Review

Role of *Withania somnifera* (Ashwagandha) in the management of male infertility

**Pallav Sengupta** a,b, **Ashok Agarwal** b,* , **Maria Pogrebetskaya** b,c, **Shubhadeep Roychoudhury** b,d, **Damayanthi Durairajanayagam** e, **Ralf Henkel** f

a Department of Physiology, Faculty of Medicine, MAHSA University, Jalan SP2, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia  
b American Center for Reproductive Medicine, Cleveland Clinic, 10681 Carnegie Avenue, Cleveland, OH 44195, USA  
c Case Western Reserve University/University Hospitals Cleveland Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106, USA  
d Department of Life Science and Bioinformatics, Assam University, Silchar 788011, India  
e Discipline of Physiology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia  
f Department of Medical Biosciences, University of the Western Cape, Bellville 7535, Cape Town, South Africa

Pallav Sengupta is a Senior Lecturer of the Department of Physiology, Faculty of Medicine at MAHSA University, Malaysia. He has a PhD in male reproductive physiology from the University of Calcutta, India and over 24 peer reviewed publications in PubMed. He is a collaborating scientist with the American Center for Reproductive Medicine at Cleveland Clinic.

**KEY MESSAGE**

*Withania sominifera* roots improve semen quality by increasing sperm count and motility, regulating reproductive hormone levels and inhibiting lipid peroxidation. Proposed mechanisms underlying these effects are direct alleviation of oxidative stress and enhancement of hormonal balance via the gamma-aminobutyric acid-like mimetic pathway. *Withania sominifera* could potentially supplement routine treatment of male infertility.

**ABSTRACT**

To manage male infertility caused by hormonal imbalance, infections and other predicaments, multifarious treatment strategies are emerging worldwide. Contemporary treatments, such as assisted reproductive techniques, are costly with low success rates of only 10–30%; however, herbal remedies are gaining more attention as an alternative or supplementary therapeutic modality for male infertility. The beneficial effects induced by oral intake of the roots of a small evergreen shrub, *Withania sominifera* (Ashwagandha) on semen quality of infertile men have previously been studied. Oral intake of Ashwagandha roots has been found to inhibit lipid peroxidation, improve sperm count and motility, and regulate reproductive hormone levels. The molecular mechanisms of these effects, however, are yet to be unveiled.
In this review, we will discuss the role of herbal medicines in male infertility; provide a detailed analysis of various human and animal studies involving *Withania somnifera*; describe a proposed direct oxidative mechanism involving mitigation of oxidative stress as well as an indirect mechanism consisting of a gamma-aminobutyric acid-like-mimetic pathway ameliorating hormonal balance through crosstalk among different endocrine glands to improve male fertility; and how *Withania somnifera* supplementation mitigates risk factor-induced male infertility as well as ameliorates male fertility.

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**Introduction**

The use of herbal medicines has persisted for thousands of years, and continues to represent a considerable area of medical treatment in many countries, particularly in Asia, Africa and South America. In recent years, the use of traditional medicine has expanded even in western countries, which is signified by its steadily growing application (Samy et al., 2008). Even well-known common and widely used medicines, such as aspirin, were originally derived from plant extracts. According to an estimation by the World Health Organization, 80% of the world’s population rely on plant-derived medicines for their health care, most of whom are from developing countries (Gurib-Fakim, 2006).

Plants and other natural resources have always been of interest to researchers, mainly for the treatment of various medical conditions as well as in the development of new drugs. It is progressively gaining importance as a mode of treatment worldwide (Kuo et al., 2017; Mills et al., 2005; Pandey et al., 2013). In India, the traditional medicinal system, termed Ayurveda, supplements more modern forms of medicine. Ayurveda is also described as the ‘science of life or longevity’ because it offers a comprehensive system to rejuvenate the body towards a long, healthy life through diet and nutrition (Pandey et al., 2013). Ayurvedic medicines have been used to treat conditions such as diabetes, high blood pressure, gastritis as well as female and male reproductive health issues (Samy et al., 2008). In the Middle East, herbal remedies have been found to improve fertility in diabetic and infertile males (Khaki and Ainehchi, 2017; Ouladsahebmadarek et al., 2016). Traditional Chinese medicine also involves various modalities of treatment, some of which include acupuncture as well as the use of herbal supplements for various types of diseases that range from minor illnesses to life-threatening conditions (Crimmel et al., 2001). In the USA, complementary and alternative medicine has been increasing in popularity among the general population and also within the medical community. It has become a multi-billion dollar industry over the past decade (Clarke and Black, 2015).

Because of the increased popularity, much research has focused on various medicinal uses of plants, including treatment of male infertility. Many herbal drugs and commonly used food-stuffs are known to have a beneficial effect on male reproductive health, including *Tribulus terrestris* (Gokhru or land caltrops), *Eurycoma longifolia* (Tongkat Ali), *Allium sativum* (garlic), *Allium cepa* (onion), Cannabis sativa (marijuana), *Capsicum frutescens* (chilli pepper), and *Zingiber officinale* (ginger) (Henkel et al., 2014; Lohiya et al., 2016; Mansouri et al., 2016; Tambi et al., 2012). A well-known herb from India that has been most commonly used in the treatment of male infertility for more than 3000 years is *Withania somnifera*, known locally known as Ashwagandha or the ‘Indian ginseng’ (Lohiya et al., 2016; Malviya et al., 2016). *Withania somnifera* has been widely used for the treatment of erectile dysfunction, oligozoospermia, reproductive endocrinological problems and other male reproductive health problems.

*Withania somnifera* is reported to have several beneficial effects on male fertility, but its mode of action based on reports from human and animal studies has not yet been properly documented. Therefore, in this review, we discuss the role of herbal medicines in male infertility; provide a detailed analysis of various human and animal studies involving *Withania somnifera*; describe a proposed direct oxidative mechanism involving mitigation of oxidative stress as well as an indirect mechanism consisting of a gamma-aminobutyric acid (GABA)-like-mimetic pathway ameliorating hormonal imbalance through crosstalk among different endocrine glands to improve male fertility; and how *Withania somnifera* supplementation mitigates risk factor-induced male infertility.

**Herbal medicine and male infertility**

Infertility is defined as a couple’s inability to conceive after 1 year of properly timed and unprotected vaginal intercourse. Worldwide, it affects about 48.5 million couples, with the male factor infertility accounting for about one-half of these cases (Mascarenhas et al., 2012). The inability to conceive may cause personal and psychological stress (Dyer et al., 2004, 2005, 2009), and can also place strain on the marital relationship and decrease quality of life (Agarwal et al., 2014a). In addition to the stress factor involved, various infertility treatments and assisted reproductive techniques are expensive and do not offer high success rates, as these range only from 10–30%. Because of the constraints of assisted reproduction techniques, patients tend to resort to alternative remedies for male fertility, including herbal treatment (Dabaja and Schlegel, 2014). Yet, clinicians and researchers have only recently begun to investigate herbal remedies as a form of treatment for male fertility issues.

