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Zinc, copper and selenium in reproduction

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Abstract. Of the nine biological trace elements, zinc, copper and selenium are important in reproduction in males and females. Zinc content is high in the adult testis, and the prostate has a higher concentration of zinc than any other organ of the body. Zinc deficiency first impairs angiotensin converting enzyme (ACE) activity, and this in turn leads to depletion of testosterone and inhibition of spermatogenesis. Defects in spermatozoa are frequently observed in the zinc-deficient rat. Zinc is thought to help to extend the functional life span of the ejaculated spermatozoa. Zinc deficiency in the female can lead to such problems as impaired synthesis/secretion of (FSH) and (LH), abnormal ovarian development, disruption of the estrous cycle, frequent abortion, a prolonged gestation period, teratogenicity, still-births, difficulty in parturition, pre-eclampsia, toxemia and low birth weights of infants. The level of testosterone in the male has been suggested to play a role in the severity of copper deficiency. Copper-deficient female rats are protected against mortality due to copper deficiency, and the protection has been suggested to be provided by estrogens, since estrogens alter the subcellular distribution of copper in the liver and increase plasma copper levels by inducing ceruloplasmin synthesis. The selenium content of male gonads increases during pubertal maturation. Selenium is localized in the mitochondrial capsule protein (MCP) of the midpiece. Maximal incorporation in MCP occurs at steps 7 and 12 of spermatogenesis and uptake decreases by step 15. Selenium deficiency in females results in infertility, abortions and retention of the placenta. The newborns from a selenium-deficient mother suffer from muscular weakness, but the concentration of selenium during pregnancy does not have any effect on the weight of the baby or length of pregnancy. The selenium requirements of a pregnant and lactating mother are increased as a result of selenium transport to the fetus via the placenta and to the infant via breast milk.

Key words. Male reproduction; female reproduction; zinc; selenium; copper; pregnancy; parturition; lactation.

Introduction

Some inorganic substances such as iron (Fe), zinc (Zn), copper, (Cu), selenium (Se), molybdenum (Mo), manganese (Mn), chromium (Cr), cobalt (Co) and iodine (I) are known to be nutritionally essential and are needed in minuscule amounts every day for optimal health. They are usually referred to as biological trace elements. When living organisms migrated to the land from sea water in the course of evolution, they had to depend on the soil for biological trace elements. Geological variations in the earth's crust as a result of glaciation, volcanic activity, erosion etc caused variations in the distribution of these essential trace elements. For example Australian soil is so poor in copper and zinc that plant growth may be inhibited. The Heilongjiang province of China, and Finland, are deficient in selenium and as a result multiple myocarditis – 'Keshan disease' – is common. On the other hand, some other parts of the world such as Caracas (Venezuela) face the problem of toxic amounts of selenium. As food for humans comes through the soil-plant-animal food chain, the variations in the levels of essential trace elements cause some serious problems for human health.

Zinc, copper and selenium in male reproduction

Zinc

Of the nine biological trace elements, zinc, copper and selenium are important in reproduction. Zinc has been extensively studied. Its deficiency leads to gonadal dysfunction[185,203,205], decreases testicular weight and causes shrinkage of seminiferous tubules[186,231,232,262], Several of the zinc deficiency states such as sickle cell anemia, chronic alcoholism, idiopathic male sterility, or toxic effects of di-(2-ethyl hexyl)phthalate (DEHP) or other phthalic acid esters (PAEs), cause atrophy of the testis and the atrophy is attributed to low availability or increased urinary excretion of zinc[47,93,96,193,203,205]. Zinc deficiency is also linked to malignant growth in the testis[106]. Zinc content is high in the adult testis compared to immature animals or those in which the efferent duct is ligated[251]. In men, the zinc concentration increases at puberty and reaches a maximum at the age of 34–40 years of age when the functional activity of the organ is at its peak[291]. Similarly in rats with a maldescended testicle, the ectopic testis has a decreased zinc content, whereas that of the other testicle is normal[50]. Zinc deficiency impairs the action of the Mullerian inhibitory factor which is essential for testicular differentiation[30].

Angiotensin converting enzyme (ACE) is closely associated with testicular development and sperm development[62,130,163]. ACE is primarily localized in the germinal cells and has very little activity in Leydig and Sertoli cells[279]. The exact role of ACE is not yet clear but reduced ACE activity in the testis of zinc-deficient rats has been reported[221,272]. It is believed that zinc deficiency first impairs ACE activity, and that in turn leads to depletion of testosterone and finally impairs spermatogenesis[222]. Although the probable site of action is believed to be primary spermatocytes, it has also been reported from Leydig cells, Sertoli cells and sperm heads[1,106,186,202,226].

Zinc appears to be an indispensable element in reproduction for another reason too[16]. The gonads are the most rapidly growing tissues in the body and vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes[206,247].

