

## **Does left varicocele impair right testicular function?**

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### **Abstract**

**Introduction:** Varicocele is considered the most common cause of infertility in men. While impairment of the ipsilateral testis has been assessed, impact on the contralateral right testis is unclear. The aim was to evaluate the activation of apoptosis and histological lesions of the right testis in a model of left varicocele and to assess changes after varicocelectomy.

**Methodology:** Twenty-one male Sprague-Dawley rats were used. After the creation of experimental left varicocele, rats were randomized for no other procedure or varicocelectomy, lasting 21 days. Sham animals were used as controls. Animals were then euthanized and expression of active caspase 3 through western blot and spermatogenic activity were assessed on the harvested right testis. Quantitative parameters were compared. A  $p < 0.05$  was considered significant.

**Results:** Active caspase 3 was present in right testis of sham at the basal level. A significant increase in active caspase 3 was found in right testis of left varicocele and weak enhancement of active caspase 3 was detected in right testis after left varicocelectomy. Jonhsen's score of right testis of sham was  $9.57 \pm 0.50$ , which was significantly higher than the value obtained from right testes of rats with left varicocele [ $8.74 \pm 1.00$ ,  $p < 0.0001$ ]. Jonhsen's score after varicocelectomy was  $9.19 \pm 0.77$ , significantly different from right testes of sham [ $p = 0.0029$ ] and from right testes of the varicocele group [ $p = 0.0088$ ]

**Discussion:** Our study shows activation of apoptosis and a decrement of spermatogenic activity in the right testis in a model of left varicocele. It supports the theory that left varicocele impairs testicular function bilaterally. Left varicocelectomy ameliorates apoptosis and testicular lesions but does not restore initial spermatogenic activity. It could explain why surgical treatment of clinical left varicocele does not always improve fertility.

**Keywords:** Varicocele, Varicocelectomy, Infertility, Apoptosis.

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### **Introduction**

Varicocele is considered the most common cause of infertility in men [1]. The exact pathophysiological mechanism by which it impairs fertility remains unknown [2]. Several potential mediators have been suggested, including testicular hypoxia by venous stasis and small vessel occlusion, increased testicular and scrotal temperature, retrograde blood flow of adrenal and renal metabolites in the spermatic vein, reduction of gonadotrophin and androgen secretion, oxidative stress, microvessel endothelial cell alteration, aquaporin 9 down-regulation [3-11]. Even if medical management could offer a potential solution, to date surgery remains the mainstay of

management of varicocele [12-19]. While the impairment of the ipsilateral testis has been assessed, impact on the contralateral right testis is still under investigation [15,16,19]. As information obtained from humans on this issue is limited, for ethical reasons, we aim to evaluate the activation of apoptosis and histological lesions of the right testis in an experimental model of left varicocele in rats, and to assess eventual changes after varicocelectomy.

### **Methodology**

The protocol was approved by the Committee of Animal Health and Care of the University of Messina, and all animal procedures were carried out according to Guide

for the Care and Use of Laboratory Animals. Twenty-one male Sprague-Dawley rats, aged 7 weeks and weighing 200–225 g, were used. The animals were maintained under controlled environmental conditions [12 h light/dark cycle, temperature approximately 23°C] and provided with standard laboratory food and water ad libitum. After induction of anaesthesia with an intra-peritoneal injection of sodium pentobarbital [50 mg/kg], varicocele was induced as previously described elsewhere [15,16,19]. After an abdominal midline incision, the left renal vein, inferior vena cava and left spermatic vein were identified. A clamp was passed behind the left renal vein just distal to the spermatic vein insertion. A 4-0 silk ligature was loosely placed around the left renal vein at this site and a rigid probe of 0.64 mm in diameter was placed on the left renal vein. The ligature was tied around the vein over the top of the probe. The probe was then withdrawn and the vein was allowed to expand to the limits of the ligature, which caused vein diameter to decrease to approximately half of its original diameter. The renal and spermatic veins in each animal dilated immediately. The midline incision was closed in 2 layers with 3-0 silk suture. Sham-operated rats underwent the same vertical midline incision, and a suture was also placed but it was not tied. Twenty-eight days after the creation of varicocele, rats were randomized for no other procedure or varicocelectomy. Twenty-one days after randomization, all animals were euthanized by sodium pentobarbital overdose, and the right testes were harvested for evaluation of active caspase 3 protein expression and for histological examination. For histological purpose, sections [5 µm] were cut and stained with hematoxylin and eosin.

### **Determination of Active Caspase 3**

Samples from each group were homogenized in lysis buffer. Protein samples were denatured in reducing buffer [62 mmol/l tris, pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate, 5% -mercaptoethanol, 0.003% bromophenol blue] and separated by electrophoresis on sodium dodecylsulfate [12%] polyacrylamide gel with prestained standard proteins [Bio-Rad Laboratories, Milan, Italy]. The separated proteins were transferred onto a nitrocellulose membrane using the transfer buffer at 200 mA for 1 hour. Membranes were stained with Ponceau S to confirm equal amounts of protein, and were blocked with 5% non-fat dry milk in tris buffered saline 0.1% polysorbate for 1 h at room temperature, washed 3 times for 10 min each in tris buffered saline 0.015% polysorbate and incubated with rabbit monoclonal antibody against active caspase 3 [Chemicon International, Temecula, California] in tris buffered saline 0.1% polysorbate overnight at 4°C. After washing 3 times for 10 min each in tris buffered saline 0.15% polysorbate the membranes were incubated with peroxidase conjugated goat anti-rabbit IgG [Pierce, Milan] for 1 h at room temperature, following which membranes were analyzed by the enhanced chemiluminescence system according to manufacturer's protocol.

