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A REVIEW ON ANTIOXIDANT THERAPY FOR VARICOCELE ASSOCIATED MALE INFERTILITY

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Abstract

Varicocele is one of the key causes of male infertility. It is a condition that needs more research regarding diagnosis and treatment. Although varicocelectomy is the most sought after option for treatment, it comes with drawbacks such as invasion, recurrence, testicular atropy and unreliability. One of the easier ways patients with varicocele prefer is Assisted Reproductive Technology (ART) wherein the cost and risk involved are higher. Its repetitive invasion and ethical issues are also a concern. Antioxidant therapy, on the other hand, has proven to have benefits with minimum side effects and is being explored as an alternate option due to increasing oxidative-stress induced infertility. In this article, we have reviewed the literature concerning antioxidants such as bioflavonoids, carnitines, selenium, vitamin C, vitamin E, kallikrein, coenzyme Q10, cinnoxicam, zinc and folic acid which are used for managing the condition ofvaricocele. It was seen that these antioxidants proved to be effective in improving sperm parameters and antioxidant levels which are major causes of infertility. Further, some antioxidants such as daflon, vitamin C, and vitamin E act as good adjuvant alongside varicocelectomy. Despite its potential, there is no concrete evidence to recommend antioxidants as a routine therapy or for replacing surgery. Hence, as a future perspective, there is a need to conduct larger studies backed with pregnancy outcome reports to confidently recommend antioxidant therapy as an alternative.

Keywords: Varicocele, Male infertility, antioxidant therapy, varicocelectomy, ART

Introduction

Male infertility accounts for 60% of infertility among humans, solely or as a couple. It is defined as the inability to conceive after 1 year of regular unprotected sexual intercourse due to modification in the male partner. This alteration maybe associated with oligospermia (lower sperm production), asthenospermia (lower sperm motility), teratospermia (poor sperm morphology) or, a combination of all, oligoasthenoteratozoospermia (OAT) (Isidori et al., 2005).

The known causes of infertility account for 70% of the cases. For the rest 30%, the reasons are unknown and are called idiopathic infertility (Esteves et al., 2019).

Varicocele is one of the most common causes of male infertility and is reported in 15% of the general male population and approximately in 40% of men with infertility (Isidori et al., 2005).It is a physiological condition manifested because of the enlargement of pampiniform venous plexus (Ferramosca et al., 2015).Symptoms of pain and discomfort, reduced fertility, and failure of ipsilateral testicular growth and development are the andrological implications of varicocele(Jungwirth et al., 2012). In the spermogram of varicocele patients, reduced sperm motility is the most commonly observed disorder.It also leads to reduced testicular activity and testicular atrophy in patients. This causesazoospermic or oligospermic conditions in 4.3% to 13.3% of varicocele patients (Ketabchi and Ketabchi, 2011).

Surgery in the form of varicocelectomy remains the most sought after management of varicocele. Various open, laparoscopic, and microsurgical techniques are explored in varicocelectomy. It can also be performed from different body parts such as inguinal, scrotal, sublingual, and retroperitoneal. Yet another treatment option is percutaneous embolization (Garg and Kumar, 2016). Varicocelectomy helps in reversing the sperm DNA damage caused due to oxidative stress and has the potential to reverse the condition (Zini and Dohle, 2011). However, these surgical procedures can lead to complications such as testicular atrophy, absence of identification of predictors for success, desire for less invasive treatments, and recurrence (Diegidio et al., 2010). Moreover, there is controversy regarding the couple's chance of conception once varicocelectomy is performed. In many randomized controlled studies, the treatment was found to be ineffective (Evers and Collins, 2003; Evers et al., 2009; Yamamoto et al., 1996).

Due to the emergence of Assisted Reproductive Technology (ART), the efforts to deal with male infertility are on the declining trend especially after the introduction of Invitro Fertilization (IVF) and Intracytoplasmic sperm injection (ICSI). Nevertheless, ART is not able to solve all the infertility problems that are widespread and it also has ethical issues while implementing (Esteves et al., 2019). It comes with added risk, cost, and the need for repetitive intervention. With the present drawbacks in both varicocelectomy and ART, medical therapy is a viable option with lesser risks for managing varicocele and its associated infertility issues.

There are three major types of therapies found to date for varicocele. First is Antioxidant therapy such as carnitines, kallikrein, zinc, folic acid, selenium, cinnoxicam, coenzymeQ10, bioflavonoids, Vitamin C and Vitamin E aimed at reducing ROS (Reactive Oxygen Species) and improving antioxidant capacity. The second involves hormonal therapy using clomiphene, menotropin, tamoxifen, and gonadotropin for restoring hormonal levels for spermatogenesis. The third therapy consists of Chinese medication with agents that have antioxidant and anti-inflammatory effects such as green tea, jingling, escin, qianjing, and guizhifuling wan (Esteves et al., 2019).

Exact pathophysiology behind male infertility caused by varicocele is yet to be elucidated, but the oxidative stress plays a major role in testicular damage induced by varicocele. Varicocele is associated with increased ROS in sperm and diminished antioxidant capacity in seminal plasma (Hendin et al., 1999). ROS, including nitric oxide and hydrogen peroxide, increases drastically in varicocele patients due to heat stress and hydrostatic pressure (Agarwal et

al., 2012). Increased expression of cytokines such as Interleukin-1 and Interleukin-6 and hormones such as leptin also add to the oxidative stress. Moreover, few studies have also confirmed a correlation between the grade of varicocele and the level of oxidative stress (Hamada et al., 2012). Antioxidants can negate this oxidative stress in men and treat varicocele associated human male infertility. Moreover, they also reduce DNA damages, protect sperm from oxidative damage, stop premature sperm maturation, and improve outcomes of ART. Hence this review deals with antioxidant therapy explored by various studies for managing varicocele associated sperm parameter damages and pregnancy outcomes (Figure 1).