The three cations zinc, calcium and magnesium stimulate or inhibit progressive motility depending on the concentration of each[252]. At high concentrations, these elements, individually or jointly, impair fertility in patients with normal sperm density[267]. On the other hand, Saito et al.[228] reported an increased sperm motility in the dog epididymis on addition of different concentration of zinc to the culture media. Although a positive correlation between seminal fluid, zinc and sperm motility and sperm density in asthenozoospermic men is reported[246] excessively high levels of this ion are related to defective motility in asthenozoospermia[44]. Some of the important enzymes of spermatozoa are zinc metalloenzymes and can thus become dysfunctional when zinc is deficient. One of these, sorbitol dehydrogenase (SoDH), utilizes sorbitol to provide spermatozoa with fructose for energy, so that SoDH activity is correlated to motility[69]. Similarly LDH-X, another zinc metalloenzyme, is also reported to have some relationship with sperm motility[82,172,283]. LDH-X deficiency leads to abnormalities in the mitochondrial region of the midpiece[169]. In the spermatozoa of rats and bulls, in addition to being present in zinc metalloenzymes, zinc is thought to be bound to SH groups of cysteine amino acids of proteins of the outer dense fibers of the spermatozoon tail[8,9,40,277]. Mitochondrial disarray in spermatids, acrosomal deformities, incomplete formation or disorganization of the axonemal complex and dense fibres of spermatozoa tail, and other defects such as decapitation, disorganization and redundant tail elements with superfluous cytoplasm, have been frequently observed in zinc-deficient rats[71,277]. Kvist[153] concluded from his in vitro experiments on human spermatozoa that one of the functions of zinc is to preserve the ability of the nuclear chromatin to undergo decondensation at the stage of male genome transfer, and zinc thus plays a crucial role in fertilization. The complete and non-delayed decondensation of the S–S cross-linked sperm chromatin in the ooplasm is necessary for normal embryonic development. Spermatozoal zinc is suggested to protect an inherent capacity of decondensation, thereby helping to extend the functional life-span of the ejaculated sperm[154,155]. The spermatozoon head accumulates a fourfold higher zinc concentration than seminal plasma, and the high-affinity zinc binding sites are present within the nuclear matrix. Zinc is related to the structural integrity of DNA, and prevents destruction of DNA by inhibiting degrading enzymes[57,184,204,243,287]. Further, an insufficient zinc level in the nucleus may destabilize the quaternary structure of chromatin, reduce the DNA content of spermatozoa and thereby reduce their fertilizing capability[153–156,213].

Zinc is also localized in the Golgi complex or secretory vesicles of interstitiotrophs (IT), folliculotrophs (FT) and lactotrophs (LT) of the pituitary gland. Thus it seems that the element plays an important role in the production and secretion of LH, FSH and prolactin, and these in turn regulate testosterone production. It is now well established that zinc deficiency depresses steroidogenesis[107,115]. Besides its effect on androgen metabolism, it interacts with steroid receptors and androgen binding protein[1,268]. The earliest cytochemical changes occur in Leydig and Sertoli cells in mice after two weeks of zinc deficiency. At this stage, although the seminiferous tubules appear to be healthy, the Sertoli and Leydig cells accumulate a large number of cholesterol-rich spherical bodies that increases with duration of zinc deficiency and extend to the germ cells and lumen of the seminiferous tubules[262]. Cholesterol and neutral lipids are precursors of sex steroids. In zinc deficiency their uptake by the germinal and non-germinal cells of the testes does not seem to be affected, but probably the cells are incapable of converting them into sex steroids. This is thought to be the reason that there is a low level of serum testosterone, in spite of high serum LH and FSH levels, after LHRH administration to zinc-deficient rats[162]. Since Sertoli cell hormone (SCH), formed by the metabolism of testosterone, triggers the formation of new cell lines in the testis, its insufficiency, because of defective metabolism of cholesterol, appears to be responsible for the arrest of spermatogenesis[162].

Zinc deficiency in lower organisms, such as *Euglena gracilis*, alters the mRNA composition but does not change the translational capacity of the organism. Therefore, zinc-deficient organisms produce a smaller number of proteins as compared to organisms with adequate zinc[270,271]. Further, *E. gracilis* like any other eukaryote, produces three RNA polymerases, but zinc-deficient organisms are capable of producing only one type of mRNA, and that is of a different type from all the three normal ones[86]. Similarly, zinc-deficient organisms have only H-1 and H-3 histone proteins, whereas organisms with adequate zinc have H2A, H2B and H4

in addition to H-1 and H-3[85]. On the other hand, zinc-deficient organisms have arginine- and asparginine-rich polypeptides that vanish on supplementation with zinc. All these observations indicate that zinc has a selective role in gene expression[86]. Whether the element has a similar selective role in the mammalian reproductive tract is not known so far. DNA and RNA polymerases of prokaryotes and eukaryotes are zinc metalloenzymes[187,247,286].