The causes of male infertility are numerous, and may include hormonal imbalances, infections, varicocele and sexual dysfunction. About 40–50% of cases are idiopathic (Chachamovich et al., 2009). Although infections and varicocele are potentially treatable, these conditions are thought to cause an increase in oxidative stress, which may be the molecular basis for the infertility associated with these conditions (Agarwal et al., 2006a). Oxidative stress occurs when an imbalance occurs between reactive oxygen species (ROS) and the antioxidant capacity of the biological system (Hall, 2015).

The high prevalence of male infertility, and the psychological toll that it can have on a couple trying to start or expand their family, has led to increasing use of pharmacological, surgical and alternative therapies. The traditional treatment for male infertility is geared towards deciphering the underlying cause of the infertility. If a cause can be elucidated, then treatment can be further subdivided into either...
surgical or non-surgical options. Non-surgical treatment options are mainly based on the cause of infertility [Hall, 2015]. Surgical treatment may be necessary to correct some forms of male infertility. It is most often used to reverse specific anatomic or pathophysiologic defects to improve semen quality. The end goal of surgical treatment is to allow couples to either conceive naturally or to have an increased chance for a successful outcome with suitable assisted reproduction techniques. For example, varicocelectomy has been associated with male infertility, and can be treated surgically by various different modalities [Esteves and Agarwal, 2016]. A meta-analysis by Bzaaem et al. (2011) indicated that varicocelectomy generally demonstrates a significant improvement in sperm concentration and total and progressive motility; however, they found no conclusive evidence of improvement in spontaneous pregnancy rates after varicocelectomy repair. In an earlier meta-analysis, our laboratory also reported that, in patients with varicocele, seminal ROS levels increase and total antioxidant capacity significantly decreases, leading to oxidative stress [Agarwal et al., 2006a]. In turn, the oxidative stress caused by the varicocele triggers nuclear DNA damage in spermatozoa [Agarwal et al., 2006b]. Other surgical interventions include treatment for ejaculatory duct obstruction, orchidopexy for an undescended testis and various sperm retrieval options for assisted reproduction techniques [Walsh and Smith, 2013].

For thousands of years, various herbal medicines have been used worldwide for the treatment of male infertility and sexual dysfunction. In China, Traditional Chinese Medicine, which includes acupuncture, massage as well as herbal supplements, is commonly used in conjunction with western medicines for infertility treatment [Crimmel et al., 2001]. In a non-randomized study comprising 202 patients with male infertility, men with oligozoospermia were treated with Sheng Jing, a herbal mixture of 15 plants twice daily for 60 days. After this treatment, the men displayed normalized levels of testosterone, LH and FSH, decrease in sperm antibody production as well as improvement in semen volume, sperm density and motility. The most notable result from this study was a high pregnancy rate of 78% in the 148 couples available for follow up [Chen and Wen, 1996]. In South Africa, a significant proportion of the population still relies on traditional herbal remedies for their primary health care, including the treatment of reproductive problems such as erectile dysfunction, infertility and for contraception [Abdilahi and Van Staden, 2012]. Recent investigations on Typha capensis, a Southern African plant, which is growing in wetlands, and popularly called ‘love reed’, has been shown to have activity to scavenge free radicals and to stimulate testosterone secretion by Leydig cells [Henkel et al., 2012; Ilfergane and Henkel, 2013].

The traditional form of medicine is now also supplementing conventional western medicine in developed countries such as the USA and Germany, similar to how India continues to practice the Ayurvedic medicine alongside Western medicine. According to Ayurvedic treatment regimen, infertile men are often given a mixture of several different plants that commonly include Mucuna pruriens (velvet bean), Withania somnifera (Indian ginseng), Tribulus terrestris (land caltrops), Glycyrrhiza glabra (licorice), Terminalia arjuna (Arjuna tree), Phyllanthus emblica (Indian gooseberry or amla), Zingiber officinale (ginger) and Piper longum (Indian long pepper) [Samy et al., 2008]. With the increasing use of complementary and alternative medicine, however, it is imperative to determine the efficacy and mechanism of action of such herbal supplementations.

The Center for Disease Control defines assisted reproduction techniques as any manipulation of both sperm and egg outside of the body [CDC, 2007]. The techniques involved include IVF, gamete intrafallopian tube transfer, zygote intrafallopian transfer, intracytoplasmic sperm injection and cryopreservation [SART, 2016]. The type of assisted reproduction technique used depends on the patient, and the treatment used is based on the cause of infertility of the male, female or both, and on their own personal preferences and beliefs. The use of herbal medicine in assisted reproduction techniques has not been as widespread as its use in the treatment of infertility in general. Plant-derived bioactive compounds, however, have been investigated in a number of animal studies on cryopreservation and in humans in IVF [Mokhber Maleki et al., 2014; Sapanidou et al., 2014; Zhang et al., 2007].

The preparation of semen for assisted reproduction techniques requires the removal of seminal plasma, which is rich in antioxidant molecules and enzymes. Therefore, spermatozoa, which essentially lack their own antioxidative protection, are at an increased risk of oxidative damage due to oxidative stress [Agarwal et al., 2014a; Sapanidou et al., 2014]. Cryopreservation of spermatozoa allows for a couple to preserve their fertility for a period of time. This technique, however, is associated with the production of ROS, which subsequently leads to lipid peroxidation (LPO) and decreased sperm parameters [Agarwal et al., 2014b]. As a result of this concern, several studies have been conducted to determine whether the use of plant-derived antioxidants would improve sperm quality, such as the polyphenol-rich grape pomace extract (obtained from white grapes, species Vitis vinifera, variety Batiki Tyrnavou) and crocin (the main apocarotenoids of saffron/ Crocus sativus extract) [Mokhber Maleki et al., 2014; Sapanidou et al., 2014]. Grape seed extracts, which contain polyphenols, are able to neutralize ROS as can be seen in a study in which bovine spermatozoa were incubated with the extract and resulted in an increase in motility and viability [Belviranli et al., 2015; Sapanidou et al., 2014]. Crocin at a concentration of 1 mm caused a decrease in ROS production with subsequently lower LPO levels and increased viability and motility in cryopreserved bovine spermatozoa [Mokhber Maleki et al., 2014].

