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The Effect of the Partial Obstruction Site of the Renal Vein on Testis and Kidney in Rats: Is the Traditional Animal Model Suitable for Varicocele Research?

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Purpose: We investigated the influence of the location of the partial renal vein obstruction on the left kidney, the bilateral testes, and cauda epididymal sperm quality and determined whether this animal model is suitable for varicocele study.

Materials and Methods: A total of 25 adult male Sprague-Dawley rats were assigned to three groups: group 1 (experimental varicocele by partial ligation medial to the internal spermatic vein for 8 weeks, n=8), group 2 (partial ligation lateral to the internal spermatic vein for 8 weeks, n=10), and group 3 (sham operation for 8 weeks, n=7). Rats in groups 1, 2, and 3 underwent a left nephrectomy and bilateral orchiectomy at 8 weeks after the operation. Histological changes and Johnsen score in both testes were analyzed. Fibrotic changes in the left kidney were assessed by quantitative image analysis. Numbers of sperm and proportions of motile sperm in the cauda epididymides were determined.

Results: Significant histological abnormalities and Johnsen score changes were observed in the testes in group 1. Renal fibrosis did not differ significantly among the groups. The proportions of motile sperm were significantly lower bilaterally in group 1 than in groups 2 and 3. However, the mean bilateral epididymal sperm count in group 1 was not significantly lower than in groups 2 and 3.

Conclusions: Our results showed that experimental varicocele in the rat, induced by partial ligation medial to the internal spermatic vein, influences epididymal sperm quality without harmful effects on the left kidney. The present study certifies that this traditional animal model is suitable for varicocele research.

Key Words: *Animal models; Physiopathology; Rats; Varicocele*

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INTRODUCTION

Varicoceles have long been recognized as a cause of male infertility. It is estimated that approximately one third of the men evaluated for infertility have this condition [1]. The literature indicates that 8% to 23% of men have clinically evident varicocele [2]. However, not all men with varicocele have abnormal semen parameters. The question of why approximately 15% to 20% of men with varicocele are infertile, whereas the rest are not infertile, remains unanswered [3]. In other words, although varicocele is accepted as a cause of male infertility, the mechanism still needs to be investigated in more detail. The reason probably lies in the pathophysiology of varicocele, although this is still unclear and requires more in-depth study. Therefore, many questions remain regarding how varicoceles develop, whether varicoceles actually cause testicular dysfunction, and, if varicoceles do cause testicular dysfunction, how they do so. These general questions are difficult to approach by investigating human patients. In

addition, specific questions on the physiological or cell biology consequences of varicocele are even more difficult to address in the human population, because it is not ethical to obtain testicular biopsy specimens from healthy or infertile males. It is therefore essential to use animal testing in the study of varicocele.

Varicocele in the animal model can be induced by surgical procedures [4]. Spermatic varicosity can be induced by partially occluding the left renal vein medial to the insertion of the left internal spermatic vein. Early reports on the effects of unilateral, experimental varicocele involved the use of monkeys and dogs to demonstrate bilateral increases in testicular temperature, morphological damage to testes, and decreases in ejaculated sperm concentrations [5,6]. However, none of the experimental varicocele models that were previously studied evaluated the effect on the left kidney. If these experimental varicocele models had any effect on the left kidney, the pathophysiological results that were previously published might not be the results of only the experimental varicocele effect.

It has been a long time since animal studies have been conducted using rats, dogs, rabbits, and monkeys. Most of these studies showed that experimental left varicocele damages the testicular histology and reduces testicular mass by increasing bilateral testicular blood flow and intrascrotal temperature [7-11]. Up to now, however, no studies have evaluated the effect of surgically induced varicocele on the left kidney. Therefore, we investigated the influence of the location of the partial renal vein obstruction on the left kidney, the bilateral testes, and cauda epididymal sperm quality and determined whether the rat model is suitable for varicocele study.

MATERIALS AND METHODS

1. Animals

The study included 30 male 8- to 9-week-old Sprague-Dawley rats. All animals were fed the same food and were maintained in a constant environment under a 12:12-hour light-dark cycle. The rats were assigned to three groups: group 1 (experimental varicocele by partial ligation medial to the internal spermatic vein for 8 weeks, n=10), group 2 (partial ligation lateral to the internal spermatic vein for 8 weeks, n=10), and group 3 (sham operation for 8 weeks, n=10). Five of the initial 30 rats were excluded because of death after surgery: two in group 1 and three in group 3.

