

## Vitamin D, the placenta and early pregnancy

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

3  
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33

34 **Abstract**

35 Pregnancy is associated with significant changes in vitamin D metabolism, notably increased  
36 maternal serum levels of active vitamin D, 1,25-dihydroxyvitamin (1,25(OH)<sub>2</sub>D). This appears  
37 to be due primarily to increased renal activity of the enzyme 25-hydroxyvitamin D-1α-  
38 hydroxylase (CYP27B1) that catalyzes synthesis of 1,25(OH)<sub>2</sub>D, but CYP27B1 expression is  
39 also prominent in both the maternal decidua and fetal trophoblast components of the  
40 placenta. The precise function of placental synthesis of 1,25(OH)<sub>2</sub>D remains unclear, but is  
41 likely to involve localised tissue-specific responses with both decidua and trophoblast also  
42 expressing the vitamin D receptor (VDR) for 1,25(OH)<sub>2</sub>D. We have previously described  
43 immunomodulatory responses to 1,25(OH)<sub>2</sub>D by diverse populations of VDR-expressing  
44 cells within the decidua. The aim of the current review is to detail the role of vitamin D in  
45 pregnancy from a trophoblast perspective, with particular emphasis on the potential role of  
46 1,25(OH)<sub>2</sub>D as a regulator of trophoblast invasion in early pregnancy. Vitamin D-deficiency is  
47 common in pregnant women, and a wide range of studies have linked low vitamin D status to  
48 adverse events in pregnancy. To date most of these studies have focused on adverse  
49 events later in pregnancy, but the current review will explore the potential impact of vitamin  
50 D on early pregnancy, and how this may influence implantation and miscarriage.

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## 58 **Introduction**

59 The human placenta is a vital organ without which the mammalian fetus cannot survive. It  
60 forms the interface between the mother and fetus, supplying the fetus with oxygen, nutrients,  
61 excreting waste products, whilst protecting against maternal immunologic attack. The main  
62 functions of the placenta can be broadly categorised into transport and metabolism,  
63 protection and endocrine (Gude, et al. 2004). The complex architecture of the placenta,  
64 bounded by the maternal aspect (basal plate) and the fetal aspect (chorionic plate), houses  
65 an abundance of the fundamental functional unit of the placenta, the chorionic villus, where  
66 all nutritional-waste exchange between the maternal blood and the fetal circulation occurs.  
67 As well as facilitating a good maternal blood supply for nutrition-waste exchange, and  
68 orchestrating endocrine mediators of pregnancy to maintain maternal physiological changes  
69 for an optimal environment for fetal development, the placenta also acts to protect the fetus  
70 from xenobiotic materials and infectious agents (Gude et al. 2004; Moore, et al. 1999;  
71 Rudge, et al. 2009; Yang 1997). Successful development of the placenta involves two  
72 distinct mechanisms: implantation of the blastocyst, initiated by attachment of the embryo to  
73 the maternal endometrial epithelium, and invasion of fetal trophoblast cells into the maternal  
74 endometrium to facilitate maternal-fetal exchange of nutrients, gases and waste. The diverse  
75 mechanisms associated with the regulation of trophoblast invasion have been well  
76 documented (Menkhorst, et al. 2016). The aim of the current review is to provide an  
77 overview of these early events in placental development, with particular emphasis on the  
78 potential role of vitamin D as a determinant of early placental development through effects  
79 on trophoblast cells, particularly via effects of vitamin D on trophoblast invasion.

