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REVIEW *Reproductive biology*

Relevance of vitamin D in reproduction

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ABSTRACT: The steroid hormone vitamin D is historically recognized for its relevance to bone health and calcium homeostasis. Recent years have witnessed a shift in focus to non-skeletal benefits of vitamin D; in this latter context, an accruing body of literature attests to a relevance of vitamin D to reproductive physiology. This article reviews the existing data about the diverse and previously underappreciated roles for vitamin D in reproductive health. A large body of available literature suggests that vitamin D deficiency may be detrimental to reproductive biology. However, given that our appreciation of vitamin D's role in reproductive physiology is almost entirely shaped by 'associative' studies and that data based on prospective interventional trials are limited, these concepts remain predominantly conjectural. Exact mechanisms whereby vitamin D may participate in the regulation of reproductive physiology remain far from clear. This review underscores a need for appropriately designed intervention trials to address the existing knowledge gaps and to delineate the specific roles of vitamin D signaling in reproductive biology.

Key words: female tract / sperm quality / oocyte quality / environmental / effects

Introduction

A role for vitamin D in bone health was first reported in the 1920's when McCollum and colleagues created a rat model of diet-induced rickets (Mellanby, 1919). Disease manifestations were found to be prevented by a fat-soluble agent that was discovered in oxidized cod liver oil, and was subsequently named *vitamin D* (Mellanby, 1919; Rajakumar, 2003). Although the importance of this vitamin in calcium-phosphate homeostasis and for bone health was established early on, an understanding of the molecular biology of vitamin D was thwarted until the late 1960's when DeLuca and colleagues (Lund and DeLuca, 1966) generated radio-labeled vitamin D, a critical step in allowing the recognition of nuclear localization of vitamin D in a variety of tissues (Rajakumar, 2003; Christakos *et al.*, 2007). A succession of strides in vitamin D research has since furthered our understandings of the myriad roles that vitamin D plays in biological responses. Non-skeletal effects of vitamin D have been the focus of much interest in the past decade and an accruing body of literature is supportive of a relevance of vitamin D for a variety of organ systems beyond the skeleton (Reichel *et al.*, 1989; Walters, 1992). This review focuses on data that are based on non-human animal models as well as clinical studies and identify a relevance of an individual's vitamin D status to reproductive biology.

Physiology

Metabolism

In humans, the predominant source of vitamin D is endogenous cutaneous synthesis, whereas dietary sources contribute to <20% of the circulating levels of vitamin D (Holick *et al.*, 1987; Lips, 2006). Endogenous synthesis of vitamin D (cholecalciferol or D_3) occurs after photolytic conversion of 7-dehydrocholesterol, located in the dermal fibroblasts and epidermal keratinocytes, by the ultraviolet B component of sunlight (wavelength between 290 nm and 315 nm; Ashwell *et al.*, 2010; Fig. 1).

Nutritional forms of vitamin D consist of D_3 (cholecalciferol), which is found in foods such as fatty fish (i.e. sardine, salmon and mackerel), eggs and calf liver, and D_2 (ergocalciferol) which is manufactured through the ultraviolet irradiation of ergosterol from yeast and fungi (i.e. mushrooms; Vieth, 1999; Moore *et al.*, 2004). Both forms of dietary vitamin D are inactive and are efficiently absorbed by the gut. The dietary vitamin D (D_3 and D_2) is nearly identical to the skin-derived form of this seco steroid; in this review, the term vitamin D is implied to reflect either of the two variants (D_3 and D_2).

The circulating vitamin D prohormone (i.e. dietary or endogenously synthesized) is further metabolized by a set of cytochrome P450 enzymes; the hepatic 25-hydroxylase (CYP27A1), converts the

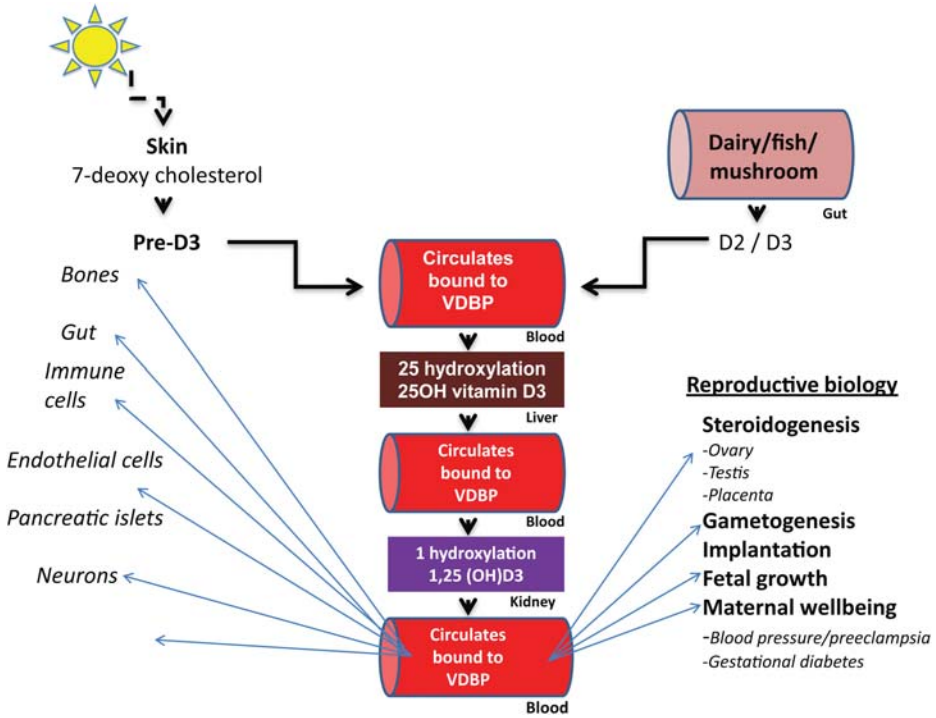

Figure 1 An overview of vitamin D metabolism and salient actions. VDBP, vitamin D binding protein; D2, ergocalciferol; D3, cholecalciferol.