It is well recognized that hormone-induced transformation of steroid receptor complexes causes them to bind to DNA. Attempts have been made to identify the acceptor sites to which the transformed receptors become attached[134]. The interaction of the receptor with its ligand appears to be mediated through a pair of 'zinc fingers' located in the highly conserved C-region of all classes of receptors[63,84,95,104,238]. The zinc finger, the DNA binding domain, is represented several times in the protein and is composed of two cysteine residues and one histidine residue that wraps around a Zn ion in a finger-like fashion[21,100]. Zinc finger proteins are a highly conserved class of eukaryotic nucleic acid binding proteins[84,144] and regulate transcription[75,101,139,225]. The ZFY DNA domain (consisting of 13 zinc fingers and identified from studies of sex reversal in man) is located on the Y chromosome, and apparently encodes a transcription factor involved in spermatogenesis[31,70,145,171,191,198,201,236,245].

Microtubules, formed during cell division, are organized through a polymerization of tubulin subunits in association with small amounts of other proteins and metal ions such as Zn^{2+}, Mg^{2+}, Mn^{2+}, and Co^{2+} [34,99,115,116,118,161,195,278]. In vitro studies have shown that tubulin binds to zinc although the number of binding sites and the strength of the binding have not been determined. Tubulin-associated proteins S100a and S100b bind zinc[11,78]. A high concentration of zinc is present in spermatozoa tail microtubules[189].

The epididymis is an important male accessory organ and gives the spermatozoa the final functional integrity for fertilization[197]. The concentration of zinc in the epididymis of pig, sheep and bull tends to be almost the same as is observed in the testis[22] but in rat it is about twice the amount[177]. ^{65}Zn uptake of the epididymis in the rat is 60% higher than that of the testis[35,282]. Thus it contains a fairly high concentration of zinc, whose uptake and maintenance is directly related to the level of circulating testosterone[105,177]. Since the testicular output of testosterone is decreased in zinc-deficient animals, the epithelial cell height, and the weight of the epididymis, are also decreased[185,231,232,259] and the values are very similar to those after castration[48,55,192]. Healthy tubules of zinc-deficient rats exhibit variations in their cytochemistry, such as an extraordinary accumulation of proteins, carbohydrates, general lipids, neutral lipids, phospholipids and cholesterol. Administration of testosterone propionate for 17 days to zinc-deficient rats reduced carbohydrate and protein to levels similar to those in zinc-supplemented controls[259]. Several of the proteins essential for epididymal maturation of spermatozoa, secreted by the epididymis, are androgen-dependent[7,25,26,91,110]. Since androgen synthesis and secretion is influenced by the availability of zinc, it is possible that the output of these proteins and eventually the maturation of spermatozoa may also be influenced.

Bértrand et al.[22] were the first to report the presence of zinc in the prostate, and the fact that this organ has a higher zinc content than any other organ of the human body. Of the dorsolateral and ventral lobes of the prostate, it is the dorsolateral lobe that has the highest concentration and uptake of zinc in rat, rabbit and man[35,177,282]. Prostatic fluids are rich in acid phosphatase. Zinc is known to influence the motility, viability and morphology[81] of spermatozoa, and a positive correlation between motility and seminal acid phosphatase is reported in bull and man[66,133,170,227]. Research indicates that the zinc and albumin secreted from the prostate form a complex that coats the sperm and thereby protects the cells[94]. Prostatic zinc may have antibacterial activity because *Trichomonas vaginalis* is readily killed at the concentration of zinc that occurs in the prostatic fluid of healthy men[158]. Seminal zinc levels are higher in the group of males with elevated acid phosphatase[269], which emphasizes the functional significance of zinc in the prostate gland. Prostatic epithelial cells accumulate large amounts of zinc in the nucleus, and the uptake studies indicate a specific zinc-binding component in the nuclear prostate. Earlier studies already indicate a similar metal binding protein in the prostate cell cytosol, but whether the two are the same is yet to be evaluated[108,220].

Zinc not only regulates in vitro uptake of androgens by the prostate, but also seems to control the intracellular concentration of these steroids. The influence is mainly achieved by means of controlling the testosterone metabolic activity of the gland, which in turn is mediated by both a non-competitive inhibition of the binding of testosterone to the 5α-reductase enzyme and by a reduction in the formation of NADPH. Zinc has also been shown to inhibit the androgen receptor binding to the prostate cytosol and nucleus, and this is another major factor in the control of hormonal distribution between subcellular fractions. By analogy to other target tissues, this inhibition is probably due to the blocking of sulphydryl groups at the binding sites, but it could also be due to further blocking at the carboxylates, imidazoles and terminal amino groups and of peptides[108]. Matusik et al.[176] clones two hormonally regulated dorsolateral prostate mRNAs: M-40 mRNA and RWB-mRNA. M-40mRNA encodes a zinc-binding protein responsible for zinc accumulation in the dorsolateral prostate.

