Urol Res (1983) 11:195–198

Urological Research

Experimental Varicocoele in the Rat – A New Experimental Model

I. Effect on Testicular Structure

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Accepted: April 20, 1983

Summary. A new experimental model of a varicocoele in the rat is proposed. The study has been carried out on the Wistar rat in which the gonadal vein enters into the iliac vein. A varicocoele was induced in different ways: by complete or imcomplete ligature of the iliac vein medial to the entry of the gonadal vein (Group I and II); by creating a venous compression by positioning the iliac artery in front of the vein, thus reproducing the so called "nut cracker" phenomenon (Group III); by creation of a small arterio-venous fistula by means of a side-to-side anastomosis between the iliac artery and vein. The results were studied macroscopically and histologically. The damage was most evident in Group III and significant in Group IV, but the absence of damage to the contralateral testis remains unexplained.

Key words: Varicocoele, Experimental surgery, Microsurgery, Infertility.

Introduction

It is well known that varicocoele is implicated as a cause of infertility in a high percentage of men [7, 12, 19]. However, the exact mechanisms by which a varicocoele affects spermatogenesis are still conjectural. We only know that various factors may be involved and implicated in the bilateral testicular damage. These are: increased scrotal temperature [8, 19], raised venous pressure with hypoxia [15, 17, 18], backflow of blood and perhaps of substances deleterious to spermatogenesis [2, 4, 5, 13], increased testicular blood inflow [16].

Further experimental investigations on the subject can therefore be justified. A critical review of the literature has convinced us that a reliable experimental model for human varicocoele is still missing. In fact, the authors that have dealt with this subject, Caldamone et al. [2], Harrison et al. [9], Kay et al. [11], Mobley et al. [14], Saypol et al. [16], have created experimental models either in the dog, in the monkey or in the rat, which are based only on the production of an obstacle to testicular venous outflow by means of a partial ligation of the left renal vein medial to the entry of the testicular vein. However, in the absence of a pressure gradient between the vena cava and the left renal vein there cannot be a reversed flow in the left testicular vein. This is thought to be a major factor in human varicocoele and it has been postulated that the compression of the left renal vein between the aorta and the superior mesenteric artery is of great importance in the pathogenesis of varicocoele (the so called "nut cracker phenomenon"). Saypol has hypothized an increased blood arterial inflow to the testis as a consequence of increased venous pressure and as a cause of infertility [16].

In the present investigation we have tried to create an experimental model which includes all the above mentioned factors.

Material and Methods

In Wistar rats in the majority of cases the tesicular vein drains into the common iliac vein in its distal portion [10]. Very rarely the testicular vein drains into the renal vein, as in man. The left adrenal and, quite frequently the ilio-lumbar vein drain into the left renal vein.

The investigation was carried out in 30 male albino adult Wistar rats weighing 250–350 g. The 5 animals which died from intra- or post-operative complications were excluded from the study. The remaining animals were subdivided into 5 groups:

Group I: (5 animals) In this group of animals anaesthesia was induced by ether on an open mask and a lower midline laparotomy was performed. The area of the aortic cross was exposed. The left common iliac artery and vein were carefully dissected and separated and some thin collateral vessels ligated or electrocoagulated. The left testicular artery and the ureter were maintained under view and carefully preserved. The dissection of the iliac vein was carried out down to the entry of the testicular vein. A complete ligation of the iliac vein above the entry of the testicular vein was then performed (Fig. 1a). The wound was closed in layers and the animals returned to the vivarium.

