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REVIEW ARTICLE



## Should the current guidelines for the treatment of varicoceles in infertile men be re-evaluated?

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### ABSTRACT

Male infertility is a major health burden worldwide. In the United Kingdom, the diagnostic and treatment pathway for male factor fertility is fragmented with wide variance in management and funding protocols. There is now a focus on potential overtreatment of couples with IVF and failure to treat male factors before considering assisted reproductive technology (ART). Despite this, contemporary Urological guidelines are not definitive in the indications for varicocele treatment, whilst the current National Institute for Health and Care Excellence (NICE) guidelines do not advocate surgical intervention. While controversy exists concerning the effects of varicocele treatment on natural pregnancy rates, there is growing evidence that varicocele treatment can have additional positive effects on fertility by reducing their impact on sperm DNA fragmentation and improving ART outcomes. Studies have demonstrated that azoospermic men may become oligospermic following varicocele intervention, obviating the need for surgical sperm retrieval. Sperm retrieval rates also increase following varicocele treatment in men with non-obstructive azoospermia. The contemporary literature demonstrates a clear clinical benefit for treating varicoceles in infertile men, which may be more cost-effective than proceeding to immediate ART. This review comprehensively evaluates the current indications for varicocele treatment, and it is proposed that these should be redefined in contemporary guidelines to reflect current advances in male fertility research.

### ARTICLE HISTORY

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### KEYWORDS

Varicocele; assisted conception; pregnancy; live birth rates

### Introduction

Infertility affects up to 15% of couples worldwide and is a major health care burden. Male infertility remains an evolving specialty with a number of subspecialists including Urologists and Gynaecologists providing care (Vivas-Acevedo, Lozano-Hernandez, & Camejo, 2014). For clinicians to provide good quality care, there must be uniformity in clinical standards and an integrated approach towards the infertile couple.

Recent studies have suggested that there has been a rise in the treatment of infertile couples with ART. For example, in the United Kingdom, there was a 3-fold increase in the use of *in vitro* fertilization (IVF) for unexplained infertility from 6204 to 19,552 cycles between 2000 and 2001 (Kamphuis, Bhattacharya, van der Veen, Mol, & Templeton, 2014). Bahadur et al. (2016) published a prediction model outlining the costs of intrauterine insemination (IUI) and IVF as approximately £800 and £8000 per cycle, respectively, implicating a potential burden on public health funding.

There are a number of recognized causes of male infertility including urogenital abnormalities, malignancy, urogenital tract infections, genetic abnormalities, endocrinopathies and varicoceles (Jungwirth et al., 2016). Although varicoceles are frequently encountered in Urological practice, the effects of varicoceles on male infertility remain a subject of much debate (Tanrikut et al., 2011). While the controversy of whether varicocele treatments improve natural pregnancy rates has been widely debated, it is now clear that they can have more far reaching effects on fertility potential in the male including effects on testosterone and sperm DNA fragmentation, with the potential to reverse azoospermia with varicolectomy.

In this article, we aim to review the current indications for varicocele treatment, pathological effects of varicoceles on seminal reactive oxygen species and sperm DNA fragmentation, the effects on natural and assisted conception and to evaluate the cost-effectiveness of varicocele treatment in comparison to

ART. We also review the potential effects of varicocelectomy in men with non-obstructive azoospermia (NOA).

### Demographics and pathophysiology

A varicocele is defined as an abnormal dilatation, tortuosity and elongation of the pampiniform plexus within the spermatic cord (Vivas-Acevedo et al., 2014). Although varicoceles are present in 15% of men, this figure increases to 40% in men with primary infertility, and up to 80% in men with secondary infertility (Alsaikhan, Alrabeeah, Delouya, & Zini, 2016; Vivas-Acevedo et al., 2014). Varicoceles are more commonly found on the left side but can occasionally occur bilaterally or even isolated on the right side (Alsaikhan et al., 2016).

Varicoceles are diagnosed during physical examination with the patient in the standing and supine positions. This allows for detection of clinically significant varicoceles, which are classified according to the Dubin and Amelar varicocele grading system outlined in Table 1 (Belay, Huang, Shen, & Ko, 2016). Clinically significant varicoceles are defined as grade I, II or III. Varicoceles are palpable or visible on standing with or without the Valsalva manoeuvre. Subclinical varicoceles are identified radiologically, although their role in the aetiopathogenesis of male factor infertility is controversial and they are not currently considered clinically significant according to the current European Association of Urology (EAU) guidelines and other authors (Belay et al., 2016; Jungwirth et al., 2016). The dynamic sonographic findings of varicoceles are commonly classified by the Sartechi and Dubin classification (Belay et al., 2016).

### Contemporary indications for varicocele treatment in the infertile male

The current EAU guidelines on male fertility recommend that varicocele treatment should only be considered in men with a clinical varicocele, oligozoospermia and subfertility, or in adolescent patients with a clinically significant varicocele and progressive failure of testicular development (Jungwirth et al., 2016). The American Urological Association (AUA) and American Society for Reproductive Medicine (ASRM) best practice guidelines advocate that treatment of varicoceles in patients attempting to conceive should be considered when all four criteria in Table 2 are met (Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine, 2004; Practice Committee of the American Society for Reproductive Medicine and Society for Male Reproduction and Urology, 2014). For adult patients with a clinically palpable varicocele and abnormal semen parameters but who are not actively attempting conception, treatment can be considered if there is a desire for further fertility (Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine, 2004).

In 2013, the National Institute for Health and Clinical Excellence (NICE) published guidelines on the management of infertility, which included medical and surgical management options. Surgical management was only recommended in cases of obstructive azoospermia and for sperm retrieval for ART, of which IVF and ICSI (intracytoplasmic sperm injection) are the preferred approaches (National Collaborating Centre for

**Table 1.** The classification of varicoceles according to the Dubin and Amelar varicocele grading system and corresponding sonographic findings (Belay et al., 2016).

