Treatment of idiopathic infertility

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Treatment of idiopathic infertility

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ABBREVIATIONS

AI	aromatase inhibitors
AMH	anti-müllerian hormone
APA	antiphospholipid antibody
ASA	antisperm antibody
ART	assisted reproductive technology
BMI	body-mass-index
CC	clomiphene citrate
CFTR	cystic fibrosis transmembrane conductance regulator
DNA	deoxyribonucleic acid
FSH	follicle-stimulating hormone
FSP	fallopian sperm perfusion
HPA	hypothalamic-pituitary axis
HSG	hysterosalpingography
HyCoSy	hystero-salpingo-contrast-sonography
ICI	intracervical insemination of sperm
IUI	intrauterine insemination
IVF	in-vitro fertilisation
LH	luteinising hormone
ORP	oxidation-reduction potential
PI	physical inactivity
RCOG	Royal College of Obstetricians and Gynaecologists
ROS	reactive oxygen species
SB	sedentary behaviour
SERM	selective oestrogen receptor modulator
WHO	World Health Organisation

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SUMMARY

TITLE: Treatment of idiopathic infertility

AUTHOR: Priyanka Ugbade

Idiopathic infertility is defined as the failure to achieve pregnancy after 12 months of unprotected sexual intercourse, without a known cause. Idiopathic infertility affects 15-30% of couples trying to conceive. Various aetiologies have been suggested as the cause of idiopathic infertility. In men and women, the immune system plays a role in conception, hence defects in this pathway could be a possible cause. Lifestyle and habits, such as having a high BMI or poor sleep quality, have also been suggested as being implicated in idiopathic infertility. Diagnosis requires a thorough history and physical examination, followed by multiple diagnostic tests for women and semen analysis for men. Despite adequate diagnostic protocol, women are often misdiagnosed with idiopathic infertility instead of age-related ovarian reserve decline. The treatment options vary for each couple. Hence, treatment is individualised and requires person-centred care. The optimal treatment strategy needs to be based on the individual patient characteristics such as age, treatment efficacy, side-effect profile, and cost considerations. Various studies have been conducted on the available treatment options. Expectant management is the starting point for women under 32 years of age with less than 2 years of infertility. Clomiphene citrate is a common first-step in anovulatory disorders but its use in idiopathic infertility has been questioned. When used in combination with intrauterine insemination, pregnancy rates are higher than when CC is used alone. Male treatment options also vary but ultimately requires assisted reproductive technology. There are various avenues for research, regarding aetiology, diagnosis and treatment. Here I summarise the treatment options available for both male and female idiopathic infertility.

Keywords: idiopathic infertility; assisted reproductive technology; in vitro fertilisation; ovarian reserve; anovulatory; clomiphene citrate; intrauterine insemination; body mass index

SAŽETAK

NASLOV: Liječenje idiopatske neplodnosti

AUTOR: Priyanka Rani Oseatunro Ugbade

Idiopatska neplodnost definira se kao neuspjeh u postizanju trudnoće nakon 12 mjeseci nezaštićenog spolnog odnosa, bez poznatog uzroka. Idiopatska neplodnost pogađa 15-30% parova koji pokušavaju začeti. Uzrok idiopatske neplodnosti nije definiran i moguće su različite etiologije. Kod muškaraca i žena, imunološki sustav ima bitnu ulogu u začeću, te se poremećaji u istom smatraju mogućim uzrokom neplodnosti. Životni stil i navike, poput visokog indeksa tjelesne mase ili loše kvalitete sna, također mogu biti povezani s idiopatskom neplodnošću. Dijagnoza zahtijeva temeljitu anamnezu i fizikalni pregled, nakon čega slijede dijagnostički testovi za žene te analiza sperme za muškarce. Unatoč odgovarajućem dijagnostičkom protokolu, ženama se često pogrešno dijagnosticira idiopatska neplodnost umjesto smanjene rezerve jajnika povezane s dobi. Mogućnosti liječenja se razlikuju za svaki par. Liječenje zahtijeva individualizirani pristup kod svakog pacijenta. Optimalna strategija liječenja mora se temeljiti na individualnim karakteristikama pacijenta, poput dobi, učinkovitosti liječenja, profila nuspojava i troškova. Provedena su razna istraživanja o dostupnim mogućnostima liječenja. Liječenje žena mlađih od 32 godine s poznatom neplodnošću unazad 2 godine, započinje promatranjem. Klomifen citrat često je lijek izbora u anovulacijskim poremećajima, no njegova uporaba kod idiopatske neplodnosti jest upitna. Kada se koristi u kombinaciji s intrauterinskom inseminacijom, stopa trudnoće jest viša nego kada se koristi isključivo klomifen citrat. Mogućnosti liječenja kod muškaraca također su različite, ali u konačnici zahtijevaju potpomognutu oplodnju. Postoje razna istraživanja na temu etiologije, dijagnoze i terapije. U ovom diplomskom radu, sažete su opcije liječenja idiopatske neplodnosti kod muškaraca i žena.

Ključne riječi: Idiopatska neplodnost; potpomognutu reproduktivnu tehnologiju; izvantjelesna oplodnja; anovulacija; klomifen citrat; unutarmaternična inseminacija; indeksa tjelesne mas

1.0 INTRODUCTION

Infertility is a complex disorder, affected by genetics and the environment. Idiopathic infertility affects 15-30% of couples unable to conceive[1]. The cause is unknown. Infertility is customarily defined as the inability to conceive after 1 year of regular unprotected intercourse. The diagnosis is made after all standard tests such as ovulation, tubal patency and semen analysis, are deemed normal. This is both frustrating and stressful for patients. The inability to conceive affects the sufferer's economics, psychology, health and well-being. Possible causes of idiopathic infertility include imbalances in the immune system and epigenetic modifications. These avenues have emerged as promising research areas in understanding idiopathic infertility.

Despite following a good diagnostic protocol, there is a high percentage of women who are misdiagnosed with idiopathic infertility. The table (table 1) below shows that after 1 year of unsuccessful conception for under 35 year olds, the false positive rate is 66.4% and after further investigations after 2 years of unsuccessful conception it falls to 9.8%. A similar trend is seen with an increase in age; the older the woman, the great the chance of being given a false diagnosis of idiopathic infertility. Thus, distinguishing between whether it is age-related diminishing ovarian reserve or otherwise, is very important.

