The Impact of Unilateral Experimental Rat Varicocele Model on Testicular Histopathology, Leydig Cell Counts, and Intratesticular Testosterone Levels of Both Testes

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Purpose: Varicocele, most treatable pathologic condition in male infertility, exerts unfavorable effects on testicular ultrastructure via various mechanisms. In this study we aimed to demonstrate adverse effects of varicocele on both testes.

Materials and Methods: Twenty one adult male Albino rats were divided into 3 groups. Sham operation was performed for group 1 (control group), and this group of rats were sacrificed 4 weeks later. Experimental varicocele model was performed for group 2 (varicocele group) and these animals were sacrificed 4 weeks after the operation. In group 3 the rats were varicocelectomized 4 weeks later. This group of rats were sacrificed at 4 weeks postoperatively. The level of testicular damage was examined, and serum testosterone and intratesticular testosterone levels were measured.

Results: Mean (±SD) damage scores of the right testes of the sham, varicocele, and varicocelectomy groups were 0, 1.64 ± 1.3, and 1.21 ± 0.3, respectively. There was no statistically significant differences between damage scores of groups 2, and 3 (P = .320), relevant scores of both groups were determined to be significantly higher than group 1 (P = .009, and P = .001). Mean (±) damage scores of the left testes of the three groups were detected to be 0.43 ± 1.13, 2.29 ± 1.15, and 1.78 ± 0.39, respectively. The difference between varicocele, and varicocelectomy groups was not statically significant (P = .112).

Conclusion: Unilateral varicocele has deleterious effects on both testes. There was no statistically significant difference as for histopathologic recovery following varicocelectomy.

Keywords: varicocele; rats; testis; parhology; spermatogenesis; animals

INTRODUCTION

Varicocele, most frequently treatable abnormality of male infertility with an incidence of 15 % in general male population, is found approximately 30-40 % of men with infertility.[1] Although defined for a long time, pathophysiology of its unfavorable effect on fertility has not been clearly elucidated yet, and thus various theories have been proposed. Well-accepted theories suggest an increase in arterial blood flow and testicular temperature induced by varicocele, venous stasis within spermatic vein, presence of toxic metabolites stemming from renal and adrenal sources, decreased levels of intratesticular and/or peripheral testosterone, and increased DNA damage in patients with varicoceles.[2-5]

Varicocele may cause progressive deterioration in Sertoli cells, and release of spermatogenic cells before their full maturation.[6] Histopathologic examinations of the cases with varicocele reveals signs of normal or arrested spermatogenesis, and Sertoli cell- only syndrome may be seen.[7, 8] Interestingly, any association between clinical grade of the varicocele, and severity of histopathologic damage could not be demonstrated.[6] Some authors, asserted that testicular biopsy is a must, and pre-varicocelectomy biopsy is of benefit in predicting postoperative outcomes, while others argue its usefulness and advocate its avoidance.[9, 10] These debates reveal insufficient knowledge in the histopathology of varicocele.

Crucial role of testosterone in male reproductive physiology is obscene and abnormalities in testosterone production are suggested to be one of the adverse mechanisms of varicocele on fertility.[4, 11] Although the impact of varicocele on serum testosterone is debatable, some investigations have been conducted on the effect of varicocele on levels of intratesticular testosterone which is acknowledged as a more influential factor on reproductive physiology.[11, 12]

The aim of this study was to create an experimental varicocele model on rats so as to compare ipsilateral and contralateral testes in varicocelectomized, non-varicocelectomized and sham groups regarding differences in histopathologic patterns, Leydig cell counts, and intratesticular testosterone levels.