80

## 81 **Vitamin D and pregnancy**

82 Despite its long-standing association with rickets and osteoporosis, vitamin D has become  
83 increasingly recognized as a pluripotent regulator of biological functions above and beyond  
84 its classical effects on bone and calcium homeostasis. Expression of vitamin D receptor

85 (VDR) for the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D), as well as the  
86 1 $\alpha$ -hydroxylase enzyme that synthesizes 1,25(OH)<sub>2</sub>D (CYP27B1), has been reported for  
87 various tissues that can be broadly termed 'barrier sites' (Jones, et al. 1998; Townsend, et  
88 al. 2005), indicating that localized responses to vitamin D may be a key feature of these  
89 tissues. Prominent amongst these barrier sites is the placenta, acting as the interface  
90 between mother and fetus. Historically, the placenta was one of the first extra-renal tissues  
91 shown to be capable of synthesizing 1,25(OH)<sub>2</sub>D, with CYP27B1 activity detectable in both  
92 maternal decidua and fetal trophoblast (Gray, et al. 1979; Weisman, et al. 1979). Initially,  
93 this was linked to the rise in maternal serum 1,25(OH)<sub>2</sub>D that occurs at the end of the first  
94 trimester of pregnancy. However, studies of CYP27B1-deficient animals and an anephric  
95 pregnant woman indicated that this is not likely to be the case (Kovacs and Kronenberg  
96 1997). Instead, the presence of VDR in the placenta suggests that vitamin D functions in  
97 tissue-specific fashion at the fetal-maternal interface (Bruns and Bruns 1983). One possible  
98 explanation is that 1,25(OH)<sub>2</sub>D acts as a regulator of placental calcium transport (Bruns and  
99 Bruns 1983), but a placental immunomodulatory function has also been proposed (Liu and  
100 Hewison 2012). Moreover, the rapid induction of VDR and CYP27B1 early in pregnancy  
101 (Zehnder, et al. 2002) suggests that vitamin D may play a more fundamental role in the  
102 process of conception, implantation and development of the placenta itself.

103

#### 104 **Vitamin D and implantation**

105 To date the precise role of vitamin D in the process of implantation remains unclear.  
106 Nevertheless, vitamin D has a biologically plausible role in female reproduction and  
107 implantation process. 1,25(OH)<sub>2</sub>D has been shown to regulate expression of the homeobox  
108 gene HOXA10 in human endometrial stromal cells (Du, et al. 2005b). HOXA10 is important  
109 for the development of the uterus during fetal life and, later in adulthood, is essential for  
110 endometrial development, allowing uterine receptivity to implantation (Bagot, et al. 2000).  
111 Interestingly, animal studies have shown that vitamin D-deficiency reduces mating success

112 and fertility in female rats. Female rats fed with a vitamin D-deficient diet are capable of  
113 reproduction, but overall fertility is reduced including the failure of implantation (Halloran and  
114 DeLuca 1980). This was shown to be corrected by administration of  $1,25(\text{OH})_2\text{D}$  (Kwiecek, et al. 1989),  
115 but also by use of diets high in calcium, phosphate and lactose (Johnson and  
116 DeLuca 2002), suggesting that the fertility effects of vitamin D may be due to indirect effects  
117 on mineral homeostasis. Other studies using knockout mouse models have further  
118 highlighted the importance of the vitamin D metabolic and signalling system in the process of  
119 implantation, with *Vdr* *-/-* and *Cyp27b1* *-/-* female mice both presenting with uterine  
120 hypoplasia and infertility (Panda, et al. 2001; Yoshizawa, et al. 1997). Conversely, injection  
121 of  $1,25(\text{OH})_2\text{D}$  has been shown to increase uterine weight and promote endometrial to  
122 decidual differentiation (Halhali, et al. 1991).