Table I Rate of false positive diagnoses of unexplained infertility according to age at initiation of pregnancy seeking[2].

Starting age (years)	% of false positives	
	At 1 year	At 2 years
<35 (reference)	66.4	9.8
35	69.7	17.8
36	75.9	26.5
37	81.1	40.6
38	85.3	55.8
39	88.7	69.4
40	91.3	80.1
41	93.3	87.6
42	94.8	92.4
43	96.0	95.4
44	96.9	96.9

Taken from: https://academic.oup.com/humrep/article/31/7/1390/1749660

Significant advances have occurred in the treatment of reproductive disorders in the last decade. The optimal treatment strategy needs to be based on individual patient characteristics such as age, treatment efficacy, side-effect profile, and cost considerations.

2.0 DEFINITION AND EPIDEMIOLOGY

Idiopathic Infertility is infertility without a definable cause. It is described as a lack of conception after 12 months of regular (2-3 or more times a week) unprotected sex, or 6 months in women over 35 years of age[1].

The percentage of couples diagnosed with idiopathic infertility after the diagnostic work-up ranges between 15-30%[2]. The cause of infertility can be found in 75% of cases. Endometriosis is a common cause of infertility (8%), and other miscellaneous factors such as endocrinological, cervical, immunological factors and genetics, make up the rest[3]. After a year of unsuccessful conception, a couple is referred to a reproductive specialist. This is an upsetting

and difficult time as the physician is unable to find a known cause for infertility. After a thorough investigation, additional stresses arise when infertility exceeds more than 3 years, as a worse prognosis is expected[2]. Indications for treatment are usually 2 years of unsuccessful conception, or if the female is over 35 years of age[3].

The cumulative pregnancy rate is higher in couples who have had a shorter period of infertility without treatment[4]. With every additional month of infertility, the chances of successful conception falls by 2%[5]. Furthermore, for each year of the female being over 30 years of age, the pregnancy rate falls by 9%[5]. The main cause of infertility over the age of 40 is reduced ovarian reserve. However, ovarian reserve is not always estimated during the diagnostics, thus many are often misdiagnosed with idiopathic infertility[5].

Of men who are of reproductive age, 10-15% suffer from idiopathic infertility[6]. There needs to be an absence of female-related infertility for the diagnosis. In 50% of these male-related cases, abnormal sperm was the cause[6]. Difficulty in confirming the partner's contribution to the lack of conception is a distinguishing characteristic of infertility.

Male infertility rates have increased over the past several decades. This is confirmed with the changing of the definition of a "normal" sperm concentration being 60 million/ml in 1940 to the present value of 20 million/ml[7]. The degree of decline is difficult to measure due to the lack of data and confirmatory testing. Studies have shown that the mean sperm count has fallen by 32.5% over the last 50 years[7].

3.0 Possible aetiology of idiopathic infertility in women

As the cause of idiopathic infertility is unknown, the following are 'possible' aetiologies, as many factors have been shown to play a part in idiopathic infertility, but further investigations must be done.

3.1 The immune system and its role in conception

For successful embryo implantation, the immune system must be in balance. Imbalances in inflammatory factors in the immune system result in altered maternal immune tolerance and infertility[8]. These factors may influence the female reproductive system.

Numerous factors are involved in maintaining a non-hostile uterine environment. Cellular immunity protects the body via T-cell mediated immunity ($CD4^+$ and $CD8^+$), macrophage and natural killer cell action and cytokine production. The over-production of $CD4^+$ and $CD8^+$ T cells is known as chronic inflammation. It is defined by the presence of lymphocytes and plasma cells in tissues. $CD4^+$ helper T-cell activation results in the secretion of Th1 and Th2 helper T-cells. During pregnancy, Th1 cells inhibit trophoblastic cell invasion, whilst Th2 cells modify the Th1 response by promoting trophoblastic invasion and maintain the foetus. Thus the immune reaction is shifted towards a Th2 response. Both subsets produce macrophages in response to a pathogen. However, the M2 macrophage subset produced by Th2 cells is able to decrease the production of the M1 macrophage produced by Th1 cells, thus contributing to a low-inflammatory environment. This theory of an imbalance was confirmed by Ozkan *et al.*, who showed that the ratio of Th1/Th2 cells was increased in females with idiopathic infertility (Figure 1)[8]. A study conducted by Wilczyński *et al.* studied Th1 and Th2 levels before and after paternal lymphocyte immunisation[9]. No differences were found between the successful and unsuccessful pregnancies, but there was a Th1 shift in women with idiopathic infertility, in comparison to the fertile women.

