

Micronutrient programming of development throughout gestation

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Vitamins and minerals serve essential roles in cellular metabolism, maintenance and growth throughout life. They are also central components of many enzymes and transcription factors. However, the need for optimum amounts of key micronutrients at critical stages during the periovulatory period and subsequent embryonic and fetal life has become the focus of sustained research activity only recently. In addition to folic acid, the minerals zinc, iron and copper and the antioxidant vitamins A and E are of particular importance during pregnancy. Both excesses and deficiencies of these micronutrients can have profound and sometimes persistent effects on many fetal tissues and organs in the absence of clinical signs of deficiency in the mother. The consequences of micronutrient imbalance on the developing conceptus may not be apparent at the time of the nutritional insult, but may be manifest later in development. However, supplementary micronutrients provided later in gestation or during postnatal life cannot completely reverse the detrimental effects of earlier micronutrient imbalance. Importantly, deficiency of a specific micronutrient, such as zinc, during pregnancy can result in a greater incidence of fetal malformation and resorptions than general undernutrition. Given the range of micronutrients that affect development, the number of developmental stages susceptible to inappropriate micronutrient status and the diverse biochemical systems and types of tissue affected, it is challenging to propose a unifying hypothesis that could explain the effects of micronutrient imbalance on programming throughout gestation. Micronutrient imbalance can affect pregnancy outcome through alterations in maternal and conceptus metabolism, as a consequence of their essential role in enzymes and transcription factors and through their involvement in signal transduction pathways that regulate development. Micronutrient-induced disturbances in the balance between the generation of free oxygen radicals and the production of antioxidants that scavenge free radicals may provide an additional mechanistic explanation. The detrimental effects of many micronutrient deficiencies, particularly zinc and copper, can be alleviated by supplementary antioxidants, whereas deficiencies of antioxidant vitamins A and E are likely to reduce defence against free radical damage.

Although the effects of macronutrients on the outcome of pregnancy have been the topic of many studies, the consequences of alterations in micronutrient (vitamin and mineral) supply on pre- and postnatal development are poorly understood. The dietary requirement for micronutrients during development is small; however, adequate amounts are essential for both the immediate and long-term well-being of the embryo, fetus and neonate. Micronutrients are involved in all stages of cell growth and differentiation, including cell signalling and protein translation, and are key elements of many enzymes and cell structures. Minerals are

important as central components of catalytic enzyme sites and as stabilizing factors in enzymes and transcription factors. In addition to their crucial roles in cellular metabolism, maintenance and growth, many vitamins also regulate the expression of key developmental genes involved in the commitment of cells to specific lineages and pattern formation, and of genes involved in growth, proliferation and in the functional attributes of specific organs. Micronutrients also affect development by modifying changes in hormones, growth factors and cell signalling pathways that affect both nutrient uptake by the conceptus and the environment in which prenatal development proceeds. Severe micronutrient deficiencies during pregnancy lead to high rates of spontaneous abortion, fetal malformation and late fetal death. However, more moderate reductions, which occur in clinical and agricultural situations, also compromise pregnancy

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outcome. Research prompted by concern over the consequences of increased environmental concentrations of certain metals on reproductive outcome has highlighted that modest increases in several essential minerals can have detrimental effects on embryo development (see Hanna *et al.*, 1997). Culture of two-cell mouse embryos in media containing various concentrations (0.05–200.0 $\mu\text{mol l}^{-1}$) of minerals, including copper, manganese and zinc, for 72 h resulted in a reduction both in the number of embryos that formed blastocysts and in embryo cell number. Hanna *et al.* (1997) proposed that high concentrations of metals in embryo culture media may compromise embryo development as a consequence of oxidative damage, occurring through either direct oxidative reactions or after activation of reactive oxygen species through secondary mechanisms.

The effect of altered micronutrient intake on maternal function and subsequent development is complicated further by the numerous interactions that occur between micronutrients. For example, copper deficiency results in the accumulation of iron in certain tissues (Keen *et al.*, 1998), lead-induced pathological changes in fetal kidney development are more pronounced in conditions of iron deficiency (Singh *et al.*, 1991), and zinc deficiency can alter vitamin A (retinol) metabolism (Christian and West, 1998).

Different organs grow, differentiate and acquire functional competence at different stages of pregnancy. Their development is more sensitive to perturbations in nutrient supply during these 'critical windows' of differentiation (Robinson *et al.*, 2000). In some situations, inappropriate nutrient supply during critical stages of pregnancy irreversibly programmes subsequent development.

