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REVIEW

Vitamin D deficiency and pregnancy: From preconception to birth

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Vitamin D is important for bone health, as well as an increasing number of other health outcomes. Here we discuss the evidence relating to vitamin D in pregnancy, from preconception to the perinatal period. During pregnancy extra calcium required for fetal skeletal growth is attained by both maternal bone resorption and increased absorption from dietary sources, necessitating increased maternal vitamin D. Many women have low vitamin D status during pregnancy and may require supplementation, although optimal serum levels and intake required to achieve those levels is not yet well defined. Evidence from animal studies, with some supportive human evidence, suggests that fertility may be impaired in mothers with low vitamin D. During pregnancy, maintaining vitamin D and calcium levels may decrease the risks of pre-eclampsia, while gestational diabetes mellitus appears to be more common in those with low vitamin D status, although there is insufficient evidence of causality. The evidence in relation to increased risks of bacterial vaginosis and caesarean section similarly requires confirmation in carefully designed observational and experimental studies. This review outlines the emerging evidence that maternal vitamin D status during pregnancy is important for the health of the mother and offspring across a range of possible health outcomes.

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1 Introduction

There is growing awareness that vitamin D status is important to health, with vitamin D inadequacy linked to an ever-increasing range of disease outcomes. The active form of vitamin D is a secosteroid hormone that is primarily derived (in humans) from irradiation of skin precursors (by the ultraviolet B (UVB) wavelengths of incoming solar radiation), followed by metabolism in the liver and kidney [1]. Vitamin D has long been recognized as essential to bone health, but it is now clear that the active form has a wide range of other functions within the body.

The focus of this review is the examination of research evidence relating to vitamin D status and a range of possible health outcomes in the period from preconception to birth (Fig. 1). Although there is evidence that prenatal and early life vitamin D status are also important to health in later life, this has been comprehensively reviewed elsewhere [2] and is not included here.

Much of the research evidence derives from animal work and human observational studies. In part, this reflects the recency of active research in this area – following a natural progression from hypotheses generated as a result of animal research and geographical and temporal patterns uncovered in ecological studies, to test in human observational studies,

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Abbreviations: AOR, adjusted odds ratio; **BV,** bacterial vaginosis; **CI,** confidence interval; **FF,** follicular fluid; **GDM,** gestational diabetes mellitus; **PCOS,** polycystic ovarian syndrome; **RCT,** randomized controlled trial; **TNF-α,** tumor necrosis factor-α; **UV,** ultraviolet; **VDR,** vitamin D receptor

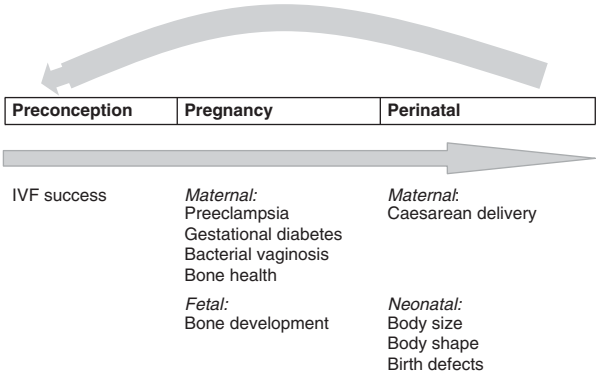

Figure 1. Manifestation of reviewed possible major outcomes related to vitamin D status related to pregnancy and birth.

and then confirming those findings in randomized controlled trials (RCTs).

In this review, we present the current evidence according to a hierarchy, from animal and laboratory studies, through observational studies and then RCT evidence where the latter is available. Specific vitamin D terminology is employed throughout the review we use vitamin D when referring to cholecalciferol or ergocalciferol; 25(OH)D to refer to 25 hydroxycalciferol (including both $25(OH)D_2$ and $25(OH)D_3$) and $1,25(OH)_2D$, the active form of the hormone, to include both $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$. Note that the pharmacokinetics of vitamin D_2 and vitamin D_3 may be slightly different; some findings in relation to vitamin D_3 are not replicated with use of vitamin D_2 [3].