Grade	Clinical examination	Sonographic findings
Subclinical	No palpable varicocele	Moderate, transient venous reflux during Valsalva manoeuvre (physiological findings)
I	Palpable only with the patient standing and performing a concurrent Valsalva Manoeuvre	Persistent venous reflux that ends before the Valsalva manoeuvre
II	Palpable with the patient standing without a Valsalva Manoeuvre	Persistent venous reflux throughout the entire Valsalva manoeuvre
III	Visible through the scrotal skin and palpable with the patient standing	Venous reflux that is present under basal conditions and does not change during the Valsalva manoeuvre

**Table 2.** AUA criteria for consideration of treatment of varicocele in the male partner of a couple attempting to conceive (Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine, 2004).

The varicocele is palpable on physical examination of the scrotum
The couple has known infertility
The female partner has normal fertility or a potentially treatable cause of infertility
The male partner has abnormal semen parameters or abnormal results from sperm function tests

Women's & Children's Health, 2013). Current NICE guidelines state that men should not be offered surgery for varicoceles as part of infertility treatment because 'it does not improve pregnancy rates' (National Collaborating Centre for Women's & Children's Health, 2013).

### Interventional techniques for the treatment of varicoceles

A full review of the techniques used in the treatment of varicoceles is beyond the scope of this article. However, established treatment options include the sub-inguinal microsurgical or inguinal approach, percutaneous radiological embolization (including sclerotherapy) or laparoscopic surgery (Baigorri & Dixon, 2016). Post-operative hydrocele and recurrent or persistent varicoceles are the most common complications, with reportedly lower rates of post-operative hydrocele formation with the microsurgical approach (0.4%), compared to the laparoscopic (2.8%), and macroscopic inguinal (7.3%) techniques. Similarly, recurrence rates appear to be lower with the microsurgical subinguinal approach (1.1%) compared to the other techniques. Radiological embolization is an alternative treatment option with reported technical success rates of 90% to 97% and recurrence rates of 2% to 24% (Lomboy & Coward, 2016). There is, however, limited outcome data comparing the success of treatment interventions in the medical literature (Baigorri & Dixon, 2016).

### The impact of varicoceles on natural conception and conception through ART

Studies have suggested that treatment of varicoceles in infertile men can increase natural pregnancy rates (PR) (Ficarra, Crestani, Novara, & Mirone, 2012; Tiseo, Esteves, & Cocuzza, 2016). However, Evers and Collins (2004) published a largely flawed meta-analysis, which concluded that the treatment of varicoceles (surgical or radiological), in couples with unexplained infertility, could not be recommended. This Cochrane review included eight randomized controlled trials (RCT) with 607 patients in total. In the updated publication (Kroese, de Lange, Collins, & Evers, 2012), the authors themselves commented on the heterogeneity of the studies included in the 2004 meta-analysis, with studies including men with normal semen analysis and three studies recruiting infertile men with subclinical varicoceles diagnosed only on thermography or Doppler ultrasound. The odds ratio (OR) comparing

varicocele treatment versus no treatment for pregnancy rate was 1.47 (95% CI 1.05 to 2.05,  $p = 0.03$ ). In a subgroup analysis of five studies that only treated infertile men with abnormal semen parameters and a clinical varicocele, the OR for pregnancy rate remained significant, at 2.39 (95% CI 1.56 to 3.66,  $p = 0.03$ ). None of the 10 studies reported on live birth rates (LBR).

Given the limitations and the heterogeneity of the studies included in the Evers and Collins (2004) meta-analysis, the Italian Society of Andrology (SIA) conducted a critical subanalysis of the data (Ficarra et al., 2006), reviewing three of the eight studies included in the Cochrane review. All three studies included patients meeting the indications for the treatment of varicoceles as outlined by the AUA guidelines. They all demonstrated that treatment of varicoceles in infertile men with abnormal semen analysis resulted in an increase in natural PR: 36.4% in the treated group compared to 20% in the control group ( $p = 0.009$ ) (Ficarra et al., 2006). In a further study, 72 couples with abnormal semen analysis and a varicocele, confirmed by ultrasonography were randomized to either treatment or control groups (Dohle, Pierik & Weber, 2003). Grade of varicocele was not documented and men with azoospermia were excluded. Within the treatment group, the natural PR was 36% within 1 year, compared to a PR of 9% in the control group.

A prospective RCT published by Abdel-Meguid, Al-Sayyad, Tayib, and Farsi (2011) concluded that varicocelectomy in infertile men significantly increased the chance of natural pregnancy within 1 year of treatment. The authors of this RCT recruited 145 infertile men with clinically significant varicoceles and at least one impaired semen parameter. Spontaneous pregnancy within 1 year was 32.9% in the treatment arm compared to 13.9% in the control arm, with an OR of 3.04 (95% CI 1.33 to 6.95,  $p = 0.01$ ). In addition to demonstrating the superiority of varicocelectomy over no treatment in infertile men, the authors also found that the semen parameters of men in the treatment arm significantly improved at 1 year compared to the control group (Abdel-Meguid et al., 2011).

Natural pregnancy aside, a further meta-analysis by Kirby, Wiener, Rajanahally, Crowell and Coward (2016) demonstrated a statistically significant increase in LBR in patients undergoing ART following varicocele repair compared to untreated varicoceles. A total of five studies within the analysis reported on LBR following IVF and found a significant increase in LBR in couples where the male partner had undergone varicocele repair compared to couples where the varicocele

remained untreated (Kirby et al., 2016). This study found that treatment of clinical varicoceles in azoospermic or oligospermic males resulted in increased PR with ART. Overall, this meta-analysis also showed improved sperm retrieval rates (SRR) in males with persistent azoospermia following varicocele repair (Kirby et al., 2016).