Primary micronutrient deficiency arises through inadequate dietary intake and, during prenatal life, from inadequate placental nutrient transfer. Genetic factors can also influence the severity of micronutrient deficiency. For example, the incidence of swayback, a recognized symptom of copper deficiency, is higher in lambs born to Blackface ewes than in lambs born to Welsh mountain ewes grazing the same low-copper pasture (Weiner *et al.*, 1978). Although these breed differences are believed to be the result of multiple genes, single gene defects are also implicated in copper deficiency. Menkes syndrome, which is characterized by progressive degeneration of the infant brain and spinal cord and failure to thrive, is thought to be due, in part, to maternal defects in a copper-transporting ATPase gene (Mercer *et al.*, 1993). Certain diseases and therapeutic or recreational drugs also alter micronutrient status through altered uptake or metabolism. Several drugs chelate micronutrients, thereby reducing circulating concentrations, whereas plasma concentrations of antioxidant vitamins are lower in smokers compared with non-smokers with similar dietary habits. Maternal alcohol consumption during pregnancy alters retinol metabolism, as shown by increased concentrations of retinol in the fetal lung and kidney after maternal ethanol ingestion (Grummer and Zachman, 1990). Micronutrient deficiencies occurring before mating or during pregnancy can perturb the

development of the preovulatory oocyte (Whaley *et al.*, 2000), embryo (Olson and Seidel, 2000), fetoplacental unit (Antipatis *et al.*, 2000) or offspring (Fisher and MacPherson, 1991) in the absence of obvious clinical signs of deficiency in the mother.

The consequences of specific micronutrient deficiencies can be more extreme and longer lasting than those occurring after general undernutrition. For example, feeding rats *ad libitum* from mating with a zinc-deficient diet increased both the number of malformations per fetus and the number of resorptions per litter compared with animals that received a control diet (Masters *et al.*, 1983).

Numerous micronutrients affect pregnancy outcome. The micronutrients that appear to have the greatest impact include the minerals zinc, copper and iron and the antioxidant vitamins A and E. Many micronutrients are stored within the maternal body, and plasma concentrations decline only when such stores are depleted. For example, plasma retinol decreases only when hepatic stores are depleted and, therefore, a short-term deficiency in vitamin A during pregnancy is unlikely to have a major impact. In contrast, evidence that zinc deficiency during only a short period of pregnancy is teratogenic indicates that there is limited mobilization of zinc stores during periods of decreased supply.

Both copper and iron may elicit changes in prenatal development through the generation of free radicals as they undergo valency changes, whereas exposure of embryos to zinc-deficient conditions stimulates the upregulation of antioxidant enzymes. This review explores the possibility that altered antioxidant status is a unifying mechanism through which several micronutrients affect pregnancy outcome. The effects of these micronutrients at key stages of periovulatory and pre- and postnatal development will be considered. As the medical impact of altered micronutrient status on prenatal growth and pregnancy outcome has been the topic of a recent review (McArdle and Ashworth, 1999), this article will focus on current published studies that describe data obtained from animal studies.

Oocyte maturation

Knowledge of the role of micronutrients in oocyte and early embryonic development is based on data obtained from alterations to the maternal diet *in vivo* or after injection of micronutrients, coupled with data describing the effects of altered micronutrient content of oocyte or embryo culture media. Oocyte maturation involves both the synthesis of cytoplasmic components (cytoplasmic maturation) and a rearrangement and reduction in the chromosomes (nuclear maturation). Meiosis begins early in fetal life but is arrested and remains at the diplotene stage until the oocyte either degenerates during atresia or resumes meiosis just before ovulation in the mature female. Essential to the successful maturation of oocytes is the breakdown of the germinal vesicle surrounding the cumulus–oocyte complex. Recent data obtained after culture of rat cumulus–oocyte

Table 1. Micronutrients that are important during the pre- or postimplantation stages of embryonic development

Stage of development	Micronutrient	
	Minerals	Vitamins
Preimplantation	Fe, Cu, Zn, Cd, Ca, Ni, Pb, Co	A, E, B12, choline, folic acid, pantothenate, riboflavin, inositol
Postimplantation	Cu, Cd, Zn, Se, Ca	A, E, B12, folic acid, pantothenate, riboflavin, inositol

Both excesses and deficiencies of micronutrients affect development.