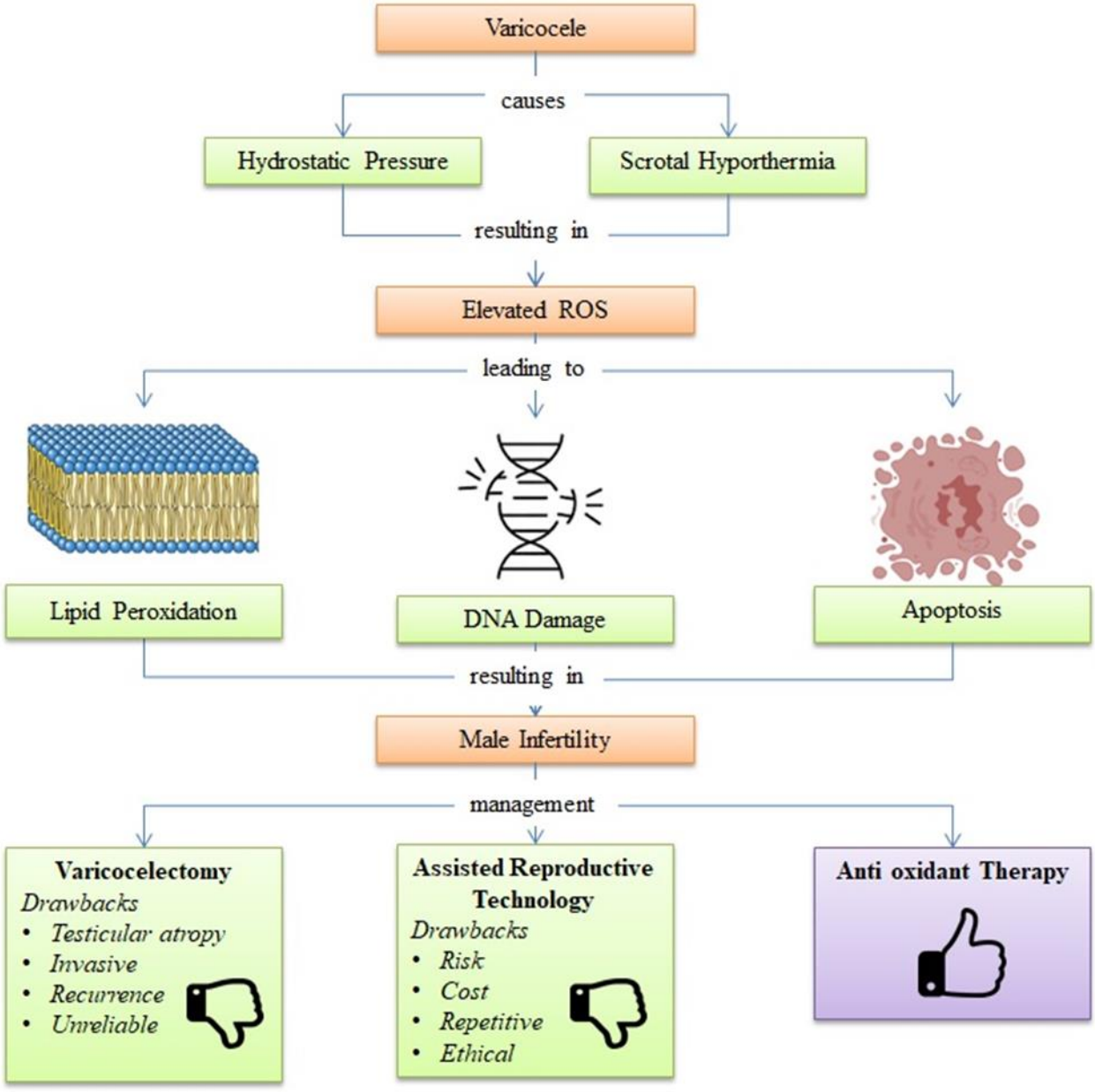

Figure 1 Causes of varicocele associated male infertility and its available treatments. Varicocele results in elevated Reactive oxygen species (ROS) which leads to male infertility. The widely used management techniques for varicocele are varicocelectomy and Assisted Reproductive

Technology, but they have drawbacks. However, Antioxidant therapy promises to overcome these drawbacks.

Bioflavonoids

Bioflavonoids, found in plants are widely distributed as phenolic groups(Tapas et al., 2008). Due to its low toxicity, this group of compound is used as medicine for mammals. Apart from showcasing abilities such as anti-inflammatory, antihepatotoxic, and anti-ulcer, bioflavonoids also have antioxidant and free radical scavenging properties (Narayana et al., 2001). Bioflavonoids have proven to possess the ability to scavenge all forms of free radicals such as superoxides, hydroxyl, and peroxyl radicals (Bors et al., 1990). The most important plant pigments for flower coloration are bioflavonoids and hence are widely distributed. They have also been reported as excellent agents for improving venous tone in varicocele. Since these are naturally occurring pigments, there are no reported side effects as well (Esteves et al., 2019). Moreover, due to its antioxidant advantages, bioflavonoid has been thoroughly studied for its therapeutic effect on various conditions, one of the being venous diseases.

Chronic venous insufficiency (CVI), a condition that affects lower extremities' venous system causes abnormalities such as pain, skin changes, swelling, edema, and ulceration (Eberhardt and Raffetto, 2014). The most possible causal factors for both CVI and varicocele are common and hence one can draw concomitance between the two conditions (Bolcal et al., 2006). Hence various studies that have proven to treat CVI have been explored for varicocele patients as well.

Hydroxyethylrutosides (HR) is a mixture of semisynthetic flavonoids. These are benxopyrone derivatives from rutin which is a naturally occurring flavonol. CVI in the lower limb has been treated therapeutically with oral administration of HR (Wadworth and Faulds, 1992).A study was conducted in 2010 that aimed to interpret the role of bioflavonoids for managing a relatively rare condition called subclinical varicocele. In this study, medical charts of 168 patients treated with HR for subclinical varicocele were analyzed. It was found that in long term results, the development of palpable varicocele from the subclinical condition was reduced. It was also found to have a higher rate of stable vein reflux and a higher resolution rate. In contrast, the onset of testicular growth arrest could not be prevented by bioflavonoid(Zampieri et al., 2010). Generally, it takes 4 years for progression from subclinical varicocele to clinical varicocele in one third adolescent children (Cervellione et al., 2008). This can be controlled through bioflavonoid therapy which has proven to be effective in the previous study described. Nevertheless, research has to be carried out with a higher number of study populations to attain solid proof for this concept.

