


Review Article

Oxidative stress, nitric oxide and inflammation in the pathophysiology of varicocele and the effect of hydrogen sulfide as a potential treatment

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Abstract

Varicocele is defined as the non-palpable enlargement of the spermatic venous plexus, which has implications on the sperm quality and fertility. The guidelines for managing varicocele in adolescents are not fully determined yet. However, based on the recent reports, during varicocele injuries to testicular tissues may be a result of the formation of reactive oxygen species (ROS) and the subsequent oxidative damage. The testis has a high metabolism rate and cell replication which itself causes excessive production of the ROS and decreases antioxidant capacity. Massive generation of ROS and their interaction with lipids, proteins and nucleic acids has adverse effects on the normal cell function. This review article describes the varicocele, its etiology, pathophysiological mechanisms and current treatment methods. An electronic search has been conducted, during 2018, via PubMed and Medline database English literature. Peer-reviewed articles were targeted and the following key-words were used: varicocele, diagnosis, etiology, cellular and molecular mechanisms. Available full-text articles were read. Related articles were also scrutinized. A hand search was also driven. Taken together, this review article mention three main pathophysiology of varicocele that can be treated by natural antioxidant. In addition, hydrogen sulfide as another factor was discussed because the reduction of this substance contribute to the male infertility which induce by varicocele.

Keywords:

Varicocele;
Diagnosis;
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Cellular and molecular
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Introduction

Varicocele is defined as non-palpable enlargement of the venous plexus of the spermatic tone which leads to infertility in men (Chen and Huang, 2010). The disease has implications on the sperm quality and fertility (Çayan et al., 2017). There are numerous methods for the treatment of varicocele. Varicocelectomy improves testicular catch-up growth

and sperm parameters in adolescents. The guidelines for managing varicocele are not fully defined, but the major surgical indications are testicular loss of volume followed by testicular pain. The most common operations are hernia surgery and varicocelectomy (Darzi et al., 2013). There is a growing interest in the use of medications and herbal medicine in the management of the varicocele. This review article describes the epidemiology and etiology as well as the diagnosis, cellular and molecular mechanism and

treatment of the varicocele in the adolescent. The main aim and logic of this review article was to gather three main pathophysiology of varicocele in addition to introduce and discuss hydrogen sulfide (H₂S) as another factor contributing the male infertility.

For this review, peer-reviewed articles with the following key-words were used: varicocele x treatment x etiology x mechanism of action x antioxidant activity. Available full-text articles were read. Related articles were also scrutinized. A hand search was also driven. The search was carried out using Biological Abstracts and Chemical Abstracts, in addition to an electronic search that has been conducted via PubMed and Medline database English literature during December 2018. The references found in the search were then studied in detail.

Male infertility

Male infertility refers to a male’s inability to induce pregnancy in a fertile female. “Male factor” infertility is

seen as an alteration in sperm concentration, motility and/or morphology. In humans, it accounts for 40–50% of the infertility (Lotti and Maggi, 2015). Male infertility is commonly due to deficiencies in the semen and the semen quality is used as a surrogate measure of male productiveness (Lotti and Maggi, 2015). The most significant of these are low sperm concentration (oligospermia), poor sperm motility (asthenospermia) and abnormal sperm morphology (teratospermia) (Harris et al., 2011). Other infertility factors include semen volume and epididymal, prostatic and seminal vesicle function (Harris et al., 2011). The problem with sperm count, motility and morphology originate from the disturbance in a normal mechanism, including pre-testicular, testicular and post-testicular factors which are listed below (Butt and Akram, 2013) (Fig. 1).

Pre-testicular factors

These are conditions that impede adequate support of the testes and include situations of poor hormonal