MATERIALS AND METHODS

After approval of the Ethics Committee, 21 adult Wistar albino rats weighing 250-300 g, were randomized into 3 groups. Seven rats in Group 1 underwent a sham operation (control group). In group 2 (n=7), and group 3 (n=7) experimental varicocele model was created on the left side. Rats in group 2, (varicocele group) were sacrificed 4 weeks after surgical induction of varicoceles. Rats in Group 3 were varicocelectomized (varicocelectomy group) 4 weeks later, and sacrificed 4 weeks after varicocelectomy. The rats were fed with standard 8 mm-pellet feeds. All animals were fed ad libitum and maintained in a constant environment with a 12:12-hour light dark cycle. Room temperature, and humidity were set at 22°C±2°C, and 5-10%, respectively

Induction of experimental varicocele models

We maintained an antiseptic environment during our surgical procedures. Body temperatures of the animals were monitored with a rectal thermometer, and kept steady around 37°C. General anesthesia was induced via intraperitoneal injection of ketamine (10 mg/100 g body weight) and chlorpromazine (1mg) mixture. An experimental varicocele model was created as described by Saypol et al.[13] After induction of standardized anesthesia, through a midline abdominal incision abdominal cavity was entered to expose the left renal vein. After a 0.8 mm-thick metal wire probe was placed parallel to the left renal vein, a 4-0 silk suture was ligated around the whole renal vein and metal wire probe. After metal wire probe was removed, a nearly 50 % narrowing of the renal vein was obtained. Finally, midline incision was closed with 3-0 silk sutures. After creation of experimental models, we waited for 4 weeks to see the effect of the induced varicocele. The external diameters of the left internal spermatic veins were measures and an increase in vein external diameter by 2-fold or more was considered varicocele. At the end of 4 weeks laparotomic exploration revealed dilated left spermatic veins in all of 14 rats. In 7 rats, dilated left spermatic veins were ligated with 4-0 silk sutures, and dissected (varicocelectomy). All rats were sacrificed using cervical dislocation method. After scarification, testes were excised. Nearly half of the testicular volumes were used for the assessments of intratesticular testosterone levels, and the other halves for his-