prohormone to an intermediary metabolite, 25-hydroxy vitamin D (25(OH)D), whereas 1-α hydroxylase (CYP27B1), primarily from the kidneys, generates the metabolically active form, 1,25-di-hydroxy vitamin D ($1,25(OH)_2D$; Schuster, 2011). The two circulating metabolites (i.e. 25(OH)D and $1,25(OH)_2D$) demonstrate distinct kinetics and physiological potency. 25(OH)D has a half-life of 2–3 weeks, and its circulating levels are recognized to reflect bodily stores of the vitamin. $1,25(OH)_2D$ in contrast, has a much shorter half-life of 4–6 h and represents the most biologically active variant of vitamin D (Vieth, 1999). An overview of metabolism and salient actions of vitamin D are summarized in Fig. 1.

Renal 1-α hydroxylase (CYP27B1) is recognized as the principal determinant and the rate-limiting enzyme in the generation of the active $1,25(OH)_2D$ metabolite. Recently, the expression of CYP27B1 has been described in a variety of non-renal tissues, suggesting the existence of local mechanisms by which the metabolically active form of the vitamin D is generated (Zehnder *et al.*, 2001; Fischer *et al.*, 2009). The activity of CYP27B1 is directly regulated by the parathyroid hormone (PTH; Lund and DeLuca, 1966; Schuster, 2011). More recently, the activity of CYP27B1 has been shown to be inhibited by fibroblast growth factor 23 (FGF-23), a component of the previously unrecognized hormonal bone–parathyroid–kidney axis that is itself modulated by PTH, $1,25(OH)_2D$ and serum phosphorus levels (Bai *et al.*, 2004; Martin *et al.*, 2012).

Mechanism of action

Vitamin D belongs to the family of steroid hormones. The cellular effects of vitamin D and its metabolites are mediated primarily through the cognate intranuclear vitamin D receptor (VDR; Christakos *et al.*, 2007), a ligand-activated transcription factor that belongs to the nuclear hormone receptor super-family. VDR is expressed in a variety of tissues other than the skeleton, including the intestines, parathyroid glands, immune cells, and more recently, the hypothalamic–pituitary axis and the reproductive tract (Christakos *et al.*, 2007; Mizwicki and Norman, 2009). The presence of VDR in the ovary, uterus, placenta and the testis suggests a regulatory role for vitamin D in reproductive physiology (Weisman *et al.*, 1979; Dokoh *et al.*, 1983; Stumpf *et al.*, 1987a; Johnson *et al.*, 1996). The VDR binds $1,25(OH)_2D$ with an affinity in the range of 0.1–1 nM compared with >100 μM for the lesser active 25(OH)D metabolite (Wecksler and Norman, 1980a,b; Wecksler *et al.*, 1980). The receptor-ligand binding initiates a cascade of events that include receptor phosphorylation and nuclear translocation; recruitment and heterodimerization with the 9-cis retinoid receptor (RXR) then ensues (Christakos *et al.*, 1996). The VDR/RXR heterodimer complexes with the steroid receptor co-activators, VDR-interacting protein and co-regulatory proteins, before binding to the vitamin D responsive elements within the promoter region of target genes, thereby allows the transcription regulation of tissue-specific genes (Christakos *et al.*, 1996).

Although the effect of active $1,25(OH)_2D$ on target cells primarily reflects genomic activity, more recent data suggest an additional non-genomic signaling mechanism via membrane associated rapid response steroid-binding receptor (MARRS, also known as Erp57/Grp58); such a mechanism is suggested in a variety of tissues including the intestine, bone, parathyroid gland, liver, monocytes and the pancreatic beta cells (Chatterjee, 2001; Erben *et al.*, 2002; Christakos *et al.*, 2007; Yu *et al.*, 2012). The function of the non-genomic signaling pathway, however, is less understood. Signaling via the VDR has additionally been linked to

CYP19 (aromatase) gene expression, functionally linking vitamin D with the family of reproductive steroid hormones (Kinuta *et al.*, 2000; for review Christakos *et al.*, 2003).

Pandemic of vitamin D deficiency

The data collected by the National Health and Nutrition Examination Surveys within North America document a 4-fold increase in the prevalence of vitamin D deficiency over the past 10–15 years with as much as 36% of the USA population being affected (Nesby-O'Dell *et al.*, 2002; Looker *et al.*, 2008). Embedded within these data are the alarming findings that populations with the greatest physiological needs for vitamin D, such as pregnant women, neonates, children and adolescents are also at highest risk for vitamin D deficiency (McCullough, 2007; Alemzadeh *et al.*, 2008; Kovacs, 2008).

Suboptimal dietary vitamin D intake, increasing environmental pollution, a shift in lifestyle with consequent reduced sun exposure, a concomitant increased use of sunscreen arising from concerns about the carcinogenic potential of sunlight are all recognized as contributors to the near-pandemic of vitamin D insufficiency (Diehl and Chiu, 2010). An inverse relationship between serum 25(OH)D levels and body mass index (BMI) is also well described (Bell *et al.*, 1988; Liel *et al.*, 1988). Although a 'cause and effect' paradigm to this relationship is unclear, obesity is recognized as an independent risk factor for hypovitaminosis D (Wortsman *et al.*, 2000); sequestration of the fat soluble vitamin within the adipose tissue is proposed as a mechanism to explain the lower circulating levels of 25(OH)D observed in the overweight and the obese (Liel *et al.*, 1988; Wortsman *et al.*, 2000).

