Antioxidants to enhance fertility: Role of eNOS and Potential Benefits

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Abstract

The use of antioxidants is now often used as a pharmacological adjunct to limit infertility. Indeed, the lay public rightly perceives oxidative stress and, thus, antioxidant treatment as important modulators of infertility. While the direct effects of antioxidant treatment on the quality of semen and oocytes are still under investigation, a significant body of evidence points to loss of vascular tone as a root-cause of erectile dysfunction and, possibly, alterations to female reproduction.

In this article, we will critically review the often neglected link between vascular dysfunction and infertility. A particular emphasis will be on the potential use of antioxidants to increase fertility and promote conception.

Keywords: antioxidants, infertility, vascular dysfunction, erectile dysfunction, pre-eclampsia, reproductive health, nitric oxide

1. Introduction

Human reproduction is known to be a highly inefficient process where as much as 50% of conceptions fail, and ~20% of clinical pregnancies end in spontaneous abortion [1]. Both male and female reproductive dysfunction is thought contribute to this high rate of failure, but few defined etiologies have been identified. Despite the multifactorial nature of human infertility, there is a growing awareness that reactive oxygen and nitrogen species (ROS/RNS) and associated oxidative damage may be potent modulators of reproductive health [2,3]. Oxidative and nitrosative stress is associated with both risk factors for infertility (e.g. smoking, diabetes, hypertension, and aging), and directly in reproductive disorders as diverse as oocyte implantation, endometriosis, and pre-eclampsia in women, and erectile dysfunction, sperm damage and motility in men [2,4]. Because of this recognition, use of antioxidants and other redox modulatory compounds as nutraceutical/pharmacological treatments for many forms of infertility has soared as a result [3]. Numerous studies point to the benefits of dietary antioxidants to limit oxidative stress, thereby potentially maintaining the quality of semen and oocytes, and also lowering the risk for endometriosis and other female reproductive disorders. However, clinical trials using antioxidants as therapies for many reproductive disorders have yielded negative or conflicting results as to their benefits on reproductive health [5-8]. While the direct effects of antioxidant treatment are still under investigation, it is interesting to note that many endpoints for both male and female infertility (e.g. erectile dysfunction in males, and preeclampsia, oocyte implantation, and endometriosis in women) actually involve vascular dysfunction, where antioxidants have provided significant benefits.

Herein we review the remarkable roles that vascular dysfunction plays as an underlying risk for many aspects of infertility as well as potential dietary antioxidant

compounds that may improve vasomotor function, thereby limiting risk for infertility and promote conception.

2. Vascular dysfunction in conditions leading to infertility

The vascular endothelium that lines the lumen of vessels are critically important in maintaining vascular tone [9]. Endothelial cells are responsive to many stimuli that either promote vasorelaxation (e.g. shear stress, nitric oxide, prostacyclins, sphingosine 1-phosphate, acetylcholine, among others) or constriction (angiotensin II, endothelin-1, thromboxane) [10,11]. Thus, any condition, like oxidative stress, which impairs vasomotor function, could have profound adverse consequences on reproductive health.

Endothelial-derived vasorelaxation is gradually lost with age [12]. Consequently, endothelial dysfunction is a primary complication of age-dependent cardiovascular diseases, including hypertension, atherosclerosis, attendant coronary artery disease, the metabolic syndrome, and is increasingly recognized as a hallmark of congestive heart failure [13,14]. Impaired vasomotion is often observed in the absence of severe atherosclerotic lesions and endothelial dysfunction often occurs long before symptoms of coronary atherosclerosis become clinically apparent. It is often at this early stage of cardiovascular disease where clinical studies link endothelial impairment and infertility. For example, erectile dysfunction is a leading indicator of CVD [15], and erectile dysfunction patients display significantly impaired brachial artery flow-mediated dilation without additional cardiovascular risk factors [16,17].