The protein signals were quantified by scanning densitometry using a bioimage analysis system [Bio-Profil, Celbio, Milan]. Equal loading of protein was assessed on stripped blots by immunodetection of β-actin with a rabbit monoclonal antibody [Cell Signaling, Celbio]. The results from each experimental group were expressed as relative integrated intensity compared to control muscle measured with the same batch. Active caspase 3 values were expressed as arbitrary units [20-22].

### **Spermatogenic Activity**

Spermatogenesis was quantified in ten tubular cross sections for each animal, using Johnsen's score system. A score of 1–10 was given to each seminiferous tubule, depending on the maturation rate of the germ cells: 10: full spermatogenesis; 9: slightly impaired spermatogenesis, many late spermatids, and disorganized epithelium; 8: less than five spermatozoa per tubule, few late spermatids; 7: no spermatozoa, no late spermatids, and many early spermatids; 6: no spermatozoa, no late spermatids, and few early spermatids; 5: no spermatozoa or spermatids, many spermatocytes; 4: no spermatozoa or spermatids, few spermatocytes; 3: spermatogonia only; 2: no germinal cells, Sertoli cells only; 1: no seminiferous epithelium [20].

### **Statistical Analysis**

The relationships between the quantitative parameters were compared by the non-parametric Mann–Whitney *U*-test with  $p < 0.05$  considered significant. Statistical analysis was carried out with Prism®, version 7.0 for Windows®.

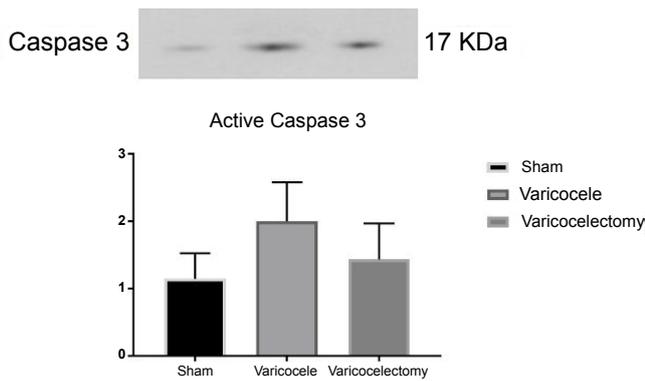
### **Results**

Determination of active caspase 3: active caspase 3 was present in the right testis of sham at the basal level. A significant increase in active caspase 3 was found in right testes of induced left varicocele. A slight, but not significant, enhancement of active caspase 3 was detected in the right testis after left varicocelectomy compared with sham.

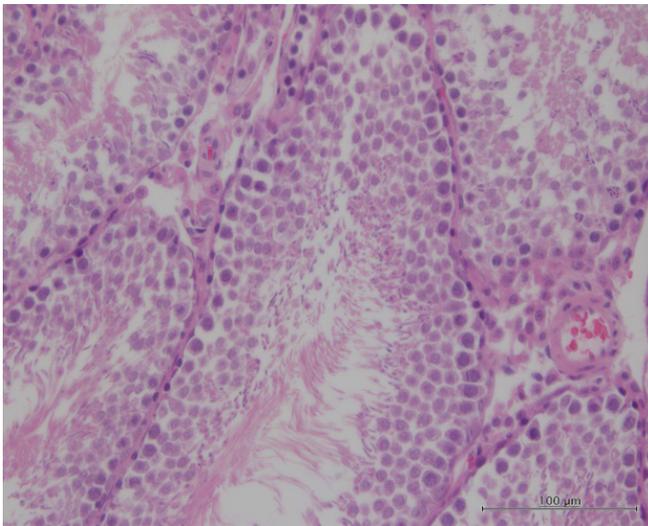
Spermatogenic activity: Johnsen's score of right testis of sham was  $9.57 \pm 0.50$ , that was significantly higher than the value obtained from right testes of rats with induced left varicocele [ $8.74 \pm 1.00$ ,  $p < 0.0001$ ]. Furthermore, Johnsen's score after varicocelectomy was  $9.19 \pm 0.77$ , which was significantly different from the right testes of sham [ $p = 0.0029$ ] and from the right testis of the varicocele-induced group [ $p = 0.0088$ ] (Figures 1-4).

