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Varicocele in the rat: a new experimental model

Effect on histology, ultrastructure and temperature of the testis and the epididymis

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Summary. With no consistent animal prototype for the study of varicocele, we set out to create a model in the rat by complete ligation of the main branch of left spermatic vein (MBSV) or by partial ligation of the left renal vein. Three months later, the histology, ultrastructure and temperature of the testis and epididymis were studied. Microscopically, spermatogenic arrest was the most frequent anomaly seen. The most frequently noted ultrastructural change of the testis was distension of smooth endoplasmic reticula in Sertoli cells. The microvilli of columnar epithelia in epididymis were sparse and showed local defects. Lesions and increased temperatures in the testis and epididymis induced by the ligation of the left MBSV were similar to those seen in partial ligation of the left renal veins, with no significant differences between left and right. Significant differences were found, however, on comparison with the controls.

Key words: Varicocele – Rat, Histology – Ultrastructure – Temperature – Infertility

It is well known that varicocele is implicated in male infertility, but the exact mechanism by which the varicocele gives rise to the infertility is still an enigma [8]. Since the mid-1970s, experimental study of this subject has intensified, and many experimental models have been created in the dog, monkey, and rat [3]. However, the studies in these models have been inconsistent. Thus, it is necessary to continue to search for an ideal model for the study of variococele.

Anatomical studies on the testicular vein system of the rat have shown that the pampiniform plexus drains up the internal inguinal ring into two efferent veins, a thick and a thin one [10, 13]. The thick efferent vein has been named the main branch of the spermatic vein (MBSV), and it leads into the common iliac vein or into the caudal end of the inferior vena cava. The thin one is named the testicular vein. On the left, 95% of the testicular veins went into the renal vein, and 5% into the inferior vena cava, while on the right, 10% of the testicular veins went into the renal vein, and 90% into the inferior vena cava. Thus, on the basis of the results of these anatomical studies, we used ligation of the left MBSV to create the varicocele, and compared it with the status following partial ligation of the left renal vein with reference to the testicular histology and ultrastructure and to the temperature of both the testis and the epididymis of the rat.

Materials and methods

Seventy-eight adult male Spraque-Dawley rats weighing 250–350 g were subdivided randomly into three groups as classified below.

Group I

The animals were anesthetized with sodium pentobarbital 40 mg/kg i.p. Through a left paramedian incision, the left renal vein was partially ligated to give an external diameter of 0.85 mm. A consistent stenosis was achieved by using a 3-0 silk suture which was tied around both the renal vein and a metal probe. The probe was carefully removed and the vein was allowed to expand against the loop of the suture. The suture was positioned at the junction of the renal vein and the inferior vena cava. The incision was closed, and the animals were returned to the vivarium.

Group II

In this group of animals the same procedure as above was carried out, the only difference being that the left MBSV was completely ligated under the operating microscope without partial ligation of the left renal vein.

Group III

This group served as controls, each rat undergoing a "sham" operation.

Three months later, the animals in groups I_1, II_1 and III_1 (10 rats each group) were anesthetized again. Biopsies were obtained from

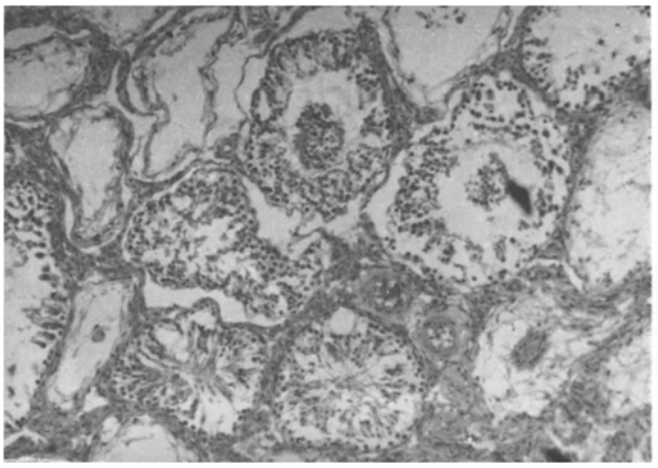

Fig. 1. Disorganization of germinal epithelia and sloughing of spermatocytes and spermatids into the lumens. No cells in some lumina. 10×10^2 X