Aside from erectile dysfunction, endothelial dysfunction also appears to be a significant factor in preeclampsia (pre-eclampsia), a hypertension syndrome

occurring in 3 to 5% of pregnant women that results in reduced placental perfusion [18]. Pre-eclampsia is a primary event that significantly increases maternal and neonatal morbidity and mortality with no current therapy other than placental delivery. A number of studies now show a significantly reduced vasomotor function in pre-eclampsia patients. For example, Aardema *et al*. used Doppler analysis to show that the abnormal flow-mediated dilation of the uterine artery was strongly associated with pregnancy complications [19,20]. Brodszki and coworkers also showed that uterine artery flow was strongly impaired in pre-eclampsia with the lowest dilation evident in women with bilateral uterine artery notches [21]. Interestingly and in keeping with evidence for erectile dysfunction in men, no significant differences in carotid or aortic vessel wall stiffness are often evident in women with pre-eclampsia versus healthy controls. In summary, while pre-eclampsia is not a major cause of infertility, endothelial dysfunction contributes to this important disorder of pregnancy and should be adequately addressed.

From the available data, it is clear that infertility is multifactorial and should be individually addressed based on careful anamnesis [22]. However, infertility in both males and females appear to be linked by a profound loss in endothelial-dependent vascular function.

3. Vascular tone is principally regulated by endothelial-derived nitric oxide

As briefly mentioned above, the vascular endothelium governs vasoresponsiveness by physio-mechanical means, hormonal stimuli, and by synthesizing and releasing factors that act on the vascular smooth muscle layer [9]. The principal vasodilatory agent is nitric oxide (NO), which induces relaxation by stimulating cGMP production in vascular smooth muscle cells (VSMC). Often, loss of

vasoresponsiveness has been attributed to insufficient endothelial-derived NO as exogenous NO donors (e.g. nitroprusside) restore adequate vasomotor function in CVD patients, the elderly, and in animal models [23]. Nitric oxide is synthesized by endothelial nitric oxide synthase (eNOS), one of a family of heme-containing, homodimeric enzymes that catalyze the 5 electron oxidation of the guanidinium moiety of L-arginine to NO and L-citrulline [24]. In endothelial cells, eNOS expression and activity is tightly governed both at the transcriptional and post-translational levels, by intracellular Ca^{+2} concentrations, and protein-protein interactions [25]. Additionally, its subcellular distribution also markedly affects overall enzyme activity. eNOS exists in at least two distinct subcellular locales in the endothelium: at caveolae of the plasma membrane and in the Golgi/perinuclear region of the cell [26]. Plasma membrane associated eNOS is considered to be more constitutively active and highly sensitive to agonist-induced intracellular Ca^{+2} fluxes. Additionally, eNOS at the plasma membrane is typically phosphorylated at Serine 1176 (*rattus* sequence) in an Akt-driven manner, which makes this caveolae-associated enzyme markedly more active in response to shear stress and other stimuli [27]. In contrast, Golgi-bound eNOS is less phosphorylated and relatively insensitive to Ca^{+2}-dependent activation; the latter may be due to inaccessibility of calmodulin to eNOS in this cellular compartment.

Besides subcellular locale, interactions of eNOS with effector proteins play a key regulatory role. eNOS in association with caveolin-1, a major protein component of endothelial caveolae, significantly inhibits eNOS activity as it prevents calmodulin binding [26,28]. Conversely, active eNOS at the plasma membrane can be co-immunoprecipitated with Hsp90 and kinases, especially Akt. Together, these regulatory mechanisms tightly govern eNOS-derived NO availability.

4. Protein effectors and the phosphorylation state of eNOS strongly affect its activity

Recent papers by Smith *et al.* [29,30] revealed that post-translational dysregulation of eNOS may play a remarkable role in endothelial dysfunction, especially in the elderly. These authors showed that aged rats displayed significant impairment in endothelial-derived vasomotor activity, but total eNOS content did not change with age. However, aging caused eNOS to disassociate from its activating proteins (Hsp90 and Akt) while increasing the levels of eNOS associating with caveolin-1, a protein known to inhibit eNOS activity. Along with this altered subcellular redistribution, these authors showed that the phosphorylation state of eNOS was significantly and persistently altered in aging rat aortic endothelia, which is consistent with its inactivation [29]. Loss of eNOS phosphorylation status was found to stem from chronically elevated sphingomyelinases (SMases) and resultant ceramide-driven protein phosphatase 2A (PP2A) activity, which specifically dephosphorylated S1176. Thus, age-related (and, likely, disease-related) changes in eNOS phosphorylation and/or its cellular association with other protein effectors may significantly alter eNOS activity and vascular function.

