

Induction of spermatogenesis in azoospermic men after varicocele repair

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BACKGROUND: The purpose of this study was to assess the treatment outcome after varicocele repair in azoospermic men and to correlate this outcome with the testicular histology patterns. **METHODS:** Medical records of 15 azoospermic men who underwent testis biopsy and microsurgical repair of clinical varicocele between July 1999 and November 2000 were reviewed. All patients had at least two semen analyses showing azoospermia taken before the surgery and two semen analyses post-operatively. Hypospermatogenesis was identified in four, maturation arrest in six, and germ cell aplasia in five men. **RESULTS:** Induction of spermatogenesis was achieved in seven men (47%). Of these, four had germ cell aplasia and three had maturation arrest. The improvement in sperm concentration and motility in patients with germ cell aplasia ranged from 1.8 to $7.9 \times 10^6/\text{ml}$, and from 32 to 76% respectively. Of these seven patients with improvement in semen quality, five relapsed into azoospermia 6 months after the recovery of spermatogenesis (four germ cell aplasia and one maturation arrest). One patient with maturation arrest established a pregnancy. **CONCLUSIONS:** Azoospermic patients may have an improvement in semen quality following varicolectomy. Semen samples should be cryopreserved after an initial improvement following varicolectomy, as relapse to a state of azoospermia may occur.

Key words: azoospermia/cryopreservation/semen/sperm/varicocele

Introduction

Clinical varicocele is observed in 10–20% of the general male population, in 35–40% of men with primary infertility and in up to 80% of men with secondary infertility (Witt and Lipshultz, 1993; Schlesinger *et al.*, 1994; Kamal *et al.*, 2001). With recent advances in diagnostic techniques and widespread application of scrotal ultrasonography and colour Doppler imaging, varicoceles are being reported in up to 91% of subfertile cases, most of whom were previously regarded as having idiopathic aetiology (Gonzales *et al.*, 1983; Resim *et al.*, 1999).

A number of theories have been proposed to explain the observed pathophysiology of varicoceles. Semen quality uniformly declines in animals with induced varicoceles, even when only a left varicocele is produced (Sofikitis and Miyagawa, 1994). The inhibition of testosterone synthesis in rats with surgically induced varicoceles was shown to be due principally to a reduction in the activity of the enzyme 17,20 desmolase (Rajfer *et al.*, 1987).

Zorgniotti and MacLeod reported that men with varicoceles have higher intratesticular temperatures than the controls (Zorgniotti and MacLeod, 1973). The reduction in scrotal temperature following varicocele ligation supports a causative

role of increased temperature on the infertility produced by the varicocele (Sofikitis *et al.*, 1992). Comhaire and Vermeulen measured spermatid catecholamine (adrenal metabolite) concentrations in infertile patients with and without varicoceles and found that although internal spermatid levels of catecholamines were higher than peripheral levels in both groups, the ratio of spermatid vein to peripheral vein catecholamine was higher in men with varicoceles than in control subjects (Comhaire and Vermeulen, 1974). Increased hydrostatic pressure in the internal spermatid vein from renal vein reflux has been proposed as a possible mechanism for varicocele-induced pathology (Shafik and Beider, 1980). Because of the 'stagnation' of blood, it has been hypothesized that varicoceles cause hypoxia, which may play a role in altering spermatogenesis in the varicocele patient. Recently, studies evaluating the role of oxidative stress in male infertility showed that infertile men with varicocele have elevated levels of sperm-derived reactive oxygen species (Hendin *et al.*, 1999; Pasqualotto *et al.*, 2000).