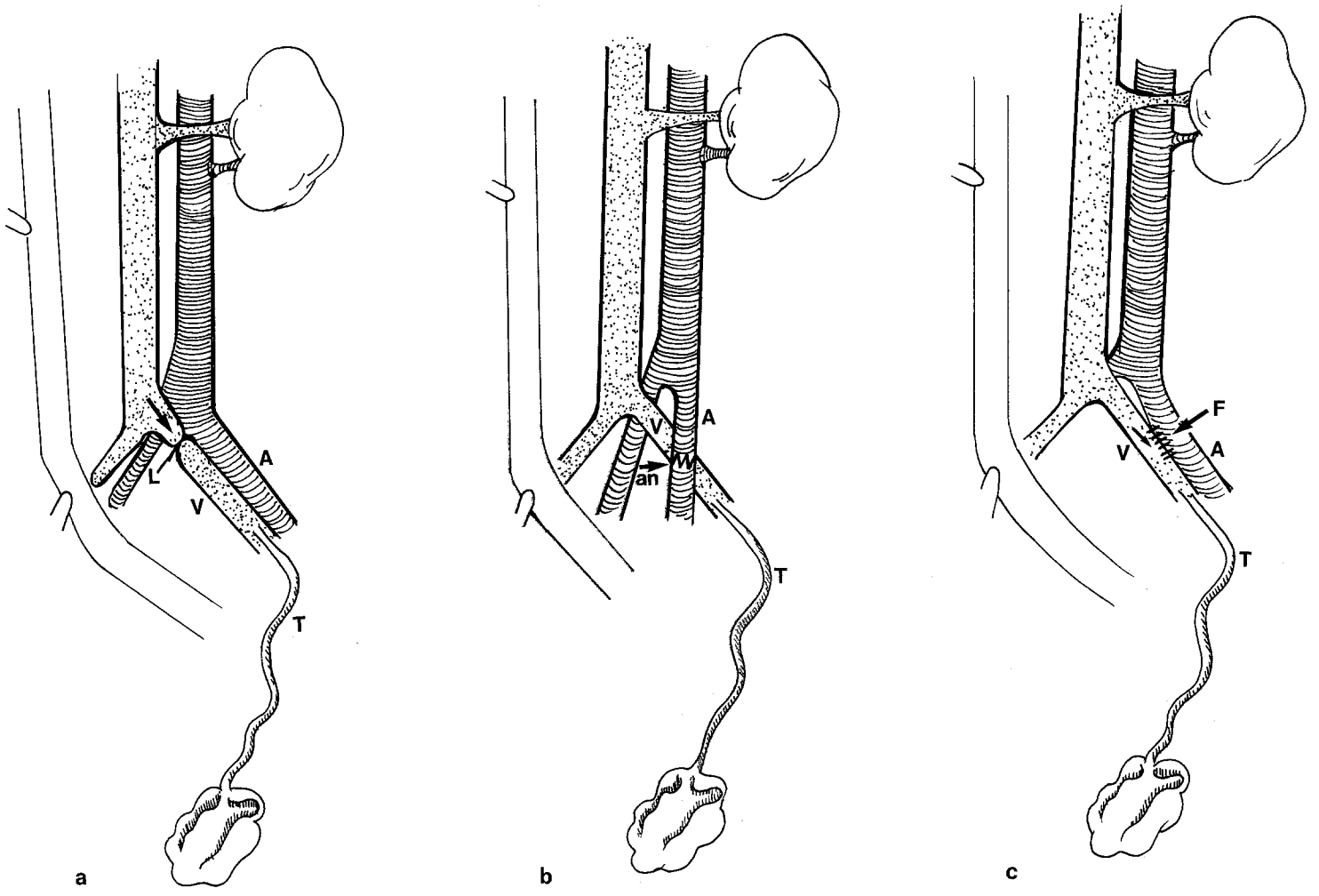

Fig. 1a–c. Group I and II (a) Complete or incomplete ligation of the iliac vein above the entry of the testicular vein. (*A*, common iliac artery; *V*, Common iliac vein; *L*, site of the ligation of the common iliac vein; *T*, testicular vein). b Group III: Anterior transposition of the iliac artery in front of the iliac vein, in order to create an arterial compass which should act as a "nutcracker". (*A*, common iliac artery anteriorly transposed; *V*, common iliac vein now lying behind the artery; *an*, microsurgical anastomosis of the common iliac artery in front of the vein; *T*, testicular vein). c Group IV: Side-to-side small arterio-venous fistula between the common iliac artery and vein. (*A*, common iliac artery; *V*, common iliac vein; *F*, side-to-side arterio-venous fistula between iliac artery and vein; *T*, testicular vein

Group II: (5 animals) In this group of animals the same procedures were carried out, with the only difference that the common iliac vein was only partially ligated.

Group III: (5 animals) In this group of animals we tried to produce venous compression by means of an arterial compass similarly to what is thought to occur in man by the aorto-mesenteric compass: the so called "nut cracker" phenomenon. To this purpose the iliac artery was separated from the vein and extensively mobilized. Then the iliac artery was cross clamped in its mid upper portion and transected. Its distal portion was slipped behind the iliac vein, anteriorly transposed and finally arterial continuity was restored in front of the vein by an end-to-end microvascular anastomosis with interrupted stitches of 10/0 Vicryl under operating microscope at 25 X magnification (Fig. 1b). Prompt engorgemet of the iliac vein distal of the arterial transposition and also of the testicular vein occurred.

Group IV: (5 animals) In this group of animals we tried to increase the blood inflow to the testis by producing a small side-to-side arterio-venous fistula between the iliac artery and vena iliaca communis (Fig. 1c). The vessels were fully mobilized and clamped together. Then a small elliptical excision of the anterior arterial wall together with a small longitudinal venotomy were made and a side-to-side anastomosis performed with two running sutures of Nylon or Prolene® 10/0. After releasing the clamps arterial backflow to the iliac vein was observed, with marked engorgement of the iliac and testicular veins.