Micronized purified flavonoid fraction (MPFF)also called daflon is yet another intriguing class of flavonoid that displays a multiplicity of actions. These are biologically active compounds that are made up of 90% diosminwhich is a derivative of flavone and 10% hesperidin which is a derivative of flavanone(Struckmann, 2000). Irrespective of severity and range of symptoms, MPPF is a proven first line medication for CVI (Katsenis, 2005). A preliminary study conducted by Kilic et al suggested the efficacy and safety of MPFF for treating the pain associated with varicocele,with almost no side effect(Kiliç et al., 2005). Though this study did not report anyimprovement in sperm morphology, concentration and count, the sperm motility increased significantly. A similar study by the authors with a higher dosage also found to

increase sperm motility in varicocele patients (So et al., 2012). This study, in particular, was a randomized placebo-controlled study which is a more reliable and strengthening reassurance of the previous preliminary study. Although surgical and radiological treatments have been proposed for treating the pain associated with varicocele, these are no doubt invasive procedures and come with side effects. In such cases, use of oral medication to alleviate the pain can be a reasonable approach especially for fertile varicocele patients who do not wish to undergo invasive procedures. This study also reports for 100% satisfaction rate among study population and it used pain scores for objective results. However, withdrawal of the drug did cause recurrence of pain. Hence there is a need for optimizing the amount of drug required to control the pain.

Further, research was also conducted on varicocele induced rats which tested markers for oxidative stress such as the MDA (malondialdehyde), MMP -2 (matrix metalloproteinase -2), MMP-9 (matrix metalloproteinase - 9) and TIMP-1 (Tissue matrix metalloprotein inhibitor - 1) (Dogan et al., 2014). MMPs are responsible for extracellular matrix degradation and TIMP – 1 inhibits this process naturally. On the other hand, MDA helps in the measurement of lipid peroxidation. Though this particular study was aimed at testing the marker levels for the surgery, MPPF was used as an adjuvant. It was observed that the daflon treatment was an effective adjuvant since it comparatively decreased the MDA levels and significantly increased the MMP 2 and MMP 9 levels.

It is necessary to increase the scope of research in these particular compounds to determine the mechanism of action and tap the right biological resources for diagnosing and treating implications in varicocele.

Cinnoxicam

Cinnoxicam is a lipophilic NSAID (Non-Steroidal Anti Inflammatory Drug) that belongs to a group of molecules called oxicam. These molecules are structurally related and possess anti-inflammatory, analgesic, and antipyretic activities. They are bound to plasma proteins extensively and are weakly acidic (Olkkola et al., 1994). Although cinnoxicam has not been much studied as a medical therapy for infertile patients, a few studies have been done to observe the improvement in seminal parameters of varicocele subjects when treated with cinnoxicam.

This NSAID was administered for 12 months and was compared with those who underwent varicocelectomy. 155 men with grade 5,4 and 3 were included as study population and were divided into 3 groups, each group containing all the grades. Group 1 underwent surgery, Group 2 was given a suppository of cinnoxicam (30mg every four days for 12 months) and Group 3 received placebo.Results revealed that group 2 had improved seminal parameters from 2^{nd} month and was stabilized by the end of 12 months, especially for grade 3 patients. Hence it concluded that cinnoxicam was a promising therapy for men with oligoasthenospermia associated with grade 3 varicocele. Whereas for the other two grades, surgery was prescribed. However, the parameters returned to baseline after cessation of the drug (Cavallini et al., 2003). Initially, when oral and injected form of drug administration was considered, the sperm count was significantly lower than suppositories. Hence more research can be conducted on the involvement and efficiency of this drug on the rectal-prostatic-lymphatic pathway.

Another combination study was conducted by the same authors with 325 patients associated with clinical and subclinical varicocele and idiopathic infertility. The study population

was divided into 3 groups with a mix of both varicocele (all grades) and Idiopathic oligoasthenospermia. Group 1 was given placebo; group 2 was supplemented with the mix of carnitines and group 3 with the combination of cinnoxicam and carnitines. Carnitines are derivatives of methionine that have potential health benefits. Despite the minor side effects, group 3 with the combination of cinnoxicam and carnitinesproved to improve seminal parameters of all the grades of varicocele except 5^{th} grade. Additionally,the pregnancy rate for group 3 was 38% as compared to 21.8% of group 2. Hence the combination of cinnoxicam and carnitines are effective for the low and moderate grades of varicocele and not the severe grades. In a few earlier studies, cinnoxicam was more active in improving the sperm count than other NSAIDs and this may be attributed to the lipophilic nature which increases lymphatic and prostatic absorption(Cavallini et al., 2004). In this study, however, the patients who could not conceive can further be tested with ART treatments given that their sperm parameters had improved.

Coenzyme Q10

Coenzyme Q10 also called Ubiquinone is an electron carrier in mitochondria for the respiratory chain. It is also a well-studied lipid-soluble antioxidant that scavenges free radicals within liposomal membranes (Frei et al., 1990). Majority of CoQ10 is present in the midpiece of the sperm cell making it available for all energy-related movements of the cell. Ubiquinol is a reduced form of CoQ10 and is an antioxidant that prevents lipid peroxidation in the cell membrane (Lewin and Lavon, 1997). Besides, the amount of CoQ10 in seminal plasma and sperm motility and count are correlated. It is hence a proven antioxidant and a promotility agent (Mancini et al., 1994).

This coenzyme helps in oxidative phosphorylation and its availability can be reduced with the prevalence of oxidative stress. Hence, the effect of CoQ10 on infertility was studied through oral supplementation therapy. One such pilot study was carried out with 38 varicocele patients for 3 monthswho were supplemented orally with 100mg exogenous CoQ10 per day and it was found to be an efficient medical treatment(Festa et al., 2014). It had improved both seminal parameters and the antioxidant capacity of the subjects. This study, however, did not report for pregnancy outcomes and also, the effect of coenzyme diminished once the medication was stopped.

Administration of CoQ10 for improving the sperm parameters among infertile patients has been studied at large(Boscaro et al., 2009). Nevertheless, given that varicocele patients exhibit different patterns in antioxidant distribution as compared to other male infertility (Mancini et al., 2012), it is necessary to extend these antioxidant treatment studies to further horizons dealing with pregnancy outputs and placebo-controlled randomized studies.

Kallikrein

Kallikrein, an antioxidant of pancreatic origin has proven to be a useful tool in treating infertility. The Kallikrein – kinin system acts as a stimulator and regulator of spermatogenesis and motility(Moreau et al., 2005). Though Kallikrein has a different mode of action as compared to other antioxidants, it accounts for improvement in intratesticular testosterone and sperm maturation (Esteves et al., 2019). A study was conducted to find out the efficacy of treatment of Kallikrein on patients with idiopathic oligospermia. The seminal parameters such as sperm

count, sperm concentration, and sperm penetration capability improved in the treatment group (P. Giovenco et al., 1987).