123

124 As well as regulating uterine and decidual development, vitamin D may also influence  
125 implantation indirectly via its well-known immunomodulatory actions. Regulation of immune  
126 function at the maternal-fetal interface involves a heterogeneous population of innate and  
127 adaptive immune cell subsets. Thus throughout pregnancy, decidual synthesis of  
128  $1,25(\text{OH})_2\text{D}$  has the potential to influence uterine natural killer cells, dendritic cells,  
129 macrophages, and T-cells (Evans, et al. 2004; Tamblyn, et al. 2015). Notable effects include  
130 inhibition of Th1 cytokines and promotion of Th2 cytokines (Gregori, et al. 2001), which are  
131 known to play a significant role in the process of implantation (Piccinni, et al. 2000; Zehnder  
132 et al. 2002). Purification of decidual cells into non-adherent stromal cells and adherent cells,  
133 which include decidual macrophages and uterine natural killer cells, has shown that  
134 adherent cells demonstrate a greater capacity for  $1,25(\text{OH})_2\text{D}$  production (Kachkache, et al.  
135 1993). Furthermore, first-trimester decidual cells treated with either  $25\text{OHD}$ - or  $1,25(\text{OH})_2\text{D}$   
136 demonstrate significant induction of antibacterial protein cathelicidin and  $\beta$ -defensins (Evans,  
137 et al. 2006; Liu, et al. 2009). Since similar effects of vitamin D are observed in peripheral  
138 monocytes, an equivalent innate antimicrobial responsivity is postulated to exist at the  
139 maternal-fetal interface (Liu and Hewison 2012).

140

141 **Vitamin D metabolism and function in trophoblast cells**

142 The organisation of maternal and fetal cells within the developing placenta has been well  
143 documented elsewhere (Oreshkova, et al. 2012; Vigano, et al. 2003), and is represented  
144 schematically in **Figure 1**. Both the maternal decidua and fetal trophoblast components of  
145 the placenta (including syncytiotrophoblast and invasive extravillous trophoblast [EVT])  
146 express CYP27B1 (Zehnder et al. 2002), and are able to produce detectable levels of  
147 1,25(OH)<sub>2</sub>D (Gray et al. 1979; Weisman et al. 1979). The resulting tissue concentrations of  
148 1,25(OH)<sub>2</sub>D appear to be significantly higher in the decidua (Tamblyn, et al. 2017), but the  
149 coincident expression of VDR in trophoblast as well as decidua (Evans et al. 2004) means  
150 that multiple cell types within the placenta are capable of responding to the locally  
151 synthesized 1,25(OH)<sub>2</sub>D, either in an autocrine or paracrine fashion.

152

153 To date, studies of the physiological impact of decidual-trophoblast 1,25(OH)<sub>2</sub>D production  
154 have focused primarily on trophoblast cells, using both primary cultures of EVT and  
155 trophoblast cells lines. Primary cultures of human syncytiotrophoblast express CYP27B1 and  
156 are able to synthesize 1,25(OH)<sub>2</sub>D (Diaz, et al. 2000), and also express VDR (Pospechova,  
157 et al. 2009). However, in choriocarcinoma trophoblast cell lines such as BeWo and JEG-3,  
158 expression of VDR is low, with analysis of the effects of chromatin remodelling agents  
159 suggesting that this may be due to epigenetic suppression of VDR in these cells  
160 (Pospechova et al. 2009). Further studies to assess the impact of differentiation of cultured  
161 trophoblast cells have been carried out using cyclic AMP (cAMP) to mimic the process of  
162 syncytialisation (Keryer, et al. 1998). Expression of hCG is elevated by cAMP in trophoblast  
163 cells, and this was associated with decreased expression of CYP27B1, with VDR expression  
164 being unaffected (Avila, et al. 2007), suggesting that presence of the vitamin D metabolic  
165 and signalling pathways in the placenta is differentiation-sensitive. The JEG-3 trophoblast  
166 cell line has also been reported to express CYP27B1, but synthesis of 1,25(OH)<sub>2</sub>D by these

167 cells appears to be significantly less than observed with primary trophoblast cells and  
168 unaffected by cAMP (Pospechova et al. 2009). In addition to cAMP, inflammatory cytokines  
169 (Noyola-Martinez, et al. 2014), and insulin-like growth factor I (Halhali, et al. 1999) also  
170 stimulate trophoblast expression of CYP27B1 and synthesis of 1,25(OH)<sub>2</sub>D.

