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INVITED REVIEW

Male Fertility

Current medical management of endocrine-related male infertility

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Male factor contributes to 50%–60% of overall infertility but is solely responsible in only 20% of couples. Although most male factor infertility is ascertained from an abnormal semen analysis, other male factors can be contributory especially if the sample returns normal. Male infertility can be due to identifiable hormonal or anatomical etiologies that may be reversible or irreversible. This manuscript will highlight existing guidelines and our recommendations for hormone evaluation for male infertility and empiric therapies including multivitamins, estrogen receptor modulators (clomiphene), estrogen conversion blockers (anastrozole), and hormone replacement.

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INTRODUCTION

Our natural instinct to propagate our genes and have offspring will always remain a definable human trait. In so being, the inability to reproduce is a very distressing concern to couples who have such intentions. It is estimated that 1 in 6 couples (15%) is unable to conceive after 12 months of regular, protected intercourse. Male factor contributes to 50%–60% of overall infertility but is solely responsible in only 20% of couples.[1] Although most male factor infertility is ascertained from an abnormal semen analysis, other male factors can be contributory especially if the sample returns normal. Male infertility can be due to identifiable hormonal or anatomical etiologies that may be reversible or irreversible. However, an identifiable cause cannot always be elucidated as is the case with 40%–50% of infertile males evaluated.[2] Azoospermia is found only in 15%–20% of infertile males, and in 10% with sperm density $<1 \times 10^6$ ml^{-1}.[3] Idiopathic infertility describes the situation faced by a male with abnormal semen analysis but unidentified etiology, while unexplained or nonclassifiable fertility describes a couple with a normal semen analysis and partner evaluation in which an origin for their inability to conceive cannot be identified. There is currently no consensus amongst infertility experts on the management of idiopathic oligospermia. This chapter will highlight existing guidelines and our recommendations for hormone evaluation for idiopathic male infertility and empiric therapies including multivitamins, estrogen receptor modulators (clomiphene), estrogen conversion blockers (anastrozole), and hormone replacement.

Recognition of irreversible causes for infertility will spare the couple the distress of unnecessary therapies while uncovering identifiable and reversible etiologies for male infertility, as well as the treatment algorithms involved for improving a couple's rate of success in conceiving a child.

In a recent survey, 25% of urologists shockingly reported using exogenous testosterone to treat low testosterone levels in conjunction with male factor infertility.[4] In fact, pharmaceutical companies have used exogenous testosterone as a form of male contraception in several trials.[5] Over the last 5 years, there has been an increase of 170% in testosterone prescriptions,[6] and concomitantly an increase in the number of couples pursuing consultation for infertility.[7] With both physicians' inappropriate use of hormone therapy and patients' lack of awareness regarding potential reproductive harms of exogenous testosterone, there exists a need for improved education regarding medical management of male factor infertility. While the FDA labels for testosterone reports that usage can result in azoospermia or spermatogenic detriment, there is a paucity of expert guidelines for appropriate hormone therapy for men of reproductive age.[8] In addition, though diagnostic and therapeutic guidelines for identifiable causes for male factor infertility are well documented in the literature,[8] there is an absence of consensus treatment pathways regarding off-label empirical medical therapies for idiopathic/unexplained infertility.[4] Despite the expert guidelines, there exist a patient need and clinician interest in treating idiopathic infertility with empiric medical therapies. Two of three US urologists would use such therapies to treat this condition, attempting the regimen for 3–6 months (61%), 12 months (24%), and >12 months (0.9%).[4] This manuscript will highlight existing guidelines and our recommendations for hormone evaluation for male infertility and empiric therapies including multivitamines, estrogen receptor modulators (clomiphene), estrogen conversion blockers (anastrozole), and hormone replacement therapy to improve reproductive potential prior to assisted reproductive techniques.