2. Experimental operation technique

Experimental varicocele (for group 1) was created by using a technique previously described [12]. Each animal was anesthetized with an intramuscular injection of zolazepam hydrochloride (10 mg/kg) and xylazine (7 mg/kg). An abdominal midline incision was made, and the abdominal contents were packed to visualize the left kidney, the left adrenal vein, the left renal vein, and the left internal spermatic vein insertion into the left renal vein. The left renal vein, inferior vena cava, and left internal spermatic vein were identified, and the external diameter of the left internal spermatic vein at the level of the iliolumbar vein was measured by using a metal micrometer to evaluate varicocele development. By careful blunt dissection, the left vein was cleared of adhering tissue in a position medial to the insertion of the left internal spermatic vein and left adrenal vein. At this site, a 4-zero silk was tied around the 0.035-inch guide wire (Terumo Guide Wire, Terumo, Frankfurt, Germany) cutting into about 4 cm, which was placed on the left renal vein (Fig. 1A). This procedure resulted in a reduction of the diameter of the left renal vein by approximately 50% and in the immediate dilation of the distal portion of the renal vein. Finally, the midline incision was closed in 2 layers with 3-zero silk sutures. After surgery, the animals were returned to their cages and managed by use of the above-mentioned presurgical protocol. The rats in group 2 underwent the same operation except that partial ligations were placed lateral to the left internal spermatic vein insertion into the left renal vein (Fig. 1B).

The rats in the control group underwent the same operation except that ligatures were only placed and not tied. The experimental protocol was approved by the Animal Care and Use Committee of our institution and was in ac-

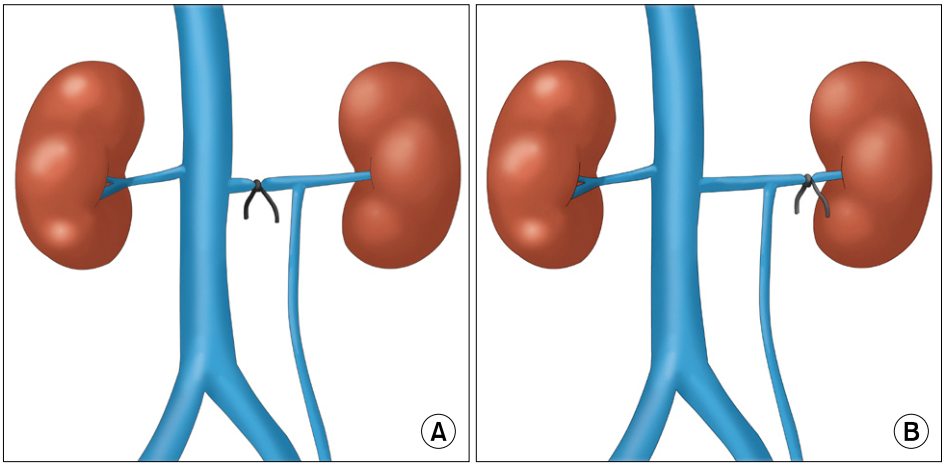

FIG. 1. Schematic illustration showing the different site of partial ligation of the left renal vein. (A) Group 1, partial ligation medial to the internal spermatic vein (B) Group 2, partial ligation lateral to the internal spermatic vein.

cordance with the declaration of Helsinki and the guideline issued by the International Association for the Study of Pain.

3. Histopathology

The rats in groups 1, 2, and 3 underwent left nephrectomy and bilateral orchiectomy at 8 weeks postoperatively. The external diameters of the left internal spermatic veins were measured at the same previous level, and an increase in vein external diameter by 2-fold or more was considered varicocele. Testes were weighed and fixed in Bouin's solution and embedded in paraffin. Histological sections of both testes of each animal were prepared and stained with H&E for histopathological examination and for determining Johnsen scores [13]. Histological changes were defined as severe when Johnsen scores were less than 6, because this score was found to be related to infertility in rats in a previous study [14]. Seminiferous tubule diameters (STDs) were measured on 25 tubules by using DP2-BSW software (Olympus, Center Valley, PA, USA) and an optical microscope (BX-51T; Olympus, Tokyo, Japan) with a x20 objective.