A human study investigated the effects of administration of a Chinese herbal mixture and the subsequent results of IVF. The herbal mixture called Bushen Shengjing Decoction was administered to patients with oligozoospermia and azoospermia for 2–3 months before ICSI. Compared with the control group, the treatment group showed an increase in sperm concentration, motility and viability. In addition, higher rates of fertilization and clinical pregnancy were also evident in the treatment group [Zhang et al., 2007]. The mixture used contained several different herbal supplements, some of which were shown to have antioxidant activity.

Additional studies need to be conducted to determine whether the use of herbal supplements for assisted reproduction techniques is warranted, and to find out what the molecular mechanisms of action are for these supplements. It is logical to hypothesize that if such supplements assist with male infertility that their subsequent use with assisted reproduction techniques would also show a beneficial effect. Until such studies are conducted, it is difficult to reach a definitive conclusion.

Withania somnifera and male infertility

Withania somnifera is commonly known as Ashwagandha in Ayurvedic medicine, Indian ginseng, poison gooseberry or winter cherry. It is a small evergreen shrub with long tuberous roots that belong to the


Solanaceae (nightshade) family of plants (Figure 1). Withania somnifera has small, greenish-yellow flowers and smooth, round fruits with numerous seeds. This plant can be found in tropical and subtropical areas, ranging from South Africa, Middle East India and China (Dar et al., 2015). In India, Withania somnifera is grown as a medicinal crop, and either the whole plant or different parts are used for its medicinal properties. Since antiquity, and to this day, the root of Withania somnifera is used as an adaptogen, diuretic, sedative, antioxidant, and aphrodisiac (Narinderpal et al., 2013). Other parts of the plant, such as the leaves and fruits, have been used as a pain reliever, memory enhancer, anti-neoplastic agent, anti-microbial agent, and anti-inflammatory agent (Narinderpal et al., 2013).

Several phytochemical studies have been conducted to determine the chemical constituents of the various parts of Withania somnifera (Kuboyama et al., 2014; Rajasankar et al., 2009). The principal bioactive compounds of Withania somnifera are withanolides, which are triterpene lactones with C28 ergosterone-based skeleton. More than 40 different withanolides, 12 alkaloids and several sitoindosides have been isolated (Mishra et al., 2000). The major biochemical constituents of Withania somnifera are withaferin-A, withanolide-D and withanone (Dar et al., 2016; Mirjalili et al., 2009) (Figure 2). Lavie et al. (1965) were the first to isolate withaferin-A from Withania somnifera. In addition to the bioactive compounds, several metabolites have also been identified, which include iron, alanine, aspartate, fructose, lactate, glutamine and many more (Chatterjee et al., 2010). Extensive toxicological studies from clinical research on Withania somnifera have shown that the plant is non-toxic in a wide range of practical doses, as well as has no reported herb-herb or herb-drug interactions (Kulkarni and Dhir, 2008; Patel et al., 2016; Prabu et al., 2013). These withanolides follow the route of intestinal epithelium absorption when administered orally. Withaferin-A is reported to have highest bioavailability among withanolides in vivo (Devkar et al., 2015).

In-vivo and in-vitro studies suggest that the root of Withania somnifera possesses anti-inflammatory properties. In Freund’s adjuvant-induced arthritis in rats, animals treated with Withania somnifera showed a significant reduction in paw size and bone degenerative changes over control rats. These improvements were also significantly better than those observed with the reference drug, hydrocortisone (Begum and Sadique, 1988; Mishra et al., 2000). In addition, a decreased release of transcription factors and cytokines associated with inflammatory reactions has also been described (Kaileh et al., 2007). Additional studies have shown that Withania somnifera roots have several effects on the central nervous system, including modulation of acetylcholinesterase activity and serotonin receptors, as well as as GABA activity (NPD, 2017). The effects of Withania somnifera as an anti-neoplastic agent have also been studied over the years, and the leaf extract has been shown to kill cancer cells via several pathways, such as through p53 signalling, death receptors signalling and apoptosis signalling (Belviranli et al., 2015). Withaferin-A is reported to activate tumour suppressor proteins (p53 and pRb) and activation of IκB kinase beta induced by tumour necrosis factor to inhibit apoptosis (Wadha et al., 2013).

Withania somnifera also possesses other pharmacological effects. Several studies have shown that it possesses antioxidant activity and inhibits LPO (Shukla et al., 2011) in spermatozoa, which is considered to be a major contributing factor to idiopathic male infertility. In addition, Withania somnifera has been associated with normalization of sex hormone levels in infertile men who are under either psychological, physiological stress, or both, further supporting the adaptogenic qualities of Withania somnifera (Mahdi et al., 2009). Withania somnifera is therefore able to overcome several different possible causes of male factor infertility by addressing multiple issues simultaneously.

Ahmad et al. (2010) described a significant increase (P < 0.01) in sperm concentration and motility in men with normozoospermia, oligozoospermia, and azoospermia treated with Withania somnifera root extract with a dose of 5 g/day orally with a cup of milk for 3 months. Earlier in 2009, Mahdi et al. (Mahdi et al., 2009) used the same dose
and duration of treatment with Withania somnifera root extract and reported improvement in semen quality in stress-related male infertility. The authors had selected men with normozoospemia (aged between 25 and 38 years) with unexplained infertility as participants, some of whom had a history of smoking or were under psychological stress. These infertile men were given Withania somnifera root powder in a single dose of 5g per day orally with a cup of skimmed milk for 3 months. Results showed a decrease in seminal LPO levels, stress and serum cortisol levels and ROS production, elevated antioxidant levels and overall improvement in sperm quality (concentration and motility), testosterone and LH levels. Sperm concentration in these three groups increased by 17, 20 and 36%, respectively, whereas sperm motility in men with normozoospemia, with a history of cigarette smoking and men with normozoospemia experiencing psychological stress was increased by 9, 10 and 13%, respectively. In addition, an average of a 14% increase in pregnancy rates was reported among the partners of the infertile males in this study (Mahdi et al., 2009).

Ambiye et al. (2013), claimed a 167% increase in sperm concentration in men with oligozoospermia (aged between 22 and 40 years) treated with Withania somnifera at a dose of 675 mg/kg three times daily for 90 days. They reported that Withania somnifera root extract improved sperm motility by 57% and semen volume by 53%, as well as testosterone and LH (Ambiye et al., 2013). In addition to these human studies, several animal experiments have also studied beneficial effects of Withania somnifera on semen quality.