AUA INFERTILITY GUIDELINES FOR HORMONE EVALUATION

The "Optimal Evaluation of the Infertile Male" is a released best practice statement based on published data from a panel of physicians and researchers released in 2010.[9] For the full initial evaluation of male

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factor infertility, the guidelines recommend a complete history and physical, focusing on an extensive reproductive history, and two semen analyses separated by at least 1 month. This should be performed if infertile (after 12 months of regular, unprotected intercourse) or earlier if known infertility risk factors exist. The reproductive history should include coital frequency/timing, duration of infertility and prior fertility, childhood illnesses and development, past medical/surgical/sexual history, and gonadal toxin or environmental exposure. Included in the medical history should be a review of medications/allergies, lifestyle exposures, family reproductive history, sexually transmitted diseases and previous infections.

The AUA guidelines do not recommend an initial hormone evaluation, but only after an abnormal sperm analysis or clinical finding suggesting an underlying endocrinological etiology for male infertility. However, most experts including ourselves believe that all infertile males should have an initial endocrine evaluation, but there is no consensus on how comprehensive this evaluation should be. Intratesticular testosterone is vital for spermatogenesis maintenance. When indicated, the minimum initial hormonal evaluation should include serum follicle stimulating hormone (FSH) and serum total testosterone (TT) levels. The AUA guidelines on infertility further state that if the TT level is low, a repeat measurement of TT along with free testosterone (FT) or bioavailable testosterone (BT), serum luteinizing hormone (LH), and prolactin level should be obtained.[9] Free testosterone assays have been used to aid the diagnosis of hypogonadism, but these tests are expensive and unreliable when suboptimal assays are utilized. However, free and bioavailable testosterone can be quickly and accurately determined using online calculators in comparison to the gold-standard testosterone dialysis assays.[10,11] Our initial hormone evaluation at the minimum typically includes TT, estradiol, FSH, and sex hormone binding globulin (SHBG) to make the calculation for free testosterone. It is the experience of our group to additionally acquire LH and prolactin lab work if the patient's initial evaluation reveals a hypogonadal state we define by FT <6.5 ng dl^{-1} We place more value and focus on the free testosterone levels as they more accurately relate with intratesticular testosterone levels than serum TT, which are crucial for the maintenance of spermatogenesis. Without evaluating free testosterone, many males will be overtreated or undertreated based on surreptitious TT levels that can't accurately portray the male's overall gonadal state given the inherently variable of SHBG across the population.

Another reason to check initial testosterone is to determine the testosterone/estrogen (T/E) ratio. This ratio has been recently identified as a good indicator for predicting normal spermatogenesis. Pavlovich *et al.* and Luboshitzky *et al.* identified an endocrinopathy in males with severely impaired sperm production to have decreased testosterone, increased estrogen, and a decreased serum T/E ratio.[12,13] In these studies, the seminal plasma levels of estrogen were higher than serum levels implying there is intratesticular production of estrogen.[12,13] Estrogen is known to have two sites of negative feedback: at the hypothalamus, decreasing GnRH pulse frequency, and at the pituitiary, decreasing GnRH responsiveness. Its function at the testicular level has been associated with stimulating sperm motility, oocyte penetration, and oxygen production.[13] More recently, it has even been shown that a decreased T/E ratio and increased estrogen may be a good indicator for specifically predicting azoospermia, especially in those with congenital absence of the vas deferens.[14] Thus, the seminal testosterone levels coupled with T/E ratio may serve as a way to predict the success of surgically retrieving spermatozoa from the testis of nonobstructive azoospermic patients.[14]

THE ROLE OF ANDROGENS IN FERTILITY

Androgens produced by the testis and adrenal gland play an essential role in the development of male reproductive organs, puberty, male sexual function, and male fertility. It is estimated that low testosterone or increased levels of luteinizing hormone (LH) are present in about 20%–30% of male infertility cases.[15] When working up male infertility, initially checking is recommended because increasing intratesticular testosterone will increase sperm count. It is well established that testosterone stimulates spermatogenesis, and the effect of LH stimulating spermatogenesis is mediated through intratesticular testosterone.[16] High levels of intratesticular testosterone is mainly bound to androgen binding protein and secreted into the seminiferous tubules.[15] Inside sertoli cells, testosterone is bound to androgen receptors. When activated, these receptors result in the initiation and maintenance of spermatogenesis and inhibition of germ cell apoptosis.[15,17] Abnormalities in androgen receptor may result in abnormal male sexual development, but also be a potential cause of male infertility.[15] It has been found that the concentration of intratesticular testosterone is 25–100 times greater than normal serum levels.[15–17] These high levels of intratesticular testosterone can never be achieved by oral or parenteral administration of androgens. Furthermore, treatment with exogenous testosterone and its metabolite, estrogen, would only suppress GnRH and LH at the hypothalamus and pituitary, respectively, which would ultimately suppress testicular testosterone production.[15,18]