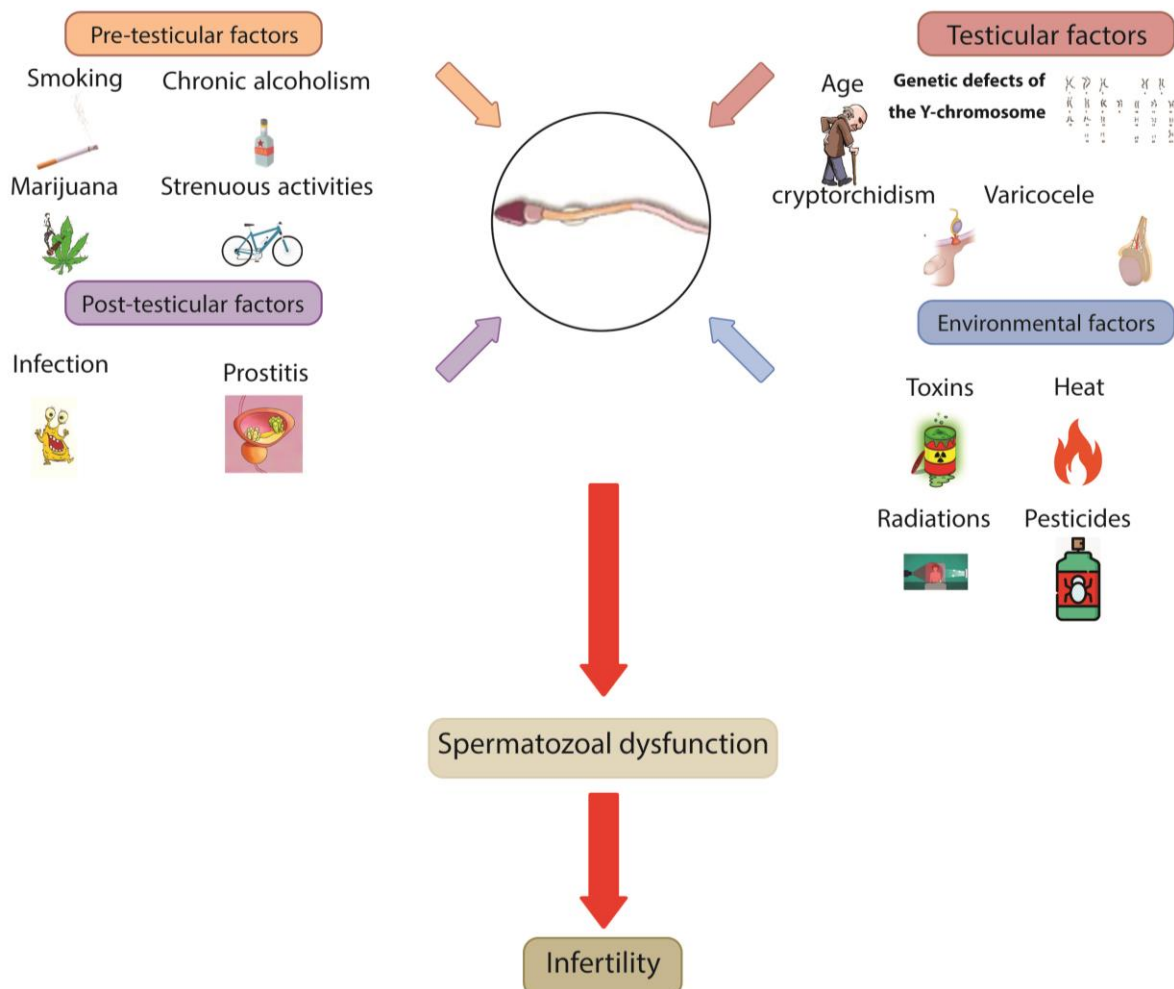


Fig.1. The summary of various factors leading to male infertility.

support and poor general health including hypogonadism, drugs such as cimetidine and spironolactone that decrease follicle stimulating hormone levels and nitrofurantoin that decreases sperm motility, adopted life style (chronic alcoholism, marijuana, cigarette smoking) and strenuous activities such as strenuous bicycle riding (Olooto, 2012).

Testicular factors

These are factors that affect quality and quantity of semen produced by the testes including age, genetic defects of the Y-chromosome (Klinefelter syndrome), neoplasm e.g. seminoma, cryptorchidism, varicocele and idiopathic factors which account for 30% of male infertility (Olooto, 2012).

Post-testicular factors

Post-testicular factors decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract problems, infection, prostatitis, ejaculatory duct obstruction and hypospadias (Olooto, 2012).

Environmental factors

The etiological importance of environmental factors in infertility has been mentioned in different studies. The implication of toxins, chemical dust and pesticides in infertility is established. Radiations and excessive heat to the genitalia have a damaging effect on the testicles. Exposure to phthalates which may occur via dietary consumption, dermal absorption or inhalation has been linked with impaired spermatogenesis and increased sperm DNA damage (Olooto, 2012).

What is varicocele?

Varicocele is defined as dilatation or tortuosity of the pampiniform plexus veins (Masson and Brannigan, 2014). Clinically, it is commonly occurring on the left side, while the prevalence of bilateral varicoceles is also high (Han et al., 2016). Even though the internal spermatic vein is responsible for varicocele formation, dilated external spermatic veins also contribute to recurrent varicoceles (Masson and Brannigan, 2014). Because the pathophysiology of testicular damage in varicocele is not completely elicited, more studies are required (Moudi, 2016). Varicoceles cause progressive impairment of spermatogenesis leading to final infertility (Masson and Brannigan, 2014).

Epidemiology

Varicocele is identified in both young and adolescent males. The frequency of the varicocele and progressive disease is higher in elderly males (Esteves et al., 2011). There is a negative correlation on the incidence of the varicocele with body mass index which lean men are more prone to varicocele than normal individuals (Esteves et al., 2011). The prevalence of varicocele in the general population varies from 4-30 percent (Han et al., 2016). The 30-50 percent of infertile males, have varicocele related infertility. Adult males with varicoceles, who have normal semen parameters, may be at risk for progressive testicular dysfunction and should be offered to monitor with semen analyses every one to two years, in order to detect the earliest sign of reduced spermatogenesis. Adolescent males with unilateral or bilateral varicoceles and decreased testicular size should be considered for varicocele (Han et al., 2016).

Diagnosis

Evaluation of a patient suspected to varicocele should include medical and reproductive history, physical examination and semen analyses. The physical examination should be performed in recumbent and upright positions. A palpable varicocele feels like a "bag of worms" and disappears when the patient is recumbent. When a suspected varicocele is not clearly palpable, the scrotum should be examined while the patient performs a Valsalva maneuver in a standing position (Hamada et al., 2016). The varicocele is defined into 3 grades. The scrotum is first visually inspected for any obvious distention around the spermatic cord (Hamada et al., 2016). If the varicocele is not palpable but the patient performs a Valsalva maneuver which distends the pampiniform plexus of veins, grade 1 varicocele is present. Subtler varicoceles may feel like a thickened or asymmetric cord. The nonvisible, but palpable varicocele is considered to be grade 2 and finally a visible varicocele is considered grade 3 (Elbendary and Elbadry, 2009). Idiopathic varicocele is more prominent in the upright position and disappears in the supine position (Agarwal et al., 2016). The volume of normal testis measures 1 to 2ml in the prepubertal male (Agarwal et al., 2016). Testicular ultrasound is the most accurate and reproducible method to assess testicular volume and size

variations (Elbendary and Elbadry, 2009).

Etiology

There are several theories that are suggested for the etiology of the varicocele. The predominance of the left side varicocele and anatomy of the left testicular vein are the basis explanation of the varicocele (Celik et al., 2016). Testicular torsion is a urologic emergency in the patients which leads to serious infertility (Parlaktas et al., 2014). The non-palpable enlargement of the venous plexus may be diagnosed by imaging techniques and is defined as subclinical varicocele (Celik et al., 2016). Detection of subclinical varicocele is not an indication for surgical repair, because there have been men with subclinical varicocele which failed to increase postoperative pregnancy rate. The left testicular vein is longer than the right, so the drop in its hydrostatic pressure is the cause for left varicocele (Kirby et al., 2016). Several studies have determined the effects of varicocele on testicular function, but there is no clear conclusion for the incidence of varicocele leading to male fertility (Agarwal et al., 2014). Numerous mechanisms such as hypoxia and stasis, testicular venous hypertension, autoimmunity, testicular temperature and oxidative stress are the main discussed causes of the varicocele (Hsieh et al., 2006). The testicular torsion detected by the circulatory failure may be caused by testis revolving around the vascular peduncle (Yuluğ et al., 2013). Testicular torsion detorsion is a kind of ischemia/reperfusion (I/R) injury to the testis (Asghari et al., 2016). Ischemia followed by reperfusion is implicated as the main pathophysiologic event in testicular torsion (Pogorelič et al., 2016). The duration and degree of twisting of the cord are closely related to the severity of the testicular injury (Sarac et al., 2017). Following unilateral torsion, the blood flow of both the ipsilateral and the contralateral internal spermatic arteries are decreased and the long-term consequences of these changes will result in impaired testicular function and fertility (Yuluğ et al., 2013). Interruption in tissue blood supply during I/R leads to cellular and tissue damage (Mirhoseini et al., 2017). Testicular torsion eventually terminates in tissue degeneration and usually requires emergency surgical intervention for reperfusion of the affected testis (Celik et al., 2016). The male infertility is routinely diagnosed by semen parameters including semen volume, sperm count,