171

172 The vitamin D catabolic enzyme CYP24A1 has been reported to be undetectable in  
173 trophoblast cells, consistent with methylation epigenetic silencing of this gene in the human  
174 placenta (Novakovic, et al. 2009). This suggests that synthesis of 1,25(OH)<sub>2</sub>D by trophoblast  
175 cells is not subject to the same catabolic feedback control observed in other VDR-expressing  
176 tissues. However, other studies have shown that trophoblast expression of CYP24A1 is  
177 increased following treatment with cAMP (Avila et al. 2007). In addition, studies using the  
178 *Hyp* mouse model, which has elevated circulating levels of the positive regulator of 24-  
179 hydroxylase fibroblast growth factor 23 (FGF23), showed elevated placental expression of  
180 CYP24A1 mRNA in these mice (Ma, et al. 2014; Ohata, et al. 2014). Likewise, direct  
181 injection of FGF23 into normal placentas from wild type mice also induced expression of  
182 CYP24A1 (Ohata et al. 2014). This appears to be mediated via trophoblast expression of  
183 fibroblast growth factor receptor 1 and its co-receptor  $\alpha$ -klotho by trophoblast, suggesting  
184 that catabolism via CYP24A1 plays an as yet undefined role in mediating trophoblast effects  
185 of vitamin D.

186

187 Despite a wide range of studies showing regulation and activity of vitamin D metabolic  
188 enzymes in primary trophoblast cells and trophoblast cell lines, the principal functional  
189 analysis of vitamin D in these cells has centered on responses to 1,25(OH)<sub>2</sub>D. Initial  
190 experiments using JEG-3 cells described stimulation of calcium uptake (Tuan, et al. 1991),  
191 and the regulation of the cytosolic calcium binding protein calbindin-D28K (Belkacemi, et al.  
192 2005) by 1,25(OH)<sub>2</sub>D, consistent with a role for vitamin D in the endocrinology of placental  
193 calcium homeostasis. However, subsequent investigations of trophoblast cells and  
194 1,25(OH)<sub>2</sub>D have explored other mechanisms associated with placental endocrine function.



195 These reports include the stimulation of human placental lactogen synthesis and release  
196 (Stephanou, et al. 1994), hCG expression (Barrera, et al. 2008), and the regulation of  
197 estradiol and progesterone synthesis (Barrera, et al. 2007).

198

199 In recent years, our perspective on vitamin D and trophoblast function has been expanded to  
200 include studies of immunomodulatory function. In primary trophoblast cells and trophoblast  
201 cell lines, 1,25(OH)<sub>2</sub>D has been shown to potently stimulate expression of the antibacterial  
202 protein cathelicidin (Liu et al. 2009), whilst also suppressing inflammatory responses to  
203 tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) (Diaz, et al. 2009). Similar anti-inflammatory responses to  
204 1,25(OH)<sub>2</sub>D have also been reported using trophoblasts from women with the inflammatory  
205 disorders of pregnancy, preeclampsia (Noyola-Martinez, et al. 2013), and antiphospholipid  
206 syndrome (APS) (Gysler, et al. 2015). In recent studies the anti-inflammatory effects of  
207 1,25(OH)<sub>2</sub>D on trophoblasts have been reported to include attenuation of oxidative stress-  
208 induced microparticle release from preeclampsia trophoblastic cells (Xu, et al. 2017), further  
209 underlining the importance of this facet of vitamin D function within the placenta. *In vivo*,  
210 studies using *Cyp27b1*<sup>-/-</sup> and *Vdr*<sup>-/-</sup> mice have shown that loss of both alleles for either of  
211 these genes on the fetal side of the placenta alone was sufficient to dramatically exacerbate  
212 anti-inflammatory responses to lipopolysaccharide (LPS) immune challenge (Liu, et al.  
213 2011). Thus, in addition to the active immune cell function classically observed in the  
214 maternal decidua, trophoblast cells also appear to make a major contribution to the  
215 regulation of placental inflammation.