RECOVERY OF SPERMATOGENESIS AFTER TESTOSTERONE CESSATION

One of the first steps in the medical management of male factor infertility is to determine if the patient who is attempting to conceive is receiving exogenous testosterone to treat symptomatic hypogonadism in conjunction with or solely and inappropriately for reproductive assistance. Testosterone inhibits the hypothalamic-pituitary axis with negative feedback to GnRH and gonadotropin (FSH, LH) release. This gonadotropin suppression ultimately inhibits spermatogenesis due to a decrease in intratesticular and overall testosterone production.[7] Anabolic steroid abusers in addition to resulting azoospermia have also been shown to incur abnormal sperm morphology and motility.[19,20] World Health Organization Task force study that 65% of men became azoospermic by 6 months with an average time azoospermia of 120 days.[21] With cessation of exogenous testosterone, 84% achieved sperm density $>20 \times 10^6$ ml^{-1} after median 3.7 months. However, only 46% of men recovered their baseline sperm density. In another study, Liu *et al.*[22] developed a probability-based recovery rate for spermatogenesis based on multivariate analysis of 30 studies. The report showed that 67% of males recover sperm densities $>20 \times 10^6$ ml^{-1} in 6 months, 90% in 12 months, 96% in 18 months, and 100% in 24 months. These data can be beneficial in counseling patients after cessation of exogenous testosterone in regards to timeline recovery expectations. In our experience, we immediately recommend cessation of exogenous testosterone in couples seeking fertility and start the patients on clomid to expedite the recovery of spermatogenesis. If patients are extremely motivated or patient LH levels are elevated, the more costly hCG therapy can also be initiated and/or added to improve the timeline for recovery.

THE ROLE OF SHBG

While it is known that testosterone levels decline with age, emerging data also suggests that genetics has a significant role in the development of hypogonadism, specifically, genetic variants in the sex hormone-binding globulin locus that may result in substantial

variation in serum testosterone levels.[23] In the serum, testosterone exists in an equilibrium between free testosterone and protein-bound testosterone. The latter can further be subdivided into the weakly albumin-bound portion which diffuses readily into target tissues and tightly SHBG-bound which is rendered inactive. While TT levels represent all forms of testosterone, 2%–3% is free, 50% is bound to albumin, and approximately 44% is tightly bound to SHBG. Like TT, SHBG can vary widely with aging or in disease states such as obesity, diabetes mellitus, hyperthyroidism, or cirrhosis. SHBG has also been shown to be an independent determinant of diabetes mellitus,[24] metabolic syndrome,[25–27] cardiovascular-related mortality,[24] cardiovascular risk profile,[28] bone mineral density,[29] verbal memory,[30] and endothelial dysfunction.[31]

Longitudinal population-based studies have shown that while SHBG levels increase with advancing age, TT levels steadily decrease.[32–34] As testosterone is commonly bound to SHBG, this could lead to an overall decrease in BT or FT. Genetic differences in the SHBG locus also spawn substantial variations of SHBG levels in the normal population and corresponding variations in available testosterone.[23] In addition, SHBG, such as TT, can vary widely in normal physiologic states such as aging or in disease states such as obesity, diabetes mellitus, hyperthyroidism, or cirrhosis. Thus, due to the degree of variation in SHGB levels, secondary to disease states and normal physiology, we recommend calculating bioavailable testosterone with TT and SHBG lab values via online calculators to more accurately describe a patient's gonadal status.[10,11] In addition, TT levels can vary up to 10% when collected over several days.[35] Review of our own data showed both over and underestimation of a low T state (TT <300 *vs* FT <6.5) 30% of the time when using TT alone compared to calculated FT in the same patient.