sperm morphology and sperm viability (Agarwal et al., 2014). Sperm characteristics are reported to significantly diminish in varicocele-induced animals comparing to normal ones.

Cellular and molecular mechanisms and new findings in varicocele treatment

Oxidative stress

During the varicocele, oxidation damage affects testicular tissues as a result of reactive oxygen species (ROS) production (Asghari et al., 2016). Testis and spermatozoa contain high levels of fatty acids which makes them vulnerable to the ROS and since they have high metabolism rate and cell replication excessive production of the ROS decreases their antioxidant capacity (Tuglu et al., 2015). ROS interacts with lipids, proteins and nucleic acids with eventually adverse effects on cell function and histology (Yuluğ et al., 2013). Malondialdehyde (MDA) is the end product of lipid peroxidation and increased MDA level has an adverse effect on sperm fertility (Asadi et al., 2017).

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) act as a complicated defense system against ROS. The enzyme SOD neutralizes superoxide anion which plays a pivotal role in Osteoarthritis inflammation (Toman et al., 2011). GPx is an important enzyme, detoxifying peroxides and hydro peroxides. Total antioxidant capacity is an indicator of the overall protective effect of antioxidants in body fluids, on cell membranes and other components of cells against oxidative injury (Yarmohammadi et al., 2014). Based on our obtained results, it was possible that the increase in TAC and activities of enzymatic antioxidants in the pancreatic porcine elastase treated group might be due to their decreased consumption for free radical detoxification or utilization which was approved by following the decrease in serum MDA (Semercioz et al., 2003). GPx is a peroxidase enzyme and protects sperm from oxidative damage and lipid peroxidation (Lee et al., 2012). In the physiologic condition, antioxidants savage free radicals while during oxidative stress, imbalance occurs between ROS and scavenges free antioxidant (Agarwal et al., 2014). Ischemia increases in intracellular hypoxanthine as a result of ATP breakdown and xanthine oxidase converts hypoxanthine and superoxide radicals.

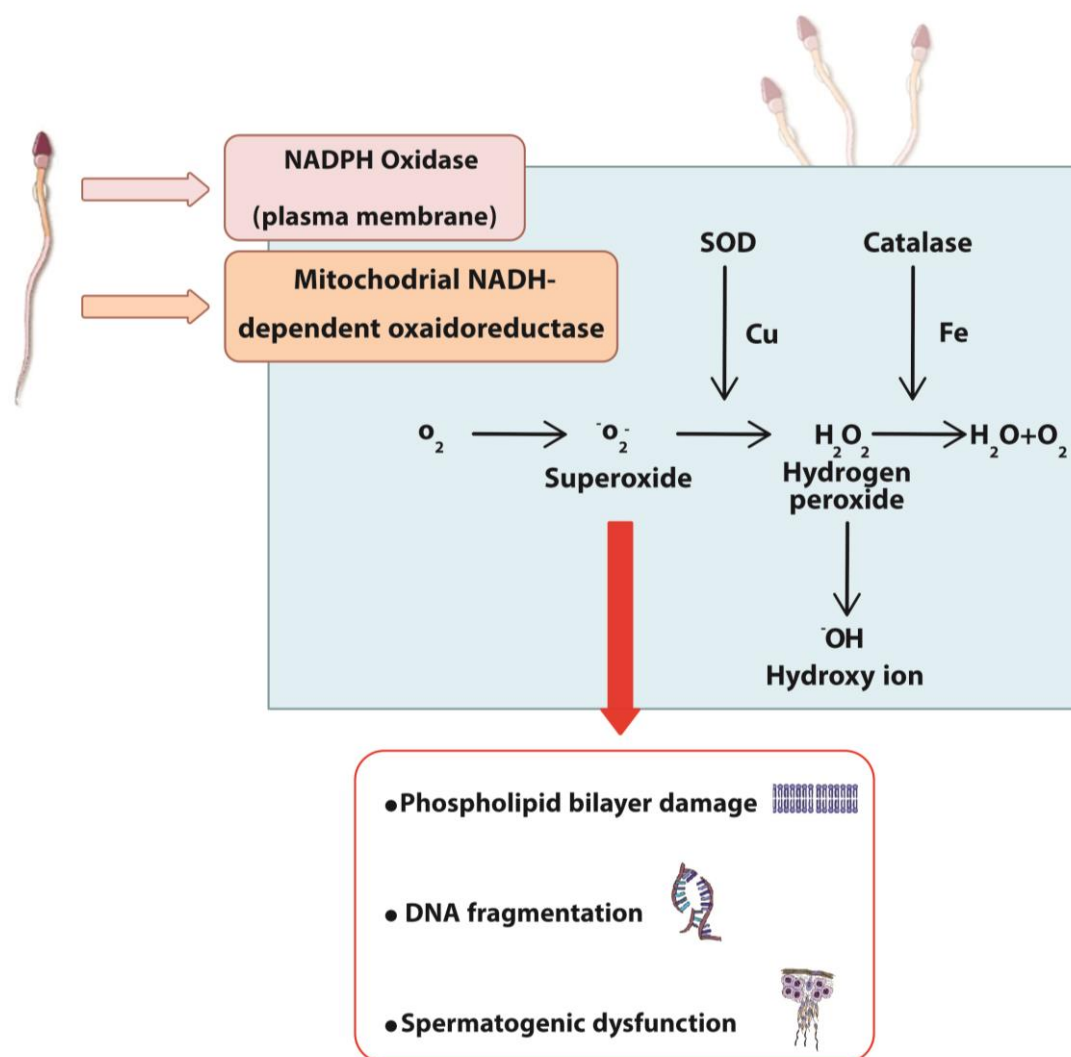


Fig.2. Molecular mechanisms of oxidative stress-induced varicocele. NADPH oxidase in plasma membrane and mitochondrial NADH-dependent oxidoreductase cause production of reactive oxygen species (e.g. superoxide) which react with various biological molecules such as DNA. In the physiologic condition, antioxidant system (e.g. SOD and catalase) can defend against reactive oxygen species while during oxidative stress, imbalance occurs between ROS and antioxidants which results in testicular tissues leading to varicocele. NAD: nicotinamide adenine dinucleotide, NADPH: nicotinamide adenine dinucleotide phosphate, SOD: superoxide dismutase, DNA: deoxyribonucleic acid.