216

### 217 **A role for vitamin D in EVT invasion?**

218 Controlled invasion of fetal cytotrophoblast and differentiated EVT cells into the maternal  
219 decidua and myometrium in the first trimester of pregnancy is a key process in placentation,  
220 and is essential for successful pregnancy. A complex network of communications among  
221 trophoblast, decidual stromal, and immune cells is reported to facilitate implantation and

222 maintenance of pregnancy, with key roles in tissue remodelling, cell trafficking, and immune  
223 tolerance being evident (Oreshkova et al. 2012). The mechanisms underpinning these  
224 processes have received increasing attention since abnormal placentation due to shallow  
225 invasion of EVT can cause important pregnancy disorders such as miscarriage (Ball, et al.  
226 2006), pre-eclampsia (Caniggia, et al. 2000), fetal growth restriction, pre-term birth, and  
227 stillbirth (Reddy, et al. 2006) (Goldman-Wohl and Yagel 2002; Kadyrov, et al. 2006;  
228 Kaufmann, et al. 2003). By contrast, unrestricted invasion resulting from a failure to restrain  
229 the invading cytotrophoblast is associated with premalignant conditions such as malignant  
230 choriocarcinomas and invasive mole (Caniggia et al. 2000; Ringertz 1970), and can lead to  
231 aberrant placentation such as pathological adhesion to the myometrium (placenta accreta),  
232 extension into the myometrium (placenta increta), or invasion through the myometrium into  
233 adjacent organs (placenta percreta) (Khong 2008).

234

235 In recent studies we have shown that human EVT isolated from first trimester pregnancies  
236 are a target for both 25(OH)D and 1,25(OH)<sub>2</sub>D (Chan, et al. 2015). In *ex vivo* experiments  
237 both vitamin D metabolites promoted the invasion of EVT through Matrigel, with zymographic  
238 analysis showing that this effect involves enhanced expression of the matrix  
239 metalloproteinases pro-MMP2 and pro-MMP9 (Chan et al. 2015). These observations are in  
240 direct contrast to previously published studies describing 1,25(OH)<sub>2</sub>D inhibition of matrix  
241 invasion by tumor cells (Bao, et al. 2006). In this case the primary mode of action for  
242 1,25(OH)<sub>2</sub>D was indirect suppression of MMPs via enhanced tissue inhibitor of  
243 metalloproteinase-1 (TIMP-1) expression. However, in other reports, low vitamin D status  
244 has been shown to be associated with elevated circulating MMP2 and MMP9 (Timms, et al.  
245 2002). Suppression of a variety of MMPs, including MMP2 and MMP9, by 1,25(OH)<sub>2</sub>D has  
246 also been described for primary cultures of human uterine fibroid cells and uterine fibroid cell  
247 lines (Halder, et al. 2013). Thus, the pro-invasive effects of vitamin D on EVTs appear to be  
248 quite distinct to pregnancy and the placenta.

249

250 The concept of vitamin D as a regulator of cellular motility and invasion is not novel and has  
251 been extensively reported in cancer states (Krishnan, et al. 2012; Leyssens, et al. 2014; Ma,  
252 et al. 2016), where effects of vitamin D have been related to modulation of epithelial  
253 mesenchymal transition (EMT) (Chen, et al. 2015; Fischer and Agrawal 2014; Hou, et al.  
254 2016). Interestingly, this effect of vitamin D has not been observed in non-pathophysiological  
255 states or during embryogenesis. For example, vitamin D is known to inhibit invasion and  
256 motility of ovarian cancer and teratocarcinoma cell lines, but does not affect these cellular  
257 characteristics in the non-neoplastic ESD3 murine embryonic cell line (Abdelbaset-Ismaïl, et  
258 al. 2016). The precise molecular mechanisms that mediate migration and invasion regulation  
259 by vitamin D remain unclear, although several different pathways have been studied.  
260 Notably, vitamin D has been shown to regulate the actin cytoskeleton in numerous cell  
261 types. In osteoblast-like cells, vitamin D promotes actin polymerisation as part of its  
262 transcriptional induction of fibroblast growth factor 23 (Fajol, et al. 2016). In endometrial  
263 cells, vitamin D treatment has also been shown to induce changes in actin architecture,  
264 through regulation of the RAc1/Pak1 axis (Zeng, et al. 2016). It is not clear if such responses  
265 are also seen in trophoblast cells during placental development, but vitamin D has been  
266 shown to rescue motility defects in fetal endothelial colony forming cell function of umbilical  
267 vein endothelial cells derived from pregnancies complicated by preeclampsia (von Versen-  
268 Hoynck, et al. 2014) and gestational diabetes (Gui, et al. 2015).