CLOMID

Low testosterone is characterized as a level below 300 or a free testosterone below 6.5 (European guidelines). When there are low testosterone levels with a normal T/E ratio, clomiphene citrate (CC) is the drug of choice. CC is a racemic mixture of two isoforms – enclomiphene and zuclomiphene.[36] It is a selective estrogen receptor modulator that blocks negative feedback at the level of the hypothalamus and the pituitary. This blockade of negative feedback indirectly enhances LH and FSH excretion from the anterior pituitary.[36] Increasing LH and FSH increases both testosterone production as well as spermatogenesis, respectively. Since CC relies on increasing FSH, it will not be as effective in patients with an elevated FSH level or in patients lacking a posttreatment FSH surge.[36] Furthermore, no improvements in semen parameters were seen when estrogen/testosterone ratio increased after CC administration or if the estrogen/testosterone ratio increased (>4.01) after a subcutaneous hCG shot.[36] Recent data has demonstrated CC causing moderate elevations in LH, FSH, and sperm concentrations in patients with pregerminal hypofertility and in those with unexplained infertility.[36] When looking at response characterized by either pregnancy or improvement in total motile sperm of 100%, Ross *et al*. found more than 50% patients (35/53 patients) responded within the first 3 months of treatment with the rest of the cohort responding after 6–15 months.[37] Another meta-analysis by Bridges *et al*. revealed a 7.7×10^6 ml^{-1} increase in sperm concentration between CC administration versus control.[38] Although sperm concentration may not be the best marker for fertility, there is a specific patient population that would most likely receive benefit – those suffering from idiopathic infertility with a sperm concentration between 10 and 20×10^6 ml^{-1}, low FSH and LH, and normal to slightly below normal motility and morphology.[38] These patients could likely attain a sperm concentration with CC therapy that would make them viable candidates for artificial insemination.[38] When examining pregnancy rate, two studies have demonstrated increased rates. Ghanem *et al*. evaluated the combination of CC and Vitamin E versus placebo finding pregnancy rates higher in treatment groups significantly higher (37% *vs* 13%, respectively $P = 0.037$).[39–41] A meta-analysis by Chua *et al*. of estrogen antagonists (clomiphene citrate 50 mg and tamoxifen) showed a statistically significant increase in sperm concentration as well as an increased pregnancy rate (OR: 2.4, NNT of 10).[38,42] Studies that did not find any significant outcomes for CC versus placebo exist, especially when looking at pregnancy outcomes. An optimal dose is also not established, with dosing ranging from 12.5 to 400 mg per day.[36,38] We recommend starting with a low 25 mg dose 3 times per week (M/W/F) and slowly titrating up to 50 mg once a day as needed. We utilize clomid in all men with a FT <6.5 that present to our infertility clinic, all men with azoospermia or severe oligospermia $<5 \times 10^6$ ml^{-1} with normal FSH levels. We consider clomid men with FT <10 with definitive symptoms of low T (low libido, poor erections, low energy).

Recently, enclomiphene citrate, one of the single isomers of CC, has been tested to help with infertility treatment. This isomer has pure estrogen antagonism compared to CC, which exhibits both agonistic and antagonistic properties.[43] So far, only one study has been completed but shows promising results demonstrating increased morning testosterone levels, LH, FSH, DHT, estrogen, while preserving spermatogenesis.[43]

Clomiphene citrate is tolerated well by most patients. More common side effects include gastrointestinal distress, dizziness, hair loss, gynecomastia, and minimal weight gain.[38,40] There is also a 1.5% risk of visual disturbances such as blurred vision, photophobia, and diplopia although they are reversible with cessation of the medication.[38,40] There have been few reports about clomiphene affecting sperm parameters. Ross *et al*. reported one of their 53 patients on high dose CC (100 mg 3 times per week) had a decrease in sperm motility, however, demonstrated rapid improvement when the dose was reduced to 50 mg every other day.[37] There was also another case report by Pasqualotto *et al*. that found three patients of their cohort becoming azoospermic after treatment, with only return to severe oligospermia after clomiphene discontinuation.[36,38] It will be interesting to note whether future studies using a nonracemic mixture of pure enclomiphene will avoid these occasional negative effects.