### Cost-effectiveness of varicocele treatment versus ART

With the encouraging evidence of varicocele treatment on natural PR, we must examine the cost-benefit analysis of varicocele treatment versus ART. While ART is becoming increasingly utilized in treating male infertility, not only does this treatment place a burden on the female partner, it also has cost implications and potential health implications for the female partner (ovarian hyperstimulation syndrome, multiple pregnancies) and offspring (Meng, Greene & Turek, 2005).

Meng et al. (2005) conducted a decision analysis model to compare the cost-effectiveness of surgical treatment of varicoceles and ART in infertile men with varicoceles. The authors compared costs of varicocelectomy with ART, which included IUI and ICSI. Overall, cost benefit analysis demonstrated that surgical repair of the varicocele was more cost-effective than ART. Table 3 outlines the pregnancy rates following varicocelectomy in relation to pre-operative total motile count (TMC), with PR being greater in men with a higher pre-operative TMC (Meng et al., 2005).

The findings of the above study complement a cost-effectiveness analysis published by Schlegel (1997). This analysis based on RCTs found that the cost per delivery following varicocelectomy was \$26,268 compared to \$89,091 following ICSI. The average delivery rate following one cycle of ICSI was 28%, compared to 30% following varicocelectomy. If ICSI were to be assumed to be as successful as possible, the cost per delivery still remained high, at \$62,263. Schlegel (1997) concluded that given the cost

implications and PR following these two treatments, surgical varicocelectomy is the most cost-effective primary treatment for varicocele-associated male infertility.

In a further review, Chiles and Schlegel (2016) considered the importance of not only the stand-alone cost of ART, but also subsequent incurred costs such as multiple pregnancies. The risk of twin births is up to 9% following ART compared to 2% following natural conception (Human Fertilisation & Embryology Authority (HFEA), 2017; Kamphuis et al., 2014). Multiple births can also impose health risks on the mother, increase the risk of premature delivery and subsequently other potential associated morbidities.

Whilst the above studies suggest that varicocele treatment is more cost-effective than ART, it is important to bear in mind that it is difficult to make generalizations on cost-effectiveness given the global variations in ART and surgical costs.

### Sperm DNA fragmentation

Varicoceles are thought to negatively impact on male fertility by increasing sperm DNA fragmentation and generating oxidative stress. Nuclear material in somatic cells consists of compacted DNA that is stabilized by nuclear proteins referred to as histones. During spermatogenesis, histones are replaced by protamines to enable the nucleus to become more highly condensed, affording protection from external damage (Hammoud et al., 2009). However, up to 15% of the histones are retained in the fully mature spermatozoon. As part of the natural process of histone replacement with protamines, sperm DNA strand breaks are temporarily induced which are subsequently repaired (Tarozzi, Bizzaro, Flamigni, & Borini, 2007).

Sperm DNA fragmentation can occur following chemotherapy or exposure to environmental or lifestyle insults (Pacey, 2010). Damage to sperm DNA has considerable consequences on sperm function and it has been suggested that sperm DNA integrity may be a better indicator of male fertility potential than current semen parameters, whose correlation to infertility is weak (O'Brien & Zini, 2005). Other theories described in the literature relating to sperm DNA fragmentation include defective chromatin packaging and abortive apoptosis, a review of which is beyond the scope of this paper (Tarozzi et al., 2007).

Various assays are used for the measurement of sperm DNA fragmentation including the COMET, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labelling), SCD (stearoyl-CoA desaturase) and

**Table 3.** Percentage pregnancy rate following varicocelectomy as demonstrated by pre-operative total motile sperm count (Meng et al., 2005).

Pre-operative TMC ( $1 \times 10^6$ )	Number	Pregnancy rate (%)
0-1.5	132	16.5
1.5-5	62	30.6
5-10	58	36.2
10-20	68	39.7
20-40	60	58.3
>40	65	61.5
Total	445	36.6

TMC: total motile count.



Halo and SCSA® (Sperm Chromatin Structure Assay) (Sharma, Masaki, & Agarwal, 2013). The SCSA® test has been developed using human and animal models over the last 35 years and is one of the most statistically robust tests available (Evenson, 2013). It measures the susceptibility of sperm DNA to denaturation when it is exposed to heat or acids. The percentage of sperm with DNA damage is expressed by the DNA Fragmentation Index (DFI). The statistically significant DFI threshold for infertility has been established at  $\geq 25\%$  (Evenson, 2013). Currently, measurement of sperm DNA fragmentation is not routinely recommended in the diagnostic evaluation of male factor infertility by international guidelines, but it may have a role in the assessment of patients who fail ART. For example, failure of embryogenesis, recurrent miscarriage (Pacey, 2018).

### Effects of varicoceles on sperm DNA fragmentation

Various mechanisms have been proposed by which varicoceles can impact on fertility in men. Proposed mechanisms include scrotal hyperthermia, hypoxia, hormonal imbalances and reflow of metabolites from renal and/or adrenal glands (Vivas-Acevedo et al., 2014). These can all generate oxidative stress, leading to DNA fragmentation. For example, Smith et al. (2006) used two separate methods for measuring DNA fragmentation and found  $7.1\% \pm 0.9\%$  DFI in the control group and  $35.5\% \pm 9.0\%$  DFI in men with varicoceles and abnormal semen parameters. In addition, Zini, Azhar, Baazeem and Gabriel (2011) published a small prospective study on infertile men with varicoceles ( $n = 25$ ) who underwent microsurgical varicocelectomy and used the SCSA® to examine the sperm DNA damage. At the start of the study, all 25 men had a sperm concentration of less than 20 million per millilitre, or  $< 50\%$  progressive motility, or both (Zini et al., 2011). Four months post-operatively, there was a mean 7% decrease in DFI ( $p = 0.0009$ ) and improvement in sperm chromatin compaction.

