REVIEW

Vitamin D, the placenta and early pregnancy: effects on trophoblast function

Ankana Ganguly[1], Jennifer A Tamblyn[1,2,3], Sarah Finn-Sell[4], Shiao-Y Chan[5], Melissa Westwood[4], Janesh Gupta[1,2], Mark D Kilby[1,2], Stephane R Gross[6] and Martin Hewison[1,3]

[1]Institute of Metabolism and Systems Research, The University of Birmingham, Birmingham, UK
[2]Fetal Medicine Centre, Birmingham Women's NHS Foundation Trust, Birmingham, UK
[3]CEDAM, Birmingham Health Partners, The University of Birmingham, Birmingham, UK
[4]Division of Developmental Biology and Medicine, Maternal and Fetal Health Research Centre, School of Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK
[5]Department of Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[6]School of Life and Health Sciences, Aston University, Birmingham, UK

Correspondence should be addressed to M Hewison: m.hewison@bham.ac.uk

Abstract

Pregnancy is associated with significant changes in vitamin D metabolism, notably increased maternal serum levels of active vitamin D, 1,25-dihydroxyvitamin ($1,25(OH)_2D$). This appears to be due primarily to increased renal activity of the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) that catalyzes synthesis of $1,25(OH)_2D$, but CYP27B1 expression is also prominent in both the maternal decidua and fetal trophoblast components of the placenta. The precise function of placental synthesis of $1,25(OH)_2D$ remains unclear, but is likely to involve localized tissue-specific responses with both decidua and trophoblast also expressing the vitamin D receptor (VDR) for $1,25(OH)_2D$. We have previously described immunomodulatory responses to $1,25(OH)_2D$ by diverse populations of VDR-expressing cells within the decidua. The aim of the current review is to detail the role of vitamin D in pregnancy from a trophoblast perspective, with particular emphasis on the potential role of $1,25(OH)_2D$ as a regulator of trophoblast invasion in early pregnancy. Vitamin D deficiency is common in pregnant women, and a wide range of studies have linked low vitamin D status to adverse events in pregnancy. To date, most of these studies have focused on adverse events later in pregnancy, but the current review will explore the potential impact of vitamin D on early pregnancy, and how this may influence implantation and miscarriage.

Key Words

- vitamin D
- pregnancy
- placenta
- trophoblast
- miscarriage

Journal of Endocrinology (2018) **236**, R93–R103

Introduction

The human placenta is a vital organ without which the mammalian fetus cannot survive. It forms the interface between the mother and fetus, supplying the fetus with oxygen, nutrients, excreting waste products, while protecting against maternal immunologic attack. The main functions of the placenta can be broadly categorized into transport and metabolism, protection and endocrine (Gude *et al.* 2004). The complex architecture of the placenta, bounded by the maternal aspect (basal plate) and the fetal aspect (chorionic plate), houses an abundance of the fundamental functional unit of the placenta, the chorionic villus, where all nutritional-waste exchange between the maternal blood and the fetal circulation occurs. In addition to facilitating a good maternal blood

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

supply for nutrition–waste exchange and orchestrating endocrine mediators of pregnancy to maintain maternal physiological changes for an optimal environment for fetal development, the placenta also acts to protect the fetus from xenobiotic materials and infectious agents (Yang 1997, Moore *et al*. 1999, Gude *et al*. 2004, Rudge *et al*. 2009). Successful development of the placenta involves two distinct mechanisms: implantation of the blastocyst, initiated by attachment of the embryo to the maternal endometrial epithelium and invasion of fetal trophoblast cells into the maternal endometrium to facilitate maternal–fetal exchange of nutrients, gases and waste. The diverse mechanisms associated with the regulation of trophoblast invasion have been well documented (Menkhorst *et al*. 2016). The aim of the current review is to provide an overview of these early events in placental development, with particular emphasis on the potential role of vitamin D as a determinant of early placental development through effects on trophoblast cells, particularly via effects of vitamin D on trophoblast invasion.

Vitamin D and pregnancy

Despite its long-standing association with rickets and osteoporosis, vitamin D has become increasingly recognized as a pluripotent regulator of biological functions above and beyond its classical effects on bone and calcium homeostasis. Expression of vitamin D receptor (VDR) for the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), as well as the 1α-hydroxylase enzyme that synthesizes $1,25(OH)_2D$ (CYP27B1), has been reported for various tissues that can be broadly termed 'barrier sites' (Jones *et al*. 1998, Townsend *et al*. 2005), indicating that localized responses to vitamin D may be a key feature of these tissues. Prominent among these barrier sites is the placenta, acting as the interface between mother and fetus. Historically, the placenta was one of the first extra-renal tissues shown to be capable of synthesizing $1,25(OH)_2D$, with CYP27B1 activity detectable in both maternal decidua and fetal trophoblast (Gray *et al*. 1979, Weisman *et al*. 1979). Initially, this was linked to the rise in maternal serum $1,25(OH)_2D$ that occurs at the end of the first trimester of pregnancy. However, studies of CYP27B1-deficient animals and an anephric pregnant woman indicated that this is not likely to be the case (Kovacs & Kronenberg 1997). Instead, the presence of VDR in the placenta suggests that vitamin D functions in tissue-specific fashion at the fetal–maternal interface (Bruns & Bruns 1983). One possible explanation is that $1,25(OH)_2D$ acts as a regulator of placental calcium transport (Bruns & Bruns 1983), but a placental immunomodulatory function has also been proposed (Liu & Hewison 2012). Moreover, the rapid induction of VDR and CYP27B1 early in pregnancy (Zehnder *et al*. 2002) suggests that vitamin D may play a more fundamental role in the process of conception, implantation and development of the placenta itself.

Vitamin D and implantation

To date, the precise role of vitamin D in the process of implantation remains unclear. Nevertheless, vitamin D has a biologically plausible role in female reproduction and implantation process. $1,25(OH)_2D$ has been shown to regulate expression of the homeobox gene *HOXA10* in human endometrial stromal cells (Du *et al*. 2005*b*). *HOXA10* is important for the development of the uterus during fetal life and, later in adulthood, is essential for endometrial development, allowing uterine receptivity to implantation (Bagot *et al*. 2000). Interestingly, animal studies have shown that vitamin D deficiency reduces mating success and fertility in female rats. Female rats fed with a vitamin D-deficient diet are capable of reproduction, but overall fertility is reduced including the failure of implantation (Halloran & DeLuca 1980). This was shown to be corrected by administration of $1,25(OH)_2D$ (Kwiecinksi *et al*. 1989), but also by use of diets high in calcium, phosphate and lactose (Johnson & DeLuca 2002), suggesting that the fertility effects of vitamin D may be due to indirect effects on mineral homeostasis. Other studies using knockout mouse models have further highlighted the importance of the vitamin D metabolic and signaling system in the process of implantation, with *Vdr*−/− and *Cyp27b1*−/− female mice both presenting with uterine hypoplasia and infertility (Yoshizawa *et al*. 1997, Panda *et al*. 2001). Conversely, injection of $1,25(OH)_2D$ has been shown to increase uterine weight and promote endometrial to decidual differentiation (Halhali *et al*. 1991).

In addition to regulating uterine and decidual development, vitamin D may also influence implantation indirectly via its well-known immunomodulatory actions. Regulation of immune function at the maternal–fetal interface involves a heterogeneous population of innate and adaptive immune cell subsets. Thus, throughout pregnancy, decidual synthesis of $1,25(OH)_2D$ has the potential to influence uterine natural killer cells, dendritic cells, macrophages and T-cells (Evans *et al*. 2004,

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

Tamblyn *et al.* 2015). Notable effects include inhibition of Th1 cytokines and promotion of Th2 cytokines (Gregori *et al.* 2001), which are known to play a significant role in the process of implantation (Piccinni *et al.* 2000, Zehnder *et al.* 2002). Purification of decidual cells into non-adherent stromal cells and adherent cells, which include decidual macrophages and uterine natural killer cells, has shown that adherent cells demonstrate a greater capacity for $1,25(OH)_2D$ production (Kachkache *et al.* 1993). Furthermore, first trimester decidual cells treated with either precursor 25-hydroxyvitamin D or $1,25(OH)_2D$ demonstrate significant induction of antibacterial protein cathelicidin and β-defensins (Evans *et al.* 2006, Liu *et al.* 2009). Since similar effects of vitamin D are observed in peripheral monocytes, an equivalent innate antimicrobial responsivity is postulated to exist at the maternal–fetal interface (Liu & Hewison 2012).