complexes with a range of antioxidants indicate that cell-permeant antioxidants inhibit germinal vesicle breakdown, thereby reducing the incidence of spontaneous resumption of meiosis (Takami *et al.*, 1999). However, not all antioxidants exhibited this effect, and ascorbic acid and vitamin E were notable exceptions. In pigs, a single injection of retinyl palmitate approximately 6 days before mating increased the percentage of oocytes that progressed to metaphase II *in vitro* and promoted follicular homogeneity (Whaley *et al.*, 2000). These observations extend earlier work from this group (Whaley *et al.*, 1997) showing that a similar retinol injection regimen before mating was associated with increased pig embryo survival and reduced within litter variability in embryo development on day 12 of pregnancy. Collectively, these data indicate that nutritional modifications that enhance embryo survival may do so by affecting oocyte maturation.

Embryo development

Accumulating evidence indicates that altered nutrient supply during early embryonic development can impart long-term consequences on the subsequent viability of the conceptus and resultant offspring. Studies *in vivo* and *in vitro* have highlighted that the micronutrient environment in which embryos develop can alter the number of embryonic cells, the rate of blastocyst development and the commitment of cells to specific lineages, and can disrupt the balance between cell proliferation and programmed cell death (apoptosis). A summary of the primary micronutrients that affect embryo development is presented (Table 1).

Early mammalian embryos are susceptible to damage from reactive oxygen species (Johnson and Nasr-Esfahani, 1994) as shown by the fact that development of bovine embryos to blastocysts *in vitro* decreases in the presence of free radicals in a dose-dependent manner (Fujitani *et al.*, 1997). Culture conditions *in vitro* appear to increase embryonic production of oxygen free radicals (Goto *et al.*, 1993). Protection of embryonic cells from free radical damage by supplementing culture media with vitamin E for 7 days immediately after fertilization increased the number of bovine zygotes that developed to expanded blastocysts *in vitro* (Olson and Seidel, 2000). Furthermore, embryos cultured in the presence of vitamin E for 5.5 days between

fertilization and transfer developed into larger blastocysts than did embryos cultured in control media, when assessed on day 12. In other studies, vitamin E partially protected early murine embryos from the effects of heat shock (Arechiga *et al.*, 1994), a cytotoxic effect thought to be mediated by free radicals. Similarly, survival and development of rat conceptuses explanted on day 8 of gestation was improved by culture with vitamin E (Steele *et al.*, 1974).

The best known example of the importance of adequate maternal micronutrient intake is the requirement for adequate periconceptual intake of folic acid to reduce the incidence of neural tube defects. However, studies of folate-related enzymes have failed to identify the metabolic defect in the neurulation stage embryo that is corrected by folic acid. Folic acid, together with vitamin B12, is an important methyl donor in many reactions, including the production of thymidine for DNA synthesis, polyamine synthesis and the biosynthesis of methionine from homocysteine. Given that homocysteine increases in folate depletion, high concentrations of this amino acid have been implicated as a possible factor associated with neural tube defects. In support of this hypothesis, treatment of avian embryos with homocysteine caused dysmorphogenesis of the heart and neural tube (Rosenquist *et al.*, 1996); these defects were prevented by folate supplementation.

If sufficient cobalt is available, ruminant species do not require an external source of vitamin B12 for peptide synthesis. Cobalt deficiency during early ovine pregnancy is known to reduce lamb vigour at birth (Fisher and MacPherson, 1991); however, the cobalt-sensitive stages during embryo development have yet to be identified. Studies of the direct effects of cobalt on embryogenesis are confined to those on mouse embryos *in vitro*. The addition of 100 $\mu\text{mol cobalt l}^{-1}$ to the culture media reduced trophoblast proliferation in expanded murine blastocysts (Paksy *et al.*, 1999). In addition to cobalt, small excesses of several metals in embryo culture media affect embryo development detrimentally (see Hanna *et al.*, 1997). The tolerance of mouse embryos to excess nickel ions appears to increase as development progresses. Storeng and Jonsen (1980) reported that 10 $\mu\text{mol nickel l}^{-1}$ had an adverse effect on the morphology of two-cell embryos, whereas concentrations of 250–300 $\mu\text{mol l}^{-1}$ were required to compromise

the development of eight-cell embryos and blastocysts (Storeng and Jonsen, 1980; Paksy *et al.*, 1999). Small excesses of cadmium, nickel and cobalt reduced trophoblast area in mouse trophoblasts (Paksy *et al.*, 1999), although the minimum effective concentrations varied depending on the metal. The morphological alterations and loss of cellular contacts observed in the blastocysts would be expected to influence adhesion and recognition events adversely. Current hypotheses indicate that metal exposure is associated with increased intracellular reactive oxygen species and associated oxidative damage that affects embryo development adversely. However, at similar molar concentrations, redox active metals were found to be less embryotoxic than non-redox active metals (Hanna *et al.*, 1997).

