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Selenium and reproductive function. A systematic review

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ABSTRACT. Selenium (Se) is an essential element involved in normal gonadal development, gametogenesis, and fertilization. Molecular studies show that the gonads actively take up and store Se, most of which is incorporated in the glutathione peroxidase enzymes. We provide a systematic review of the original molecular studies, prospective observational data and randomized controlled trials on the role of Se in reproductive function conducted in the past 30 years. A critical appraisal of these findings suggests that Se supplementation produces a bell-shaped response curve, with negative effects observed for both low and high concentrations. The few available clinical trials support the use of Se supplementation (<200 µg/d) to improve male infertility, although their pre-treatment assessment of Se levels in enrolled subjects is inconsistent and their quality and size are insufficient to enable general recommendations. In females, a putative role in oocyte maturation and fertilization is suggested, but no large controlled trials have yet been performed. The role of Se supplementation on pregnancy outcomes is promising, and ongoing studies and meta-analysis should soon enable proper recommendations to be suggested. How best to assess Se in terms of cut-off value, sample type (serum, semen, other fluids) and the specific outcome of interest remains to be clarified. In the meantime, assessment of serum Se levels followed by low-dose replacement therapy when necessary is a reasonable approach to improve male idiopathic infertility and gestational outcome.

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INTRODUCTION

Selenium (Se) is a trace element present in organic and non-organic forms in food. The daily recommended daily allowance is 55 µg in adults and 60 µg in pregnant women. However, Se intake appears to be generally low in Europe (range 11-70 µg per day) (1).

In some Chinese provinces, severe endemic Se deficiency occurs in the form of chronic osteochondropathy (Kashin-Beck disease) and dilated congestive cardiomyopathy with high case-fatality (Keshan disease), which may be reversed by Se supplementation (2). Besides these dramatic conditions, the role of Se has also been investigated in various aspects of human health, from cancer to coronary heart disease (3). However, meta-analysis does not provide any conclusive evidence (4).

The gonads, and especially the testis, have high concentrations of Se, leading to the investigation of its role in reproduction in various experimental animal studies. Unfortunately, in humans, only a few small interventional studies have investigated the clinical efficacy of Se supplements to improve fertility, while nothing is currently known about the fertility of patients with Kashin-Beck or Keshan disease.

We provide a systematic review of the original molecular studies, prospective observational data and randomized controlled trials performed in the past 30 years on the role of Se in reproductive function.

SE IN MALE REPRODUCTIVE TISSUES

Se is an essential element for normal testicular development, spermatogenesis, sperm motility and function. Its potential role in idiopathic infertility has been empirically tested (alongside other trace elements), with some positive results (5).

Animal studies evaluating the retention of ip injected radiolabeled Se (^{75}Se) in the form of H_2SeO_3 in different male and female rat tissues have demonstrated that testicular tissue contains a high amount of Se. Three weeks after the injection, authors showed that the testis-epididymis complex contained 41.8% of the total body ^{75}Se, while the high-

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est retention rate in the ovaries was approximately 0.3% (6). This study clearly demonstrates that male gonads actively take up and store Se.
Kehr et al. used X-ray fluorescence microscopy to better understand the cellular and subcellular distribution of Se (Se topography) in mice testes, finding it to be specifically concentrated in the spermatids rather than in earlier stages of spermatogenesis. They also found that the specific enrichment of Se in the sperm midpiece was due to the high levels of the mitochondrial form of type 4 glutathione peroxidase enzyme (GPx4) (7). The GPx family accounts for most of the Se uptake in reproductive tissue and offers a potential target for the beneficial effects of Se supplementation. These enzymes are most known for their role as reactive oxygen species (ROS) scavengers but also exert other metabolic actions (Table 1) and deserve their own description.