While increasing the prevalence of obesity may partly explain the escalating trends in vitamin D insufficiency (Nesby-O'Dell *et al.*, 2002; Looker *et al.*, 2008), the latter in turn may itself be contributory to the burgeoning pandemic of obesity (Foss, 2009). Secondary hyperparathyroidism that occurs consequent to hypovitaminosis Dis suggested to stimulate 1-α hydroxylase activity, contributing to a compensatory elevation in $1,25(OH)_2D$ levels (Arunabh *et al.*, 2003). Recent *in vitro* experiments suggest that $1,25(OH)_2D$ causes an increase in the intra-adipocyte calcium ion concentration, which in turn can stimulate lipogenesis and inhibit lipolysis (Shi *et al.*, 2001; Zemel, 2002). Obesity is hypothesized to be linked to abnormal as well as reduced leptin receptor signaling (for review, Gautron and Elmquist, 2011). Interestingly, studies in transgenic leptin deficient and leptin receptor knockout mice suggest that leptin and its cognate receptor may also regulate renal CYP27b1 and $1,25\ (OH)_2D$ synthesis (Matsunuma and Horiuchi, 2007).

Vitamin D and reproduction

The importance of vitamin D for the reproductive biology is supported by several rodent studies; an appreciation that vitamin D signaling may be relevant for reproductive health in humans, however, is relatively recent (Table I). The goal of this review is to summarize a relatively underappreciated body of literature that highlights the diverse physiological roles for vitamin D and its metabolites in reproductive physiology.

Lessons from animal models

The majority of the experimental data defining a role for 1,25 (OH)2D, the active metabolite of vitamin D, in reproduction are either derived from diet-induced vitamin D-deficient rodent models or from transgenic Vdr or Cyp27b1 null mice (Halloran and DeLuca, 1979; Johnson and DeLuca, 2001; Sun *et al.*, 2010; Dicken *et al.*, 2012). The available evidence makes a strong case for the importance of vitamin D for procreative success, while underscoring the need for additional studies to allow a better understanding of the mechanistic relevance of vitamin D in reproduction.

Vitamin D and male reproductive physiology: animal models

The expression of Vdr on sperm and throughout the male testes, the presence and activity of Cyp27b1 in the testes and the evidence of local synthesis of $1,25(OH)_2D$ within the Sertoli and Leydig cells all attest to a relevance of vitamin D in male reproduction (Stumpf *et al.*, 1987b; Osmundsen *et al.*, 1989; Kwiecinski *et al.*, 1989a). Perturbations in male behavior, spermatogenesis and fertility are described in the context of vitamin D deficiency (Kinuta *et al.*, 2000). Inseminating wild-type females with sperm collected from diet-induced vitamin D deficient male rats resulted in 65% fewer sperm deposited in the genital tract, and 73% fewer pregnancies in vitamin D sufficient female rats (Osmundsen *et al.*, 1989; Kwiecinski *et al.*, 1989a). Likewise, oligoasthenospermia, hypergonadotrophic hypogonadism and altered tissue expression of aromatase activity are described in the transgenic Vdras well as in the Cyp27b1 null mice; these data suggest a regulatory role for vitamin D signaling in gonadal function. Of interest,the reproductive and gonadal phenotypes of transgenic estrogen receptor alpha and aromatase null mice are similar to the Vdrnull male mutant (Eddy *et al.*, 1996; Mahato *et al.*, 2000; Couse *et al.*, 2001). These latter observations may suggest that theimpaired spermatogenesis and subfertility that are apparent in Vdrnull male mice may partly be mediated through defects in estrogen signaling, a hypothesis that merits detailed assessment. Others, however, maintain that hypocalcemia and hypophosphatemia resulting from vitamin D deficiency are the primary mechanisms that underlie the observed defects in spermatogenesis. Vitamin D deficiency related hypocalcemia is hypothesized to compromise capacitation and acrosome reactions required for fertilization(Breitbart, 2002).

Table I Vitamin D and reproduction.

Parameter	Experimental models	Human studies
Folliculogenesis	+	±
Spermatogenesis	+	+
Steroidogenesis	+	−
Implantation	+	+
Relevance in pregnancy	+	+
Relevance for progeny	+	+

A comparison between experimental models and human studies with regard to recognized target effects of vitamin D on specified reproductive parameters. +Evidence for involvement; −no evidence for involvement; ±contradictory evidence.

Consistent with this hypothesis high calcium-phosphorous diets rescue male fertility in Vdrtransgenic null mice and in male rats with diet-induced vitamin D deficiency (Uhland *et al*., 1992; Kinuta *et al*., 2000; Johnson and DeLuca, 2001).

Vitamin D and female reproductive physiology: animal model

Similar to the observations invitamin D deficient males, several lines of evidence suggest that vitamin D deficiency disrupts female reproductive physiology(Halloran and DeLuca, 1980; Kwiecinksi *et al*., 1989b; Panda *et al*., 200; Dicken *et al*., 2012). Nuclear localization of $1,25(OH)_2D$ and VDR are described in a variety of female reproductive organs including the hypothalamus, pituitary gland, uterus, oviduct, ovary, mammary gland and the placenta. Diet-induced vitamin D deficiency in female rats results in severely compromised fertility: a 45–70% reduction in the probability of becoming pregnant, 67–100% reduction in the number of viable pups and 0–33% probability of rearing normal sized and healthy litters are described in experimental models of vitamin D deficiency (Halloran and DeLuca, 1980; Kwiecinksi *et al*., 1989b).