5. Redox-dependent modifications that affect eNOS activity or NO bioavailability

Apart from the aforementioned post-translational regulatory mechanisms governing eNOS function, redox changes in the endothelium can substantially modify both enzyme activity as well as the enzyme's product, NO, thereby adversely affecting vasomotion. For the latter, eNOS synthetic activity is dependent on

maintaining tetrahydrobiopterin (BH4) in a highly reduced state. When the tetrahydrobiopterin/biopterin ratio is high eNOS readily produces NO; however, when the BH4 redox ratio declines, the internal electron transport chain of eNOS becomes uncoupled, which actually generates superoxide instead of NO. Thus, in a pro-oxidative milieu, eNOS may not only become a target of oxidants, but also may exacerbate oxidative stress and endothelial dysfunction. In keeping with this concept, incubating endothelial cells with oxidized LDL (oxLDL) constitutively downregulates eNOS activity and causes loss of eNOS in the plasma membrane [31]. Thus, a pro-oxidant imbalance in the vasculature may affect both eNOS activity, thereby contributing to lower synthesis of endothelial-derived NO.

In addition to eNOS uncoupling, reactive oxygen species (ROS) may limit NO bioavailability by converting it into non-vasoactive reactive nitrogen species (RNS) [32]. Indeed, production of ROS in excess to the antioxidant defense mechanisms plays an important role in endothelial dysfunction. Thus far, the three most widely studied sources of vascular ROS are catalyzed by xanthine oxidoreductase, NADH/NADPH oxidase, and NO synthase (NOS) [33]. For the vascular endothelium, substantial evidence shows that NADH/NADPH oxidase is quantitatively the predominant producer of superoxide, a relatively innocuous but ubiquitous type of ROS (Fig 1) (see below for the role of NADPH oxidase in spermatozoa). However, despite its relatively benign nature, superoxide readily reacts with NO at rates approaching diffusion limitations to produce peroxynitrite ($OONO^-$), a powerful reactive nitrogen species which would not only limit NO bioactivity, but increase endothelial oxidative stress and damage [23,34]. Chronic limiting levels of low molecular weight antioxidants, which would promote sustained increases in superoxide would be expected to exacerbate peroxynitrite formation. Thus,

conversion of NO to deleterious reactive nitrogen species represents a plausible means for limiting NO bioavailability and its contribution to the altered vessel function in infertility.

6. Vascular dysfunction: potential therapeutic agents

Because oxidative stress appears to be an underlying factor in endothelial-dependent decline in vasoactivity, numerous studies have attempted to ameliorate lower endothelial redox state, and hence improve vascular responsiveness through increasing antioxidant levels. We will now briefly review many of the antioxidants used for these studies and their potential benefits both to vascular health and infertility.

6.1 Vitamin C

Vasomotor dysfunction in cardiovascular patients can be reversed by vitamin C treatment, which appears to facilitate endothelial-derived NO (EDNO) bioavailability [35-37]. Ascorbic acid stimulates EDNO synthesis through increasing intracellular levels of BH4 and its reduced redox state, thereby maintaining eNOS coupling [38,39]. Data from Visioli *et al*. is instructive as to the effectiveness of ascorbate to maintain eNOS synthetic activity [40]. This study showed that ascorbic acid did not work through maintaining glutathione (GSH) levels or its reduced redox state in HAEC, indicating that it is unlikely that ascorbic acid increased EDNO synthesis simply by markedly improving the intracellular redox environment. Rather, maintenance or improvement of intracellular BH4 levels (by reducing dihydro- or trihydrobiopterin to BH4) appeared to be a more likely mechanism. These results were in keeping with previously published studies showing ascorbic acid can maintain biopterin in a highly

reduced state [38,41]. As such, ascorbic acid (500 mg/d) is considered a useful addition to the pharmacological therapy of hypertension [42] and might be explored as a potential adjunct to the treatment of impaired vasomotion and associated erectile dysfunction.