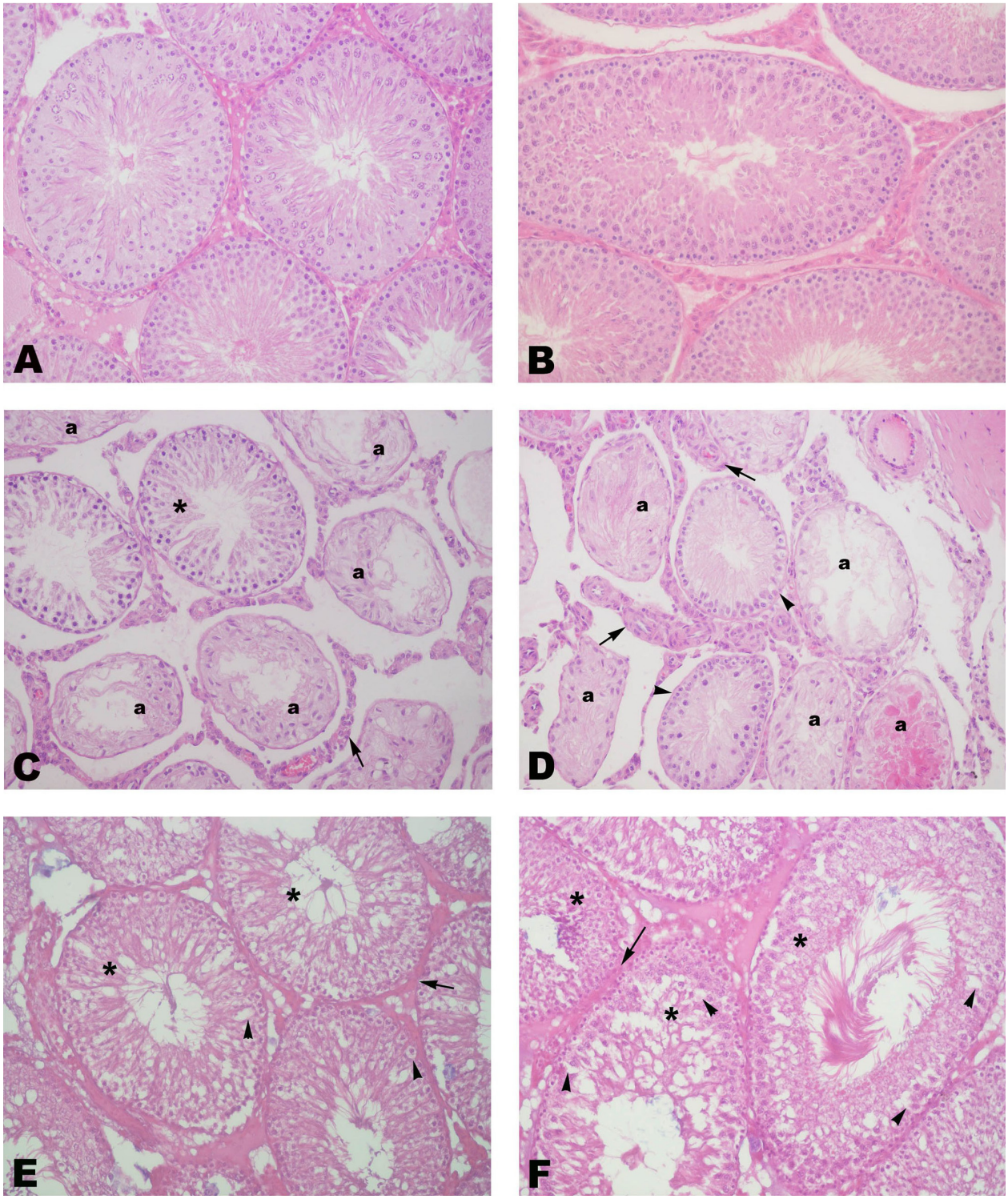

Figure 1. In the right (A) and left (B) testis of a sham operated rat (group 1) evenly distributed spermatogenetic cell sequences in seminiferous tubules, and normal interstitial tissue are observed. In the right testis (C) of a varicocele- induced rat (group 2) spermatogenic cell-specific sequence in scarce number of seminiferous tubules (*), and atrophic cells in numerous seminiferous tubuli (a) Increased connective tissue (arrow). In the left testis (D), one or at most two arrays of cell lines of the spermatogenic series on the walls of majority of seminiferous tubuli (arrow head), while in some tubulis with decreased diameters (a) only single cell lines are seen. Increased connective tissue, and narrowed seminiferous tubule (arrow). In the right and left testis (E and F) of a varicocelectomized rat with experimentally induced varicocele (group C), on the walls of the majority of seminiferous tubuli (*) multilayered spermatogenic cell- specific series, and spermiums in their lumens. Formation of numerous vacuoli (arrow head) on the walls of some seminiferous tubuli. Thicker connective tissue relative to the right testis (arrow).

Table 1. Mean testicular damage scores, intratesticular, and serum testosterone levels of sham, left varicocele /varicocelectomy groups of rats.

Groups	No.	Mean (± SD) testicular damage scores	Intratesticular testosterone levels (mean ± SD) ng/mL	Number of Leydig cells	Serum testosterone levels (mean ± SD) ng/dl
Sham group- right testes	7	0.00 ± 0.00	11.29 ± 3.69	18.3 ± 2.4	0.616 ± 0.2728
Sham group- left testes	7	0.43 ± 1.13	13.62 ± 4.83	18.2 ± 2.6	
Varicocele group -right testes	7	1.64 ± 1.28	12.53 ± 3.38	13.0 ± 2.5	0.587 ± 0.258
Varicocele group - left testes	7	2.29 ± 1.15	6.76 ± 2.09	12.6 ± 2.3	
Varicocelectomy group-right testes	7	1.21 ± 0.27	10.56 ± 2.70	15.3 ± 2.2	0.599 ± 0.278
Varicocelectomy group – left testes	7	1.79 ± 0.39	9.34 ± 1.54	13.2 ± 2.2	
P values between group 1 and 2		$P_{right} = .009$ $P_{left} = .001$	$P_{right} > .05$ $P_{left} = .001$	$P_{right} = .022$ $P_{left} = .012$	$P > .05$
P values between group 1 and 3		$P_{right} = .001$ $P_{left} = .001$	$P_{right} > .05$ $P_{left} = .024$	$P_{right} = .026$ $P_{left} = .024$	$P > .05$
P values between group 2 and 3		$P_{right} = .320$ $P_{left} = .112$	$P_{right} > .05$ $P_{left} = .044$	$P_{right} > .05$ $P_{left} > .05$	$P > .05$

