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

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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$(OH)_2$D). 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$(OH)_2$D, 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$(OH)_2$D remains unclear, but is likely to involve localised tissue-specific responses with both decidua and trophoblast also expressing the vitamin D receptor (VDR) for 1,25$(OH)_2$D. We have previously described immunomodulatory responses to 1,25$(OH)_2$D 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$(OH)_2$D 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.

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, whilst protecting against maternal immunologic attack. The main functions of the placenta can be broadly categorised 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. As well as facilitating a good maternal blood 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 (Gude et al. 2004; Moore, et al. 1999; Rudge, et al. 2009; Yang 1997). 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$(OH)_2$D), as well as the 1α-hydroxylase enzyme that synthesizes 1,25$(OH)_2$D (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 amongst 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$(OH)_2$D, 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$(OH)_2$D 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 and 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 and Bruns 1983). One possible explanation is that 1,25$(OH)_2$D acts as a regulator of placental calcium transport (Bruns and Bruns 1983), but a placental immunomodulatory function has also been proposed (Liu and 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$(OH)_2$D has been shown to regulate expression of the homeobox gene HOXA10 in human endometrial stromal cells (Du, et al. 2005b). 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 and DeLuca 1980). This was shown to be corrected by administration of 1,25$(OH)_2$D (Kwiecinksi, et al. 1989), but also by use of diets high in calcium, phosphate and lactose (Johnson and 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 signalling system in the process of implantation, with *Vdr* -/- and *Cyp27b1* -/- female mice both presenting with uterine hypoplasia and infertility (Panda, et al. 2001; Yoshizawa, et al. 1997). Conversely, injection of 1,25$(OH)_2$D has been shown to increase uterine weight and promote endometrial to decidual differentiation (Halhali, et al. 1991).

As well as 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$(OH)_2$D has the potential to influence uterine natural killer cells, dendritic cells, macrophages, and T-cells (Evans, et al. 2004; 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$(OH)_2$D production (Kachkache, et al. 1993). Furthermore, first-trimester decidual cells treated with either 25OHD- or 1,25$(OH)_2$D 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 and Hewison 2012).

Vitamin D metabolism and function in trophoblast cells

The organisation of maternal and fetal cells within the developing placenta has been well documented elsewhere (Oreshkova, et al. 2012; Vigano, et al. 2003), and is represented schematically in **Figure 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$(OH)_2$D (Gray et al. 1979; Weisman et al. 1979). The resulting tissue concentrations of 1,25$(OH)_2$D 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$(OH)_2$D, either in an autocrine or paracrine fashion.

To date, studies of the physiological impact of decidual-trophoblast 1,25$(OH)_2$D 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$(OH)_2$D (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 remodelling agents suggesting that 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 syncytialisation (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 signalling 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$(OH)_2$D by these

cells appears to be significantly less than 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$(OH)_2$D.

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$(OH)_2$D 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$(OH)_2$D. 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$(OH)_2$D, consistent with a role for vitamin D in the endocrinology of placental calcium homeostasis. However, subsequent investigations of trophoblast cells and 1,25$(OH)_2$D 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$(OH)_2$D has been shown to potently stimulate expression of the antibacterial protein cathelicidin (Liu et al. 2009), whilst also suppressing inflammatory responses to tumor necrosis factor α (TNFα) (Diaz, et al. 2009). Similar anti-inflammatory responses to 1,25$(OH)_2$D 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$(OH)_2$D 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 remodelling, cell trafficking, and immune tolerance being evident (Oreshkova et al. 2012). The mechanisms underpinning these processes have received increasing attention since abnormal placentation due to shallow invasion of EVT can cause important pregnancy disorders such as miscarriage (Ball, et al. 2006), pre-eclampsia (Caniggia, et al. 2000), fetal growth restriction, pre-term birth, and stillbirth (Reddy, et al. 2006) (Goldman-Wohl and Yagel 2002; Kadyrov, et al. 2006; Kaufmann, et al. 2003). 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 (Caniggia et al. 2000; Ringertz 1970), 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$(OH)_2$D (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$(OH)_2$D inhibition of matrix invasion by tumor cells (Bao, et al. 2006). In this case the primary mode of action for 1,25$(OH)_2$D 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$(OH)_2$D 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) (Chen, et al. 2015; Fischer and Agrawal 2014; 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 polymerisation 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$(OH)_2$D 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$(OH)_2$D has also been shown to stimulate hCG expression and secretion via a cAMP/PKA-mediated signalling 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 (Du, et al. 2005a), 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. 2011a). 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. 2011b). 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 and 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 which 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. 2007b), 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 and 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* fertilisation (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 et al. 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 >50nmol/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 et al. 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 to 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$(OH)_2$D 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$(OH)_2$D 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$(OH)_2$D 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 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 5,000 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 signalling (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. 2007a; 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 anti-microbial 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 (**Figure 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$(OH)_2$D, 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.