Smit et al. (2013) reported on a further small prospective study on 49 infertile men with diagnosed varicocele and at least 1-year history of infertility.

Surgical repair techniques of varicoceles included high inguinal spermatic vein ligation ( $n = 36$ ) and microsurgical varicocelectomy ( $n = 8$ ). The results demonstrated a significant improvement in DFI post-operatively ( $35.2\%$  pre-op vs.  $30.2\%$  post-op;  $p = 0.019$ ) as well as an improvement in total sperm count, sperm concentration and progressive motility. However, this study was not controlled, making it difficult to draw a firm conclusion. The authors described 63% of patients as positive responders, defined as a 50% or more improvement in sperm concentration post-operatively. Within this group of positive responders, DFI reduced from  $35.3\%$  pre-operatively to  $28.6\%$  post-operatively ( $p = 0.009$ ). There was no significant difference in luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone level (Smit et al., 2013). Interestingly, the non-responders demonstrated a significant increase in LH and FSH levels post-operatively. The findings of these two studies are summarized in Table 4.

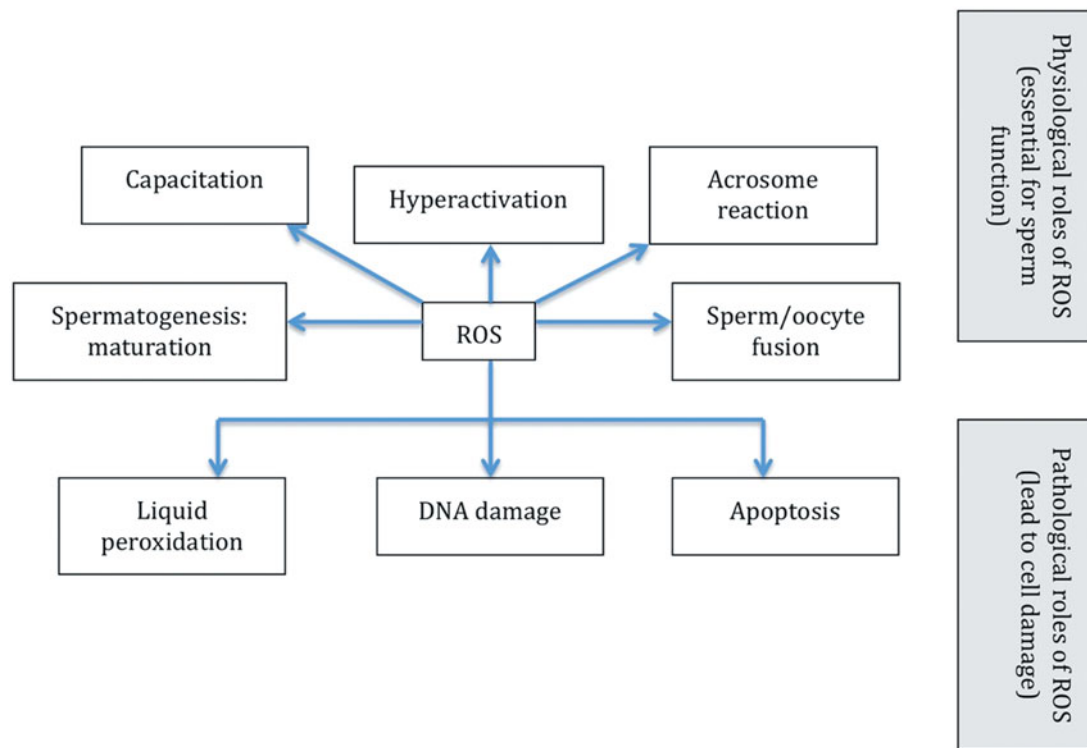
Ni et al. (2016) investigated the association of oxidative stress and DNA damage in 88 men with varicocele compared to 25 normal fertile controls without varicocele. Those with varicocele consisted of 15 infertile men with subclinical varicocele, 51 men with oligoasthenozoospermia with clinical varicocele who underwent microsurgical varicocelectomy, and 22 normozoospermic with clinical varicocele. DFI was significantly elevated in all patients with clinical varicocele but not in those with subclinical varicocele compared to controls. There was a significant improvement in semen parameters following varicocele repair and there were indications that oligoasthenozoospermic men with clinical varicocele who did not undergo surgery experienced a deterioration in semen parameters and further increases in DFI levels, which was not observed in those with subclinical varicocele (Ni et al., 2016).

Given the limited high quality data in the literature, evidence from the small studies discussed, suggests that clinical varicoceles may be associated with increased oxidative stress and subsequent sperm DNA damage; and that varicocelectomy may reduce the damage by reducing oxidative stress.

**Table 4.** Summarized findings of studies demonstrating improvement in sperm DFI after varicocele repair in infertile males.

Study	Number	Pre-op sperm DFI (%)	Post-op sperm DFI (%)	Method of assay	Pregnancy rate assessed?
Zini et al. (2011)	25	$18 \pm 11$	$10 \pm 5$ (4 months)	SCSA	No
Smit et al. (2013)	49	35.2	30.2	SCSA	Yes, 37% spontaneous conception
	positive responders (31)	35.3	28.6		

DFI: DNA Fragmentation Index; SCSA: sperm chromatin structure assay.



**Figure 1.** The physiological and pathological impact of reactive oxygen species (ROS) on spermatozoa. Adapted from Aitken et al., 2005.

