexus[3] which itself is the result of missing and/or incomplete valves[4]. It is thought that the retrograde blood flow leads to varying degrees of increased hydrostatic pressure and in turn this impairment increases the temperature within the testis.[4-6] Thus after considerable temperature alterations in varicocele patients, the germinal cells apoptosis is predictable, which in turn leads to testicular atrophy and depletion.[4,7-9] On the other hand some of the studies showed the range of sperm concentrations from azoospermia to very low volume among patients with varicocele.[10,11]

There are controversial reports about the effect of one sided varicocele effect on both testes. Some believe that following one side varicocele the other side is also affected, however some disagree with this and are suggesting monolateral impact of induced varicocele [12]-[15]. Meanwhile the exact simultaneous impact of varicocele on right and left testes has not been fully clarified yet. For example, different reports on animal models indicated that, an induced unilateral varicocele (left varicocele) on the models resulted in remarkably lower sperm count than those of the control animals.[13,14] These studies suggested that left sided varicocele could able to induce bilateral detrimental effects. In contrast, Turek and co-workers believe that spermatogenesis damage or testes failing may differ within a single testis resulting in focal areas or `patches' of sperm production within an organ.[15] The first aim of the present study was to compare the histological alterations in left and right testicular tissues during long time unilateral-induced varicocele. It has been well established that varicocele is classified into three grades of I, II and III though the clinical signs mainly depend on the age conditions.[16,17] Therefore, the age-dependent effect of varicocele on the histological changes in both left and right testis along with sperm quantity and quality assessment were subjected to the present study too.

Materials and Methods

Animals. Twenty four mature male Wistar rats, 10 weeks old and weighing between 200 to 220 g were used. The rats were obtained from the Animal House of Faculty of Veterinary Medicine, Urmia University (Iran) and were acclimatized in an environmentally controlled room (temperature, 20 - 22°C with 12h light/12h dark). Food and water were given *ad libitum*. In this study all experiments were in accordance with the Urmia University guidelines for research on laboratory animals. Following week acclimatization, the animals were assigned into four groups (n = 6) as control-sham and test groups. The test subgroups nominated upon the time of termination of the study as 4 months, 6 months and 8 months, after the varicocele induction.

Varicocele induction. In test groups left varicocele was induced as previously reported[16]. In brief, following induction of anesthesia with ketamine 5% (Razak, Iran), 40 mg kg^{-1}, i.p. and xylazine 2% (Trritau, Germany) 5 mg kg^{-1}, i.p. the diameter of renal vein was reduced to 1 mm, left renal vein ligation was performed at a direct medial to the junction of the adrenal and spermatic veins. Then the anastomotic branch between the left testicular vein and the left common iliac vein was ligated. The animals in control-sham group were anesthetized and only underwent to a simple laparatomy and no

vein ligation was performed on these animals.

Animals mating. After 4, 6 and 8 months the animals were adjoined to 10 weeks old female rats. Two mature female rats were adjoined to one varicocelized male rat for 2 weeks in order to identify their natural mating abilities.

Histological analyses. Following 4, 6 and 8 months varicocele induction, the animals were euthanized by special CO_2 device and one half of the testes were dissected out and fixed in 10% formalin for histological investigations and subsequently embedded in paraffin. Sections (5-6 µm) were stained with Iron-Weigert (Pajohesh Asia., Iran) and were analyzed under light microscope by multiple magnifications (400× and 1000×). A hundred cross sections from testes were analyzed to clarify the vascular dilatation, thrombosis and mononuclear immune (MNI) cells infiltration.

Identifying the spermatogenesis stages. The STs classified into two groups in which they were presented stages I-VIII and IX-XIV of spermatogenic cycle, respectively. In order to evaluate the percentage of each stage 100 STs in 100 cross sections of the testicular tissue from the test and control-sham groups were analyzed. Furthermore those tubules with lower than 3 layers of germinal epithelium were classified in the group of tubules with arrested spermatogenesis.[18]

Spermiogenesis index determination (SPI). The STs with normal luminal spermatozoa were considered as tubules with positive SPI and those tubules with empty lumen (without any spermatozoa) were marked as tubules with negative SPI. In order to evaluate the SPI, 100 STs in 100 cross sections for each rat were evaluated.

Assessment of the first and second maturation divisions. As the spermatocyte type II is a short-lived cell, detecting of these cells under light microscope is very hard. Thus in the current study the preleptotene spermatocytes, which are the largest spermatogenic cells were counted in 100 tubules of 100 testicular cross sections for each rat. The number of these cells computed in order to obtain the number of cells, which were candidated to have first maturation divisions. Furthermore the number of spherical and elongated spermatids was counted with already mentioned method and considered as the second maturation division markers.[19]

Histochemical analyses. The remained half of the testes were dissected out and immediately transferred to a nitrogen vapor and after 10 minutes the specimens were cut in 10µm with cryostat (LMC, England). The alkaline phosphatase (ALP) and Oil-Red-O staining were conducted. The SCs with cytoplasmic lipid accumulation and with positive cytoplasmic ALP were considered as abnormal cells. The number of lipidophilic SCs per one seminiferous tubule was counted in 100 tubules of 20 cross sections for each rat.