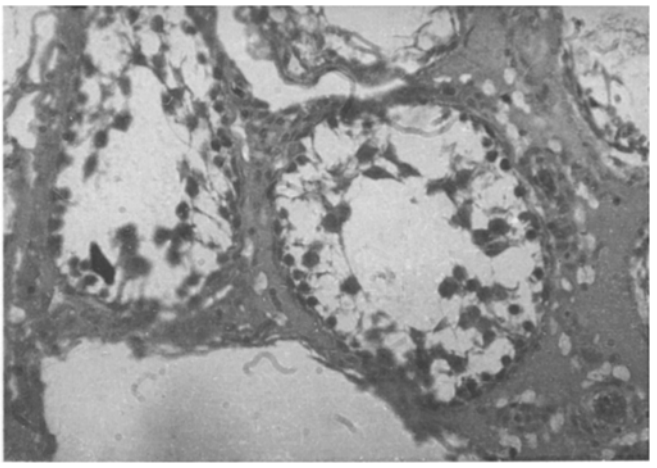

Fig. 2. Only Sertoli cells in the lumina and vacuolar degeneration of the Leydig cells. 2.5×10^2 X

the center of each testis and from the tail of each epididymis. These specimens were immediately fixed by immersion in 4% gluteraldehyde in 0.2 M phosphate buffer, then post-fixed in 1% osmium tetroxide. Next, they were dehydrated, first in ethanol and then in acetone, and finally embedded in Epon 812-Araldite. Thin sections were taken and double-stained with uranyl acetate and lead citrate, then viewed with H-600 transmission electron microscopy (Tokyo, Japan). The remains of the testis and epididymides were completely removed, fixed in Bouin's liquid and embedded in paraffin. Two sections were taken from each testis and each epididymis for viewing with light microscopy. One specimen was stained with hematoxylin and eosin, while the other was stained with periodic acid-Schiff. In each section of the right and left testes, 50 tubules were randomly selected, for examination and a record kept as to whether the tubules were normal or abnormal in configuration. The square roots of the percentages of abnormal tubules were then subjected to arc-sine transformation prior to statistical evaluation by t, t' and q tests.

The animals in groups $I_{2,3}$, $II_{2,3}$ and $III_{2,3}$ (8 rats per group) were positioned in the supine position under anesthesia. The temperatures of the testes and epididymides (both right and left) were measured by puncturing the centers of the testes and the tails of the epididymides with a 23 gauge microprobe (CDT-I, Chengdu, China). The temperature in the laboratory was 23 ± 0.5°C and the rectal temperatures of the rats were controlled at 37 ± 0.1°C. Statistical analyses involved analysis of variance and Dunnett's test.

Results

Histology and ultrastructure

In groups I_1 and II_1, all the rats with left testicular vein dilatation had abnormal histology bilaterally except two of the rats in group I_1 (one showed no alteration, the other showed only a slight lesion of the left testis, both without testicular vein dilatation). Slight abnormalities were also noted in the tubules near the tunica albuginea in group III_1.

Lesions of the testes observed by light microscopy showed that both impaired and normal tubules intermingled and impaired tubules presented "patchy" alteration among normal tubules. The predominant lesion was that of spermatogenic arrest at the spermatid and preliminary spermatocyte phases and the next frequent lesion was that of premature sloughing of spermatogenic cells (spermatids and spermatocytes) into the lumina of the tubules. Some rats showed minimal atrophy of the lumen and a decreased number of cells in the lumen. Most rats had significant atrophy of the lumen, disorganization of the germinal epithelia, and sloughing of spermatocytes and/or spermatids into the lumen. In the more severe lesions no cells were noted in the lumina. The Leydig cells showed vacuolar degeneration (Figs. 1, 2). The tubular wall was normal in every case. No significant histological alterations in the epididymides were observed by light microscopy.

Table 1. Rats: Abnormal tubules in the testes (mean ± SD)

Group	*n*	Left	Right
I_1	*10*	52.24 ± 13.25*	53.35 + 12.24*
II_1	*10*	56.01 ± 13.86*	51.93 ± 9.02*
III_1	*10*	27.71 ± 3.45	27.23 ± 4.06

* $P < 0.01$ vs III_1

The semi-quantitative data of abnormal tubules are shown in Table 1. There were no significant differences between left and right testes in any group, and no significant difference was found between groups I_1 and II_1 ($Ps > 0.05$). It is obvious that there are many more abnormal tubules of the testes in group I_1 and group II_1 than in group III_1 ($Ps < 0.01$).