2 Synthesis and action of $1,25(OH)_2D$

The vitamin D precursor, 7-dehydrocholesterol, is a normal intermediary in the cholesterol pathway and is present in the skin within the plasma membranes of epidermal and dermal cells [4]. UVB irradiation causes molecular instability resulting in a chemical rearrangement, with the formation of previtamin D_3. A subsequent temperature-dependent [4] thermal isomerization results in the formation of vitamin D_3 (cholecalciferol) which is released into the circulation, transported by the vitamin D-binding protein. In general, there is also a small contribution to the body's total vitamin D from diet and/or supplements [5]. This may be vitamin D_2 (ergocalciferol), derived from plants, or vitamin D_3, found principally in fatty fish such as salmon, sardines and mackerel and, to a certain extent, egg yolks [6].

Whether ingested or sunlight derived, vitamin D (D_2 or D_3) is transported to the liver, where it is hydroxylated by a number of mitochondrial and microsomal P450 enzymes [7] acting as 25 hydroxylases (primarily *CYP27A1*), to form 25(OH)D. This is the main circulating form of ''vitamin D'' and serum 25(OH)D is the usual measure of vitamin D status [8]. Serum 25(OH)D circulates attached to the vitamin D-binding protein, and may be further hydroxylated in the kidney by *CYP27B1* (1α hydroxylase), a P450 mitochondrial enzyme [7], to form the active hormone, 1,25-dihydroxy-vitamin D ($1,25(OH)_2D$) [8], depending on the calcium requirements. Many other tissues also possess the 1 α-hydroxylase enzyme, allowing the local conversion of 25(OH)D to the active $1,25(OH)_2D$ [7]. Production of the active metabolite is tightly regulated: with upregulation of *CYP27B1* by parathyroid hormone and downregulation, in the kidney only, by a fibroblast growth factor [7]. In addition, there is negative feedback control through $1,25(OH)_2D$-induced upregulation of a vitamin D 24 hydroxylase (*CYP24A1*), resulting in the catabolism of 25(OH)D and $1,25(OH)_2D$ to less active metabolites [9].

The active $1,25(OH)_2D$ exerts effects predominantly through a genomic pathway leading to changes in gene transcription, that takes hours or days [10]. The active hormone enters the cells by passive diffusion and binds to a nuclear vitamin D receptor (VDR) [7, 9]. This causes a conformational change in the VDR, allowing it to dimerize with the retinoid X receptor and subsequently interact with specific DNA sequences, vitamin D response elements, on target genes to activate or repress gene transcription [7, 9].

A more rapid response pathway, taking seconds to minutes, may occur *via* interaction with a cell surface receptor and second messengers such as mitogen-activated protein kinase or cyclic adenosine monophosphate[10]. This allows transmission of extracellular signals to intracellular targets, resulting in initiation of myogenesis, cell proliferation, differentiation or apoptosis [11]. Such pathways have been demonstrated in muscle [11] and may be the route for some of the $1,25(OH)_2D$ effects on pancreatic β-cells, vascular smooth muscle, the intestine, and monocytes [10].

The well-known calcitropic functions of $1,25(OH)_2D$ include the physiological regulation of calcium transport and bone mineralization by increasing intestinal calcium absorption [1], suppressing parathyroid secretion [7] and promoting mineralization of the skeleton [12]. It is now being increasingly recognized that vitamin D has important non-calcitropic actions, involving VDR activation by locally produced $1,25(OH)_2D$ in a number of tissues in a paracrine and autocrine manner [13].

The pleiotropic effects of $1,25(OH)_2D$ include stimulation of insulin production [14], thyroid-stimulating hormone secretion [15],] and improvement of myocardial contractility [16]. An important role for $1,25(OH)_2D$ in increasing differentiation and suppressing proliferation of monocytes was reported in 1981 [17] and was followed by work that showed that $1,25(OH)_2D$ could induce suppression of cancer cell growth and improved differentiation [18]. The application of vitamin D analogs in the management of rapidly proliferating cells became immediately apparent [19], indicating these as a therapy for psoriasis [20].