SD: Standard deviation.

topathologic examination. Blood samples were drawn from tail veins for the measurement of serum testosterone.

Preparation of light microscopic sections

Testicular samples obtained for light microscopic examination were fixed in Bouin's solution for 3 days, and left in 4 % aqueous lithium carbonate solution for 3 days to remove picric acid. Tissue samples were dehydrated in graded ethanol, and dissolved in toluene to obtain a pellucid solution. 5 µm-thick sections were stained with hematoxylin-eosin (H&E) for general morphologic evaluation, and examined under Olympus B × 51 photomicroscope. For histopathologic scoring, every section was assessed in 5 similar fields of view under 100X magnification. Histopathologic scoring was performed using similar method as previously described by Hess et al.[14] Undamaged: grade 0; Normal intercellular junctions, but presence of cellular damage indicators as loose cellular organization, eosinophilic cytoplasm, and picnotic nuclei, grade1; disorganized germ, and Sertoli cells, and impaired intercellular junctions, grade 2; only Sertoli cells or scarce number of germ, and Sertoli cells with impaired junctions, grade 3.

Leydig cells in interstitial connective tissue were counted under 40X magnification in five distinct microscopic fields for each rat, and their means (± SD) were calculated. Testosterone levels were measured using electrochemoluminescence immunoassay (ECLIA) analytical method according to the manufacturer's protocol with Roche Modular analytics E 170 device (Roche diagnostics Gmbh, Germany). For intratesticular testosterone assays, testicular tissue was homogenized using the procedure as previously described by Rajfer and associates.[15]

Mann-Whitney *U* test was used for statistical analysis. $P < .05$ was accepted as a cut-off value for statistically significance.

RESULTS

Light microscopic examination of the right and left testicular tissues of rats in the sham group morphologically demonstrated seminiferous tubuli, and interstitial connective tissue with regularly arrayed spermatogenic cell series (Figure 1). In the right testes of the varicocele group, spermatogenic cells which maintained their spermatogenic activities were detected only in a scarce number of seminiferous tubuli, and also a few, irregularly arrayed, spermatogenic cell-specific series on the walls of these tubuli were observed. Interstitial connective tissue was increased. In the left testes of varicocele model group, most of the seminiferous tubuli contained one or at most two arrays of spermatogenic cell-specific series on their walls. In some tubuli only one spermatic cell line was

observed with constricted tubular lumen, and proliferation of interstitial connective tissue (Figure 1). In the varicocelectomy group multilayered spermatogenic cells were seen on the walls of most of the seminiferous tubuli, and spermiums were encountered in the lumens of some tubuli. However vacuoles were detected on the walls of some tubuli. Interstitial connective tissue was somewhat increased and also in left testes of varicocelectomized rats, multilayered spermatogenic cell-specific series and intraluminal spermiums were observed on the walls of some seminiferous tubuli. Numerous vacuole formations were seen on the walls of some seminiferous tubuli. Compared with the right testes, interstitial connective tissue of the left testes was thicker (Figure 1).