Vitamin D metabolism and function in trophoblast cells

The organization of maternal and fetal cells within the developing placenta has been well documented elsewhere (Vigano *et al.* 2003, Oreshkova *et al.* 2012) and is represented schematically in Fig. 1. Both the maternal decidua and fetal trophoblast components of the placenta (including syncytiotrophoblast and invasive extravillous trophoblast (EVT)) express CYP27B1 (Zehnder *et al.* 2002) and are able to produce detectable levels of $1,25(OH)_2D$ (Gray *et al.* 1979, Weisman *et al.* 1979). The resulting tissue concentrations of $1,25(OH)_2D$ appear to be significantly higher in the decidua (Tamblyn *et al.* 2017), but the coincident expression of VDR in trophoblast as well as decidua (Evans *et al.* 2004) means that multiple cell types within the placenta are capable of responding to the locally synthesized $1,25(OH)_2D$, either in an autocrine or paracrine fashion.

To date, studies of the physiological impact of decidual-trophoblast $1,25(OH)_2D$ production have focused primarily on trophoblast cells, using both primary cultures of EVT and trophoblast cells lines. Primary cultures of human syncytiotrophoblast express CYP27B1 and are able to synthesize $1,25(OH)_2D$ (Diaz *et al.* 2000) and also express VDR (Pospechova *et al.* 2009). However, in choriocarcinoma trophoblast cell lines such as BeWo and JEG-3, expression of VDR is low, with analysis of the effects of chromatin remodeling agents suggesting that

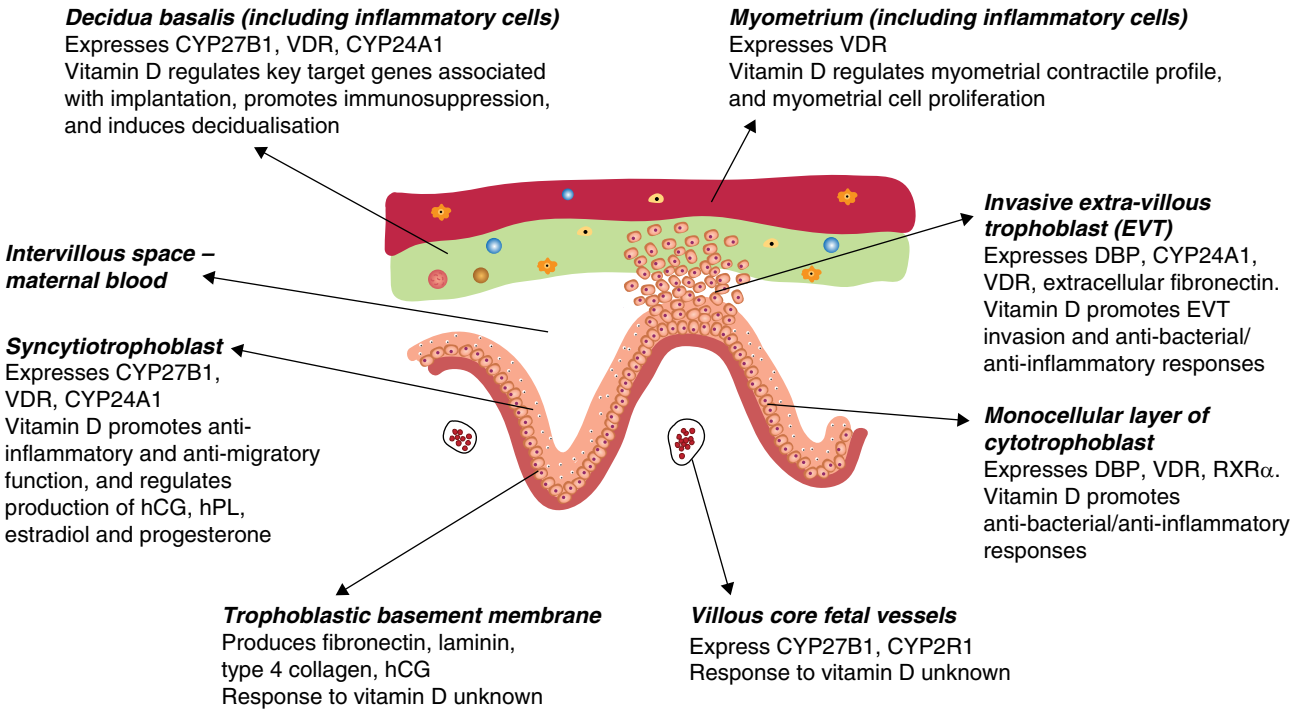

Figure 1
Vitamin D pathway components at the maternal–fetal interface associated with implantation. Schematic showing key cell types involved in implantation and associated expression of components of the vitamin D system: CYP2R1, vitamin D-25-hydroxylase; CYP24A1, vitamin D-24-hydroxylase; CYP27B1, 25-hydroxyvitamin D-1α-hydroxylase; DBP, vitamin D binding protein; hCG, human chorionic gonadotropin; hPL, human prolactin; RXR, retinoid X receptor; VDR, vitamin D receptor.

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

this may be due to epigenetic suppression of VDR in these cells (Pospechova *et al.* 2009). Further studies to assess the impact of differentiation of cultured trophoblast cells have been carried out using cyclic AMP (cAMP) to mimic the process of syncytialization (Keryer *et al.* 1998). Expression of hCG is elevated by cAMP in trophoblast cells, and this was associated with decreased expression of CYP27B1, with VDR expression being unaffected (Avila *et al.* 2007), suggesting that presence of the vitamin D metabolic and signaling pathways in the placenta is differentiation sensitive. The JEG-3 trophoblast cell line has also been reported to express CYP27B1, but synthesis of $1,25(OH)_2D$ by these cells appears to be significantly less than that observed with primary trophoblast cells and unaffected by cAMP (Pospechova *et al.* 2009). In addition to cAMP, inflammatory cytokines (Noyola-Martinez *et al.* 2014) and insulin-like growth factor I (Halhali *et al.* 1999) also stimulate trophoblast expression of CYP27B1 and synthesis of $1,25(OH)_2D$.

The vitamin D catabolic enzyme CYP24A1 has been reported to be undetectable in trophoblast cells, consistent with methylation epigenetic silencing of this gene in the human placenta (Novakovic *et al.* 2009). This suggests that synthesis of $1,25(OH)_2D$ by trophoblast cells is not subject to the same catabolic feedback control observed in other VDR-expressing tissues. However, other studies have shown that trophoblast expression of CYP24A1 is increased following treatment with cAMP (Avila *et al.* 2007). In addition, studies using the *Hyp* mouse model, which has elevated circulating levels of the positive regulator of 24-hydroxylase fibroblast growth factor 23 (FGF23), showed elevated placental expression of CYP24A1 mRNA in these mice (Ma *et al.* 2014, Ohata *et al.* 2014). Likewise, direct injection of FGF23 into normal placentas from wild-type mice also induced expression of CYP24A1 (Ohata *et al.* 2014). This appears to be mediated via trophoblast expression of fibroblast growth factor receptor 1 and its co-receptor α-klotho by trophoblast, suggesting that catabolism via CYP24A1 plays an as yet undefined role in mediating trophoblast effects of vitamin D.