Copper

It is well established that a trace amount of copper is needed for proper functioning of biological systems, and it is essential in human nutrition[88,136,137,147,210,223,242,257]. Copper is an important component of numerous metalloenzymes and metalloproteins, so lack of copper leads to defects in the hemopoietic, cardiovascular, nervous, skeletal, integumentary, immune and reproductive systems. Of the several copper-metalloenzymes, cytochrome-c-oxidase and superoxide dismutase have been extensively studied in different organs, and the relative decline in each of these two enzymes in deficient animals is found to be organ specific[209,211,212]. The level of copper is decreased in the testis of rats fed low copper diets[141], and the level of testosterone in the male has been suggested to play a role in the severity of copper deficiency[89]. The presence of testosterone could predispose male rats to the lethal effects of copper deficiency. Male rats, whether castrated or not, are anemic, exhibit heart hypertrophy and die when deficient in copper[89]. Castration of the male reduced testosterone levels by 50%, and this reduction improved the copper deficiency anemia and delayed death by two weeks compared to intact males, thus it ameliorated the severity of the copper deficiency. However, the protection was only temporary[89]. There are sexual differences in the expression of copper-deficiency in rats[89]. If male rats are fed on a starch-based copper-deficient diet, the level of copper is similar to that of fructose-based copper deficiency, but they survive[90]. Female rats, on the other hand, are protected against the fructose-induced mortality of copper deficiency[90]. Thus it has been concluded that unless fructose is fed to copper-deficient male rats, copper deficiency per se is not sufficient to produce anemia, heart hypertrophy, pathology and mortality[89]. This is further supported by the fact that feeding a fructose-based copper-deficient diet to rats from weaning delays testicular development compared to that of rats fed a starch-based diet[142].

Selenium

A variety of malfunctions, such as heart disease and increased cancer risk, have been correlated with selenium deficiency. The biological function of selenium is as a cofactor of glutathione peroxidase, which protects the cell from damage by free radicals. Farm animals fed on selenium-deficient grasses exhibit poor growth and low fertility[114]. On the contrary, rats fed on a selenium-deficient diet reproduced normally, but the offspring were devoid of hair, grew more slowly and failed to reproduce. However, if their diet was supplemented with 0.1 ppm of selenium, hair coat, growth and reproductive capabilities were restored[179]. The selenium content of male gonads increases considerably during pubertal maturation. The amount of selenium taken up by the testes accounts for about 5% of the amount deposited in muscles and liver, whereas before and after the pubertal period the selenium level is about 10%[18,19]. After several studies on rats, Behne et al.[19,20] concluded that: 1) during insufficient selenium-intake the supply of the element to the testes has priority over the supply to other tissues; 2) the decrease in the testes' selenium content after hypophysectomy, and the subsequent rise after administration of either PMS or testosterone, indicates that hormones responsible for spermatogenesis are involved either directly or indirectly in the maintenance of the testicular selenium level; 3) stimulation with LHRH or hCG results in a less marked increase in serum testosterone concentration in selenium-deficient animals, and 4) when animals are fed a selenium-deficient diet, the requirement of the testes is initially met by selenium transferred from other tissues, and unless and until these pools are depleted, the testicular level is not decreased. This led to authors to postulate the involvement of selenium in the biosynthesis of testosterone[19,20]. Selenium-deficient rats also suffer from oligospermia, a decline in the ratio of motile to immotile spermatozoa, and increase in abnormal spermatozoa. The abnormalities are confined to disorganization of the mitochondrial sheath of the mid-piece, because of increased fragility and reduced stability of the mitochondrial sheath[38,41,276,288,289]. Tracer studies with ^{75}Se have demonstrated that selenium is localized in the mitochondrial capsule protein (MCP, mitochondrial capsule – a specialization of the outer mitochondrial membrane) of the mid-piece[28,38,41,199,200]. MCP is a cysteine- and proline-rich selenoprotein, where selenium occurs near the amino-terminal end. Isolated MCP retains the curved shape of sperm mitochondria, therefore it is suggested that it functions in organizing mitochondria into the helical sheath around the flagellum in the mid-piece of spermatozoa[200]. SDS-PAGE studies indicate that it is composed of polypeptides of Mr 15000 to 17000 that seem to have been derived from a precursor molecule of high molecular weight (Mr 47000 to 54000), available in the free form even to immature germ cells[37,39]. Further, the maximum incorporation of selenium in MCP occurs at step 7 and step 12, and the uptake decreases by step 15 of spermatogenesis[37–39], whereas recent studies of Shih and Kleene[241] indicate that MCP mRNA is first detected in step 3 round spermatids and persists at high concentration until step 16. This mRNA could not be detected even in low concentrations in pachytene primary spermatocytes from 18-day prepubertal mice, hence the authors concluded that its expression is probably restricted to haploid cells only[37–39].

LH is known to control the secretion of testosterone from Leydig cells. It has been suggested that selenium-deficiency causes some changes in the LH receptors of Leydig cells and thus affects testosterone secretion[17–20].