Although the pathogenesis of the varicocele remains enigmatic, gross testicular alterations associated with varicocele are well documented. The effects of the varicocele vary but may often result in a generalized impairment of sperm production,

characterized by abnormal semen quality, ranging from oligozoospermia to complete azoospermia. Furthermore, the varicocele influences not only the physiology and the reproductive potential of the sperm, but also the fertilizing capacity of the haploid male gamete (Sofikitis *et al.*, 1996). The observation of azoospermia or severe oligoasthenoospermia in association with varicocele is common and is reported to range from 4.3 to 13.3% (Czaplicki *et al.*, 1979; Matthews *et al.*, 1998). In a prospective, controlled study, varicocele repair was demonstrated to improve semen quality and fertility potential in men with oligozoospermia. A few reports have independently found that varicocele repair in men with azoospermia and severe oligoasthenoospermia resulted in induction or enhancement of spermatogenesis in 40–60% of the patients, thus demonstrating the benefit of performing a varicocele repair in men with azoospermia (Matthews *et al.*, 1998; Esteves and Glina, 1999; Kim *et al.*, 1999; Kadioglu *et al.*, 2001).

There is clinical evidence to suggest that spermatogenesis in damaged or failing testes may vary within a single testis, resulting in focal areas or 'patches' of sperm production within an organ largely devoid of germinative cells (Turek *et al.*, 1997). It is assumed that a testis of a man with non-obstructive azoospermia might show a homogeneously or a non-homogeneously distributed spermatogenesis. In the former case, a large piece of testicular tissue will represent the whole testicular parenchyma, whereas in the later case a large piece of testicular tissue might be negative for focal advanced spermatogenesis.

The purpose of this study was to evaluate the improvement in semen quality and pregnancy outcome after varicocelectomy in men with azoospermia. We also sought to correlate the testicular histology patterns of a group of azoospermic men with varicocele with the treatment outcome after varicocele repair.

Materials and methods

This study was approved by our institution review board and the patients involved granted their informed consent. In this prospective study, 15 azoospermic men with varicocele underwent a microsurgical varicocele repair between July 1999 and November 2000. All of them had primary infertility. The mean age of the wife at presentation was 31 ± 4.5 years (range 25–39). All women were normal based on history, hormonal levels and hysterosalpingogram. The minimum duration of infertility required was defined as a failure to establish a pregnancy within 1 year with unprotected intercourse. A basic infertility evaluation including a detailed history and a complete physical examination was undertaken. Only patients with varicocele identified on physical examination were included. Patients who were taking antioxidants including vitamins C and E were excluded from the study.

Testicular size was evaluated in all patients with a caliper or by scrotal ultrasound. Testicular atrophy was bilateral in five patients (33.3%) and unilateral in three (20%). The mean (\pm SD) right testis was 22.4 ± 4.11 cm³ and left testis was 20.33 ± 4.40 cm³. Mean pre-operative hormone levels were FSH 18 ± 12 mIU/ml (range 9–38), LH 13 ± 8 mIU/ml (range 6–43), and testosterone 432 ± 135 ng/dl (range 268–567).

All patients had at least two semen analyses confirming azoospermia obtained by masturbation after 2–5 days of abstinence before the surgery. Sperm concentration and motility were evaluated according to published criteria (World Health Organization, 1999) and sperm morphology according to Kruger's strict criteria. Patients with pyospermia were treated before varicocele repair. Repair was bilateral in 12 and unilateral in three patients using a subinguinal approach and a microsurgical technique. Using the microsurgical approach, we ligated the pampiniform plexus, leaving intact the cremasterium plexus as well as the gubernaculum veins.

Each patient underwent open diagnostic testis biopsy at the same time as the varicocele repair under general anaesthesia. Biopsies were performed on both testes, irrespective of which appeared healthier whether by size or consistency. All biopsies were analysed by an experienced pathologist (L.B.S.).

Two post-operative semen analyses were performed on each patient at 6 and 12 months.

The biopsy results, post-operative semen analysis and the correlation between the induction of spermatogenesis and the testis biopsy were studied. We also evaluated the pregnancy outcome following the varicocele repair.