Embryos recovered from mice fed a zinc-deficient diet for a 3 or 6 day period encompassing oocyte maturation and fertilization had fewer cells and delayed blastocyst development *in vitro* (Peters *et al.*, 1991). In preimplantation mouse embryos recovered from dams fed a zinc-deficient diet for 3 days from the day before mating, zinc supplementation of the culture media did not improve blastocyst development (Peters *et al.*, 1991). These data indicate that the effects of periconceptual zinc deficiency cannot be overcome by subsequent zinc supplementation. The mechanism by which zinc deficiency impairs embryo development is unclear. Zinc deficiency does not appear to affect the function of oocyte and embryonic membranes (Peters *et al.*, 1993) or embryonic cell cycle characteristics (Rogers *et al.*, 1995); however, recent data indicate that zinc deficiency induces apoptosis of embryonic cells in rats (Jankowski-Hennig *et al.*, 2000). One possible mechanism underlying zinc deficiency-induced tissue alterations is excessive cellular oxidative damage after changes in free radical defence mechanisms. In a study using 3T3 cells, Oteiza *et al.* (2000) demonstrated that exposure to zinc-deficient conditions triggers intracellular responses associated with oxidative stress.

Evidence for the importance of copper for prenatal development arose from studies of enzootic ataxia (swayback) in lambs. This disease, which also occurs in pigs, goats and guinea-pigs, is characterized by spastic paralysis, severe lack of co-ordination and anaemia. In a series of studies Bennets *et al.* (1948) showed that the frequency and severity of the disease could be reduced markedly when cows were given supplementary copper either before or during early pregnancy. More recent studies involving culture *in vitro* of preimplantation murine embryos collected from dams that received a copper-deficient diet for 2 months before mating, showed that maternal copper deficiency reduced the number of blastocysts able to hatch from the zona pellucida (Menino *et al.*, 1986). Rat embryos recovered from dams fed a copper-deficient diet for 4 weeks before mating were characterized at day 11 by an oedematous hindbrain (Jankowski *et al.*, 1995). These effects were exacerbated by culture in copper-deficient media (Hawk *et al.*, 1998). Rat

embryos cultured in copper-deficient medium have impaired hindbrain and cardiac development, coupled with reduced antioxidant enzyme activity compared with those cultured in complete media (Hawk *et al.*, 1998). In contrast to zinc deficiency, copper deficiency does not appear to promote apoptosis. Supplementation of copper-deficient media with antioxidants reduced the teratogenic effect, again implicating antioxidant status as a key factor in mediating the effects of micronutrients on embryo development.

It is well known that small amounts of copper from intrauterine contraceptive devices can prevent embryogenesis by blocking implantation and blastocyst development. Small excesses (50–100 $\mu\text{mol l}^{-1}$) of copper in media used to culture mouse preimplantation embryos are also embryotoxic (Hanna *et al.*, 1997), although the underlying mechanisms for the toxicity are unclear.

Retinoic acid, the most biologically active form of retinol, is an important regulator of cell division and differentiation in embryonic tissues. The effects of vitamin A deficiency and excess on embryo development are remarkably similar, indicating that embryonic cells must carefully regulate endogenous retinoic acid concentrations. In the nucleus, retinoic acid acts as a ligand to activate two families of transcription factors, the RA receptors (RAR) and the retinoid X receptors (RXR), which heterodimerize and bind to upstream responsive sequences of RA-responsive genes. To date, gene knockout studies have failed to identify the distinct roles of each RAR and RXR receptor subtype in embryogenesis, although fetuses carried by RXR α null mutant mice have ocular and cardiac malformations and die from cardiac failure during mid-gestation, indicating that this receptor plays a vital role in heart development (Kastner *et al.*, 1994).