269

270 Effects of vitamin D on EVT invasion and migration may also be mediated indirectly via  
271 effects on other known EVT regulators.  $1,25(\text{OH})_2\text{D}$  has been shown to abolish S1P  
272 mediated inhibition of migration via suppression of S1PR2 in trophoblast cell lines Swan-71  
273 and JEG-3 (Westwood 2017).  $1,25(\text{OH})_2\text{D}$  has also been shown to stimulate  
274 hCG expression and secretion via a cAMP/PKA-mediated signalling pathway (Barrera et al.  
275 2008). Although hCG is a potent regulator of trophoblast motility and invasion (Chen, et al.  
276 2011; Evans 2016), it is unclear whether changes in hCG expression are specifically

277 required for effects of vitamin D on trophoblast invasion. In a similar fashion,  $1,25(\text{OH})_2\text{D}_3$   
278 has been shown to positively regulate progesterone synthesis by human trophoblast cells  
279 from term placenta (Barrera et al. 2007). In HTR8/SVneo trophoblast cells, which have been  
280 reported to consist of a mixed population of cells, progesterone appears to suppress  
281 trophoblast motility and invasion (Chen et al. 2011). Thus,  $1,25(\text{OH})_2\text{D}$  may exert indirect  
282 effects on trophoblast invasion, although it is still not clear whether these effects are pro-  
283 migratory. Indirect actions of vitamin D on EVT function may also stem from effects on  
284 placental cell differentiation. Recent studies have shown that inactivation of VDR in  
285 trophoblastic BeWo cells resulted in increased trophoblast differentiation and syncytium  
286 formation (Nguyen, et al. 2015). In a similar fashion vitamin D may also influence EVT  
287 invasion and motility indirectly by targeting the development of cells on the maternal side of  
288 the placenta. Endometrial stromal cells treated with  $1,25(\text{OH})_2\text{D}$  have elevated expression of  
289 specific genes, including HOXA10 (Du, et al. 2005a), which are known to be involved in the  
290 regional development of uterine decidualization and embryo implantation by controlling  
291 downstream target genes. The complex circuitry of vitamin D metabolism and function  
292 involved in mediating direct or indirect effects on EVT invasion and migration has still to be  
293 fully elucidated and is likely to be a key component of future studies of vitamin D in  
294 pregnancy.

295

### 296 **Vitamin D and trophoblast function: clinical implications**

297 Irrespective of proposed functional targets, vitamin D-dysregulation during pregnancy has  
298 been linked to adverse effects on placental function and pregnancy in general. In 2010 the  
299 Institute of Medicine (IOM) defined vitamin deficiency as serum concentrations of  $25(\text{OH})\text{D}$   
300 less than 20 ng/ml (50 nM) (Holick, et al. 2011a). Subsequently the Endocrine Society  
301 issued slightly different guidelines, defining vitamin D-insufficiency as being serum  $25(\text{OH})\text{D}$   
302 levels below 30 ng/ml (75 nM) (Holick, et al. 2011b). Against this backdrop, several recent  
303 publications have highlighted the prevalence of low serum concentrations of  $25(\text{OH})\text{D}$  (less

304 than 25 nM) in pregnant women: 20% of pregnant women in the UK (Javaid, et al. 2006),  
305 25% in the UAE (Dawodu, et al. 1997), 80% in Iran (Bassir, et al. 2001), 45% in northern  
306 India (Sachan, et al. 2005), 60% in New Zealand (Eagleton and Judkins 2006) and 60–84%  
307 of pregnant non-Western women in the Netherlands (van der Meer, et al. 2006). It remains  
308 unclear if this reflects simply a normal physiological drop in vitamin D concentrations during  
309 pregnancy or if pregnancy is a stress test which can exacerbate and unmask pathological  
310 vitamin D deficiency.