Results

Germ cell aplasia was identified in five (Figures 1 and 2), hypospermatogenesis in four, and early maturation arrest (arrest at the primary spermatocyte stage) in six of the men (Table I). Induction of spermatogenesis was achieved in seven of them (46.6%). Of these seven men, four had germ cell aplasia and three had maturation arrest. No improvement was seen in patients with hypospermatogenesis. The improvement was seen in six patients with bilateral and in one with unilateral varicocele. Following the surgical procedure, the mean (\pm SD) sperm concentration in the patients with germ cell aplasia was $2.1 \pm 3.4 \times 10^6$ /ml and with maturation arrest was $3.2 \pm 2.9 \times 10^6$ /ml. Also, the mean \pm SD sperm motility and morphology in the patients with germ cell aplasia was 15.1 ± 17.0 and $2 \pm 2.44\%$, and with maturation arrest was 37.6 ± 27.2 and $3.4 \pm 2.7\%$ respectively. The improvement in sperm concentration and sperm motility achieved in patients with germ cell aplasia ranged from 1.8 to 7.9×10^6 /ml and from 32

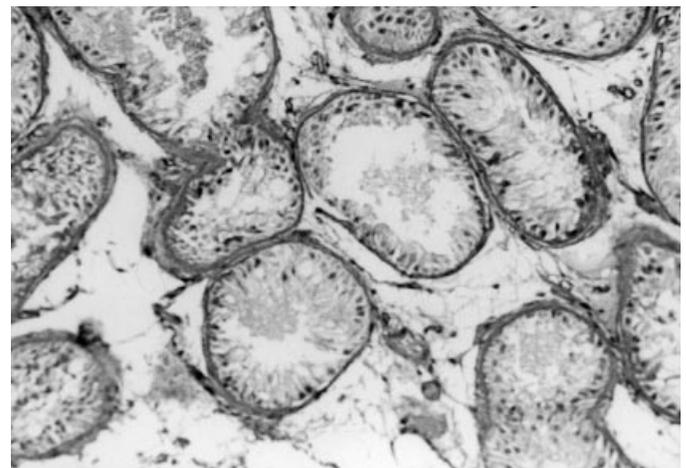
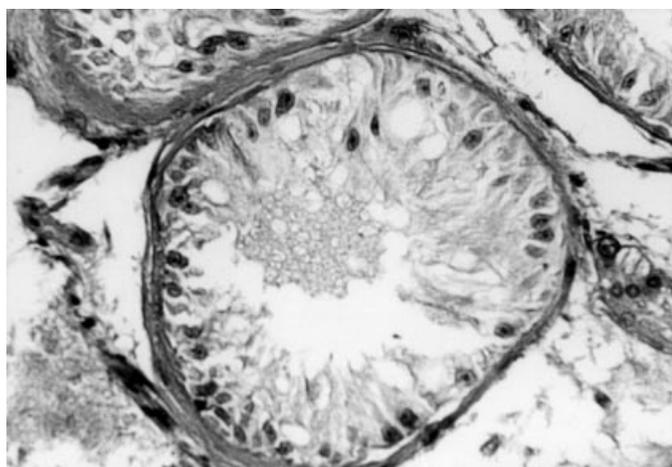
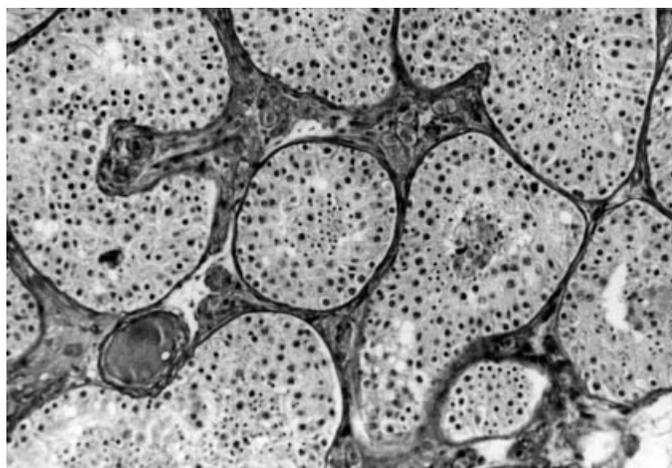


Figure 1. Patient 13. Histology showing germ cell aplasia. Original magnification $\times 400$.