An important immune regulatory role for $1,25(OH)_2D$ in both innate and adaptive immunity has also been established [21] and recently, $1,25(OH)_2D$ has been shown to inhibit the development and function of proinflammatory Th17 cells [22].

The above actions of $1,25(OH)_2D$ indicate the possible roles it may play in pregnancy and during fetal development, a period of immense cellular differentiation and proliferation. This will be reviewed in the following sections.

3 Maternal and fetal vitamin D during pregnancy

3.1 Physiology

During pregnancy, maternal $1,25(OH)_2D$ requirements can increase up to four- to five-fold to facilitate the availability of extra calcium required for fetal skeletal growth [23]. Approximately, 25–30 g of calcium is transferred to the fetus by the end of pregnancy with the majority of this occurring in the last trimester [24, 25]. Calcium levels in the third trimester fetus are higher than in the maternal plasma (reviewed in [26]) with maternal total serum calcium concentrations declining as the pregnancy progresses [27], highlighting the role of active transport across the placenta.

If changes in sunlight exposure and vitamin D intake are taken into consideration, maternal 25(OH)D levels during pregnancy do not differ markedly from nonpregnant women over the same time period [28]. However, serum $1,25(OH)_2D$ concentrations increase 50–100% over the nonpregnant state during the second trimester and by 100% during the third trimester [29]. Such increases could be explained by increasing synthesis and/or decreasing catabolism of $1,25(OH)_2D$. There is convincing evidence (reviewed in [30]) of increasing $1,25(OH)_2D$ synthesis in the maternal kidney. Evidence in relation to $1,25(OH)_2D$ synthesis in decidual and placental tissue is more contentious (reviewed in [31]). There is an increased expression of 1α-hydroxylase and VDR genes [9] and high levels of 1α-hydroxylase [32] in human placental and decidual tissues during the first and early second trimesters. However, as there is inconsistent evidence of transplacental transfer of $1,25(OH)_2D$ at physiological levels [33, 34] (although it is clear that 25(OH)D crosses the placental barrier [25]), this may not contribute to maternal $1,25(OH)_2D$ levels, but be required locally for induction of immune tolerance of implantation and successful maintenance of pregnancy, *via* dampening of Th1 immune function [9, 32]. Decreased catabolism may also contribute to higher placental levels of $1,25(OH)_2D$, as there is some evidence of specific epigenetic downregulation of the *CYP24A1* (24-hydroxylase) gene [35] in the placenta.

The pregnant woman derives the majority of the calcium required for fetal calcification from her diet *via* enhanced calcium absorption largely induced by the rise in maternal $1,25(OH)_2D$ [36]. However, as the rise in calcium absorption occurs in the first trimester, before the rise in $1,25(OH)_2D$ begins [26], it seems likely that an additional mechanism is at play.

The level of serum 25(OH)D in cord blood correlated highly with the maternal serum levels with *r*-values of 0.89 [34] and 0.71 [37] in two studies comparing paired samples. Fetal $1,25(OH)_2D$ derives mainly from the fetal kidney [25, 27], possibly with some contribution from other sites such as the placenta, as noted above.

Possible immunoregulatory roles for placental $1,25(OH)_2D$ are currently under investigation [35]. Although the role of $1,25(OH)_2D$ in the development of central tolerance during the first trimester is not known, higher 25(OH)D levels in cord blood are associated with higher levels of the immunosuppressive cytokine IL-10 [38] and inhibition of Th1 and Th2 differentiation [39]. $1,25(OH)_2D$ appears to function as an intracrine regulator of the antimicrobial protein cathelicidin in the trophoblasts, thereby boosting innate immunity in the placenta [40]. Indirect mechanisms may also be involved, for example, through $1,25(OH)_2D$-induced alterations in cytokines which themselves have important effects, for example, maternal IL-6 is an important mediator of the adverse impact of maternal immune activation on neurodevelopmental outcomes [41] and $1,25(OH)_2D$ downregulates IL-6 [42]. Through such effects, higher $1,25(OH)_2D$ levels during pregnancy could possibly also lower the risks of infection for the developing fetus. This is an area of active research.