Sahin et al. (2016), compared the potency of different herbal plants in the enhancement of semen quality, and they described that Withania somnifera significantly improves sperm count and motility (P < 0.05) when given at a dose of 300 mg/kg for 8 weeks to Sprague-Dawley rats. Kumar et al. (2015) and Bhargavan et al. (2015) described the effects of Withania somnifera root extract on reversal of male fertility in arsenic and alcohol-induced testicular impairments respectively in rats. They observed significant augmentation of sperm count and motility [P < 0.05] when given at a dose of 300 mg/kg for 8 weeks to Sprague-Dawley rats. Dhumal et al. (2013) reported that the herbal drug, Afrodet Plus containing Withania somnifera and other herbs showed an improvement of semen quality in Holtzman rats administered with 90 mg/kg AP containing 100 mg Withania somnifera root extract for 21 days (Table 1).

Figure 2 – Principal biochemical constituents of Withania somnifera. Major withanolides present in Withania somnifera are withaferin-A, withanolide-D and withanone. They exhibit antioxidative property by increasing the activities of cellular antioxidant enzymes [superoxide dismutase (SOD); catalase (CAT); glutathione S-transferase (GST); heme oxygenase 1 (HO-1); NADPH dehydrogenase, quinone 1 (NQO1); nuclear factor E2-related factor 2 (Nrf2)] as well as anti-inflammatory effects by inhibiting prostaglandin (PGE2) synthesis by cyclooxygenase-2 (COX-2) and nitric oxide production by inhibiting nitric oxide synthase (iNOS) and release of interleukins (IL-6 and IL-1β) and nuclear factor κB light chain enhancer of activated B cells (NFκB). Withanoids also possess apoptosis-inducing activity by stimulating reactive oxygen species, BCL2-associated X protein (Bax), Death receptor 5 (DR5), Mitogen-activated protein kinases (MAPK), protease-activated receptor 4 (PAR-4), tumour suppressor proteins (p53 and p21) and major caspases (–3, –8 and –9). Withanoids prevent cellular proliferation by regulating cellular division through cyclin-dependent kinases (CDKs), signal transducer and activator of transcription 3 (STAT3), B-cell lymphoma 2 (Bcl2), heat shock protein 90 (Hsp90), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), inhibitor of nuclear factor κB kinase subunit β (IκKB), proliferating cell nuclear antigen (PCNA) and survivin.
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Mechanism of action of *Withania somnifera*

The mechanisms by which *Withania somnifera* is able to exert its effects on the reproductive system and fertility can be broadly divided into oxidative and non-oxidative mechanisms. The oxidative mechanism involves modulation of antioxidant activity, along with the regulation of antioxidant enzymes and the co-factors required for the proper functioning of antioxidant enzymes. The non-oxidative mechanisms mainly include the effect of *Withania somnifera* on the hypothalamic-pituitary-gonadal (HPG) axis, as well as its anti-stress activities via the hypothalamic-pituitary-adrenal (HPA) axis. On administration, *Withania somnifera* extract is metabolized to its major constituents: withaferin-A, withanolide-D, withanone and other withanolide derivatives. These biochemical constituents either act directly on testicular or other male reproductive cells, or indirectly on endocrine homeostasis to improve male fertility. On the basis of these findings, the postulated general mechanism of action of *Withania somnifera* is presented in Figure 3.

**Oxidative mechanisms**

Reactive oxygen species are highly reactive compounds that have one or more unpaired electrons [Griveau and Lannou, 1997] with half-
lives in the nano- to milli-second range. Although molecular oxygen is chemically a di-radical, it is relatively inert, but reacts in certain physiological processes with organic molecules and thus forms ROS. In normal physiological processes, energy is produced in the mitochondria through the oxidation of biological molecules, which require oxygen as a terminal electron acceptor. In the male germ cell, a sperm-specific oxidoreductase, dependent on nicotinamide adenine dinucleotide phosphate-oxidase, is located in the midpiece of the flagellum ([Gavella and Lipovac, 1992]). A major part of ROS production occurs because of disruption and subsequent leakage of electrons from the mitochondrial electron transfer chain from Complex I or III ([Finkel and Holbrook, 2000; Koppers et al., 2008]). Even under normal physiological aerobic conditions, about 1–5% of the consumed oxygen is converted into free radicals ([Boveris and Chance, 1973; Chance et al., 1979]), which is an unavoidable consequence of metabolism. The production of ROS via this mechanism are regarded as cytotoxic byproducts that are involved in the cause of disease and ageing ([Raha and Robinson, 2000]). Superoxide anions, which are a type of ROS, dismutate to hydrogen peroxide ([Sharma and Agarwal, 1996]). Hydrogen peroxide is a reactant that has been associated with male infertility since the 1940s, when John MacLeod ([1943]) observed a decrease in motility when spermatozoa were incubated under high oxygen tension. A small and controlled concentration of ROS, however, is required for normal sperm function as ROS are also important mediators of signal transduction that lead to hyperactivation, capacitation and acrosome reaction ([de Lamirande et al., 1997]). Therefore, a delicate balance between ROS (oxidation) and ROS scavenging enzymes and antioxidants (reduction) must be maintained to prevent damage to the spermatozoa. This physiological damage leading to dysfunction of the male germ cell is a result of the plasma membrane containing an extraordinarily high amount of polyunsaturated fatty acids ([Parks and Lynch, 1992]). Elevation of ROS has been associated with decreased sperm motility owing to cellular depletion of adenosine pyrophosphate, cellular membrane damage via LPO, and DNA damage ([Agarwal et al., 2014a]). In addition, ROS can directly oxidize and damage the sperm DNA ([Agarwal et al., 2014a]), and also the end products of LPO, such as malondialdehyde, 4-hydroxy-2-alkenals or 2-alkenals are also genotoxic and mutagenic ([Luczaj and Skrzydlewska, 2003]).

To combat the deleterious effects of ROS, seminal plasma contains numerous enzymatic and non-enzymatic antioxidants ([Agarwal et al., 2004]). In addition, spermatozoa have, to a limited extent, antioxidant enzymes, including superoxide dismutase (SOD), catalase as well as glutathione peroxidase ([Agarwal et al., 2014a]). Each of the enzymes have distinct functions: SOD functions by converting superoxide anions into hydrogen peroxide and oxygen, catalase causes the dismantlement of hydrogen peroxide into oxygen and water, and glutathione peroxidase reduces hydrogen and lipid hydroperoxides via glutathione as an electron donor ([Harris, 1992]). The non-enzymatic antioxidants include substances such as ascorbic acid, alphatoxopherol, pyruvate and glutathione ([Zini and Al-Hathal, 2011]).

Reactive oxygen species that are present in seminal plasma emanate from various sources, some of which are endogenous, whereas others are exogenous. The main endogenous sources include neutrophils, macrophages and immature spermatozoa. Exogenous sources encompass mostly environmental and lifestyle factors, such as smoking, alcohol and radiation ([Agarwal et al., 2014a]). Therefore, potential treatment for male infertility owing to oxidative stress must address the ROS themselves and the underlying cause of their production. If the causes of the oxidative stress cannot be altered, however, then the treatment must be able to at least buffer it.