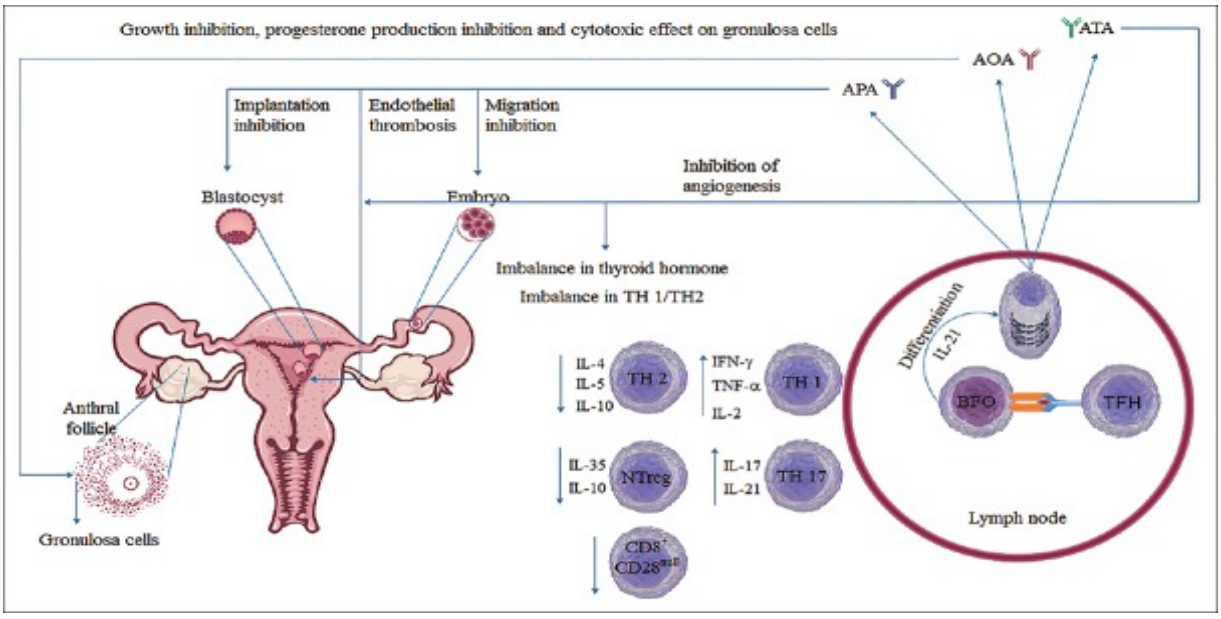

Figure 1: immunological changes in the female reproductive system[10]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6937763/

The Th17 subset of $CD4^+$ T- cells are pro-inflammatory. Studies have suggested that Th17 cells play a key role in rejection or implantation of the foetus. Ozkan *et al.,* recognised that IL-17 serum levels were increased in females with idiopathic infertility[8]. Th17 concentrations in the serum and decidua have been shown to be higher in infertility, compared to the early stages of a healthy pregnancy[10].

Interleukin-21 and T follicular helper cell production are also increased (see Figure 1). Interleukin-21 production leads to the production of autoantibody, such as antiphospholid antibodies (APAs). High levels of APA results in inhibition of migration and implantation of the embryo. Sauer *et al.* found increased APAs in females with idiopathic infertility[11].

In an earlier study, a large number of $CD8^+$ $CD28^-$ T cell populations were found in the decidua, induced by trophoblast cells. This occurs during the early stages of pregnancy, in the first trimester. This subset of $CD8^+$ cells regulate self-reactive T-cells and natural killer cell

function, hence they aid in providing a hospitable immunogenic environment for the semi-allogenic foetus, whilst preventing rejection of the foetus[8]. This is supported by a recent study that showed low levels of $CD8^+$ T cells at the maternal-foetal boundary which might result in over-activity of the immune system and resultant inflammation, leading to infertility. Hill *et al.,* reported a decreased amount of $CD8^+$ $CD28^-$ T cells in women with idiopathic infertility[12].

3.2 Melatonin

Melatonin is usually present at high levels in follicular fluid in the oocyte. Intra-follicular melatonin concentrations have been shown to be low in women with idiopathic infertility with resultant oxidative imbalance[12]. Thus, melatonin protects the oocyte against oxidative stress. Oxidative imbalance results in the over-production of reactive oxygen species (ROS). In a study, low follicular levels of melatonin was associated with higher reactive oxygen species (ROS) levels and reduced oocyte quality in infertile women[13]. Melatonin supplementation has been shown to improve the oxidative imbalance and oocyte quality, resulting in a slight increase in pregnancy success rates[12]. Melatonin has the capability to neutralise oxidative stress by scavenging ROS. A randomised pilot study investigating exogenous melatonin supplementation and its effects on oxidative stress and in vitro fertilization (IVF) showed that melatonin supplementation ameliorated intra-follicular oxidative imbalance. This resulted in a slight increase in the number of live births in the idiopathic infertile patients[14].

4.0 Possible aetiology of idiopathic infertility in men

4.1 Lifestyle

Physical inactivity (PI) and sedentary behaviour (SB) are associated with infertility in men and women. PI is considered less than 150 minutes of physical activity per week. SB is any task that requires low levels of energy. In men, moderate physical activity has shown a positive association with semen quality. In women, moderate PA increased live birth rates [15]. However, excessive PA has shown to be associated with lower semen quality in men and lower fertility rates in women[15].

Body mass index (BMI) has also been shown to impact fertility. Obesity (BMI>25 kg/m^2) can have a detrimental effect on male and female fertility[15]. However, body composition has not been as intensely investigated. Body composition is a better parameter than BMI as BMI does not differentiate visceral and subcutaneous fat. A French case-control multi-centric observational study showed that men who were physically inactive with fat mass greater than the reference values for their age, had a greater chance of infertility[15]. In women, physical inactivity and low fat-free mass were associated with idiopathic infertility[15].

Sleep plays an important role in controlling hormone production via the hypothalamic-pituitary axis (HPA). Sleep problems are associated with hypertension, diabetes, obesity, and many other health conditions. In an Italian cross-section study on 382 men seeking fertility help, semen volume was lower in those with problems initiating sleep[16]. Furthermore, it is now recognised that sleeping problems are associated with erectile dysfunction and low testosterone levels[17]. Also, in overweight men, semen volume was lower in patients with problems initiating sleep[16].

Similarly, it has been shown that in women who work shifts with altered sleep cycles, melatonin production is greatly reduced. This causes the HPA to go in to over-drive causing 'early pregnancy loss, failed embryo implantation, anovulation and amenorrhea'[18].

Dupont *et al.*, carried out the ALIFERT case-control study, which investigated idiopathic infertile and fertile men under the age of 45 years[15]. Metabolic syndrome criteria were checked, along with smoking status. It was found that infertile men were less healthy (met the criteria for metabolic syndrome) than the fertile men, and are more likely to be smokers[15]. The results of this study suggested metabolic syndrome and smoking to be risk factors for idiopathic infertility.

4.2 Autoimmune infertility

Antisperm antibodies (ASAs) have been shown to play a role in idiopathic infertility [13]. ASAs are immunoglobulins directed against sperm antigens. ASAs are found in fertile men and fertile women, but are found in higher concentrations in infertile patients (9-12%) [19]. Thus, the presence alone of ASAs does not cause infertility. The presence of multiple ASAs can lead to the immobilization and/or agglutination of spermatozoa, which blocks sperm-egg interaction. ASAs may have a negative impact on sperm maturation and sperm motility. ASAs also impair sperm morphology, acrosome reaction and DNA fragmentation. This could be a result of the higher levels of ROS found in patients positive for ASA[19]. Causes of ASA production are genital tract infections and testicular trauma. ASAs can also be found in the female reproductive system, induced by trauma to the vaginal mucosa.