## Oxidative stress

While the aetiology of sperm DNA fragmentation appears to be multifactorial, it is widely accepted that the mechanism by which most of these factors cause DNA damage is through excessive generation of reactive oxygen species (ROS) (Aitken, Smith, Jobling, Baker, & De Iuliss, 2014; Gharagozloo & Aitken, 2011; Vessey, Perez-Miranda, Macfarquhar, Agarwal, & Homa, 2014). ROS contain unpaired electrons in their outer shell and as such are highly reactive and damaging to susceptible molecules. ROS are normally maintained at low levels by effective antioxidant pathways, but if the production of ROS overwhelms the capacity of these pathways, then oxidative stress occurs, leading to pathological effects as seen in Figure 1 (Aitken et al., 2014; Aitken, Gibb, Baker, Drevet, & Gharagozloo, 2016; Tremellen, 2008). The sperm plasma membrane is enriched in polyunsaturated fatty acids, which are exquisitely sensitive to peroxidation. ROS induced peroxidation of membrane lipids, proteins and DNA leads to the formation of potentially genotoxic and mutagenic adducts, damaging membrane function as well as interfering with DNA methylation (Menezo, Silvestris, Dale, & Elder, 2016; Morielli & O'Flaherty, 2015; Wright, Milne, & Leeson, 2014).

## Oxidative stress and infertility

There is now growing evidence to support a link between oxidative stress and male infertility (Aitken et al., 2014; Tremellen, 2008). Oxidative stress occurs with conditions that are known to be associated with poor semen parameters and DNA damage such as testicular torsion, cryptorchidism, male accessory gland infection, urogenital tract infection and also varicoceles (Hendin, Kolettis, Sharma, Thomas, & Agarwal, 1999; Ochsendorf, 1999; Pasqualotto et al., 2000; Turner & Lysiak, 2008). Oxidative stress is cytotoxic to sperm, manifesting in impairment of semen parameters, in particular a reduction in motility and vitality (Aitken et al., 2014; Morielli & O'Flaherty, 2015). Seminal oxidative stress can be assessed by measuring ROS directly using a chemiluminescence assay or by measuring oxidation reduction potential (Agarwal et al., 2017; Aitken, Buckingham, & West, 1992; Homa, Vessey, Perez-Miranda, Riyait, & Agarwal, 2015; Vessey et al., 2014). Several studies show that there is a significant increase in ROS levels and a reduced antioxidant capacity in infertile men compared with fertile controls, irrespective of semen parameters (Aitken, 1995; Tremellen, 2008). Indeed, high levels of ROS have been observed in 30% to 80% of infertile men

(Agarwal et al., 2014). High ROS levels impair semen parameters and fertilization, adversely affect blastocyst development and negatively affect pregnancy rates after IVF (Agarwal et al., 2014; Aitken et al., 2014; Chen, Allam, Duan, & Haidl, 2013; Ghaleno, Valojerdi, Hassani, Chehrizi, & Janzamin, 2014; Zorn, Vidmar, & Meden-Vrtovec, 2003). Furthermore, elevated seminal ROS levels are correlated with an increased time to natural conception, as well as risk of miscarriage (Imam, Shamsi, Kumar, Deka, & Dada, 2011; Tremellen, 2008).

In a case-controlled study of 20 infertile couples with recurrent miscarriage compared to 20 couples that had achieved a recent healthy live birth, seminal ROS levels were assessed by chemiluminescence. Sperm DFI assessed by SCSA<sup>®</sup> was significantly elevated in the male partners with recurrent miscarriage, and total antioxidant capacity (TAC) scores were significantly lower (Imam et al., 2011). They concluded that both oxidative stress and DNA damage impair embryo development, reducing the chances of healthy live births.

### Varicocele repair and oxidative stress

There is considerable evidence implicating oxidative stress as the key mechanism responsible for varicocele associated male infertility (Agarwal, Prabakaran, & Allamaneni, 2006; Cho, Esteves, & Agarwal, 2016). Several studies have shown that both infertile men and fertile men with varicoceles have significantly elevated seminal ROS and lower TAC compared to those without varicoceles (Agarwal et al., 2006; Hendin et al., 1999; Sakamoto, Ishikawa, Kondo, Yamaguchi, & Fujisawa, 2008; Sharma, Pasqualotto, Nelson, Thomas, & Agarwal, 1999).

Two prospective studies evaluated ROS and TAC scores in relation to fertility, one of which evaluated 21 infertile men with varicoceles and 17 healthy controls (Hendin et al., 1999) and the other study compared results from 56 infertile men with varicocele to 24 healthy men with proven fertility (Sharma et al., 1999). Both studies revealed significantly raised ROS activity and decreased TAC scores in the men with varicoceles. A further meta-analysis including four studies demonstrated that infertile men with varicoceles had increased ROS levels compared to control groups (Agarwal et al., 2006).

An alternative approach to measuring oxidative stress is to assess products of peroxidation or antioxidant levels. Mostafa, Anis, Imam, El-Nashar, and Osman (2009) assessed levels of malondialdehyde (MDA) and hydrogen peroxide as well as five antioxidants

(superoxide dismutase, catalase, glutathione peroxidase, vitamin E, vitamin C) in the semen of 45 healthy fertile men and compared them to those of 45 fertile men with varicocele, 44 infertile men with oligoasthenozoospermia without varicocele and 42 infertile men with oligoasthenozoospermia with varicocele. The estimated seminal ROS was significantly higher and estimated antioxidants were significantly lower in all groups compared to healthy controls without varicocele.