The GPx and Catalase are the first line of cellular defense against oxidative stress (Agarwal et al., 2014). During testicular torsion and detorsion, lipid peroxidation is an indicator of enhanced formation of ROS. Following treatment, improvements were detected in the sperm penetration assay, strict morphology quantification, oxidant determination (ROS) and DNA fragmentation (Miyaoka and Esteves, 2012). Testicular volume upsurge after varicocelectomy in adolescents with preoperative testicular volume which involved increased left testis size to normal within 12 months after surgery (Cocuzza, 2017) (Fig. 2).

Male fertility is a complex phenomenon and physical or chemical damage and toxins or pathophysiologic

conditions such as prostate (Aliramaji et al., 2014) can affect. Correlation between varicocele and fertility is well known (Aliramaji et al., 2014). There is still an ongoing debate among researchers to the extent varicocele affects semen parameters, which usually vary from normal to mild asthenospermia, teratospermia or asthenoteratospermia. Initially, sperm concentration is not seriously affected; though later all three sperm parameters can gradually deteriorate, resulting in azoospermia in very few cases (Damsgaard et al., 2016). The low sperm concentration is attributed to the high germ cell apoptosis usually observed in those men, while the low motility is attributed to the increased concentration of ROS or to the presence of anti-

sperm antibodies (Damsgaard et al., 2016).

The ROS are essential for sperm processes leading to successful fertilization, such as capacitation, hyperactivated motility and acrosomal reaction (Agarwal et al., 2014). Changes lead to abnormal sperm function and infertility (Masson and Brannigan, 2014). So, the neutral levels of ROS are critical for normal fertilization, capacitation, hyperactivation and motility (Agarwal et al., 2014). Varicocelectomy reduces ROS levels increase in the antioxidant capacity of semen in infertile men (Masson and Brannigan, 2014). Varicocele that develops in early life is a progressive but surgically correctable disease that deteriorates testicular function and semen mortality (Damsgaard et al., 2016).

ROS-mediated lipid peroxidation facilitates sperm adhesion to the oocyte (Agarwal et al., 2014). Beyond physiologic conditions increased ROS production leads to oxidative stress. Semen samples of infertile males with varicocele have elevated ROS levels. Varicocele is associated with increased DNA fragmentation index which will be improved post-varicocelectomy (Ni et al., 2014). Thus, the imbalance between oxidant load and antioxidant defense system plays a key role in varicocele-induced infertility (Ni et al., 2014). ROS-induced oxidative damage destroys mitochondrial membrane potential and decreases sperm motility (Dorostghoal et al., 2017).

It is important to notice that ROS is also able to damage sperm nucleic acids. Oxidative stress has the potential to induce various types of DNA damage-DNA base pair oxidation, single- or double stranded DNA breaks, chromosomal rearrangements and gene mutations which include deletions, point mutations and polymorphisms (Dorostghoal et al., 2017). Sperms use these enzymatic mechanisms to protect and repair damaged DNA. Yet, these protective mechanisms can be overwhelmed, especially in the presence of excessive ROS, leaving sperms with defective DNA.

New studies are needed to determine the role of oxidative stress in the development of varicocele associated infertility (Malivindi et al., 2018). Proteomic expression analysis in men with varicocele has demonstrated a decreased expression of the SOD1, a gene that encodes for the SOD1 responsible for counteracting free superoxide radicals in cells (Hosseinfar et al., 2013). Differences in gene

expression during varicocele are related to imbalances in the pro and anti-oxidant species in sperm. SOD1, glutathione S-transferases are family of cytosolic and mitochondrial enzymes that act in the removal of oxidative species from cells. Deletions in the glutathione S-transferase M1 (GST-M1) and GST-T1 enzymes during varicocele have been associated with impaired sperm motility and elevated levels of 8-hydroxy-2-deoxyguanosine in sperm DNA (Dorostghoal et al., 2017). Certain genetic lesions disrupt the balance of pro- and antioxidant species in cells. In men with varicocele, such lesions could lead to greater ROS exposure that mediates more significant oxidative damage to the testes, contributing to varicocele-associated infertility.

L-carnitine in combination with the nonsteroidal anti-inflammatory agent cinnoximicam has been studied; however, it failed to show improvement in seminal parameters in men with clinical varicocele (Huang, 2015). Early use of menotropin in combination with surgical repair provided additional improvement in semen parameters when compared with varicocelectomy alone (Weedin et al., 2010). It is reported that melatonin administration, as a potent free radical scavenger, might be helpful in the prevention or at least, in the delay of the severe effects of varicocele on testicular tissue (Saalu et al., 2013). Another experimental study showed that flaxseed, a fat soluble antioxidant, reduced ROS in prepubertal rats with varicocele (Sohrabipour et al., 2013). It is showed that chrysin, a flavonoid in honey, improved sperm parameters via its antioxidant effect (Missassi et al., 2017). Silymarin can ameliorate varicocele decreased spermatogenesis by up-regulating testicular TAC, SOD and GSH-px levels (Mazhari et al., 2018).

Although, the main mechanism for drugs and medications are not fully elicited but it is clear that almost all of these drugs or supplements such as vitamin A, E, C and minerals e.g. magnesium and zinc oxide improve cellular defense system by protection of antioxidant activity via increasing the cellular SOD, GPx and total antioxidant status.