Several studies have looked at the effects of oral antioxidant supplements and their effects on male factor infertility ([Charagooloo and Aitken, 2011]). Simply using antioxidants, however, may not alleviate the problem because the cause is not always known. In addition, considering that the specific balance between oxidation and reduction is essential for normal sperm function, an oversupply of antioxidants would lead to so-called ‘reductive stress’, which is as dangerous for cells and organs as oxidative stress ([Castagné et al., 1999; Henkel and Solomon, 2017]). This paradoxical phenomenon is called the ‘antioxidant paradox’, a term that was coined by Halliwell ([2000]). On this basis, researchers have begun to look at herbal supplements for the treatment of male-factor infertility.

Studies reporting the relationship between Withania somnifera treatment and oxidative parameters are presented in Table 2. Semen from infertile men has been found to contain decreased antioxidant activity. For example, one of our recent studies has established the cut-off value of total antioxidant capacity of 1947 μM in seminal plasma that can diagnose infertile males with oxidative stress ([Roychoudhury et al., 2016]). Decreased antioxidant activity occurs when concentrations of antioxidants or functional antioxidative enzymes decrease; dysfunctional enzymes increase, or the amount of ROS increase, which overwhelms the natural antioxidant capacity. As mentioned, the root powder of Withania somnifera ([5g/day], when administered daily to infertile men with normozoospermia for 3 months, had decreased ROS production in the seminal plasma and improved sperm count ([Shukla et al., 2011]). In several studies investigating the effects of Withania somnifera on semen quality in infertile males, enzymatic antioxidant activity was determined by measuring LPO and protein carbonyl groups in seminal plasma, which indirectly depicts the enzymatic activity of SOD and catalase. Compared with pretreatment, the amount of LPO was found to decrease after administration of Withania somnifera ([Ahmad et al., 2010; Mahdi et al., 2009; Shukla et al., 2011]), which is most likely attributed to a synergistic relationship between the increase in the enzymatic activity of SOD and catalase and the innate antioxidant activity of Withania somnifera. Therefore, compounds of the Withania somnifera extract are able to donate electrons and halt the destructive chain reaction of free radicals, thereby lowering the overall ROS burden ([Misra et al., 2000]).

In addition to the reduction in LPO seen in infertile men treated with Withania somnifera, several studies investigated the concentration of metals ions such as arsenic ([Kumar et al., 2015]), copper, zinc, iron and gold in seminal plasma, and how they were altered by Withania somnifera treatment. Zinc in conjunction with copper is required as co-factors for SOD, whereas catalase and glutathione peroxidase require iron and selenium, respectively ([Shukla et al., 2011]). Although gold does not participate directly with antioxidant enzymes, gold treatment has been shown to improve steroidogenesis and gametogenesis in immature Wistar rats. When gold chloride was injected sub-cutaneously at a dose of 0.5 mg/kg body weight per day into immature rats for 26 days, plasma levels of testosterone were found to be significantly elevated from baseline, which led researchers to conclude that this treatment is associated with a stimulatory effect on testicular function ([Biswas et al., 2004]). On this basis, Shukla et al. ([2011]) investigated the effects of Withania somnifera on combating oxidative stress and cell death in spermatozoa, and its effects on the essential metal (copper, zinc, iron and gold) concentrations within seminal plasma of infertile men ([Shukla et al., 2011]). Therefore, it is reasonable to conclude that the root extract of Withania somnifera can exert its antioxidant activity via the supplementation of co-factors that are required for the proper functioning of antioxidant
Table 2 – Studies that investigated the relationship between *Withania somnifera* (root powder given at a dose of 5 g/day for 3 months) and either pro-oxidants or antioxidants.

<table>
<thead>
<tr>
<th>Study</th>
<th>Fertility status of participants</th>
<th>Age of the participants</th>
<th>Number of participants</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withania somnifera effect on intracellular ROS</td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>n = 75; normozoospermic: 25; oligozoospermic: 25; asthenozoospermic: 25</td>
<td>ROS generation was diminished in men with normozoospermia (32.56% decrease; ( P &lt; 0.05 )), oligozoospermia (49.76% decrease; ( P &lt; 0.01 )) and asthenozoospermia (44.33% decrease; ( P &lt; 0.01 )) compared with pretreatment.</td>
<td>Shukla et al., 2011</td>
</tr>
<tr>
<td>Withania somnifera and ROS in seminal plasma</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60); treated group (n = 60) divided into three sub-groups: normozoospermic: 20, under psychological stress: 20; smoker: 20; unknown cause</td>
<td>LPO in seminal plasma of infertile men, men with normozoospermia who are cigarette smokers and men with normozoospermia under psychological stress decreased by 29, 27, 23% respectively, compared with baseline.</td>
<td>Mahdi et al., 2009</td>
</tr>
<tr>
<td>Withania somnifera and antioxidant vitamins</td>
<td>Normozoospermic; oligozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25, oligozoospermic: 25, asthenozoospermic: 25</td>
<td>LPO and protein carbonyl groups in seminal plasma after treatment reversed in men with normozoospermia, oligozoospermia, and asthenozoospermia (( P &lt; 0.01 )).</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Withania somnifera and essential metal concentration</td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>n = 75</td>
<td>Levels of vitamins A, E and C were reduced in all infertile men before and after treatment levels recovered in men with normozoospermia (( P &lt; 0.01 )), oligozoospermia (( P &lt; 0.01 )) and asthenozoospermia (( P &lt; 0.01 )).</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Withania somnifera and antioxidant enzymes in seminal plasma of infertile men</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60); treated group (n = 60) divided into three sub-groups: normozoospermic: 20 under psychological stress, 20 under smoking, 20 under unknown cause</td>
<td>Increased the seminal plasma concentrations of Cu(^{2+}), Zn(^{2+}), Fe(^{2+}) and Au(^{2+}) in infertile men with normozoospermia (( P &lt; 0.01 )), oligozoospermia (( P &lt; 0.01 )) and Fe(^{2+}), Zn(^{2+}) and Au(^{2+}) and asthenozoospermia (( P &lt; 0.01 )).</td>
<td>Shukla et al., 2011</td>
</tr>
<tr>
<td>Withania somnifera and antioxidant enzymes in seminal plasma of infertile men</td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25, oligozoospermic: 25, asthenozoospermic: 25</td>
<td>SOD activity in seminal plasma of men with normozoospermia, men with normozoospermia who smoke, and men with normozoospermia under psychological stress by 8, 18 and 17%, and catalase activity by 6, 3 and 11%, respectively, compared with the pretreatment parameters.</td>
<td>Mahdi et al., 2009</td>
</tr>
</tbody>
</table>

LPO, lipid peroxidation; ROS, reactive oxygen species; SOD, super oxide dismutase.
enzymes. Hence, the ability of *Withania somnifera* root extract to decrease ROS and oxidative stress experienced by spermatozoa can be surmised to stem from two mechanisms: its potent antioxidant activity as well as its ability to enhance antioxidant enzymes via the supplementation of required co-factors (depicted as the direct effect in Figure 3).