4.3 Oxidative stress

At normal physiological levels, ROS regulate intracellular signalling cascades. Oxidative stress occurs when the production of ROS exceeds the antioxidant defences, resulting in cellular damage. ROS are products of normal cellular metabolism, but excess

production has been shown to affect sperm motility and capacitation. ROS also cause sperm membrane and DNA damage. ROS-induced sperm DNA damage, negatively affects the paternal genomic contribution to the embryo. In a study by Pasqualotto *et al.,* ROS was negatively correlated with sperm quality[18]. ROS formation has been found to be higher in idiopathic infertile men[20].

4.4 Epigenetic modifications

Epigenetics refers to heritable forms of gene activity and expression without any DNA sequence changes. These epigenetic modifications can be inherited through mitotic and meiotic divisions. Many studies have suggested that defects in spermatogenesis could be associated with epigenetic regulation of imprinting in the germ line[21, 22]. A recent study by Tang *et al.*, showed that aberrant DNA methylation patterns of imprinted genes were more prevalent in idiopathic infertile males[23]. Aberrant imprinting in spermatozoa is therefore a risk factor for congenital diseases in children conceived with assisted reproduction techniques (ART) [24].

5.0 DIAGNOSTICS

Idiopathic infertility is a diagnosis of exclusion. This diagnosis is given after testing and confirmation of tubal patency, normal uterine cavity parameters, normal ovulatory function and normal semen function. All parameters are deemed normal, because no abnormalities were found during the normal diagnostic protocol.

The initial process of history taking and physical examination can be very revealing. History taking will define the duration of conception issues, menstrual cycle length, gynaecological and obstetric history (i.e. pelvic infections, ectopic pregnancies). This will help direct diagnosis and treatment. Family history such as first degree relatives with fertility issues,

genetic mutations and birth defects, can highlight possible causes of infertility, i.e. Turner's syndrome. Thus, many possible conditions can be recognised before coming to the conclusion of idiopathic infertility. Personal and lifestyle history such as age, occupation, exercise, stress, dieting/weight changes, smoking, and alcohol use are important as these factors can affect fertility.

Along with the history and physical examination, diagnostic procedures are also carried out. Progesterone testing is carried out at day 21 for women with regular menstrual cycles, and for those with irregular cycles, testing is carried out 7 days after the presumed date of onset of menses, and every week after, until the onset. Women with irregular menstrual cycles should be offered a blood test to measure serum gonadotropins; follicle-stimulating hormone (FSH) and luteinising hormone (LH). An FSH level between 10 to 20 IU/L, taken on the third day of the menstrual cycle, is associated with infertility[25]. The Clomiphene challenge test, antral follicle count and anti-müllerian hormone (AMH) levels are used to predict the response to ovarian stimulation with exogenous gonadotropins and assisted reproductive technology.

Further testing may be necessary in the case of anovulation, involving thyroid function tests and prolactin measurements.

Women with no known risk factors for tubal occlusion should be offered hysterosalpingography (HSG). HSG is a reliable and minimally-invasive method for diagnosis and offers potential therapeutic effect. Hystero-salpingo-contrast-sonography (HyCoSy) is able to recognise pelvic conditions that may be responsible for infertility and are not detectable by HSG. A hysteroscopy or laparoscopy should be offered to women with risk factors for tubal occlusion, such as endometriosis. These procedures are invasive but allow for uterine abnormalities such as fibroids to be delineated.

The evaluation of male infertility begins with a good history and physical examination. The focus is on pelvic surgeries, systemic diseases, occupational exposure and previous fertility. The next step involves semen analysis. The male patient should abstain from ejaculation for 48 to 72 hours. The World Health Organisation (WHO) has provided guidelines for semen parameters: semen volume should be 1.5 ml or more, pH: 7.2 or more; sperm concentration: 15 million spermatozoa/ml or more; total sperm number: 39 million spermatozoa per ejaculate or more; total motility (percentage of progressive motility and non-progressive motility): 40% or more motile or 32% or more with progressive motility; vitality: 58% or more live spermatozoa; sperm morphology (percentage of normal forms): 4% or more[25]. If necessary, repetition of semen analysis should be done after 3 months to allow for the completion of the sperm cycle.

For further diagnosis, scrotal ultrasound measures the testicular volume. Hormone levels are also measured: serum testosterone, oestradiol, LH, FSH, prolactin, and HbA1c[26]. Morning total testosterone and FSH levels can differentiate between primary (low testosterone levels with high FSH) and secondary disorders (low testosterone levels with low FSH). Genetic assays such as karyotyping and CFTR analysis are key to diagnosis for genetic abnormalities.

For more in-depth semen analysis, oxidation-reduction potential (ORP) assay has been suggested[17]. ORP can measure the amount of antioxidants and oxidants, thus helping determining whether oxidative stress is the cause of infertility. However, it is both expensive and time-sensitive.

6.0 TREATMENT

6.1 Female treatment

6.1.1 Expectant Management

The Royal College of Obstetricians and Gynaecologists (RCOG) recommend suitable couples try expectant treatment first. The chance of a successful pregnancy outcome is dependent on factors such as the woman's age, infertility duration and pregnancy history.

Couples with a good prognosis, based on age (under 32 years) and duration of infertility (<2 years) can be offered expectant management[27]. Women over the age of 37 years with unexplained infertility, have a 1% or less chance of pregnancy via expectant management, thus this is not option for these patients[27]. Thus, expectant management is not offered to women over 32 years of age, or those with suspected decreased ovarian reserve.

Steures *et al.*, carried out a trial of 253 couples with a good-prognosis; median duration of infertility was 2 years and the mean age was 33 years. 127 couples were given immediate treatment whilst 126 couples started expectant management. With the expectant management cohort, there was a 27% chance of a live birth in couples after 6 months without intervention[28].