Retinoic acid is believed to confer positional orientation during development. It is involved in the directional differentiation of the nervous system and is released by the 'zone of polarizing activity', which assigns positional value to limb bud cells. Studies using retinoid-synthesizing enzymes and their knockouts have revealed precise spacial distribution patterns of these enzymes in the early mouse embryo (reviewed by Maden, 2000). The expression of retinol dehydrogenases begins at the primitive streak stage, in which it is predominant in posterior tissues and the mesoderm. Retinoic acid also appears to be required for the left–right asymmetry of visceral organs during development. Addition of a retinoic acid receptor agonist to cultured headfold stage mouse embryos inhibits expression of genes that are normally expressed on the left side of the embryo. This treatment also leads to defects in cardiac antero-posterior patterning (Chazaud *et al.*, 1999).

In domestic animals, retinol supplementation before mating has beneficial effects on embryo development. In superovulated ewes, injection of all-*trans* retinol increased the number of embryos that formed blastocysts *in vitro* (Eberhardt *et al.*, 1999), whereas retinol injection of gilts before mating increased embryo survival and reduced intra-

Table 2. Micronutrients that play a role in the development or function of fetal and neonatal organs

Organ	Micronutrient	
	Minerals	Vitamins
Liver	Fe, Se, Cu, Zn, Cd	A, B12, choline, folic acid
Heart	Cu, Zn	A, D
Kidney	Fe, Cd, Zn, Pb	A
Brain	Fe, Cu, Zn, I	A, B6, B12, folic acid, biotin
Lung	Cu, Zn, Cd	A
Bone	Ca, Mg	D, E, C

Both excesses and deficiencies of micronutrients affect development.

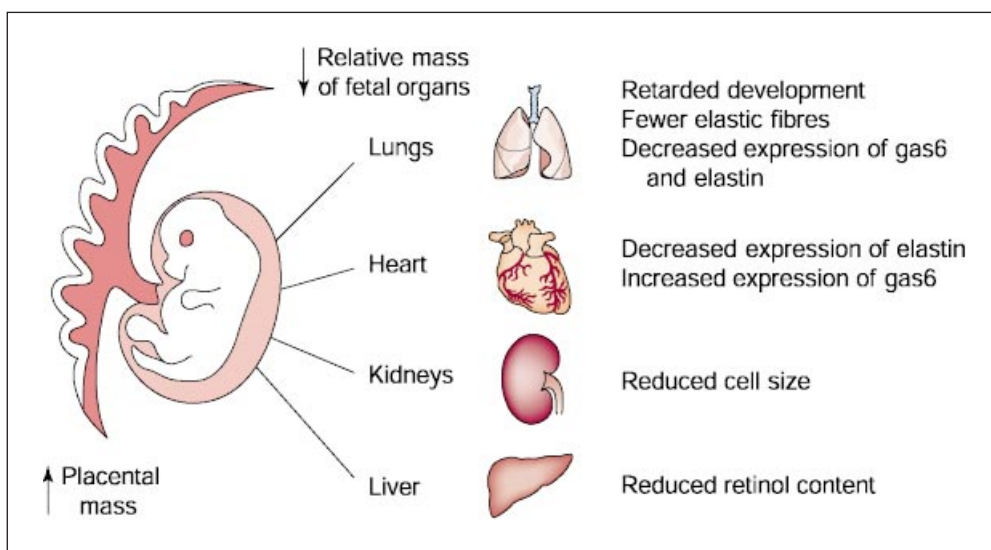


Fig. 1. Schematic representation of the effects of a 50% reduction in maternal plasma retinol concentrations on late fetal development in rats.

litter variability in blastocyst development on day 12 (Whaley *et al.*, 1997). However, these studies did not distinguish whether the primary effect was an enhancement of oocyte quality or an increase in embryo development *per se*.

Culture of hamster blastocysts in medium containing 11 water-soluble vitamins and growth factors or in medium lacking one of these micronutrients indicated that the omission of only pantothenate, choline or inositol reduced the number of blastocysts that hatched (Kane and Bavister, 1988). More recently, McKiernan and Bavister (2000) demonstrated that the addition of pantothenate to culture media increased the size of hamster blastocysts. The percentage of live fetuses recovered after transfer of embryos cultured in supplementary pantothenate was greater than that after culture in control media.

Effects on fetal development

In addition to gross effects on fetal growth and survival, micronutrient deficiencies during fetal life are known to

affect relative organ growth and can have profound and sometimes persistent effects on the molecular, cellular, immunological and morphological development of a range of fetal and neonatal tissues (summarized in Table 2).

