

# Varicocele and Retrograde Adrenal Metabolites Flow

## An Experimental Study on Rats

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### Key Words

Adrenal metabolites · Experimental varicocele · Male sterility · Varicocele

### Abstract

**Background:** Idiopathic varicocele is one of the causes of potentially correctable male subfertility. The mechanisms causing spermatogenesis impairment have yet to be clarified. The aim of this study is to analyze the effects of renal and adrenal metabolite reflux on testicular exocrine function in a rat experimental model. **Materials and Methods:** In the study, 45 male Lewis Stock adult rats, each weighing 300 g, were used. The rats were subdivided into three groups of 15 rats. In group A (control group) testicular volume and basal follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured at the beginning of the study and after 9 months. In group B, varicocele was induced by means of rings introduced in the left renal vein in order to cause a renospermatic reflux. In group C, similarly to group B, varicocele was induced after removal of left adrenal gland. The effects of varicocele on testicular function were then analyzed 3, 6 and 9 months after surgery. After 9 months, all rats underwent testicular biopsy. **Results:** Both groups B and C showed a reduction in testicular volume, an increase in FSH and a decrease in testosterone

levels. These levels were higher in group B. Testicular histological assessment showed important structural abnormalities in group B rats. **Conclusions:** These data support the hypothesis that renal and adrenal metabolites enhance varicocele-induced testicular damage. This theory is supported both by hormonal impairment and testicular histological analysis.

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### Introduction

Idiopathic varicocele is one of the most common causes of male subfertility, with prevalence in the general population of approximately 23%.

The most accepted physiopathologic hypotheses on gonadal damage include poor testicle oxygenation and an increase in intratesticular temperature caused by blood stasis and spermatic venous reflux of adrenal metabolites towards the gonad. However, the mechanism causing progressive spermatogenesis impairment in idiopathic varicocele cases still needs to be clarified [1–13].

In this study, the authors used an animal model (Lewis Stock male adult rats) to clarify the role played by adrenal metabolites renospermatic reflux on the testicular damage, as shown by the gonad histological assessment carried out on the different groups of animals used for this study.

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**Fig. 1.** Left adrenal gland isolated for excision.



**Fig. 2.** Stenosis of the distal renal vein obtained with a C-shaped silver ring.

## Materials and Methods

We used 45 Lewis Stock male adult rats, each weighing 300 g, subdivided into three groups of 15 rats (A, B, and C). In each rat follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone basal values were assessed via femoral vein blood sampling. We then measured (a) testicular volume, assessed by means of Prader's orchidometer; (b) blood FSH, LH and testosterone basal values, and (c) left renal vein basal pressure, after cannulation with a 22-G needle [14].

The first group (group A) was used as control group. We induced varicocele in the second group (group B), while in the third group (group C) varicocele was induced and left adrenalectomy was performed (fig. 1). Induction of varicocele was achieved using an origi-

**Table 1.** Hormone assay in group B rats

Period	FSH, U/l <sup>1</sup>	LH, U/l <sup>1</sup>	Testosterone mmol/l <sup>1</sup>
Preoperative	1.31 ± 0.05	0.85 ± 0.05	0.92 ± 0.03
3 months	1.36 ± 0.03	0.88 ± 0.03	0.90 ± 0.02
6 months	1.83 ± 0.06	0.90 ± 0.02	0.85 ± 0.04
9 months	2.39 ± 0.04	0.98 ± 0.03	0.71 ± 0.05

<sup>1</sup> Baseline hormone assay in normal rats: FSH 1.31 U/l, LH 0.85 U/l, testosterone 0.92 nmol/l.

Hormone values of group A rats (control group) show no significant increases in FSH.