Transmission electron microscopy showed that the most frequent change was distension or vacuolization of the smooth endoplasmic reticula in Sertoli cells, even when there was no spermatogenetic disorder (Fig. 3). The extension of vacuolization could cause breaks in the plasma membrane, resulting in the release of immature germ cells, whereas the junctions between Sertoli cells remained intact. The nuclei vacuoles of early spermatids often occurred, and there were particularly large vacuoles

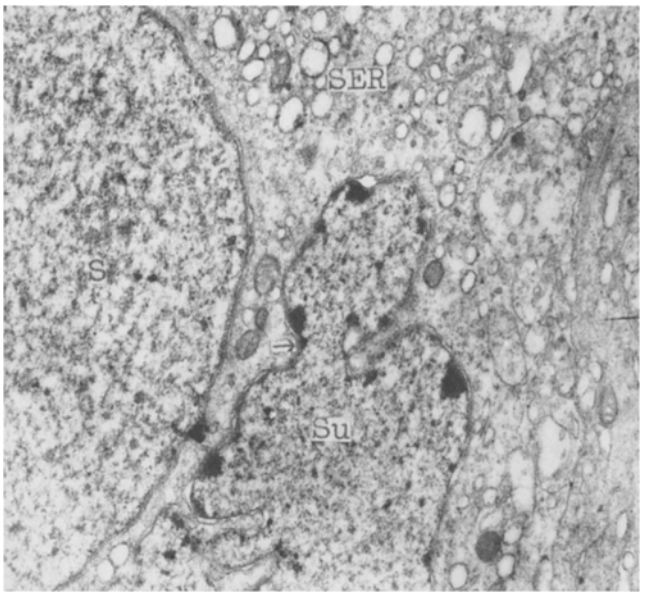

Fig. 3. Distension of the smooth endoplasmic reticula (*SER*) in the Sertoli cells (*Su*). 6×10^3 X

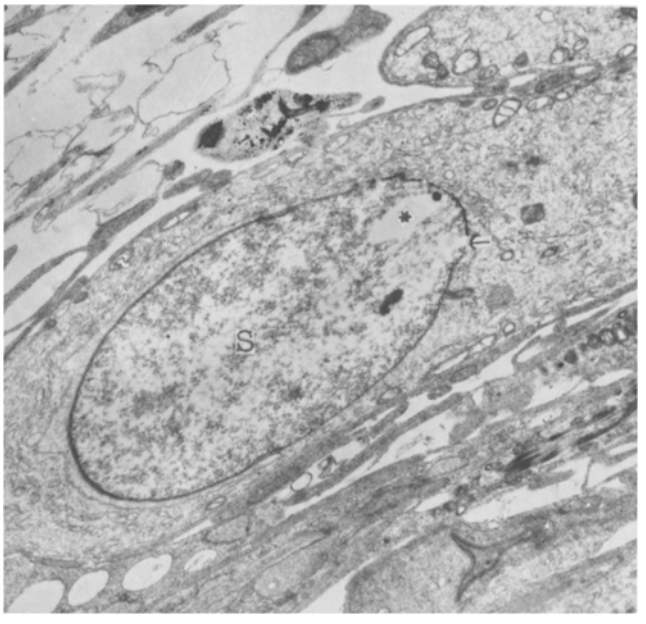

Fig. 4. A large area of vacuolization near the nucleus membrane (*) and a break of the nucleus membrane (←) of the early spermatid. 4×10^3 X

Table 2. Rats: testicular temperature (°C) (mean + SD)

Group	*n*	Left	Right
I_2	8	34.60 ± 0.39*	34.61 ± 0.30*
II_2	8	34.49 ± 0.56*	34.54 ± 0.46*
III_2	8	34.01 ± 0.24	34.04 ± 0.26

*$P < 0.05$ vs III_2

Table 3. Rats: temperature in the caudal end of the epididymis (°C) (mean ± SD)

Group	*n*	Left	Right
I_3	8	32.59 ± 0.51*	32.65 ± 0.37*
II_3	8	32.65 ± 0.63*	32.63 ± 0.58*
III_3	8	31.93 ± 0.29	32.09 ± 0.36

*$P < 0.05$ vs III_3

near the nucleus membrane, which could possibly cause rupture of the nuclear membranes (Fig. 4). Smooth endoplasmic reticula of the spermatids were also noted to be dilated. In late spermatids, the acrosomes became distorted, while local defects in the mitochondria sheaths of spermatids were also noted. The spermatogonia, spermatocytes, and basal membranes were intact. The Leydig cells were not studied in full detail, but distension of the smooth endoplasmic reticula was often observed. The microvilli of columnar epithelia in the epididymides were sparse and showed local defects.