Experimental reductions in maternal vitamin A deficiency during pregnancy are often achieved after consumption of a diet inadequate in vitamin A for several weeks before mating. In rats, consumption of a vitamin A-free diet for 7 weeks before mating and throughout pregnancy results in a 50% reduction in maternal plasma vitamin A concentrations during late pregnancy (Antipatis *et al.*, 2000). In both rats (Antipatis *et al.*, 2000) and pigs (Ashworth and Antipatis, 2001), such moderate maternal vitamin A deficiency is associated with asymmetrical fetal organ growth and reductions in the relative masses of fetal lungs, heart and liver during late pregnancy. In addition, studies in rats have shown that functional characteristics of the developing lung, kidney, heart and nervous system are also affected by maternal vitamin A status (Antipatis *et al.*, 2000; Fig. 1). The developing lung is particularly sensitive

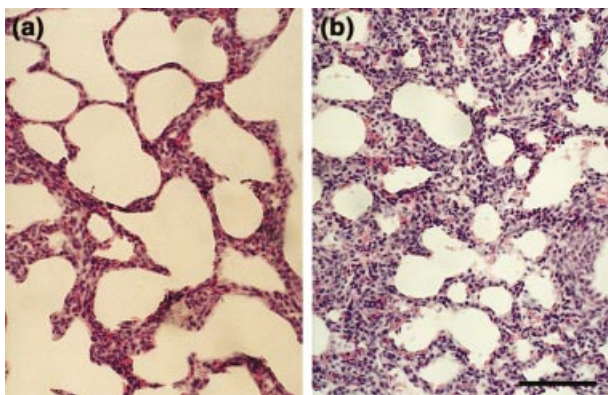


Fig. 2. Histological analysis of neonatal lung development in rats. (a) Typical lung morphology in a neonate carried by a control-fed animal. Saccular development is well advanced. (b) Typical lung morphology in a neonate carried by a vitamin A-deficient mother. Sacculi are more infrequent and less advanced compared with those in the neonate carried by the control animal. Scale bars represent 50 μm .

to changes in maternal vitamin A supply. The lungs of fetuses and neonates carried by vitamin A-deficient mothers are relatively lighter and morphologically immature, showing less branching of the bronchial passageways and reduced staining for elastic fibres compared with those from fetuses and neonates carried by control-fed dams (Fig. 2; Antipatis *et al.*, 1998). In a subsequent experiment, dietary repletion with vitamin A from day 7 of pregnancy was used to restore maternal plasma retinol concentrations fully in rats fed a vitamin A-free diet for the preceding 9 weeks. Dietary repletion restored relative fetal and neonatal lung masses to values typical of fetuses and neonates carried by control-fed dams (Antipatis and Ashworth, 1999), indicating that some effects of maternal vitamin A deficiency are reversible. Furthermore, expression of the key structural and developmental genes, elastin and growth arrest specific gene 6 (Gas6) in lungs from vitamin A-deficient fetuses is decreased and these effects persist into perinatal life. Elastin synthesis is essential for the maturation of the alveoli, and the number and size of alveoli in turn determine the total lung capacity of the neonate. Other studies have shown that rat neonates born to vitamin A-deficient dams have reduced lung surfactant phospholipids accompanied by reduced expression of the gene for fatty acid synthetase, a key enzyme in the synthetic pathway for surfactant phospholipid precursors (Chailley-Heu *et al.*, 1999). Moderate maternal vitamin A deficiency also affects the maturation of the fetal and neonatal kidney. Maternal retinol concentrations are correlated with the number of nephrons in the fetal rat kidney at day 21 (Lelievre-Pegorier *et al.*, 1998) and modulate nephron endowment at birth. Although relative fetal and neonatal brain masses are unaffected by maternal vitamin A deficiency in rats (Antipatis *et al.*, 2000), deficiency can lead to shortening of the caudal hindbrain

and to perturbations associated with abnormal patterning of the posterior hindbrain and expansion of the anterior portion of the hindbrain in this species (White *et al.*, 2000).

The beneficial effects of folate are not confined to events in early pregnancy. Marginal concentrations of maternal folate throughout gestation impair cellular growth and replication; women with low folate concentrations at week 28 of gestation have an approximately twofold greater risk of preterm delivery and low infant birth weight (Scholl *et al.*, 1996). In pigs, folic acid injection at weaning, mating and during the first 12 weeks of gestation improves the number of live piglets at birth (Matte *et al.*, 1984). The potential for folic acid supplementation to be more effective in pregnancies carrying greater numbers of embryos is consistent with the contention that folic acid may act by supporting DNA synthesis at critical stages of embryo development (Lindemann, 1993). In addition, folic acid treatment is associated with increased fetal protein content (Trembley *et al.*, 1989; Harper *et al.*, 1996).

