Selenium in reproductive health

Hiten D. Mistry PhD¹, Fiona Broughton Pipkin DPhil², Christopher W. G. Redman MD³ & Lucilla Poston PhD¹

London, Nottingham and Oxford, United Kingdom

¹ Maternal and Fetal Research Unit, Division of Women’s Health, King’s College London, London, SE1 7EH, UK; ² School of Clinical Sciences, Department of Obstetrics & Gynaecology, University of Nottingham, Nottingham, NG5 1PB, UK; ³ Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK.

Correspondence name & address: Dr. Hiten D. Mistry

Maternal and Fetal Research Unit
Division of Women’s Health
King’s College London
St Thomas’ Hospital
Westminster Bridge Road
London, UK
SE1 7EH
Tel: +44(0)20 7188 8151
Fax: +44(0)20 7620 1227
Email: hiten.mistry@kcl.ac.uk

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This is a topical review summarising the increasing evidence for an association between inadequate dietary antioxidant selenium intake and several disorders of reproduction.

Short Title: Selenium in reproductive Health
Abstract

Hiten D. Mistry PhD¹, Fiona Broughton Pipkin DPhil², Christopher W. G. Redman MD³ & Lucilla Poston PhD¹

Selenium is an essential trace element of importance to human biology and health. Increasing evidence suggests that this mineral plays an important role in normal growth and reproduction in animals and humans, and selenium supplementation is now recommended as part of public health policy in geographical areas with severe selenium deficiency in soil. Here, the biological functions of selenium are addressed prior to a detailed review of associations between selenium status and reproductive health. In many countries, selenium dietary intake falls below the recommended nutrient intakes (RNIs) and is inadequate to support maximal expression of the selenoenzymes. Numerous reports implicate selenium deficiency in several reproductive and obstetric complications including male and female infertility, miscarriage, pre-eclampsia, fetal growth restriction, preterm labour, gestational diabetes and obstetric cholestasis. Currently, there is inadequate information from the available small intervention studies to inform public health strategies. Larger intervention trials are required to reinforce or refute a beneficial role of selenium supplementation in disorders of reproductive health.

Keywords: Antioxidant, pregnancy, reproduction, selenium
**Introduction**

Selenium was first discovered in 1817 by Jöns Jacob Berzelius when investigating the chemicals responsible for outbreaks of ill health amongst workers in a Swedish sulphuric acid plant, which had switched from expensive, imported sulphur to a local product (Oldfield, 1987). The local product contained a contaminant which he named Selēnē, after the Greek goddess of the moon (McKenzie et al., 1998). Selenium lies directly below sulphur in the periodic table and above tellurium, and has similar chemical properties, as it binds with equal affinity to metals and non-metals, both directly and hydrochemically (Bauer, 1997, Burk and Levander, 2006). In 1957, Klaus Schwarz proved that selenium is an essential nutrient necessary for both normal growth and reproduction in animals through experiments demonstrating that minute amounts of selenium were protective against a form of liver necrosis in laboratory rats fed diets containing torula yeast as a protein source (Schwarz and Foltz, 1957). Dietary supplementation, by means of selenium-enriched fertilizer in crop production, foliar spraying of staple crops such as rice or soya beans or, directly, through multi-vitamin supplementation is now an accepted practice in areas of selenium deficiency, worldwide (Oldfield, 2002, Yang et al., 1988).

Selenium, amino acids and selenoproteins

The amino acids methionine and cysteine contain sulphur in the form of thiol groups. Selenium can replace the sulphur to form selenomethionine ([Se]Met) or selenocysteine ([Se]Cys or Sec) as a normal physiological process. A selenoprotein is any protein that includes a Sec or [Se]Met residue, which confers specific biological function. Dietary
selenium, initially taken up from the soil and concentrated by plants, is absorbed in the small intestine and incorporated into proteins by complex mechanisms which remain unclear (Reilly, 2006). The majority of selenium in the human diet is derived from [Se]Met in plant materials and both [Se]Met and Sec in animal products (Combs, 2001, Sunde, 1990). [Se]Met cannot be synthesised by higher animals, including humans, but after ingestion is non-specifically incorporated into proteins (e.g. haemoglobin, albumin) in place of methionine (Thomson et al., 1993). Selenophosphate is synthesised from selenide and ATP through the action of selenophosphate synthetase 2 (SEPHS2) and is the source of selenium from which Sec is then formed and co-translationally incorporated into selenoproteins at in-frame UGA codons. Sec has a lower $pK_a$ than Cys and is more nucleophilic, so is more reactive. During protein catabolism, Sec is rapidly broken down to elemental selenium, leaving no free pool of cellular Sec. This has a biological advantage, since Sec can react with oxygen, thioredoxin and thioredoxin reductase, giving rise to rapid NADPH oxidation and the formation of damaging reactive oxygen species (ROS) (Lu and Holmgren, 2009).

The form in which selenium is present in food affects bioavailability and expression of the different selenoproteins. Organic selenium sources such as Se[Met] are more efficient at increasing the blood selenium concentration than inorganic selenium, such as selenite and selenate, but they appear to be equally adept at raising whole-blood glutathione peroxidase (GPx) activity in the long-term (Thomson et al., 1993). Bioavailability from the different selenium sources is also tissue dependent. Dietary protein is more effective than other sources in increasing measurable selenium status. High selenium consumption
leads to higher selenium content of proteins in the form of [Se]Met and Sec (Kohrle et al., 2005).