3.2 Issues relevant to maternal vitamin D status: Diet, skin color, sun avoidance, location

Increased vitamin D requirements in pregnancy must be met through dietary intake, supplements, and sun exposure. The efficiency and amount of UVB-induced production of vitamin D depends on the dose of relevant wavelengths of UVR to skin precursors. That dose, in turn, depends on ambient UVB (variable by season, time of day, and location), skin pigmentation (with darker skin reducing the effective UVB dose to epidermal cells), and barriers (sun protection measures and clothing) [43–45]. Women pregnant at high latitude locations, during winter or effectively sun protected, *e.g.* darkly pigmented and/or veiled [46], are at increased risk of vitamin D insufficiency, the latter even in high ambient UVR environments [47, 48].

3.3 Vitamin D supplementation

In a recent benefit-risk assessment of vitamin D supplementation that reviewed RCT and prospective cohort data in relation to falls, fracture prevention, cardiovascular health and colorectal cancer, serum 25(OH)D levels of 75–110 nmol/L provided optimal benefits for these outcomes without increasing risks [49]. These levels can be obtained in nonpregnant individuals with daily doses of 1800–4000 IU [49]. For pregnant and lactating women, optimal serum 25(OH)D levels have not yet been defined [50–52].

A 2009 review has recommended that women at risk of vitamin D deficiency should be monitored at the beginning of gestation and at midgestation [23], so that vitamin D deficiency can be corrected. A woman who is vitamin D deficient at the start of pregnancy may remain so even with daily doses of 1000 IU/day [53, 54]. The Canadian Paediatric Society has recommended a daily dose of 2000 IU in pregnant and lactating women [55]. However, doses over 2000 IU may be required to maintain serum 25(OH)D levels over 80 nmol/L [56]. A RCT involving a comparison of maternal antenatal supplementation of 4000 IU D_3 compared with 400 IU D_3 has recently concluded in the US with results from a conference presentation published in the media (*Times* web site: http://www.timesonline.co.uk/tol/news/uk/scotland/article6868729.ece). The complete findings are keenly awaited.

It is also important to note that there are currently few data on the possible adverse health implications of maintenance of relatively high 25(OH)D levels, *e.g.* >75 nmol/L for long periods of time [57]. Although short-term trials attest to the safety of even very high levels of vitamin D supplementation [58], the results of the above-mentioned clinical trials of vitamin D supplementation during pregnancy will provide important data in this respect.

4 Vitamin D and obstetric outcomes

4.1 Prepregnancy: Conception/fertility

The potential role of vitamin D in conception and fertility has not been extensively investigated to date and most studies have involved animal models. In vitamin D deficient rats ovarian function (vitamin D modulated) and spermatogenesis (calcium modulated) were impaired, with fertility reduced by 75% and litter size by 30% [59]. The reduction in fertility was associated with reduced probability of impregnation as well as an increased likelihood of complications during pregnancy. Further analysis of the data suggested that the decreased litter size may not have been caused directly by vitamin D deficiency but related to the smaller size of the vitamin D-deficient parents [59]. In a *CYP27B1* (1α hydroxylase) deficient mouse model, female null mutants were infertile, exhibited uterine hypoplasia and absent corpora lutea [60]. Furthermore, in VDR null mutant mice, sperm count and motility were reduced in males and there were histological abnormalities of the testis [61]. Females were infertile, but developed normal fertility if fed a calcium-rich diet. It is thus postulated that defective reproduction is the result of hypocalcemia that ensues after vitamin D deficiency [62], rather than directly due to vitamin D deficiency.

In humans, a prospective cohort study has shown that higher 25(OH)D levels in both serum and follicular fluid (FF) predicted the success of *in vitro* fertilization techniques [63]. This finding persisted after statistical adjustment for maternal age, BMI, ethnicity, and the number of embryos transferred (each 2.5 nmol/L increase in FF 25(OH)D increased the likelihood for achieving a clinical pregnancy by 7% ($p = 0.01$) [63]. As the study did not reveal any relationship between 25(OH)D levels and ovarian response, it was postulated that, as in animal studies [59], endometrial receptivity and implantation may be the beneficiaries of higher 25(OH)D levels [63]. Serum and FF levels of 25(OH)D were highly correlated [63, 64], paving the way for 25(OH)D measurement during *in vitro* fertilization cycles in the future to allow vitamin D supplementation if required.