Epididymal sperm count, viability, motility and sperm abnormalities. Epididymides were separated carefully from the testicles under a 20-fold magnification provided by a stereo zoom microscope (model TL2, Olympus Co., Tokyo, Japan). The tail of epididymis was separated, trimmed and minced in 5 mL Hams F10 medium. After 20 minutes the grinded epididymal tissue was separated from the released spermatozoa. The sperm count was performed according to standard hemocytometric lam method and up to 20 drops were used in order to prepare eosin-nigrosin stained slides from each sample to evaluate dead and abnormal sperms.[20,21] The abnormalities of the sperms were classified in three classes of head, tail and duplicate abnormalities. To investigate the epididymal sperm motility each sperm sample was diluted in Hams F10 (1/2) and the motility of the sperms characterized

into three immotile, non-progressive and progressive motility classes.[22]

Statistical analyses. The statistical analyses were performed on all numerical data by using two-way ANOVA and using Origin software version 6.0. All values were expressed as the mean ± SD. To compare the graded histological findings between groups, the Kruskal-Wallis test was used. Correlations between the percentage of STs with dissociated germinal epithelium with Oil-red-O positive Sertoli cells were analyzed on an Indigo-2 O2 work station (Silicon Graphics, Mountain View, CA) using Matlab (MathWorks Inc., Natick,MA). $P < 0.05$ was considered to be statistically significant.

Results

Total body and testicular weight gain. Macroscopic observations revealed that the testes in all varicocele groups were significantly ($P < 0.05$) decreased in size and weight in comparison with the control-sham animals. Body weight showed no significant alterations in total body weight gain of the animals in different test and control groups (Table1).

Neonatal from natural mating. The rate of born neonates decreased significantly ($P \leq 0.05$) in all test groups. Accordingly, the 8 months varicocelized rats demonstrated the lowest number of neonates in comparison with other test and control-sham groups (Table 2).

Table 1. Mean average of testicular weight gain, length and wide in different varicocele and control-sham groups. All data are presented in Mean ± SD.

LEFT TESTES				
Groups	**Control-sham**	**4 months varicocele**	**6 months varicocele**	**8 months varicocele**
Testicular Weight Gain (gr)	0.88 ± 0.02	0.76 ± 0.04^{*a}	0.65 ± 0.03*	0.61 ± 0.05^{*f}
Testicular length (Mm)	19.870 ± 1.259	15.35 ± 1.98^{*b}	10.67 ± 1.21^{*d}	8.35 ± 0.81^{*g}
Testicular wide (Mm)	12.42 ± 0.86	9.16 ± 1.32^{*c}	8.00 ± 1.26*	5.83 ± 1.16^{*h}
RIGHT TESTES				
Groups	**Control-sham**	**4 months varicocele**	**6 months varicocele**	**8 months varicocele**
Testicular Weight Gain (gr)	0.89 ± 0.05	0.82 ± 0.02$^{*a'}$	0.72 ± 0.03*	0.71 ± 0.02$^{*f'}$
Testicular length (Mm)	19.78 ± 1.06	16.36 ± 0.77$^{*b'}$	14.59 ± 1.03$^{*d'}$	11.78 ± 1.09$^{*g'}$
Testicular wide (Mm)	12.85 ± 0.69	11.83 ± 1.32$^{*c'}$	9.67 ± 1.21*	7.83 ± 1.49$^{*h'}$

Stars are indicating significant differences ($P \leq 0.05$) between different test groups with control-sham animals data in the same row. Superscripts are presenting significant differences between left and right testes of different test and control-sham animals in the same column.

Table 2. The number of enjoined total rats and the born neonates after 2 weeks. All data are presented in Mean ± SD.

Groups	**Total male rats (No)**	**Total Female Rats(No)**	**Total Born neonate (No)**
Control-sham	6	12	84*
4 months varicocele	6	12	21*
6 months varicocele	6	12	12*
8 months varicocele	6	12	4*

Stars are indicating significant difference between the numbers of born fetuses in different varicocele groups with each other and with control-sham animals.

Table 3. Mean average for Tunica albuginea thickness, seminiferous tubules diameter, germinal epithelium height, dilated vessels number per one section and the percentage of vessels with thrombosis in one section in different varicocele and control-sham groups. All data are presented in Mean ± SD.