While the exact mechanisms are far from understood, disrupted estrogen signaling is hypothesized to contribute to impaired reproductive physiology in vitamin D deficient females (Yoshizawa *et al*., 1997; Panda *et al*., 2001).Similar to the males, female Vdrnull mice also exhibit hypergonadotropic hypogonadism accompanied by reduced aromatase activity (Kinuta *et al*., 2000). While hypergonadotropic hypogonadism is common to aromatase deficient as well as the transgenic Vdror Cyp27b1 nullfemale mice, the ovarian phenotypes for these animal models are quite different. Ovarian morphology in the aromatase null females is characterized by large hemorrhagic cysts, suggestive of an ovulatory defect (Britt *et al*., 2000, 2001). In contrast, in the Vdras well as in Cyp27b1 transgenic null females, the ovaries are devoid of corpora lutea, exhibit hypertrophied interstitial cells, and early follicular development appears to be arrested (Panda *et al*, 2001; Dicken *et al*., 2012). Hypergonadotropic hypogonadism is evident in the Vdr transgenic null females, suggesting gonadotrophin resistant (Kinuta *et al*., 2000). The transgenic Cyp27b1 null females in contrast are eugonadotropic and exhibit a robust ovarian response to exogenous gonadotrophins (Dicken *et al*., 2012). While these data identify that neither ovarian failure nor gonadotrophin resistance accounts for the abnormal reproductive phenotype of the Cyp27b1 females, some degree of hypothalamic–pituitary axis dysfunction is, however, suggested by the follicular response to exogenous gonadotrophins that is described in the Cyp27b1 null mice (Dicken *et al*., 2012). Contrary to Dicken *et al*., however, Sun *et al*., observed reduced numbers of oocytes retrieved from the oviducts in the Cyp27b1 null mice compared with WT controls following ovulation induction in 24–25-old mice (Sun *et al*., 2010). Differences in the age of experimental animals may account for the disparity in observations reported in the latter two studies; Dicken *et al*., focused on the young adult animals, whereas Sun *et al*., superovulated females who most likely were developmentally peri-pubertal. Thus, it is possible that the pool of gonadotrophin responsive oocytes is reduced in the Cyp27b1 null peri-pubertal animals compared with the young adult females (Sun *et al*., 2010; Dicken *et al*., 2012).

In a recent study by Malloy *et al*., the authors provide novel implications of Vdr-mediated signaling for ovarian function. An up-regulation of the luciferase activity was observed in HeLA cells (cervical cancer cell line) that were transfected with wild-type (WT) VDR cDNA expressing vector and anti-Mullerian hormone (AMH) reporter plasmid; these observations suggest that the promoter for AMH has a putative domain for the vitamin D response element (Malloy *et al*., 2009). AMH, a member of the transforming growth factor (TGF–β) family, is produced by the ovarian granulosa cells and is recognized to regulate follicular recruitment and selection, as well as inhibit the aromatase activity (Durlinger *et al*., 2002; Visser *et al*., 2006). Given that vitamin D signaling can directly modulate ovarian AMH expression, it is plausible that vitamin D deficiency in females may disrupt ovarian physiology via altering AMH signaling.

In contrast to the males, the ability of calcium to rescue female reproductive physiology and fertility in vitamin D deficient animal models is far from consistent; different studies have reported complete, partial or no rescue (Johnson and DeLuca, 2002; Sun *et al*., 2010; Dicken *et al*., 2012). While Johnson *et al*. have suggested that calcium supplementation in the VDR null mice rescued female fertility, the magnitude of effect in the described model is difficult to interpret because the control group (wild type litter mates) was fed a high calcium diet and both groups had equally small litter sizes (5.29 ± 2.1 versus 3.5 ± 1.3; $n = 4–7$; Johnson and DeLuca, 2001).

VDR is expressed in the uterine endometrial cells as well as in the immune cells residing within the uterine endometrium (Evans *et al*., 2006). In addition to uterine hypoplasia, impaired bone formation and skeletal compromise are also evident in the Vdr null mice (i.e. phenotypes consistent with hypoestrogenism). The uteri of the Vdr null mice are responsive to estrogen priming suggesting the uterine defect in the Vdr null is in part a consequence of hypogonadism (Zarnani *et al*., 2010). However, neither estradiol priming nor calcium supplementation suppressed gonadotrophins nor rescued the reproductive phenotype in Vdr null females.

Heterogeneity in the phenotypes of females with vitamin D deficiency caused by Vdr and Cyp27b1 gene deletion suggest that our understanding of the regulatory role of vitamin D is not complete. In addition to abnormal reproductive phenotypes, Vdr null mice exhibit early aging that is characterized by cognitive dysfunction and alopecia; these latter features are not apparent in the Cyp27b1 transgenic null mice, nor in comparably aged, diet-induced vitamin D deficient rats (Lester *et al*., 1982). It is possible that the disruption of the Vdr gene and the resultant inability to form VDR/RXR heterodimers results in physiological effects that are distinct from those specific to vitamin D deficiency alone (Keisala *et al*., 2009).