Selenoproteins in reproductive organs: GPx4 and Sepp1

The GPx family comprises eight different enzyme isoforms (GPx 1-8), which play a key role in redox reactions. GPx 1-4 and GPx6 are Se-dependent enzymes. Their main function is to catalyze the reduction through glutathione of hydrogen peroxide, organic hydroperoxides and lipid peroxides and protect cells against oxidative stress. Each isoform is tissue-specific and seems to have a sexually dimorphic expression (8) and possible additional tissue-specific functions. GPx4, also known as phospholipid hydroperoxide GSH Px (PHGPx), is a good example. It is specifically expressed in the testis and has not only an antioxidant action but also a structural function, given that it comprises over 50% of the mitochondrial capsule in the midpiece of mature sperm. The complete disruption of GPx4 causes early embryonic lethality in homozygous Gpx4 knockout (KO) mice, whereas heterozygote mice are viable, fertile and appear normal, despite their decreased levels of GPx4 mRNA and protein (9).
The Gpx4 gene encodes three isoforms with different N-terminal amino acid sequences that are localized specifically in the mitochondria, cytosol, and nucleus. All three efficiently catalyze the reduction of phospholipid hydroperoxides. The mitochondrial (mGPx) and cytosolic (cGPx) forms make up almost half the capsule material embedding the sperm's mitochondrial helix. The nuclear variant (snGPx) appears to be involved in the stabilization of condensed chromatin during sperm maturation. DNA condensation and protection from chromatin fragmentation have been extensively suggested as important factors in unexplained male infertility (5). Conrad et al. demonstrated that mice with a targeted deletion of the snGPx4 gene were not only viable, but also fully fertile, in contrast with full KO mice (10).
Invalidation of the cytosolic GPx4 variant leads to early developmental defects (11). Schneider showed that deletion of mitochondrial GPx4 (mGPx4) allows both normal embryogenesis and postnatal development but causes male infertility. In this mouse model, infertility was associated with impaired sperm quality and multiple severe structural abnormalities. These included bent sperm, sperm heads detached from the midpiece, sliding of mitochondria along the midpiece and extrusion of microtubules and outer dense fibers from the midpiece. Ultrastructural analysis revealed irregular alignment of mitochondria, swollen mitochondria, bends between the midpiece and the tail and structural abnormalities between the head and the midpiece. Interestingly, intracytoplasmic sperm injection of mGPx4-/- sperm bypassed infertility and produced viable offspring (12).
The importance of GPx4 for male fertility has also been demonstrated in humans. Imai et al. found GPx4 to be abundantly distributed in late spermatocytes and spermatids and localized in the sperm midpiece, specifically in the mitochondria. This study also found that about 10% of the infertile men examined showed a dramatic decrease in the level of GPx4 expression in sperm. This rose to 35% in infertile males with oligoasthenozoospermia, whereas no abnormal GPx4 expression was found in sperm from fertile males (13).
Foresta et al. confirmed these results, showing that both sperm motility and morphological integrity are directly correlated with GPx4 concentration. Similarly, residual GPx4 activity measured after reductive sperm solubilization was significantly lower in infertile men than in controls, and even more so in oligoasthenozoospermic men. This activity was directly cor-

Table 1 - *Effects on fertility and reproduction in mice model with KO for glutathione peroxidase (GPx) isoforms.*

Imai 2003 (9)	Mice with KO for GPx4 (all the isoforms)	Homozygosis: lethal at embryo stage Heterozygosis: viable and fertile, normal phenotype
Conrad 2005 (10)	Mice KO for nuclear snGPx4 isoform	Viable and fully fertile
Chabory 2010 (11)	Mice KO for cytosolic cGPx4 isoform	Present development defects
Schneider 2009 (12)	Mice KO for mitochondrial mGPx4 isoform	Present male infertility