6.1.2 Fallopian tube sperm perfusion (FSP)

Tubal/ utero flushing could possibly increase the number of sperm that reach the Pouch of Douglas and ampulla of the fallopian tube[29]. Sperm from the donor partner is concentrated and the debris is removed to maximise the number of healthy, motile spermatozoa. In a trial, sperm perfusion was shown to yield a higher pregnancy rate than IUI[30]. IUI gave a pregnancy rate of 7.6 per cycle and 15.6% per patient; in the FSP group, 14 ongoing pregnancies occurred, giving a pregnancy rate of 21.2% per cycle and 42.4% per patient[30].

6.1.3 Clomiphene citrate

Clomiphene citrate (CC) is a common first step in treatment in anovulatory infertility. Many have mixed views towards CC. In a 2010 meta-analysis of treatment of idiopathic infertility and subfertility with CC versus a placebo, CC alone did not increase the pregnancy rate per woman[27]. However, when used in combination with IUI, pregnancy rates are improved rather than when used alone[31].

Complications of CC include multiple gestations[7], fertility impairments and endometrial dysfunction. However, if administered in 50mg doses, these side-effects are prevented and the efficacy is still similar to that of a 100mg dose[27].

CC was shown to be less effective alone, than gonadotropins, in a comparative study[29].

6.1.4 Intrauterine insemination

Intrauterine insemination (IUI) is the process by which processed and concentrated motile sperm are directly inserted in to the uterine cavity. It is often recommended for infertility that does not involve the fallopian tubes. For those with idiopathic infertility and hostile cervical environments, IUI improved pregnancy rates when combined with another treatment modality[31]. It is an intermediate and cost-effective procedure, proceeding IVF. Studies have shown that having the correct balance of microbiome of the vagina, especially dominance of vaginal *L crispatus,* enhances IUI success rates[32]. Physiologic homeostasis is necessary for an environment ready for pregnancy and implantation.

The efficacy of IUI for the treatment of idiopathic infertility was examined in a large clinical trial, run by the National Institute of Health. 900 infertile couples were given one of the following treatments: Intracervical insemination of sperm (ICI) mimicking natural intercourse, IUI of sperm increasing the number of ejaculate in the female reproductive tract, or FSH injections plus ICI or IUI; FSH to increase follicular maturity and improve ovulation.

The controlled ICI group saw a 2% pregnancy rate per cycle; comparatively, IUI saw a 5% pregnancy rate per cycle[33]. Therefore, alone, IUI treatment is only marginally better than the compared treatments.

A 2011 study which compared expectant management, CC and IUI treatments for 6 months, suggested that treatment such as clomiphene citrate and IUI do not provide better birth rates than expectant management in cases of idiopathic infertility[31]. This provides a baseline for future treatment options.

6.1.5 Clomiphene plus IUI

The combination of an ovulation inducing agent plus IUI may be used to overcome mild ovarian, oocyte or fallopian dysfunction. It is a good first-line treatment due to its low cost, low multiple gestation rate and clinically good pregnancy rate[31].

In a study of treatment for 900 women with idiopathic infertility, the patients were randomly assigned treatment with letrozole, clomiphene and gonadotropins; all of which were given in combination with IUI, produced pregnancy rates of 22, 28, 36% respectively[31]. Although clomiphene and IUI rates were lower than the gonadotropins, CC plus IUI gives lower multiple gestation rates; 9% compared to 32%[31].

6.1.6 Aromatase inhibitors (AI) plus IUI

An AI plus IUI combined treatment may result in pregnancy for women with unexplained infertility who do not respond to CC plus IUI, and who cannot or choose not to use IVF or gonadotropin therapy. AIs have similar incidences of clinical pregnancy, multiple gestation, and live birth rates compared with CC[34, 35].

6.1.7 Gonadotropins plus IUI

In a meta-analysis of eight trials comparing gonadotropins versus oral agents with IUI for patients with idiopathic infertility, gonadotropin use did not result in improved live birth rates[36]. Thus, gonadotropins are not a good option for idiopathic infertility.

6.1.8 In vitro fertilisation

In vitro fertilisation (IVF) gives the highest pregnancy rate in the shortest time, per cycle[6]. However, it is the most costly intervention and also has the highest multiple pregnancy rate[6]. The average success rate for IVF treatment using fresh eggs in the UK is 29% for women <35 years of age, 23% for women aged 35–37 years, 15% for women aged 38–39years and 9% for women aged 40–42 years[37].

IVF is able to help with the following conditions that affect fertility: ovarian dysfunction, cervical factors, spermatozoa and egg interaction and sperm and egg transport[38].

A possible complication of IVF treatment is ovarian hyper-stimulation, and the chances of this are approximately 6-14% in a gonadotrophin-releasing hormone agonist cycle[38].

A 2012 Cochran review of randomised trials which compared the effectiveness of IVF versus other treatment modalities in couples with unexplained infertility, showed higher live birth rates (45.8%) in comparison to expectant management (3.7%)[39]. After a single cycle of IVF, the live birth rate is higher than that of expectant management[39].

Another trial that compared two cycles of CC or IUI against two cycles of gonadotropin injections/ IUI, or immediate IVF resulted in live birth rates of 8%, 7% and 16% respectively. 84% of all pregnancies were a result of IVF[40]. The cohort consisted of 154 randomised couples with ≥6 months of idiopathic infertility, with the female aged between 38-42 years. Thus,

immediate IVF is the best option for this age group. However, IVF is much more costly in comparison to the other treatment modalities.

The need for a methodical approach was highlighted in a cohort study of couples with unexplained infertility[41]. The initial treatment involved gonadotropin injections plus IUI for up to three cycles and then IVF for those who did not conceive. The pregnancy rate with gonadotropin injections plus IUI was 15.7 % per cycle and 29.8 % per patient and the pregnancy rate in those who went on to IVF was 36.7%[42].

6.2 Male treatment

6.2.1 Gonadotropins

Gonadotropins are not shown to be helpful in cases of male idiopathic infertility. Gonadotropins are approved for treatment for hypogonadotropic hypogonadism[42].

6.2.2 Aromatase inhibitors

Aromatase inhibitors (AIs) block the conversion of testosterone to oestradiol (T/E). AIs reduce the effects of oestrogen on spermatogenesis. Letrozole and anastrazole increase endogenous testosterone production and serum testosterone levels, without increasing oestrogen levels, as with SERMs like clomiphene[31]. Aromatase inhibitors are mostly of use in men with low serum testosterone and elevated oestradiol levels[43].