Later, this treatment was extended to varicocele patients and the study rectified the issue of sperm quality for 38 men who were administered with Kallikrein 600 units per day orally for 3 months duration (Micic et al., 1990). These were compared with a control group of 27 oligoasthenospermia men with left varicocele. It reported an increase in sperm motility (24% to 35%) and sperm morphology (58% to 71%). The study considered it either as an alternative treatment for patients refusing to undergo surgery or to be used as an associate therapy along with surgery. This study was conducted almost three decades back and no further studies have been reported since then on the kallikrein's effectiveness as a potential drug for treating varicocele. With the ability of this antioxidant and lack of studies, there is a wide scope for its research on a larger study group, placebo-controlled, and pregnancy outcome studies.

Vitamin C

Vitamin C (Ascorbic acid) can be obtained by humans only exogenously. It is majorly found in fruits and vegetables and is a good antioxidant agent for the body. Vitamin C is necessary to keep the correct balance between oxidants and antioxidants. Vitamin C can be oxidized by many species due to which many human diseases have emerged(Padayatty et al., 2003). The concentration of Vitamin C in seminal plasma is 10 times higher than that of blood serum (Jacob et al., 2018). Ascorbic acid can scavenge and neutralize the oxidative radicals such as superoxide, hydroxyl, and hydrogen peroxide protecting from oxidative damage (Fraga et al., 1991). In one of the studies, a combination of vitamin C, vitamin E, and glutathione was used for improving sperm count and reducing 8-OHdG (8 hydroxy 2 deoxyguanosine) (Kodama and Ph, 1997). Although many studies have shown improved sperm parameters due to Vitamin C supplementation in infertile men, some studies have proved otherwise as well (Agarwal and Majzoub, 2017; Lopes-Santiago et al., 2012).

Due to such controversial results with supplementation of Vitamin C as a therapy, Cyrus et al conducted a double-blind placebo-controlled clinical trial for reviewing the effect of this antioxidant supplemented to patients aftervaricocelectomy, as an adjuvant treatment. 115 men with clinical varicocele and infertility along with abnormal sperm parameters were included in the study. After varicocelectomy, the study population was divided into two groups. The intervention group which received 250mg of vitamin C and the control group that received a placebo were followed up for three months.This therapy was found to improve the quality of sperm such asmotility and morphology whereas the count saw no such improvement(Cyrus et al., 2015). This advancement in qualitative and not quantitative parameters of sperm can be attributed to the fact that some of the study populations considered in this study had normal sperm count even before the surgery. Hence there is a need for a more sophisticated and diverse study population to confirm this pattern of effectiveness.

A recent study was conducted in which a combination of antioxidants including vitamin C was used to treat oligoasthenospermic patients with or without varicocele. This was a 6 months long randomized double-blind placebo-controlled trial in which 104 subjects participated. The outcome of improved sperm parameters was more evident in varicocele patients as compared to others (Busetto et al., 2018).

Vitamin C,in general,is a safe and cheap oral supplement that has therapeutic effects on male infertility. Since this antioxidant is also largely available in fruits and vegetables, it comprises a healthy diet to patients. If the studies are further explored on vitamin C as either an adjuvant or a therapy in itself for varicocele, it can be a blessing in disguise for alternative treatment in infertility.

Vitamin E

Vitamin E also known as α- tocopherol, is a fat-soluble compound. It is a chain breaking antioxidant and can quench free radicals such as hydroxyl and superoxide anion contributing to reduced oxidative damage in cell membranes (Thérond et al., 1996). Suleiman et al had investigated the protective role of Vitamin E in sperm cells of patients with asthenospermia and found that it had significantly decreased the MDA concentration and improved sperm motility leading to pregnancy as well (Suleiman et al., 1996).

Although supplementation of Vitamin E individually for treating varicocele has not been studied, it has been explored with other combinations in affected subjects. One such integrated treatment method was formulated in combination with Chinese medicine and dexamethasone. It was used as an adjuvant after the surgery was performed and, the medical therapy alone was treated as control. The experimental method worked better than the control and proved that it can be a good adjuvant treatment(Guo et al., 2006).

Yet another research was done with rats using the combination of testosterone and Vitamin E for 60 days. The subjects were divided into 5 groups; Group 1 was control and the rest of the rats were induced with varicocele. Group 3 was treated with vitamin E (150mg/kg orally), group 4 was treated with testosterone (400µg/kg, intraperitoneal) and group 5 with a combination of both the treatments. This combination (group 5) exhibited protective functions against varicocele induced rats by enhancing the testicular antioxidant status and endocrine activities including HSP70-2 chaperon expression(Khosravanian et al., 2014). Heat shock proteins (HSP) are molecular chaperones that help in spermatogenesis by aiding proteins in folding, assembly, and transport (Marmar, 2001). Hence upregulation of HSP70-2 due to treatment of vitamin E and testosterone may have reversed the varicocele induced arresting of spermatogenesis. Also, group 3 and group 4 showed appreciative results inhibiting damages due to varicocele although through different mechanisms. Vitamin E inhibited these damages partly by enhancing both the enzymatic and non-enzymatic antioxidant content. This antioxidant treatment could also be the reason for observing the upregulation of HSP70-2 and inturn protection of RNA in group 4 subjects. It was finally concluded that Vitamin E and testosterone administration protected DNA and RNA by two different mechanisms, one by improving antioxidant status and the other by improving chromatin condensation and protamine stability by upregulating HSP70-2 molecular chaperon.

Selenium

Selenium is an essential trace element that is involved in various biological functions. It protects humans from diseases like cancer and oxidative stress in the body (Bartel et al., 2007). The importance of Selenium for male fertility had been studied since long and one of the studies has shown that Selenium in semen is significantly higher as compared to seminal plasma. The element also has a positive correlation to sperm concentration and morphology (Noack-Füller et

al., 1993). Selenium exerts its antioxidant action through glutathione peroxidase enzymes and thus helps in spermatogenesis and testis development(Moslemi and Tavanbakhsh, 2011). With the establishment of selenium's importance in semen, investigations were carried out to explore its ability as medical therapy for infertility.

Selenium as a therapy along with N-acetyl-cysteine (individually and together) was carried out ina double-blind placebo-controlled study with 468 infertile men for 26 weeks. Selenium therapy proved to improve sperm parameters such as concentration, motility, and morphology in cells (Safarinejad and Safarinejad, 2009).