4. Quantitative assessments of renal fibrosis

Sections in groups 1, 2, and 3 fixed in 10% formalin were stained by the Masson's trichrome method to detect renal fibrosis as follows [15]: nuclear staining was achieved by staining slides with Celestin blue for 5 minutes and then washing in water. Sections were then incubated in Mayer's acid hematoxylin for 5 minutes, washed in water, and blued in Scott's tap water for 1 minute. For counterstaining, slides were immersed in Biebrich scarlet acid fuchsin for no longer than 10 seconds and quickly rinsed in water. Sections were then oxidized in 5% phosphotungstic acid for 20 minutes, transferred to aniline blue for 30 minutes to stain any fibrosis present, washed in water, dehydrated in absolute alcohol for 10 seconds three times, and cleared in xylene for 10 seconds three times. Slides were then mounted in DPX and allowed to dry overnight.

Fibrosis was quantified as follows: sections were analyzed under an optical microscope equipped with a x10 objective, as mentioned above. Five fields per section were assessed. For evaluation purposes, fields that included renal cortex structures such as the glomerulus and the proximal and distal tubules were included. Fibrosis was quantified by using Leica Qwin software (Leica Imaging Systems, Camb- ridge, England) by expressing blue stained areas as percentages of total areas.

5. Cauda epididymal spermatozoa evaluation

Cauda epididymides were minced in 5 ml of normal saline containing 0.5% bovine serum albumin at 37°C and were then filtered. Sperm suspensions were placed on glass slides that had been prewarmed at 37°C. Percentages of motile sperm were determined by counting more than 200 spermatozoa in randomly selected fields under a light microscope. Sperm counts were expressed as the number of motile spermatozoa per gram of cauda epididymis tissue [16]. Samples were evaluated by one expert investigator unaware of sample identities.

6. Statistical analysis

SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analysis. The Mann-Whitney test was used to detect differences between Groups 1, 2, and 3. All values are expressed as means±SD. p-values of <0.05 were considered statistically significant.

RESULTS

1. Varicocele evaluation

At the time the rats were killed, the mean diameters of the left internal spermatic vein in group 1, group 2, and group 3 were 1.8±0.3, 0.4±0.1, and 0.4±0.1 mm, respectively. All 8 rats that underwent partial ligation of the left renal vein showed objective dilatation of the left internal spermatic vein when killed, whereas the animals in groups 2 and 3 did not show such dilatations (Fig. 2).

2. Body weight

At the time the rats were killed, the mean body weights in group 1, group 2, and group 3 were 571.1±32.5 g, 580.1±61.8 g, and 575.2±35.9 g, respectively. No significant differences were found between the groups.

3. Histopathology

1) Testicular weight: Mean testes weights are shown in Table 1. No significant differences were observed between the groups.

2) Seminiferous tubule diameters: Mean STDs are listed in Table 1. Mean STDs of both testes in group 1 were significantly lower than in groups 2 and 3.

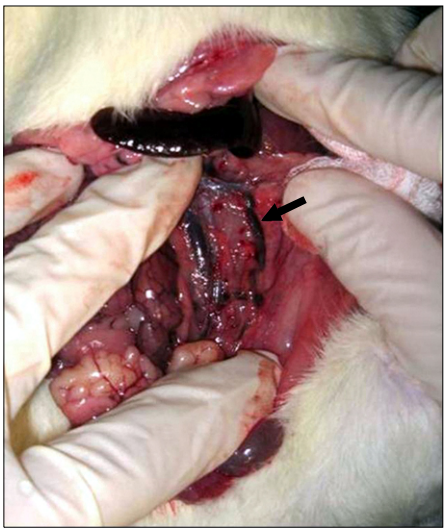

Fig. 2. Dilatation of the internal spermatic vein after partial ligation of the left renal vein medial to the internal spermatic vein insertion site (arrow).