LEFT TESTES				
Groups	**Control-sham**	**4 months varicocele**	**6 months varicocele**	**8 months varicocele**
Tunica Albuginea Thickness (µm)	90.02 ± 1.41	101.66 ± 4.22^{*a}	112.34 ± 2.06^{*f}	121.83 ± 1.32^{*k}
Tubules Diameter (µm)	406.20 ± 3.76	349.40 ± 5.22^{*b}	271.00 ± 5.43^{*g}	226.20 ± 9.41^{*l}
Germinal Epithelium Height (µm)	172.00 ± 2.60	112.67 ± 3.20^{*c}	99.83 ± 5.45^{*h}	67.35 ± 2.06^{*m}
Dilated vessels (NO)	1.25 ± 0.61	5.34 ± 0.81^{*d}	6.50 ± 1.37^{*i}	7.83 ± 1.47^{*n}
Thrombosis (%)	0	24.00 ± 1.22^{*e}	33.81 ± 1.48^{*j}	37.20 ± 1.92^{*o}
RIGHT TESTES				
Groups	**Control-sham**	**4 months varicocele**	**6 months varicocele**	**8 months varicocele**
Tunica Albuginea Thickness (µm)	96.67 ± 2.42	104.00 ± 3.84$^{*a'}$	105.00 ± 3.87$^{*f'}$	113.84 ± 1.94$^{*k'}$
Tubules Diameter (µm)	407.80 ± 3.11	370.60 ± 4.39$^{*b'}$	311.40 ± 5.98$^{*g'}$	282.4 ± 5.31$^{*l'}$
Germinal Epithelium Height (µm)	173.50 ± 2.73	122.51 ± 4.03$^{*c'}$	115.53 ± 3.39$^{*h'}$	99.67 ± 6.41$^{*m'}$
Dilated vessels (NO/1cross section)	1.08 ± 0.49	3.41 ± 0.49$^{*d'}$	4.35 ± 0.81$^{*I'}$	5.83 ± 1.16$^{*n'}$
Thrombosis (%/1 cross section)	0	19.60 ± 1.14$^{*e'}$	30.62 ± 1.67$^{*j'}$	32.80 ± 1.92$^{*o'}$

Stars are indicating significant differences ($P \leq 0.05$) between different test groups with control-sham data in the same row. Superscripts are presenting significant differences between left and right testes of different test and control-sham animals in the same column.

Histological observation. Although both of the testicular tissues showed the increased tunica albuginea thickness and subcapsular edema, the left testes however showed the damages much substantiated. In the STs of left testicles a remarkable atrophy with a severe edema in the interstitial connective tissue was observed, while the right testes manifested lower edema. Histopathological observations revealed the vascular thrombosis in varicocelized rats. Moreover the vascular dilatation was illustrated in varicocelized animals and developed time-dependently as in animals with 8 months varicocele the highest number of vessels with vasodilatation was computed. Comparing of the vessels dilatation and thrombosis between right and left testicles clarified that the right testes contained lower dilated vessels and thrombosis in comparison to left testes. The data for histomorphometric analyses are presented in table3. High infiltration of MNI cells was observed in connective tissue of the testes in both right and left testicles. The right testes were manifested with significantly ($P < 0.05$) lower number of MNI cells per one mm^2 of the interstitial regions (Fig 1.).

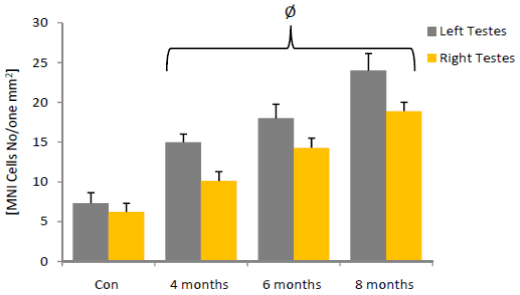

Fig 1. Mean average of mononuclear immune (MNI) cells numbers per one mm^2 of the interstitial connective tissue, all data are presented in Mean ± SD. Ø is indicating significant differences ($P \leq 0.05$) between all test groups with each other and at the same time presents the considerable differences ($P \leq 0.05$) between all test groups with control-sham.

Observations demonstrated that the germinal epithelium height was reduced by the time in all test groups. Accordingly the animals in 8 months varicocelized group were manifested with arrested spermatogenesis in more than 30% of the STs (Fig 2.). No spermatogenesis arrest and/or germinal cells degeneration revealed in control-sham animals.

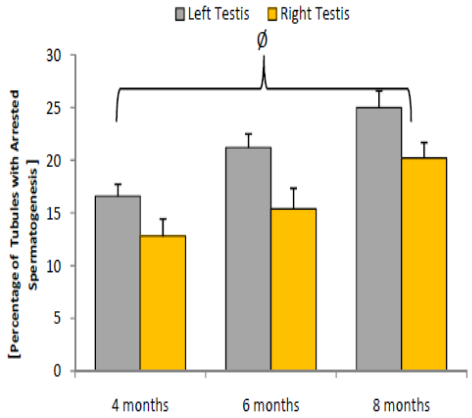

Fig 2. Mean average of seminiferous tubules with arrested spermatogenesis process, all data are presented in Mean ± SD. Ø is indicating significant differences ($P \leq 0.05$) between all test groups with each other. No tubules with arrested spermatogenesis were manifested in control-sham group.