AROMATASE INHIBITORS

Aromatase inhibitors (AIs) are the medication of choice in infertile males with normal testosterone levels but abnormal T/E ratios. Aromatase, a cytochrome p450 enzyme, is present in the testes, prostate, brain, bone, and adipose tissue of men; it converts testosterone and androstenedione to estradiol and estrone, respectively.[44,45] Estradiol negatively feeds back on the hypothalamus and pituitary to reduce gonadotropic secretions ultimately affecting spermatogenesis.[45] AIs decrease estrogen production by reversibly inhibiting cytochrome p450 isoenzymes 2A6 and 2C19 of the aromatase enzyme complex.[36] Inhibiting the negative feedback of estrogen on the hypothalamus allows for stronger GnRH pulses that stimulate the pituitary to increase production of FSH. This increased FSH finally leads to increased spermatogenesis within the Sertoli cells.[36] Since AIs increase testosterone levels without impacting estrogen levels, unlike CC, they are better medications for men with abnormal T/E ratios.[36] AIs can be classified in two different ways – steroidal versus nonsteroidal

or by generation. Steroidal AIs mimic androstenedione and cause irreversible enzyme inhibition, whereas nonsteroidal AIs compete with endogenous substrates causing reversible inhibition.[45] AIs can also be described by generation according to their selectivity and potency with the third generation AIs being most selective and potent.[45] The main AIs used for infertility are Letrozole and Anastrazole, both third generation nonsteroidal AIs that show improved outcomes in early studies.[45] Raman and Schlegel treated 140 patients who had abnormal T/E ratios with Anastrazole and found treatment groups had statistically significant increases in their ratio as well as improved semen parameters.[44] Interestingly, in the study, obese patients did not have a greater benefit than originally expected.[44] Side effects are rare in Anastrazole, with <5% of men reporting changes in libido and 7.4% of men with transient and clinically insignificant alterations in liver function tests.[44]

Letrozole is a more potent AI than Anastrazole that has also been shown in clinical trials to help with male infertility. Cavallini *et al*. demonstrated Letrozole to markedly increase in FSH and serum testosterone in patients with nonobstructive azoospermia.[46] Testosterone rose from 331 to 1117 ng dl^{-1} with all hormone levels returning to baseline within a week after discontinuing therapy.[44] Sayalam *et al*. treated oligospermic men with low testosterone and low T/E ratios and observed an increase serum testosterone, sperm concentration, motility, and morphology with an expected decrease in estradiol. Two of 10 oligospermic patients conceived while 4 of 17 of the azoospermic cohort had sperm return to their ejaculate during treatment.[44,45] Another study by Greorgiou *et al*. observed similar findings with increases in serum testosterone, sperm concentration, motility and morphology when treating with either Letrozole or Anastrazole.[47]

There is still debate over the optimal dose of Letrozole. Drastic changes in estrogen/testosterone ratio have been observed, up to 6-fold, with variable dosing ranges ranging from 2.5 mg daily to 2.5 mg 3 times a week.[45] One study by Loves *et al*. even observed supraphysiologic testosterone levels with Letrozole 2.5 mg once a week for 6 weeks.[45] Side effects include decreased libido, headache, and transient liver enzyme abnormalities (in <10% of men).[44] Like most of these oral therapies for male infertility, better randomized controlled trails are still needed to help define the role of AIs in male infertility.

Combining the rationale behind the use of either CC or AIs in men with abnormal testosterone or abnormal T/E ratio, respectively, it is reasonable to extrapolate that using both oral therapies in men where testosterone and T/E ratio are abnormal. There have been no studies to date looking at using clomiphene and AIs simultaneously. We utilize Anastrozole 1 mg 3/week (M/W/F) in men with T >6.5 but T/E ratios <10/1. We often combine clomid with Anastrozole to optimize T levels and T/E ratios simultaneously.