related above all with forward motility, but also with viability and morphological integrity (14).
The impact of sperm GPx expression on fertilization ability, embryo quality and reproductive outcome in *in vitro* fertilization (IVF) has also been assessed. Sperm samples with lower mRNA expression for GPX4 yielded more asymmetric embryos. However, GPx4 levels had no effect on the later phase of *in vitro* development or on pregnancy rate (15).
Selenoprotein P (Sepp1) is also involved in male reproductive function. This is a transport protein for Se and is also expressed in vesicle-like structures in the basal region of the Sertoli cells (16). Male Sepp1 KO mice had markedly reduced fertility, lower Se levels and glutathione peroxidase activity (17). However, a high-Se diet did not restore testis Se levels or normal sperm phenotype in these mice (16).
All this evidence suggests that Se plays a part in the male reproductive system and is modulated by the selenoproteins GPx4 and Sepp1. Plasma Sepp1 concentration has been suggested as a possible marker for Se status in humans (18). However, the correlation between Sepp1 concentration in semen or serum and semen parameters and reproductive function has never been studied.

SE AND MALE FERTILITY

The experimental data reported herein suggest that Se and selenoproteins have an important role in male fertility. However, the clinical trials performed in humans had various biases and inconsistencies and were mainly performed on an empirical basis (5), despite the solid rationale from experimental data. For these reasons, a rigorous comparison of *in vivo* and *in vitro* controlled studies is necessary to draw reliable conclusions.

Animal models

Several studies in male animal models have supported the hypothesis that Se deficiency affects male reproductive capacity *via* oxidative stress and that Se replacement is beneficial for the testes. Sperm from Se-deficient mice showed impaired chromatin condensation, reduced fertilization capacity and increased lipid peroxidation in both the testes and sperm cells, indicating that Se deficiency induces oxidative stress (19).
Se supplementation reduced the harmful effects of cadmium in rat testes by preventing the decline in serum testosterone and partially reversing the effects of cadmium toxicity (i.e. degeneration of seminiferous tubules, with increased interstitial space associated with degeneration of interstitial cells and interstitial fibrosis). The theory is that Se counteracts the cadmium-triggered overproduction of ROS, increasing GSH-Px activity (20).
Another study demonstrated that supplementation of inorganic Se or Se-enriched probiotics significantly alleviated the adverse effects of hyperlipidemia on the fertility of male mice fed a high-fat diet. Se compounds reduced testicular tissue injury, increased serum testosterone levels and improved sperm indexes in Se-treated animals. This improved fertility index was attributed to the antioxidant action of Se (21). The study also confirmed that decreased levels of Se contribute to oxidative stress in the testis. Mice fed with a low-Se diet have a significantly reduced number of pachytene spermatocytes and young and mature spermatids and significantly lower sperm numbers compared with animals on a normal or high-Se diet. Interestingly, the high-Se diet did not produce any further improvements in fertility over the normal diet (22), suggesting that while Se replacement can be beneficial, over-replacement does not offer a therapeutic advantage. As further confirmation of this, Kaushal et al. found a highly significant increase in lipid peroxidation and ROS generation in both Se-deficient and Se excess-fed mice, with reduced sperm concentration, motility and overall fertility, suggesting that Se deficiency and excess both increase oxidative stress and reproductive outcome (23). In fact, although excessive ROS generation leads to sperm DNA damage/apoptosis, membrane peroxidation and reduced sperm motility, a controlled level is required for some sperm functions, such as capacitation and acrosome reaction.

Human studies

Several recent studies have suggested that oxidative stress also plays a major role in male fertility in humans (24-26). Ideally, the reference range for Se sufficiency/deficiency should be defined before investigating whether or not supplementation could be used to improve male fertility. The concentration of Se largely depends on the tissue analyzed. Circulating serum levels <60 µg/l could be considered as corresponding to "low Se status". Values of between 58 µg/l and 80 µg/l can be regarded as a gray area that might benefit from Se replacement (27-29). However, there is little available information on the relationship between serum Se and the levels in the target organs (in our case the reproductive tissues). In fact, it is not yet clear which biological fluid (blood, serum, seminal plasma, sperm cells, follicular fluid) gives the most accurate picture of Se concentration in terms of its role in reproduction.
A panel of selenoproteins concentration assessed in semen could help define Se status as sufficient or insufficient (27).