TABLE 1. Comparisons of the groups in the experimental varicocele model

Group	No. of rats	Testicular weight (g)[d]		Seminiferous tubule diameters (μm)		Johnsen score (points)		% Fibrosis[e]	x10^6/g of cauda[f]		% of motile sperms	
		Left	Right	Left	Right	Left	Right	Left kidney	Left	Right	Left	Right
Group 1[a]	8	1.61±0.29	1.69±0.11	246.2±39.5[g]	253.1±18.0[g]	8.1±0.35[g]	8.7±0.47[g]	2.1±0.3	223.6±67.4	194.1±56.4	59.9±5.3[g]	60.4±4.8[g]
Group 2[b]	10	1.65±0.35	1.67±0.23	275.3±15.3	293.0±17.3	10±0	10±0	1.9±0.2	231.4±58.3	261.0±41.8	71.2±6.3	76.4±7.0
Group 3[c]	7	1.69±0.09	1.71±0.09	280.0±14.1	291.0±25.8	10±0	10±0	2.2±0.4	233.1±27.8	255.0±19.2	73.7±8.2	75.5±7.9

[a]: experimental varicocele by partial ligation medial to the internal spermatic vein for 8 weeks, [b]: partial ligation lateral to the internal spermatic vein for 8 weeks, [c]: sham operation for 8 weeks, [d]: no significant differences between the groups, [e]: no significant differences between the groups, [f]: number of motile spermatozoa per gram of cauda epididymis tissue; no significant differences between the groups, [g]: $p < 0.05$, compared with group 2 and 3

3) Histologic change and Johnsen score: The main histological changes observed in group 1 were reduced spermatogenesis, germ cell desquamation, maturation arrest, increased congestion, and perivascular fibrosis (Fig. 3A). Mean Johnsen scores are shown in Table 1. The mean Johnsen scores of the left and right side testes in group 1 were significantly lower than in groups 2 and 3. Left and right testes sections in groups 2 and 3 did not show any significant histological abnormalities (Fig. 3B, C).

4. Quantitative assessment of renal fibrosis

Renal fibrosis did not differ significantly among the groups (Table 1, Fig. 4). The structures of the glomerulus and of the proximal and distal tubules were well preserved regardless of the operation type.

5. Sperm count and motility

The mean sperm counts and percentages of motile spermatozoa in the left and right epididymides are shown in Table 1. Sperm motility in group 1 was significantly lower than in groups 2 and 3. However, no significant differences in sperm counts were found among the groups.

DISCUSSION

The prevalence of varicocele in the general male population is approximately 15%, but the rate is higher than 35% in infertile men [10]. These lesions are common, and varicocele treatment is a traditional part of therapy for male infertility. Varicocele is characterized by stasis and high pressure in the veins that constitute the pampiniform plexus and a limited capacity of the available channels for venous return [11]. Therefore, research on the pathophysiology of varicocele has been essential for a long time, but specific questions on the biological or physiological consequences of varicocele are difficult to address in the human population. The acquisition of tissue, surgical interventions for experimental purposes, and the availability of appropriate numbers of control patients and of varicocele patients of desired ages with appropriate durations of varicocele and other characteristics have hampered this kind of research. Accordingly, studies in animal models of varicocele have contributed enormously to our understanding of this lesion in men.

The rat is a good candidate for the development of an animal model of varicocele. Saypol et al developed a model of varicocele in rats by partially ligating the left renal vein [17]. Besides the adequate size of the left renal vein of the rat for varicocele-inducing surgery, many data on testicular pathology and spermatogenesis are available because this species has been used most frequently in animal testing [18]. Nevertheless, some points concerning the surgical procedures should be borne in mind. The left internal spermatic vein inserts into the left renal vein medially, almost at the point where the left renal vein and inferior vena cava meet. Accordingly, in order to ligate the portion medial to the insertion of the left internal spermatic vein, it