MALE OBESITY AND SUBFERTILITY

The increased worldwide incidence of obesity has seen a larger cohort of these patients seeking treatment for male infertility. The mirrored increasing prevalence of obesity with an associated decline in semen parameters and subfertility has been thought to be due to negative feedback loops of decreased testosterone and increased estrogen in this sub-population that alters the hypothalamic-pituitary-gonadal axis. Despite the common thinking, a recent review highlighted the controversial data substantiating these claims with only 21 of 40 studies showing decreased sperm counts, 13 of 35 with reduced motility, and 9 of 29 with abnormal morphology. This discrepancy can partly be explained by uncontrolled confounders, however, the increased cohort of obese men with oligospermia and azoospermia in comparison to the general population gives credence to higher rates of infertility in this sub-population. In addition, this is supported by the further pathogenesis of increased reactive oxygen series and associated disruptions in DNA integrity in obese males in 10 of 12 studies.[48] All obese male patients should be encouraged to induct lifestyle changes including increased physical activity, diet modifications, and weight loss. These lifestyle changes have been shown to improve semen parameters in multiple studies.[49–53] Pharmacologically, aromatase inhibitors can be beneficial in correcting the testosterone-estrogen ratio and improving overall testosterone levels. See the aromatase inhibitors section for more details.

hCG

Human chorionic gonadotropin (hCG) is an LH analog derived from urine or recombinant sources that stimulate intratesticular Leydig cell testosterone production. hCG thus increases intratesticular and serum testosterone levels to improve spermatogenesis.[8] This IM or subcutaneous hormone replacement therapy is the only on-label pharmaceutical for the treatment of male infertility. hCG alone has shown to be able to maintain spermatogenesis only for short periods of time, as Depenbusch *et al*.[54] showed in males receiving the formulary for 2 years who eventually showed decreasing sperm counts after 12 months on the monotherapy. This is believed to highlight the necessary role of FSH in long-term maintenance of spermatogenesis. Additional drawbacks to hCG hormone replacement include the invasive injections required for delivery and high expense of the therapy.

hCG has also been combined with testosterone to improve intratesticular and serum testosterone levels. Although the combination therapy was initially studied as a form of male contraceptive, Coviello *et al*.[55] showed that males on exogenous testosterone with 94% decreased concentrations of intratesticular testosterone recovered with concomitant administration of IM hCG. An additional study correlated this effect with maintenance of spermatogenesis in a small series with 10 males.[56] Overall, a cochrane review of 6 RCT's with males on gonadotropins, which includes formularies other than hCG, showed 456 participants to have a live birth rate of 27% versus 0% and spontaneous pregnancy rate per couple of 16% versus 7% in placebos, purporting an overall beneficial effect on male infertility.[57] A recent case series assessed 49 men on testosterone supplementation with azoospermia or severe oligospermia ($<1 \times 10^6$ sperm ml^{-1}) on 3000 units hCG subcutaneously injected every other day for mean 14 months with supplementation of clomphine citrate, tamoxifen, anastrozole, or rFSH. Spermatogenesis improved in 47 men (95.9%) as defined by $>1 \times 10^6$ ml^{-1} sperm density with mean recovery in spermatogenesis of 4.6 months. Nineteen of these men achieved a pregnancy with no adverse effects documented, showing the feasibility of hCG combination therapy.[58]

In treating infertility, hCG hormone therapy is a very effective but less utilized regimen due to the great expense of the drug and more invasive nature of its IM delivery. It is usually recommended to a couple with idiopathic male infertility who have not had success achieving functional eugonadal levels on clomid and/or anastrozole, in patient who have developed clomid syndrome, or for motivated couples who would like to start with hCG therapy and willing to pay the expenses. When prescribed, we recommend 1500 units of hCG IM 3 times a week as a starting dose. The dose is escalated up to 3500 units 3 times per week to achieve high normal TT levels (TT >500). Subsequently, we would obtain a semen analysis in 3 months to monitor the patient's

response. We have experienced high rates of success with hCG hormone therapy and utilize to expedite T recovery in body builders or men on traditional T who have failed a clomid trial. Recombinant-FSH can be added if hCG alone is not successful. We have not traditionally employed additional therapies including GnRH, or HMG therapy. If not responsive to these alternative therapies, we recommend pursuing other means for achieving fertility via donor sperm or adoption.