Zinc deficiency is teratogenic in all species in which it has been examined. However, the severity of the perturbation of embryo development is not necessarily directly related to the degree of dietary deficiency. In an elegant experiment performed by Masters *et al.* (1983), fetal development was compared in rats offered zinc-sufficient or zinc-deficient diets *ad libitum*, or reduced quantities of the zinc-deficient diet. Consumption of the restricted amounts of the deficient diet led to tissue catabolism of zinc and resulted in fewer malformations and resorptions than seen in litters from dams fed the zinc-deficient diet *ad libitum*. Feeding rats a zinc-deficient diet from day 1 or day 3 of pregnancy led either to developmental retardation or to extensive apoptosis in the visceral arches, neural tube and somites by day 11 (Rogers *et al.*, 1995). Removal of dietary zinc for as little as 4 days during week 2 of pregnancy increased apoptosis in neural crest cells. One of the possible mechanisms proposed to explain the effects of zinc deficiency on brain development is an impairment in the formation of the cytoskeletal network.

An inadequate fetal supply of selenium and vitamin E during pregnancy is a contributory cause of fetal nutritive muscular dystrophy in calves, lambs and foals. Inadequate placental transport or metabolism of these nutrients has been implicated in addition to inadequate maternal intake (Bostedt and Schramel, 1990). However, more recent studies investigating selenium deficiency over six generations of rats indicate that there are regulatory mechanisms to conserve selenium in critical tissues (Bates *et al.*, 2000). Evidence for such mechanisms was provided by observations that most tissues maintained concentrations of the selenium-rich iodothyronine deiodinase enzymes, and hence thyroid hormone production.

Effects on placental development and function

Micronutrient deficiencies can also alter placental development and the expression of key signalling molecules that are

involved in establishing the balance between growth promotion and regulation at the fetal–placental interface. For example, vitamin A deficiency in rats increases the ratio of placental:fetal mass during late pregnancy (Antipatis *et al.*, 2000); rats deficient in iron or vitamin A show increased placental expression of tumour necrosis factor α (TNF- α) and leptin (Lea *et al.*, 2000).

Mouse fetuses with a targeted disruption of the RXR α gene develop placental defects localized predominantly in the labyrinthine zone of the chorioallantoic placenta (Sapin *et al.*, 1997). Reduction in maternal retinol status as a consequence of controlled periods of dietary withdrawal also alters placental function. Placentae from vitamin A-deficient rats show a marked infiltrate of neutrophils that are immunopositive for TNF- α and leptin. The number of apoptotic trophoblast cells increases in the tissue surrounding the neutrophils. Maternal vitamin A deficiency also alters the balance in apoptotic regulatory genes in the placenta. Trophoblast giant cell bax (pro-apoptosis) immunoreactivity was reduced as a consequence of vitamin A deficiency, whereas bcl-2 (anti-apoptosis) remained unchanged (Antipatis *et al.*, 1997). These data indicate that the aberrant effects of maternal vitamin A deficiency on the fetal–placental unit may be associated with abnormal placental apoptosis induced by high concentrations of TNF- α produced by infiltrating neutrophils or a change in the ratio of bcl-2:bax in the trophoblast giant cells.

Maternal iron deficiency does not appear to elicit morphological changes in the rat placenta; however, TNF- α and leptin were increased significantly in the spongio-trophoblast/giant cell region of the placenta (Lea *et al.*, 2000). These results indicate that different mechanisms underlie the effects of micronutrients on cytokine production at the maternal–fetal interface.

Effects on neonatal development

Micronutrient deficiencies during pregnancy are often reflected in retarded neonatal organ maturation or in reduced neonatal vigour or cognitive development. Nutrition *in utero* can influence the ontogeny of the immune system and an individual's subsequent immunocompetence. For example, marginal zinc deficiency during pregnancy in rhesus monkeys was associated with reduced infant immune responsiveness, as measured by numbers of peripheral lymphocytes (Keen *et al.*, 1989). This association occurred in the absence of marked reductions in infant plasma of soft tissue zinc concentrations. In ewes, subclinical cobalt deficiency during early pregnancy extends the time taken for lambs to stand, find the udder and suck (Fisher and MacPherson, 1991). The deficiency is also associated with a depression in passive immunity, reflected in lower lamb IgG concentrations. In pigs (Antipatis *et al.*, 2001; Bland *et al.*, 2001), maternal concentrations of fat-soluble vitamins during pregnancy are also related to neonatal IgG concentrations. Further work is required to clarify whether these associations reflect increased IgG in colostrum or