This selenium therapy was subsequently extended by other researchers for treatingvaricocele induced rats. In the study, sodium selenite was administered to bothvaricocelized rats and normal rats. Sodium selenite administration was found to normalize testes CAT (catalase), GPX (glutathione peroxidase), SOD (superoxide dismutase), and MDA after therapy. Histopathological reports also suggested that sodium selenite had protective effects on testicular damage in varicocelized rats as compared to normal ones (Taghizadeh et al., 2017). This study reported no toxicity symptom or death due to the treatment proving the safety of optimum dosage of selenium therapy.

In yet another study, a combination of drugs (Vitamin A, and E, selenium, L-Carnitine, pentoxifylline, clomiphene citrate, and antioxidants) was administered to 107 patients alongside varicocelectomy for 3-6 months. Apart from regular sperm parameters, even natural pregnancy was recorded. Sperm concentration was reported to increase in both surgery and drug therapy. Simultaneously, pregnancy was observed in 47% of surgery group patients and 21% of medical therapy group patients (Gamidov et al., 2012).

Zinc and folic acid

Zinc is an essential mineral necessary for the human body. It is involved in the metabolism of DNA and RNA. Zinc, in particular to the male reproduction system, is important for its antiapoptotic and antioxidant properties. It also plays a major role in spermatogenesis (Agarwal and Majzoub, 2017). Zinc deficiency is associated with numerous complications in sperm such as flagellar abnormalities (hypertrophy and hyperplasia), abnormal or absent midpiece, axonemal disruption, and defects of inner microtubular dynein arms (Paper, 2008). Due to its importance, Zinc has been studied as a therapeutic agent to treat infertility and varicocele as well. One such preliminary study was conducted with zinc sulfate for treating 101 varicocelemen with or without surgery but having low zinc concentration in seminal plasma. They were administered with 440mg of zinc sulphate daily from two months to two years. The group that was treated with zinc sulphate alone witnessed successful impregnation of 27.7%, while the group that preceded the treatment with surgery witnessed 50% impregnation (Cosentino and Cockett, 1987). Although the seminal zinc level increased significantly in both the groups within 2 months, the group with zinc sulfate treatment did not witness any significant change in sperm motility or count in contrast to the group that underwent surgery and the oral treatment. This study hence concluded that the zinc sulphate treatment is effective for varicocele patients with lower seminal zinc concentration, especially after the surgery.

Along with zinc as an oral supplementation to improve semen quality, folate which is the reduced form of folic acid is also an essential micronutrient for infertility. It is a well-known fact

that folate plays an important role in DNA and RNA synthesis and its deficiency causes impairment of the remethylation cycle and elevation of homocysteine(Ebisch et al., 2007; Wallach et al., 2000). Hence zinc and folate have been used as supplement therapy alongside the surgery. In one of the studies, 102 patients who underwent varicocelectomy for grade 3 stage were divided into four groups and given supplementation treatment with group 1 receiving zinc sulfate (66mg/day), group 2 receiving folic acid (5mg/day), group 3 combinational therapy and group 4 as control. The study was conducted for 6 months and was observed that the group that underwent combination therapy had improved sperm parameters as compared to the other 3 groups (Azizollahi et al., 2013). In this study, although the authors observed improvement in sperm parameters after varicocelectomy, it wasn't of great significance. But with the treatment of zinc and folic acid, these sperm parameters increased to a significant level along with an increase in acrosomal integrity and protamine content. Further studies must be carried out to study long term effects of folate if any and pregnancy outcomes of this medication.

In yet another similar study with the same study groups, the terminal blood hormonal levels were also evaluated after 6 months. It was evident that the group that underwent combination therapy had elevated levels of blood inhibin B levels and superoxide dismutase (SOD) in comparison to others (Azizollahi et al., 2014). Inhibin B has an important role in testicular hormonal functions such as FSH and testosterone (Fujisawa et al., 2001). More precisely, this study has concluded for greater effects of zinc with or without folate as compared to the effects with folate alone, implicating the role of zinc in these mechanisms which has to be established thoroughly through future studies.

There was also a study conducted with a daily dose of this antioxidant combination (5mg folic acid and 66mg zinc sulfate) and pentoxifylline (1200mg) for 3 months. This was done to test the efficiency of oral therapy for preceding the ART procedure (Review and Garibaldi, 2009). Pentoxifylline is involved in oxidative metabolism and preserves antioxidant activities. It has vascular and rheological properties that help in the improvement of seminal volume and motility (Samlaska and Winfield, 1994). In the study, however, among the semen parameters, the most significant improvement seen at the early stage (within 4 weeks) was the sperm morphology whereas seminal volume and sperm motility slightly increased at the later stage of treatment (8 to 12 weeks). Nevertheless, with regard to pregnancies recorded, it summed up to a total of 55% where 33% were spontaneous pregnancies, 28% were pregnancies after undergoing ART post oral therapy. Discussions can also be extended about pregnancy rates recorded by other similar studies with varicocelectomy as an alternative surgical procedure. Spontaneous pregnancy was recorded at 28% to 40% for post varicocelectomy patients. When varicocelectomy was combined with ART, the overall pregnancy rate of 53% was recorded [68,69]. There is hence a positive correlation that can be established between the oral treatment, varicocelectomy and ART procedures. It is also worthy to note here that the improvement in sperm morphology lasted until 4 weeks of cessation of therapy as compared to other therapies where the parameters return to baseline almost soon after the treatment is stopped.

Carnitine

Carnitine is a widely popularized group of elements that have potential health benefits. L-Carnitine (LC) and L-Acetyl-Carnitine (LAC) help in protecting the DNA and plasma membrane from the damage induced by oxygen radicals (Zhou et al., 2007). LC which is a derivative of

methionine and lysine helps in beta-oxidation of fatty acids in mitochondria and thus in energy production (Gandini et al., 2004.). It also serves to suppress the macrophages in semen by imitating glucocorticoid (Adel et al., 2009). Numerous studies have been carried out in infertile male subjects to determine the effect of oral medical treatment of carnitine on semen parameters and reversal of fertility [70,73].

With specific to varicocele, a study was conducted in 2016 to compare the effects of varicocelectomy and oral LC supplementation on varicocele grade 2 patients until 6 months. 62 patients were considered for this double-blind clinical trial without randomization. It was concluded that the medical therapy was as effective as the surgery in terms of improving sperm parameters such as count, volume, motility, and morphology (Sofimajidpour et al., 2016). However, randomization of the trial with a higher number of subjects is needed for conclusive data representation on the same.