Average number of Leydig cells in the left testes of the sham, varicocele, and varicocelectomy groups were estimated to be 18.2 ± 2.6, 12.6 ± 2.3, and 13.2 ± 2.2, respectively. There was no statistically significant difference between left testes of the groups with varicocele, and varicocelectomized rats ($P > .05$), while in the left testes of the sham group Leydig cell counts were significantly higher than those of the other groups ($P = .012$). Average number of Leydig cells in the right testes of the sham, varicocele, and varicocelectomy groups were estimated to be 18.3 ± 2.4, 13.0 ± 2.5, and 15.3 ± 2.2, respectively. In the right testis of the sham group Leydig cell counts were significantly higher than varicocele groups ($P = .022$). There was no statistically significant difference between right testes of the groups with varicocele, and varicocelectomized rats, and also there was no statistically significant difference between right testes of the sham group and varicocelectomized rats ($P > .05$) (Figure 2).

Mean (± SD) damage scores of the right testes of the sham, varicocele, and varicocelectomy groups were 0, 1.64 ± 1.3, and 1.21 ± 0.3, respectively. The difference between varicocele, and varicocelectomy groups did not attain a statistically significant level ($P = .320$), while mean damage scores of both groups were significantly higher than those of the sham group ($P = .009$, and $P = .001$). Mean (±) damage scores of the left testes of the sham, varicocele, and varicocelectomy groups were detected to be 0.43 ± 1.13, 2.29 ± 1.15, and 1.78 ± 0.39, respectively. The difference between varicocele, and varicocelectomy groups was not statically significant ($P = 0.112$).