Selenite ($\text{SeO}_3^{2-}$; inorganic form of selenium) crosses the plasma membrane, and reacts with cytoplasmic thiols in the reduction pathway; this forms selenide, which is then methylated, giving rise to methylated selenium derivatives that are excreted in urine, in expired air via the lungs and in faeces (Fig. 1) (Sunde, 1990, Ip, 1998). In humans these products of selenium metabolism are predominantly excreted in urine (Yang et al., 1989, Oster and Prellwitz, 1990). The proportion of selenium intake excreted in this manner depends on dietary intakes; when this is high, urinary excretion will also be high and vice versa (Robinson et al., 1973, Thomson and Robinson, 1986, Oster and Prellwitz, 1990).

There appears to be no homeostatic control of selenium absorption, which is unusual, in contrast, for example, to the complex regulation of iodine absorption (Kohrle et al., 2005, Reilly, 2006, Fairweather-Tait et al., 2010). Selenium is stored in the tissues in varying density: 30% in the liver, 30% in muscle, 15% in the kidney, 10% in the plasma, and the remaining 15% throughout other organs (Levander, 1987, Reilly, 2006). Concentrations of free selenium are greatest in the renal cortex and pituitary gland, followed by the thyroid gland, adrenals, testes, ovaries, liver, spleen, and cerebral cortex (Drasch et al., 2000, Kohrle et al., 2005).

Selenoproteins, coded by twenty five selenoprotein genes in humans (Table 1) (Kryukov et al., 2003), exert multiple actions on endocrine, immune and inflammatory functions
(Beckett and Arthur, 2005, Thomson, 2004), in part because they have powerful antioxidant functions. The selenoenzymes have a Sec group at their active sites, which enables the formation of disulphide bonds (Burk and Levander, 2006); these function as a redox centre, participating in transfer of electrons between molecules (Flohe et al., 2000).

Of the identified selenoproteins, three are iodothyronine deiodinases, which catalyse the removal of iodine from the 5 or 5’ positions of iodothyronine substrates. This regulates the activation and inactivation of thyroid hormones in all tissues (Beckett and Arthur, 2005). A further three are the thioredoxin reductase family (TrxR1, TrxR2 and TrxR3). Their substrates, thioredoxin and thioredoxin peroxidase do not contain selenium. These constitute a powerful dithiol-disulphide system that regulates the cellular redox state (Hill et al., 2003, Burk et al., 2003, Mostert et al., 2003) (Table 1). The Trx system also regulates other antioxidants (such as heme oxygenase-1, methionine sulfoxide reductases, ascorbate (Vit C), tocopherol (Vit E), ubiquinone (Q10)), modulates several transcription factors (eg those involved in the maturation of p53) and regulates apoptosis and protein phosphorylation (Surai, 2006, Arner, 2009, Mostert et al., 2003).

Of particular importance to reproduction and pregnancy are the 6 antioxidant GPxs which play a pivotal role in reducing hydrogen peroxide (H$_2$O$_2$) and lipid peroxides to harmless products (water and alcohols; Fig. 2), thereby dampening the propagation of damaging ROS (Rotruck et al., 1973, Brigelius-Flohe et al., 2003). This important pathway of cellular protection has been demonstrated in all mammalian tissue examined (Allan et al., 1999, Knapen et al., 1999). As antioxidants, the GPxs help maintain membrane integrity, protect prostacylin production, and limit the propagation of oxidative damage to lipids,
lipoproteins, and DNA (Brigelius-Flohe et al., 2003). This pathway may also offer protection against development of several chronic diseases in which oxidative damage has been implicated, including atherosclerosis and certain cancers (Rayman, 2002, Combs, 2001, Brigelius-Flohe, 2008). However, the claims that selenium supplements contribute to the prevention of chronic disease currently lack substantial evidence based proof of efficacy. Indeed some of the larger trials have been negative, for example the recent randomised, placebo-controlled cancer chemoprevention trial (selenium and vitamin E cancer prevention trial; SELECT) demonstrated no benefit of supplements of selenium (200 μ/day) and vitamin E (400 IU/day) in prevention of prostate cancer in a total of 35,533 men (Lippman et al., 2009).

Dietary selenium

Plant foods are the major dietary sources of selenium in most countries (Rayman, 2000, Combs, 2001). Surveys suggest that wheat is the most efficient selenium accumulator of the common cereals, and is one of the most important selenium sources for man (Lyons et al., 2003, Reilly, 2006). The content in food depends on the selenium content of the soil where plants are grown or animals are raised. For example, the selenium content in the soil of the high plains of northern Nebraska and the Dakotas is very high, and the inhabitants have the highest selenium intakes in the US (Longnecker et al., 1991). Whether this degree of high intake has any positive health benefit is not known, but toxic effects supervene when intake exceeds ~ 850 μg/day (Goldhaber, 2003).
Other foods make a substantial contribution to selenium intake in northern Europe, particularly meat, poultry, and fish (a total of about 36% in the UK) (Ministry Of Agriculture Fisheries and Food, 1997). Thus it has been predicted that vegetarians or vegans are at specific risk of selenium deficiency (Reilly, 2006, Judd et al., 1997), but this claim is not fully substantiated.