In a prospective study, Chen, Huang, Chang, and Wei (2008) used polymerase chain reaction to measure the 8-OHdG (8-hydroxy-2'-deoxyguanosine) content in spermatozoa DNA and used seminal plasma protein thiols and ascorbic acid to examine oxidative damage to spermatozoa and alterations in antioxidant capacity. A total of 30 infertile patients with varicocele were assessed before and 6 months after subinguinal microsurgical varicocelectomy. 22 patients (73.3%) showed significant improvement in sperm motility, morphology and sperm concentration after varicocelectomy. The incidence of sperm mitochondrial DNA damage was reduced after surgery (12 to 4 patients), as were the levels of 8-OHdG ( $10.27 \pm 2.24 \times 10^5$  2'-deoxyguanosine before versus  $5.95 \pm 1.46 \times 10^5$  2'-deoxyguanosine after). In contrast, antioxidant levels increased after surgery (seminal plasma protein thiols  $0.77 \pm 0.75$  nmol/ml before vs  $3.12 \pm 0.94$  mg/dl after; ascorbic acid  $1.87 \pm 0.40$  mg/dl before vs  $3.00 \pm 1.17$  nmol/ml after). These data demonstrate a reduction in oxidative sperm DNA damage and improvement in antioxidant capacity as a result of varicocelectomy.

### Effects of varicocelectomy on natural pregnancy and ART

The importance of treating varicoceles becomes more evident when one considers the effect of varicoceles on pregnancy, live birth and miscarriage rate (Ficarra et al., 2012; Tiseo et al., 2016). Over the years, several studies have reported a detrimental effect of varicoceles on the prospects for pregnancy. For example, in a case-controlled study of 224 infertile men with clinical varicocele, Okuyama et al. (1988) demonstrated significantly higher pregnancy rates following varicocelectomy compared to the control group with expectant management of varicocele (30.6% vs 18.1%,  $p < 0.01$ ). In a further study on 136 couples with recurrent miscarriage with varicoceles, 68 underwent inguinal varicocelectomy while the remainder were managed with expectant therapy (Mansour Ghanaie et al., 2012). Patients in both groups were matched for age, grade of varicocele and smoking status and the



couples were followed up for 12 months. During this time the PR was 44.1% in the group treated with varicocelectomy and 19.1% in those untreated ( $p = 0.003$ ). Furthermore, of the women who conceived, 13.3% developed miscarriage in the varicocelectomy group compared to 69.2% in those that did not undergo surgery ( $p = 0.001$ ). More recently, two meta-analyses have been published analysing pregnancy rates from ART and in patients with varicoceles (Esteves, Roque, & Agarwal, 2016; Yuan et al., 2016).

The meta-analysis by Yuan et al. (2016) included 538 patients who underwent varicocelectomy followed by ART or ART alone without prior treatment of the varicocele. Overall, the results of the 7 included studies showed a significant OR of 1.76 (95% CI 1.35 to 2.29,  $p = 0.000$ ) for an increase in clinical pregnancy and an OR of 0.65 (95% CI 0.42 to 0.99,  $p = 0.042$ ) for a decrease in miscarriage rate in those undergoing varicocelectomy prior to ART. Whereas the systematic review and meta-analysis by Esteves, Roque, et al. (2016) was performed on 4 retrospective studies involving 870 ICSI cycles performed on non-azoospermic infertile men with clinical varicoceles. A total of 438 cycles were performed on men who had previous varicocele repair and 432 without. The clinical pregnancy rate (OR = 2.07) favoured the group having undergone varicocelectomy but this did not reach statistical significance. Despite this, the study did show a significantly higher successful SRR in those with a varicocele repair compared to the control group, OR 2.65 (95% CI 1.69 to 4.14,  $p < 0.001$ ).

In a further study by Gokce et al. (2013), ICSI was performed in 306 couples where all male partners were diagnosed with clinical varicoceles. Following varicocelectomy in 168 men, semen parameters showed a significant improvement but more importantly, there was a clear association with pregnancy and live birth rate. Both the PR (62.5% vs 47.1%,  $p = 0.001$ ) and LBR (47.6% vs 29.0%,  $p = 0.0002$ ) were significantly higher in this group of patients compared to those without varicocele repair and logistic regression analysis for viable pregnancy was OR 2.02 (95% CI 1.25 to 3.87,  $p = 0.032$ ) and for live births was OR 2.12 (95% CI 1.26 to 3.97,  $p = 0.026$ ).

A meta-analysis from 2015 has also reported that the LBR after IVF or ICSI was significantly higher in men with low DFI compared to those with high DFI (RR 1.27,  $p = 0.01$ ) (Osman, Alsomait, Seshadri, El-Toukhy, & Khalaf, 2015). In addition, a prospective study confirmed that high levels of sperm DNA fragmentation were a negative predictor of outcome from IUI. The study reporting on 119 couples who

underwent IUI found that none of the successful pregnancies from ART had sperm DFI of more than 12% (Duran, Morshedi, Taylor, & Oehninger, 2002).

The aforementioned studies strongly suggest that surgical treatment of varicoceles in infertile men decreases sperm DFI, with compelling evidence that varicocele treatment improves natural PR and improves PR with subsequent positive outcomes from ART (Esteves, Roque, et al., 2016; Gokce et al., 2013; Yuan et al., 2016).

Smit et al. (2013) reported that 37% of couples conceived naturally after varicocele repair was undertaken in the infertile male. Of their cohort of 49 infertile males, 19 couples proceeded to ART (IUI, IVF and ICSI) following failure of natural conception post-operatively. In those with successful ART pregnancies, conception was achieved within a mean of 14.6 months following varicocele repair. Overall, the study found that the post-operative DFI was significantly lower in couples who were able to conceive naturally or with ART compared to the couples who failed to conceive at all (Smit et al., 2013). This study provides further compelling evidence of improvement in DFI and sperm parameters in infertile men following treatment of varicoceles leading to increased success in both natural and subsequent assisted conception should natural pregnancy not occur.