311

312 Vitamin D deficiency in pregnant women has been shown to be associated with increased  
313 risk for pregnancy complications (Lewis, et al. 2010). These include preeclampsia (Bodnar,  
314 et al. 2007b), fetal growth restriction, small for gestational age fetus (Bodnar, et al. 2010),  
315 bacterial vaginosis (Bodnar, et al. 2009), and gestational diabetes mellitus (Maghbooli, et al.  
316 2008; Zhang, et al. 2008). Maternal vitamin D-deficiency has also been linked to adverse  
317 effects in offspring, including reduced bone density (Javaid et al. 2006) and childhood rickets  
318 (Wagner and Greer 2008), as well as increased risk of asthma (Camargo, et al. 2007), and  
319 schizophrenia (McGrath 2001).

320

321 The impact of vitamin D status on early events in pregnancy has also been studied. In  
322 northern countries, where there is a strong seasonal contrast in light exposure and UVB-  
323 induced vitamin D production in skin, conception rates are decreased during winter months,  
324 with rates rising during summer and an increased birth rate in spring (Rojansky, et al. 1992).  
325 Interestingly, ovulation rates and endometrial receptivity also appear to be reduced during  
326 long dark winters in northern countries (Rojansky, et al. 2000), which may be explained in  
327 part by seasonal variations in vitamin D levels. With this in mind, several observational  
328 studies have investigated the potential impact of vitamin D on *in vitro* fertilisation (IVF), albeit  
329 with largely conflicting outcomes. In a study of infertile women undergoing IVF, those with  
330 higher levels of 25(OH)D in serum and follicular fluid, were more likely to achieve pregnancy  
331 following IVF, and high vitamin D levels were also shown to improve the parameters of

332 controlled ovarian hyperstimulation (Ozkan, et al. 2010). Aleyasin et al. found no significant  
333 association between 25(OH)D levels in serum and follicular fluid with IVF outcomes  
334 (Aleyasin, et al. 2011). However, this did not include any women with a serum vitamin D  
335 level >50nmol/L. In another study of 100 women undergoing IVF, serum concentrations of  
336 25(OH)D were positively associated with fertilization rate (Abadia, et al. 2016). However,  
337 serum 25(OH)D was unrelated to the probability of pregnancy or live birth after IVF (Abadia  
338 et al. 2016). Anifandis et al. investigated 101 women who received IVF-intracytoplasmic  
339 sperm injection (ICSI) ovarian stimulation cycles. In this study, women with vitamin D-  
340 sufficiency (25(OH)D level >30 ng/ml in follicular fluid) had a lower quality of embryos and  
341 were less likely to achieve clinical pregnancy, compared with women with insufficient  
342 (follicular fluid 25(OH)D level 20.10 to 30 ng/ml) or deficient vitamin D status (follicular fluid  
343 25(OH)D level <20 ng/ml) (Anifandis, et al. 2010).