Selenium incorporation into plants (initially), and then into animal tissues, not only depends on soil selenium content or geochemistry but also on soil pH, rainfall, land contour, the use of high-sulphur fertilisers and microbial activity; some bacteria can convert insoluble forms of selenium to soluble forms, which can then be taken up by plants (Diplock, 1993, Lyons et al., 2003). Selenium tends to be more concentrated in the soils of the drier regions of the world, where soil tends to be more alkaline; in acidic poorly aerated soils, selenium is relatively unavailable to plants as it is present mainly as insoluble selenite complexes (Lyons et al., 2003, Reilly, 2006).

In addition, in wetter regions, rain leaches selenium from the soil (Reilly, 2006). Selenium forms both inorganic and organic compounds and can be an oxidant as well as a reductant, an important factor in soil formation (Van Dorst and Peterson, 1984). Selenium’s chemical adaptability accounts for its widespread occurrence in soils, plants, animals and humans (Bauer, 1997). Soil selenium concentrations range from 0.1 to more than 100 mg/Kg. However, most soils contain between 1.0 to 1.5 mg/Kg (0.1-0.6 mg/Kg is considered deficient) (Lyons et al., 2003, Combs, 2001).
Selenium deficiency

The optimal range of selenium intake to ensure biological benefit appears to be narrow and has still not been determined with certainty; however selenium deficiency has been studied in animals and humans (Van Vleet, 1980, Zachara et al., 1993a, Hurst et al., 2010). Selenium deficiency as assessed by dietary intake and/or blood selenium concentrations has been identified in people inhabiting geographical regions notable for low soil selenium content, such as volcanic regions and in Finland and New Zealand, where the reported average selenium intake is approximately 30-40 µg/day (Levander and Burk, 1994, Thomson, 2004). Animal selenium deficiency diseases have been routinely identified since the 1950s in livestock in countries that have low selenium soil conditions (Oldfield, 1997, Koller and Exon, 1986).

Human selenium deficiency diseases have been recognised in China and Tibet (Moreno-Reyes et al., 2003, Levander and Beck, 1997). Keshan disease, a reversible endemic cardiomyopathy, is characterised by focal myocardial necrosis often associated with inflammatory infiltrates and calcification. The disorder is exclusively endemic in selenium-deficient rural areas of China e.g. Keshan (Beck et al., 2003) and supplementation with selenium tablets (as sodium selenite) in pregnancy (Moore et al., 2000) provides highly effective protection against its development in susceptible women (Beck et al., 2003).

In Northern Karelia (Finland) very low blood selenium concentrations have also been reported in men with a high risk of myocardial infarction (MTT Agrifood Research...
Finland, 2005). Smoking further compromises selenium status by decreasing the serum concentration of selenium, and erythrocyte GPx activity (Northrop-Clewes and Thurnham, 2007, Duthie et al., 1993). Low selenium status may exacerbate disease progression in conditions not otherwise associated with selenium-deficiency e.g. human immunodeficiency virus (HIV) infection and hepatitis C virus, although the mechanism which affords protection by selenium is not known (Rayman, 2000).

Dietary selenium intake in most parts of Europe is considerably lower than in the USA, mainly due to the European soils providing a poorer source of selenium (Thomson, 2004, Rayman, 2008). The reduction in consumption of wheat imported from the US in the European Union from the 1980s, as a result of the European Common Agricultural Policy, has been associated with a fall in daily selenium intake in the UK and other Western European countries over the last 20 years (Jackson et al., 2004).

Assessments of requirements, adequacy and intakes of selenium have been reviewed previously in detail (Rayman, 2008, Thomson, 2004). The recommended daily allowances (RDA) for both men and women in USA is 55 µg/day, rising to 60 µg/day for pregnant women (Institute of Medicine, 2000). The UK is still using the 1991 reference nutrient intakes (RNI) of 75 µg/day for adult men, 60 µg/day for adult women and 75 µg/day for lactating women (Department of Health, 1991). The Department of Health reviewed whether selenium intake should be higher in 1998 and then again in 2009, but concluded that the original figures were still applicable (Department of Health, 1998, Department of Health, 2009). The World Health Organisation (WHO) set its normative
requirement estimate (NR) at a lower value of 40 µg/day for men and 30 µg/day for women (WHO/FAO/IAEA, 1996). The RDA/RNI values have been determined from the intake believed necessary to maximise the activity of the antioxidant GPx in plasma, whereas the NR is based on selenium intake needed to achieve two-thirds of maximum activity of erythrocyte GPx (Thomson, 2004).

Selenium intake appears on average to be at or above the RDA in the US or Canada. A study in Maryland in 1981 reported that adults consumed an average of 81 µg/day of selenium (Welsh et al., 1981) and recently this has been estimated to be 108 µg/day for all US adults and 89 µg/day for women (Chun et al., 2010). A Canadian survey in 1975 reported intakes of 113 to 220 µg/day (Thompson et al., 1975); this was followed in 1998 by a report indicating consumption of between 98-224 µg/day (Gissel-Nielsen, 1998). Conversely, the UK selenium dietary intake is generally below the RNI; a dietary survey published by the UK Government over the period 1994 to 1995 indicated that the average intake was as low as 30–40 µg per day (Ministry Of Agriculture Fisheries and Food, 1997) a figure which had not improved in a survey conducted between 2008 and 2009 (Department of Health, 2009). Although it has been argued that UK intakes are sufficiently low to warrant government intervention (Rayman, 2000), a UK government expert committee concluded in 1998 that intervention was, at that time, not warranted (Department of Health, 1998). Whether, this conclusion pertains to the dietary intake in 2010 is uncertain and is worthy of investigation.