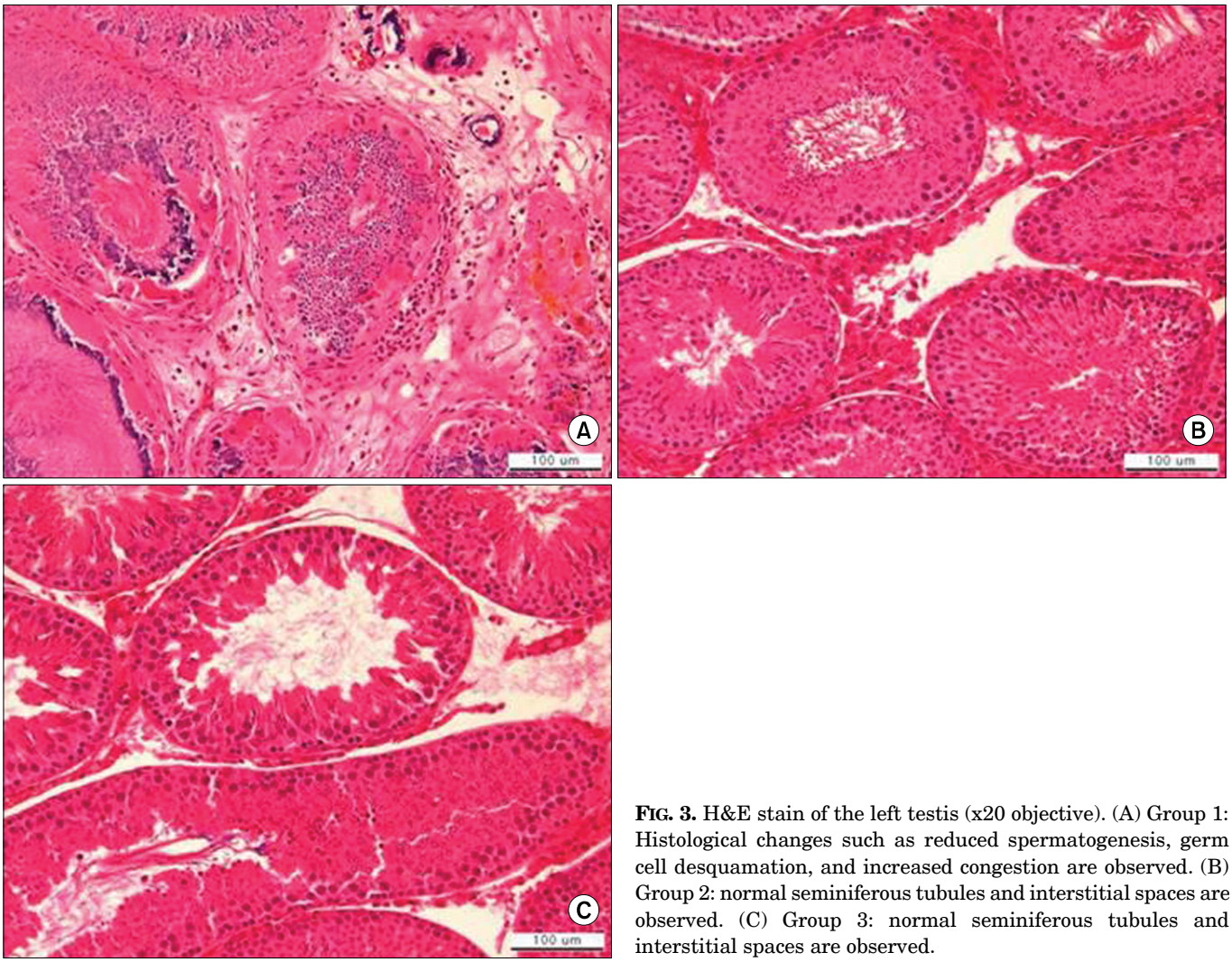

FIG. 3. H&E stain of the left testis (x20 objective). (A) Group 1: Histological changes such as reduced spermatogenesis, germ cell desquamation, and increased congestion are observed. (B) Group 2: normal seminiferous tubules and interstitial spaces are observed. (C) Group 3: normal seminiferous tubules and interstitial spaces are observed.

is necessary to partially dissect the inferior vena cava in most cases. However, blind dissection behind the vein can sometimes result in inadvertent puncture or tearing of the vein. Although this bleeding might seem excessive, the tears are often small and a few minutes of topical compression usually stops the bleeding. In addition, it should be emphasized that a correct, consistent degree of obstruction is necessary for the reliable development of varicocele in rats. As stated in a previous report, excessive reduction in renal vein size will result in actual occlusion and eventual necrosis of the kidney. On the other hand, insufficient constriction cannot produce the increase in lateral intrarenal vein pressure required to force the development of left internal spermatic vein varicosity. To avoid this problem, we inserted a 0.035 inch Terumo guide wire when ligating the renal vein in the preliminary experiment. Using this method, we successfully created a varicocele model in all experimental animals.