Despite a wide range of studies showing regulation and activity of vitamin D metabolic enzymes in primary trophoblast cells and trophoblast cell lines, the principal functional analysis of vitamin D in these cells has centered on responses to $1,25(OH)_2D$. Initial experiments using JEG-3 cells described stimulation of calcium uptake (Tuan *et al.* 1991), and the regulation of the cytosolic calcium-binding protein calbindin-D28K (Belkacemi *et al.* 2005) by $1,25(OH)_2D$, consistent with a role for vitamin D in the endocrinology of placental calcium homeostasis. However, subsequent investigations of trophoblast cells and $1,25(OH)_2D$ have explored other mechanisms associated with placental endocrine function. These reports include the stimulation of human placental lactogen synthesis and release (Stephanou *et al.* 1994), hCG expression (Barrera *et al.* 2008) and the regulation of estradiol and progesterone synthesis (Barrera *et al.* 2007).

In recent years, our perspective on vitamin D and trophoblast function has been expanded to include studies of immunomodulatory function. In primary trophoblast cells and trophoblast cell lines, $1,25(OH)_2D$ has been shown to potently stimulate the expression of the antibacterial protein cathelicidin (Liu *et al.* 2009), while also suppressing inflammatory responses to tumor necrosis factor α (TNFα) (Diaz *et al.* 2009). Similar anti-inflammatory responses to $1,25(OH)_2D$ have also been reported using trophoblasts from women with the inflammatory disorders of pregnancy, preeclampsia (Noyola-Martinez *et al.* 2013) and antiphospholipid syndrome (APS) (Gysler *et al.* 2015). In recent studies, the anti-inflammatory effects of $1,25(OH)_2D$ on trophoblasts have been reported to include attenuation of oxidative stress-induced microparticle release from preeclampsia trophoblastic cells (Xu *et al.* 2017), further underlining the importance of this facet of vitamin D function within the placenta. *In vivo*, studies using *Cyp27b1*–/– and *Vdr*–/– mice have shown that loss of both alleles for either of these genes on the fetal side of the placenta alone was sufficient to dramatically exacerbate anti-inflammatory responses to lipopolysaccharide (LPS) immune challenge (Liu *et al.* 2011). Thus, in addition to the active immune cell function classically observed in the maternal decidua, trophoblast cells also appear to make a major contribution to the regulation of placental inflammation.

A role for vitamin D in EVT invasion?

Controlled invasion of fetal cytotrophoblast and differentiated EVT cells into the maternal decidua and myometrium in the first trimester of pregnancy is a key process in placentation and is essential for successful pregnancy. A complex network of communications among trophoblast, decidual stromal and immune cells is reported to facilitate implantation and maintenance of pregnancy, with key roles in tissue remodeling, cell trafficking and immune tolerance being evident (Oreshkova *et al.* 2012). The mechanisms underpinning these processes have received increasing attention since

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

abnormal placentation due to shallow invasion of EVT can cause important pregnancy disorders such as miscarriage (Ball *et al.* 2006), preeclampsia (Caniggia *et al.* 2000), fetal growth restriction, preterm birth and stillbirth (Goldman-Wohl & Yagel 2002, Kaufmann *et al.* 2003, Kadyrov *et al.* 2006, Reddy *et al.* 2006). By contrast, unrestricted invasion resulting from a failure to restrain the invading cytotrophoblast is associated with premalignant conditions such as malignant choriocarcinomas and invasive mole (Ringertz 1970, Caniggia *et al.* 2000) and can lead to aberrant placentation such as pathological adhesion to the myometrium (placenta accreta), extension into the myometrium (placenta increta) or invasion through the myometrium into adjacent organs (placenta percreta) (Khong 2008).

In recent studies, we have shown that human EVT isolated from first trimester pregnancies are a target for both 25(OH)D and $1,25(OH)_2D$ (Chan *et al.* 2015). In *ex vivo* experiments, both vitamin D metabolites promoted the invasion of EVT through Matrigel, with zymographic analysis showing that this effect involves enhanced expression of the matrix metalloproteinases pro-MMP2 and pro-MMP9 (Chan *et al.* 2015). These observations are in direct contrast to previously published studies describing $1,25(OH)_2D$ inhibition of matrix invasion by tumor cells (Bao *et al.* 2006). In this case, the primary mode of action for $1,25(OH)_2D$ was indirect suppression of MMPs via enhanced tissue inhibitor of metalloproteinase-1 (TIMP-1) expression. However, in other reports, low vitamin D status has been shown to be associated with elevated circulating MMP2 and MMP9 (Timms *et al.* 2002). Suppression of a variety of MMPs, including MMP2 and MMP9, by $1,25(OH)_2D$ has also been described for primary cultures of human uterine fibroid cells and uterine fibroid cell lines (Halder *et al.* 2013). Thus, the pro-invasive effects of vitamin D on EVTs appear to be quite distinct to pregnancy and the placenta.

The concept of vitamin D as a regulator of cellular motility and invasion is not novel and has been extensively reported in cancer states (Krishnan *et al.* 2012, Leyssens *et al.* 2014, Ma *et al.* 2016), where effects of vitamin D have been related to modulation of epithelial–mesenchymal transition (EMT) (Fischer & Agrawal 2014, Chen *et al.* 2015, Hou *et al.* 2016). Interestingly, this effect of vitamin D has not been observed in non-pathophysiological states or during embryogenesis. For example, vitamin D is known to inhibit invasion and motility of ovarian cancer and teratocarcinoma cell lines, but does not affect these cellular characteristics in the non-neoplastic ESD3 murine embryonic cell line (Abdelbaset-Ismail *et al.* 2016). The precise molecular mechanisms that mediate migration and invasion regulation by vitamin D remain unclear, although several different pathways have been studied. Notably, vitamin D has been shown to regulate the actin cytoskeleton in numerous cell types. In osteoblast-like cells, vitamin D promotes actin polymerization as part of its transcriptional induction of fibroblast growth factor 23 (Fajol *et al.* 2016). In endometrial cells, vitamin D treatment has also been shown to induce changes in actin architecture, through regulation of the RAc1/Pak1 axis (Zeng *et al.* 2016). It is not clear if such responses are also seen in trophoblast cells during placental development, but vitamin D has been shown to rescue motility defects in fetal endothelial colony-forming cell function of umbilical vein endothelial cells derived from pregnancies complicated by preeclampsia (von Versen-Hoynck *et al.* 2014) and gestational diabetes (Gui *et al.* 2015).

Effects of vitamin D on EVT invasion and migration may also be mediated indirectly via effects on other known EVT regulators. $1,25(OH)_2D$ has been shown to abolish S1P-mediated inhibition of migration via suppression of S1PR2 in trophoblast cell lines Swan-71 and JEG-3 (Westwood 2017). $1,25(OH)_2D$ has also been shown to stimulate hCG expression and secretion via a cAMP/PKA-mediated signaling pathway (Barrera *et al.* 2008). Although hCG is a potent regulator of trophoblast motility and invasion (Chen *et al.* 2011, Evans 2016), it is unclear whether changes in hCG expression are specifically required for effects of vitamin D on trophoblast invasion. In a similar fashion, $1,25(OH)_2D_3$ has been shown to positively regulate progesterone synthesis by human trophoblast cells from term placenta (Barrera *et al.* 2007). In HTR8/SVneo trophoblast cells, which have been reported to consist of a mixed population of cells, progesterone appears to suppress trophoblast motility and invasion (Chen *et al.* 2011). Thus, $1,25(OH)_2D$ may exert indirect effects on trophoblast invasion, although it is still not clear whether these effects are pro-migratory. Indirect actions of vitamin D on EVT function may also stem from effects on placental cell differentiation. Recent studies have shown that inactivation of VDR in trophoblastic BeWo cells resulted in increased trophoblast differentiation and syncytium formation (Nguyen *et al.* 2015). In a similar fashion, vitamin D may also influence EVT invasion and motility indirectly by targeting the development of cells on the maternal side of the placenta. Endometrial stromal cells treated with $1,25(OH)_2D$ have elevated expression of specific genes, including *HOXA10*

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

(Du *et al.* 2005*a*), which are known to be involved in the regional development of uterine decidualization and embryo implantation by controlling downstream target genes. The complex circuitry of vitamin D metabolism and function involved in mediating direct or indirect effects on EVT invasion and migration has still to be fully elucidated and is likely to be a key component of future studies of vitamin D in pregnancy.