Selenium toxicity
Whilst selenium deficiency is prevalent and therefore the more predominant health issue, there is also a moderate to high health risk of selenium toxicity, first discovered in animals grazing in areas with high selenium content in the soil (Twomey et al., 1977). Chronic toxicity of selenium in humans results in selenosis, a condition characterised by brittleness or loss of hair and nail loss, gastrointestinal problems, rashes, garlic breath odour, and nervous system abnormalities (Yang et al., 1983). In China, it has been reported that selenosis occurs with increased frequency in people who consumed selenium at levels above 850 µg/day (Yang and Zhou, 1994). The Institute of Medicine, USA, has set a tolerable upper intake level for selenium at 400 µg/day for adults to prevent the risk of developing selenosis (Institute of Medicine, 2000). The European Commission and WHO have proposed the lower daily upper limit of 300 µg/day for adults (European Commission Health and Consumer Protection Directorate, 2000, WHO/FAO/IAEA, 1996).

**Selenium in Reproductive Health**

The role that selenium plays in both male and female reproduction is well recognised in animal husbandry (Reilly, 2006). Selenium is essential for male fertility, being required for testosterone biosynthesis and the formation and normal development of spermatozoa (Behne et al., 1996, Flohe, 2007). Studies using selenoprotein P-knockout mice support a requirement for selenium in testicular function (Hill et al., 2003) and animals fed selenium-deficient diets show impaired spermatozoan motility with flagellar defects localised primarily to the midpiece, decreasing the chance of fertilisation (Behne et al., 1996, Wu et al., 1973).
Testicular tissue contains high concentrations of selenium, predominantly as GPx4 and this provides the pivotal link between selenium, sperm quality and male fertility since GPx4 is a fundamental determinant of the architecture of the spermatozoan midpiece (Beckett and Arthur, 2005, Knapen et al., 1999), and is considered to shield developing sperm cells from oxidative DNA damage (Ursini et al., 1999, Safarinejad and Safarinejad, 2009). ROS have been implicated in male infertility because, through attack of the spermatozoa membrane, sperm viability is decreased.

Some evidence suggests that increasing selenium dietary intake increases antioxidant GPx activity, thereby increasing male fertility (Irvine, 1996). Bleau et al.’s study in 1984 was one of the first indications, in humans, that selenium deficiency may be related to male fertility, reporting an optimal range between 50 - 60 μg/ml in semen and a positive correlation between sperm count and semen selenium concentration in 125 men from couples being investigated for infertility (Bleau et al., 1984). In Scotland (where mean selenium intakes are below requirements, ~30-40 μg per day) a placebo-controlled randomised control trial (RCT) of 64 men demonstrated that sperm quality and fertility improved after selenium supplementation (Scott et al., 1998). A placebo-controlled RCT from Tunisia of 54 infertile and 54 men on placebo also demonstrated the beneficial effects of a combination of vitamin E (400 mg) and selenium (225 μg) daily supplements for 3 months on improving sperm motility (Keskes-Ammar et al., 2003). In another recent placebo–controlled RCT in Iran of 468 infertile men, supplementation with 200 μg selenium orally daily for 26 weeks improved semen quality including sperm count,
concentration, morphology and motility, as well as plasma and semen selenium concentrations (Safarinejad and Safarinejad, 2009). A recent review of the effect of oral antioxidants (including selenium) on male subfertility concluded that supplementation could improve sperm quality and/or pregnancy rates but recommended that large adequately powered trials using individual antioxidants are required (Ross et al., 2010).

Data regarding selenium and female fertility are sparse. Paszkowski et al., completed a study of 135 follicular fluid samples collected from 115 patients during transvaginal oocyte retrieval; patients with unexplained infertility had significantly decreased follicular selenium concentrations compared to those with tubal infertility or a known male related cause of infertility (Paszkowski et al., 1995). A recent case-controlled study from Turkey also found lower serum and follicular fluid selenium concentrations in 30 women undergoing IVF treatment compared to 13 age-matched non-pregnant control women (Ozkaya et al., 2010). Another rather indirect indication of a role for selenium in fertility comes from a small study of women with a history of unexplained infertility. In 6 of the 12 women investigated the red-cell magnesium content failed to normalises after 4 months of magnesium supplementation and was associated with a lower red-cell GPx activity than that observed in the remaining 6 women whose red-cell magnesium regained normality (Howard et al., 1994). Subsequent supplementation with magnesium and selenium for 2 months achieved red-cell magnesium normalisation and increased red-cell GPx activity and the women later (within 8 months) conceived with a healthy pregnancy outcome (Howard et al., 1994). The authors theorised that failure to maintain cellular
magnesium homeostasis result from ROS induced cell permeability secondary to poor selenium status (Howard et al., 1994).