Some of the important findings from the many studies that have been done by partially occluding the left renal vein are that testicular temperature increases bilaterally by varicocele and that spermatogenesis decreases bilaterally. Most of the representative studies were done in rats; however, the results of other studies that used rabbits or dogs were similar. As with the previous studies, our study also showed a decrease in sperm count and motility and in the diameter of the seminiferous tubule. More significantly, there were no changes in the structure of the left kidney because of the surgically made partial occlusion of the left renal vein. To our knowledge, no studies that evaluated the effect on the left kidney by surgically induced varicocele have yet been published. The time it takes for the development of varicocele after varicocele-inducing surgery can vary depending on the surgeon and the laboratory; however, most studies showed the average time to be approximately 4 to 8 weeks. Thus, it is reasonable to deduce that this would be the appropriate time for subsequent study [11,18,19].

Nutcracker syndrome is the clinical manifestation of the situation in which the left renal vein suffers from pressure when passing through the angle between the abdominal aorta and the superior mesenteric artery [20]. This syndrome is very similar to our experimental varicocele model, which is related to the increasing pressure of the left renal

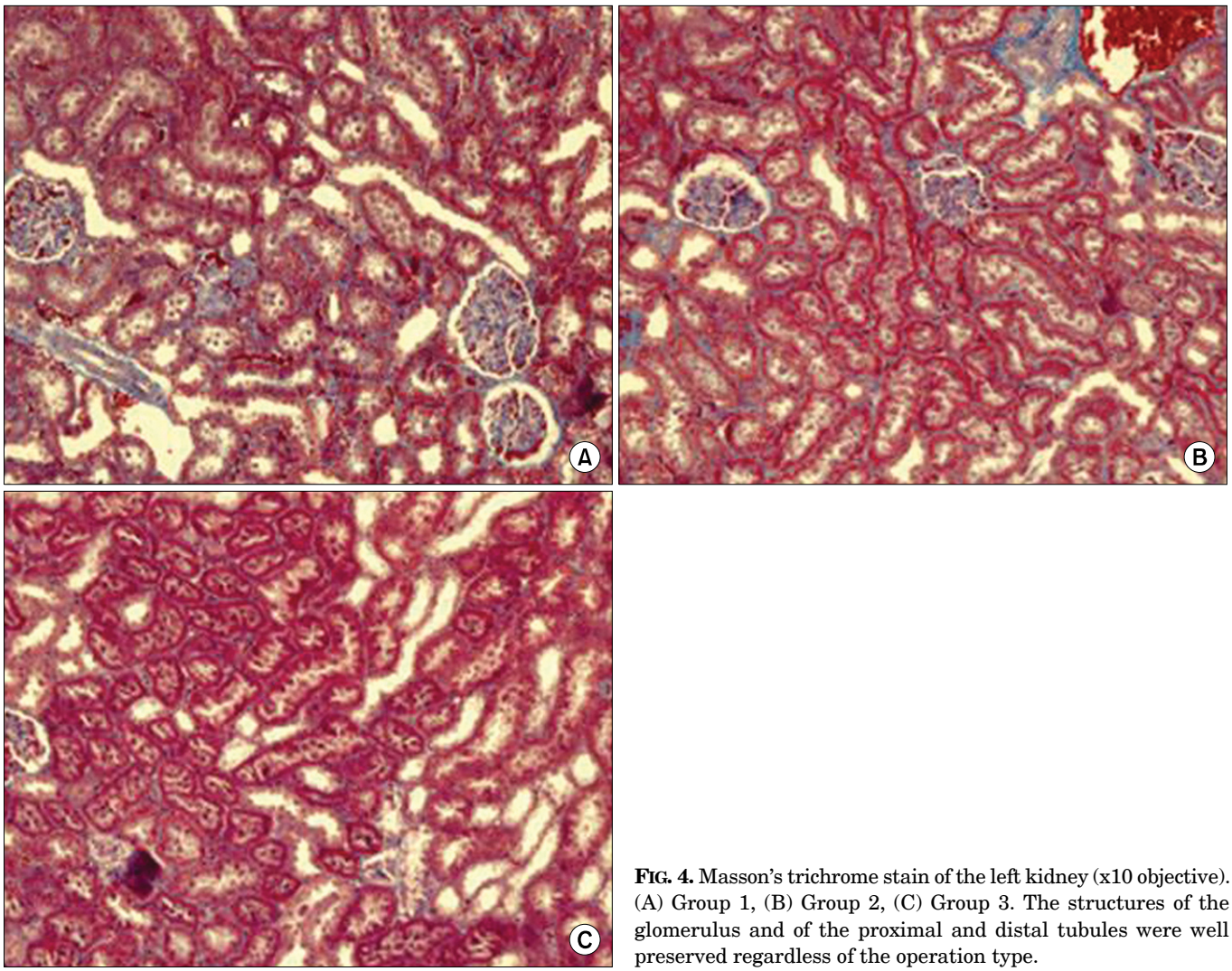

FIG. 4. Masson's trichrome stain of the left kidney (x10 objective). (A) Group 1, (B) Group 2, (C) Group 3. The structures of the glomerulus and of the proximal and distal tubules were well preserved regardless of the operation type.

vein. Takemura et al performed a renal biopsy in three patients with nutcracker syndrome [21]. They found no remarkable glomerular or interstitial injuries in any of the patients and no deposition of immunoglobulins or complement components in any part of the renal tissues. These findings are consistent with our results.