Table I. Semen analysis after varicocele repair and the histology pattern of the testis

| Patient | Sperm concentration ($\times 10^6/\text{ml}$) | % motile | Normal morphology, Kruger (%) | Testis biopsy | Pregnancy | Azoospermia |
|---------|---|----------|-------------------------------|---------------------|-----------|-------------|
| 1 | 0 | 0 | 0 | Hypospermatogenesis | No | Yes |
| 2 | 0 | 0 | 0 | Hypospermatogenesis | No | Yes |
| 3 | 0 | 0 | 0 | Hypospermatogenesis | No | Yes |
| 4 | 0 | 0 | 0 | Hypospermatogenesis | No | Yes |
| 5 | 0 | 0 | 0 | Maturation arrest | No | Yes |
| 6 | 0 | 0 | 0 | Maturation arrest | No | Yes |
| 7 | 0 | 0 | 0 | Maturation arrest | No | Yes |
| 8 | 8.9 | 24 | 6 | Maturation arrest | Yes | No |
| 9 | 1.2 | 37 | 3 | Maturation arrest | No | Yes |
| 10 | 2.6 | 29.5 | 3 | Maturation arrest | No | No |
| 11 | 0 | 0 | 0 | Germ cell aplasia | No | Yes |
| 12 | 1.8 | 45.5 | 4 | Germ cell aplasia | No | Yes |
| 13 | 7.9 | 75.7 | 5 | Germ cell aplasia | No | Yes |
| 14 | 2.5 | 35 | 3 | Germ cell aplasia | No | Yes |
| 15 | 3.8 | 32 | 5 | Germ cell aplasia | No | Yes |

**Figure 2.** Patient 13. Histology showing germ cell aplasia with basal membrane thickening. Original magnification $\times 400$.**Figure 3.** Patient 8. Histology showing maturation arrest with Leydig cell hyperplasia. This patient achieved pregnancy. Original magnification $\times 400$.

to 75.7% respectively. In patients with maturation arrest, the improvement in sperm concentration ranged from 1.2 to $8.9 \times 10^6/\text{ml}$ and sperm motility ranged from 24 to 37%

respectively. According to Kruger's strict criteria sperm morphology, the improvement ranged from 3 to 6%. In Table I, we show the highest sperm density, motility and morphology between the two semen analyses obtained after the surgical procedure.

Of the seven patients with improved semen quality, five relapsed into azoospermia 6 months after the recovery of spermatogenesis (four germ cell aplasia and one maturation arrest). One patient with maturation arrest (Figure 3) established a pregnancy. The other couple with recovery of spermatogenesis did not establish a pregnancy.

Two of the five patients (40%) with bilateral testicular atrophy and high FSH levels had sperm in the semen. Also, one of the three patients (33.3%) with unilateral atrophy with high FSH levels, and four of the seven patients with normal testicular size and FSH levels, had sperm in the semen.

Discussion

Only a few studies have shown that non-obstructive azoospermic patients with varicocele identified on physical examination may benefit from varicocele repair (Tulloch, 1952; Kim *et al.*, 1999; Matthews *et al.*, 1998; Esteves and Glina, 1999; Kadioglu *et al.*, 2001). Interestingly, the first study on the importance of varicolectomy to male infertility (Tulloch, 1952) reported spontaneous pregnancy after varicocele repair in an azoospermic man. Since that time, varicolectomy has become the most commonly performed surgery in male infertility. The few studies published on completely azoospermic men have consistently shown an improvement of semen parameters in up to 50% of the cases and of spontaneous pregnancies after microsurgical inguinal varicolectomy (Tulloch, 1952; Matthews *et al.*, 1998; Kim *et al.*, 1999; Esteves and Glina, 1999; Kadioglu *et al.*, 2001). Recently, one study of 28 patients (Kim *et al.*, 1999) and another of 10 patients (Esteves and Glina, 1999) demonstrated that testicular histopathology was the most important predictive factor of outcome. They concluded that patients with germ cell aplasia and maturation arrest at the spermatocyte stage did not improve semen quality. However, 50% of the completely azoospermic men with maturation arrest at the spermatid stage and 55.6% of