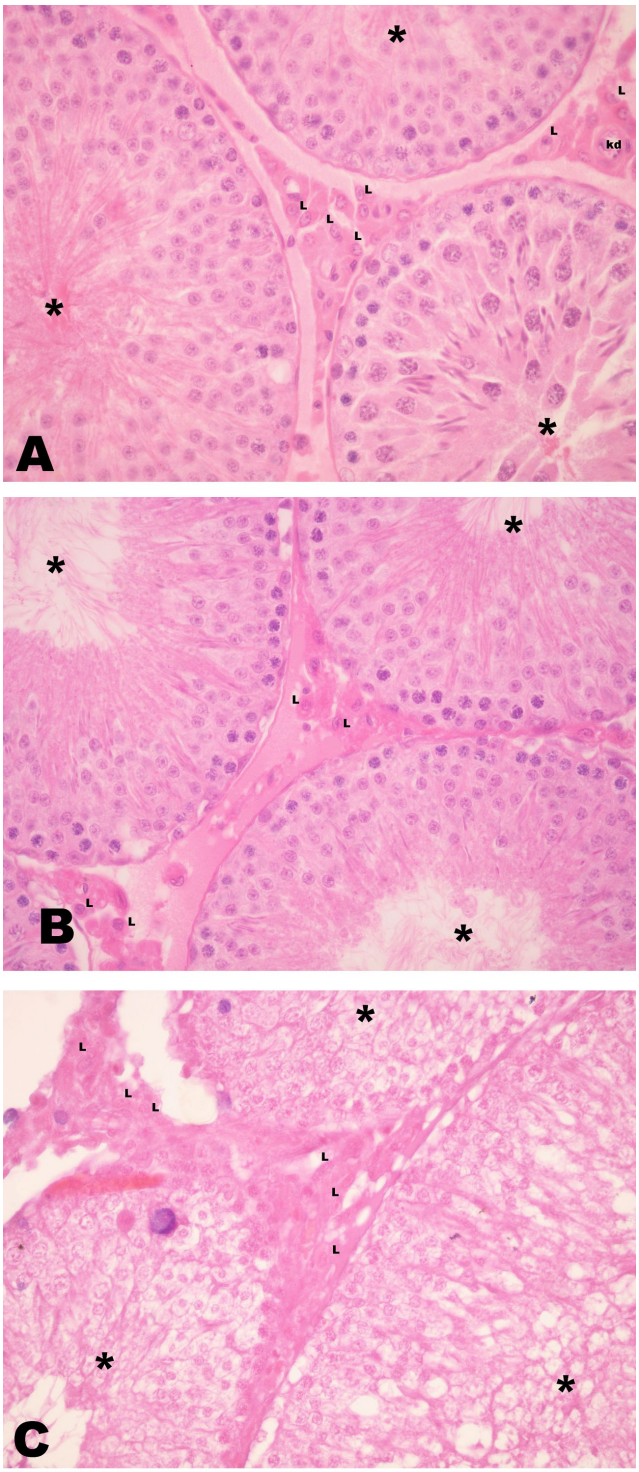

Figure 2. Leydig cells (L) in the seminiferous tubuli (*), and blood vessels (bv) in the interstitial tissue of the left testis (A) of a sham-operated rat are seen. In the left testis (B) of a varicocele-induced rat relatively fewer number of Leydig cells (L) are present in seminiferous tubuli (*), and interstitial connective tissue when compared with the sham group. After creation of varicocele, and then application of left varicocelectomy, in the left testis of a rat (C) mild increase in Leydig cells localized in seminiferous tubules (*) , and interstitial connective tissue (L) were observed (magnification: 40×).

When the extent of destructive changes in the right, and left testes of 3 groups were compared, the difference between the sham, and varicocele groups was not statistically significant (P = 0.317, P = 0.282), while varicocelectomy group was significantly different from the other two groups (P = 0.01). In the varicocelectomy group, recovery in right testes was significantly more than left testes (Table 1).

Intratesticular testosterone levels of the groups are given in Table 1. There was no statistically significant difference between 3 groups as for right testes (P > .05), by contrast there was statistically significant difference between 3 groups for left testes (P < .05). Average serum testosterone values (mean ± SD) in the sham, varicocele, and varicocelectomy groups were found to be 0.616 ± 0.273 ng/dl, 0.587 ± 0.258 ng/dl, and 0.599 ± 0.278 ng/dl respectively, without any statistically significant difference among three groups (P > .05).

DISCUSSION

Although varicocele is a well-defined entity for decades, its adverse mechanisms on infertility are not clear yet.[16] Besides, controversies concerning its diagnostic tools, incidence, therapeutic indications, and optimal management still remain.[16-19] Men with varicoceles can have normal semen parameters, besides they can present with signs of azoospermia.[20] In the testes of patients with impaired semen parameters, different degrees of histopathologic damage have been defined. However, there are confounding data about prognostic value of these histopathologic changes.[9, 10, 21] The aim of this study was to compare variations in histopathologic damage scores, Leydig cell counts, serum, and intratesticular testosterone levels measured in experimental rat varicocele models with those of the sham, and varicocelectomy groups. Experimental left varicocele model can be produced in rats. Increased testicular blood flow and temperature, decrease in intratesticular testosterone production, and impairment in spermatogenesis develops similar to those observed in human beings.[4] Testicular histopathology in men with varicoceles can be only evaluated with testicular biopsies. However in damaged testis, it is acknowledged that characteristic features can range from patchy areas of normal spermatogenesis, and also Sertoli cell-only abnormalities.[6] Therefore, biopsy material can yield only limited information. Thus we used experimental rat varicocele model with the intention of more extensive histopathologic examination of testes.

Özgür and associates performed bilateral testicular biopsies in 6 patients during, 3, and 6 months after varicocelectomies, and examined ultrastructure of testicular tissue under electron microscope. They revealed histopathologic damage in both testes despite the presence of left varicoceles.[22] Gat and associates evaluated patients with unilateral varicoceles previously detected only during physical examination in detail using more sophisticated diagnostic tools like venography, and reported an incidence of 80 % for bilateral varicoceles which they had implicated for bilateral testicular damage.[18] Also in our study we found similar histopathologic changes in the right testes of rats with induced left varicoceles and observed histopathologic improvement in both testes following varicocelectomy. There was no statistically significant difference between right and left testes. This outcome reinforces the assumption that unilateral varicocele is a bilateral disease, and suggests that bilateral testicular damage develops secondary to overlooked mechanisms unrelated to the presence of bilateral varicoceles.