Over the years, researchers have proposed several theories for the mechanisms by which varicocele may impair male infertility. They include scrotal hyperthermia [22], hypoxia [23-25], retrograde flow of adrenal blood [19], endocrine and testicular paracrine imbalances [3], and most recently apoptosis [4], angiogenesis [9], and DNA damage and related abnormal spermatogenesis [26]. However, because much of the pathophysiology of varicocele is still unknown, more studies using animal models should be performed and experiments to verify the validity of the animal model must be required.

We did not investigate sperm morphology in the present study, because no accepted classification scheme has been developed for its assessment [27]. Several previous studies have followed the scheme proposed by Linder et al [28], but the utility of this scheme has been shown to be problematic. First, classification schemes devised to date have been highly subjective, and this is particularly true for categories like "small headed sperm." Because head size is a continuous variable, "normality" is difficult to define. Second, many classification schemes allow an individual sperm to be classified in different ways. Finally, no successful statistical comparative method has been devised [27].

We did observe fibrotic changes to the left kidney according to the location of the renal vein partial obstruction. It would be more informative to perform various kinds of tests using genetics or molecular biology to detect renal damage. However, because fibrotic change is the most representative outcome in a poorly circulated organ [29], we decided on this as an experimental variable. Further research using the other modalities should be performed to reveal the pathophysiology of the varicocele.

CONCLUSIONS

The results of this study showed that partial ligation of the left renal vein medial to the internal spermatic vein influences testicular spermatogenesis and epididymal sperm

motility without harmful effects on the left kidney. On the contrary, partial ligation lateral to the internal spermatic vein or sham operation does not affect testicular spermatogenesis, epididymal sperm motility, or fibrotic change in the left kidney. Our results certify that this traditional animal model is suitable for varicocele research.

Conflicts of Interest

The authors have nothing to disclose.