In Turkish men with idiopathic infertility, sperm ROS and seminal plasma malondialdehyde (MDA) – an important marker of lipid peroxidation – showed a significant correlation with sperm quality. Significant positive correlations were also found between the percentage of DNA fragmentation in spermatozoa and MDA levels of seminal plasma (30). MDA is formed when ROS degrade polyunsaturated lipids. It reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts (30).

Similarly, in infertile Tunisian men semen MDA levels were inversely correlated with sperm motility and sperm concentration, while there was a positive correlation between seminal lipid peroxidation and the percentage of abnormal forms (31). In this population elevated seminal MDA was accompanied by decreased Se concentration in seminal plasma.

This observation focuses on the possible measurement of Se status not only in serum but also in other organic fluids. In fact, there was a correlation between seminal plasma Se concentration and sperm motility, with mean values of 64±20.64 µg/l in fertile subjects and 56.00±22.81 µg/l in asthenozoospermics (31). However, serum Se concentration was not assessed, so any correlation between seminal and serum Se levels could not be evaluated.

Se levels in seminal plasma were lower in patients with varicocele (38.2±36.4 µg/l) than in the normozoospermic group (56.1±48.1 µg/l). Se correlated with sperm concentration, motility and normal morphology according to Camejo et al. (32). Conversely, other studies found higher seminal plasma Se levels in men with sperm abnormalities. In a small Chinese population, seminal plasma Se levels were lower (73.16±20.35 ng/ml) in the control group than in those with abnormal sperm concentration and motility (103.79±37.89 ng/ml), while no correlation was found between Se concentration and sperm morphology (33). In Nigerian men, the seminal plasma Se concentrations were significantly higher in azoospermic (244.88±5.51 µg/l) than oligospermic subjects (81.89±7.09 µg/l), although the latter value was also significantly lower than in normal subjects and controls (144.88±9.45 µg/l). Overall, a significant negative correlation was found between seminal plasma Se and total sperm count, but also a significant positive correlation between seminal plasma Se and sperm motility.

The authors of this study also investigated circulating serum Se concentration, finding that mean levels were significantly higher in oligospermic (330.71±14.96 µg/l) than azoospermic subjects (127.56±7.87 µg/l) and controls (137.80±12.60 µg/l). The dissociation of serum/seminal plasma ratios observed in the infertile men in this study suggests that a critical balance in Se concentration in either blood and seminal plasma is necessary for normal spermatogenesis, but also that there may be defective Se incorporation into spermatozoa in infertile men, with azospermic men being an extreme example.

Another interesting isolated finding is that serum Se levels show a significant positive correlation with serum and seminal testosterone levels but not with any other hormone. This suggests a possible positive influence of Se on Leydig cells (34), which deserves further investigation. In summary, these studies show that both high and low Se levels may have a negative influence on sperm count and motility, as also described in mouse models (23).

Few clinical trials have evaluated the effects of antioxidant supplementation on male fertility. The comparison of different studies is complicated by the heterogeneity of their designs and inconsistencies in enrollment (5). Table 2 summarizes the findings of the major clinical trials investigating the role of Se on male fertility.

Se supplements at dosages from 100 µg to 200 µg daily, taken alone (28, 29) or combined with vitamin E (35-37), were found to improve sperm motility. Some of these studies also found an improvement in sperm count and morphology (28, 36) and pregnancy rate (35). In contrast, another study found no change in 33 subfertile men after 12 weeks of 200 µg/d Se supplementation (27). Hawkes et al. (38) studied 11 healthy subjects and found reduced sperm motility in the Se-rich (297 µg/d) group; in a subsequent study, however, the same authors (39) failed to find any difference between groups treated with Se supplements (300 µg/d) and placebo (Table 2). Safarinejad et al. also found an increase in testosterone after Se supplementation (28). The variable results could be explained by differences in the supplemented dose, inclusion criteria and baseline Se status, possibly depending on geographic area. In addition, Se supplementation seems to have more effect on motility (28, 29, 35, 36) than on other seminal parameters. A significant improvement was seen in patients with baseline markedly reduced sperm motility (i.e. ≤20%). Above these values, the response is variable: both an improvement [in a group of patients in whom serum Se was not measured (37)] and no change [in a group of patients with "gray area" serum Se levels (27)] have been reported for patients with sperm motility of around 30%.