Marmar and associates suggested that varicocele might be a secondary lesion associated with an underlying genetic disorder adversely contributing to infertility.[1] In our study histopathologically demonstrated testicular damage as a consequence of experimental varicocele induced in healthy rats does not support this viewpoint. In our study, we have demonstrated that increase in histopathologic damage scores, and decrease in the number of germ cells were noted in both testes, being more marked in the left side. However improvements in all these impairments achieved after varicocelectomy did not reach the level observed in the sham group. These observations are in accordance with the outcomes obtained by Özgür and associates in human studies. In the above-mentioned study, the authors had detected only incomplete ultrastructural improvement during histopathologic examination of testicular biopsies performed 3, and 6 months after varicocelectomies.[22] This condition may be due, as proposed by Cozzolino and Lipshultz, varicocele is a progressive lesion eventually leading to irreversible infertility or it just might be because of inadequate time period following varicocelec-

tomy.[20]

One of the interesting outcomes of our study is that improvement in the right testes are relatively more prominent after left sided varicocelectomies. However because of the limitations of methodology, we cannot determine whether this improvement is due to a rapid postoperative recovery or an additional favorable effect of the induced-varicocele. Post-varicocelectomy studies performed in various time periods will aid us to interpret this outcome.

Testosterone secreted from Leydig cells has an irrefutable impact on reproductive physiology. In the literature, controversial reports on serum or intratesticular testosterone levels in men with varicoceles are encountered. Pasqualotto and associates asserted that varicocele has no effect on serum testosterone levels, while Tanrıkut and associates reported significantly lower serum testosterone levels in patients with varicoceles, and pointed to a significant rise in their levels after varicocelectomy.[23, 24] Shaik and associates reported a significant decrease in serum testosterone levels in varicocele-induced dogs.[25] Luo and associates had reported that they had not found a decrease in serum testosterone levels in their varicocele-induced experimental rats, while a significant decrease in intratesticular levels was seen only at 8 weeks postoperatively.[26] In our study, we detected a significant decrease in the varicocele group relative to the sham group as for Leydig cell counts. This phenomenon can be explained by increased Leydig cell apoptosis demonstrated by Luo and associates in their experimental rat varicocele model.[26] In our study any significant change in serum testosterone levels in all 3 groups was not detected. Although in the literature various studies where experimental varicocele models have been used, 4 weeks of waiting period was allowed for the varicocele to exert its effects, different outcomes obtained at 8 weeks in a study by Luo and associates might question the validity of a 4-week watchful waiting period.[26-28] Therefore, we thought that the validity of our waiting period should be also confirmed by studies with longer observational periods. Besides, in investigations where serum free, and bioavailable testosterone are evaluated might importantly contribute to our funds of knowledge. In the varicocele group we detected a significant decrease in the levels of intratesticular testosterone. In the varicocelectomy group, intratesticular testosterone levels increased somewhat without reaching those in the sham group. In the right testes of three groups any difference in the intratesticular testosterone levels could not be found. This might be secondary to late-onset or milder deterioration in the right testes.

Our investigation had some weak points in that we studied limited number of rats because of ethical concerns, and couldn't evaluate ultrastructural architecture of the testes under electron microscope, and measure bioavailable testosterone levels as well.

CONCLUSION

In conclusion, unilateral varicocele has deleterious effects on both testes. Following varicocelectomy, histopathologic recovery was seen without any statistically significant difference. This phenomenon might be attributed to scarce number of experimental animals included in the investigation because of ethical concerns, shorter postoperative period or incompletely reversible nature of the damage induced by varicocele. Studies with larger groups on this subject are obviously needed.

CONFLICT OF INTEREST

None declared.

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