6.2 Glutathione (GSH) analogs and GSH synthesis inducers

Glutathione (GSH) is a major low molecular weight cellular antioxidant that directly terminates ROS, or acts as a substrate for GSH-dependent antioxidant enzymes (e.g. GSH-dependent peroxidases, glutaredoxin, and GSH-S transferases) [43]. Even though GSH is not readily taken up by endothelial cells directly, a number of compounds increase intracellular levels of GSH either by increasing cysteine availability (e.g. *N*-acetyl-cysteine), which is the rate-limiting substrate for GSH synthesis, or by inducing expression of GSH synthesis genes (e.g. lipoic acid, sulforaphane, and other Phase II detoxication gene inducers) [44-46]. As mentioned, GSH does not appear to directly maintain biopterin in a highly reduced and active state, but does markedly improve EDNO bioavailability. One purported mechanism for this increased availability may be through formation of GSH-NO conjugates, which can enhance VSMC cGMP production and vasorelaxation [47,48].

Additionally, endothelial GSH levels may strongly influence eNOS activity by maintaining phosphorylation status, especially at the S1176 residue. As previously alluded, oxidative stress induced by pro-inflammatory cytokines (TNFα) [49], redox cycling agents (doxorubicin), or by ROS [10] lowers GSH levels and activates PP2A via induction of sphingomyelinases and concomitant increases in ceramide levels [50-52]. As PP2A specifically dephosphorylates the S1176 site of eNOS, oxidative stress may thus lead to inactivation of the enzyme. In this regard, the importance of

maintaining cellular GSH status is exemplified in *in vitro* studies where pretreating MCF7 cells with GSH, GSH-methylester, or *N*-acetylcysteine prior to TNFα prevented cellular GSH loss and also limited sphingomyelinase-mediated ceramide generation [53]. We followed up on this indirect evidence and showed that by improving tissue and cellular GSH levels, age-associated impaired vascular compliance could be reversed and eNOS phosphorylation state maintained [44,54-58]. This reversal was achieved by using (*R*)-α-lipoic acid (LA), a dithiol compound that is readily absorbed from the diet and increases cellular GSH by increasing the expression of GSH synthesis genes. Specifically, aortic EDNO-dependent vasomotor function was markedly improved 12 hrs following intraperitoneal administration of LA (40 mg/kg), and this improvement was achieved through enhanced eNOS *and* Akt phosphorylation [59]. Thus, pharmacological agents that maintain or improve endothelial GSH status may markedly improve EDNO bioavailability and vascular tone through preserving eNOS phosphorylation and activation. In particular, LA appears to be a promising pharmacological agent for this purpose with little clinical side-effects [60] as it would induce a long-term elevation in GSH synthetic capacity rather than an acute, limited effect supplied by compounds that transiently raise cysteine availability. While the use of LA in the treatment of vascular dysfunction, specifically related to conditions leading to infertility, requires ad-hoc investigations, the vasomodulating properties of LA provide solid bases for future clinical trials.

In summary, there appears to be a link between inflammation, oxidative stress, and generation of ceramide, which can be governed by thiols, namely GSH, status. Hence, modulation of GSH status may be employed to reverse the age- or disease-related decline in cellular GSH.

6.3 Polyphenols

Polyphenols are being actively investigated as vasomodulators. In particular, administration of flavonoids, especially from tea and cocoa, ameliorates endothelial dysfunction and restores proper vasomotion [41]. This is likely as a consequence of increased endothelial NO synthase (eNOS) activity. The association between flavonoid intake and maintenance of vascular tone is strongest in the Mediterranean area, where the traditional diet is rich in plant-based foods and in wild vegetables [61]. It is noteworthy that, based on scientific evidence and targeted marketing, several polyphenol-containing supplements are being sold to infertile men and women; hence, the need to clarify, in vivo, the role of these molecules in ameliorating fertility.