Vitamin D and trophoblast function: clinical implications

Irrespective of proposed functional targets, vitamin D dysregulation during pregnancy has been linked to adverse effects on placental function and pregnancy in general. In 2010, the Institute of Medicine (IOM) defined vitamin deficiency as serum concentrations of 25(OH)D less than 20 ng/mL (50 nM) (Holick *et al.* 2011). Subsequently, the Endocrine Society issued slightly different guidelines, defining vitamin D insufficiency as being serum 25(OH) D levels below 30 ng/mL (75 nM) (Holick *et al.* 2011). Against this backdrop, several recent publications have highlighted the prevalence of low serum concentrations of 25(OH)D (less than 25 nM) in pregnant women: 20% of pregnant women in the UK (Javaid *et al.* 2006), 25% in the UAE (Dawodu *et al.* 1997), 80% in Iran (Bassir *et al.* 2001), 45% in northern India (Sachan *et al.* 2005), 60% in New Zealand (Eagleton & Judkins 2006) and 60–84% of pregnant non-Western women in the Netherlands (van der Meer *et al.* 2006). It remains unclear if this reflects simply a normal physiological drop in vitamin D concentrations during pregnancy or if pregnancy is a stress test that can exacerbate and unmask pathological vitamin D deficiency.

Vitamin D deficiency in pregnant women has been shown to be associated with increased risk for pregnancy complications (Lewis *et al.* 2010). These include preeclampsia (Bodnar *et al.* 2007*b*), fetal growth restriction, small-for-gestational-age fetus (Bodnar *et al.* 2010), bacterial vaginosis (Bodnar *et al.* 2009) and gestational diabetes mellitus (Maghbooli *et al.* 2008, Zhang *et al.* 2008). Maternal vitamin D deficiency has also been linked to adverse effects in offspring, including reduced bone density (Javaid *et al.* 2006) and childhood rickets (Wagner & Greer 2008), as well as increased risk of asthma (Camargo *et al.* 2007) and schizophrenia (McGrath 2001).

The impact of vitamin D status on early events in pregnancy has also been studied. In northern countries, where there is a strong seasonal contrast in light exposure and UVB-induced vitamin D production in skin, conception rates are decreased during winter months, with rates rising during summer and an increased birth rate in spring (Rojansky *et al.* 1992). Interestingly, ovulation rates and endometrial receptivity also appear to be reduced during long dark winters in northern countries (Rojansky *et al.* 2000), which may be explained in part by seasonal variations in vitamin D levels. With this in mind, several observational studies have investigated the potential impact of vitamin D on *in vitro* fertilization (IVF), albeit with largely conflicting outcomes. In a study of infertile women undergoing IVF, those with higher levels of 25(OH)D in serum and follicular fluid, were more likely to achieve pregnancy following IVF, and high vitamin D levels were also shown to improve the parameters of controlled ovarian hyperstimulation (Ozkan *et al.* 2010). Aleyasin and coworkers found no significant association between 25(OH)D levels in serum and follicular fluid with IVF outcomes (Aleyasin *et al.* 2011). However, this did not include any women with a serum vitamin D level >50 nmol/L. In another study of 100 women undergoing IVF, serum concentrations of 25(OH)D were positively associated with fertilization rate (Abadia *et al.* 2016). However, serum 25(OH)D was unrelated to the probability of pregnancy or live birth after IVF (Abadia *et al.* 2016). Anifandis and coworkers investigated 101 women who received IVF-intracytoplasmic sperm injection (ICSI) ovarian stimulation cycles. In this study, women with vitamin D sufficiency (25(OH)D level >30 ng/mL in follicular fluid) had a lower quality of embryos and were less likely to achieve clinical pregnancy, compared with women with insufficient (follicular fluid 25(OH)D level 20.10–30 ng/mL) or deficient vitamin D status (follicular fluid 25(OH)D level <20 ng/mL) (Anifandis *et al.* 2010).

Elucidation of the immunomodulatory effects of $1,25(OH)_2D$ has led to the suggestion that vitamin D might have a role in protecting against spontaneous abortion (Bubanovic 2004). This was supported by *ex vivo* analyses showing that $1,25(OH)_2D$ is able to suppress inflammatory cytokine production by endometrial cells from women with unexplained recurrent spontaneous abortions (Tavakoli *et al.* 2011). More recently, $1,25(OH)_2D$ has been shown to potently regulate natural killer cells from women with recurrent miscarriage (Ota *et al.* 2015). Considering these observations, the impact of maternal vitamin D status on pregnancy outcome has been studied in several cohorts. In a large prospective cohort study of 1683 pregnant women donating serum before

http://joe.endocrinology-journals.org
https://doi.org/10.1530/JOE-17-0491

gestational week 22, serum concentrations of 25(OH)D less than 50 nM were associated with a >2-fold increase in first miscarriage rate, although no significant effect was observed for second trimester miscarriage (Andersen *et al.* 2015). In a prospective study of pre-conceptual vitamin D, maternal serum 25(OH)D levels were not found to be associated with chances of conceiving or overall risk of miscarriage (Moller *et al.* 2012). However, women with miscarriage in the second trimester had lower first trimester serum concentrations of 25(OH)D than those women who did not miscarry (Moller *et al.* 2012). In a much larger, nested case-control study of over 5000 women did not reveal any adverse effects of low serum 25(OH)D on pregnancy outcomes (Schneuer *et al.* 2014). A recent meta-analysis and systematic review concluded that vitamin D deficiency is not associated with increased risk of spontaneous recurrent abortion (Amegah *et al.* 2017). Thus, the possible impact of sub-optimal vitamin D on implantation and adverse pregnancy outcomes such as miscarriage still remains unclear. Interestingly, in endometrial tissue from women with unexplained recurrent spontaneous abortion, expression of key components in the vitamin D metabolic (CYP27B1/CYP24A1) and signaling (VDR) systems was found to be comparable to endometrial tissue from healthy fertile women (Tavakoli *et al.* 2015). By contrast, recent studies of women with recurrent miscarriage showed that expression of mRNA and protein for CYP27B1 in villous and decidual tissue was lower than in control tissues from normal healthy pregnancies (Wang *et al.* 2016). In future studies it will be important to clarify how variations in the vitamin D system within the placenta and fetal trophoblast cells affect implantation and the maintenance of a successful healthy pregnancy.

A major contributing factor to vitamin D status in pregnant women is obesity, with lower circulating levels of 25(OH)D being reported in in pregnant women with high body mass index (BMI), relative to pregnant women with a normal BMI (Bodnar *et al.* 2007*a*, Karlsson *et al.* 2015). Maternal obesity is associated with adverse health effects for both mother and child, with increased inflammation has been proposed as an important pathological mechanism for the detrimental effects of obesity during pregnancy (Denison *et al.* 2010, Pantham *et al.* 2015). A role of vitamin D in the process is still unclear. However, given the established anti-inflammatory effects of vitamin D at the fetal–maternal interface (Tamblyn *et al.* 2015), it is possible that some pregnancy effects of obesity are mediated via low circulating maternal vitamin D.