A recent study using the Cyp27b1 transgenic null females compared with WT littermates demonstrated that mice fed regular mouse chow (deficient of active vitamin D) for 3 months post-weaning exhibited irregular estrous cycles, reduced fertility and decreased pup survival that appeared to be rescued by calcium–phosphate diet supplementation (Sun *et al*., 2010). However, this study was limited because the authors did not study females weaned onto a truly vitamin D-deficient diet; instead the females were weaned onto a regular chow diet which is fortified with the vitamin D prohormone. The significance of this latter point is that when circulating 25(OH)D levels are high, as occurs in the Cyp27b1 and Vdr null experimental models (Panda *et al*., 2004), the 25(OH)D itself can activate Vdr (Rowling *et al*., 2007). Thus, it is likely that calcium supplementation rescued the reproductive phenotype of these females because high circulating levels of 25(OH)D allowed the recovery of VDR signaling

and consequently a less dramatic phenotype. Consistent with this hypothesis, Dicken *et al*., demonstrated that the estrous cycle irregularity was not rescued in Cyp27b1 null females weaned onto a diet devoid of vitamin D but supplemented with calcium (Dicken *et al*., 2012).

Vitamin D, pregnancy, parturition and lactation: animal models

The importance of vitamin D in embryo implantation is also suggested. HOXA 10 is a transcription factor that is recognized as being critical for embryo-endometrial cross talk which initiates the process of implantation. Endometrial expression of HOXA10 is up-regulated by vitamin D, thereby raising a possibility that vitamin D signaling contributes to successful embryo implantation and to immune tolerance (Curtis Hewitt *et al*., 2002; Du *et al*., 2005).

Alterations in maternal feeding behavior, milk production and lactation are described in vitamin D deficient and hypocalcemic post-partum females (dams). Reduced maternal feeding and milk production are evident in experimental models of vitamin D deficiency (Brommage and DeLuca, 1984b). Dams with diet-induced vitamin D deficiency, exhibit anorexia, abnormal maternal behavior and reduced milk production (Brommage and DeLuca, 1984a; Brommage *et al*., 1984). Poor maternal nutrition and reduced milk production are hypothesized to contribute to failure in the pups to thrive and survive under conditions of maternal vitamin D deficiency (Brommage and DeLuca, 1984b). Despite the above-summarized evidence linking maternal vitamin D deficiency to poor outcomes in the offspring, it is difficult to ascertain if the poor neonatal outcomes reflect impaired vitamin D signaling, calcium–phosphate dysregulation, or if the sequelae were consequent to maternal malnutrition and/or reduced maternal capacity to lactate.

As currently understood, while reproductive compromise is clearly evident in the animal models of vitamin D deficiency, the exact downstream mechanisms whereby reproductive physiology is affected is minimally understood.

Vitamin D and reproduction: relevance in humans

As discussed in the preceding section, ample solid evidence exists supporting a role for vitamin D in the regulation of reproductive physiology in the non-human models. In contrast, literature describing a role for vitamin D in the reproductive biology of humans is, however, sparse and almost entirely correlative.

Vitamin D and male reproductive physiology: human data

Calcium is recognized as a critical intracellular signal in male physiology with defined roles during spermatogenesis, sperm motility, hyperactivation and acrosome reaction (Yoshida *et al*., 2008). VDR is detected in the human testis, prostate and in human spermatozoa (Corbett *et al*., 2006). More recently, vitamin D metabolizing enzymes are described in the human testis, the ejaculatory tract, mature spermatozoa and in Leydig cells (Blomberg *et al*., 2012; Foresta *et al*., 2010). CYP24A1 is a vitamin D-metabolizing enzyme that acts as a check on vitamin D signaling; 24-hydroxylation is generally the first step in the catabolism of active metabolites of vitamin D. CYP24A1 is induced by $1,25(OH)_2D$, and offers a feedback mechanism to avoid vitamin D toxicity and to titrate cellular responsiveness to vitamin D. CYP24A1 expression has recently been described in the human sperm annulus (Blomberg *et al*., 2012). In a study comparing the expression of CYP24A1 in the ejaculated sperm from 77 subfertile and 50 healthy young men, the authors observed significantly reduced CYP24A1-expressing spermatozoa in the subfertile men compared with the healthy group (1 versus 25%, $P < 0.001$). CYP24A1 expression was further observed to positively correlate with sperm concentration, motility and morphology ($P < 0.004$ for each parameter). The authors further noted that the presence of >3% CYP24A1-positive spermatozoa distinguished the healthy men from the subfertile men with a sensitivity of 66.0%, a specificity of 77.9% and a positive predictive value of 98.3% (Blomberg *et al*., 2012). To understand if vitamin D signaling was causative to the observed associations, these authors further conducted *in vitro* functional studies on sperm from an additional 40 men (22 young, 18 subfertile); exposure to $1,25(OH)_2D$ increased intracellular calcium concentration and improved motility in the sperm from healthy young subjects, but failed to impact on sperm from the subfertile men (Blomberg *et al*., 2012). A recent review by the same group of investigators offers a comprehensive coverage of the non-genomic effect of vitamin D in human spermatozoa (Bloomberg *et al*., 2012b).In a cross-sectional study of 307 men, Ramlau-Hansen *et al*., however, failed to identify any clinical relevance of vitamin D deficiency on sperm parameters (Ramlau-Hansen *et al*., 2011).

While the aforementioned data imply a role for vitamin D and VDRsignaling in spermatogenesis, sperm maturation and testicular endocrine function, additional studies are required to clearly define the importance of VDR signaling in male gamete and gonadal physiology.

Vitamin D and female reproductive physiology: human data

Several recent studies explore the role of vitamin D and its metabolites in overall health and in specific reproductive disorders of women. VDR mRNA and protein are detected in the human endometrium, myometrium, ovarian, cervical and breast tissues (Friedrich *et al*., 2003; Vienonen *et al*., 2004). Vitamin D deficiency is hypothesized to contribute to the pathophysiology of a spectrum of gynecological disorders, of which polycystic ovary syndrome (PCOS) appears to be most well studied.