Overall, these studies suggest that the effects of varicocele on both natural and assisted conception as well as miscarriage rates are highly likely to be attributed to an elevation in oxidative stress and subsequent sperm DNA fragmentation. In couples who have been unable to conceive naturally or may be embarking upon ART or who have experienced failed cycles of ART, consideration should be given to treatment of the varicocele. Although this has been shown to improve success rates in SRR, there is a lack of high level evidence to suggest it has an overall significant improvement in LBR, which is the ultimate outcome in fertility treatment.

### Non-obstructive azoospermia (NOA) and varicoceles

NOA occurs in up to 10% of infertile men and at present, surgical sperm retrieval and ICSI is the only method to achieve paternity (Esteves, Miyaoka, Roque, & Agarwal, 2016). Yet, with the increasing costs of ART, the PR for these men is only quoted to be 20% to 40% (Esteves, Miyaoka, et al., 2016). A US cohort study found that after 1 cycle of IVF with or without the use of ICSI, the LBR was 30.4% and this decreased

to 22.5% by cycle 5 (Stern et al., 2010). Considering these low figures of success and the cost implications of each cycle, it would be intuitive that any factors that may influence success should be optimized.

Although the treatment of varicoceles in men with NOA is not routinely undertaken in our practice, it has been concluded that elevated oxidative stress and raised scrotal temperature from varicoceles may lead to sperm DNA damage and germ cell apoptosis, leading to azoospermia (Esteves, Miyaoka, et al., 2016). There is some evidence that treatment of varicoceles in these men may not only improve SRR in men with NOA but sperm may appear in the ejaculate in up to 43% of men post-operatively and may obviate the need for sperm retrieval.

Esteves, Miyaoka, et al. (2016) conducted a meta-analysis of 18 published articles comparing outcomes in azoospermic men who underwent varicocele treatment and those with untreated varicoceles (see Table 5). Most studies were retrospective studies without a control group, but they found that the OR for successful sperm retrieval was 2.65 (95% CI 1.69 to 4.14,  $p < 0.0001$ ) when comparing men with treated varicoceles to those with untreated varicoceles. The largest cohort within the series reported a 60.80% successful SRR in varicocele treated men compared to 38.46% in untreated men ( $p = 0.01$ ) (Esteves, Miyaoka, et al., 2016; Haydardedeoglu, Turunc, Kilicdag, Gul, & Bagis, 2010). The largest cohort by Haydardedeoglu et al. (2010) also demonstrated a 22.1% ( $p = 0.03$ ) increase in PR and 23% increase in LBR when comparing varicocele treated and untreated men, respectively. Despite the improved SRR, meta-analysis of both studies that reported on PR and LBR, found no statistically significant difference between varicocele treated and untreated men with NOA (Esteves, Miyaoka, et al., 2016). A total of 16 of the included studies reported on the presence of sperm in the ejaculate post-operatively. The mean sperm count was 1.82 million, with motility of 22.9% (Esteves, Miyaoka, et al., 2016). The mean time from varicocele repair to appearance of sperm in the ejaculate ranged from 4.5 to 11 months.

The presence of sperm in the postoperative ejaculate was also found to correlate with testicular histopathology. In the meta-analysis by Esteves, Miyaoka, et al. (2016), 8 of the studies reported on testicular pathology, obtained pre-operatively or intra-operatively. A total of 161 patients were followed up for a period of 13.3 months and the data showed that those with hypospermatogenesis were most likely to have sperm in their postoperative ejaculate (Esteves, Miyaoka, et al., 2016). The presence of sperm in the

post-operative ejaculate was detected in 56.2% of those with hypospermatogenesis, 35.3% of those with maturation arrest and only 9.7% of those with Sertoli cells only. In 88 patients with sperm in the postoperative ejaculate, 13.6% had a successful natural pregnancy within the follow up period of 12.7 months. A total of 26.1% of this cohort succeeded in conception either naturally or with ART: 7 studies within the series reported on ART, from which 18.9% of couples who underwent ICSI following varicocele treatment had successful pregnancies (Esteves, Miyaoka, et al., 2016).

The above data would suggest that varicocele treatment in men with NOA is correlated with a positive outcome for SRR and subsequent ART. In some studies, the treatment of varicoceles has demonstrated an increase in natural and assisted PR post-operatively, allowing patients to achieve biological parenthood (Esteves, Miyaoka, et al., 2016). Treatment may also result in sperm appearing in the ejaculate post-operatively and can lead men to transition from azoospermia to oligozoospermia after 4.5 to 11 months. Despite an increase in SRR success, the small studies included in meta-analysis by Esteves, Miyaoka, et al. (2016) overall, did not show statistically significant improvement in PR and LBR following varicocele treatment. The meta-analysis has limitations as only 4 of the 18 studies reported on the primary outcomes of SRR and PR. However, there is an argument that treatment of varicoceles in men with NOA may obviate the need for surgical sperm retrieval.

A review undertaken by Chiba and Fujisawa (2016) found that the time interval between varicocele repair and improvement in semen parameters was 3 months, with no significant subsequent improvement. Therefore, even in female partners with limited ovarian reserve, it could be argued that it may be beneficial for the male partner to undergo varicocele repair prior to ART. Currently, there is no consensus on the optimal interval following varicocele repair at which sperm retrieval should be performed. This review has shown that prognostic factors for sperm in the postoperative ejaculate include testicular histology with hypospermatogenesis. Hypospermatogenesis, along with late maturation arrest was also demonstrated in 2 of 6 men with NOA who had spermatogenesis induced following varicolectomy in Ishikawa, Kondo, Yamaguchi, Sakamoto, and Fujisawa's (2008) retrospective study.