A combination of insulin, transferrin and selenium (ITS) is widely used as an adjuvant mixture in culture media for studies of ovarian and early pregnancy tissue, including human pre-antral follicles (Roy and Treacy, 1993, Abedelahi et al., 2010) or human fetal ovaries (Roig et al., 2006). The addition of selenium is reported to increase total antioxidant capacity and GPx activity, and decrease the levels of ROS, thus improving the in vitro development of follicles (Abedelahi et al., 2010).

In reinforcing the antioxidant properties of selenium, these studies highlight a potential role in female reproductive function. As concluded in a recent review, the relationship between oxidative stress, decreased female fertility, and selenium deficiency is an association which warrants further research activity (Ruder et al., 2009).

Selenium and disorders of pregnancy

Miscarriage

Miscarriage, a clinically detectable pregnancy that fails to progress past 24 weeks’ gestation, occurs in 10-20% of all pregnancies (Bradley and Hamilton-Fairley, 1998). Genetic (chromosomal) abnormalities explain at least half of all miscarriages. Although anatomical, endocrine, immune, infective and thrombophilic conditions are other possible causes, most chromosomally normal miscarriages remain unexplained or idiopathic (Hirschfeld et al., 2007).
Miscarriages have been associated with selenium deficiency in veterinary practice (Stuart and Oehme, 1982), and selenium supplements prevent early pregnancy loss in sheep (Hidiroglou, 1979). In humans, a UK observational study reported significantly lower serum selenium concentrations in 40 women with 1st trimester miscarriage compared to 40 age-matched non-pregnant and 40 healthy gestation-matched women (Barrington et al., 1996). A similar finding was reported in another observational study from Turkey of 20 women with 1st trimester miscarriage compared to controls (Kocak et al., 1999). Red-cell and hair selenium concentrations are also reported to be lower in women with recurrent miscarriage (Al-Kunani et al., 2001, Kumar et al., 2002). Early pregnancy loss has been linked to reduced antioxidant protection of biological membranes and DNA and also to low concentrations of the selenium-dependent GPx (Barrington et al., 1997, Zachara et al., 2001, Jauniaux et al., 2006), and although speculative, women with recurrent pregnancy loss could potentially benefit from optimisation of selenium status.

**Normal Pregnancy**

During normal pregnancy, the selenium requirement is increased as a result of demands from the growing fetus (Smith and Picciano, 1986) and both inorganic and organic forms of selenium cross the placenta in humans and experimental animals (Shennan, 1987, Shennan, 1988, Nandakumaran et al., 2003, Nandakumaran et al., 2002). The RDA of selenium in pregnancy in the USA, calculated based on a fetal deposition of 4 μg/day throughout pregnancy, is 60 μg/day (Institute of Medicine, 2000).
In countries such as Poland and Yugoslavia where soil selenium content and dietary intake are low, maternal selenium concentrations and GPx activity fall during pregnancy, being the lowest at delivery compared with non-pregnant controls (Mihailovic et al., 2000, Zachara et al., 1993b). In contrast, in areas of very high soil selenium content e.g. South Dakota, it would appear that there is no gestational trend in serum selenium concentrations (Kundu et al., 1985). Babies generally have lower selenium concentrations compared to the mother (Gathwala et al., 2000, Mistry et al., 2008), which might be anticipated as selenium is transported via the placenta across a concentration gradient via an anion exchange pathway, (Shennan, 1987, Shennan, 1988).

**Pre-eclampsia**

Pre-eclampsia (*de novo* proteinuric hypertension) is estimated to occur in ~3% of all pregnancies and is a leading cause of maternal and perinatal mortality and morbidity in the Western world (Sibai et al., 2005, Steegers et al., 2010); together with other hypertensive disorders of pregnancy, pre-eclampsia is responsible for approximately 60,000 maternal deaths each year (Broughton Pipkin, 2001) and increases perinatal mortality five-fold (Roberts and Lain, 2002). Optimal outcome for the mother and child often dictates that the infant is delivered early leading to increased preterm delivery and low infant birthweight rates. Placental and maternal systemic oxidative stress are components of the syndrome (Poston, 2004) and contribute to a generalised maternal systemic inflammatory activation (Redman and Sargent, 2003). Placental ischaemia-
Reperfusion injury has been implicated in excessive production of ROS, causing release of placental factors that mediate the inflammatory responses (Hung and Burton, 2006).

Endothelial cell dysfunction has been implicated in the many clinical manifestations of pre-eclampsia including hypertension and altered haemodynamics (Hubel, 1999, Poston, 2006). There is increased interest in the association between selenium status and pre-eclampsia. In light of the association between oxidative stress and the prevalence of low dietary selenium status worldwide, several studies have suggested that selenium deficiency may be linked to pre-eclampsia.

The recent appreciation that nutrient-gene interactions may play a major role in manifestation of hereditary disease traits (Hesketh, 2008) could be of relevance to the association between selenium status and pre-eclampsia. Several genes which encode selenoproteins demonstrate functional polymorphisms. Examples include GPx3, functional polymorphisms of which decrease transcriptional activation, gene expression and plasma protein activities (Voetsch et al., 2007a, Voetsch et al., 2007b). A single nucleotide polymorphism within the 3’UTR of the *GPx4* gene (*GPx4c718t*) affects GPx protein concentration and activity but also has differential effects on GPx3 and GPx1 when selenium supplementation is stopped (Meplan et al., 2008).