Diets rich in plant foods are associated with better vascular compliance [61]. As an example, recent studies using isolated rat vessels showed that *Cynara cardunculus* (wild artichoke) exerts vasorelaxant effects. In particular, feeding studies - using doses of polyphenols that are compatible with human consumption - confirmed that *Cynara c.* can restore proper vascular function in aged Fisher 344 x BN rats fed the extract (10 mg polyphenols/Kg) for five days [62]. These data confirm *in vivo* the results that were observed *in vitro* [63] and thus supply the basis for formulating nutraceuticals aimed at maintaining vascular motility, especially in the elderly. Also, this adds further experimental evidence to the body of basic research supporting the epidemiological evidence of a lower CHD incidence in the Mediterranean area. In particular, these data suggest that maintenance of correct vasomotion by bioactive components from plants in various Mediterranean regions (Lucania - Southern Italy, Crete, Segura Valley – Southern Spain [61]) might contribute to its cardioprotective properties. The precise mechanism(s) of action responsible for the enhancement of NO production are still elusive. However, in vitro

data exclude any effect on eNOS levels [63] and are in agreement with other data that suggest how water-soluble antioxidants and thiol agents maintain an intracellular reduced environment and preserve cofactors such as BH_4, hence preventing eNOS uncoupling [33] and facilitating its activity [64]. Finally, facilitated hydroxylation of eNOS, which is the first step in NO production by this enzyme [9], might also explain the potentiating activities of wild plant extracts, which are rich in ortho-diphenols (as an example, we have identified luteolin-7-glicoside, apigenin, rosmaric acid, and ursolic acid in wild artichoke [63]).

Other polyphenols that are attracting considerable research are those contained in cocoa and in tea.

Indeed, cocoa and chocolate products have a much higher content – on a per-weight basis - of polyphenols and total antioxidant activity than other polyphenol-containing food and beverages such as red wine, green tea and black tea [65]. There are interventional studies with cocoa in humans, that demonstrated significant improvements of arterial dilatation, very likely as consequence of cocoa polyphenols' ability to enhance the bioactivity of NO [66]. At the cellular level, the use of platelets as a tool to clarify the role of cocoa's polyphenols on NO, Pignatelli et al. observed that polyphenols are able to both reduce the production of superoxide anion and to increase platelet NO; an inverse correlation between these two effects was also observed [67]. Mechanistically, these effects have been attributed to the ability of polyphenols to inhibit the activation of NADPH oxidase [67,68]. The same group underlined the synergism among the various cocoa polyphenols in exerting an antioxidant effect [68]. From a potential therapy viewpoint, these findings shed some light on the antioxidant effect of polyphenol-rich beverages (including those containing cocoa) because several studies documented blood concentrations of

single polyphenols < 1 μM, i.e. much lower than those necessary to exert an antioxidant effect *in vitro*. In synthesis, a mixture of polyphenols is able to reduce superoxide anion and to increase NO generation, in turn suggesting that *in vivo* a combination of more than one polyphenols (such as that present in cocoa) is likely responsible for the beneficial effect on arterial dilatation observed with polyphenol-rich foods.

More recently, by using noninvasive ultrasound measurements of flow mediated vasodilation (FMD), Hermann *et al*. [69] showed that dark chocolate, but not white chocolate improves endothelial function and platelet function in young healthy smokers, a population known to have impaired endothelial function and platelet hyper-reactivity. Smoking is also a risk factor for erectile dysfunction [70]. Flow-mediated dilation by high-sensitive ultrasonography of the brachial artery, shear stress-dependent platelet adhesion, and total antioxidant status were assessed in 25 persons before and after ingestion of 40 gr of either dark (74% cocoa) or white chocolate (4% cocoa). Two hours after intake of dark chocolate, FMD (and plasma total antioxidant capacity) improved significantly. The beneficial effect of dark chocolate on FMD lasted for eight hours. On the other hand, white chocolate ingestion did not have any effect neither on FMD nor on platelet function.

The other beverage that is currently being investigated for its vasomodulating activities is tea [65]. Starting from the landmark experiments of Duffy *et al*. [71], the role of tea flavonoids – in particular epigallocatechin gallate - in maintaining proper vasomotion are being clarified. In addition to their vasomodulating activities, tea catechins exert other biological activities that might be relevant to fertility/infertility and their associated risk factors. Among such activities, there are those on the prostate [72] and those on plasma lipids. The latter suggest a salubrious role of

catechins in limiting the progression of the metabolic syndrome [73], which brings about vascular alterations that have being associated with erectile dysfunction [74]. In synthesis, regular tea drinking is associated with improvements of vascular reactivity and plasma lipid profile, which might ameliorate erectile function and, in turn, fertility.