Various studies have found Se supplementation to have a positive effect on the semen parameters of patients with baseline blood and semen Se values lower than those of non-responders (Table 2). It should be noted that not all studies on the repro-

Table 2 - *Effects of selenium supplementation on sperm parameters.*

Author	Number of subjects (age)	Characteristics	Selenium basal concentration	Basal sperm motility %	Treatment	Time of treatment	Resuts
Moslemi et al. 2011 (35) (Prospective single-arm study)	690 Age: 20-45 yr (mean 28.5)	Idiopatic asthenoteratospermia (Iran)	Not measured	10-30%	200 µg/d L-selenomethionine + 400 IU/d Vit E	3 months	Improvements in sperm motility, morphology, pregnancy rate
Vézina et al. 1996 (36) (Prospective open-label study)	9 Age: 28-36 yr (mean 31.3)	OAT (Canada)	Seminal plasma: 68.3 µg/l (mean value)	12.1%	1st month: 100 µg/d Organic Se + 400 mg/d Vit E 2nd-6th month: 200 µg/d Organic Se + 400 mg/d Vit E	6 months	Improvements in sperm motility, vitality and morphology
Scott et al. 1998 (29) (Double blind RCT)	69 Mean age: 33.3, SD 0.64 yr	Reduced sperm motility (UK)	Blood plasma: 81.0±1.8 µg/l	21%	100 µg/d L-selenomethionine Or 100 µg/d L-selenomethionine + Vitamins A (1 mg), C (10 mg), E (15 mg) Or placebo	3 months	Improvements in sperm motility but no effect on sperm count
Iwaner et al. 1995 (27) (Prospective study)	33 Age: 19-38 yr (mean 31)	Subfertile men (Poland)	Seminal plasma: 28.0±9.5 µg/l Blood plasma: 62.4±8.8 µg/l Whole blood: 80.4±9.7 µg/l	30.2%	200 µg /d Se-rich-yeast (16 subjects) Or 200 µg/d Sodium selenite mixed baker's yeast subject (17 subjects)	3 months	No effect on sperm count, motility and morphology
Keskes-Ammar et al. 2003 (37) (RCT open label)	54	Voluntary and infertile men (Tunisie)	Not measured	34 %	225 µg selenium + Vit E 400 mg (28 subject) Or 4.5 g/d vit B (26 subjects)	3 months	Improvements in sperm motility
Safarinejad et al. 2009 (28) (Double blind RCT)	468 Age: 25-48 yr (mean 31)	Idiopatic OAT (Iran)	Seminal plasma: 26.6±4.4 µg/l Blood plasma: 77.7±6.8 µg/l	22%	200 µg Se/d (116 subjects), Or 600 mg NAC/d (118 subjects), Or 200 µg Se+ 600 mg NAC/d (116 subjects) Or placebo (118 subjects)	6 months	Improvements in sperm count, motility and morphology (in both Se+NAC and Se alone groups)
Hawkes et al. 2001 (38) (Double blind RCT)	11 Age: 20-45 yr	Healthy men (USA)	Seminal plasma: 52±17 µg/l Blood plasma: 130±20 µg/l	81.6-90%	First 21 days: Dietary Se 47 µg/d Remaining 99 days: 297 µg/d Dietary selenium or 13 µg/d Dietary selenium	4 months	Decreased fraction of motile sperm in the high-selenium group
Hawkes et al. 2009 (39) (Double blind RCT)	42 Age: 18-45 yr	Healthy men (USA)	Seminal plasma: 48.12±11.84 µg/l Blood plasma: 146.13±18.96 µg/l	62%	300 µg/d Se yeast Or Placebo	11 months	No effects on seminal parameters
Lombardo et al. 2012 (70) (Prospective open-label study)	60 Age: 30-55 yr	Chronic Prostatis (bacterial or not CPPS) (Italy)	Not measured	18%	82.3 µg selenium + lycopene (1.5 mg) + epigallocatechin gallate (250 mg) + ellagic acid (250 mg) + zinc (20 mg) (30 subjects) *vs* No treatment (30 subjects)	6 months	Improvements in progressive sperm motility, decreased atypical morphology. Improvement in leucocytospermia and sympthoms