Analyses for spermatogenesis stages showed that after varicocele induction the left testicles were identified with higher determination in stages of I-VIII and IX-XIV in comparison to right testes (Fig 3.).

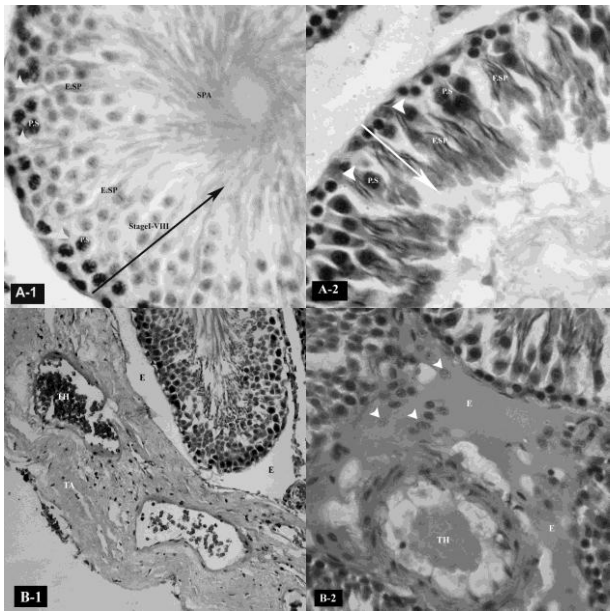

Fig 3. Cross section from testes; (**A-1**) control-sham group, seminiferous tubule with stage I-VIII of the spermatogenesis (*arrow*), normal Sertoli cells (*head arrow*), note the prileptotene spermatocytes (*P.S*), enlongated spermatids (*E.SP*) and spermatozoa (*SPA*). (**A-2**) control-shm group, seminiferous tubule with stages of IX-XIV (*arrow*), note the normal Sertoli cells (head arrows), prileptotene spermatocytes (*P.S*), enlongated spermatids (*E.SP*). (**B-1**) 8 months varicocelized group, note the tunica albuginea which increased in thickness (*TA*), severe sub-capsular edema (*E*) and vascular thrombosis of tunica's vascular network. (B-2) 8 months varicocelized group, note the abnormal leydig cells with granulated cytoplasms (head arrows) and the vascular thrombosis (TH) associated with severe edema in the contective tissue. Iron-Weigert staining, (A-1, A-2 and B-2 400×), (B-1 100×).

Comparing the damages in both mentioned stages showed that the major impact of the varicocele influenced the stages I-VIII in both testes (Fig 4-A, 4-B).

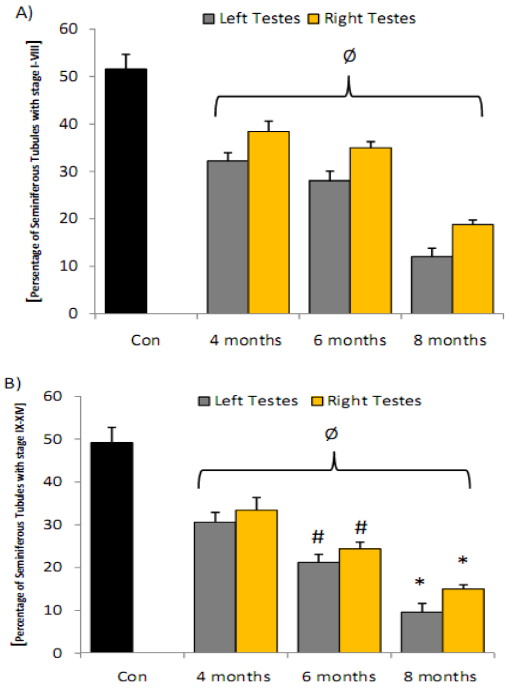

Fig 4. Mean average for the percentage of seminiferous tubules which were manifested with normal stages of I-VIII (**A**) and IX-XIV (**B**) of spermatogenic cycle in different test and control-sham groups, all data are presented in Mean ± SD. Ø is indicating significant differences ($P \leq 0.05$) between all test groups with control-sham animals and between all test groups with each other in Fig A. # and * are indicating significant difference between left and right testes data in the same months.

Light microscopic analyses showed that the SPI was negative in more than 20% of the tubules. Accordingly the left testes of the 8 months varicocelized animals showed the highest negative SPI percentage in comparison to same side testes and as well in comparison to opposite testicular tissues in different test groups (Fig 5). More than 30% of the STs were revealed with germinal epithelium dissociation in varicocelized animals and in particular in the left testes. Histological analyses showed that the numbers of preleptotene spermatocytes type I, spherical and elongated spermatids per one ST were significantly ($P < 0.05$) decreased in varicocelized animals with high impact on left testes. No histological alterations were revealed in the control-sham animals. The data for type I spermatocytes, spherical and elongated spermatids are presented in Fig 6-A, 6-B.