A small number ofobservational studies identify an inverse association between serum 25(OH)D levels with insulin resistance, features of hyperandrogenism and circulating androgens in women with PCOS (Panidis *et al*., 2005; Hahn *et al*., 2006; Mahmoudi *et al*., 2010; Ngo *et al*., 2011; Wehr and Pilz). In an observational study, Thys-Jacobs *et al*. describednormalization of menstrual cyclicity in 7 out of 9 oligomenorrheic women with PCOS who underwent supplementation with vitamin D and calcium over a 6 month period; the authors implied therapeutic efficacy of vitamin D for women with PCOS (Thys-Jacobs *et al*., 1999). Others report dietary supplementation with vitamin D or an analog improves insulin sensitivity (Kotsa *et al*., 2009; Selimoglu *et al*., 2010), circulating testosterone (Selimoglu *et al*., 2010) and parameters of ovarian folliculogenesis and ovulation (Rashidi *et al*., 2009).In a pilot study of 12 overweight and vitamin D deficient women with PCOS, we observed a significant lowering in circulating androgens (total testosterone and androstenedione) following three month intervention with vitamin D and calcium (Pal *et al*., 2012).

Insulin resistance, obesity, inflammation and dyslipidemia, i.e. metabolic phenomenon that are commonly encountered in PCOS, are well

described in the setting of vitamin D insufficiency (Botella-Carretero et al., 2007; Holick, 2007; Foss, 2009);vitamin D insufficiency is thus theorized to underlie the pathophysiology of PCOS. However, because the majority of women with PCOS are either overweight or obese it is difficult to determine if vitamin D deficiency independent of excess body mass contributes to the pathogenesis of PCOS.

A limited number of studies relate vitamin D deficiency to premenstrual syndrome, uterine fibroids, dysmenorrhea and more recently to early menarche (Bertone-Johnson et al., 2010; Sharan et al., 2011; Villamor et al., 2011; Lasco et al., 2012). Compared with placeboa significant improvement in severity of dysmenorrhea was observed over a two month period following a single dose of 300 000 IU vitamin D; this latter study is one of the few instances where a cause-effect relationship between low vitamin D status and the studied outcome is supported by the randomized controlled study design (Lasco et al., 2012).

A variety of reproductive tract tissues express VDR and messenger RNA for 1CYP27B1. Expression of VDR is reported in normal and neoplastic cervical (Friedrich et al., 2003), ovarian and breast tissue (Friedrich, 2000). While the role of vitamin D signaling incervical and ovarian cancer isunclear, a significant body of epidemiological data suggest vitamin D deficiency is a risk for non-inherited forms of breast cancer (Shao et al., 2012). Interestingly endometriosis has the distinction of being one of the few disorders identified as resulting from arelative excess of vitamin D (Somigliana et al., 2007). A recent study on proteomic analysis of serum collected from patients with endometriosisdemonstrated a significantly higher expression of vitamin D-binding protein (VDBP) in sera of patients with endometriosis (Faserl et al., 2011). Others have similarly demonstrated a increased plasma and peritoneal fluid VDBP levels in patients with endometriosis compared with controls (Ferrero et al., 2005).An overexpression of VDR and vitamin D metabolizing enzymes is also described in the peritoneal lesions and in endometrial tissue of women with endometriosis compared with healthy controls (Agic et al., 2007). The authors suggest that activity of immune cells and cytokines that are thought to play pathogenic roles in the development and maintenance of endometriosis may be modulated by the higher than normal peritoneal levels of vitamin D and its metabolites (Agic et al., 2007). While these data imply that VDBP and vitamin D signaling may play a role in the pathogenesis of endometriosis, the underlying mechanisms are minimally investigated. It is notable that existing data describing abnormalities in vitamin D metabolism and levels in disorders of reproductive tract are almost entirely associative; a direct cause and effect of the observed relationship requires substantiation.

Beyond the suggested associations with specific metabolicdisorders a roleforvitamin D is also implied for procreative success in women. Serum 25(OH)D levels are reported to predict ovarian response in women undergoing ovulation induction with clomiphene citrate (CC). Low levels of 25(OH)D and vitamin D deficiency ($<$25 nmol/l or $<$10 ng/ml) were found to be associated with lower rates of follicledevelopment and pregnancy after ovarian stimulation with 50 mg CC (Ott et al., 2012); of note, the threshold taken to define vitamin D insufficiency in this latter study is consistent with a state of severe vitamin D deficiency (Hollis et al., 2011). Implications of vitamin D status for reproductive success have also been suggested in women undergoing *in vitro* fertilization (IVF). In a prospective study of 84 women undergoing IVF, Ozkan et al.assessed 25(OH)D levels in the ovarian follicular fluid and determined an association between vitamin D status and cycle outcome. Follicular fluid levels of 25(OH)D were observed to be significantly higher in women achieving clinical pregnancy following fresh embryo transfer compared with those with failed outcome (34.42 $\pm$ 15.58 versus 25.62 $\pm$ 10.53 ng/ml, $P = 0.013$). Highest implantation rates were observed in women with a follicular fluid level of 25(OH)D in the highest tertile (43.01 $\pm$ 10.65 ng/ml) compared with those with follicular fluid 25(OH)D levels in the lower tertiles ($P = 0.041$). While these data relate vitamin D status with reproductive success in otherwise healthy, albeit infertile, women undergoing IVF (Ozkan et al., 2010), others fail to confirm these associations (Aleyasin et al., 2011). Additionally another group of investigators reported adverse impact of higher 25(OH)D levels on embryo quality (Anifandis et al., 2010). Additional studies are needed to explore these associations better and to study the direct effects of vitamin D and its metabolites on the endometrium and implantation.