Selenoprotein S (also known as SEPS1 or VIMP), which contains a Sec residue at its active site, is an anti-inflammatory protein that acts primarily to limit the damaging consequences of endoplasmic reticulum stress (Ye et al., 2004), which has recently been
suggested to contribute to the development of pre-eclampsia (Burton et al., 2009). A polymorphic variant in the SEPS1 locus has been associated with increased cardiovascular disease morbidity in Finnish females (Alanne et al., 2007) and a 105G>A promoter polymorphism associated with reduced function has been defined and is significantly but not strongly associated with pre-eclampsia (Moses et al., 2008). Given that pre-eclampsia has a familial component (Cincotta and Brennecke, 1998, Lie et al., 1998, Chappell and Morgan, 2006), a high prevalence of these polymorphisms could, in association with selenium deficiency be a major determinant of impaired antioxidant defence in this disorder, through altered selenoprotein activity, and thereby contribute to development of the disease through ‘nutrigenomic’ pathways. Genome wide association studies of adequate size, such as that currently underway (Wellcome Trust case-control consortium (WTCCC3) – pre-eclampsia; http://www.wtccc.org.uk/ccc3/projects/ccc3_eclampsia.shtml) will be valuable in determination of the prevalence of these and similar functional polymorphisms in women affected by pre-eclampsia.

In the UK, where selenium dietary intake is low, our group and others have reported selenium concentrations in pre-eclamptic pregnancies to be reduced in sera from the mother (Atamer et al., 2005, Mistry et al., 2008) and fetus (Mistry et al., 2008) as well as in amniotic fluid (Dawson et al., 1999) and in toenails (reflecting longer term selenium stores) (Rayman et al., 2003), when compared to normal pregnant controls. A recent retrospective study from Iran reported lower plasma selenium concentrations in 40 pre-eclamptic compared to 40 control women (Maleki et al., 2011). Conversely, others have
shown no differences (Rayman et al., 1996) and in one study from the USA, higher sera selenium concentrations have been reported in women with pre-eclampsia (Mahomed et al., 2000). However, a reported lack of sensitivity of the assays used (Rayman et al., 1996), or dependence of the maternal leucocyte selenium content in estimation of selenium status (Mahomed et al., 2000) may confound interpretation of these studies.

Selenoprotein GPx activities in both maternal and cord plasma have also been shown to be lower in pre-eclamptic pregnancies. A retrospective study of plasma taken from 25 pre-eclamptic and 15 healthy pregnant Turkish women in their 3rd trimester observed significantly lower GPx levels in pre-eclampsia compared to controls (Yildirim et al., 2004). A similar study, also from Turkey, retrospectively collected maternal blood just before delivery, from 30 mild pre-eclamptic (defined as blood pressure $\geq 140/90$ mm Hg plus $\geq 300$ mg/24 hours proteinuria); 30 severe pre-eclamptic (defined as $\geq 160/110$ mm Hg plus 5 g proteinuria in 24 hours) and 30 normal pregnant women. This study reported lower concentrations of GPx in both pre-eclampsia groups compared to the controls (Bulgan Kilicdag et al., 2005).

Several other retrospective studies from the USA (Wang and Walsh, 1996, Walsh and Wang, 1993), Turkey (Atamer et al., 2005) and Australia (Vanderlelie et al., 2005) of placental tissue collected from normal pregnancy and pre-eclampsia report a reduction in GPx activity in pre-eclampsia. Our group recently conducted a retrospective cross-sectional study in the UK of 25 pre-eclamptic women and 27 healthy controls, in which maternal blood samples were collected before delivery, as well as cord blood and
placental tissue immediately after delivery (Mistry et al., 2008). Plasma concentrations of thiobarbituric acid reactive substances (TBARS; a marker for lipid peroxidation) were increased in maternal and cord plasma in the pre-eclamptic group. Moreover, total GPx activity in plasma and in placental tissue were significantly reduced in pre-eclampsia (Mistry et al., 2008). Further prospective, longitudinal studies are required to elucidate a ‘cause or effect’ relationship. If selenium deficiency is confirmed in women suffering from pre-eclampsia, and this continues to be linked with GPx inadequacy, selenium supplementation in pregnancy may be of benefit in prevention or amelioration of pre-eclampsia, a hypothesis which is currently being addressed in a RCT (see below).

Some small studies have attempted to assess the influence of selenium supplementation on the incidence of pregnancy related hypertensive disorders, Han et al., conducted a small placebo-controlled RCT in Beijing, China, a population with a high risk of pregnancy-induced hypertension (PIH) and between 26%-27% and selenium deficiency. 52 women with known risk factors for PIH were randomised to selenium (100 μg/day) for 6-8 weeks during late pregnancy, and 48 were randomised to placebo (Han and Zhou, 1994). The selenium supplemented group had a reduced incidence of development of PIH (7.7%; 4/52) compared to the placebo group (22.7%; 11/48), and significantly increased maternal and cord blood selenium concentrations. Another very small prospective double-blind, placebo-controlled RCT study in Indonesia, reported lower rates of pre-eclampsia and/or PIH in women who were at increased risk of developing these conditions, after supplementation (n = 29) with a range of antioxidants and cofactors including selenium (100 μg) (Rumiris et al., 2006). Neither study adequately addressed
the role of supplementation on the incidence of pre-eclampsia. Recently however, Tara et al, investigated selenium supplementation of Iranian women in their first trimester (100 μg selenium per day) in a small pilot RCT and concluded that supplementation may be associated with a lower frequency of pre-eclampsia although this didn’t quite reach statistical significance (Tara et al., 2010).