**Table 2.** Hormone assay in group C rats

Period	FSH, U/l	LH, U/l	Testosterone mmol/l
Preoperative	1.31 ± 0.05	0.85 ± 0.05	0.92 ± 0.03
3 months	1.38 ± 0.04	0.86 ± 0.08	0.90 ± 0.03
6 months	1.58 ± 0.08	0.92 ± 0.06	0.87 ± 0.05
9 months	1.96 ± 0.02	0.96 ± 0.05	0.85 ± 0.04

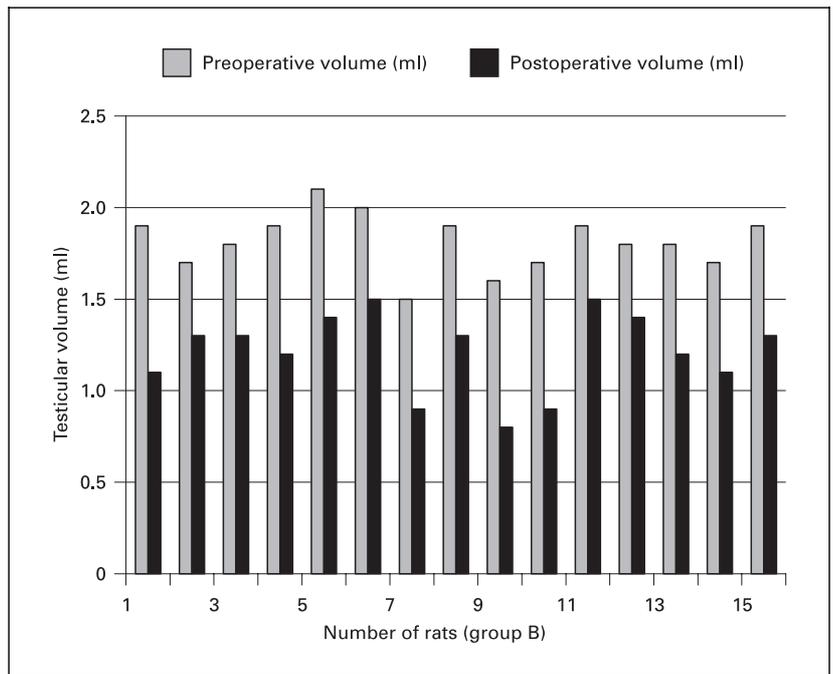
Explanations as in table 1.

**Table 3.** Hormone assay in group A rats

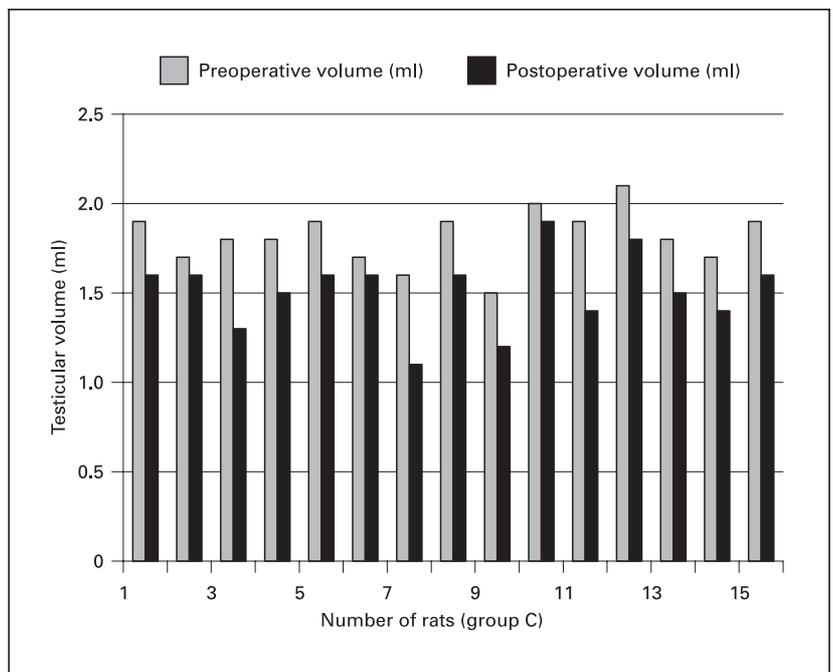
Period	FSH, U/l	LH, U/l	Testosterone mmol/l
Basal value (0 months)	1.31 ± 0.05	0.85 ± 0.05	0.92 ± 0.02
9 months	1.33 ± 0.11	0.85 ± 0.12	0.91 ± 0.07

nal technique: after median laparotomy, carried out under general anesthesia (0.8 cm<sup>3</sup>/100 g chloralium hydrate intraperitoneal infusion), a C-shaped silver ring was applied at the confluence of the left renal vein into the inferior vena cava (fig. 2). After renal vein cannulation with a 22-G needle, the venous pressure basal value was 5 cm H<sub>2</sub>O (as found in the control group).