completely azoospermic men with hypospermatogenesis achieved post-operative improvements in semen quality. It might be argued that azoospermic men with post-operative improvements were also those most likely to be successful with testicular sperm extraction procedures for an ICSI cycle. In our study, induction of spermatogenesis was achieved in seven men (46.6%). Of these seven men, four had a germ cell aplasia and three had maturation arrest. Other known and unknown causes of male infertility, such as Y-microdeletions, must be considered in cases with persistent azoospermia following varicocele repair (Cayan *et al.*, 2001).

The fact that 12 out of the 15 patients with left varicocele had right varicocele is of clinical importance. One very elegant study using animal models (Sofikitis *et al.*, 1992) observed that the surgical repair of the secondary right varicocele improved all semen parameters indicating the harmful consequences of the primary induced left varicocele on the right testis. Therefore, it appears that the primary left varicocele leads to a development of a secondary right varicocele due to activation of a tension reception within the wall of the left testicular vein.

It is important to notice here that sperm morphology according to the Kruger strict criteria varied from 3 to 6%, demonstrating that varicocele repair may cause an improvement in sperm function. However, despite post-operative improvement in semen parameters in our small series, assisted reproductive techniques may still be required to enable the majority of couples to initiate a pregnancy (Palermo *et al.*, 1992; Aboulghar *et al.*, 1997). However, the clinical importance of varicocele repair is that a substantial number of completely azoospermic men destined to undergo invasive testicular sperm retrieval procedures involving repeated open or needle biopsies in combination with ICSI now have the potential to provide sperm by ejaculation (Turek *et al.*, 1997; Silber, 2000). When a choice is possible, using motile sperm from a fresh ejaculate is preferable to using testicular sperm extraction (TESE) in preparation for ICSI and IVF (Aboulghar *et al.*, 1997). Freshly ejaculated live sperm are associated with ICSI success rates superior to those achieved with sperm retrieved via TESE and, additionally, an invasive and potentially damaging procedure is avoided. The use of fresh, motile, ejaculated sperm is technically much easier and provides better results than sperm harvested by testicular sperm extraction procedures. However, couples need to be counselled that they will have to wait for at least 12 months following varicocelectomy before proceeding to any type of assisted reproduction treatment. Longer follow-up after varicocele repair will determine whether or not these azoospermic patients improve their sperm concentration, motility and morphology.

Of the utmost importance, our study demonstrated that five out of seven patients (71.4%) who had an improvement in the quality of their semen 6 months after a varicocele repair returned to their previous azoospermic state 12 months after surgery. We believe that varicocele repair in those patients who relapsed to azoospermia might have had a temporary effect resulting in the induction of spermatogenesis which, over a small period of time, returned to their original status of azoospermia. Therefore, whatever mechanisms varicocelect-

omy induced spermatogenesis, they were not able to sustain it for a long time.

These alarming data suggest that a semen cryopreservation should be performed once the patient has sperm in the semen (Pasqualotto and Agarwal, 2001).

A recent study showed that in infertile couples undergoing intrauterine insemination in whom the female partner was normal and the male had a varicocele, pregnancy and live birth rates were significantly higher if the man underwent varicocele treatment (Daitch *et al.*, 2001). The authors conclude that men should be screened for varicocele before intrauterine insemination is initiated for male factor infertility. Therefore, a functional factor not measured in routine semen analysis may affect pregnancy rates in this setting.