Temperature

From Tables 2 and 3, it can be seen that there were no significant differences in temperature between the left and right testes and epididymides in groups $I_{2,3}$, $II_{2,3}$, and $III_{2,3}$ ($Ps > 0.05$). However, the temperatures of both testes and epididymides were higher in groups $I_{2,3}$ and $II_{2,3}$ than in groups $III_{2,3}$.

Discussion

Varicocele is one of the most common clinical diseases associated with male infertility and occurs only in the human being. Human studies were obviously limited in understanding the effect of varicocele on germinal epithelium and spermatogenesis. Because of this, animal models of varicocele have become particularly valuable tools even though there are a number of anatomical and physiological differences between man and animals. Experimental studies where varicocele was created by means of partial ligation of the left renal vein include those done on monkeys by Harrison et al. [7] and Kay et al. [9], as well as those done on dogs and rats by Saypol et al. [11] and Al-Juburi et al. [1], have demonstrated impairment to spermatogenesis. Unfortunately, these studies were not able to show consistent results. In order to imitate possible causes of human varicocele, Carmignari's group [3, 4] created a series of methods to induce varicoceles in rats, but again, the results were disappointing. This has stimulated our continued search for an ideal model in the study of varicocele.

Our study showed that experimental varicocele in rats, induced either by means of partial ligation of the left renal vein or by ligation of the left MBSV, could cause testicular lesions similar to the testicular alterations in human varicocele. The spermatogenic arrest at the spermatid and preliminary spermatocyte phases and the premature

sloughing of spermatogenic cells into the lumina of the tubules were the most common lesions observed in our study, and these were also the most frequent abnormalities found in human varicocele, even though they were not specific for varicocele alone [15]. The question to be answered is why the histological lesion presented "patchy" alteration. It is suspected that some specific changes in testicular microcirculation might be caused by the varicocele. In 1985 Chakraborty et al. [5], with the use of electron microscopy, found alterative microcirculatory vessels in the testes of men with varicocele. They showed that the luminal diameters of arterioles were significantly smaller in the affected testes than in controls, but no change was noted in the overall diameter of the arterioles and venules. Hence, it is necessary to understand the detailed microcirculation of the testes in future studies of varicocele.

The semi-quantitative data in our study indicate that the induced varicocele damaged the testes bilaterally, the right testis showing the same abnormality as the left testis. Although there are theories about a relationship between left varicocele and bilateral testicular lesion in human beings, the exact mechanism of such a connection still remains obscure. Perhaps vascular communications between the two testes could explain why a unilateral varicocele can result in bilateral testicular disfunction. Although we have been able to demonstrate vascular communications between both testicular vein systems in rats anatomically [13], the existence of anatomical communications between the two testicular vascular systems in human beings remain to be proven.

Numerous articles have shown ultrastructural alterations of the testis in human primary varicocele, in addition to those created experimentally in animal models [2, 12]. The major damage resulting from varicocele occurred in the adluminal area. The lesion of the basal lumina has not been observed in most investigations, including our study [14], but Fussell [6] demonstrated ultrastructural changes in the basal lumina in the monkey in 1981. If the change in the basal lumina represents the loss of the biochemical blood-testis barrier, then the permanent loss of this barrier could result in irreversible infertility. Unfortunately, there is no published work involving investigation of the possibility of reversion of ultrastructural changes of the basal lumina following surgical repair of varicocele and the possibility that a return of male fertility might occur as a result. It is worth noting that there are a number of studies about the ultrastructural alteration of the testis resulting from varicocele, but nothing has yet been published about ultrastructural lesions of the epididymis. Our study has provided preliminary evidence of ultrastructural change in the epididymis. Perhaps there is an important relationship between the disorder of the epididymis and male infertility resulting from the varicocele.

Our study also showed increased temperatures both in the testes and in the epididymides after induced varicocele. Many clinical and experimental studies have indicated that testicular temperature is increased by a varicocele [9]. It is now well known that the higher temperature will damage spermatogenesis in the testis. This is the basis of one of the theories about male infertility resulting from varicocele. In addition, it has been confirmed that increased temperature in the epididymis decreases the vitality of the sperm. This may be another reason for the impaired male fertility caused by varicocele.

According to our morphology and temperature studies, we believe that in many respects, experimental varicocele induced in the SD rat by means of ligation of the left MBSV or partial ligation of the left renal vein is a useful and practicable model for studying the effect of varicocele on germinal epithelium and spermatogenesis.

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