Conclusions

Expression of placental CYP27B1 and VDR at early stages of pregnancy suggests an important role for vitamin D in placental physiology. In previous studies, we have hypothesized that placental vitamin D may function, at least in part, to promote antimicrobial and anti-inflammatory immune activity, with both the maternal decidua and fetal trophoblast contributing to these actions. However, analysis of trophoblast cells *ex vivo* and *in vitro* indicates that vitamin D may have a much broader role in placental function, including the regulation of trophoblast differentiation and EVT invasion of the decidua and myometrium (Fig. 1). Thus, effects of vitamin D may occur earlier in pregnancy than previously appreciated, underlining the requirement for adequate vitamin D status across gestation. To date, studies of vitamin D status (maternal serum 25(OH)D) in pregnancy have tended to focus on later stages of pregnancy, and associated adverse events such as preterm birth, gestational diabetes and preeclampsia. Likewise, supplementation trials for vitamin D in pregnancy have focused on women between 10 and 18 weeks of pregnancy. However, the responsiveness of trophoblast cells to $1,25(OH)_2D$, notably effects on EVT invasion, suggests that further studies of vitamin D and adverse events in early pregnancy are required. To date, there have been a limited number of reports of vitamin D deficiency and miscarriage, but these need to be expanded to include more rigorous supplementation trials. The review we present is supportive of early, pre-conceptual, supplementation with vitamin D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This study was supported by funding from Action Medical Research (#1949 to M K & M H), and Wellbeing of Women (RTF401, J A T), Medical Research Council grant MR/M02296X/1 (M W and S F-S), and a Royal Society Wolfson Merit Award (WM130118 to M H).

References

Abadia L, Gaskins AJ, Chiu YH, Williams PL, Keller M, Wright DL, Souter I, Hauser R, Chavarro JE & Environment and Reproductive Health Study Team 2016 Serum 25-hydroxyvitamin D concentrations and treatment outcomes of women undergoing assisted reproduction.

American Journal of Clinical Nutrition **104** 729–735. (https://doi.org/10.3945/ajcn.115.126359)

Abdelbaset-Ismail A, Pedziwiatr D, Suszynska E, Sluczanowska-Glabowska S, Schneider G, Kakar SS & Ratajczak MZ 2016 Vitamin D3 stimulates embryonic stem cells but inhibits migration and growth of ovarian cancer and teratocarcinoma cell lines. *Journal of Ovarian Research* **9** 26. (https://doi.org/10.1186/s13048-016-0235-x)

Aleyasin A, Hosseini MA, Mahdavi A, Safdarian L, Fallahi P, Mohajeri MR, Abbasi M & Esfahani F 2011 Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **159** 132–137. (https://doi.org/10.1016/j.ejogrb.2011.07.006)

Amegah AK, Klevor MK & Wagner CL 2017 Maternal vitamin D insufficiency and risk of adverse pregnancy and birth outcomes: a systematic review and meta-analysis of longitudinal studies. *PLoS ONE* **12** e0173605. (https://doi.org/10.1371/journal.pone.0173605)

Andersen LB, Jorgensen JS, Jensen TK, Dalgard C, Barington T, Nielsen J, Beck-Nielsen SS, Husby S, Abrahamsen B, Lamont RF, *et al*. 2015 Vitamin D insufficiency is associated with increased risk of first-trimester miscarriage in the Odense Child Cohort. *American Journal of Clinical Nutrition* **102** 633–638. (https://doi.org/10.3945/ajcn.114.103655)

Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S & Messinis IE 2010 Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reproductive Biology and Endocrinology* **8** 91. (https://doi.org/10.1186/1477-7827-8-91)

Avila E, Diaz L, Barrera D, Halhali A, Mendez I, Gonzalez L, Zuegel U, Steinmeyer A & Larrea F 2007 Regulation of vitamin D hydroxylases gene expression by 1,25-dihydroxyvitamin D3 and cyclic AMP in cultured human syncytiotrophoblasts. *Journal of Steroid Biochemistry and Molecular Biology* **103** 90–96. (https://doi.org/10.1016/j.jsbmb.2006.07.010)

Bagot C, Troy P & Taylor H 2000 Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. *Gene Therapy* **7** 1378. (https://doi.org/10.1038/sj.gt.3301245)

Ball E, Bulmer JN, Ayis S, Lyall F & Robson SC 2006 Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. *Journal of Pathology* **208** 535–542. (https://doi.org/10.1002/path.1927)

Bao BY, Yeh SD & Lee YF 2006 1alpha,25-Dihydroxyvitamin D3 inhibits prostate cancer cell invasion via modulation of selective proteases. *Carcinogenesis* **27** 32–42. (https://doi.org/10.1093/carcin/bgi170)

Barrera D, Avila E, Hernandez G, Halhali A, Biruete B, Larrea F & Diaz L 2007 Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. *Journal of Steroid Biochemistry and Molecular Biology* **103** 529–532. (https://doi.org/10.1016/j.jsbmb.2006.12.097)

Barrera D, Avila E, Hernandez G, Mendez I, Gonzalez L, Halhali A, Larrea F, Morales A & Diaz L 2008 Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts. *Reproductive Biology and Endocrinology* **6** 3. (https://doi.org/10.1186/1477-7827-6-3)

Bassir M, Laborie S, Lapillonne A, Claris O, Chappuis MC & Salle B 2001 Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. *Acta Paediatrica* **90** 577–579. (https://doi.org/10.1111/j.1651-2227.2001.tb00802.x)

Belkacemi L, Zuegel U, Steinmeyer A, Dion JP & Lafond J 2005 Calbindin-D28k (CaBP28k) identification and regulation by 1,25-dihydroxyvitamin D3 in human choriocarcinoma cell line JEG-3. *Molecular and Cellular Endocrinology* **236** 31–41. (https://doi.org/10.1016/j.mce.2005.03.002)

Bodnar LM, Catov JM, Roberts JM & Simhan HN 2007*a* Prepregnancy obesity predicts poor vitamin D status in mothers and their neonates. *Journal of Nutrition* **137** 2437–2442.

Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW & Roberts JM 2007*b* Maternal vitamin D deficiency increases the risk of preeclampsia. *Journal of Clinical Endocrinology and Metabolism* **92** 3517–3522. (https://doi.org/10.1210/jc.2007-0718)

Bodnar LM, Krohn MA & Simhan HN 2009 Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. *Journal of Nutrition* **139** 1157–1161. (https://doi.org/10.3945/jn.108.103168)

Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, Marazita ML & Simhan HN 2010 Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *Journal of Nutrition* **140** 999–1006. (https://doi.org/10.3945/jn.109.119636)

Bruns ME & Bruns DE 1983 Vitamin D metabolism and function during pregnancy and the neonatal period. *Annals of Clinical and Laboratory Science* **13** 521–530.

Bubanovic I 2004 1alpha,25-dihydroxy-vitamin-D3 as new immunotherapy in treatment of recurrent spontaneous abortion. *Medical Hypotheses* **63** 250–253. (https://doi.org/10.1016/j.mehy.2003.11.037)

Camargo CA, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K & Gillman MW 2007 Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 years of age. *American Journal of Clinical Nutrition* **85** 788–795.

Caniggia I, Winter J, Lye SJ & Post M 2000 Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta* **21** (Supplement A) S25–S30. (https://doi.org/10.1053/plac.1999.0522)

Chan SY, Susarla R, Canovas D, Vasilopoulou E, Ohizua O, McCabe CJ, Hewison M & Kilby MD 2015 Vitamin D promotes human extravillous trophoblast invasion in vitro. *Placenta* **36** 403–409. (https://doi.org/10.1016/j.placenta.2014.12.021)

Chen JZ, Wong MH, Brennecke SP & Keogh RJ 2011 The effects of human chorionic gonadotrophin, progesterone and oestradiol on trophoblast function. *Molecular and Cellular Endocrinology* **342** 73–80. (https://doi.org/10.1016/j.mce.2011.05.034)

Chen S, Zhu J, Zuo S, Ma J, Zhang J, Chen G, Wang X, Pan Y, Liu Y & Wang P 2015 1,25(OH)2D3 attenuates TGF-beta1/beta2-induced increased migration and invasion via inhibiting epithelial-mesenchymal transition in colon cancer cells. *Biochemical and Biophysical Research Communications* **468** 130–135. (https://doi.org/10.1016/j.bbrc.2015.10.146)

Dawodu A, Agarwal M, Patel M & Ezimokhai M 1997 Serum 25-OHD and calcium homoeostasis in United Arab Emirates mothers and neonates: a preliminary report. *Middle East Paediatrics* **2** 9–11.