One may argue that testicular sperm retrieved from azoospermic patients with varicocele might have intrinsic defects, thus reinforcing the idea that these azoospermic patients should undergo a varicocelectomy before initiating any type of assisted reproduction procedure (Cayan *et al.*, 2001). Again, the importance of these findings is that a significant number of azoospermic men destined to undergo invasive testicular sperm retrieval procedures involving repeated open or needle biopsies in combination with ICSI now have the potential of providing sperm via ejaculation or even of establishing a pregnancy without technical assistance.

In addition, the presence of Y-microdeletion, observed in 15% of completely azoospermic men, may render assisted reproduction treatment necessary (Mak and Jarvi, 1996; Pryor *et al.*, 1997). Thus, azoospermic men with palpable varicocele should themselves make the decision as to whether to undergo varicocele repair or testicular sperm extraction and IVF, after genetic counselling (Cayan *et al.*, 2001).

It is well known that the age of the female partner is a critical factor that needs to be taken into account prior to varicocele repair. When the female partner is older than 37 years, varicocelectomy may not be performed on the male partner because even if the semen quality improves after some months, the female fertility potential will be decreased. However, in our study, one female patient was 39 years old and could not afford an ICSI procedure. Since varicocele repair is covered by the government health insurance, the patient chose for her partner to undergo a varicocele repair.

We strongly recommend that azoospermic men with a palpable varicocele should undergo varicocele repair. Once the patient has sperm detected in the semen analysis after induction of spermatogenesis following a varicocele repair, he should be warned of the possibility of a relapse into azoospermia and offered the possibility of sperm cryopreservation.

We suggest that a varicocele repair must be considered for all men with azoospermia who have a palpable varicocele. A single testis biopsy showing the germ cell aplasia may not reflect the overall testis histology but just a focal area. Therefore, azoospermic patients with germ cell aplasia in a single large testis biopsy may have an improvement in semen quality following varicocelectomy. Due to the considerable risk of their relapsing into azoospermia after an initial improvement in semen quality following varicocelectomy,

patients should be informed of the benefits of sperm cryopreservation.