Significantly higher ^{75}Se retentions are reported from the pituitary gland, the caput and corpus epididymis, and the bulbourethral and prostate[32,265] glands. Variations in epididymal selenium concentrations are attributed to variations in the spermatozoal concentrations in the epididymis[32,37,138,178]. Willett et al.[285] demonstrated an association between low serum selenium levels and gastrointestinal and prostate cancer. Although selenium has a definite role in the prevention of cancer, and several explanations have been put forward, the exact mechanism by which selenium inhibits the growth of tumor cells is not known[17]. One of the suggested causes of prostate cancer is the high level of cadmium in the prostate[10], and in vitro studies have demonstrated that cadmium stimulates the growth of human prostatic epithelium[280]. Selenium, by its interaction with cadmium, has a protecting effect against cadmium-induced toxicity[17,196,239]. Also, selenium inhibits DNA, RNA and protein synthesis, and thus at non-toxic levels it could help in the inhibition of the growth of tumour cells[58].

Zinc, copper and selenium in females

Zinc deficiency in female reproduction; pregnancy, parturition and lactation

Food, feeding habits and deficiency of trace elements can affect the fertility of a population, and was the main cause of a decline in fertility in Europe between 1875 and 1913[175]. During that period, Europeans mainly consumed roller-mill processed white bread and potatoes that were deficient in zinc. Zinc deficiency, in females, leads to impaired synthesis/secretion of FSH and LH, abnormal ovarian development, disruption of the estrous cycle, frequent abortion, gross congenital malformation of fetuses (depending upon the reproductive stage when the zinc deficiency sets in), a prolonged gestation period, teratogenic effects, still-births, and delayed and prolonged deliveries accompanied by excessive bleeding, difficult parturition, uncoordinated uterine impulses or poor uterine activity, pre-eclampsia, toxemia and low birth weights of infants. Fluctuations of zinc and magnesium concentrations in a phase-related fashion in plasma have been reported in normally menstruating women. Whether these changes are hormonally mediated or reflect metabolic changes has not been worked out[68]. Zinc-levels in women using oral contraceptive agents (OCAs) have been of concern since 1968, when it was observed that women using OCAs had lower plasma zinc levels than women not using OCAs[2,24,54,112,119,207,208,274]. On the other hand, King[143] estimated that there was no increased endogenous zinc loss and the zinc-dependent functions were not compromised in OCA-users. However, OCA use may 1) alter post-absorptive utilization of zinc, 2) reduce circulating zinc, and 3) increase levels in some tissues or depress the release of the element from others, but there is no evidence to suggest that these changes alter the dietary zinc requirement[16].

Taneja and Kaur[260,261], working on virgin female mice fed on a zinc-deficient diet, observed mainly the I–VI type follicles rather than the VIII type, retardation of ovarian follicular growth with varying degrees of atresia, lack of preovulatory Graafian follicles, a reduced and shrunken corpus luteum and a fragmented zona pellucida and vitelline membrane. In addition, there was an excessive accumulation of coarse granules of phospholipids, triglycerides and cholesterol in the cells of the granulosa, theca interna and externa and interstitium. All these indicated cessation of oogenesis and ovulation. Similar abnormal ovarian development has been observed in zinc-deficient rhesus and bonnet monkeys[127]. Zinc deficiency, even if it is marginal, has been reported to affect oocyte maturation by doubling the number of degenerating oocytes and increasing chromosomal anomolies (hyperhaploidy and hypohaploidy) in metaphase II oocytes[279]. This led the authors to suggest that for all preconceptional women special precautions are necessary to ensure a sufficient intake of zinc. Maternal zinc deficiency may be a result of dietary inadequacy, or may be secondary to a disease such as diabetes, and carries a substantial risk for the developing offspring[279].

The estrous cycle in zinc deficiency exhibited a gradual prolongation of duration of successive cycles, (especially of proestrus and estrus). Prolonged proestrus and estrus are followed by diestrus and anestrus phases (after 6 weeks of zinc deficiency). Vaginal smears in the anestrus phase are comprised of leucocytes with a few isolated nucleated epithelial cells, whereas in the estrus phase non-nucleated cornified cells are altogether absent[103,129,261]. Zinc may regulate events in the menstrual cycle through its association with the regulation of progesterone[109], prolactin and opiate receptor binding in the CNS[146,165,253,264]. Plasma zinc concentrations are high during mensus and the follicular phase, and then decline during the ovulation and luteal phases. This change in elemental concentration is correlated to 1) hormonal changes or changes in the distribution of specific carrier protein[249], 2) variations in plasma albumen[249], 3) regulation of the activity or binding of selected hormones[109,165] and 4) interleukin-1[42]. Since zinc is associated with immunological responses, both pregnant and non-pregnant women with low plasma zinc level suffer from vaginitis three times more frequently than those with a higher level of plasma zinc[79]. Zinc-deficient mice exhibit decreased immunity that runs parallel to infection by *Candida ablicans*[229].