Denison FC, Roberts KA, Barr SM & Norman JE 2010 Obesity, pregnancy, inflammation, and vascular function. *Reproduction* **140** 373–385. (https://doi.org/10.1530/REP-10-0074)

Diaz L, Sanchez I, Avila E, Halhali A, Vilchis F & Larrea F 2000 Identification of a 25-hydroxyvitamin D3 1alpha-hydroxylase gene transcription product in cultures of human syncytiotrophoblast cells. *Journal of Clinical Endocrinology and Metabolism* **85** 2543–2549. (https://doi.org/10.1210/jcem.85.7.6693)

Diaz L, Noyola-Martinez N, Barrera D, Hernandez G, Avila E, Halhali A & Larrea F 2009 Calcitriol inhibits TNF-alpha-induced inflammatory cytokines in human trophoblasts. *Journal of Reproductive Immunology* **81** 17–24. (https://doi.org/10.1016/j.jri.2009.02.005)

Du H, Daftary GS, Lalwani SI & Taylor HS 2005*a* Direct regulation of HOXA10 by 1,25-(OH)2D3 in human myelomonocytic cells and human endometrial stromal cells. *Molecular Endocrinology* **19** 2222–2233. (https://doi.org/10.1210/me.2004-0336)

Du H, Daftary GS, Lalwani SI & Taylor HS 2005*b* Direct regulation of HOXA10 by 1,25-(OH) 2D3 in human myelomonocytic cells and human endometrial stromal cells. *Molecular Endocrinology* **19** 2222–2233. (https://doi.org/10.1210/me.2004-0336)

Eagleton C & Judkins A 2006 Vitamin D deficiency in pregnant New Zealand women. *New Zealand Medical Journal* **119** U2144.

Evans J 2016 Hyperglycosylated hCG: a unique human implantation and invasion factor. *American Journal of Reproductive Immunology* **75** 333–340. (https://doi.org/10.1111/aji.12459)

Evans KN, Bulmer JN, Kilby MD & Hewison M 2004 Vitamin D and placental-decidual function. *Journal of the Society for Gynecologic Investigation* **11** 263–271. (https://doi.org/10.1016/j.jsgi.2004.02.002)

Evans KN, Nguyen L, Chan J, Innes BA, Bulmer JN, Kilby MD & Hewison M 2006 Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. *Biology of Reproduction* **75** 816–822. (https://doi.org/10.1095/biolreprod.106.054056)

Fajol A, Honisch S, Zhang B, Schmidt S, Alkahtani S, Alarifi S, Lang F, Stournaras C & Foller M 2016 Fibroblast growth factor (Fgf) 23 gene transcription depends on actin cytoskeleton reorganization. *FEBS Letters* **590** 705–715. (https://doi.org/10.1002/1873-3468.12096)

Fischer KD & Agrawal DK 2014 Vitamin D regulating TGF-beta induced epithelial-mesenchymal transition. *Respiratory Research* **15** 146. (https://doi.org/10.1186/s12931-014-0146-6)

Goldman-Wohl D & Yagel S 2002 Regulation of trophoblast invasion: from normal implantation to pre-eclampsia. *Molecular and Cellular Endocrinology* **187** 233–238. (https://doi.org/10.1016/S0303-7207(01)00687-6)

Gray TK, Lester GE & Lorenc RS 1979 Evidence for extra-renal 1 alpha-hydroxylation of 25-hydroxyvitamin D3 in pregnancy. *Science* **204** 1311–1313. (https://doi.org/10.1126/science.451538)

Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM & Adorini L 2001 Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. *Journal of Immunology* **167** 1945–1953. (https://doi.org/10.4049/jimmunol.167.4.1945)

Gude NM, Roberts CT, Kalionis B & King RG 2004 Growth and function of the normal human placenta. *Thrombosis Research* **114** 397–407. (https://doi.org/10.1016/j.thromres.2004.06.038)

Gui J, Rohrbach A, Borns K, Hillemanns P, Feng L, Hubel CA & von Versen-Hoynck F 2015 Vitamin D rescues dysfunction of fetal endothelial colony forming cells from individuals with gestational diabetes. *Placenta* **36** 410–418. (https://doi.org/10.1016/j.placenta.2015.01.195)

Gysler SM, Mulla MJ, Stuhlman M, Sfakianaki AK, Paidas MJ, Stanwood NL, Gariepy A, Brosens JJ, Chamley LW & Abrahams VM 2015 Vitamin D reverses aPL-induced inflammation and LMWH-induced sFlt-1 release by human trophoblast. *American Journal of Reproductive Immunology* **73** 242–250. (https://doi.org/10.1111/aji.12301)

Halder SK, Osteen KG & Al-Hendy A 2013 Vitamin D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells. *Human Reproduction* **28** 2407–2416. (https://doi.org/10.1093/humrep/det265)

Halhali A, Acker GM & Garabedian M 1991 1,25-Dihydroxyvitamin D3 induces in vivo the decidualization of rat endometrial cells. *Journal of Reproduction and Fertility* **91** 59–64. (https://doi.org/10.1530/jrf.0.0910059)

Halhali A, Diaz L, Sanchez I, Garabedian M, Bourges H & Larrea F 1999 Effects of IGF-I on 1,25-dihydroxyvitamin D(3) synthesis by human placenta in culture. *Molecular Human Reproduction* **5** 771–776. (https://doi.org/10.1093/molehr/5.8.771)

Halloran BP & DeLuca HF 1980 Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. *Journal of Nutrition* **110** 1573–1580.

Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM & Endocrine Society 2011 Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* **96** 1911–1930. (https://doi.org/10.1210/jc.2011-0385)

Hou YF, Gao SH, Wang P, Zhang HM, Liu LZ, Ye MX, Zhou GM, Zhang ZL & Li BY 2016 1alpha,25(OH)(2)D(3) suppresses the migration of ovarian cancer SKOV-3 cells through the inhibition of epithelial-mesenchymal transition. *International Journal of Molecular Sciences* **17** 1285. (https://doi.org/10.3390/ijms17081285)

Javaid M, Crozier S, Harvey N, Gale C, Dennison E, Boucher B, Arden N, Godfrey K, Cooper C & Group PAHS 2006 Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* **367** 36–43. (https://doi.org/10.1016/S0140-6736(06)67922-1)

Johnson LE & DeLuca HF 2002 Reproductive defects are corrected in vitamin d-deficient female rats fed a high calcium, phosphorus and lactose diet. *Journal of Nutrition* **132** 2270–2273.

Jones G, Strugnell SA & DeLuca HF 1998 Current understanding of the molecular actions of vitamin D. *Physiological Reviews* **78** 1193–1231.

Kachkache M, Rebut-Bonneton C, Demignon J, Cynober E & Garabedian M 1993 Uterine cells other than stromal decidual cells are required for 1,25-dihydroxyvitamin D3 production during early human pregnancy. *FEBS Letters* **333** 83–88. (https://doi.org/10.1016/0014-5793(93)80379-9)

Kadyrov M, Kingdom JCP & Huppertz B 2006 Divergent trophoblast invasion and apoptosis in placental bed spiral arteries from pregnancies complicated by maternal anemia and early-onset preeclampsia/intrauterine growth restriction. *American Journal of Obstetrics and Gynecology* **194** 557–563. (https://doi.org/10.1016/j.ajog.2005.07.035)

Karlsson T, Andersson L, Hussain A, Bosaeus M, Jansson N, Osmancevic A, Hulthen L, Holmang A & Larsson I 2015 Lower vitamin D status in obese compared with normal-weight women despite higher vitamin D intake in early pregnancy. *Clinical Nutrition* **34** 892–898. (https://doi.org/10.1016/j.clnu.2014.09.012)

Kaufmann P, Black S & Huppertz B 2003 Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biology of Reproduction* **69** 1–7. (https://doi.org/10.1095/biolreprod.102.014977)

Keryer G, Alsat E, Tasken K & Evain-Brion D 1998 Cyclic AMP-dependent protein kinases and human trophoblast cell differentiation in vitro. *Journal of Cell Science* **111** 995–1004.