Some autoimmune diseases are associated with decreased fertility [65], *e.g.* Antiphospholipid Syndrome and Systemic Lupus Erythematosus, and these disorders have also been associated with low levels of 25(OH)D [66]. In addition, difficulties with fertility is common in many women with polycystic ovarian syndrome (PCOS), a condition characterized by hyperandrogenic chronic anovulation, arrested follicular development, and metabolic disturbances such as insulin resistance [67, 68]. Treatment with vitamin D and calcium has been demonstrated to normalize menstrual cycles in some women with PCOS [68] and treatment with a vitamin D_3 analog (alphacalcidol) significantly increased the first phase of insulin secretion in another study of women with PCOS [67]. No studies to date appear to have examined how these separate findings might relate to one another.

4.2 During pregnancy

4.2.1 Pre-eclampsia

Pre-eclampsia, a common complication affecting up to 10% of pregnancies, presents as hypertension, proteinuria, and endothelial dysfunction in the mother and can result in fetal growth restriction, premature delivery, and low birth weight. It is a heterogeneous disorder for which the pathogenesis may vary, depending on the risk factor profile. Risk factors include, but are not limited to, maternal chronic hypertension, maternal obesity, previous and/or family history of pre-eclampsia, and multifetal pregnancy [69]. Women with pre-eclampsia demonstrate abnormalities in calcium metabolism [70], which could induce several of the processes resulting in the phenotype of the disorder [23, 71]. In a meta-analysis of intervention trials, calcium supplementation halved the risk of pre-eclampsia, with the greatest effect seen in women at high risk of pre-eclampsia and women who had a low baseline level of calcium [71]. However, not all studies were consistent and in two intervention trials, calcium supplementation did not reduce the risk of pre-eclampsia [72, 73], although in one of these [73] it did reduce the severity of the disorder.

Through its role in the regulation of calcium transport, or through one of its many noncalcaemic regulatory roles [74],

vitamin D may also be involved in the pathogenesis of pre-eclampsia. In ecological studies, seasonal variation in the incidence of pre-eclampsia in the northern hemisphere has been demonstrated, with highest incidence in winter (a time of low potential for vitamin D synthesis) and lowest in summer/early autumn [75–77]. In addition, pre-eclampsia is more common in dark-skinned women [78, 79] and in women with some autoimmune diseases (which may be associated with vitamin D insufficiency, such as type I diabetes and rheumatoid arthritis ([80–82], reviewed in [83]).

Pre-eclampsia has been associated with an increased release of Th1 cytokines such as tumor necrosis factor-α (TNF-α) [84, 85] and increased TNF-α levels have been found in the placenta and amniotic fluid of pregnancies affected by pre-eclampsia [86]. In normal cultured trophoblasts, TNF-α expression is inhibited by $1,25(OH)_2D$ and TNF-α increases the gene expression of *CYP24A1* [87], *i.e.* TNF-α and $1,25(OH)_2D$ are mutually inhibitory. However, pre-eclamptic placentas may have decreased ability to convert 25(OH)D to $1,25(OH)_2D$ [88] (despite increased 1α hydroxylase (*CYP27B1*) and decreased 25-hydroxylase (*CYP27A1*) and 24-hydroxylase gene expression (*CYP24A1*), compared with normal placental tissue [89]. These findings are consistent with a role for TNF-α in pre-eclampsia to increase $1,25(OH)_2D$ catabolism, leading to the low levels of circulating $1,25(OH)_2D$ observed [87] that in turn could contribute to the lower calcium levels seen in patients with pre-eclampsia.

Evidence from observational human studies supports a role of vitamin D in the pathogenesis of pre-eclampsia. In a nested case–control study, maternal serum 25(OH)D concentration of <37.5 nmol/L during early pregnancy (<22 wk gestation) was associated with a fivefold increase in the odds of developing pre-eclampsia (adjusted odds ratio (AOR) = 5.0; 95% confidence interval (CI) 1.7–14.1; adjusted for maternal race/ethnicity, prepregnancy BMI, maternal education, season, and gestational age when the serum sample was taken). The risk of pre-eclampsia more than doubled for each 50 nmol/L decrease in maternal 25(OH)D level [75].