Lowered serum zinc levels are related to risk factors during pregnancy, and labour complications. Significantly lower plasma zinc levels are reported in women experiencing hypertension and/or toxemia and hypoalbumina of pregnancy[5,6,52,131,273], and supplementation

with zinc results in fewer complications[5,52,190]. It is estimated that the total zinc needed during the last half of human pregnancy corresponds to 2.6 mg absorbed zinc/day, and this may need special adaptations such as 1) an increase in zinc absorption, 2) reduced endogenous zinc loss, 3) redistribution of tissue zinc and 4) an efficient maternal-fetal zinc transfer for zinc utilization during pregnancy. A decline in circulating (serum/plasma) zinc concentrations begins early in pregnancy and continues to term. It is associated with complications in the antenatal period such as mild toxemia, vaginitis and post-dates, while the intrapartum period complications include a prolonged latent phase, a protracted active phase and cervical and vaginal lacerations[76,215–219]. The elemental deficiency can also lead to congenital anomalies and hence Swanson and King[256] and Lazebnik et al.[161] suggested the screening of plasma zinc levels in patients so that incidences of dysfunctional labour pattern could be reduced. However, this decline is considered to be to some extent secondary to plasma volume expansion[131,132], plasma protein changes[102] and hormonal effects[112]. Jameson[131] speculated that plasma volume expansion during pregnancy is more important than hormonal effects in decreasing the plasma zinc concentration. Romeu and Arola[224] are of the opinion that the maternal zinc stores are enough to supply the fetus during starvation, despite significant reductions in maternal reserves, whereas Jameson[132] pointed out that some pregnancy-related complications can be reduced by zinc sulphate therapy. Further, when a zinc-deficient woman becomes pregnant and is exposed to the nutritional demands of the fetus and to the influence of progesterone, she is likely to develop the manifestations of cadmium toxicity[53] or toxicity of lead, mercury and certain drugs and alcohols[132]. Sheldon et al.[240] suggested that decrease in concentration of zinc and magnesium is a normal physiological adjustment to pregnancy and that iron supplementation does not influence these changes. On the other hand, Lao et al.[159] suggested combined measurements of plasma and erythrocytic zinc and perhaps carbonic anhydrase concentration for management of complicated pregnancies, and stressed the importance of an adequate daily supply of zinc in pregnancy[85].

Zinc deficiency in early pregnancy, in rats, is also reported to produce abnormal blastocysts[128], increased rates of resorption and a high incidence of congenital malformation and teratogenicity, particularly fetal neural tube defects such as anencephaly[2,9,49,73,124,126,128,135,174,230,250]. Further it is established that mice are much more sensitive to dietary deficiency of zinc than are rats, and that during organogenesis in rats cellular deaths are probably responsible for observed terata, whereas in mice it is necrosis which is probably responsible for dysmorphologies[218].

Pregnant women suffering from acrodermatitis enteropathica (AE), an inborn error of zinc metabolism, exhibit high frequencies of fetal deaths and malformed infants, particularly with neural tube defects[113,126,129,131,250]. The pathogenesis of AE is the result of impaired intestinal zinc absorption, and the patients exhibit low serum lipid and arachidonic acid, increased IgA, and defective prostaglandin synthesis[113,166]. Hambidge[113], and Lonnerdal et al.[166] recognized that AE can be treated by oral zinc supplementation. With zinc supplementation, AE women were able to maintain normal plasma zinc levels and had normal pregnancies and deliveries. Similarly, infants from diabetic females exhibit skeletal abnormalities[127], and immunological defects which persist for at least three generations[12–15].

Zinc is thought to be related to some factors governing the length of gestation, since low zinc levels are reported in both preterm and post term deliveries[5,6], whereas in zinc-deficient rats, delayed and prolonged deliveries are accompanied by excessive bleeding, and the neonates and placenta are ignored[167].

Zinc is known to be involved in the normal response to estrogen instructions. The successful transition from gestation to labour and delivery requires a carefully regulated and harmonized series of events. It requires the removal of progesterone and the achievement of estrogen dominance[164]. The rapid formation and appearance of gap junctions are believed to be a significant factor in the initiation and progress of parturition. One possible relationship between decreased zinc levels and prolonged gestation may be with the hormone-dependent formation of myometrial gap junctions. The appearance of gap junctions at term has been linked to the withdrawal of the inhibitory effect of progesterone and the increase in the stimulting impact of estrogen[167]. Lytton and Bunce[167] observed that the uterine pressure cycle pattern was abnormal in zinc-deficient pregnant rats during oxytocin-induced labor. Both contractile synchrony and propagation appeared to be diminished and, in particular, the birth of individual pups was prolonged and accompanied by intense abdominal straining. The number and size of myometrial gap junctions increase enormously during the last 48 h before delivery, as the uterus comes under estrogen dominance[97,98]. However, in zinc-deficient rats, the number of gap junctions detected were 49 or 39% of those in controls fed (ad libitum). It has been emphasized that this may contribute to the irregular and poorly synchronized uterine pressure cycle patterns and may indicate poor compliance of estrogen-controlled gene expression[77].