Prior to this research, a combination study was conducted on varicocele patients. The patients were divided into 3 groups; Group 1 was given a placebo and the other two groups were administered with LC+LAC and LC+LAC+cinnoxicam respectively. As mentioned earlier, in the cinnoxicam section, it was concluded that the combination of all three antioxidants proved to be effective for treating low-grade varicocele. This was concluded by pregnancy rates as well which were 38% for group 3 as compared to 1.7% and 21.8% for groups 1 and 2 respectively (Cavallini et al., 2004). Nevertheless, these values returned to baseline after cessation of treatment. It was also observed that no drug was effective for grade 5 varicocele. The higher the degree of varicocele, the lower was the effect of drugs. Similar to this study, another double-blind placebo-controlled combination study was conducted with 104 infertile subjects with or without varicocele. They were administered with LC+LAC+other micronutrients for 6 months. All the positive changes in sperm parameters were more evident in varicocele patients as compared to the ones without (Busetto et al., 2018). However, with respect to the previous study discussed, this treatment was not compared with the effects of varicocelectomy.

As an overall future prospective, studies that can give solid evidence affirming the replacement of surgery with oral supplementation is necessary for effectively treating varicocele. If not replacement therapy, an association between the two should be more conclusively studied for addressing the challenges of varicocele treatment. A summary of the studies conducted so far in this arena is put forth in Table 1.

Table 1: Timeline of Clinical studies performed for management of varicocele using various antioxidant therapies

Sl.No	Year & Reference	Antioxidant	Combination	Varicocelectomy	Study Design	Results
1	1987	Zinc	None	Adjuvant with varicocelectomy	Prospective uncontrolled study	Increase in sperm motility improvement in pregnancy rate
2	1990	Kallikrein	none	sole therapy	Randomised placebo controlled study	Increase in sperm motility and morphology
3	2003	Cinnoxicam	None	Compared with	RCT	Effective in lower grades

				varicocelectomy		varicocele
4	2004	Cinnoxicam	L-carnitine + acetyl L-carnitine	Sole therapy	Prospective controlled study	Improvement in seminal parameters
						Improvement in pregnancy rate
5	2005	MPPF	None	Sole therapy	Open uncontrolled study	Treated pain associated with varicocele
						Improvement in Sperm motility
6	2006	Vitamin E	Chinese medicine + dexamethasone	Adjuvant with varicocelectomy	Prospective uncontrolled study	Improvement in sperm density and motility
						Improvement in pregnancy rate
7	2009	Folic acid	Zinc + Pentoxifyllin	Sole therapy	Open uncontrolled study	Improvement in sperm morphology
8	2010	HR	None	Sole therapy	Open controlled study	Development of palpable varicocele from subclinical condition was reduced
9	2012	MPPF	None	Sole therapy	RCT	Increase sperm motility
						Alleviate pain associated with varicocele
						Withdrawal of drug did not cause recurrence of pain
10	2012	Selenium	Vitamin A + Vitamin E + L-carnitine + Pentoxifylline + clomiphene citrate + antioxidants	Compared with varicocelectomy	Prospective controlled study	Improvement in sperm concentration
						Comparitively lesser pregnancy outcomes
11	2013	Folic acid	Zinc	Adjuvant with varicocelectomy	Prospective RCT	Improvement in semen parameters
						Increase in acrosome integrity
						Increase in protamine content
12	2014	MPPF	None	Adjuvant with varicocelectomy	RCT (Rats)	Effective adjunct with varicocelectomy
13	2014	Coenzyme Q10	None	sole therapy	Open prospective uncontrolled study	Improvement in seminal parameters
						Improvement in antioxidant capacity
14	2014	Vitamin E	Testosterone	Sole therapy	RCT (Rats)	Improvement in antioxidant capacity
						Upregulation of HSP70-2

15	2014	Folic acid	Zinc	Adjuvant with varicocelectomy	RCT	Elevated blood inhibin B level
						Increase in SOD
16	2015	Vitamin C	none	Adjuvant with varicocelectomy	DBPCT	Improvement in sperm motility and morphology
17	2016	L - carnitine	None	Compared with varicocelectomy	DBPCT	As effective in improving sperm parameters
18	2017	Selenium	None	Sole therapy	RCT(Rats)	Improvement in antioxidant capacity
						Protection against testicular damage
19	2018	Vitamin C	Other antioxidants	Sole therapy	DBPCT	Improvement in sperm parameters
20	2018	L - carnitine	acetyl L-carnitine + micronutrients	Sole therapy	DBPCT	Improvement in sperm parameters

Conclusion

Despite the benefits available in medical management of varicocele, there are very few studies being done to concretely prove a certain antioxidant to be promisingly reliable for its treatment. There is a need for conducting bigger and diverse sample studies for developing an effective treatment backed with pregnancy outcome reports as well. Exploring various possibilities such as optimizing combination approaches, using antioxidant treatment as an adjuvant to surgery and to conduct grade-wise studies can be future prospects in treating varicocele.

Conflict of interest

The authors declare that they have no conflict of interests

References

1. Abd-Allah AR, Helal GK, Al-Yahya AA, Aleisa AM, Al-Rejaie SS, Al-Bakheet SA. Pro-inflammatory and oxidative stress pathways which compromise sperm motility and survival may be altered by L-carnitine. Oxidative medicine and cellular longevity. 2009 Apr 1;2.
2. Majzoub A, Agarwal A. Antioxidant therapy in idiopathic oligoasthenoteratozoospermia. Indian journal of urology: IJU: journal of the Urological Society of India. 2017 Jul;33(3):207.
3. Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: part 1. Nature Reviews Urology. 2012 Dec;9(12):678.
4. Al-kandari AM, Shabaan H, Ibrahim HM, Elshebiny YH, Shokeir AA. Comparison of Outcomes of Different Varicocelectomy Techniques: Open Inguinal, Laparoscopic, and Subinguinal Microscopic Varicocelectomy: A Randomized Clinical Trial. Urology. 2007;417–420.

5. Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, Santulli M, Agarwal A, Gandini L. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. Fertility and sterility. 2004 Jun 1;81(6):1578-84.
6. Azizollahi G, Azizollahi S, Babaei H, Kianinejad M, Baneshi MR, Nematollahi-mahani SN. Effects of supplement therapy on sperm parameters, protamine content and acrosomal integrity of varicocelectomized subjects. Journal of assisted reproduction and genetics. 2013 Apr 1;30(4):593-9.
7. Azizollahi GH, Nematollahi-Mahani SN, Baneshi MR, Safari Z, Azizollahi S. Effect of folic acid and zinc sulphate on endocrine parameters and seminal antioxidant level after varicocelectomy. Andrologia. 2014 Apr;46(3):240-5.
8. BARTEL J, BARTZ T, WOLF C, CHARKIEWICZ E, KÜHBACHER M, POHL T, KYRIAKOPOULOS A. Activity of the glutathione peroxidase-2. Differences in the selenium-dependent expression between colon and small intestine. Cancer Genomics-Proteomics. 2007 Sep 1;4(5):369-72.
9. Bolcal C, Sargin M, Mataraci I, Iyem H, Doganci S, Kilic S, Temizkan V, Demirkilic U, Tatar H. Concomitance of varicoceles and chronic venous insufficiency in young males. Phlebology. 2006 Jun 1;21(2):65-9.Bors, W., Heller, W., Michel, C., and Saran, M. (1990). Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. Methods Enzymol.
10. Balercia G, Buldreghini E, Vignini A, Tiano L, Paggi F, Amoroso S, Ricciardo-Lamonica G, Boscaro M, Lenzi A, Littarru G. Coenzyme Q10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. Fertility and sterility. 2009 May 1;91(5):1785-92.
11. Busetto GM, Agarwal A, Virmani A, Antonini G, Ragonesi G, Del Giudice F, Micic S, Gentile V, De Berardinis E. Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study. Andrologia. 2018 Apr;50(3):e12927.
12. Cavallini G, Biagiotti G, Ferraretti AP, Gianaroli L, Vitali G. Medical therapy of oligoasthenospermia associated with left varicocele. BJU international. 2003 Apr;91(6):513-8.
13. Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. Journal of andrology. 2004 Sep 10;25(5):761-70.
14. Cervellione RM, Corroppolo M, Bianchi A. Subclinical varicocele in the pediatric age group. The Journal of urology. 2008 Feb;179(2):717-9.
15. Takihara H, Cosentino MJ, Cockett AT. Zinc sulfate therapy for infertile male with or without varicocelectomy. Urology. 1987 Jun 1;29(6):638-41.
16. Cyrus A, Kabir A, Goodarzi D, Moghimi M. The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: a double blind placebo controlled clinical trial. International braz j urol. 2015 Apr;41(2):230-8.
17. Diegidio P, Jhaveri JK, Ghannam S, Pinkhasov R, Shabsigh R, Fisch H. Review of current varicocelectomy techniques and their outcomes. BJU international. 2011 Oct;108(7):1157-72.
18. Dogan F, Armagan A, Oksay T, Akman T, Aylak Fİ, Bas E. Impact of micronised purified flavonoid fraction on increased malondialdehyde and decreased

metalloproteinase-2 and metalloproteinase-9 levels in varicocele: outcome of an experimentally induced varicocele. Andrologia. 2014 May;46(4):380-5.
19. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. Circulation. 2014 Jul 22;130(4):333-46..
20. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Human reproduction update. 2007 Mar 1;13(2):163-74.
21. Esteves SC, Cho CL, Majzoub A, Agarwal A, editors. Varicocele and Male Infertility: A Complete Guide. Springer Nature; 2019 Sep 11.
22. Evers JL, Collins JA. Assessment of efficacy of varicocele repair for male subfertility: a systematic review. The Lancet. 2003 May 31;361(9372):1849-52.
23. Evers JL, Collins J. Surgery or embolisation for varicocele in subfertile men. Cochrane Database of Systematic Reviews. 2004(3).
24. Ferramosca A, Albani D, Coppola L, Zara V. Varicocele negatively affects sperm mitochondrial respiration. Urology. 2015 Oct 1;86(4):735-9.
25. Festa R, Giacchi E, Raimondo S, Tiano L, Zuccarelli P, Silvestrini A, Meucci E, Littarru GP, Mancini A. Coenzyme Q10 supplementation in infertile men with low-grade varicocele: an open, uncontrolled pilot study. Andrologia. 2014 Sep;46(7):805-7.
26. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proceedings of the National Academy of Sciences. 1991 Dec 15;88(24):11003-6.
27. Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. Proceedings of the National Academy of Sciences. 1990 Jun 1;87(12):4879-83.
28. Fujisawa M, Dobashi M, Yamasaki T, Kanzaki M, Okada H, Arakawa S, Kamidono S. Significance of serum inhibin B concentration for evaluating improvement in spermatogenesis after varicocelectomy. Human Reproduction. 2001 Sep 1;16(9):1945-9.
29. Gamidov SI, Ovchinnikov RI, Popova AI, Tkhagapsoeva RA, Izhbaev SK. Current approach to therapy for male infertility in patients with varicocele. Therapeutic archive. 2012 Oct 15;84(10):56-61.
30. Garg H, Kumar R. An update on the role of medical treatment including antioxidant therapy in varicocele. Asian journal of andrology. 2016 Mar;18(2):222.
31. Guo YJ, Wang ZJ, Cao M, Kang GJ. An integrated method works well on varicocele. Zhonghua nan ke xue= National journal of andrology. 2006 Sep;12(9):800.
32. Hamada A, Esteves SC, Agarwal A. Insight into oxidative stress in varicocele-associated male infertility: part 2. Nature Reviews Urology. 2013 Jan;10(1):26.
33. Hendin BN, KOLETTIS PN, SHARMA RK, THOMAS JR AJ, AGARWAL A. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. The Journal of urology. 1999 Jun 1;161(6):1831-4.
34. Isidori A, Latini M, Romanelli F. Treatment of male infertility. Contraception. 2005 Oct 1;72(4):314-8.
35. Jacob RA, Planalto FS, Agee RE. Cellular ascorbate depletion in healthy men. The Journal of nutrition. 1992 May 1;122(5):1111-8.
36. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, Krausz C, EAU Working Group on Male Infertility. European Association of Urology guidelines on Male