Group V: (5 animals) This group which served as a control, underwent a "sham" operation.

No postoperative deaths were recorded in these 25 animals and all recovered well from the operation. All the animals were controlled 30 days after surgery and sacrificed. Controls included harvesting of the testes, which were macroscopically examined and weighed. Then a careful histological examination was carried out: a longitudinal incision was made in the tunica albuginea of both testes which were then fixed in 2.5% glutaraldehyde in phosphate buffer at pH 7.4 and post fixed in osmium tetroxide; then the pieces were embedded in Durcupan ACM and semithin sections were obtained with an LKB III Ultramicrotome and stained with toluidine blue 1%.

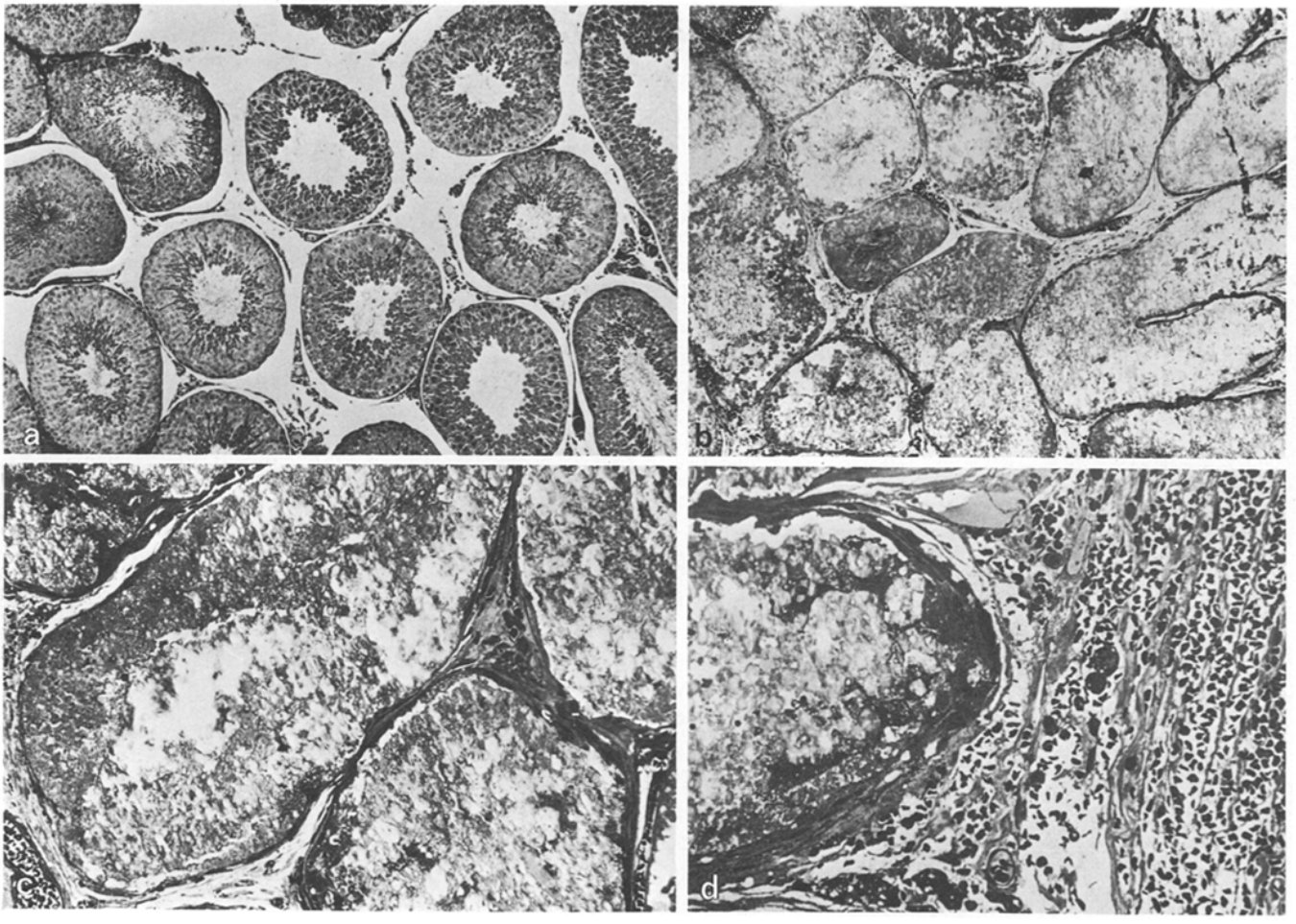

Fig. 2a–d. Group I and II (**a**): Abnormal pattern of the testis 30 days after creation of varicocele by complete or incomplete ligation of the iliac vein. **b** Group III: Severe degenerative pattern of the left testis 30 days after creation of varicocele by anterior iliac artery transposition. Diffuse necrosis of the germinal epitheliuu, with presence of exfoliated cells in the lumen. **c** At high power magnification the damages are more evident, with capillary engorgement and extravasation of red cells in the interstitium. **d** A detail showing the extravasation of the red cells in the interstitium

Results

The results must be discussed in detail according to the various Groups of animals.

In Group I and II no significant alteration of the germinal epithelium was found in the left testis as well as of the Sertoli's cells or of the membrana propria or of the interstitial compartment (Fig. 2a).

Also the contralateral testis appeared normal in all cases.

In Group III there was significant structural damage to the left testis in all cases. The histological pattern showed epithelial disorganisation of the seminiferous tubules, with severe degenerative changes of the germinal epithelium and a considerable amount of exfoliated cells commonly found in the lumen of tubules (Fig. 2b, 2c).

Numerous polynuclear cells could also be observed in the tubular lumen. The Sertoli cells as well showed minor but significant alterations.

The membrana propria appeared to be considerably thickened in most cases and a considerable amount of extravasated red cells could also be observed in the interstitium (Fig. 2d).

The contralateral testis showed a normal histological pattern in all cases.

In Group IV there were minor alterations of the germinal epithelium, but considerable interstitial oedema with marked engorgement of the peritubular capillaries was noted in all the cases. No contralateral changes were seen.

In Group V finally, no alterations were noted in either testis.

Discussion

The association between male subfertility and varicocoele has been proved by several authors [7, 12, 19] and may be considered as definitively accepted.

However, it is not clear why the venous anomaly causes bilateral testicular damage in a certain percentage of men

and even whether this damage can be related with certainty to the venous abnormality.