Khong TY 2008 The pathology of placenta accreta, a worldwide epidemic. *Journal of Clinical Pathology* **61** 1243. (https://doi.org/10.1136/jcp.2008.055202)

Kovacs CS & Kronenberg HM 1997 Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. *Endocrine Reviews* **18** 832–872. (https://doi.org/10.1210/edrv.18.6.0319)

Krishnan AV, Swami S & Feldman D 2012 The potential therapeutic benefits of vitamin D in the treatment of estrogen receptor positive breast cancer. *Steroids* **77** 1107–1112. (https://doi.org/10.1016/j.steroids.2012.06.005)

Kwiecinksi GG, Petrie GI & DeLuca HF 1989 1,25-Dihydroxyvitamin D3 restores fertility of vitamin D-deficient female rats. *American Journal of Physiology: Endocrinology and Metabolism* **256** E483–E487.

Lewis S, Lucas RM, Halliday J & Ponsonby AL 2010 Vitamin D deficiency and pregnancy: from preconception to birth. *Molecular Nutrition and Food Research* **54** 1092–1102. (https://doi.org/10.1002/mnfr.201000044)

Leyssens C, Verlinden L & Verstuyf A 2014 The future of vitamin D analogs. *Frontiers in Physiology* **5** 122.

Liu NQ & Hewison M 2012 Vitamin D, the placenta and pregnancy. *Archives of Biochemistry and Biophysics* **523** 37–47. (https://doi.org/10.1016/j.abb.2011.11.018)

Liu N, Kaplan AT, Low J, Nguyen L, Liu GY, Equils O & Hewison M 2009 Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway. *Biology of Reproduction* **80** 398–406. (https://doi.org/10.1095/biolreprod.108.073577)

Liu NQ, Kaplan AT, Lagishetty V, Ouyang YB, Ouyang Y, Simmons CF, Equils O & Hewison M 2011 Vitamin D and the regulation of

placental inflammation. *Journal of Immunology* **186** 5968–5974. (https://doi.org/10.4049/jimmunol.1003332)

Ma Y, Samaraweera M, Cooke-Hubley S, Kirby BJ, Karaplis AC, Lanske B & Kovacs CS 2014 Neither absence nor excess of FGF23 disturbs murine fetal-placental phosphorus homeostasis or prenatal skeletal development and mineralization. *Endocrinology* **155** 1596–1605. (https://doi.org/10.1210/en.2013-2061)

Ma Y, Johnson CS & Trump DL 2016 Mechanistic insights of vitamin D anticancer effects. *Vitamins and Hormones* **100** 395–431.

Maghbooli Z, Hossein-nezhad A, Karimi F, Shafaei AR & Larijani B 2008 Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. *Diabetes/Metabolism Research and Reviews* **24** 27–32. (https://doi.org/10.1002/dmrr.737)

McGrath J 2001 Does 'imprinting'with low prenatal vitamin D contribute to the risk of various adult disorders? *Medical Hypotheses* **56** 367–371. (https://doi.org/10.1054/mehy.2000.1226)

Menkhorst E, Winship A, Van Sinderen M & Dimitriadis E 2016 Human extravillous trophoblast invasion: intrinsic and extrinsic regulation. *Reproduction, Fertility, and Development* **28** 406–415. (https://doi.org/10.1071/RD14208)

Moller UK, Streym S, Heickendorff L, Mosekilde L & Rejnmark L 2012 Effects of 25OHD concentrations on chances of pregnancy and pregnancy outcomes: a cohort study in healthy Danish women. *European Journal of Clinical Nutrition* **66** 862–868. (https://doi.org/10.1038/ejcn.2012.18)

Moore JM, Nahlen BL, Misore A, Lal AA & Udhayakumar V 1999 Immunity to placental malaria. I. Elevated production of interferon-γ by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. *Journal of Infectious Diseases* **179** 1218–1225. (https://doi.org/10.1086/314737)

Nguyen TP, Yong HE, Chollangi T, Borg AJ, Brennecke SP & Murthi P 2015 Placental vitamin D receptor expression is decreased in human idiopathic fetal growth restriction. *Journal of Molecular Medicine* **93** 795–805. (https://doi.org/10.1007/s00109-015-1267-1)

Novakovic B, Sibson M, Ng HK, Manuelpillai U, Rakyan V, Down T, Beck S, Fournier T, Evain-Brion D, Dimitriadis E, *et al*. 2009 Placenta-specific methylation of the vitamin D 24-hydroxylase gene: implications for feedback autoregulation of active vitamin D levels at the fetomaternal interface. *Journal of Biological Chemistry* **284** 14838–14848. (https://doi.org/10.1074/jbc.M809542200)

Noyola-Martinez N, Diaz L, Avila E, Halhali A, Larrea F & Barrera D 2013 Calcitriol downregulates TNF-alpha and IL-6 expression in cultured placental cells from preeclamptic women. *Cytokine* **61** 245–250. (https://doi.org/10.1016/j.cyto.2012.10.001)

Noyola-Martinez N, Diaz L, Zaga-Clavellina V, Avila E, Halhali A, Larrea F & Barrera D 2014 Regulation of CYP27B1 and CYP24A1 gene expression by recombinant pro-inflammatory cytokines in cultured human trophoblasts. *Journal of Steroid Biochemistry and Molecular Biology* **144** 106–109. (https://doi.org/10.1016/j.jsbmb.2013.12.007)

Ohata Y, Yamazaki M, Kawai M, Tsugawa N, Tachikawa K, Koinuma T, Miyagawa K, Kimoto A, Nakayama M, Namba N, *et al*. 2014 Elevated fibroblast growth factor 23 exerts its effects on placenta and regulates vitamin D metabolism in pregnancy of Hyp mice. *Journal of Bone and Mineral Research* **29** 1627–1638. (https://doi.org/10.1002/jbmr.2186)

Oreshkova T, Dimitrov R & Mourdjeva M 2012 A cross-talk of decidual stromal cells, trophoblast, and immune cells: a prerequisite for the success of pregnancy. *American Journal of Reproductive Immunology* **68** 366–373. (https://doi.org/10.1111/j.1600-0897.2012.01165.x)

Ota K, Dambaeva S, Kim MW, Han AR, Fukui A, Gilman-Sachs A, Beaman K & Kwak-Kim J 2015 1,25-Dihydroxy-vitamin D3 regulates NK-cell cytotoxicity, cytokine secretion, and degranulation in women with recurrent pregnancy losses. *European Journal of Immunology* **45** 3188–3199. (https://doi.org/10.1002/eji.201545541)

Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C & Pal L 2010 Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertility and Sterility* **94** 1314–1319. (https://doi.org/10.1016/j.fertnstert.2009.05.019)

Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN & Goltzman D 2001 Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *PNAS* **98** 7498–7503. (https://doi.org/10.1073/pnas.131029498)

Pantham P, Aye ILMH & Powell TL 2015 Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta* **36** 709–715. (https://doi.org/10.1016/j.placenta.2015.04.006)

Piccinni MP, Scaletti C, Maggi E & Romagnani S 2000 Role of hormone-controlled Th1- and Th2-type cytokines in successful pregnancy. *Journal of Neuroimmunology* **109** 30–33. (https://doi.org/10.1016/S0165-5728(00)00299-X)

Pospechova K, Rozehnal V, Stejskalova L, Vrzal R, Pospisilova N, Jamborova G, May K, Siegmund W, Dvorak Z, Nachtigal P, *et al*. 2009 Expression and activity of vitamin D receptor in the human placenta and in choriocarcinoma BeWo and JEG-3 cell lines. *Molecular and Cellular Endocrinology* **299** 178–187. (https://doi.org/10.1016/j.mce.2008.12.003)