MULTIVITAMINS

The latest Cochrane meta-analysis examined if supplementary oral antioxidants improve fertility outcomes for subfertile men. When looking at live birth rates, the meta-analysis found that antioxidants may be effective. A subfertile male with an expected live birth rate of 5% can increase up to 10%–31% with the most associated antioxidant being Vitamin E.[59] Antioxidants were also associated with increasing clinical pregnancy rates in subfertile males from a rate of 6% to within 11%–28%. The antioxidants most associated with increased clinical pregnancy rates were Vitamin E and zinc.[59] Both studies in the meta-analysis looking at DNA fragmentation demonstrated lowered fragmentation rates when compared to placebo.[59] One study in the meta-analysis examined Vitamin C plus Vitamin E versus placebo while the other study compared DHA versus placebo.[59] When examining sperm parameters, combined antioxidants had an association with increased sperm motility after 3 months. Vitamin C plus Vitamin E did not show any association with increased sperm motility nor sperm concentration at 3 months. Carnitines also found no association with increased sperm concentration after 6 months compared with placebo.[59] When looking specifically at Coenzyme Q10, a previous review by Lafuente *et al.* illustrated that Coenzyme q10 helps improve pregnancy rate, sperm concentration, and sperm motility. The current Cochcrane meta-analysis did note an improvement on sperm motility and concentration with Coenzyme Q10 at 6 months although no conclusions could be drawn because of high heterogeneity amongst studies.[59] A more recent meta-analysis by Garg and Kumar concluded similar findings – coenzyme Q10 supplementation resulted in improved semen parameters in several randomized control trials, however, the data is still poor and insufficient.[60] None of the antioxidants revealed any conclusive adverse events or an increased miscarriage risk. Like the Coenzyme Q10 data, the Cochrane meta-analysis also cited that the studies reviewed showed large heterogeneity with low quality of evidence indicating the need for larger placebo-controlled trials as well as head to head trials on antioxidants.[59] Given the evidence supporting vitamins in subfertile men, we recommend vitamins to healthy men attempting pregnancy given their low risk and cost.

It is thought that varicoceles increase scrotal temperature, reflux of blood blow, and damaged microcirculation, all of which act together to increase germ cell death and produce high amounts of reactive oxygen species leading to impaired fertility.[59,61] Varicocele grade has also been shown to correlate with the degree of oxidative stress.[61] Although surgery is still the standard of care in managing varicoceles, antioxidants could offer a potential low-risk solution in treating varicocele-induced infertility. Due to the heterogeneity of current studies, the lack of larger placebo-controlled studies, and lack of studies using pregnancy as an end point, there is currently insufficient data to recommend the use of antioxidants solely as medical management. However, the initial studies are promising.

Multiple studies have focused on antioxidants as sole therapy in men with varicoceles. Cavallini *et al.* reported significant improvements in sperm motility (24%–35%) and morphology (58%–71%) in patients with grade III varicoceles when given cinnoxicam, an anti-inflammatory agent that inhibits reactive oxygen species and prostaglandin synthesis.[46] Cavallini *et al*. also compared the effects of combination therapy between L-carnitine + acetyl-L-carnitine + cinnoxicam (group 1), L-carnitine + acetyl-L-carnitine (group 2), and a control group (group 3) for 6 months. Dosages were 2 g per day for L-carnitine, 1 g per day for acetyl-L-carnitine and 30 mg per 4 days for cinnoxicam. Group 1 had significantly increased semen parameters in all patients except in grade V varicocele and consistently had higher semen parameters than group 2. Group 2 also had significantly increased semen patterns except in grades IV and V varicocele. Pregnancy rates were also highest in group 1 (38% *vs* 21.8% in group 2 *vs* 1.7% in group 3). Although promising, the study groups were extremely heterogenous and composed of patients with varying degrees varicoceles and idiopathic infertility (all groups had a majority of patients with varicoceles *vs* idiopathic infertility).[46]

In an open uncontrolled study, Oliva *et al*. reported significant improvements of sperm morphology that lasted 4 weeks after the end of treatment when using combination therapy of pentoxifylline (1200 mg per day) + zinc (66 mg per day) + folic acid (5 mg per day) in 36 men with clinical varicoceles.[61]