Nitric oxide (NO)

Varicocele associated with a number of factors, including microcirculation disturbance of the testis, vasoactive substance reflux and increase in NO concentration (Li et al., 2015). NO has a role in tonus

regulation, whereas in the male reproductive tract nitric oxide synthase (NOS) has been found in the testes, epididymis and vascular endothelial cells. NO in the physiologic level improves sperm motility and function but excessive NO in sperm causes detrimental effects (Wu et al., 2004).

Endothelial nitric oxide synthase (eNOS) is localized in human Sertoli cells, Leydig cells, premature spermatocytes and early spermatids as well as in degenerated and apoptotic intraepithelial germ cells (Zini et al., 1996). Leydig cells highly express nNOS, expression of which is lower in the cells such as spermatogonia, spermatocytes and spermatids. Inducible NOS is predominantly expressed in peritubular testicular macrophages and reduced expression has been detected in Sertoli, Leydig and germ cells. In the epididymis, epithelial localization of eNOS has also been shown (Zini et al., 1996).

In adolescents with left-sided varicocele, demonstrating that, compared with controls, iNOS expression was increased in Leydig cells. Higher NO levels in the intratesticular fluid and higher iNOS expression in testicular biopsy samples from men with grade 2 and grade 3 varicocele. iNOS is the main source of NO generation in varicocele (Agarwal et al., 2012). Irrespective of the generating mechanism, NO produced by the spermatic veins and testicular tissues can diffuse by the membranes of various germ cells. NO react with the SOD anion generated inside the mitochondria of the germ cells to produce active metabolites which are strong oxidants (Jourdeuil et al., 2001). High levels of NO can interfere with mitochondrial oxygen respiration, particularly through S-nitrosylation of thiol groups of the respiratory chain and leads to the inhibition of ATP production (Poderoso et al., 1996). Excessive NO decreases intracellular reduced glutathione (an antioxidant peptide) by inhibition of glutathione reductase, which exacerbates the oxidative stress (Poderoso et al., 1996).

New experimental pharmacological interventions using NOS inhibitors such as aminoguanidine and N ω -nitro-L-arginine methyl ester (L-NAME) in experimental rat models of varicocele and are recognized to decrease apoptosis and sperm DNA fragmentation as well as improve testicular spermatogenesis, epididymal sperm motility, morphology and vitality (Agarwal et al., 2012). It is reported that curcumin decreased NO levels and improved sperm

parameters in varicocele male rats (Izadpanah et al., 2015). Another study showed that celecoxib ameliorate sperm functions by inhibiting iNOS expression and NO in varicocele rats (Mazhari et al., 2018). Pentoxifylline improves sperm motility by increasing NO production in human spermatozoa (Banihani et al., 2018).

Inflammation

Inflammation has been recognized as an important factor in the onset and development of varicocele (Salama et al., 2003). Tumor necrosis factor- α (TNF- α) and interleukin (IL)-1 β are key inflammatory cytokines involved in the pathological process of varicocele. Germ cell-derived TNF- α increases tissue damage and the inflammatory response (Khosravian et al., 2015) and may control physiological spermatogenic cell apoptosis by regulating Fas ligand levels (Musalam et al., 2010). IL-1 β is an immune-derived cytokine and promotes its own secretion under ischemia and hypoxia and the increased expression of IL-1 β induces detrimental effects in the testes of infertile males with varicocele (Khosravian et al., 2015).

IL-6 is a T cell-derived factor, specifically inducing the differentiation of B lymphocytes into active antibody-producing plasma cells. IL-6 is an early marker of systemic inflammatory responses and tissue damage and is considered to be a central mediator in the cytokine cascade. Interleukins (IL-1, IL-6) may act as a spermatogonial growth factor, identified in Sertoli and germ cells. It was reported that in immature rats with varicocele the IL-6 and interferon-gamma (INF- γ) levels increased progressively (Habibi et al., 2015). INF- γ and TNF- α have inhibiting effects on sperm acrosin activity and the rate of acrosome reaction, which could be attributed to their influence on the activities of Na-K-ATPase, Ca²⁺-ATPase, SOD and NO concentration. INF- γ induces apoptosis and decreases sperm motility in the varicocele. Some cytokines affect sperm motility, viability, capacity and varicocele and decrease testosterone and fertility by decreasing cytokine levels and the number of Sertoli, spermatogonia and Leydig cells (Perdichizzi et al., 2007).

Hydrogen sulfide

H₂S is a colorless, water-soluble, volatile gas with a characteristic smell of rotten eggs which was initially

Table 1: The summary of main factors and possible cellular mechanisms related to varicocele.

Effective factor	Main factors	Cellular function	Cellular mechanism
Oxidative stress (Ni et al., 2015; Ni et al., 2014)	ROS	↑MDA, ↓SOD, ↓GPx, ↓SOD	Damage-DNA base pair oxidation
Inflammation (Perdichizzi et al., 2007)	Elevated inflammatory factors	TNF- α , ↑IL-1 β	Fas ligand
H₂S (Perdichizzi et al., 2007)	H ₂ S	Damage-DNA base pair oxidation	inhibiting NF- κ B nuclear translocation ↓ROS
NO (Agarwal et al., 2012)	NO	nNOS, iNOS, eNOS	mitochondrial oxygen respiration ↓ROS

considered to be a toxic gas. H₂S is the third type of endogenous gaseous transmitter, such as carbon monoxide and NO, and may induce antioxidant, anti-inflammatory and anti-apoptotic effects in various systems. H₂S generated by three enzymes: cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST) which express in different organs. CBS and 3MST are highly expressed in the brain and nervous system, whereas CSE is reported to be predominant in the cardiovascular system (Kimura, 2013). It is also reported that both CBS and CSE are expressed in rat testes but little is known about 3MST expression, and its role in the reproductive system is still unknown (Sugiura et al., 2005). Sodium hydrosulfide (NaHS) has been widely used in clinical trials to evaluate the biological effects of H₂S. Morpholin-4-ium 4 methoxyphenyl (morpholino) phosphonodithioate (GYY4137) releases H₂S in organic solvents which showed protective effects in varicocele rats (Li et al., 2008).