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Declaration of Interests

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

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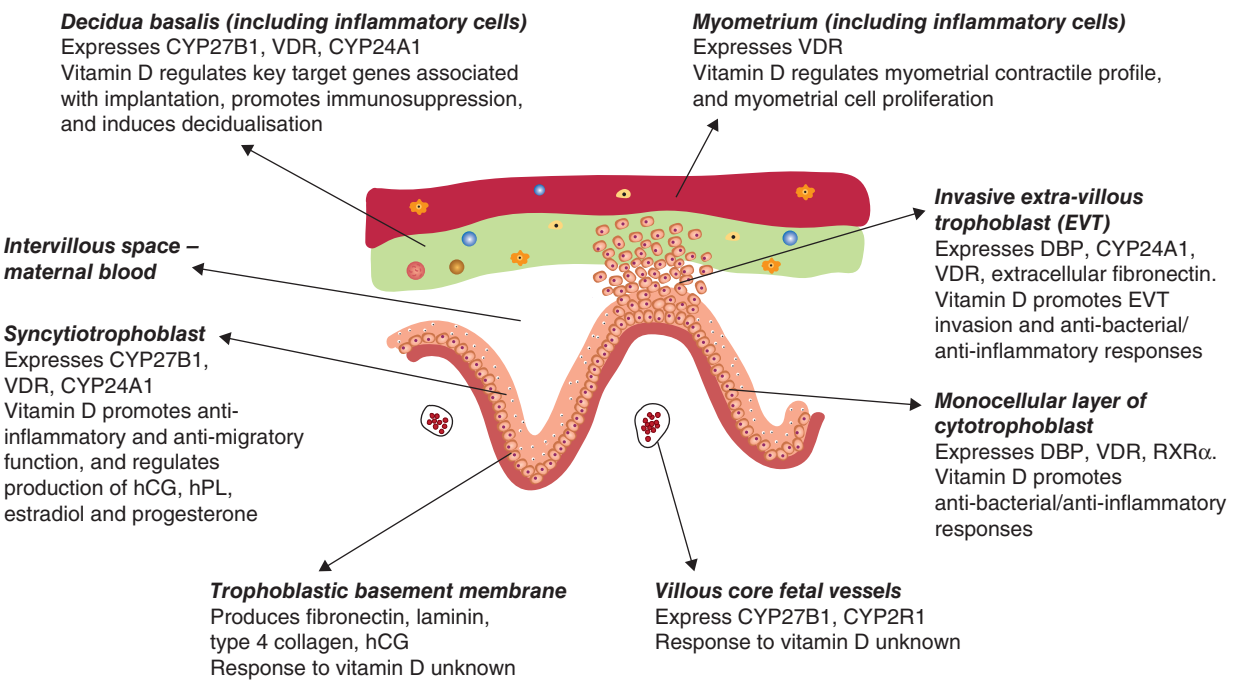
Legend to figure

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: vitamin D binding protein (DBP); vitamin D receptor (VDR); retinoid X receptor (RXR); vitamin D-25-hydroxylase (CYP2R1); 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1); vitamin D-24-hydroxylase (CYP24A1); human chorionic gonadotropin (hCG); human prolactin (hPL).


Decidua basalis (including inflammatory cells)
Expresses CYP27B1, VDR, CYP24A1
Vitamin D regulates key target genes associated with implantation, promotes immunosuppression, and induces decidualisation
Myometrium (including inflammatory cells)
Expresses VDR
Vitamin D regulates myometrial contractile profile, and myometrial cell proliferation
Invasive extra-villous trophoblast (EVT)
Expresses DBP, CYP24A1, VDR, extracellular fibronectin.
Vitamin D promotes EVT invasion and anti-bacterial/ anti-inflammatory responses
Intervillous space – maternal blood
Syncytiotrophoblast
Expresses CYP27B1, VDR, CYP24A1
Vitamin D promotes anti-inflammatory and anti-migratory function, and regulates production of hCG, hPL, estradiol and progesterone
Monocellular layer of cytotrophoblast
Expresses DBP, VDR, RXRα.
Vitamin D promotes anti-bacterial/anti-inflammatory responses
Trophoblastic basement membrane
Produces fibronectin, laminin, type 4 collagen, hCG
Response to vitamin D unknown
Villous core fetal vessels
Express CYP27B1, CYP2R1
Response to vitamin D unknown