As already quoted, various mechanisms have been postulated as capable of interfering with spermatogenesis. Experimental studies have been performed by Caldamone et al. [2] in dogs, and by Saypol et al. [16] in both dogs and rats and by Harrison and Kay in monkeys [9–11]. In these studies the authors, who created an experimental varicocoele by means of partial ligation of the left renal vein medially to the entry of the testicular vein, could demonstrate an impairment to spermatogenesis, but were not able to show any evidence of structural testicular damage, except in some isolated cases in dogs with marked dilation of the testicular vein after ligation [2, 14]. The reason for this finding is not clear. However in the above-mentioned studies only venous pressure was increased, without achieving reversal of the blood flow as is demonstrated to occur in man. In fact, in order to reproduce this phenomenon, a pressure gradient between the inferior vena cava and the left renal vein would have been necessary. In man this situation is thought to be created by the arterial compass exerted by the superior mesenteric artery on the left renal vein. In this way not only a high venous pressure is produced, but also an actual inversion of the venous flow down to the testis.

Thanks to the peculiar anatomical disposition of the gonadal venous system in the Wistar rats we were able to reproduce such a situation by the transposition of the iliac artery anterior to the vein with creation of a situation very similar to that encountered in human pathology. Curiously only in this group did we find significative testicular alterations which can be ascribed to raised pressure, to backflow and to increased temperature, but not to reflux of deleterious substances from the kidney or the adrenals because of the peculiar anatomical disposition of Wistar rats. The finding of a contralateral normal testis remains to be explained.

Whether these reported changes may cause infertility remains to be acsertained and a paper on the effect of this situation of semen quality and on impregnating capability is running now in our laboratory and will be soon presented.

Finally we have also wanted to investigate the effect of an increased blood inflow to the testes since it was demonstrated by Saypol et al. [16] that the whole blood supply to the testes was considerably increased in cases of experimentally induced varicocoele. To this purpose we have created a small arterio-venous fistula, with resulting increased arterial inflow to the testes. Such a condition allows the study of the effect of increased inflow to the testis ruling out the possible effects of deleterious substances to spermatogenesis transported by the refluxing venous system. We have found that an increased arterial blood supply to the testis causes only minor alterations, such as interstitial oedema or capillary engorgement, but does not affect in any way the germinal epithelium.

In conclusion we think we have described a new experimental model of varicocoele in animals which resembles as closely as possible the alterations encountered in man and perhaps can provide additional useful information on the pathophysiology of the alterations encountered in subfertile men with varicocoele.

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