Reddy UM, Ko C-W & Willinger M 2006 Maternal age and the risk of stillbirth throughout pregnancy in the United States. *American Journal of Obstetrics and Gynecology* **195** 764–770. (https://doi.org/10.1016/j.ajog.2006.06.019)

Ringertz N 1970 Hydatidiform mole, invasive mole and choriocarcinoma in Sweden 1958–1965. *Acta Obstetricia et Gynecologica Scandinavica* **49** 195–203. (https://doi.org/10.3109/00016347009158054)

Rojansky N, Brzezinski A & Schenker JG 1992 Seasonality in human reproduction: an update. *Human Reproduction* **7** 735–745. (https://doi.org/10.1093/oxfordjournals.humrep.a137729)

Rojansky N, Benshushan A, Meirsdorf S, Lewin A, Laufer N & Safran A 2000 Seasonal variability in fertilization and embryo quality rates in women undergoing IVF. *Fertility and Sterility* **74** 476–481. (https://doi.org/10.1016/S0015-0282(00)00669-5)

Rudge CV, Rollin HB, Nogueira CM, Thomassen Y, Rudge MC & Odland JO 2009 The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. *Journal of Environmental Monitoring* **11** 1322–1330. (https://doi.org/10.1039/b903805a)

Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK & Bhatia V 2005 High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *American Journal of Clinical Nutrition* **81** 1060–1064.

Schneuer FJ, Roberts CL, Guilbert C, Simpson JM, Algert CS, Khambalia AZ, Tasevski V, Ashton AW, Morris JM & Nassar N 2014 Effects of maternal serum 25-hydroxyvitamin D concentrations in the first trimester on subsequent pregnancy outcomes in an Australian population. *American Journal of Clinical Nutrition* **99** 287–295. (https://doi.org/10.3945/ajcn.113.065672)

Stephanou A, Ross R & Handwerger S 1994 Regulation of human placental lactogen expression by 1, 25-dihydroxyvitamin D3. *Endocrinology* **135** 2651–2656. (https://doi.org/10.1210/endo.135.6.7988455)

Tamblyn JA, Hewison M, Wagner CL, Bulmer JN & Kilby MD 2015 Immunological role of vitamin D at the maternal-fetal interface. *Journal of Endocrinology* **224** R107–R121. (https://doi.org/10.1530/JOE-14-0642)

Tamblyn JA, Susarla R, Jenkinson C, Jeffery LE, Ohizua O, Chun RF, Chan SY, Kilby MD & Hewison M 2017 Dysregulation of maternal and placental vitamin D metabolism in preeclampsia. *Placenta* **50** 70–77. (https://doi.org/10.1016/j.placenta.2016.12.019)

Tavakoli M, Jeddi-Tehrani M, Salek-Moghaddam A, Rajaei S, Mohammadzadeh A, Sheikhhasani S, Kazemi-Sefat GE & Zarnani AH 2011 Effects of 1,25(OH)2 vitamin D3 on cytokine production by endometrial cells of women with recurrent spontaneous abortion.

Fertility and Sterility **96** 751–757. (https://doi.org/10.1016/j.fertnstert.2011.06.075)

Tavakoli M, Salek-Moghaddam A, Jeddi-Tehrani M, Talebi S, Kazemi-Sefat GE, Vafaei S, Mohammadzadeh A, Sheikhhassani S & Zarnani AH 2015 Comparable vitamin D3 metabolism in the endometrium of patients with recurrent spontaneous abortion and fertile controls. *Molecular Reproduction and Development* **82** 356–364. (https://doi.org/10.1002/mrd.22486)

Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, Aganna E, Price CP & Boucher BJ 2002 Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM* **95** 787–796. (https://doi.org/10.1093/qjmed/95.12.787)

Townsend K, Evans KN, Campbell MJ, Colston KW, Adams JS & Hewison M 2005 Biological actions of extra-renal 25-hydroxyvitamin D-1alpha-hydroxylase and implications for chemoprevention and treatment. *Journal of Steroid Biochemistry and Molecular Biology* **97** 103–109. (https://doi.org/10.1016/j.jsbmb.2005.06.004)

Tuan RS, Moore CJ, Brittingham JW, Kirwin JJ, Akins RE & Wong M 1991 In vitro study of placental trophoblast calcium uptake using JEG-3 human choriocarcinoma cells. *Journal of Cell Science* **98** 333–342.

van der Meer IM, Karamali NS, Boeke AJP, Lips P, Middelkoop BJ, Verhoeven I & Wuister JD 2006 High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. *American Journal of Clinical Nutrition* **84** 350–353.

Vigano P, Mangioni S, Pompei F & Chiodo I 2003 Maternal-conceptus cross talk—a review. *Placenta* **24** S56–S61. (https://doi.org/10.1016/S0143-4004(03)00137-1)

von Versen-Hoynck F, Brodowski L, Dechend R, Myerski AC & Hubel CA 2014 Vitamin D antagonizes negative effects of preeclampsia on fetal endothelial colony forming cell number and function. *PLoS ONE* **9** e98990. (https://doi.org/10.1371/journal.pone.0098990)

Wagner CL & Greer FR 2008 Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* **122** 1142–1152. (https://doi.org/10.1542/peds.2008-1862)

Wang LQ, Yan XT, Yan CF, Zhang XW, Hui LY, Xue M & Yu XW 2016 Women with recurrent miscarriage have decreased expression of 25-hydroxyvitamin D3-1alpha-hydroxylase by the fetal-maternal interface. *PLoS ONE* **11** e0165589. (https://doi.org/10.1371/journal.pone.0165589)

Weisman Y, Harell A, Edelstein S, David M, Spirer Z & Golander A 1979 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta. *Nature* **281** 317–319. (https://doi.org/10.1038/281317a0)

Westwood MA, Al-Saghir K, Finn-Sell S, Tan C, Cowley E, Berneau S, Adlam D & Johnstone E 2017 Vitamin D attenuates sphingosine-1-phosphate (S1P)-mediated inhibition of extravillous trophoblast migration. *Placenta* **60** 1–8. (https://doi.org/10.1016/j.placenta.2017.09.009)

Xu J, Jia X, Gu Y, Lewis DF, Gu X & Wang Y 2017 Vitamin D reduces oxidative stress-induced procaspase-3/ROCK1 activation and MP release by placental trophoblasts. *Journal of Clinical Endocrinology and Metabolism* **102** 2100–2110. (https://doi.org/10.1210/jc.2016-3753)

Yang K 1997 Placental 11 beta-hydroxysteroid dehydrogenase: barrier to maternal glucocorticoids. *Reviews of Reproduction* **2** 129–132. (https://doi.org/10.1530/ror.0.0020129)

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, *et al*. 1997 Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nature Genetics* **16** 391–396. (https://doi.org/10.1038/ng0897-391)

Zehnder D, Evans KN, Kilby MD, Bulmer JN, Innes BA, Stewart PM & Hewison M 2002 The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua. *American Journal of Pathology* **161** 105–114. (https://doi.org/10.1016/S0002-9440(10)64162-4)

Zeng N, Salker MS, Zhang S, Singh Y, Shi B, Stournaras C & Lang F 2016 1alpha,25(OH)2D3 induces actin depolymerization in endometrial carcinoma cells by targeting RAC1 and PAK1. *Cellular Physiology and Biochemistry* **40** 1455–1464. (https://doi.org/10.1159/000453197)

Zhang C, Qiu C, Hu FB, David RM, Van Dam RM, Bralley A & Williams MA 2008 Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. *PLoS One* **3** e3753. (https://doi.org/10.1371/journal.pone.0003753)

Received in final form 24 October 2017
Accepted 6 November 2017
Accepted Preprint published online 6 November 2017