However, further prospective controlled studies are needed to define the optimum interval at which post-operative sperm retrieval should be undertaken if necessary and most importantly, appropriately

**Table 5.** Adapted table from meta-analysis by Esteves, Miyaoka, et al. (2016) outlining post-operative semen parameters of men with NOA following varicocele repair and natural and ART pregnancy rates with the use of post-operative ejaculated sperm.

Study	Mean age (years)	Mean follow up (months)	Presence of sperm in postoperative ejaculate, n (%)	Mean postoperative sperm count ( $\times 10^6/\text{ml}$ )	Mean interval between varicocele repair and sperm in ejaculate (months)	Mean postoperative sperm motility (%)	Natural pregnancy (n)	Pregnancy rates by ART (n)
Matthews, Matthews, and Goldstein (1998)	NR	10.3	12/22 (55.0)	2.20	NR	NR	2/12	1/10
Kim, Leibman, Grinblat, and Lipshultz (1999)	35	15	14/28 (50.0)	1.18	8	44.0	0/14	2/14
Kadioglu et al. (2001)	30.1	13.4	5/24 (20.8)	0.04	NR	NR	0/5	NR
Çakan and Altüg (2004)	29	9	3/13 (23.1)	0.73	4.5	26.0	0/3	0/1
Esteves and Glina (2005)	32	18.9	6/17 (35.3)	0.8	5	NR	1/6	NR
Gat, Bachar, Everaert, Levinger, and Gornish (2005)	34.1	12	18/32 (52.6)	3.81	NR	1.2	4/18	5/14
Pasqualotto, Sobreiro, Hallak, Pasqualotto, and Lucon (2006)	NR	12	9/28 (33.3)	4.06	NR	37.6	1/9	NR
Poulakis, Ferakis, de Vries, Witzsch, and Becht (2006)	33.8	24.8	7/14 (50.0)	3.10	NR	2.2	2/7	1/5
Ishikawa et al. (2008)	33.3	>6	2/6 (33.3)	0.07	NR	NR	NR	NR
Cocuzza et al. (2009)	29.4	9	3/10 (30.0)	5.50	NR	36.6	NR	NR
Lee, Park, and Seo (2007)	32	7.4	7/19 (36.8)	0.24	NR	30.2	1/7	NR
Abdel-Meguid (2012)	34.9	19.3	10/30 (32.3)	2.30	NR	15.3	NR	NR
Kirac, Deniz, and Biri (2013)	31.7	11.4	7/23 (30.4)	1.34	NR	37.5	1/7	2/6
Zampieri, Bosaro, Costantini, Zaffagnini, and Zampieri (2013)	33	6	17/35 (48.6)	0.6	6	11.0	NR	0/4
Aboutaleb, Elsherif, Omar, and Abdelbaky (2014)	29.9	17.3	6/20 (30.0)	2.00	NR	NR	NR	NR
D'Andrea et al. (2015)	37	6	11/23 (47.8)	1.30	6	10.0	NR	NR

NR: not recorded.

**Table 6.** Summary of current international fertility guidelines on the indications for varicocele treatment in male factor infertility.

	Indication for varicocele treatment in male factor infertility
ASRM/AUA (last updated in 2014)	When ALL of below are present: <ol style="list-style-type: none"> <li>1. Varicocele is palpable</li> <li>2. Documented couple infertility</li> <li>3. One/more abnormal semen parameters or sperm function results</li> <li>4. Female partner has normal fertility or potentially correctable infertility</li> </ol>
EAU (last updated in 2016)	Only for men with clinical varicocele, oligozoospermia and infertility.
NICE (last updated in 2017)	<ol style="list-style-type: none"> <li>1. Treatment for varicocele is not advocated</li> <li>2. There is currently no indication that men should undergo a physical examination.</li> </ol>
<b>Authors proposed indications</b>	<ol style="list-style-type: none"> <li>1. At least a physical examination for all men, which should lead to further diagnostic imaging in those with clinical varicoceles</li> <li>2. Non-obstructive azoospermia</li> <li>3. Those undergoing ART</li> <li>4. Those having had previously failed IVF/ICSI.</li> </ol>

ASRM: American Society for Reproductive Medicine; AUA: American Urological Association; EAU: European Association of Urology; NICE: National Institute for Health and Care Excellence.

powered RCTs focusing on LBR should be conducted in men with varicocele and NOA.

## Conclusion

There is now emerging evidence in the literature that varicocele treatment will increase natural pregnancy rates and live birth rates. Furthermore, there is evidence suggesting that varicocele treatment may be more cost-effective than ART in certain healthcare systems, but the applicability of these data to other countries is uncertain.

Varicocele treatment should not be restricted to men with oligozoospermia and significant clinical varicoceles as suggested by current guidelines, but should also be considered in men undergoing ART or had failed treatments such as IVF/ICSI, when female factors have been corrected. These data would suggest that varicocele treatment to reduce DNA fragmentation may improve the outcome from IVF/ICSI and live birth rates in this cohort of patients.

In men with NOA, a number of recent meta-analyses suggest that varicocele treatment will not only result in the spontaneous appearance of sperm in the ejaculate, and thus avoiding the need for invasive sperm retrieval, but also increase the probability of successful sperm retrieval.

Table 6 summarizes the current international fertility guidelines with our suggested revised indications for the treatment of varicoceles in male infertility. Current fertility guidelines do not include or review the above novel indications for the treatment of varicoceles and we advocate that the indications of varicocele treatment should be redefined in contemporary guidelines to reflect current advances in male fertility research and provide a more cost-effective and patient

focused approach to the treatment of male factor infertility.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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