References

- Aboulghar, M.A., Mansour, R.T., Serour, G.I., Fahmy, I., Kamal, A., Tawab, N.A. and Amin, Y.M. (1997) Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm. *Fertil. Steril.*, **68**, 108–111.
- Cayan, S., Lee, D., Black, L.D., Reijo Pera, R.A. and Turek, P.J. (2001) Response to varicocelectomy in oligospermic men with and without defined genetic infertility. *Urology*, **57**, 530–535.
- Comhaire, F. and Vermeulen, A. (1974) Varicocele sterility: cortisol and catecholamines. *Fertil. Steril.*, **25**, 88–95.
- Czaplicki, M., Bablock, L. and Janczewski, Z. (1979) Varicocelectomy in patients with azoospermia. *Arch. Androl.*, **3**, 51–55.
- Daitch, J.A., Bedaiwy, M.A., Pasqualotto, E.B., Hendin, B.N., Hallak, J., Falcone, T., Thomas, A.J. Jr, Nelson, D.R. and Agarwal, A. (2001) Varicocelectomy improves intrauterine insemination success rates in men with varicocele. *J. Urol.*, **165**, 1510–1513.
- Esteves, S.C. and Glina, S. (1999) Recovery of spermatogenesis after microsurgical varicocele repair in azoospermic men is related to testicular histology. *J. Urol.*, **161** (Suppl.), **1199**, 311.
- Gonzales, R., Reddy, P., Kaye, K.W. and Narayan, P. (1983) Comparison of Doppler examination retrograde spermatic venography in the diagnosis of varicocele. *Fertil. Steril.*, **40**, 96–99.
- Hendin, B.N., Kolettis, P.N., Sharma, R.K., Thomas, A.J. Jr and Agarwal, A. (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J. Urol.*, **161**, 1831–1834.
- Kadioglu, A., Tefkeli, A., Cayan, S., Kendirali, E., Erdemir, F. and Tellaloglu, S. (2001) Microsurgical inguinal varicocele repair in azoospermic men. *Urology*, **57**, 328–333.
- Kamal, K.M., Javeri, K. and Zini, A. (2001) Microsurgical varicocelectomy in the era of assisted reproductive technology: influence of initial semen quality on pregnancy rates. *Fertil. Steril.*, **75**, 1013–1016.
- Kim, E.D., Leibman, B.B., Grinblat, D.M. and Lipshultz, L.I. (1999) Varicocele repair improves semen parameters in azoospermic men with spermatogenic failure. *J. Urol.*, **162**, 737–740.
- Mak, V. and Jarvi, K.A. (1996) The genetics of male infertility. *J. Urol.*, **156**, 1245–1257.
- Matthews, G.J., Matthews, E.D. and Goldstein, M. (1998) Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia. *Fertil. Steril.*, **70**, 71–75.
- Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A.C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.
- Pasqualotto, F.F. and Agarwal, A. (2001) Should we offer semen cryopreservation to men with testicular cancer? *Clev. Clin. J. Med.*, **68**, 101–102.
- Pasqualotto, F.F., Sharma, R.K., Nelson, D.R., Thomas, A.J. Jr and Agarwal, A. (2000) Relationship between oxidative stress, semen characteristics and clinical diagnosis in men undergoing infertility investigation. *Fertil. Steril.*, **73**, 459–464.
- Pryor, J.L., Kent-First, M. and Muallen, A. (1997) Microdeletions in the Y chromosome of infertile men. *New Engl. J. Med.*, **336**, 534–539.
- Rajfer, J., Turner, T.T., Rivera, F., Howards, S.S. and Sikka, S.C. (1987) Inhibition of testicular testosterone biosynthesis following experimental varicocele in rats. *Biol. Reprod.*, **36**, 933–937.
- Resim, S., Cek, M., Fazlioglu, A., Caskurlu, T., Gurbuz, G. and Sevin, G. (1999) Echocoulour doppler ultrasonography in the diagnosis of varicocele. *Int. Urol. Nephrol.*, **31**, 371–382.
- Schlesinger, M.H., Wilets, I.F. and Nagler, H.M. (1994) Treatment outcome after varicocelectomy. *Urol. Clin. North. Am.*, **21**, 517–529.
- Shafik, A. and Beider, G.A. (1980) Venous tension patterns in cord veins. In normal and varicocele individuals. *J. Urol.*, **123**, 383–385.
- Silber, S.J. (2000) Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. *Hum. Reprod.*, **15**, 2278–2284.
- Sofikitis, N. and Miyagawa, I. (1994) Bilateral effect of varicocele on testicular metabolism in the rat. *Int. J. Fertil.*, **39**, 239–247.
- Sofikitis, N.V., Takahashi, C., Nakamura, I., Hirakawa, S. and Miyagawa, I. (1992) Surgical repair of secondary right varicocele in rats with primary left varicocele: effects on fertility, testicular temperature, spermatogenesis, and sperm maturation. *Arch. Androl.*, **28**, 43–52.
- Sofikitis, N.V., Miyagawa, I., Incze, P. and Andrighetti, S. (1996) Detrimental effect of left varicocele on the reproductive capacity of the early haploid male gamete. *J. Urol.*, **156**, 267–270.
- Tulloch, W.S. (1952) Consideration of sterility; subfertility in the male. *Edinb. Med. J.*, **59**, 29–34.
- Turek, P.J., Cha, I. and Ljung, B-M. (1997) Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ 'mapping' for mature sperm in azoospermic men. *Urology*, **49**, 743–748.
- Witt, M.A. and Lipshultz, L.I. (1993) Varicocele: a progressive or static lesion? *Urology*, **42**, 541–543.
- World Health Organization (1999) *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, Cambridge University Press, Cambridge, England.
- Zorgniotti, A.W. and MacLeod, J. (1973) Studies in temperature, human semen quality, and varicocele. *Fertil. Steril.*, **24**, 854–863.

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