In groups B and C, the C-shaped ring was adequately clamped in order to increase renal vein pressure up to 7 cm H<sub>2</sub>O [15–17]. This increase in left renal vein pressure was sufficient to cause a reno-sper-matic reflux (with a consequent dilation of the left spermatic vein). Blood FSH, LH and testosterone were measured at the beginning of the study and then 3, 6 and 9 months after varicocele induction in groups B and C. In group A, the same values were measured at 0 and 9 months.



**Fig. 3.** Testicular volume preoperatively and after 9 months in group B rats (mean testicular volume reduction 0.6 ml).



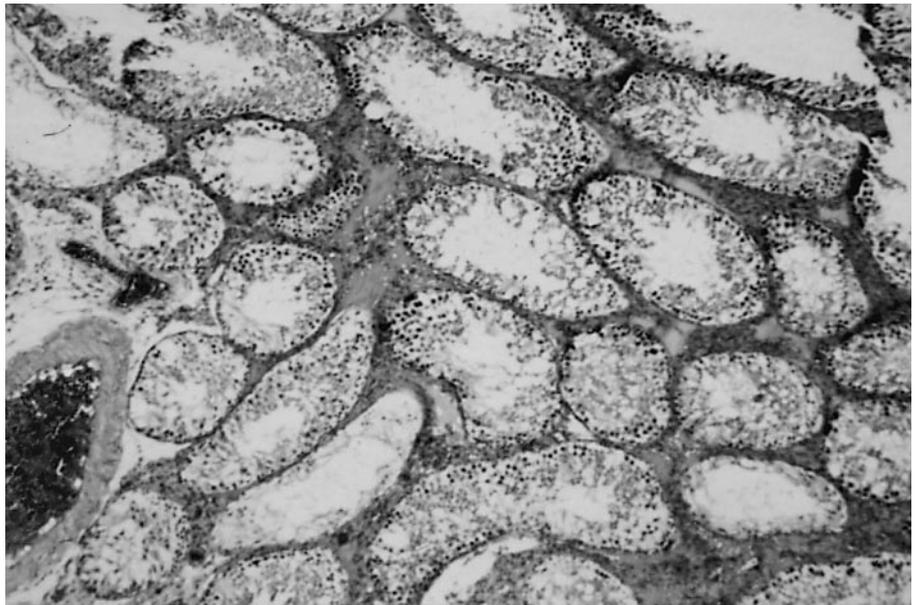
**Fig. 4.** Testicular volume preoperatively and after 9 months in group C rats (mean testicular volume reduction 0.3 ml).

Testicular volume was measured in all rats using Prader's orchidometer at the beginning and at the end of the investigation (0 and 9 months). All rats underwent testicular biopsy on both testicles 9 months after varicocele induction. Testicular tissue was fixed with 20% formalin for 48 h and embedded in paraffin.