6.2.3 Selective oestrogen receptor modulators (SERMs)

The current SERMs in use are clomiphene and tamoxifen, which act by negative feedback at the level of the hypothalamus and pituitary. This negative feedback causes an increase in secretion of FSH and LH, leading to an increase in testosterone production and

spermatogenesis. SERMs have not been studied in great detail, yet are part of empiric treatment. SERM administration has been shown to improve sperm count and quality however it does not lead to an increase in sperm concentration. The data is scant thus a definitive conclusion has not been made[44]. CC has been used for decades as a treatment for infertility but its efficacy has not been determined as there is not enough evidence from randomized placebo-controlled trials.

6.2.4 Androgens

Despite the negative effect of exogenous testosterone, causing a decrease in endogenous sperm production and resulting in a decrease in testosterone levels, 25% of American urologists prescribe testosterone to male patients with idiopathic infertility[45].

6.2.5 Anti-inflammatory treatment

Glucocorticoids are the choice of treatment for anti-sperm antibodies (ASA). Oral agents are often used to suppress antibody production. High levels of corticosteroids have a detrimental effect on the reproductive system, as with the rest of the body. However, mixed results have been shown for its treatment against immunologic infertility[46]. Assisted reproductive therapy (IUI and IVF) has been suggested, as immune suppressive treatment has shown varying results[16]. Shin *et al*., conducted a retrospective review on 75 sub-fertile men with reproductive obstructive and associated ASAs. Couples were treated with low-dose prednisone or not pre-treated with prednisone. Fertilisation rates and pregnancy rates were higher in the group pre-treated with prednisone[47].

6.2.6 Antioxidants

An alternative approach for males with idiopathic infertility, is to move away from empiric therapy and look for any possible causes of oxidative stress infertility. Studies have shown that antioxidants have the ability to counteract the effects of reactive oxygen species.

Recently, Micic *et al.,* conducted a study on the treatment of idiopathic oligoasthenozoospermia in males who failed to impregnate their female partner within 1 year. Male patients were treated with L-carnitine and L-acetylcarnitine with micronutrients. After 6 months of treatment, sperm motility, vitality and volume improved[48]. In another study, idiopathic infertile men were given antioxidant therapy once a day for 6 months[49]. Proteins for spermatogenesis and reproductive hormones were upregulated. This novel finding suggests that antioxidants may be beneficial in the treatment of male-related infertility.

6.2.7 Vitamin supplementation

Vitamins may also be prescribed to support and enhance the effects of current treatments. Carotenoids help maintain cell membrane integrity, aid with epithelial cell proliferation, and are involved in spermatogenesis[50]. Poor sperm quality has been linked to low serum retinol concentrations[51]. It is currently an over the counter addition to treatment.

Seminal ascorbic acid levels are positively correlated to the percent of normal spermatozoa, and it is negatively correlated to the DNA fragmentation index[40]. Studies have shown that 1g daily of Vitamin C helps increase the mean sperm count and concentration[52].

Vitamin E provides protection for the sperm cell membrane against oxidative stress-induced damage. A positive association has been found between Vitamin E dietary intake and total sperm motility[52].

6.2.8 Assisted reproductive technology (ART)

Ultimately, ART is the final treatment choice. ICSI has often been the treatment choice for patients with poor sperm parameters[43]. IVF with ICSI has been shown to produce pregnancy rates in men with sperm autoantibodies comparable to men without autoantibodies[43]. IVF has shown to have higher fertilisation rates when immunosuppressive therapy is used[53].

Also, antioxidants such as vitamin E and L-carnitine have been shown to increase live birth rates when used in conjunction with ART[48].

The highest frequency of genetic anomalies is observed in severe spermatogenic impairment, which can be treated with IVF. However, given the risk of transmitting genetic disorders to the future offspring through IVF, the diagnosis of known genetic abnormalities and the discovery of novel genetic factors in idiopathic infertility is of the outmost clinical importance.

7.0 CONCLUSION

Idiopathic infertility poses a tremendous clinical issue as genetics and the environment play an important role in its pathology and treatment. Many aetiologies have been suggested, providing interesting avenues for further research.

A personalised approach involving different aspects of medicine is necessary for management of idiopathic infertility. It is important for couples with idiopathic infertility to receive individualised treatment plans on the basis of their predicted chance of spontaneous live birth, as well as anticipated success rates, costs and complications of treatment. Conception is strongly influenced by female age and the duration of infertility, thus these factors must be considered when creating a treatment plan.

The workup for men may be prematurely stopped due to normal semen parameters and hormone profile. However, further investigations are needed if the female partner is fertile and if the couple have a decreased possibility of spontaneous conception. Novel techniques and methods for sperm analysis may tailor the use of various treatment options. Currently, ART is the optimal treatment for male and female idiopathic infertility.

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9.0 REFERENCES

1. Quaas A, Dokras A. Diagnosis and treatment of unexplained infertility. Reviews in Obstetrics and Gynecology. 2008 Spring; 1(2):69-76.

2. Somigliana E, Paffoni A, Busnelli A, Francesca Filippi, Pagliardini L, Vigano P, Vercellini P. Age-related infertility and unexplained infertility: an intricate clinical dilemma. Human Reproduction. 2016 Apr; 31(7):1390-1396. doi:10.1093/humrep/dew066

3. Collins JA, Burrows EA, Willan AR. The prognosis for live birth among untreated infertile couples. Fertility and Sterility. 1995 Jul; 64(1): 22-28.

4. Glazener CMA, Kelly NJ, Weir MJA, David JSE, Cornes JS, Hull MGR. The diagnosis of male infertility–prospective time-specific study of conception rates related to seminal analysis and post-coital sperm-mucus penetration and survival in otherwise unexplained infertility. Human Reproduction. 1987; 14(2): 665–671.

5. Ray A, Shah A, Gudi A, Homberg R. Unexplained infertility: an update and review of practice. Reproductive Biomedicine online. 2012 Mar; 24(6): 591-602.