Histochemical observations. Histochemical observations demonstrated that the number of ALP and oil-red-o positive SCs increased in the varicocele positive groups in a time-dependent manner (Fig 7.). Comparing of STs in the left and right testes showed that, statistically ($P < 0.05$) more tubules with damaged SCs were identified in the left testes in comparison to the right testicular tissues. Evaluating the correlation between damaged SCs with dissociated germinal epithelium clarified that by increasing the number of degenerated SCs, the percentage of germinal epithelium increased in same tubules (Fig 8-A, B and 9-A, B).

Sperm characteristics. Results from sperm count assay showed that the sperm samples delivered from varicocelized animals exhibited significantly ($P < 0.05$) lower sperm count in comparison to the control-sham group. Comparing the left and right epididymal sperm samples illustrated that the sperm number of the right epididymis was remarkably ($P \leq 0.05$) higher than that in the left ones and lower than that in the control-sham group. Light microscopic analyses revealed that sperm abnormalities increased in varicocele induced groups in a time-dependent fashion.

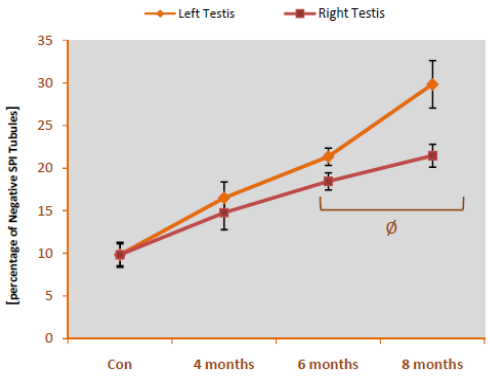

Fig 5. Mean average of the percentage of the seminiferous tubules with negative spermiogenesis index in different test and control-sham animals, all data are presented in Mean±SD. There are significant differences ($P \leq 0.05$) between all test groups with control-sham rats. Ø is indicating remarkable differences ($P \leq 0.05$) between data for 6 and 8 months varicocelized animals.

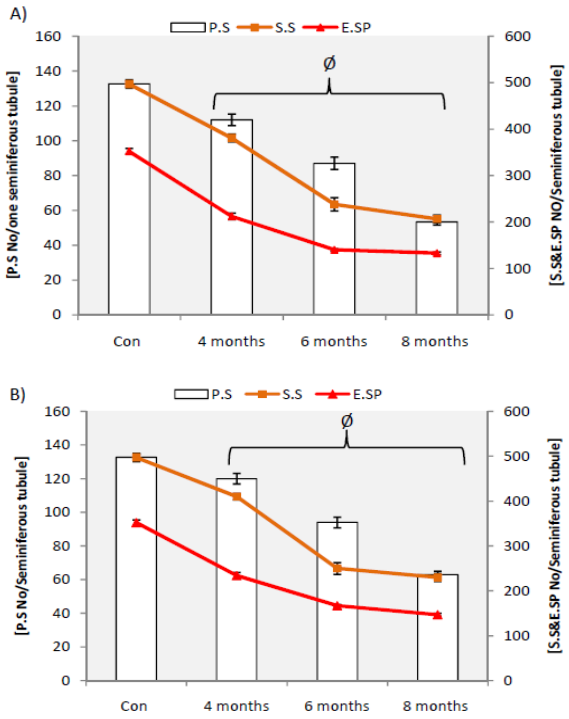

Fig 6. Mean average of Preleptotene spermatocytes (*P.S*), spherical (*S.S*) and elongated (*E.SP*) spermatids number per one seminiferous tubule in left (**A**) and right (**B**) testicles, all data are presented in Mean ± SD. Ø is indicating significant differences ($P \leq 0.05$) between all test groups with each other and at the same time presents the considerable differences ($P \leq 0.05$) between all test groups with control-sham.