There is no current consensus on the optimal dietary selenium supplement for use in clinical supplementation, since bioavailability and effects on expression of the various selenoproteins depend on the form of selenium product used (Rayman, 2008). A small UK based RCT of selenium supplementation (selenium in pregnancy; SPRINT) conducted by the Universities of Surrey and Oxford is ongoing. Although not powered to demonstrate clinical benefit this study is designed to assess the impact of selenium supplements on pre-eclampsia related biomarkers. Unselected primiparae are recruited between 12 and 16 weeks’ gestation. The active treatment is 60 μg a day of selenium-enriched yeast, which is intended to normalise blood selenium concentrations. Most selenium in selenium-enriched yeast is in the form of [Se]Met, and supplementation with this yeast has, in the majority of reported studies been shown to increase the activity of the selenoenzymes (Rayman, 2004). If successful, a larger multicentre RCT adequately powered to detect differences in rates of pre-eclampsia will be needed to assess potential clinical benefit.

Preterm labour
Preterm labour (labour < 37 weeks’ gestation) is a major cause of perinatal morbidity and mortality occurring in 6-7% pregnancies in the developed world and up to 25% in undeveloped countries (Steer, 2005) and is likely to be of complex origin. Amongst the few studies to have investigated selenium and preterm labour, Dobrzynski et al from Poland reported lower maternal selenium concentrations and reduced maternal and cord plasma GPx activities in 46 women who delivered preterm compared to 42 women delivering at term (Dobrzynski et al., 1998). The low selenium concentrations and GPx activities in the blood of the preterm infants were proposed to contribute to respiratory distress syndrome, retinopathy of prematurity, increased haemolysis or other prematurity related conditions (Dobrzynski et al., 1998). A study from Germany of formula-fed preterm infants (gestational age < 32 weeks, birthweight < 1500 g) observed significantly lower mean plasma selenium concentrations compared to healthy term infants who were also formula-fed (Sievers et al., 2001). Another recent report from Iran of 30 preterm (gestational age <34 weeks) and 30 term infants (gestation age >37 weeks) also revealed significantly lower serum selenium concentrations in the preterm infants compared to term controls (Iranpour et al., 2009). A study from the USA of 13 preterm and 15 term infants found no differences in maternal plasma selenium concentrations, but also reported that preterm infants had lower selenium concentrations compared to term infants (Mask and Lane, 1993). As might be anticipated, the daily dietary selenium intake was 2-3 times higher (96-134 μg) than in the subjects reported in the Polish population (Dobrzynski et al., 1998). Evidently, population selenium intake may explain some variation between studies.
Preterm premature (pre-labour) rupture of membranes (PPROM) is a major initiating factor in preterm labour and affects 10-12% of all pregnancies. PPROM is defined as premature rupture of chorioamniotic membranes before the onset of labour and is associated worldwide with increased rates of neonatal and maternal morbidity and mortality (Parry and Strauss, 1998, ACOG, 2007). Increased generation of ROS as well as antioxidant deficiency may play a important role in the pathophysiology of PPROM, which has been associated with enhancement of collagen degradation and subsequent damage to fetal membrane integrity (Wall et al., 2002, Woods, 2001, Woods et al., 2001). A potential association with selenium has been highlighted through a recent small prospective double blind, placebo-controlled RCT in Iran randomised 166 primigravid pregnant women in the first trimester of pregnancy to receive 100 μg/day selenium or placebo until delivery (Tara et al., 2010). The supplemented group demonstrated a significant increase in the mean serum selenium concentration and a reduction in the incidence of PPROM (Tara et al., 2010).

Fetal growth restriction

Fetal growth restriction or delivery of a small for gestational age infant (SGA) is defined as an individualised birthweight ratio below the 10th percentile, and is associated with increased perinatal mortality and morbidity (Cetin et al., 2004). Some studies of SGA deliveries report a reduced placental selenium concentrations (Klapec et al., 2008), whereas others report higher (Osada et al., 2002, Zadrozna et al., 2009) or unchanged concentrations (Llanos and Ronco, 2009). Strambi et al., demonstrated that in 81 SGA (both term and preterm) retrospective cases from Italy, infant plasma selenium
concentrations were significantly lower compared to adequate-for-gestational age (AGA) infants (Strambi et al., 2004). Again geographical differences may explain the difference between the selenium status in the different studies.

A recent investigation by our group in a cohort of adolescent pregnant women from two UK inner cities (Baker et al., 2009) found lower plasma selenium concentrations in mothers who delivered SGA infants compared to mothers who delivered AGA infants (Mistry et al., 2010). A recent series of papers from North Dakota State University suggest some protective effect of high selenium intake in nutrient-restricted pregnant ewes on fetal birthweight and placental development (Lekatz et al., 2010). We are not aware of any ongoing studies investigating maternal and fetal selenium status in relation to fetal growth restriction although these observations would warrant a larger prospective study especially focusing on adolescent pregnant women and those residing in selenium-deficient populations.