**Non-oxidative mechanisms**

Apart from oxidative stress, other causes, such as hormonal imbalance caused by physiological or psychological causes, are also linked to male infertility. Stress-related hormones, in particular glucocorticoids, have a deleterious effect on the hypothalamic–pituitary–gonadal (HPG) axis and subsequently on spermatogenesis ([Chandra et al., 2012](#)). Gonadotrophin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary to release FSH and LH. They both subsequently act on the gonads, regulating spermatogenesis and testosterone production. Therefore, when the HPG axis is disrupted by hormones, such as gonadotrophin-inhibiting hormone, prolactin (PRL) and cortisol, spermatogenesis is negatively affected ([Nargund, 2015](#)). As *Withania somnifera* root extract is considered to be an adaptogen, it is able to promote homeostasis within the body, by decreasing the stress response, and normalizing the levels of cortisol, thereby alleviating infertility to some extent. The adaptogenic qualities of *Withania somnifera* were demonstrated in a study that analysed its effects on fertile men with normozoospermia, who were under psychological, environmental stress (heavy smokers) or had infertility of unknown cause. The root powder of *Withania somnifera*, when administered for 3 months at a dosage of 5g per day with skimmed milk, resulted in a decrease in morning and afternoon levels of cortisol in the experimental groups when compared with pretreatment levels ([Mahdi et al., 2009](#)). The normalization of the cortisol levels in the treatment group was also accompanied by an increase in testosterone and LH levels along with a decrease in FSH and PRL ([Mahdi et al., 2009](#)). These hormonal profiles were comparable to those of fertile males. In addition, the above study measured pregnancy outcomes in individuals treated with *Withania somnifera*, and revealed an average increase of 14% compared with controls. An earlier similar study that analysed the reproductive hormonal profiles of infertile men after treatment with *Withania somnifera* root powder at a dose of 5g per day for 3 months with milk showed an increase in the levels of testosterone and LH with an accompanying decrease in FSH and PRL ([Ahmad et al., 2010](#)). These results demonstrate that *Withania somnifera* can regulate the levels of serum sex hormones through manipulation of the HPG axis, thereby enhancing fertility (Table 3).

The augmentation of the HPA and the HPG axis may take place either at the adrenal cortex, gonads, pituitary or hypothalamic levels. Several studies have shown that *Withania somnifera* root powder exhibits its effect at the hypothalamic level ([Bhattarai et al., 2010; Dar et al., 2016; Kataria et al., 2015; Ray and Jha, 2001](#)). Regulation at other sites, however, has not been analysed, and therefore cannot be excluded. To study the effect that *Withania somnifera* exerts on the hypothalamus, *Kataria et al. (2015)* used GnV-3 cells which are neuronal cells from GnRH clonal cell line derived from rat hypothalamic cells. These investigators were able to demonstrate that aqueous extract of *Withania somnifera* leaves stimulated GnRH neuronal activity via differentiation and upregulation of the release of GnRH ([Kataria et al., 2015](#)). They hypothesized that an increase in GnRH neuronal activity was caused by *Withania somnifera*’s GABA-mimetic activity. This hypothesis was confirmed by subsequent in-vitro studies with mice brain hypothalamic slices by the same group. Methanolic extract of *Withania somnifera* was shown to affect GnRH neuronal activities by activating GABA<sub>A</sub> receptors via direct interaction with the membrane, rather than through action potential-mediated mechanisms. Therefore, this extract was able to exhibit GABA-mimetic action via interaction with the GABA<sub>A</sub> receptors. These receptors are ligand-gated Cl<sup>-</sup> channels that activate Cl<sup>-</sup> currents in GnRH neurons when bound to GABA ([Bhattarai et al., 2010](#)). Although GABA is most commonly considered to be an inhibitory neurotransmitter in the central nervous system, most mature GnRH neurons showed an unusual characteristic, in that they are excited by GABA activation ([DeFazio et al., 2002; Watanabe et al., 2014](#)). Therefore, one could conclude that *Withania somnifera* exerts its effects via regulation of GnRH neurons within the hypothalamus and stimulates its release, thereby inducing the production of downstream hormones such as LH, FSH and testosterone. In addition, although whole root or leaf extracts were used in these experiments, it is believed by many that the major bioactive compounds within the plant are Withaferin A ([Ray and Jha, 2001](#)) and Withanone ([Dar et al., 2016](#)).

If *Withania somnifera* root extract does in fact exert its effects at the level of the hypothalamus, it would support the results that were seen in the previous studies that reported a normalization of pituitary as well as gonadal hormones ([Nargund, 2015](#)). By normalizing the pulsatile nature of GnRH, all downstream hormones would be altered as well, leading to increased fertility (depicted as the indirect effect in Figure 3). In-vivo studies, however, need to be conducted to truly understand the complex mechanisms by which *Withania somnifera* functions to improve the fertility of infertile men.

Thus far, our discussion has mainly focused on sperm quality parameters and hormonal balance. An additional topic that needs to be addressed to fully comprehend the various components that interact with one another to aid in reproduction is the seminal plasma. Semen consists of spermatozoa that are suspended in a fluid called the seminal plasma. Seminal plasma is secreted by the seminal vesicles, prostate and bulbo-urethral glands. The various components of seminal plasma facilitate sperm function ([Juypena and Stelletta, 2012](#)). Numerous chemical components make up seminal plasma and include inorganic ions, albumin, enzymes, hormones and peptides ([Gershein and Thielten, 1988](#)). As the seminal plasma is intimately associated with spermatozoa, it is safe to assume that its components have a direct effect on the viability of spermatozoa. The metabolites of seminal plasma of fertile and infertile men have been assessed in a number of studies, and several differences have been highlighted ([Gershein and Thielten, 1988](#)). In infertile patients, the activities of the enzymes alanine aminotransferase, lactate dehydrogenase (LDH) and isocitrate dehydrogenase were significantly decreased compared with fertile men. In addition to the decline in the above-mentioned enzymes, the following compounds were also found to decrease: glycerophosphocholine, citrate, glutamate, alanine and histidine. Levels of phenylalanine, however, were elevated in infertile men compared with fertile men ([Gupta et al., 2013](#)).