A similar apparent risk reduction was found in a recent cohort study of 23 423 nulliparous women in Norway. There was a 27% reduction in the risk of pre-eclampsia in women who took 10–15 μg/day (400–600 IU/day) of vitamin D supplements compared with women who took no supplements (AOR = 0.73, 95% CI 0.58–0.92; adjusted for BMI, maternal height, maternal age, maternal education, season of birth, and smoking) [90]. Another cohort study reported lower maternal $1,25(OH)_2D$ serum levels in pre-eclamptic pregnancies compared with normal pregnancies (107 ± 20 *versus* 125 ± 20 nmol/L, $p = 0.003$) [91]; however, the circulating levels of $1,25(OH)_2D$ did not differ before the development of eclampsia [91]. Intriguingly, in a northern Finland birth cohort the risk of pre-eclampsia was halved (AOR = 0.49, 95% CI 0.26–0.92) in the first pregnancies of women who received vitamin D supplementation during their own first year of life [92].

Two RCT studies have examined the effect of combined preparations (fish oil and minerals [93], and vitamin D and calcium [94]) ingested from midgestation, on blood pressure outcomes in pregnancy. The first showed a 31.5% (95% CI 11–47%) decrease in the odds of development of pre-eclampsia [93], while in the second study, although blood pressure at 32 and 36 wk was lower in the supplemented group, there was no significant difference in the proportion of who developed pre-eclampsia (6% in the supplemented group compared with 9% in the nonsupplemented group [94]]. It is not possible to detangle the separate importance of vitamin D and calcium from these studies and the RCTs of antenatal vitamin D supplementation without calcium will be needed to do so.

4.2.2 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM), which affects 3–8% of all pregnancies depending on the population studied, is a glucose intolerance that has its onset, or is first recognized, during pregnancy [95]. The known risk factors for developing GDM include maternal obesity or being overweight, being of a particular race/ethnicity, prior history of GDM, family history of type II diabetes, history of previous fetal death, previous delivery of a macrosomic infant, and increasing maternal age [96].

As $1,25(OH)_2D$ is known to stimulate insulin production [14] and improve insulin sensitivity [97], it has been postulated that vitamin D deficiency may be involved in the pathogenesis of GDM [98]. To date, a limited number of studies have examined this issue.

In two cross-sectional studies, serum 25(OH)D levels (measured at 28.7 (± 3.3) wk [99] and 24–28 [100] wk gestation) were lower in women with GDM than in normal controls (48.6 *versus* 55.3 nmol/L, $p = 0.04$ [99] and 16.49 *versus* 22.97 nmol/L, $p = 0.009$ [100], respectively). Differences remained after accounting for age, weight, and ethnicity [99]. Although there was an increased odds of being diagnosed with GDM in those with 25(OH)D levels <50 nmol/L (presumably compared with those with 25(OH)D levels ≥ 50 nmol/L, although the reference group is not specifically stated), this was not statistically significant (AOR = 1.92, 95% CI 0.89–4.17). In contrast, a third cross-sectional, hospital-based study in India, where the prevalence of vitamin D deficiency (<50 nmol/L) was high (66%), found no association between 25(OH)D levels (measured at 30-wk gestation) and the diagnosis of GDM [52].

There are two important points to note in relation to the above studies. Firstly, as is typical of cross-sectional studies, it is not possible to be confident of the temporal association of two factors measured at the same time point and it is possible that there is incomplete control of confounding due

to pre-pregnancy BMI or other risk factors for GDM. Secondly, as in each of these specific studies the samples for 25(OH)D measurement were taken well into the pregnancy, in late second or early third trimester, one might speculate that GDM had already developed and that the temporal pattern of measurement was flawed.