Vitamin D and pregnancy: human data

Maternal calcium metabolism undergoes dramatic modulation so as to maintain the skeletal mineralization needs of the developing fetus (Abrams, 2007). The gravid woman's physiology adapts to the escalating fetal requirements with advancing gestation; increasing gastrointestinal calcium absorption is evident early in pregnancy, and maximized in the last trimester (Cross et al., 1995; Ritchie et al., 1998). The increasing fetal demand for calcium is further met by the increased production of 1,25$(OH)_2$D via the maternal kidneys and placenta (Ritchie et al., 1998). Compared with pre-pregnancy levels, there is a 2-fold increase in both the total plasma levels of 1,25$(OH)_2$D and VDBP in the first and second trimesters. In the third trimester, however, a continued rise in 1,25$(OH)_2$D occurs without a concomitant increase in VDBP, resulting in increased levels of free (unbound) 1,25$(OH)_2$D (Urrutia and Thorp, 2012). Placental hormones including parathyroid hormone-related protein (PTHrP) and placental lactogen may be particularly relevant in modulating vitamin D homeostasis during pregnancy (Hosking, 1996; Kovacs, 2012). PTHrP, placental lactogen, as well as prolactin stimulate renal CYP27B1 activity, contributing to the increased circulating levels of 1,25$(OH)_2$D in pregnancy (Hosking, 1996; Kovacs, 2012).The significance of vitamin D for maternal and fetal wellbeing is thus suggested by the aforementioned phenomena that ensure an increased availability of the active form of vitamin D to the mother and the fetus during pregnancy, particularly in the third trimester (Urrutia and Thorp, 2012).

Several pregnancy-related disorders that have been hypothesized to relate to maternal vitamin D deficiency includ pre-eclampsia, gestational diabetes mellitus (GDM) and a risk for delivery by Cesarean section (Fischer et al., 2007; Merewood et al., 2009; Shand et al., 2010; Soheilykhah et al., 2010). Pre-eclampsia is one of the most common obstetric complications, and a significant contributor to maternal and fetal morbidity and mortality. While the etiology is not entirely clear, abnormal placentation, poor placental perfusions, endothelial dysfunction and oxidative stress are recognized mechanisms underlying pre-eclampsia. The presence of vitamin D and its receptors in the placenta as well as the ability of vitamin D to modulate immune, inflammatory and vascular responses has suggested a causative role of maternal vitamin D deficiency in the pathogenesis

of pre-eclampsia (Bodnar *et al*., 2007a). Higher maternal vitamin D levels are associated with a lower incidence of pre-eclampsia and with lower blood pressure readings (Shand *et al*., 2010; Ringrose *et al*., 2011). Others have explored the relationship between maternal D levels earlier in pregnancy with the likelihood of developing pre-eclampsia during the course of pregnancy. A Norwegian study of >23 000 nulliparous pregnant women found that supplemental vitamin D intake protects against the development of pre-eclampsia (Haugen *et al*., 2009). The effects of maternal vitamin D supplementation on lowering blood pressures in the third trimester are reported in a randomized control trial of vitamin D supplementation versus control (Marya *et al*., 1987). In this study, 400 gravid women were randomly selected to either receive calcium (375 mg/day) and vitamin D (1200 IU/day) at 20–24 weeks onward or non-supplemented throughout the pregnancy. At 32 and 36 weeks of pregnancy, the systolic and diastolic blood pressure of the vitamin D supplementation group was significantly lower than the non-supplemented group. However, the incidence of pre-eclampsia in the supplemented group (6%) was not significantly different from the non-supplemented group (9%; Marya *et al*., 1987). The existing literature relating maternal vitamin D status with the risk of pre-eclampsia is equivocal. In a case–control study, Yu *et al*. (2012) quantified maternal serum vitamin D level in the first trimester and explored its relevance for risk of developing early (<34 weeks) or late (≥34 weeks) pre-eclampsia. The authors failed to identify any relationship between maternal serum vitamin D levels with biochemical (pregnancy-associated plasma protein) or biophysical (uterine artery pulsatility index and mean arterial pressure) markers of impaired placental perfusion or function (Yu *et al*., 2012).

Although a relationship between vitamin D deficiency and risk for type 2 diabetes mellitus is well described in non-pregnant populations (Chiu *et al*., 2004), the association of vitamin D with GDM, however, has shown mixed results. In one case–control study by Zhang *et al*., women with vitamin D deficiency early in pregnancy had a 2.66-fold (confidence interval (CI): 1.01–7.02) increased chance of developing GDM compared with women with normal levels (Zhang *et al*., 2008). Two additional case–control studies, however, failed to identify any relationship between maternal vitamin D levels and subsequent maternal risk for GDM (Baker *et al*., 2012; Makgoba *et al*., 2011).

Maternal rickets and concomitant pelvic deformities are a recognized contributor to inefficient labor and difficulties with parturition. A recent study of 253 women identified a 4-fold increase in likelihood for delivery by Cesarean section in women with low vitamin D (<37.5 nmol/l) at the time of delivery compared with those with higher (>37.5 nmol/l) vitamin D levels (Merewood *et al*., 2009). The increased risk of Cesarean section (emergent or elective) was attributed to negative effects of low vitamin D levels on uterine musculature and contractibility. However, the relationship between vitamin D and the route of delivery is not consistent. Moreover, others researchers fail to observe any effect of maternal vitamin D status (assessed early or mid-pregnancy or at the time of delivery) on the route of delivery (Dror *et al*., 2011; Savvidou *et al*., 2012).