NAC: N-acetyl-cysteine; RCT: randomized controlled trial; CPPS: chronic pelvis pain syndrome; OAT: oligo-astheno-teratozoospermia.

ductive effects of Se supplementation measured serum and/or semen Se at the baseline.

It can be assumed that there is an ideal level of circulating or semen Se that enables the optimal function of the selenoproteins involved in fertility (especially GPx4, which has a crucial role in sperm motility).

The relationship between Se status and cancer of the reproductive tissues is fascinating, albeit beyond the scope of this review. No data are available on Se and

testicular cancer; however, Se supplementation has been claimed to reduce the risk of prostate cancer in men with reduced plasma Se levels, perhaps through an antiangiogenic effect (40).

FEMALE FERTILITY AND PREGNANCY

There are comparatively few studies of the role of Se in female fertility, and only recently has some light been shed on its possible role in ovarian physiology. However, many encouraging studies have found a beneficial effect on pregnancy outcomes. This aspect goes beyond the scope of this review, although it is briefly summarized below. In any case it has been recently reviewed (41).

Evidence for the role of Se in ovarian function comes from Grazul-Bilska et al., who found that maternal Se dietary intake could be involved in the regulation of early folliculogenesis and cellular proliferation of the follicles, blood vessels and stromal tissues of fetal ovaries in sheep (42).

In addition to its possible influence on embryonic gonadal development, Se has also been claimed to protect female reproduction from oxidative stress. ROS produced by the pre-ovulatory follicles are considered important inducers for ovulation. Nevertheless an imbalance between pro- and antioxidants has been reported in a number of reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS) and unexplained infertility (43). A significant increase in the expression of ROS and MDA in follicular fluid was observed in endometriosis patients, compared to controls. In addition, Se levels were found to be significantly lower in infertile women with endometriosis than in women with tubal infertility (44).

Free radicals also have a crucial role in the pathogenesis of PCOS in both rats (45) and humans, and are directly correlated with androgen levels (46).

New evidence suggests that "IMOD", a Se-based herbal medicine, prevents histopathologic, endocrine and biochemical ovarian alterations induced by hyperandrogenism in rats with letrozole-induced PCOS. Rats treated with IMOD showed decreased ovarian atresic and cystic follicles, normal testosterone levels and an estrus cycle similar to PCOS-free controls (45). Recently, lower plasma Se levels were found in women with PCOS than in a control group, while there was a negative correlation between Se and total testosterone (47).

Taken as a whole, these data support a direct correlation between Se status and oxidative stress. A possible explanation lies in the inflammatory hypothesis: ROS overproduction in endometriosis, such as in PCOS, could induce an excessive consumption of Se, due to its antioxidant properties. On the other hand, a primary low Se status leads to an antioxidant imbalance with a secondary accumulation of ROS, a predisposing factor for PCOS.

As with males, some evidence is emerging for the putative protective role of Se in female reproductive cancers (ovarian cancer). The average Se concentration and GPx activity in the plasma of patients affected by benign neoplasia and cancer of the uterine cervix, uterine corpus or ovary was significantly lower than in the plasma of healthy women, suggesting that lower Se-related antioxidant capacity might favor the development of neoplastic diseases of the reproductive system (48).