Using a different, more rigorous methodology, a prospective nested case–control study, maternal 25(OH)D deficiency (<50 nmol/L) at 16-wk gestation was found to be associated with increased risk of GDM development, after accounting for known risk factors including maternal age, race/ethnicity, family history of diabetes, and prepregnancy BMI (AOR = 2.66, 95% CI 1.01–7.02) [98]. Each 5 ng/mL (12.5 nmol/L) decrease in plasma 25(OH)D was associated with a 29% increase in the odds of developing GDM (AOR = 1.29, 95% CI 1.05–1.60).

Although these studies are relatively consistent in showing that low levels of 25(OH)D are associated with increased risk of GDM, RCT evidence is required to clarify the direction and magnitude of effect.

4.2.3 Bacterial vaginosis

Bacterial vaginosis (BV) is a vaginal infection that affects nearly a third of reproductive-aged women [101]. During pregnancy, BV identified before 20-wk gestation is strongly associated with low birth weight and indicated in preterm delivery and clinical chorioamnionitis [102]. In a cross-sectional study of low-income pregnant women (with a high percentage of black women), there was a linear inverse dose–response relationship between 25(OH)D level and the occurrence of BV, after adjustment for race and sexually transmitted diseases [103]: 25(OH)D concentrations of 20 and 50 nmol/L were associated with a 65 and 26% higher prevalence of BV at <16-wk gestation, compared with a serum concentration of 75 nmol/L (adjusted prevalence ratio = 1.65, 95% CI 1.01–2.69; adjusted prevalence ratio = 1.26, 95% CI 1.01–1.57, respectively). In a second study, involving pregnant African American adolescents, after adjustment for season, presence of BV was associated with vitamin D deficiency (<37.5 nmol/L) (OR 4.4, $p = 0.02$) [104].

Again, one must be mindful that both of these studies were cross sectional and it is not clear that the results were fully adjusted for possible confounders such as socio-economic status. The findings are biologically plausible through the effect of $1,25(OH)_2D$ on immune function and the antimicrobial effects of cathelicidin, but require confirmation by further studies.

4.2.4 Maternal bone health

During pregnancy there is an overall maternal bone loss of between 2 and 5% [105, 106]. We were unable to find any studies directly assessing whether vitamin D deficiency affected the maternal bone health during pregnancy. One study showed that there was greater bone loss (assessed by calcaneal quantitative ultrasound) in women who were in their first trimester during the winter months compared with those whose first trimester was in the summer [107]. Additionally, higher levels of estimated UVB exposure in early pregnancy were associated with greater baseline calcaneal width ($r = 0.32$, $p < 0.001$) and change in calcaneal width during pregnancy ($r = 0.36$, $p < 0.001$). Although this is suggestive of a possible vitamin D effect, vitamin D status was not measured, and definitive results will have to come from future work.

4.2.5 Fetal bone health

To date, any possible association between vitamin D deficiency and fetal bone health *in utero* has been primarily investigated in animal models, with most studies showing normal skeletal mineral content in vitamin-D-deficient and -insufficient animals [30]. However, as the majority of these studies have been undertaken in rodents, which are nocturnal, and are known to have requirements for vitamin D that differ from humans, they may not be the most suitable model to investigate such effects [36].

Using recent advances in prenatal ultrasound technology, a longitudinal cohort study of pregnant women found that a low 25(OH)D level during pregnancy (at 19- or 34-wk gestation) was associated with splaying of the distal metaphysis of the fetal femur (*i.e.* increased cross-sectional area), which is directly analogous to that seen in childhood rickets, and is seen as early as 19-wk gestation [108]. Compared with fetuses whose mothers were 25(OH)D replete (>50 nmol/L), the metaphyseal cross-sectional area was 5 and 14% greater in those whose mothers were 25(OH)D insufficient (25–50 nmol/L) and 25(OH)D deficient (<25 nmol/L), respectively. No association was found between maternal vitamin D concentration and fetal femur length at 19- or 34-wk gestation [108].

4.3 During the perinatal period

4.3.1 Caesarean delivery

Recent data suggest an association between vitamin D deficiency and an increased risk of caesarean section [109]. Women with 25(OH)D levels <37.5 nmol/L were almost four times more likely to have a caesarean section compared with women with levels 37.5 nmol/L, after adjustment for race, age, education level, insurance status, and alcohol use (AOR = 3.84; 95% CI 1.71–8.62). This finding clearly requires further investigation including RCTs of vitamin D supplementation during pregnancy.