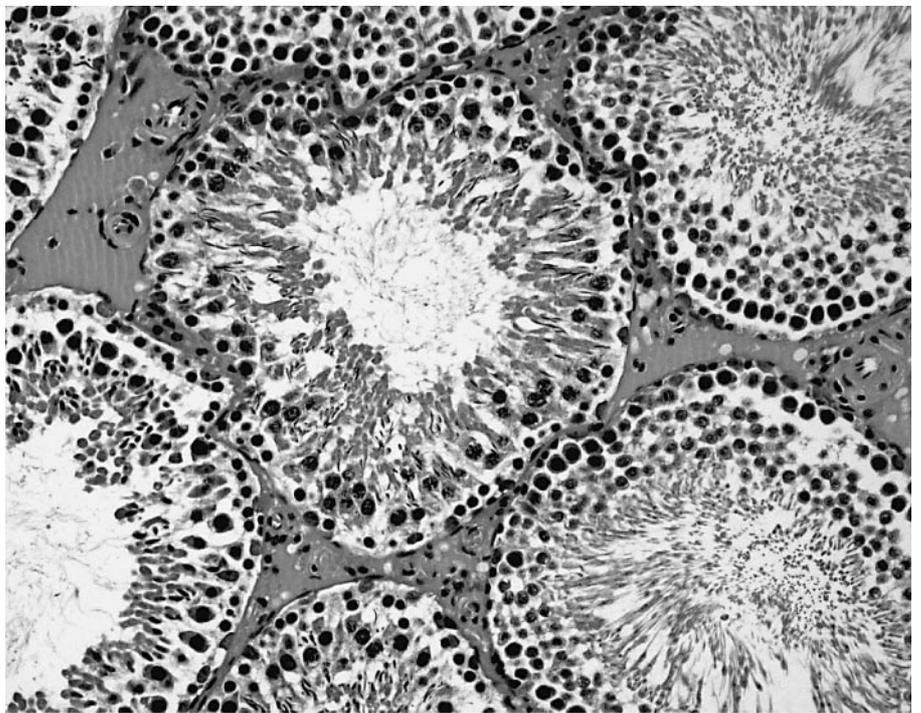
No animal died during the postoperative period and all 45 rats used in this investigation were then sacrificed.

## Results

Variables observed after 3, 6 and 9 months (tables 1–3) show a progressive increase in FSH and a decrease in testosterone blood levels, especially in group B rats. Both groups B and C showed a reduction in testicular volume



**Fig. 5.** Histological testicular features in the postadrenalectomy group.



**Fig. 6.** Normal histological appearance in right testicular biopsies of group B and C rats.

(normal mean value 1.8 ml in rats weighing 300 g). The reduction was higher in the first group (mean reduction of 0.6 ml in group B and of 0.3 ml in group C) (fig. 3, 4).

Testicular histological assay revealed morphostructural abnormalities characterized by interstitial fibrosis,

small blood vessel degeneration, seminal tubuli sclerosis and by a decrease in Leydig and, most importantly, Sertoli cells (fig. 5).

All these abnormalities were more evident in group B biopsies: 13/15 in group B vs. 4/15 in group C. No abnor-

mality was revealed by biopsies of group A rats or by right testicular biopsies of group B and C rats (fig. 6).

### Statistical Analysis

Statistical analysis was performed using one-way analysis of variance ( $p < 0.05$ ) followed by Duncan's multiple-range tests ( $p < 0.05$ ) to analyze intergroup differences. All values are expressed as mean  $\pm$  SD.  $p < 0.05$  was considered statistically significant.

## Discussion

This study shows the role played by venous reno-spermatoc reflux in the multifactorial pathogenesis of the varicocele-induced testicular damage. Data analysis suggests that testicular adrenal metabolite reflux surely enhances parenchymal gonadal damage induced by venous stasis.

This is shown in group B, where a higher decrease in testosterone level, associated with gonadal parenchyma degenerative phenomena, can be observed. These degenerative events are clinically more important in group B than in group C. The consequent increase in FSH and decrease in testosterone levels give evidence of testicular discomfort.

In the current study, we attempted to investigate the influence of retrograde adrenal metabolites flow in the development of testicular damage induced by varicocele.

It is known that optimal concentrations of intratesticular and intraepididymal testosterone are important for activating and maintaining the spermatogenesis and epididymal sperm maturation process. Considering that Leydig cell function influences Sertoli cell secretory function, which in turn affects spermatogenesis and the epididymal sperm maturation process, defects in Sertoli cell secretory function may be present in rats with varicocele further impairing spermatogenesis and the epididymal sperm maturation process.

These data allow us to say that the negative action of adrenal metabolites plays an important role in the early development of morphostructural abnormalities leading to testicular damage, and thence, subfertility in varicocele-affected patients [18–27].

## Authors' Declaration

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