REFERENCES

1. Choi H, Kim KS, Kim KM. The effect of experimental varicocele on the testis of adolescent rats. J Urol 1990;144:499-501.
2. Steeno O, Knops J, Declerck L, Adimoelja A, van de Voorde H. Prevention of fertility disorders by detection and treatment of varicocele at school and college age. Andrologia 1976;8:47-53.
3. Skoog SJ, Roberts KP, Goldstein M, Pryor JL. The adolescent varicocele: what's new with an old problem in young patients? Pediatrics 1997;100:112-21.
4. Kim KS, Choi H. The effects of experimental varicocele on testis in adolescent rats. Korean J Urol 1989;30:19-28.
5. Kay R, Alexander NJ, Baugham WL. Induced varicoceles in rhesus monkeys. Fertil Steril 1979;31:195-9.
6. Al-Juburi A, Pranikoff K, Dougherty KA, Urry RL, Cockett AT. Alteration of semen quality in dogs after creation of varicocele. Urology 1979;13:535-9.
7. Simsek F, Türkeri L, Cevik I, Bircan K, Akdas A. Role of apoptosis in testicular tissue damage caused by varicocele. Arch Esp Urol 1998;51:947-50.
8. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995;92:5510-4.
9. Vukovic V, Haugland HK, Nicklee T, Morrison AJ, Hedley DW. Hypoxia-inducible factor-1alpha is an intrinsic marker for hypoxia in cervical cancer xenografts. Cancer Res 2001;61:7394-8.
10. Greenberg SH. Varicocele and male fertility. Fertil Steril 1977; 28:699-706.
11. Verstoppen GR, Steeno OP. Varicocele and the pathogenesis of the associated subfertility. A review of the various theories. III: Theories concerning the deleterious effects of varicocele on fertility. Andrologia 1978;10:85-102.
12. Kilinc F, Kayaselcuk F, Aygun CM, Guvel S, Egilmez T, Ozkardes H. Experimental varicocele induces hypoxia inducible factor-1alpha, vascular endothelial growth factor expression and angiogenesis in the rat testis. J Urol 2004;172:1188-91.
13. Johnsen SG, Agger P. Quantitative evaluation of testicular biopsies before and after operation for varicocele. Fertil Steril 1978;29: 58-63.
14. Asci R, Sarikaya S, Büyükalpelli R, Yilmaz AF, Yildiz S. The effects of experimental varicocele on testicular histology and fertility in monorchic adult rats. BJU Int 1999;83:493-7.
15. Raleigh D, O'Donnell L, Southwick GJ, de Kretser DM, McLachlan RI. Stereological analysis of the human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval. Fertil Steril 2004;81:1595-603.
16. Kim SJ, Lee MS. Effect of Sertoli cell changes on germ cells in experimentally produced varicocele in rats. Korean J Urol 1992;33: 404-17.
17. Saypol DC, Howards SS, Turner TT, Miller ED Jr. Influence of surgically induced varicocele in testicular blood flow, temperature, and histology in adult rats and dogs. J Clin Invest 1981;68: 39-45.
18. Turner TT. The study of varicocele through the use of animal models. Hum Reprod Update 2001;7:78-84.
19. Comhaire F, Vermeulen A. Varicocele sterility: cortisol and catecholamines. Fertil Steril 1974;25:88-95.
20. Zhang H, Li M, Jin W, San P, Xu P, Pan S. The left renal entrapment syndrome: diagnosis and treatment. Ann Vasc Surg 2007; 21:198-203.
21. Takemura T, Iwasa H, Yamamoto S, Hino S, Fukushima K, Isokawa S, et al. Clinical and radiological features in four adolescents with nutcracker syndrome. Pediatr Nephrol 2000;14:1002-5.
22. Zorgniotti AW. Testis temperature, infertility, and the varicocele paradox. Urology 1980;16:7-10.
23. Chakraborty J, Hikim AP, Jhunjhunwala JS. Stagnation of blood in the microcirculatory vessels in the testes of men with varicocele. J Androl 1985;6:117-26.
24. Nahoum CR, Fontes EA, Freire FR. Semen analysis in sickle cell disease. Andrologia 1980;12:542-5.
25. Oztürk H, Tander B, Aydin A, Okumus Z, Cetinkursun S. The effects of chemical sympathectomy on testicular injury in varicocele. BJU Int 2001;87:232-4.
26. Cho WJ, Yeo MH, You HJ, Chang IY, Moon HY, Kim CS. The expression of p53 and phosphorylation of H_2AX in germ cells of varicocele rats. Korean J Urol 2006;47:1354-60.
27. Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. Reprod Toxicol 1996;10:237-44.
28. Linder RE, Strader LF, Slott VL, Suarez JD. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 1992;6:491-505.
29. Bechtel W, Zeisberg M. Twist: a new link from hypoxia to fibrosis. Kidney Int 2009;75:1255-6.