Obstetric cholestasis

Obstetric cholestasis (OC) is a serious complication of pregnancy and affects approximately 4,500 women per year in the UK. Affected women develop itching, otherwise-unexplained elevation of plasma liver enzymes and of serum bile acids and occasionally jaundice. OC is associated with an increased risk of premature delivery and fetal distress and is believed to be an important cause of stillbirth (Gurung et al., 2009).

Selenium was first linked with OC in 1987 when Kauppila et al demonstrated that serum selenium concentrations were significantly lower in 12 Finnish women with OC when
compared to 12 normal pregnancies during the last trimester and postpartum (Kauppila et al., 1987). Furthermore they also showed GPx activities to be decreased, showing a significant positive correlation with selenium concentration (Kauppila et al., 1987). Thus, it has been hypothesised that inadequate antioxidant protection may lead to hepatocyte oxidative damage and reduce excretion of bile (Akerboom et al., 1984). These initial results have been confirmed and extended in a study of 21 women with OC in Chile, also showing that the decrease in prevalence of OC in Chile during the last decade coincided with an increase in plasma selenium concentrations (Reyes et al., 2000).

**Gestational diabetes mellitus**

Gestational diabetes mellitus (GDM) is one of the more common diseases in pregnancy, affecting between 2% and 5% of pregnant women and is associated with birthweights above the 90th centile, increased levels of primary Caesarean deliveries and neonatal hypoglycaemia (Gilmartin et al., 2008). GDM is defined as a deficient insulin supply relative to the increased demands that are characteristic of pregnancy (Metzger et al., 2007). The causes are not known but are closely related to a constitutional risk of type 2 diabetes in later life and strongly associated with obesity. A significant proportion of GDM women develop type 2 diabetes 5-16 years after pregnancy (17-63% risk) (Kjos et al., 1995, O'Sullivan and Mahan, 1964, Mestman et al., 1972).

A link between selenium and glucose metabolism has been observed previously in animal studies (Becker et al., 1996, McNeill et al., 1991, Ezaki, 1990) and selenium administered to streptozotocin-diabetic rats showed a restoration of glycemic control and a
modification of the activity of a range of enzymes involved in hepatic glycolysis and glyconeogenesis (Becker et al., 1996). Several studies from China, Kuwait, Turkey and the USA have shown a decrease in maternal plasma selenium concentrations in women with GDM (Tan et al., 2001, Hawkes et al., 2004, Kilinc et al., 2008, Al-Saleh et al., 2004). Bo et al completed a retrospective study investigating selenium intakes through dietary questionnaires in 504 pregnant women (210 with hyperglycemia and 294 healthy controls) as well as measuring serum concentrations in a second cohort (71 hyperglycemic and 123 controls) (Bo et al., 2005). A lower dietary intake of selenium was observed in the hyperglycaemic group and in the second cohort, selenium concentrations were significantly lower in the women who had impaired glucose tolerance; both dietary intakes and selenium concentration were negatively associated with gestational hyperglycemia in a multiple regression model (odds ratio 0.97 and 0.92 respectively) (Bo et al., 2005).

An inverse relationship between selenium concentrations and blood glucose concentrations has also been observed (Kilinc et al., 2008, Tan et al., 2001, Hawkes et al., 2004), but was not accompanied by changes in insulin (Hawkes et al 2004) suggesting that selenium may affect glucose metabolism downstream from insulin, or possibly through independent energy regulating pathways such as thyroid hormones (Hawkes et al., 2004). This relationship is unique to pregnancy: diabetes in non-pregnant subjects is associated with higher blood selenium concentrations (Laclaustra et al., 2009).

Conclusions
There are wide differences in selenium intake across diverse populations, depending on the selenium content of the soil, and hence the selenium content in staple foodstuffs, as well as on variations in individuals’ diets. Both deficiency and excess are damaging to health. In turn, varying intakes are associated with differences in selenoprotein and selenoenzyme expression in different tissues. This must be taken into account when comparing data from different countries or populations. Evidently, the balance between intake, tissue concentration and selenoenzyme synthesis is a very delicate one. This review illustrates the potential influence that selenium status has on many disorders relating to both animal and human reproduction and pregnancy. While persuasive evidence already exists to suggest that additional selenium would be beneficial in some of these disorders, results from intervention trials underway or planned have the potential to reinforce or refute the argument for increasing selenium intake.
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Figure legends

Figure 1: Selenium metabolic pathway. This diagram illustrates how selenoproteins can be produced in the body from a variety of selenium sources. Glutathione (GSH) is considered to be the main component of the selenium metabolism pathway taking part in the first of a series of reduction reactions which convert selenite to hydrogen selenide (H$_2$Se). [Se]Met: selenomethionine; [Se]Cys: selenocysteine. Adapted from (Sunde, 1990, Ip, 1998, Patrick, 2004).

Figure 2: Major pathways of reactive oxygen species generation and metabolism. Superoxide can be generated by specialized enzymes, such as the xanthine or NADPH oxidases, or as a byproduct of cellular metabolism, particularly the mitochondrial electron transport chain. Superoxide dismutase (SOD) then converts the superoxide to hydrogen peroxide (H$_2$O$_2$) which has to be rapidly removed from the system. This is generally achieved by catalase or peroxidases, such as the selenium dependent glutathione peroxidases (GPxs) which use reduced glutathione (GSH) as the electron donor.