It has been demonstrated that H₂S serves a key role in reducing ROS production and has therapeutic potential in preventing oxidative stress damage in different types of organs. In a recent study, treatment with the novel H₂S donor, phosphonodithioate, alleviated oxidative stress in varicocele testes by reducing the expression of MDA and increasing the expression of SOD (Xia et al., 2019). Furthermore, it has been demonstrated that exogenous H₂S inhibits the activation of caspase-3 to induce an anti-apoptotic effect in renal ischemia-reperfusion injury (Sivarajah et al., 2009).

H₂S is generated in the endocrine and reproductive organs and has different functions for example, H₂S

modulates insulin secretion in pancreatic islets. Adipose tissues have the ability to produce H₂S, which regulates local insulin sensitivity and vascular responsiveness. H₂S also acts on the hypothalamic–pituitary–adrenal axis and is involved in the stress responses. The effects of H₂S on male and female reproductive system have also attracted great interest for their potential therapeutic implications in reproductive disorders. Alterations of H₂S biosynthesis are associated with various endocrine disorders, and hormones may be important factors in the regulation of H₂S production. Understanding the regulatory mechanisms for H₂S synthesis pathways may help develop new therapeutic strategies (Zhu et al., 2011).

We know that H₂S regulates the signal pathway that is related to both inflammation and oxidative stress. H₂S treatment significantly suppresses the increasing of pro-inflammatory factors after the lipopolysaccharide (LPS) injection. The anti-inflammatory effect of H₂S in the testes was most likely mediated by means of inhibiting NF- κ B nuclear translocation, as H₂S likewise prevented the loss of blood-testis barrier integrity in the LPS-treated mice. It was shown that excess production of ROS and a higher level of MDA were inhibited by exogenous administration of H₂S. The antioxidant activity of H₂S may be due to scavenging of ROS, likely mediated by the stimulation of Nrf2 nuclear localization. Nrf2 activates the transcription of multiple genes that encode for proteins to protect cells against oxidative stress (Wang et al., 2018). Reduction in H₂S levels in testis may help to infertility caused by varicocele. We measured H₂S levels in varicocele rats in a recent study (unpublished data). The H₂S levels significantly reduced and NaHS administration increased H₂S

levels and improved sperm function (unpublished data). The summary of the main factors and possible cellular mechanisms related to varicocele has been shown in Table 1.

There are two approaches to varicocele repair: surgery and percutaneous embolization. The ideal technique for varicocele repair should include preservation of optimal testicular function, elimination of the varicocele and lower complication rates (Huang et al., 2015). Treatment of varicocele in infertile men aims to restore or improve testicular function (Ni et al., 2014). The gold standard treatment currently accepted for varicocele is surgical repair either by open approach with or without magnification, laparoscopy or through percutaneous embolization of the internal spermatic vein. Regardless of the chosen technique, the ultimate goal relies on the occlusion of the dilated veins that compose the pampiniform plexus.

The high retroperitoneal (Palomo), radiologic and laparoscopic approaches allow the ligation of the gonadal vein. The inguinal (Ivanissevich) and subinguinal approaches permit ligation of the external spermatic and cremasteric veins that may contribute to the varicocele and may play a role in recurrence (Huang et al., 2015). Open surgical varicocelectomy is performed by retroperitoneal, inguinal or subinguinal approaches. The retroperitoneal high ligation of the internal spermatic vein (Palomo's technique) although easy to perform, it is associated with high recurrence and hydrocele formation rates. Inguinal and subinguinal approaches allow for the ligation of external spermatic vessels. Non-surgical treatment modalities for varicocele-related infertility are poorly studied; thus there is a need for well-designed trials.

Conclusion

In conclusion, the negative effects of varicocele on fertility are widely recognized. Studies have shown that varicocele-mediated spermatozoa damage might be the major cause of infertility in these patients. Although different mechanisms have been proposed as the cause of spermatozoa injury, varicocele-induced oxidative stress could be the leading etiologic factor. Several investigators have demonstrated that ROS production is elevated in patients with varicocele. Other investigators have

reported a low level of non-enzymatic antioxidants in patients with varicocele, decreasing their ability to encounter the increased ROS production. We have reported the pathologic mechanisms of ROS production through various metabolic pathways in patients with varicocele. Some compensatory molecular mechanisms lead to oxidative stress and some might protect from it. Patients should be evaluated after varicocele treatment for the persistence or recurrence of the varicocele. If the varicocele persists or recurs, internal spermatic venography may be performed to identify the site of persistent venous reflux. Either surgical ligation or percutaneous embolization of the refluxing veins may be used. Semen analyses should be performed after varicocele treatment at about three-month intervals for at least one year or until pregnancy is achieved (Lee et al., 2012). In this review article we introduce H₂S as the contributor factor in the male infertility and more studies are needed to confirm H₂S donors as the effective treatment in varicocele. In addition, main pathophysiological mechanism of varicocele is not clearly understood yet. More studies are needed to discover various mechanism in this disease. Gap of literature is lack of enough works to show different mechanism that contribute to infertility which induced by varicocele. Moreover, different natural and antioxidant treatment should be investigating in treatment of varicocele.

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Conflict of interest

There is no conflict of interest in this study.

References

- Agarwal A, Durairajanayagam D, Halabi J, Peng J, Vazquez-Levin M. Proteomics, oxidative stress and male infertility. *Reprod Biomed Online* 2014; 29: 32-58.
- Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: part 1. *Nat Rev Urol* 2012; 9: 678-90.
- Agarwal A, Sharma R, Durairajanayagam D, Cui Z, Ayaz A, Gupta S. Spermatozoa protein alterations in infertile men with bilateral varicocele. *Asian J Androl* 2016; 18: 43-53.
- Aliramaji A, Pasha YR, Kasaeian AA, Amani N, Yaghini M,