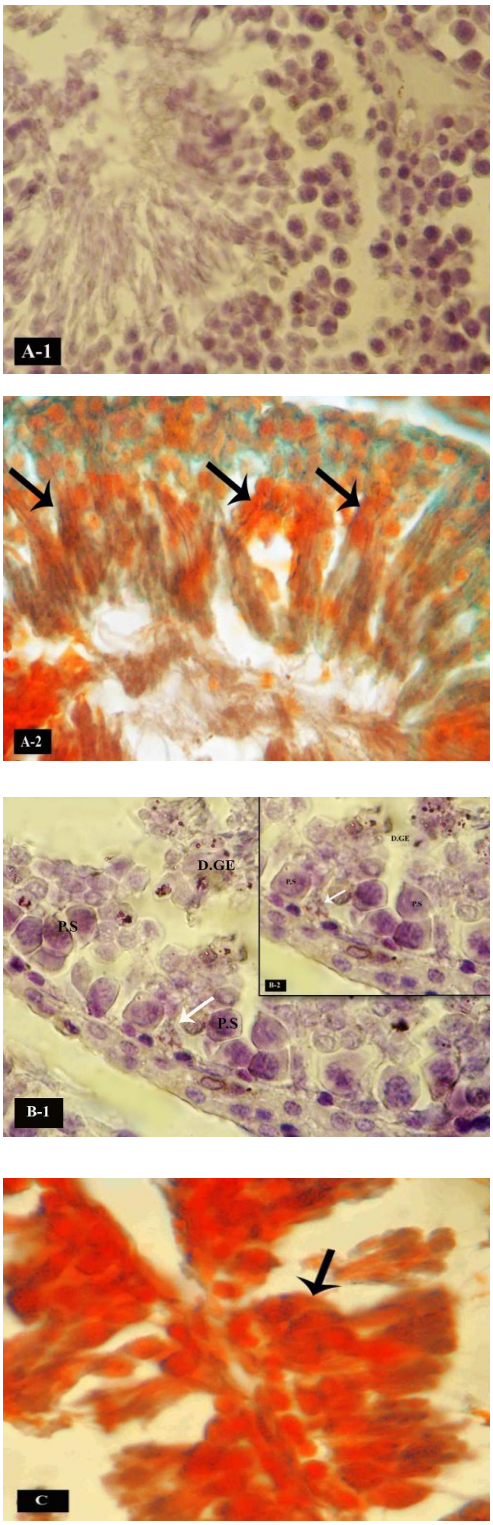

Fig 7. Cross section from testes; (**A-1**) control-sham group, no alkaline phosphatase positive cell are presented in control-sham Sertoli and germinal cells. (**A-2**) control-sham group, the Sertoli cells are oil-red-o negative while the second three layers of the germinal epithelium are lipid positive (*arrows*). (**B-1**) Varicocele group, note the Sertoli cells with positive alkaline phosphatase stained cytoplasm (*white arrow*) and dissociated germinal epithelium cells (*D.GE*) with alkaline phosphatase positive cytoplasm. (**B-2**) High magnification, alkaline phosphatase positive Sertoli cell (*white arrow*) with dissociated germinal cells (*D.GE*) which are presented with alkaline phosphatase positive cytoplasms. (**C**) Varicocele group, note the lipid positive Sertoli cells (*arrow*) which are presented with light orange cytoplasm. Alkaline phosphatase (A-1, B-1 and B-2) and Oil-Red-O staining (A-2 and C), 400×.

The percentage of dead sperms elevated by the time in all test groups. Meanwhile the percentage of abnormal sperms (head, tail and duplicate abnormalities) and dead sperms was significantly ($P < 0.05$) lower in the right testes.

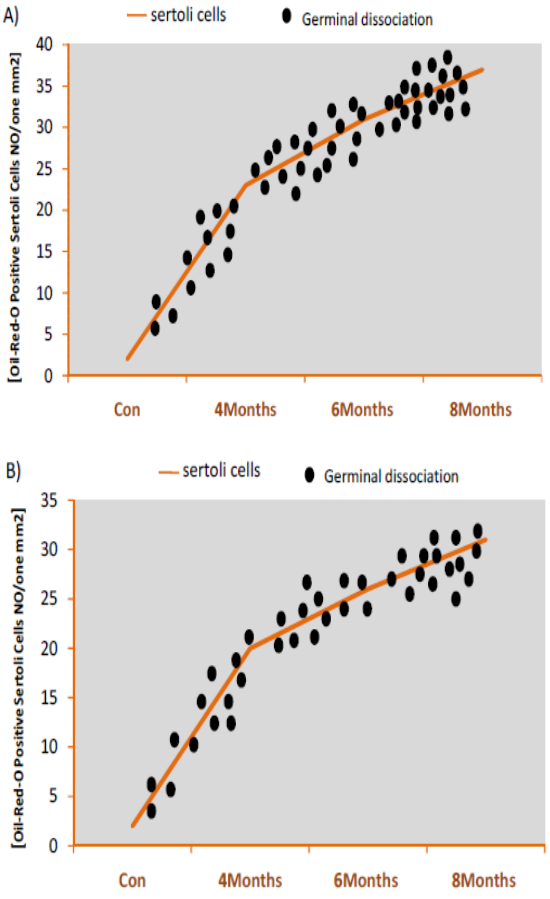

Fig 8. Correlation between lipid positive Sertoli cells number per one mm^2 with percentage of seminiferous tubules with dissociated germinal epithelium in left (**A**) and right (**B**) testes. Total number of lipid positive Sertoli cells correlates positively with germinal epitheliums dissociation. Sertoli cells; $r^2 = 0.078$; $P \leq 0.05$ and percentage of seminiferous tubules with dissociated germinal epithelium; $r^2 = 0.69$; $P \leq 0.05$