Prostaglandins are involved in the regulation of numerous physiological processes such as body temperature, blood pressure, platelet aggregation and parturition[194]. Luteolysis and induction of labour are mediated by

$PGF_{2\alpha}$ near term[163]. Exogenous administration on day 20 of gestation causes premature delivery on day 21 in female rats[254]. There is an increase in the number of binding sites in the ovarian membrane of zinc-deficient pregnant rats[80]. This may be because of alterations in membrane composition which in turn may change receptor function[43]. Zinc deficiency causes alterations in fatty acids and phospholipids[51,61] and enhances lipid peroxidation[23,255]. It is possible that membrane structural changes associated with zinc deficiency interfere with $PGF_{2\alpha}$ receptor-mediated phase changes and consequently the luteolytic process. Thus the higher number of binding sites may represent non-functional receptors leading to longer gestation period.

Maternal zinc is related to low birth weights. Women given supplementary zinc showed a lower frequency of pregnancy complications and of low-birth-weight babies than women without the supplement[157,181,244]. Birth weight, crown-rump and femur length of male newborns are reported to be reduced in male newborns as compared to female newborns from zinc-deficient rhesus monkey[126]. Other abnormalities of newborn monkeys of low birth weights included osteoporotic effects and a wide epiphysis[127].

The process of lactation is nutrient-demanding, and therefore nutritional requirements of lactating mothers are usually very high. In lactation, the maternal body probably has an enhanced and efficient mechanism for the absorption of trace elements and their utilization in milk synthesis. The adequacy of lactation is indexed by the growth of the infant, and the occurrence of low plasma trace-element levels during lactation tends to suggest that milk biosynthesis has priority in the distribution of trace elements. Zinc requirements are relatively high in very young infants and decrease with growth. The marked decrease in human milk zinc concentration, as lactation progresses, thus does not inevitably result in suboptimal zinc intake. Rather, this pattern may represent a control mechanism for zinc secretion by the mammary gland, which meets the infant's need without imposing an unnecessary burden on maternal zinc status[46,59,149]. On the other hand, Donangelo[72] observed slightly lower total zinc concentrations and a lower in vitro availability of serum zinc in mothers nursing newborns which did not maintain adequate weights in the first months of life, and concluded that the zinc status of the mother can affect the adequacy of zinc supply and limit the rate of growth of exclusively breast-fed infants.

Copper deficiency in female reproduction; pregnancy and infant abnormalities

Copper-deficient female rats are protected against mortality resulting from copper deficiency[90]. It has been suggested that this protection is provided by the presence of endogenous estrogens, as estrogens have been shown to alter the subcellular distribution of copper in liver, and increase plasma copper levels by inducing the synthesis of ceruloplasmin[45,226,233]. However, even ovariectomized females are protected against the severity of copper deficiency. Although in ovariectomized females total plasma estrogen is reduced by 48%, this has no effect on the symptoms of copper deficiency. Thus, whether the females are ovariectomized or intact, they are not susceptible to severe copper deficiency. Estrogens do increase ceruloplasmin levels in females, but in copper deficiency the incorporation of copper into ceruloplasmin is inhibited, and possibly the levels of estrogen in intact prepubertal females are too low to stimulate ceruloplasmin activity[45,89]. Increases in the serum copper and ceruloplasmin levels of female rats and other animals with estrogen treatment are clear, and depend upon the duration of hormonal treatment[56,60,83,92,117,182,183,274,290]. The increases occurred at the expense of hepatic copper and were not due to increased intestinal absorption, so prolonged estrogen treatment leads to an alteration in the distribution of copper. Copper is also known to affect the level of norepinephrine and dopamine in brain by synthesis and/or release of neurotransmitters[212]. Thus, elevated copper levels in the brains of females taking oral contraceptives might alter brain amine levels, thereby causing physiological and behavioral changes, as observed in a number of cases[87]. That copper requirements may be sex-dependent is further revealed by studies in men and postmenopausal women. Males have abnormally high levels of circulating triglycerides and fatty acid if fed on a fructose-based copper-deficient diet, but this response is absent in females of reproductive age[90,168,223].

The growth of the fetus imposes a severe drain on the essential metal homeostasis of the maternal organisms. The demands of the conceptus for nutrients grow enormously towards the completion of its development at the end of gestation[111]. Copper is not significantly withdrawn from maternal storage tissues such as liver during pregnancy, despite the fact that important secretions of this metal in fetal organs are supplied from maternal stores. The transient increase in plasma copper level observed during mid-pregnancy of rats is caused by increased food consumption and increased intestinal absorption of copper, and not directly by pregnancy, as the needs of fetus are low at this stage[55,74,125,173]. This results in increased copper deposition in the maternal tissues that is later on used for fetal and postnatal development[284].

Infants completely dependent upon parenteral nutrition, without supplementation of copper, develop hypochromic normocytic anemia, neutropenia and skeletal abnormalities in association with profound hypocupremia[140], and these abnormalities responded well to oral copper supplementation[242]. Hypomyelination is also reported in pups of copper-deficient rat dams[211].