- Moudi E. Fertility after varicocele surgery in Babol clinic and Shahid Beheshti hospitals during 2001-2011. *Casp J Appl Sci Res* 2014; 3.
- Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *J Clin Diagn Res* 2017; 11: IE01-IE05.
- Asghari A, Akbari G, Beigi AM, Mortazavi P. Tramadol reduces testicular damage of ischemia-reperfusion rats. *Anim Reprod* 2016; 13: 811-9.
- Banihani SA, Abu-Alhayjaa RF, Amarin ZO, Alzoubi KH. Pentoxifylline increases the level of nitric oxide produced by human spermatozoa. *Andrologia* 2018; 50.
- Butt F, Akram N. Semen analysis parameters: experiences and insight into male infertility at a tertiary care hospital in Punjab. *J Pak Med Assoc* 2013; 63: 558-62.
- Çayan S, Şahin S, Akbay E. Paternity rates and time to conception in adolescents with varicocele undergoing microsurgical varicocele repair vs observation only: a single institution experience with 408 patients. *J Urol* 2017; 198: 195-201.
- Celik E, Oguzturk H, Sahin N, Turtay MG, Oguz F, Ciftci O. Protective effects of hesperidin in experimental testicular ischemia/reperfusion injury in rats. *Arch Med Sci* 2016; 12: 928-934.
- Chen SS, Huang WJ. Differences in biochemical markers and body mass index between patients with and without varicocele. *J Chin Med Assoc* 2010; 73: 194-8.
- Cocuzza M. Editorial comment: use of indocyanine green angiography in microsurgical subinguinal varicocelectomy - lessons learned from our initial experience. *Int Braz J Urol* 2017; 43: 980-81.
- Damsgaard J, Joensen UN, Carlsen E, Erenpreiss J, Blomberg Jensen M, Matulevicius V, et al. Varicocele is associated with impaired semen quality and reproductive hormone levels: a study of 7035 healthy young men from six european countries. *Eur Urol* 2016; 70: 1019-29.
- Darzi AA, Hashemi SR, Noorbaran A, Pasha AR, Modaress SR, Nikbakhsh N, et al. Electronic database annual report on abdominal wall hernias, 2010-2011, Shahid Beheshti University Hospital, Babol, Iran. *World Appl Sci J* 2013; 21: 1084-8.
- Dorstghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M, Jabbari A. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia* 2017; 49.
- Elbendary MA, Elbadry AM. Right subclinical varicocele: how to manage in infertile patients with clinical left varicocele? *Fertil Steril* 2009; 92: 2050-3.
- Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. *Clinics* 2011; 66: 691-700.
- Habibi B, Seifi B, Mougahi SM, Ojaghi M, Sadeghipour HR. Increases in interleukin-6 and interferon-gamma levels is progressive in immature rats with varicocele. *Iran J Med Sci* 2015; 184: 531-7.
- Hamada A, Esteves SC, Agarwal A. Association between varicocele and infertility. In: *Varicocele and male infertility* 2016; Springer, pp. 19-35.
- Han H, Zhou XG, Qian XS, Feng SJ, Tian L, Zhang XD, et al. Significant alterations of serum hormone levels in the spermatic vein plexus of patients with varicoceles. *Andrologia* 2016; 48: 1108-12.
- Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. *Rev Urol* 2011; 13: e184-90.
- Hosseinifar H, Gourabi H, Salekdeh GH, Alikhani M, Mirshahvaladi S, Sabbaghian M, et al. Study of sperm protein profile in men with and without varicocele using two-dimensional gel electrophoresis. *Urology* 2013; 81: 293-300.
- Hsieh YY, Chang CC, Lin CS. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *Int J Biol Sci* 2006; 2: 23-9.
- Huang IS. 182 The application of real-time testicular touch print smear in testicular sperm extraction-intracytoplasmic sperm injection treatment for non-obstructive azoospermia. *Eur Urol Suppl* 2015; 14: e182.
- Izadpanah M, Alizadeh R, Minaee MB, Heydari L, Babatunde A, Abbasi M. The effects of curcumin on sperm parameters and nitric oxide production in varicocele rats. *Int J Morphol* 2015; 33.
- Jourd'heuil D, Jourd'heuil FL, Kutchukian PS, Musah RA, Wink DA, Grisham MB. Reaction of superoxide and nitric oxide with peroxynitrite. Implications for peroxynitrite-mediated oxidation reactions in vivo. *J Biol Chem* 2001; 276: 28799-805.
- Khosravanian H, Razi M, Farokhi F, Khosravanian N. Simultaneous Administration of dexamethasone and vitamin E reversed experimental varicocele-induced impact in testicular tissue in rats; correlation with Hsp70-2 chaperone expression. *Int Braz J Urol* 2015; 41: 773-90.
- Kimura H. Physiological role of hydrogen sulfide and polysulfide in the central nervous system. *Neurochem Int* 2013; 63: 492-7.
- Kirby EW, Wiener LE, Rajanahally S, Crowell K, Coward RM. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis. *Fertil Steril* 2016; 106: 1338-43.
- Lee B, Jung JH, Kim HS. Assessment of red onion on antioxidant activity in rat. *Food Chem Toxicol* 2012; 50: 3912-9.
- Li G, Xie ZZ, Chua JM, Wong PC, Bian J. Hydrogen sulfide protects testicular germ cells against heat-induced injury. *Nitric Oxide* 2015; 46: 165-71.
- Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, et al. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. *Circulation* 2008; 117: 2351-60.
- Lotti F, Maggi M. Ultrasound of the male genital tract in relation to male reproductive health. *Hum Reprod*