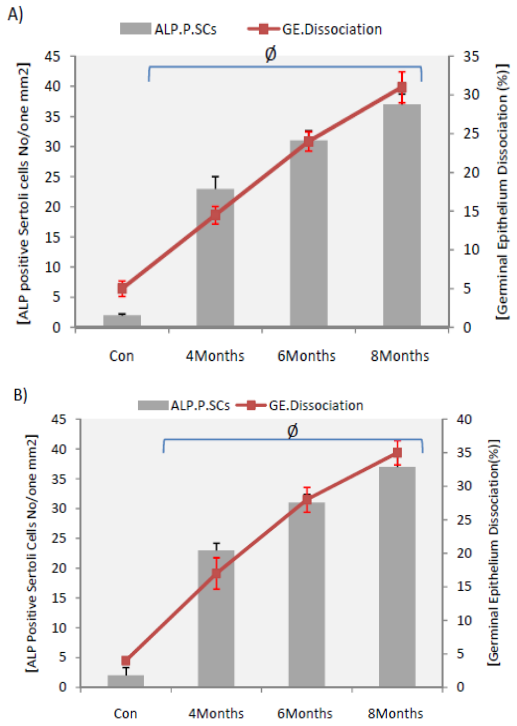

Fig 9. Mean average of ALP positive Sertoli cells per one mm^2 and percentage of seminiferous tubules with dissociated germinal epithelium in left (A) and right (B) testes in different test and control-sham groups, all data are presented in Mean ± SD. Ø is indicating significant differences ($P \leq 0.05$) between all test groups with each other and at the same time presents the considerable differences ($P \leq 0.05$) between all test groups with control-sham.

Although the sperms motility decreased in all varicocelized rats, the sperms from right epididymis however were manifested with fair motility in comparison to those from left side. The data for sperm parameters are depicted in Table 4.

Discussion

A high incidence of varicocele in men with primary and/or secondary infertility problems and the development of varicocele at the period of puberty suggest that this disorder is able to cause a progressive reduction in fertility. There is an increasing interest in clarifying the details of varicocele-induced impact on the testicular tissues.[20,22] Previous studies have focused on the effect of varicocele on fertilizing abilities, total semen contents and quality in varicocele positive patients, while the exact bilateral effect of varicocele on testicular tissues had not been fully understood. Our histological investigations revealed that the detrimental effect of varicocele influenced the testicular tissues in both sides and led to severe damage on spermatogenesis activity in STs associated with remarkable defect in I-VIII and IX-XIV stages of spermatogenic cycle.[4-16] Other findings of current study uncovered that the varicocele induction could significantly influence sperms' quality and quantity from both testes. Our histological analyses showed a severe edema in the interstitial connective tissue, vasodilatation accompanying with thrombosis and remarkable MNI cells infiltration. Previous findings confirmed that all mentioned features were typical characteristics of chronic inflammation.[23-25] While the vascular impairments and immune cells infiltration were remarkably less in the right testes than in left testes, suggesting a weaker inflammatory impact of varicocele on right testicles. On the other hand histochemical staining for cytoplasmic lipid foci showed that the number of SCs with dense cytoplasmic lipid accumulation increased in left and right testicular tissues of varicocele positive animals.It is interesting to note that the lipid supplementation in SCs depends on different intensity of spermatogenesis cells degeneration and the amount of detached residual bodies of spermatids.[19,20]Moreover our ALP staining as a marker for inflammation showed that a lower number of ALP-positive SCs were manifested in right testes of the varicocelized animals. It is has been shown that the SCs have nutritive, protective and supportive functions for spermatogenesis cells and they play an essential role in maintenance of the spermaiogenesis[26] Any inflammatory detrimental effects on SCs would be able to influence the spermatogenesis process.[20,21,25,27]
Thus it may suggested that a slight germinal cells degeneration happened in

Table 4. Mean average of sperm count, total percentages of abnormal sperm, head, tail and duplicate abnormalities of sperms, immotile sperm, non-progressive and progressive motilities percentages in different test and control-sham animals. All data are presented in Mean ± SD.