4.3.2 Preterm delivery and birth weight

Low vitamin D status during pregnancy may be associated with shorter gestation (by 0.7 wk; 95% CI −1.3, −0.1, one study only) [110], lower weight at birth [111–113] or postnatally [114],] and poorer intrauterine long bone growth [110]. In contrast, one study noted greater weight and length in newborns with vitamin D deficiency [115]. Recently, Morley and colleagues reported that the relationship between maternal 25(OH)D levels and offspring birth size varied according to offspring's VDR genotype, suggesting effect modification by infant *fok*I genotype [116]. Babies of deficient mothers had lower birth weight with FF or Ff but not ff genotype (p-value for interaction after adjustment for potential confounding factors = 0.02).

There has been a preliminary report from the unpublished US RCT that ''premature babies born to women taking high doses of vitamin D were reduced by half at both 32 and 37 wk, and there were also fewer babies who were born ''small for dates'' – that is smaller than would be expected considering the length of time spent in the womb'' (*Times* web site). This is potentially a very exciting and important finding and has not been reported elsewhere.

4.3.3 Birth defects

There is evidence from animal studies that vitamin D regulates cell differentiation and proliferation in the heart and brain [117, 118]. Cardiac muscle is a target tissue for $1,25(OH)_2D$ which exerts a direct action on the heart by affecting cardiac muscle contractility [119, 120]. It has been suggested that low maternal vitamin D results in a significant slowing of neonatal cardiac development [119]. In rats, being born to vitamin D-deficient mothers affects brain morphology and gene expression at birth, with maternal vitamin D deficiency leading to persistent changes into adulthood [121–123]. Intriguingly, maternal vitamin D deficiency in rats appears to affect vascular function by stimulating nephrogenesis, although whether any renal functional advantage ensues is not known [124]. The role of vitamin D in pregnancy in humans, in terms of these aspects of human fetal development, is largely unexplored.

5 Future directions for research

We have reviewed the existing evidence for a range of possible adverse maternal, fetal, and perinatal health outcomes that may relate to low vitamin D status in pregnancy. To date, the evidence is based largely on animal and laboratory work, and ecological and observational studies, rather than experimental/intervention designs. Such evidence needs to be treated with caution. Although there are examples where observational studies, with supportive animal and experimental data, have provided sufficient evidence of a causal association to stimulate public health action, *e.g.* smoking as a risk factor for lung cancer [125], and prone sleeping position as a risk factor for sudden infant death syndrome [126], there are notable examples where RCTs have not only not supported the observational evidence, but shown opposite effects, *e.g.* hormone replacement therapy as protective for cardiovascular disease [127].

RCTs have established the importance of adequate folic acid to prevent the formation of neural tube defects [128], but few such trials have occurred for vitamin D supplementation in relation to specific clinical outcomes. Even with these trials (mostly of high-risk women, many years ago) being systematically reviewed, it appears that there is not enough evidence to evaluate the effects of vitamin D supplementation in pregnancy [129]. Women should not be vitamin D deficient, including during pregnancy and we support the view that women at risk of vitamin D deficiency should be monitored and appropriately supplemented during pregnancy [23].

High quality large-scale RCTs are required to assess the optimal serum 25(OH)D levels in pregnancy, and to further evaluate the possible adverse outcomes of maternal, fetal or infant vitamin D insufficiency. Such studies are underway, but there are some caveats: RCTs will be feasible for common short-term outcomes such as birth weight but less feasible for rarer long-term outcomes among offspring; and treatment contamination of the control arm has to be considered, whether by self supplementation or sun exposure leading to UVR-derived vitamin D generation. Furthermore, careful attention will be required before extrapolating the findings to regions of differing UVR levels or for mothers of differing skin types. No one study will be sufficient, and the confirmation, if any, of causality between low vitamin D levels in pregnancy and the range of possible adverse health outcomes will need to be considered in the light of coherence across the biological, observational, and RCT evidence.

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