344

345 Elucidation of the immunomodulatory effects of 1,25(OH)<sub>2</sub>D has led to the suggestion that  
346 vitamin D might have a role in protecting against spontaneous abortion (Bubanovic 2004).  
347 This was supported by *ex vivo* analyses showing that 1,25(OH)<sub>2</sub>D is able to suppress  
348 inflammatory cytokine production by endometrial cells from women with unexplained  
349 recurrent spontaneous abortions (Tavakoli, et al. 2011). More recently, 1,25(OH)<sub>2</sub>D has  
350 been shown to potently regulate natural killer cells from women with recurrent miscarriage  
351 (Ota, et al. 2015). Considering these observations, the impact of maternal vitamin D status  
352 on pregnancy outcome has been studied in several cohorts. In a large prospective cohort  
353 study of 1683 pregnant women donating serum before gestational week 22, serum  
354 concentrations of 25(OH)D less than 50 nM were associated with a >2-fold increase in first  
355 miscarriage rate, although no significant effect was observed for second trimester  
356 miscarriage (Andersen, et al. 2015). In a prospective study of pre-conceptual vitamin D,  
357 maternal serum 25(OH)D levels were not found to be associated with chances of conceiving  
358 or overall risk of miscarriage (Moller, et al. 2012). However, women with miscarriage in the  
359 second trimester had lower first trimester serum concentrations of 25(OH)D than those

360 women who did not miscarry (Moller et al. 2012). In a much larger, nested case-control study  
361 of over 5,000 women did not reveal any adverse effects of low serum 25(OH)D on  
362 pregnancy outcomes (Schneuer, et al. 2014). A recent meta-analysis and systematic review  
363 concluded that vitamin D-deficiency is not associated with increased risk of spontaneous  
364 recurrent abortion (Amegah, et al. 2017). Thus, the possible impact of sub-optimal vitamin D  
365 on implantation and adverse pregnancy outcomes such as miscarriage still remains unclear.  
366 Interestingly, in endometrial tissue from women with unexplained recurrent spontaneous  
367 abortion, expression of key components in the vitamin D metabolic (CYP27B1/CYP24A1)  
368 and signalling (VDR) systems was found to be comparable to endometrial tissue from  
369 healthy fertile women (Tavakoli, et al. 2015). By contrast, recent studies of women with  
370 recurrent miscarriage showed that expression of mRNA and protein for CYP27B1 in villous  
371 and decidual tissue was lower than in control tissues from normal healthy pregnancies  
372 (Wang, et al. 2016). In future studies it will be important to clarify how variations in the  
373 vitamin D system within the placenta and fetal trophoblast cells affect implantation and the  
374 maintenance of a successful healthy pregnancy.

375

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

386

## 387 **Conclusions**

388 Expression of placental CYP27B1 and VDR at early stages of pregnancy suggests an  
389 important role for vitamin D in placental physiology. In previous studies we have  
390 hypothesized that placental vitamin D may function, at least in part, to promote anti-microbial  
391 and anti-inflammatory immune activity, with both the maternal decidua and fetal trophoblast  
392 contributing to these actions. However, analysis of trophoblast cells *ex vivo* and *in vitro*  
393 indicates that vitamin D may have a much broader role in placental function, including the  
394 regulation of trophoblast differentiation and EVT invasion of the decidua and myometrium  
395 (**Figure 1**). Thus, effects of vitamin D may occur earlier in pregnancy than previously  
396 appreciated, underlining the requirement for adequate vitamin D status across gestation. To  
397 date, studies of vitamin D status (maternal serum 25(OH)D) in pregnancy have tended to  
398 focus on later stages of pregnancy, and associated adverse events such as preterm birth,  
399 gestational diabetes and preeclampsia. Likewise, supplementation trials for vitamin D in  
400 pregnancy have focused on women between 10 and 18 weeks of pregnancy. However, the  
401 responsiveness of trophoblast cells to 1,25(OH)<sub>2</sub>D, notably effects on EVT invasion,  
402 suggests that further studies of vitamin D and adverse events in early pregnancy are  
403 required. To date there have been a limited number of reports of vitamin D-deficiency and  
404 miscarriage, but these need to be expanded to include more rigorous supplementation trials.  
405 The review we present is supportive of early, pre-conceptual, supplementation with vitamin  
406 D.

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411 **Declaration of Interests**

412 The authors declare that there is no conflict of interest that could be prejudicing  
413 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-1 $\alpha$ -hydroxylase (CYP27B1); vitamin D-24-hydroxylase (CYP24A1); human chorionic gonadotropin (hCG); human prolactin (hPL).