Menke's disease, a human X-chromosome linked disorder characterized by mental retardation and peculiar hair, is related to abnormal copper metabolism. The patients have low liver copper and plasma ceruloplasmin levels. The disorder is a result of a mutation on the X-chromosome close to band q-13[65,122]. This mutation is expressed in all cells, resulting in altered copper homeostasis. Low levels of copper induce metallothionein in some organs and make copper unavailable for other cuproenzymes. The overproduction in Menke's cells is likely to be due to the deficiency or absence of a copper regulatory factor. This putative factor would normally fix copper in the liver as it enters from the portal blood en route to ceruloplasmin synthesis. Changes in this disease are similar to those observed in nutritional copper deficiency[64]. Abnormal cells prevent copper from reaching functional sites and children die in most cases before the age of three years, mainly from bronchopneumonia.

Wilson's disease is an autosomal recessive disorder in which copper accumulates in liver and secondarily in other organs. Serum ceruloplasmin levels are usually below normal. The molecular basis of this disease is not known. An elevated liver copper level, urinary copper excretion and low ceruloplasmin levels are good indicators of the disease[235].

Other human mutations leading to alterations in copper metabolism are 1) albinism (congenital loss of activity of tyrosinase, a cuproenzyme); 2) Down's syndrome (overproduction of Cu-Zn SoDM[27]); 3) cytochrome-c-oxidase deficiency[284] and 4) Cutis-laxa (X-linked, defects in cross linking of collagen due to decreased lysyl oxidase activity)[36].

Various mutations related to copper metabolism have also been reported in mice. An X-chromosome linked disorder analogous to Menke's disease of human beings is reported in mice, and the pups die when two weeks old unless copper is administered at day 7 of postnatal life[123]. Similarly an analogy to the Cutis laxa mutation of humans is also found in mice. Other mutants are dappled, tortoise-shell, viable brindled, and toxic milk. Toxic milk mutants accumulate copper in the liver and produce milk deficient in copper, and the milk is therefore toxic to suckling pups[214].

Selenium deficiency, female reproduction, pregnancy and lactation

Selenium deficiency in females results in infertility, abortion and retained placenta, and the newborns from selenium-deficient mothers suffer from muscular weakness[3,120,234,237,258,281]. Neither the age of the mother, her parity, smoking, iron supplementation, toxemia of pregnancy, abortive ovum or early uterine contractions are reported to have any effect on selenium concentration during pregnancy, nor does the concentration of selenium during pregnancy have any effect on the weight of the baby or the length of pregnancy[4,33]. However, the selenium requirement of pregnant and lactating mothers is increased as a result of selenium transport to the fetus via the placenta and to the infant via the breast milk[121,152,248]. The level of selenium in human milk is strongly affected by maternal intake and status[151,152]. The level of the element in cow's milk is one half of that found in human milk of unsupplemented women living in the same area[248]. That is the reason why Keshan's disease, an endemic cardiomyopathy, affects predominantly young children and women of child-bearing age in selenium-deficient areas like Heilongjiang province of the Peoples Republic of China[121,151,152,248]. Colostrum and transitional milk have a higher selenium concentration than mature milk[248]. Most of the selenium in human milk is protein-bound, and at least some selenoproteins have been detected in dialyzed milk samples following molecular sieve chromatography[188].

Selenium requirements of infants and young children are high due to their rapid growth. The erythrocyte and plasma GSH-Px activities, as well as serum selenium concentration, are lower in newborns than in their mothers and other adults, and newborns are therefore at risk of becoming severely selenium-deficient if maintained on a commercial infusion solution with low selenium. Hence, a continuous monitoring of selenium status and supplementation, if necessary, has been recommended[152,180,275]. Studies of TPN infants given supplements such as sodium selenite and L (+) selenomethionine revealed that selenite-Se rapidly normalizes plasma GSH-Px but is not retained as well as is selenomethionine-Se[275]. The selenium content of mature human milk ranges between 10–20 µg/litre, and considering an average milk requirement of at least 750 ml/day at 3 months of age, an exclusively breast-fed infant would get approximately 8–15 µg selenium per day. Since the present 'safe and adequate' intake range for the 0–6 month-old infant ranges between 10 and 40 µg/day, it seems likely that the intake of certain infant populations, for example those from selenium-poor areas e.g. in China and New Zealand, will be below the recommended limits especially if they are exclusively breast fed[150]. Selenium levels of human milk can effectively be increased by supplements of 100 µg selenium/day as yeast selenium whereas maternal supplementation with 100 µg/selenium/day as selenite was less effective in increasing milk selenium concentration[150]. Kumpulainen[150] suggested the supplementation of infant formulas to provide 10 µg selenium/day, or supplementation of fertilizers with selenite (which was shown in experiments in Finland to lead to a 10-fold increase in selenium contents), to ensure an adequate supply of selenium to lactating mothers and their infants.

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