Infertility: the 2012 update. European urology. 2012 Aug 1;62(2):324-32.
37. Katsenis K. Micronized purified flavonoid fraction (MPFF)*: a review of its pharmacological effects, therapeutic efficacy and benefits in the management of chronic venous insufficiency. Current Vascular Pharmacology. 2005 Jan 1;3(1):1-9.
38. Ketabchi AA, Ketabchi M. Psychological effects of infertility in clinical varicocele patients. Health. 2011 Aug 10;3(8):504-6.
39. Khosravanian N, Razi M, Farokhi F, Khosravanian H. Testosterone and vitamin E administration up-regulated varicocele-reduced Hsp70-2 protein expression and ameliorated biochemical alterations. Journal of assisted reproduction and genetics. 2014 Mar 1;31(3):341-54.
40. Kiliç S, Güneş A, Ipek D, Dusak A, Güneş G, Balbay MD, Baydinç YC. Effects of micronised purified flavonoid fraction on pain, spermiogram and scrotal color Doppler parameters in patients with painful varicocele. Urologia internationalis. 2005;74(2):173-9.
41. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertility and sterility. 1997 Sep 1;68(3):519-24.
42. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function. Molecular Aspects of medicine. 1997 Jan 1;18:213-9.
43. Zini A, Boman J, Baazeem A, Jarvi K, Libman J. Natural history of varicocele management in the era of intracytoplasmic sperm injection. Fertility and sterility. 2008 Dec 1;90(6):2251-6.
44. Lopes-Santiago BV, Monteiro GA, Bittencourt R, Arduino F, Ovidio PP, Jordão-Junior AA, Araùjo Jr JP, Lopes MD. Evaluation of sperm DNA peroxidation in fertile and subfertile dogs. Reproduction in Domestic Animals. 2012 Dec;47:208-9.
45. Mancini A, Conte B, De Marinis L, Hallgass ME, Pozza D, Oradei A, Littarru GP. Coenzyme Q10 levels in human seminal fluid: diagnostic and clinical implications. Molecular aspects of medicine. 1994 Jan 1;15:s249-55.
46. Mancini A, Festa R, Raimondo S, Silvestrini A, Giacchi E, Littarru GP, Pontecorvi A, Meucci E. Biochemical alterations in semen of varicocele patients: a review of the literature. Advances in urology. 2012 Jan 1;2012.
47. Marmar JL. Varicocele and male infertility: Part II: The pathophysiology of varicoceles in the light of current molecular and genetic information. Human Reproduction Update. 2001 Sep 1;7(5):461-72.
48. Mićić S, Tulić C, Dotlić R. Kallikrein Therapy of Infertile Men with Varicocele and Impaired Sperm Motility: Therapie mit Kallikrein bei infertilen Männern mit Varicocele und reduzierter Spermatozoenmotilität. Andrologia. 1990 Mar 4;22(2):179-83.
49. Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A. The kallikrein-kinin system: current and future pharmacological targets. Journal of pharmacological sciences. 2005;99(1):6-38.
50. Moslemi MK, Tavanbakhsh S. Selenium–vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. International journal of general medicine. 2011;4:99.
51. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian journal of pharmacology. 2001 Feb;33(1):2-16.

52. Noack-Füller G, De Beer C, Seibert H. Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. Andrologia. 1993 Jan 2;25(1):7-12.
53. Olkkola KT, Brunetto AV, Mattila MJ. Pharmacokinetics of oxicam nonsteroidal anti-inflammatory agents. Clinical pharmacokinetics. 1994 Feb 1;26(2):107-20.
54. Giovenco P, Amodei M, Barbieri C, Fasani R, Carosi M, Dondero F. Effects of kallikrein on the male reproductive system and its use in the treatment of idiopathic oligozoospermia with impaired motility. Andrologia. 1987 Jun;19(S1):238-41.
55. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American college of Nutrition. 2003 Feb 1;22(1):18-35.
56. Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Medical Principles and Practice. 2008;17(2):108-16.
57. Oliva A, Dotta A, Multigner L. Pentoxifylline and antioxidants improve sperm quality in male patients with varicocele. Fertility and sterility. 2009 Apr 1;91(4):1536-9.
58. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. The Journal of urology. 2009 Feb;181(2):741-51.
59. Samlaska CP, Winfield EA. Pentoxifylline. Journal of the American Academy of Dermatology. 1994 Apr 1;30(4):603-21.
60. Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. Fertility and sterility. 2006 May 1;85(5):1409-14.
61. Söylemez H, Kiliç S, Atar M, Penbegül N, Sancaktutar AA, Bozkurt Y. Effects of micronised purified flavonoid fraction on pain, semen analysis and scrotal color Doppler parameters in patients with painful varicocele; results of a randomized placebo-controlled study. International urology and nephrology. 2012 Apr 1;44(2):401-8.
62. Sofimajidpour H, Ghaderi E, Ganji O. Comparison of the effects of varicocelectomy and oral L-carnitine on sperm parameters in infertile men with varicocele. Journal of clinical and diagnostic research: JCDR. 2016 Apr;10(4):PC07.
63. Struckmann JR. Clinical efficacy of micronized purified flavonoid fraction: an overview. Journal of vascular research. 1999;36(Suppl. 1):37-41.
64. Suleiman SA, Ali ME, Zaki ZM, El-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. Journal of andrology. 1996 Sep 10;17(5):530-7.
65. Taghizadeh L, Eidi A, Mortazavi P, Rohani AH. Effect of selenium on testicular damage induced by varicocele in adult male Wistar rats. Journal of Trace Elements in Medicine and Biology. 2017 Dec 1;44:177-85.
66. Tapas A, Sakarkar D, Kakde R. The Chemistry and Biology of Bioflavonoids. Research Journal of Pharmacy and Technology. 2008;1(3):132-43.
67. Therond P, Auger J, Legrand A, Jouannet P. α-Tocopherol in human spermatozoa and seminal plasma: relationships with motility, antioxidant enzymes and leukocytes. MHR: Basic science of reproductive medicine. 1996 Oct;2(10):739-44.
68. Wadworth AN, Faulds D. Hydroxyethylrutosides. A review of its pharmacology, and therapeutic efficacy in venous insufficiency and related disorders. Drugs. 1992 Dec 1;44(6):1013-32.

69. Wong WY, Thomas CM, Merkus JM, Zielhuis GA, Steegers-Theunissen RP. Male factor subfertility: possible causes and the impact of nutritional factors. Fertility and sterility. 2000 Mar 1;73(3):435-42.
70. Yamamoto M, Hibi H, Hirata Y, Miyake K, Ishigaki T. Effect of varicocelectomy on sperm parameters and pregnancy rate in patients with subclinical varicocele: a randomized prospective controlled study. The Journal of urology. 1996 May;155(5):1636-8.
71. Zampieri N, Pellegrino M, Ottolenghi A, Camoglio FS. Effects of bioflavonoids in the management of subclinical varicocele. Pediatric surgery international. 2010 May 1;26(5):505-8.
72. Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. Asia Pacific journal of clinical nutrition. 2007;16:383.
73. Zini A, Dohle G. Are varicoceles associated with increased deoxyribonucleic acid fragmentation?. Fertility and sterility. 2011 Dec 1;96(6):1283-7.