increased absorption of immunoglobulins across the neonatal gut. Supplementing ewes with vitamin E at 40 days before parturition increased lamb weight at day 45 of life, but this effect was not apparent by day 90 of life (Schultz *et al.*, 1999). However, maternal vitamin E supplementation does not appear to affect the immune status of lambs, as measured by colostrum or lamb serum IgG concentrations (Hatfield, *et al.*, 1999; Schultz *et al.*, 1999).

Maternal micronutrient status also affects brain function in offspring. For example, vitamin B6 deficiency during pregnancy in rats alters the function of *N*-methyl-D-aspartate receptors, which are thought to play an important role in learning and memory (Guilarte, 1993). Furthermore, feeding rats zinc-deficient diets throughout pregnancy and lactation compromises microtubule formation in pup brains (Oteiza *et al.*, 1990) and marginal maternal iron deficiency can impair postnatal motor development (Kwik-Urbe *et al.*, 1999). Studies in rats have shown that iron deficiency anaemia during brain development leads to behavioural deficits that cannot be alleviated by iron treatment after weaning (Felt and Lozoff, 1996). As mentioned earlier, inadequate copper supply during early pregnancy is associated with a failure of co-ordination in lambs and other species. The biochemical factors associated with such altered brain development are unclear at present, but may involve excessive cellular oxidative damage or a reduction in brain concentrations of cuproenzymes (reviewed by Keen *et al.*, 1998). Offspring of rat dams fed a copper-deficient diet during pregnancy and lactation have delayed maturation of the hippocampus (Hunt and Idso, 1995), the area of the brain responsible for learning and declarative memory. However, this study did not distinguish the relative contribution of inadequate maternal copper during the pre- and postnatal period.

In humans, severe maternal iodine deficiency can result in brain damage and mental retardation of offspring (reviewed by Delange, 2000). This damage is a consequence of the requirement of iodine for the synthesis of thyroid hormones, which in turn regulate the metabolic pattern of most organs, especially the brain. In rats, thyroid hormones are found in embryonic and fetal tissues before the onset of fetal thyroid function, and receptors for triiodothyronine (T3) are present in the brain by day 14 of gestation. Recent data demonstrate that iodine and selenium interact to alter thyroid function and that endemic cretinism may result from combined selenium and iodine deficiency, rather than iodine deficiency alone (for a review, see Arthur *et al.*, 1999). In ewes, maternal iodine deficiency during late pregnancy induced low plasma thyroxine (T4) concentrations in newborn lambs, which was associated with increased susceptibility to hypothermia and decreased viability at birth (Caple and Nugent, 1982).

As mentioned previously, maternal retinoid status affects the development of the neonatal lung. Neonates from vitamin A-deficient rats (Antipatis *et al.*, 2000) and pigs (Antipatis *et al.*, 2001) have relatively smaller lungs than neonates from mothers fed normally. Respiratory failure is a

common cause of perinatal mortality in rat pups born to vitamin A-deficient mothers (Antipatis *et al.*, 1998). In pups that do survive, the lungs have less air space, smaller sacculi and fewer elastic fibres than do the lungs of contemporary pups from control-fed dams. Maternal copper deficiency during pregnancy is also associated with lung abnormalities. Lungs from neonatal rabbits born to dams fed copper-deficient diets have high proportions of poorly crosslinked elastin and collagen and low concentrations of surfactant phospholipids (Abdel *et al.*, 1994). In rats, lungs from neonates carried by copper-deficient mothers show thickening of the air–blood barrier (Sarricolea *et al.*, 1993), which reduces the efficiency of gaseous exchange.

Conclusion

Both micronutrient deficiencies and excesses have profound and sometimes persistent effects on the developmental competence of oocytes, conceptuses and neonates in all species studied to date. The consequences of a single micronutrient deficiency can be more severe than the effects of more general undernutrition, which can be overcome, at least partially, by maternal homeostatic mechanisms. Greater understanding of the role of key micronutrients and the impact of antioxidant status at key stages of development will provide new opportunities to refine nutritional guidelines designed to enhance pregnancy outcome.

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