On the basis of these differences in the metabolites of fertile and infertile men, [Gupta et al. (2013)](#) endeavoured to determine the efficacy of *Withania somnifera* on seminal plasma metabolites of infertile men by using high-resolution proton nuclear magnetic resonance spectroscopy ([Gupta et al., 2013](#)). In their study, infertile men were treated with 5g per day of powdered *Withania somnifera* root extract for 3
Table 3 – Studies that investigated the relationship between *Withania somnifera* (root powder given at a dose of 5 g/day for 3 months) and reproductive hormone profile.

<table>
<thead>
<tr>
<th>Hormones Studied</th>
<th>Fertility status of participants</th>
<th>Age of the participants</th>
<th>Number of participants</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Testosterone</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60); treated group (n = 60) divided into three sub-groups: normozoospermic: 20; under psychological stress: 20; smoker: 20; unknown cause.</td>
<td>Pre-treatment serum T decreased among infertile men with normozoospermia by 10% among; men with normozoospermia who smoke by 8% and among men with normozoospermia who are under stress by 14%. Post-treatment serum T improved by 13%, 10% and 22%, respectively.</td>
<td>Mahdi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25; oligozoospermic: 25; asthenozoospermic: 25</td>
<td>Serum T post-treatment in men with normozoospermia, asthenozoospermic and oligozoospermia recovered significantly [P &lt; 0.01] compared with pre-treatment levels.</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Serum LH</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60); treated group (n = 60) divided into three sub-groups: normozoospermic: 20; under psychological stress: 20; smoker: 20; unknown cause.</td>
<td>Pre-treatment LH levels low: in men with normozoospermia who are infertile 11%; in men with normozoospermia who smoke 16%; and in men with normozoospermia who are under stress 21%. Post-treatment LH increased by 5%, 14% and 22%, respectively.</td>
<td>Mahdi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25; oligozoospermic: 25; asthenozoospermic: 25</td>
<td>Serum LH after treatment in men with normozoospermia, asthenozoospermia and oligozoospermia recovered significantly [P &lt; 0.01] compared with pre-treatment levels.</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Serum prolactin</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60), treated group (n = 60) divided into three sub-groups: normozoospermic: 20 under psychological stress, 20 under smoking, 20 under unknown cause.</td>
<td>Pre-treatment prolactin levels in infertile men with normozoospermia and men with normozoospermia who smoke and men with normozoospermia who are under stress were elevated by 12, 17 and 20%. Post-treatment levels decreased by 12.5%.</td>
<td>Mahdi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25; oligozoospermic: 25; asthenozoospermic: 25</td>
<td>Serum prolactin levels in men with oligozoospermia and asthenozoospermia were significantly greater then the control group of fertile men; after treatment prolactin levels in men with oligozoospermia and men with asthenozoospermia significantly decreased [P &lt; 0.05] compared with before treatment.</td>
<td>Ahmad et al., 2010</td>
</tr>
<tr>
<td>Serum FSH</td>
<td>Normozoospermic but infertile</td>
<td>25–38 years</td>
<td>Control (n = 60), treated group (n = 60) divided into three sub-groups: normozoospermic: 20 under psychological stress, 20 under smoking, 20 under unknown cause.</td>
<td>Pre-treatment prolactin levels in infertile men with normozoospermia, men with normozoospermia who smoke and men with normozoospermia who are experiencing stress was elevated by 8, 10, 13%. Post-treatment prolactin levels decreased by 9.5%.</td>
<td>Mahdi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Normozoospermic; oligozoospermic; asthenozoospermic</td>
<td>Not mentioned</td>
<td>Control (n = 75); treated group (n = 75) divided into three sub-groups: normozoospermic: 25; oligozoospermic: 25; asthenozoospermic: 25.</td>
<td>Serum FSH levels in men with oligozoospermia and asthenozoospermia were significantly greater then the control group of fertile men; after treatment, FSH levels in the oligozoospermic and asthenozoospermic groups significantly decreased [P &lt; 0.05] compared with before treatment.</td>
<td>Ahmad et al., 2010</td>
</tr>
</tbody>
</table>
Several elevated BMI (testosterone, sex hormone binding globin and free testosterone with productive hormones in males found a negative relationship between In addition, a meta-analysis that analysed the effect tof BMI on re-
determinethat,aftertreatment,levelsoftheabovemetabolitesshifted inmale-factorinfertility (environmental and lifestyle factors have been observed to play a role these data that the higher the BMI of an individual, the greater the its enzymatic activity is associated with a decrease in sperm motility of a LDH-controlled reaction. It has been shown that a decrease in concentrations of lactate, citrate, alanine, histidine and phenylalanine in one to stimulate testicular germ cell development by spermatogenesis. Withania somnifera used in different systems, most of the studies have used diverse doses, and there is no ‘standard dose’ of Withania somnifera used in different systems, most of the studies conducted in humans have used 5g/d as a dose (Ahmad et al., 2010; Mahdi et al., 2009; Shukla et al., 2011). Animal and in-vitro studies have used diverse doses (Dhumal et al., 2013; Kumar et al., 2015; Sahin et al., 2016). For future research work, however, using animals as experimental models may require a ‘standard dose’. In this aspect, the formula of dose translation based on body surface area (BSA) can be applied (Reagan-Shaw et al., 2007). This formula uses human equivalent dose, animal dose and animal and human Km factors (the ratio of body weight and BSA). The Center of Drug Evaluation and Research and the Center for Biologics Evaluation and Research (2002) have suggested that the extrapolation of animal dose is correctly carried out only through normalization to BSA.

Studies on Withania somnifera used 5g per day Withania somnifera to treat the patients without clearly indicating the body weights of the subjects, but most of them continued the treatment as a single dose for 3 months (Mahdi et al., 2009; Shukla et al., 2011). As the participants of these studies are adults, if the average weight is considered as 70 kg for an adult human being, the human equivalent dose (mg/ kg) becomes 71.43 mg/kg. If the researchers are using rats as an experimental model (Km value 6) and converts it from human dose, the single dose for a rat will be 154.14 mg/kg. This dose is similar to the doses used in animal studies mentioned above (Mahdi et al., 2009; Shukla et al., 2011). As previously discussed, several studies have been conducted using Withania somnifera root powder or root extract on antioxidantive, antiproliferative as well as spermatogenic effects of the plant (Ambiye et al., 2013; Mahdi et al., 2009; Shukla et al., 2011). Although different studies have used different doses, and there is no ‘standard dose’ of Withania somnifera used in different systems, most of the studies conducted in humans have used 5g/d as a dose (Ahmad et al., 2010; Mahdi et al., 2009; Shukla et al., 2011). Animal and in-vitro studies have used diverse doses (Dhumal et al., 2013; Kumar et al., 2015; Sahin et al., 2016). For future research work, however, using animals as experimental models may require a ‘standard dose’. In this aspect, the formula of dose translation based on body surface area (BSA) can be applied (Reagan-Shaw et al., 2007). This formula uses human equivalent dose, animal dose and animal and human Km factors (the ratio of body weight and BSA). The Center of Drug Evaluation and Research and the Center for Biologics Evaluation and Research (2002) have suggested that the extrapolation of animal dose is correctly carried out only through normalization to BSA.