- Update 2015; 21: 56-83.
- Malivindi R, Rago V, De Rose D, Gervasi MC, Cione E, Russo G, et al. Influence of all-trans retinoic acid on sperm metabolism and oxidative stress: Its involvement in the physiopathology of varicocele-associated male infertility. *J Cell Physiol* 2018; 233: 9526-37.
- Masson P, Brannigan RE. The varicocele. *Urol Clin North Am* 2014; 41: 129-44.
- Mazhari S, Razi M, Sadrkhanlou R. Silymarin and celecoxib ameliorate experimental varicocele-induced pathogenesis: evidences for oxidative stress and inflammation inhibition. *Int Urol Nephrol* 2018; 50: 1039-52.
- Mirhoseini M, Talebpour Amiri F, Karimpour Malekshah AA, Rezanejad Gatabi Z, Ghaffari E. Protective effects of melatonin on testis histology following acute torsion-detorsion in rats. *Int J Reprod Biomed (Yazd)* 2017; 15: 141-146.
- Missassi G, Dos Santos Borges C, de Lima Rosa J, Villela E Silva P, da Cunha Martins A Jr, Barbosa F Jr. Chrysin administration protects against oxidative damage in varicocele-induced adult rats. *Oxid Med Cell Longev* 2017; 2017: 2172981.
- Miyaoka R, Esteves SC. A critical appraisal on the role of varicocele in male infertility. *Adv Urol* 2012; 2012: 597495.
- Moudi E. Varicocele in adolescent: a literature review. *Int J Adv Biotechnol Res* 2016; 7: 1501-6.
- Musalam AO, Eid RA, Al-Assiri M, Hussein MR. Morphological changes in varicocele veins: ultrastructural study. *Ultrastruct Pathol* 2010; 34: 260-8.
- Ni K, Steger K, Yang H, Wang H, Hu K, Chen B. Expression and role of leptin under hypoxic conditions in human testis: organotypic in vitro culture experiment and clinical study on patients with varicocele. *J Urol* 2015; 193: 360-7.
- Ni K, Steger K, Yang H, Wang H, Hu K, Chen B. Sperm protamine mRNA ratio and DNA fragmentation index represent reliable clinical biomarkers for men with varicocele after microsurgical varicocele ligation. *J Urol* 2014; 192: 170-6.
- Olooto WE. Infertility in male; risk factors, causes and management-A review. *J Microbiol Biotechnol Res* 2012; 2: 641-5.
- Parlaktas BS, Atilgan D, Ozyurt H, Gencten Y, Akbas A, Erdemir F, et al. The biochemical effects of ischemia-reperfusion injury in the ipsilateral and contralateral testes of rats and the protective role of melatonin. *Asian J Androl* 2014; 16: 314-8.
- Perdichizzi A, Nicoletti F, La Vignera S, Barone N, D'Agata R, Vicari E, et al. Effects of tumour necrosis factor-alpha on human sperm motility and apoptosis. *J Clin Immunol* 2007; 27: 152-62.
- Poderoso JJ, Carreras MC, Lisdero C, Riobó N, Schöpfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. *Arch Biochem Biophys* 1996; 328: 85-92.
- Pogorelič Z, Mustapić K, Jukić M, Todorčić J, Mrklič I, Meštrović J, et al. Management of acute scrotum in children: a 25-year single center experience on 558 pediatric patients. *Can J Urol* 2016; 23: 8594-8601.
- Saalu Linus C., Akunna Gabriel G., Enye Linus A., Ogunmodede Oluwaseyi S., Akingbade Adebajani M. Pathophysiology of varicocele: evidence for oxidative stress as a mechanism pathway. *Eur. J. Anat.* 2013;17:82-91.
- Salama N, Bergh A, Damber JE. The changes in testicular vascular permeability during progression of the experimental varicocele. *Eur Urol* 2003; 43: 84-91.
- Sarac M, Bakal U, Tartar T, Kuloglu T, Yardim M, Artas G, et al. Ghrelin and NUCB2/Nesfatin-1 expression in unilateral testicular torsion-induced rats with and without N-acetylcysteine. *Cell Mol Biol* 2017; 63: 40-45.
- Semercoz A, Onur R, Ogras S, Orhan I. Effects of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuroendocrinology letters* 2003; 24: 86-90.
- Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, et al. Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. *Shock* 2009; 31: 267-74.
- Sohrabipour S, Jafari A, Kamalinejad M, Sarrafnejd A, Shahrestany T, Sadeghipour HR. The role of flaxseed and vitamin E on oxidative stress in prepubertal rats with experimental varicocele: An experimental study. *Iran J Reprod Med* 2013; 11: 459-66.
- Sugiura Y, Kashiba M, Maruyama K, Hoshikawa K, Sasaki R, Saito K, et al. Cadmium exposure alters metabolomics of sulfur-containing amino acids in rat testes. *Antioxid Redox Signal* 2005; 7: 781-7.
- Toman HA, Nasir A, Hassan R, Hassan R. Skeletal, dentoalveolar, and soft tissue cephalometric measurements of Malay transfusion-dependent thalassaemia patients. *Eur J Orthod* 2011; 33: 700-4.
- Tuglu D, Yuvanc E, Yilmaz E, Gencay IY, Atasoy P, Kisa U, et al. The antioxidant effect of dexmedetomidine on testicular ischemia-reperfusion injury. *Acta Cir Bras* 2015; 30: 414-21.
- Wang J, Wang W, Li S, Han Y, Zhang P, Meng G, et al. Hydrogen sulfide as a potential target in preventing spermatogenic failure and testicular dysfunction. *Antioxid Redox Signal* 2018; 28: 1447-62.
- Weedin JW, Kherra M, Lipshultz LI. Varicocele repair in patients with nonobstructive azoospermia: a meta-analysis. *J Urol* 2010; 183: 2309-15.
- Wu TP, Huang BM, Tsai HC, Lui MC, Liu MY. Effects of nitric oxide on human spermatozoa activity, fertilization and mouse embryonic development. *Arch Androl* 2004; 50: 173-9.
- Xia YQ, Ning JZ, Cheng F, Yu WM, Rao T, Ruan Y, et al. GYY4137 a H₂S donor, attenuates ipsilateral epididymis injury in experimentally varicocele-induced rats via activation of the PI3K/Akt pathway. *Iran J Basic Med Sci* 2019; 22: 729-35.
- Yarmohammadi S, Amirsardari M, Akbarzadeh A, Sepidarkish M, Hashemian AH. Evaluating the

- relationship of anxiety, stress and depression with sleep quality of students residing at the dormitories of Tehran University of Medical Sciences in 2013. *World J Med Sci* 2014; 11: 432-8.
- Yuluğ E, Türedi S, Alver A, Türedi S, Kahraman C. Effects of resveratrol on methotrexate-induced testicular damage in rats. *Sci World J* 2013; 2013: 489659.
- Zhu XY, Gu H, Ni X. Hydrogen sulfide in the endocrine and reproductive systems. *Expert Rev Clin Phar* 2011; 4: 75-82.
- Zini A, O'Bryan MK, Magid MS, Schlegel PN. Immunohistochemical localization of endothelial nitric oxide synthase in human testis, epididymis, and vas deferens suggests a possible role for nitric oxide in spermatogenesis, sperm maturation, and programmed cell death. *Biol Reprod* 1996; 55: 935-41.