Coenzyme Q10 has also been shown to play a potential role in managing male infertility. Festa *et al*. demonstrated significant improvements in sperm concentration, sperm motility, and total antioxidant capacity when giving 100 mg per day of coenzyme Q10 for 12 weeks in patients with low-grade varicocele. Although promising, the value of these results is limited because the study was a short, open labeled, uncontrolled trial.[61]

MPFF or Daflon, a derivative of bioflavonoid, has also been studied by Kilic *et al.*[62] and Soylemez *et al*.[63] It is thought that these agents prolong the activity of peripheral norepinephrine and ultimately improving venous tone in varicoceles. In both studies, 1 g per day of MPFF for 6 months demonstrated significant improvement in varicocele-associated pain, improvement in sperm motility and color Doppler parameters (decrease in reflux time of the left spermatic vein during Valsalva). However no improvements in sperm count, concentration, morphology was shown.[61]

When examining antioxidants versus surgery, two studies demonstrated that both treatment modalities were beneficial to sperm quality, but surgery had better outcomes. Cavallini *et al*. compared 61 patients undergoing varicocelectomy to 41 patients treated with 30 mg per 4 days with cinnoxicam. Surgery significantly improved sperm values within 4 months in all graded varicoceles where cinnoxicam only improved sperm quality in patients with moderate varicoceles.[46] Gamidov *et al.*[64] compared 728 patients with bilateral varicocelectomies, 107 patients with antioxidant therapy (combination of clomphene citrate, Vitamins A and E, selenium, L-carnitine, and pentoxifylline), and a control group. They reported increased sperm concentrations in both surgical and medical therapy groups, however the surgery group increase was much larger (70% *vs* 30%). When comparing natural pregnancy, patients who underwent surgery (47%) had better outcomes than medical treatment (21%) and control groups (3.6%).[61]

Antioxidants have also been used as adjuvant therapy after surgical management of varicocele. Zinc and folic acid have been studied the most and seem to have a positive impact on semen parameters after surgery. Takihara *et al.*[65] treated 36 men postvaricocelectomy with zinc (440 mg per day) and compared to 65 infertile men on zinc therapy. There was significant improvement in sperm motility at 2 and 12 months of therapy with 50% of the postoperative patients impregnating their partners whereas only 28% of the medical therapy group successfully

impregnated their partners.[61] Azizollahi *et al*.[66] further demonstrated the potential use of zinc and additionally folic acid by comparing 102 infertile patients postvaricocelectomy. Thirty-two patients received 66 mg zinc sulfate, 26 received 5 mg folic acid, 29 received combination therapy, and 25 received placebo for 6 months after surgery. Patients receiving zinc sulfate demonstrated significant increases in sperm morphology; patients receiving folic acid demonstrated significant increases in sperm concentration. The patients on the combination therapy showed significantly improved sperm concentration (30×10^6 ml^{-1} to 47×10^6 ml^{-1}), morphology (normal sperm increased from 46% to 57%), and motility (28.7%–43.0%).[59,61] Furthermore, a significant rise in peripheral blood inhibin B and seminal plasma activity was detected in patients on zinc and folic acid.[61,67]

Vitamin C has also been shown to play a role in adjuvant treatment after varicocelectomy in infertile men. Cyrus *et al*. demonstrated that men ($n = 115$) after varicocelectomy receiving 250 mg bid for 3 months had statistically significant increases in sperm motility and morphology than placebo.[68] No effect was shown on sperm count.[68] Although this is promising, more studies are needed to confirm the efficacy of Vitamin C as adjuvant therapy.

Definitive evidence is lacking to determine role of antioxidants in patients with varicoceles. Although the initial studies are promising, larger randomized control studies that use pregnancy as an endpoint are needed before antioxidants can be recommended as treatment.

CONCLUSIONS

This manuscript described existing guidelines and our recommendations for hormone evaluation for idiopathic male infertility. We utilize SHBG levels in addition to TT levels and FSH on initial patient screening given its ability to better quantify hypogonadism. Empiric therapies including multivitamins are safe and have support in the literature. Estrogen receptor modulators (clomiphene), hCG and aromatase inhibitors can be used to effectively augment and normalize low TT and potentially improve semen parameters.

COMPETING INTERESTS

Lipocine and Abbvie for consulting (testosterone related).

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