**Comparison of human and animal studies: an estimation of Withania somnifera dosage**

As previously discussed, several studies have been conducted using Withania somnifera root powder or root extract on antioxidantive, antiproliferative as well as spermatogenic effects of the plant (Ambiye et al., 2013; Mahdi et al., 2009; Shukla et al., 2011). Although different studies have used different doses, and there is no ‘standard dose’ of Withania somnifera used in different systems, most of the studies conducted in humans have used 5g/d as a dose (Ahmad et al., 2010; Mahdi et al., 2009; Shukla et al., 2011). Animal and in-vitro studies have used diverse doses (Dhumal et al., 2013; Kumar et al., 2015; Sahin et al., 2016). For future research work, however, using animals as experimental models may require a ‘standard dose’. In this aspect, the formula of dose translation based on body surface area (BSA) can be applied (Reagan-Shaw et al., 2007). This formula uses human equivalent dose, animal dose and animal and human Km factors (the ratio of body weight and BSA). The Center of Drug Evaluation and Research and the Center for Biologics Evaluation and Research (2002) have suggested that the extrapolation of animal dose is correctly carried out only through normalization to BSA.

**Amelioration of modifiable risk factors-induced male infertility**

Male-factor infertility, solely accounting for 20–30% of infertility cases and a contributory factor in one-half of the overall infertility cases, behooves clinicians to counsel their patients in various lifestyle modifications that may aid in restoring fertility (Agarwal et al., 2015). Several environmental and lifestyle factors have been observed to play a role in male-factor infertility (Sengupta, 2013). Associated exposure to cigarette smoke, psychological stress, mobile phone usage and elevated body mass index (BMI) have been studied with relation to semen quality (Jurewicz et al., 2014). When obese men were compared with average men, the former were shown to have a threefold higher incidence of infertility. A BMI above 25 kg/m² was subsequently associated with a 25% reduction in both sperm count and motility (Jurewicz et al., 2014). In addition, a meta-analysis that analysed the effect of BMI on re-productive hormones in males found a negative relationship between testosterone, sex hormone binding globin and free testosterone with elevated BMI (MacDonald et al., 2010). It can be extrapolated from these data that the higher the BMI of an individual, the greater the decrease in sperm parameters and subsequently in fertility. Therefore, as with many other disease states, the simple act of increasing physical activity and decreasing one’s weight can lead to improved fertility and decrease need for subsequent treatment. Cigarette smoking and its association with male infertility has been extensively studied over the years. Results, however, have been contradictory where some researchers show a negative association with sperm parameters and others do not (Harlev et al., 2015). Smoking has been associated with an increase in ROS and leukocytospermia, both of which have been associated with male infertility (MacDonald et al., 2010). Cessation of smoking is therefore expected to aid in decreasing the oxidative stress and in improving sperm quality, and may improve fertility. Another factor that should be addressed when dealing with male infertility is psychological stress, which has been shown to affect spermatogenesis mainly by altering testosterone levels via the HPA–HPG axis (Nargund, 2015). Counselling patients about their stress levels and its potential detrimental effects that could lead to infertility would likely prove to be a difficult process, and should probably be reserved for clinicians who are well-trained in the area. Other factors, such as exposure to certain metals and environmental toxins, have also been associated with infertility; however, such factors would be near impossible to alter. Therefore, some aspects of infertility can be modified by altering several lifestyle choices, and should be included in the overall assessment and treatment of males who present with infertility.
Factors like age, life stages and organ of interest for treatment which may demand alteration of dosage in animal models. Therefore, before calculating the final dose of treatment, all these factors should be given due consideration.

Future perspectives

The usage of herbal medicine for the treatment of male infertility has increased over the past several decades by modern day clinicians. This is simply a re-discovery of what traditional medicines have been using since antiquity. Numerous studies have shown the various pharmacological effects that Withania somnifera has on sperm parameters and also on sex hormones. In this review, we have attempted to provide a molecular mechanism of action, to have a better understanding of how Withania somnifera exerts its effects. The studies conducted on infertile males thus far, have not looked specifically at pregnancy rates, implantation rates or live birth rates. As live birth is the end-point of fertility treatments, it should be set as a primary outcome in future studies. Although double-blinded, randomized, placebo-controlled trials are considered the gold standard, it can be recognized that future studies should focus more on the synergistic effect, if any, of Withania somnifera with standard medical treatment in improving fertility. Additional studies that look at Withania somnifera and assisted reproduction techniques would be beneficial, as the use of assisted reproduction techniques in the future is likely to increase. In particular, it would be interesting to observe whether the beneficial properties of Withania somnifera persist if added to cryopreservation media. Despite the limited data, Withania somnifera does exhibit antioxidant properties, improve semen parameters and regulate sex hormones. These effects may greatly affect men seeking treatment for infertility, as it poses a low-cost alternative and does not yet seem to show any adverse side-effect. By combining Withania somnifera with lifestyle modification and standard medical treatment in patients with infertility, the clinician may be able to maximize the chances of a successful clinical pregnancy leading to a live birth. In the present review, we have only summarized the outcomes of already-published reports on the ‘non-toxic’ herb Withania somnifera [Kulkarni and Dhir, 2008]; however, we have not carried out any dose–response relationship or toxicological study. Therefore, this review is unable to declare Withania somnifera as ‘safe’ or ‘toxic’ drug. Yet, from the results of available reports, it can be appraised that Withania somnifera is beneficial for male fertility in various aspects. As treatment of patients with Withania somnifera in a clinical trial showed effective enhancement of male fertility, it can be used as a treatment in future.

Acknowledgement

This research study was supported by funds from the American Center for Reproductive Medicine, Cleveland Clinic.

Article info

Article history:
Received 6 June 2017
Received in revised form 21 November 2017
Accepted 22 November 2017

Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
Withania somnifera
Ashwagandha
Male infertility
Semen quality

References


ARTICLE INFO


Center of Drug Evaluation and Research and the Center for Biologics Evaluation and Research, 2002. Estimating the safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Food and Drug Administration, Rockville, Maryland, USA.