Se in assisted reproductive technologies

Se appears to be reduced in the serum and follicular fluid of women undergoing IVF. Se depletion in the follicular fluid of women with unexplained fertility has been reported (49), while reduced Se-dependent GPx activity in the follicular fluid collected during oocyte pick-up for IVF has been associated with subsequent non-fertilization of the oocytes (44). Multivitamin/mineral supplementation normalized the trace element levels in the serum and follicular fluid of women undergoing IVF (50). Dickerson et al. showed that Se levels in hair correlated positively with follicle number and oocyte yield after ovarian stimulation, suggesting that Se has a positive effect on ovarian response to gonadotropin therapy for IVF (51). However, the few studies conducted to date involve small caseloads and are inadequate to address specific outcomes.

Se in pregnancy

An increased requirement for Se is observed during pregnancy, triggered by its avid uptake by the developing tissue of the fetus. This phenomenon leads to a drop in maternal blood and tissue Se concentrations.

A significant number of well-conducted recent studies have shown that Se deficiency and supplementation have an impact on obstetric complications, especially in difficult pregnancies (52). Oxidative stress has been associated with various complications, such as spontaneous abortion, recurrent pregnancy loss, pre-eclampsia, and intrauterine growth restriction (43). Se depletion has been consistently associated with recurrent miscarriage (53-55), pre-eclampsia (56), pre-term birth (57), and small for gestational age infants (58, 59). Se supplementation alone (60) or combined with other antioxidants (61) has been associated with a lower frequency of pre-eclampsia, premature (pre-labor) membrane rupture (62) and *post-partum* depression (63).

The role of Se supplementation on disthyroidism in pregnancy has recently attracted great interest. Negro et al. (64) found that Se administration (200 µg/d) during pregnancy reduced thyroperoxidase antibody titers and decreased the incidence of *post-partum* thyroiditis or persistent hypothyroidism. However, whether this could have an effect on gestational outcome remains unclear. An ongoing multicenter randomized control trial, the Serena Study (NIH Clinical Trial ID: NCT01465867 "SElenium supplementation in the management of thyroid autoimmunity during pREgNAncy") will address this issue.

Attention has recently focused on the potential effect of Se supplementation during pregnancy on gestational diabetes (GDM). Observational studies show that Se levels in women with GDM or glucose intolerance are significantly lower than in pregnant controls (65-67). Serum Se levels and Se intake were negatively correlated with gestational hyperglycemia (defined as one abnormal oral glucose tolerance test value in women with GDM) in 504 Italian pregnant women, even after adjustment for known GDM risk factors (68). Zeng et al. found that a high-Se (3.0 mg/kg) diet induced a moderate GDM and *post-partum* insulin resistance in gestating rats (69). In humans, however, there is insufficient evidence to advocate or exclude any role of Se supplementation on GDM incidence.

CONCLUSIONS

This literature review shows that Se is required for the development and function of the reproductive tissues in both sexes, possibly due to its key role in the modulation of antioxidant balance. In males, there is also a large body of molecular evidence indicating that it has a structural function, exerted in the sperm through GPx4 selenoprotein and affecting sperm motility, chromatin integrity and fertility rate. The response curve for Se seems to be bell-shaped, with negative effects observed for both low and very high concentrations. However, the definition of "low Se status" in terms of cut-off values, detection method and biological source is yet to be clarified. There is also room for improvement in the assessment of Se status-related biomarkers, while it has not yet been established which biological fluid gives the most reliable measurement of Se concentrations. Finally, new possible markers for Se status such as Sepp1 still require validation. The few available clinical trials support the use of Se supplementation (<200 µg/d) to improve male infertility, although the quality and size of the studies are insufficient to enable general recommendations. In females, some evidence is emerging for a possible role in oocyte maturation and fertilization, but no large controlled trials are yet available. The role of Se supplementation on pregnancy outcomes is promising, and ongoing studies and meta-analysis should soon enable the development of proper recommendations. In the meantime, assessment of Se status in at-risk populations followed by low-dose replacement when necessary is a reasonable approach to improve male idiopathic infertility and gestational outcome.

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