6. Kothandaraman N, Agarwal A, Abu-Elmagd M, Al-Qahtani MH. Pathogenic landscape of idiopathic male infertility: new insight towards its regulatory networks. Genomic Medicine. 2016 Aug; 16023.

7. Sengupta P, Borges E, Dutta S, Krajewska-Kulak E. Decline in sperm count in European men during the past 50 years. Human and Experimental Toxicology. 2017 Apr; 37(3): 247-255.

8. Ozkan ZS, Deveci D, Kumbak B, Simsek M, Ilhan F, Sekercioglu S, Sapmaz E. What is the impact of Th1/Th2 ratio, SOCS3, IL17, and IL35 levels in unexplained infertility? Journal of Reproductive Immunology. 2014 Jun; 103:53-8. doi: 10.1016/j.jri.2013.11.002

9. Wilczyński JR, Radwan P, Tchórzewski H, Banasik M. Immunotherapy of patients with recurrent spontaneous miscarriage and idiopathic infertility: does the immunization-dependent Th2 cytokine overbalance really matter? Archivum Immunologiae et Therapiae Experimentalis. 2012 Apr; 60(2):151-60.

10. Ehsani M, Mohammadnia-Afrouzi M, Mirzakhani M., Esmaeilzadeh S, Shahbazi M. (2019). Female Unexplained Infertility: A Disease with Imbalanced Adaptive Immunity. Journal of human reproductive sciences. 2019 Oct-Dec; 12(4), 274–282.

11. Sauer R, Roussev R, Jeyendran RS, Coulam CB. Prevalence of antiphospholipid antibodies among women experiencing unexplained infertility and recurrent implantation failure. Fertility and Sterility. 2010 May; 93(7):2441-2443. doi:10.1016/j.fertnstert.2009.08.062

12. Hill JA, Faris HM, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertility and Sterility. 1988 Aug; 50(2):216-222.

13. Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A et al. Journal of Pineal Research. 2008 Apr; 44(3):280-7. doi: 10.1111/j.1600-079X.2007.00524.x 918

14. Espino J, Macedo M, Lozano G, Ortiz Á, Rodríguez C, Rodríguez AB (2019). Impact of Melatonin Supplementation in Women with Unexplained Infertility Undergoing Fertility Treatment. Antioxidants (Basel). 2019 Aug; 8(9), 338. https://doi.org/10.3390/antiox8090338

15. Foucaut AM, Faure C, Julia C, Czernichow S, Levy R, Dupont C; ALIFERT collaborative group. Sedentary behavior, physical inactivity and body composition in relation to idiopathic infertility among men and women. PLoS One. 2019 Apr; 14(4):e0210770. doi: 10.1371/journal.pone.0210770.

16. Viganò P, Chiaffarino F, Bonzi V, Salonia A, Ricci E, Papaleo E et al. Sleep disturbances and semen quality in an Italian cross sectional study. Basic Clinical Andrology. 2017 Aug; 21; 27:16. doi: 10.1186/s12610-017-0060-0.

17. Liu PY. A Clinical Perspective of Sleep and Andrological Health: Assessment, Treatment Considerations, and Future Research. The Journal of clinical endocrinology and metabolism. 2019 Oct; 104(10):4398-4417. doi:10.1210/jc.2019-00683

18. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. Fertility and Sterility. 2000 Mar; 73 (3):459-464. doi:10.1016/s0015-0282(99)00567-1

19. Bozhedomov VA, Nikolaeva MA, Ushakova IV, Lipatova NA, Bozhedomova GE, Sukhikh GT. Functional deficit of sperm and fertility impairment in men with antisperm antibodies. Journal of Reproductive Immunology. 2015 Nov; 112: 95-101. doi:10.1016/j.jri.2015.08.002

20. Gunes S, Arslan MA, Hekim G, Asci R. The role of epigenetics in idiopathic male infertility. Journal of assisted reproduction and genetics. 2016 Mar; 33(5), 553-569. https://doi.org/10.1007/s10815-016-0682-8

21. Agarwal A, Sharma RK, Sharma R, Assidi M, Abuzenadah AM, Alshahrani S, Durairajanayagam D, Sabanegh E. Characterizing semen parameters and their association with reactive oxygen species in infertile men. Reproductive Biology and Endocrinology. 2014 May; 12:33. doi: 10.1186/1477-7827-12-33

22. Kitamura A, Miyauchi N, Hamada H, Hiura H, Chiba H, Okae H, Sato A, John RM, Arima T. Epigenetic alterations in sperm associated with male infertility. Congenital Anomalies (Kyoto). 2015 Aug; 55(3):133-44. doi: 10.1111/cga.12113

23. Tang Q, Pan F, Yang J, Fu Z, Lu Y, Wu X, Han X, Chen M, Lu C, Xia Y, Wang X, Wu W. Idiopathic male infertility is strongly associated with aberrant DNA methylation of imprinted loci in sperm: a case-control study. Clinical Epigenetics. 2018 Oct; 10(1):134. doi: 10.1186/s13148-018-0568-y

24. Giahi L, Mohammadmoradi S, Javidan A, Sadeghi MR. Nutritional modifications in male infertility: a systematic review covering 2 decades. Nutrition reviews. 2016 Feb; 74(2), 118–130. https://doi.org/10.1093/nutrit/nuv059

25. Firth HV, Hurst JA. Oxford Desk Reference: Clinical Genetics and Genomics. Oxford University Press, Sep 13, 2017.

26. Punab M, Poolamets O, Paju P, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Human Reproduction. 2017 Jan; 32(1):18-31. doi:10.1093/humrep/dew284

27. Sovino H, Sir-Petermann T, Devoto L. Clomiphene citrate and ovulation induction. Reproductive Biomedicine Online. 2002 Jun; 4(3):303-310.

28. Steures P, van der Steeg JW, Hompes PG, Habbema JD, Eijkemans MJ, Broekmans FJ, Verhoeve HR, Bossuyt PM, van der Veen F, Mol BW. Collaborative Effort on the Clinical Evaluation in Reproductive Medicine. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. Lancet. 2006 Jul; 368(9531):216-21. doi: 10.1016/S0140-6736(06)69042-9

29. Cantineau AEP, Cohlen BJ, Heineman MJ, Marjoribanks J, Farquhar C. Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility. Cochrane Database of Systematic Reviews. 2013 Oct; (10). doi: 10.1002/14651858.CD001502.pub4

30. Ricci G, Nucera G, Pozzobon C, Boscolo R, Giolo E, Guaschino S. A simple method for fallopian tube sperm perfusion using a blocking device in the treatment of unexplained infertility. Fertility and Sterility. 2001 Dec; 76(6):1242-1248. doi:10.1016/s0015-0282(01)02913-2

31. Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. Journal of Urology. 2001 Mar; 165(3):837-841.