### **Discussion**

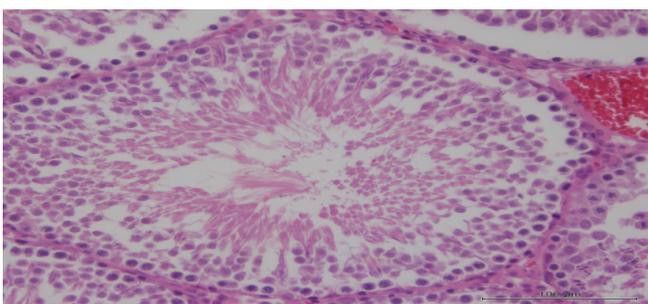
Even if varicocele is considered a predominantly left side abnormality, medical literature reports articles demonstrating inconsistent and sometimes contradictory data, which have led some authors to dissociate varicocele from male infertility. In 2012, the Cochrane Gynaecology and Fertility Group evidenced that treatment of varicocele



**Figure 1.** Western blot analysis of active caspase 3 expression (17 kDa) in specimens of sham, right testis of induced left varicocele and right testis after left varicocelectomy. Bars represent mean  $\pm$  SD (Asterisk indicates  $p=0.0251$  Sham vs. Varicocele)

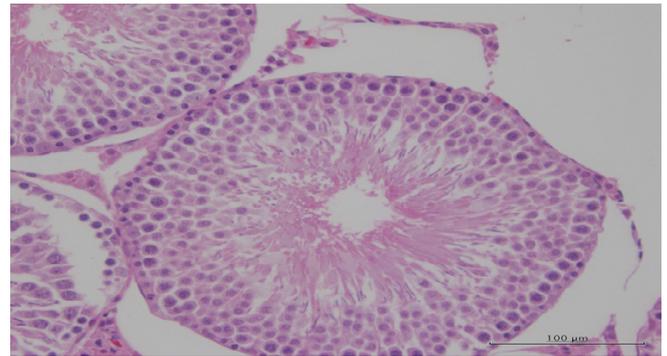


**Figure 2.** Right testis of a sham is shown. Many spermatozoa are present in a normal tubule (H & H, original magnification 400x)



**Figure 3.** Right testis of a left varicocele. No spermatozoa are noted in the central tubule. Moderate extent of intra and extratubular oedema is present, with venular ectasia (H & H, original magnification 400x)

in men from couples with otherwise unexplained subfertility may improve a couple's chance of pregnancy [23]. However, according to the same Cochrane data, 17 men would need to be treated to achieve one additional pregnancy. As varicocele may lead to a progressive deterioration over time in semen quality and testicular function, ranging from oligoteratoasthenozoospermia



**Figure 4.** Right testis of a left varicocele having undergone varicocelectomy. Few spermatozoa are present. Morphology of the tubule is normal but a moderate extratubular oedema can be seen (H & H, original magnification 400x)

to azoospermia and, on the converse, male fertility is preserved with only one healthy testis, it has been suggested that alteration of spermatogenesis in varicocele affected patients represents a bilateral testicular dysfunction [24-28]. The findings from venography combined with fluid mechanics analysis proved that in the majority of infertile men suffering from varicocele the right side is also affected [86%] but cannot be easily detected [28]. It seems to be due to retroperitoneal bypasses and collateral veins [28]. These vascular interconnections between left and right side expose the right testis to the same unclear physiopathological agents that lead to left testicular lesion in some patients affected by left varicocele. In our study, we documented significantly high levels of active caspase 3 as a marker of activation of the apoptotic machinery [21]. Moreover, activation of apoptosis was coupled with minimal but statistically significant impairment of testicular texture, implying a reduction of sperm cells. It suggests that, even if experimental varicocele was induced in the left side, there is a contralateral involvement. Furthermore, our data suggest that surgical correction improves spermatogenic activity, but does not perfectly restore initial testicular function. This result is consistent with the Cochrane Gynaecology and Fertility Group statement, and substantiates that varicocele might be considered as a bilateral abnormality even in monolateral clinical involvement. Nevertheless, right histological lesions in left varicocele affected patients were described by Gat [28]. As clinical data support that sometimes surgical treatment of left varicocele for male infertility is not effective, it has been supposed that monolateral varicocelectomy is an inadequate procedure for varicocele; indeed, it does not improve fertility in affected males, based on the evidence that varicocele can be considered a bilateral vascular disease [28]. For this reason, in the recent past, percutaneous transvenous occlusion or bilateral microsurgery of the entire impaired testicular venous drainage system has been indicated as effective, eliminating increased hydrostatic pressure [28]. In our opinion, on the basis of current controversial knowledge, bilateral treatment of a clinical monolateral varicocele is excessive, above all in paediatric age where

highly specific and sensible markers of impaired infertility have not yet been assessed. Given that a multitude of components are probably involved in varicocele related infertility, protecting the sperm cell membrane from damage and reducing apoptosis, we believe that medical management of varicocele related infertility represents an ongoing perspective.

In conclusion, our study shows activation of apoptosis and a decrement of spermatogenic activity in the right testis in an experimental model of left varicocele. It supports the theory that left varicocele bilaterally impairs testicular function. Left varicocelectomy ameliorates apoptosis and testicular lesions but does not restore initial spermatogenic activity. It could explain why surgical treatment of clinical left varicocele rarely improves fertility.

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