Summing up, polyphenolic compounds are a promising class of antioxidant nutrients, which has prospects to limiting cardiovascular oxidative stress and thereby limiting risks for certain forms of infertility. While it is still controversial as to the bioavailability of flavonoids, accruing evidence points to sufficient absorption to affect endothelial antioxidant profiles, especially in conditions that limit endogenous antioxidant defenses. In this regard, cocoa and chocolate polyphenols may have high potential to lower oxidant-induced infertility because of their high content, palatability, and their aforementioned effectiveness in improving flow-mediated vascular dilation. Thus, clinical studies using polyphenol compounds, either specific flavonoids or foods high in polyphenol content, are warranted if we want to prove their benefits to improve conditions such as erectile dysfunction, pre-eclampsia, and potentially sperm motility.

In synthesis, we propose EDNO as a major target to treat infertility, given the role that a correct vascular response plays both in men and women, via ensuring erectile and tissutal functions.

7. Antioxidant uses in infertility outside of that associated with vascular dysfunction: focus on semen

Spermatozoa are very rich in polyunsaturated fatty acids, namely docosahexaenoic acid (DHA). As such, they are susceptible to oxidation, even

though the human semen is rich in vitamin C and omega 3 fatty acids exert cellular anti- rather than pro-oxidant actions [75,76]. Indeed, there is evidence of decreased sperm viability associated with increased lipid peroxidation; this phenomenon becomes more relevant with age. It is noteworthy that – at least in developed countries – more and more men postpone fatherhood, increasing chances of having oxidatively-damaged semen. It is also noteworthy that spermatozoa per se produce elevated fluxes of ROS, which need to be counterbalanced by an appropriate antioxidant network to avoid damage to macromolecules, namely lipids, proteins, and nucleic acids [77]. The discovery of a NADPH oxidase in sperm [78] opens a potential field of research aimed at reducing ROS generation by sperm itself [79]. In this respect, provision of DHA (an inhibitor of NADPH oxidase in endothelial cells [76] might prove beneficial both because it provides essential fatty acids indispensable for sperm survival and because it would lower production of ROS once sperms are produced. Accordingly, it is of note that infertile men had lower concentrations of omega-3 fatty acids in spermatozoa than fertile men [80].

Among the antioxidant machinery that is currently being investigated in semen as potential target for therapy, glutathione peroxidases (GPX) are attracting much attention [81]. Indeed, several GPX have been found to be present on and around epididymal transiting sperm cells and the precise localization of the various GPXs in, on and around sperm cells argues in favor of specific roles for these enzymes [82]. In particular, GPXs could function as hydrogen peroxide sensors to regulate its concentration and to find a proper balance between the physiological actions of ROS on sperm cells and their detrimental activities on cell physiology [82]. It must be underlined that the mere antioxidant role played by GPX in mature sperm has been questioned [83] and it has been proposed that mature spermatozoa depend on

GPX as a structural protein, to maintain the proper integrity and stiffness of the midpiece [83]. In synthesis, the nature and precise role(s) of GPX in maintaining "healthful" spermatozoa is still to be fully clarified [84], but the field is moving rapidly and in the near future some opportunities for therapy, e.g. with selenium supplementation [85], might arise.

The use of vitamin C has also been advocated as a tool to ameliorate semen quality [86]. The rationale is that some investigators found lower ascorbate concentrations in samples from infertile men, as compared with fertile individuals [87]. To the best of our knowledge, there is a limited number of trials that tested the effects of vitamin C supplementation on semen quality [88,89]. Hence, while adequate consumption of vitamin C are recommended and exert positive actions on the vascular system, its direct effect on spermatozoa viability are suggestive, but as yet to be fully established. It is also noteworthy that the addition of vitamin C to semen decreases freezing-thawing damage induced by cryopreservation [90].

Other antioxidant molecules or plants extracts that have been proposed to increase semen quality via their antioxidant actions include pycnogenol® [91] and vitamin E [92]. Both await confirmation from randomized and double blind trials.