32. Amato V, Papaleo E, Pasciuta R, Viganò P, Ferrarese R, Clementi N, Sanchez AM, Quaranta L et al. Differential Composition of Vaginal Microbiome, but Not of Seminal Microbiome, Is Associated With Successful Intrauterine Insemination in Couples With Idiopathic Infertility: A Prospective Observational Study. Open Forum Infectious Diseases. 2019 Dec 10; 7 (1):ofz525. doi: 10.1093/ofid/ofz525.

33. Ko EY, Siddiqi K, Brannigan RE, Sabanegh ES Jr. Empirical medical therapy for idiopathic male infertility: a survey of the American Urological Association. Journal of Urol. 2012 Mar; 187(3):973-978. doi:10.1016/j.juro.2011.10.137

34. Watson A, Vandekerckhove P, Lilford R, Vail A, Brosens I, Hughes E. A meta- analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result? Fertility and. Sterility. 1994 Mar; 61, 470–477.

35. ACOG Committee Opinion No. 738: Aromatase Inhibitors in Gynecologic Practice. Obstetrics and Gynecology. 2018 Jun; 131 (6):e194-e199. doi:10.1097/AOG.0000000000002640

36. Zolton JR, Lindner PG, Terry N, DeCherney AH, Hill MJ. Gonadotropins versus oral ovarian stimulation agents for unexplained infertility: a systematic review and meta-analysis. Fertility and Sterility. 2020 Feb; 113(2):417-425.e1. doi:10.1016/j.fertnstert.2019.09.042

37. Ricci G, Nucera G, Pozzobon C, Boscolo R, Giolo E, Guaschino S. A simple method for fallopian tube sperm perfusion using a blocking device in the treatment of unexplained

infertility. Fertility and Sterility. 2001 Dec; 76(6):1242-1248. doi:10.1016/s0015-0282(01)02913-2

38. Al-Azemi MK, Omu AE, Fatinikun T, Mannazhath N, Abraham S. Factors contributing to gender differences in serum retinol and alpha-tocopherol in infertile couples. Reproductive Biomedicine Online. 2009 Oct; 19 (4):583-590. doi:10.1016/j.rbmo.2009.05.005

39. Eskenazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Human Reproduction. 2005 Apr; 20(4):1006-1012. doi:10.1093/humrep/deh725

40. Thiele JJ, Friesleben HJ, Fuchs J, Ochsendorf FR. Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. Human Reproduction. 1995 Jan; 10(1):110-115. doi:10.1093/humrep/10.1.110

41. Aboulghar MA, Mansour RT, Serour GI, et al. Management of long-standing unexplained infertility: A prospective study. American Journal of Obstetrics and Gynecology. 1999 Aug; 181(2):371-375. doi:10.1016/s0002-9378(99)70564-8

42. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. International Journal of Andrology. 2006 Dec; 29(6):569-575. doi:10.1111/j.1365-2605.2006.00700.x

43. Check ML, Check JH, Katsoff D, Summers-Chase D. ICSI as an effective therapy for male factor with antisperm antibodies. Archives of Andrology. 2000 Nov-Dec; 45(3):125-130. doi:10.1080/01485010050193887

44. Cannarella R, Condorelli RA, Mongioì LM, Barbagallo F, Calogero AE, La Vignera S. Effects of the selective estrogen receptor modulators for the treatment of male infertility: a systematic review and meta-analysis. Expert Opinion on Pharmacotherapy. 2019 Jan; 20(12):1517-1525. doi:10.1080/14656566.2019.1615057

45. Ko EY, Siddiqi K, Brannigan RE, Sabanegh ES. Empirical medical therapy for idiopathic male infertility: a survey of the American Urological Association. Journal of Urology. 2012 Mar; 187(3):973-978. doi:10.1016/j.juro.2011.10.137

46. Naz RK. Modalities for treatment of antisperm antibody mediated infertility: novel perspectives. American Journal of Reproductive Immunology. 2004 May; 51(5):390-397. doi:10.1111/j.1600-0897.2004.00174.x

47. Shin D, Palermo GD, Goldstein M, Rosenwaks Z, Schlegel PN. Indications for corticosteroids prior to epididymal sperm retrieval. International journal of fertility and women's medicine. 1998 May-Jun; 43(3):165-70.

48. Micic S, Lalic N, Djordjevic D, Bojanic N, Bogavac-Stanojevic N, Busetto GM, Virmani A, Agarwal A. Double-blind, randomised, placebo-controlled trial on the effect of L-carnitine and L-acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. Andrologia. 2019 Jul; 51(6):e13267. doi: 10.1111/and.13267

49. Agarwal A, Panner Selvam MK, Samanta L, Vij SC, Parekh N, Sabanegh E, Tadros NN, Arafa M, Sharma R. Effect of Antioxidant Supplementation on the Sperm Proteome of

Idiopathic Infertile Men. Antioxidants (Basel). 2019 Oct; 16; 8(10):488. doi: 10.3390/antiox8100488

50. Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. Central European Journal of Urology. 2013 Apr; 66 (1):60-7. doi: 10.5173/ceju.2013.01.art19.

51. Al-Azemi MK, Omu AE, Fatinikun T, Mannazhath N, Abraham S. Factors contributing to gender differences in serum retinol and alpha-tocopherol in infertile couples. Reproductive Biomedicine Online. 2009 Oct; 19 (4):583-590. doi:10.1016/j.rbmo.2009.05.005

52. Eskenazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Human Reproduction. 2005 Apr; 20(4):1006-1012. doi:10.1093/humrep/deh725

53. Lindsay TJ, Vitrikas KR. Evaluation and treatment of infertility. American Family Physician. 2015 Mar; 92 (6):437

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