LEFT EPIDIDYMIS				
Groups	**Control-sham**	**4 months Varicocele**	**6months Varicocele**	**8 months Varicocele**
Sperm count (×10^6)	72.50 ± 3.78	42.50 ± 2.08^{*a}	38.00 ± 2.16^{*b}	31.75 ± 2.36^{*c}
Total abnormal sperms-%	16.20 ± 4.43	50.80 ± 1.64^{*a}	59.20 ± 1.66^{*b}	70.20 ± 1.78^{*c}
Head abnormality-%	8.50 ± 1.64	16.83 ± 1.32^{*a}	19.50 ± 2.42^{*b}	23.34 ± 1.36^{*c}
Tail abnormality-%	6.83 ± 1.47	17.35 ± 1.63^{*a}	20.83 ± 1.47^{*b}	25.83 ± 1.84^{*c}
Duplicate abnormality-%	3.50 ± 1.87	12.33 ± 1.50^{*a}	18.16 ± 1.94^{*b}	19.51 ± 1.87^{*c}
Immotile sperm -%	7.50 ± 1.04	43.83 ± 1.32^{*a}	41.63 ± 1.57^{*b}	48.26 ± 1.07^{*c}
Non-progressive motility-%	3.16 ± 0.72	11.50 ± 1.04^{*a}	20.34 ± 1.03^{*b}	18.50 ± 1.12^{*c}
Progressive motility-%	92.25 ± 1.70	45.00 ± 1.41^{*a}	39.75 ± 1.70^{*b}	33.75 ± 2.62^{*c}
RIGHT EPIDIDYMIS				
Groups	**Control-sham**	**4 months Varicocele**	**6months Varicocele**	**8 months Varicocele**
Sperm count (×10^6)	73.75 ± 3.30	54.50 ± 3.41$^{*a'}$	43.75 ± 2.06$^{*b'}$	37.25 ± 1.70$^{*c'}$
Total abnormal sperms-%	15.80 ± 5.63	48.60 ± 1.67$^{*a'}$	55.60 ± 2.60$^{*b'}$	62.80 ± 2.77$^{*c'}$
Head abnormality-%	7.75 ± 1.54	13.83 ± 1.47$^{*a'}$	16.67 ± 1.04$^{*b'}$	19.83 ± 1.16$^{*c'}$
Tail abnormality-%	6.41 ± 1.68	14.00 ± 1.26$^{*a'}$	15.85 ± 2.14$^{*b'}$	18.83 ± 1.16$^{*c'}$
Duplicate abnormality-%	3.16 ± 1.48	11.50 ± 1.04$^{*a'}$	16.50 ± 1.04$^{*b'}$	17.00 ± 1.41$^{*c'}$
Immotile sperm -%	6.84 ± 0.75	31.34 ± 1.50^{*a}	36.83 ± 1.16$^{*b'}$	42.83 ± 1.72$^{*c'}$
Non-progressive motility-%	3.35 ± 0.83	13.50 ± 1.08$^{*a'}$	21.35 ± 1.50$^{*b'}$	21.66 ± 1.39$^{*c'}$
Progressive motility-%	91.25 ± 1.50	56.75 ± 1.70$^{*a'}$	44.00 ± 3.65$^{*b'}$	36.00 ± 4.08$^{*c'}$

Stars are indicating significant differences ($P \leq 0.05$) between different test groups with control-sham data in the same row. Superscripts are presenting significant differences between left and right testes of different test and control-sham animals in the same column.

right testes of varicocelized rats is resulted in a lower rate of SCs phagocytosis thus after phaocytosis of residuals from germinal cells degeneration a small number of these cells presented with lipid positive cytoplasms. Furthermore a few numbers of SCs were inflamed (lower number of cells with cytoplamic ALP site) thus lower spermatogenesis cells were influenced from SCs fall. This finding suggested that there was a mutual relationship between SCs and germinal epithelium in varicocelized testes.

Our histological examinations showed that the percentage of STs with dissociated germinal epithelium and arrested spermatogenesis increased by the time. This impairment suggests that following chronic inflammation and SCs fail, the nutritive correlation and adhesion between SCs and type I spermatocytes disrupted, which in turn led to severe reduction in the number of spermatocyte type I cells (cells which are candidate for first maturation divisions). It is well documented that the spermatocyte type I cells are the precursors in spermatogenesis cells process.[28,29] Any detrimental effect on these cells can reduce first maturation division ratio and consequently decrease the second division maturation rate.

Other findings of this study suggested that due to the degeneration of a smaller number of SCs in right testes, the remained cells mediated the FSH and testosterone-dependent reactions. They also may play a critical protective role to keep the spermatocyte type I cells alive in order to participate in different

spermatogenesis stages (I_VIII and IX-XIV). The results of current study supported this claim, where we found a high percentage of STs with normal I -VIII and IX-XIV stages, higher number of spherical and elongated spermatids (indicating better rate of second maturation deviation) in right testes in comparison with left side.

We showed that the sperm motility decreased in samples from both left and right epididymis. The relationship between varicocele and reduced motility of sperms can be explained by two hypothesis; a waves of events that result in an intensive decrease in axonemal protein phosphorylation and consequently the sperm immobilization and secondly, varicocele-induced free radicals such as H_2O_2 can diffuse across the membranes into the cells and inhibit the activity of enzymes such as G6PDH.[30,31] High degenerated precursor cells in spermatogenesis cell series increased the number of damaged spermatids and simultaneously decreased the percentage of stages I-VIII which in turn resulted in a negative SPI. Consequently these impairments led to reduce sperm content with enhanced sperm abnormality in varicocele cases. The low rate of successfully fertilizing female rats corroborated these findings.

In conclusion; Current findings suggested that a long-term varicocele-induction could influence bilaterally left and right testes by causing a chronic inflammation in testicular tissues, which in turn affected the SCs and spermatogenesis cell series function. Therefore first and second maturation divisions stop by the time which influenced tubular SPI, total sperm count, sperm abnormality, motility and ultimately fertilizing abilities.

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