The final compound that bears discussion is lycopene. In fact, several lines of evidence (mostly focused on chemoprevention) indicate that lycopene plays a role in maintaining prostate function and health, mostly as an anti-inflammatory agent [93]. However, conclusive evidence that it works solely through this means is inconclusive [94]. Based on the purported activities of lycopene on the prostate, some researchers are starting to investigate its role on quality of semen [95]. Indeed, lycopene possesses protective properties with respect to DNA damage and the rationale

behind testing its activities on spermatozoa is sound. However, the evidence is once again very limited and human trials are needed to confirm or disprove this hypothesis.

8. Concluding remarks

As briefly reviewed herein, many forms of infertility are associated with oxidative stress, and many types (e.g. erectile dysfunction, pre-eclampsia) are also caused by or exacerbated from significant impairment of vascular endothelial function. Thus, there is a compelling theoretical rationale to limit oxidative stress associated with these conditions by the use of antioxidant therapies or pharmacological agents, which induce endogenous antioxidant defenses. While the promise for antioxidants to limit conditions of infertility is great, most evidence to date showing therapeutic efficacy of antioxidants are from experimental animal models or in vitro studies. Table 1 reports the most common commercial preparations (antioxidants and others) that are being sold accompanied by various claims to treat infertility. Where small-scale clinical trials have been performed [96], the outcomes have largely proven inconclusive. While this may seemingly limit enthusiasm for antioxidant therapies for infertility, there is an equally good argument for the design and implementation of clinical trials using specific antioxidants to treat underlying vascular dysfunction associated with many types of infertility. In this regard, "non-traditional" antioxidants, such as lipoic acid or polyphenols, may hold significant promise. These compounds may not only directly improve EDNO, but also have additional long-term benefits to enhance antioxidant defenses, thereby providing potent means of lowering vascular dysfunction and attendant infertility. While current evidence is still controversial, future trials will help clarify this issue and might provide scientific bases for the use of antioxidants in the treatment of the various facets of infertility.

Acknowledgments

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Figure legends

Figure 1. Mitochondria are the major contributors to free radical production by

Human Aortic Endothelial Cells (HAEC). Confluent HAEC were challenged with the

calcium ionophore A23187 in the presence or absence of apocyanin (an inhibitor of

NADPH oxidase), allopurinol (an inhibitor of xanthine oxidase), or rotenone (an

inhibitor of mitochindrial complex I). Superoxide production was continuously

assessed by the SOD-inhibitable cytochrome c reduction assay, using a plate reader

(Tecan, Mannedorf, Switzerland). Absorbance was read at 550 nm and superoxide

production was determined using the extinction coefficient of cytochrome c at 550 nm

(ε : 21 mM-1cm-1). Unpublished data.

Table 1 Commercial preparations currently sold for enhancing fertility.

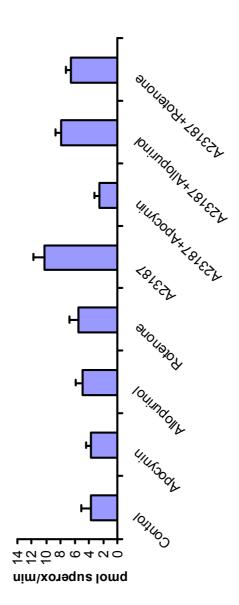| Compound	Comment
Sold as antioxidants	
Vitamin C	Ameliorates vasomotion through several antioxidant and non-antioxidant mechanisms. Unclear activity on semen quality.
Vitamin E	Unclear actions on endothelium and semen quality.
Zinc	High (~200 μg/ml) quantity in semen. Rationale behind supplementation unclear.
Sold as fertility-enhancers	
Arginine	eNOS substrate might augment vasomotion. Anecdotally reported to increase semen production.
Ginseng (Panax ginseng)	Tonic according to ethnopharmacology. No evidence of fertility-improving activities.
Astragalus (*Astragalus membranaceus*)	Attributed with sperm motility-enhancing properties. Unproven.
Maca (*Lepidium meyenii*)	Increases sexual potency according to ethnopharmacology. One animal study showed increased spermatogenesis [97]

Figure 1