Maternal vitamin D deficiency has been linked to a predisposition to a spectrum of infectious etiologies including periodontal disease (Boggess *et al*., 2011), bacterial vaginosis (BV; Bodner *et al*., 2009; Hensel *et al*., 2011) and HIV morbidity and mortality associated with opportunistic illnesses (French *et al*., 2011; Mehta *et al*, 2011). In a case–control study of 117 pregnant women diagnosed with moderate-to-severe periodontal disease at <26 weeks of gestation compared with 118 periodontally healthy controls, Boggess *et al*. identified a 2-fold increase in the prevalence of vitamin D insufficiency (serum 25(OH)D < 75 nmol/l [i.e. <30 ng/ml]) in women with periodontal disease (Boggess *et al*., 2011). Cause-specific mortality attributable to HIV, disease progression and disease-related anemia were significantly higher in a cohort of HIV-positive gravid Tanzanian women with low vitamin D levels (serum 25(OH)D < 32 ng/ml) compared with those with normal vitamin D levels (Findelstein *et al*., 2011; Mehta *et al*., 2011). In a randomized, double-blind, placebo-controlled trial by Mehta *et al*., 884 Tanzanian HIV-infected gravid women received either vitamin supplementation (including vitamin D) or placebo. Women with low 25(OH)D levels (<32 ng/ml) had significantly higher upper respiratory infections [odds ratio (OR): 1.27 (1.04–1.54)], and thrush [OR: 2.74 (1.29–5.83)] diagnosed during the first 2 years of follow-up (Mehta *et al*., 2011). Additionally, women with low vitamin D status were at higher risk for HIV-related wasting (Mehta *et al*., 2011). The relationship between BV and vitamin D status has been explored in a study of >3500 women (pregnant and non-pregnant; Hensel *et al*., 2011); vitamin D deficiency (serum 25(OH)D < 30 ng/ml) was identified as an independent determinant of risk for BV (OR: 2.9, 95% CI: 1.1–7.3) in the pregnant populations. Similarly, Bodnar *et al*. showed in a prospective cohort study of 469 pregnancy women in the first trimester that the mean serum 25(OH)D concentration was lower among BV cases (29.5 nmol/l) compared with women with normal vaginal flora (40.1 nmol/l; Bodner *et al*., 2009). Approximately 57% of women with a low serum 25(OH)D level (<20 nmol/l) had BV compared with 23% of women with normal serum 25(OH)D levels (>80 nmol/l; Bodner *et al*., 2009). These two studies clearly show an association between vitamin D deficiency and BV in pregnant women.

As evident, the existing literature relating maternal status of vitamin D with pregnancy-related outcomes is almost exclusively 'associative'. While the data suggest that insufficient maternal levels of vitamin D may increase the likelihood of common obstetric conditions such as pre-eclampsia, GDM and infectious entities such as BV, these concepts require additional investigation. If maternal vitamin D deficiency modulates maternal risk for the afore-discussed morbidities, aggressive vitamin D supplementation in pregnancy would offer a simple, inexpensive and safe strategy that could hold the potential to positively impact maternal and neonatal health. Additional studies are needed to bridge gaps in knowledge regarding mechanism and the ability to rescue the pathophysiology with dietary vitamin D supplementation.

Maternal Vitamin D status and implications for fetal development and neonatal well-being: human data

Maternal and cord blood levels of 25(OH)D have been shown to closely correlate across populations (Bassir *et al*., 2001; Wang *et al*., 2010; Dror *et al*., 2011; Hossain *et al*., 2011). Given the fetal dependence on maternal stores of 25(OH)D freely crossing the placental barrier, maternal vitamin D deficiency is likely to affect the fetus and the health of the newborn.

Maternal vitamin D status may hold long-term implications for the health of the progeny. Recently, a prospective cohort study of 424

pregnant women demonstrated that maternal vitamin D insufficiency may affect fetal femoral bone development as early as 19 weeks of gestation (Mahon et al., 2010); lower maternal 25(OH)D concentrations relate to greater femoral bone metaphyseal cross-sectional area and higher femoral splaying index in the fetuses. Additionally a longitudinal cohort study, Javaid et al., observed that lower maternal 25(OH)D serum levels correlated with a lower whole-body and lumbar spine bone mineral content in children at the age of 9 years (Javaid et al., 2006).

Small randomized clinical trials and observational studies also suggest a relationship between higher maternal vitamin D levels in the third trimester of pregnancy with neonatal birthweight (Ertl et al., 2012). A reduced risk for small for gestational age (weight less than 10 percentile, Gairdner standards) babies was observed in 59 women randomized to vitamin D supplementation (1000 IU D_3 daily) initiated in the third trimester, compared with 67 gravid women given placebo. Nearly twice as many infants in the control group were small for gestational age compared with those women receiving vitamin D supplementation (29 versus 15%; Brooke et al., 1980). Higher birthweights were similarly observed in another randomized control study of vitamin D supplementation (a bolus each of 600 000 IU administered at gestational months 7 and 8, plus 1200 IU D_2/day during the third trimester) versus placebo (Marya et al., 1987). More recently, Hollis et al. (2011) conducted the largest randomized trial of nearly 500 pregnant women who were randomized to supplementation with 400, 2000 or 4000 IU of vitamin D. Although a significant improvement in circulating 25(OH)D levels was seen across all groups, the investigators did not observe any differences in birthweights across the dose categories (Hollis et al., 2011).

Higher maternal serum levels of 25(OH)D have been related to a reduced likelihood of reactive airway disease in the children. Litonjua (2012) observed a 40% increased risk of asthma in children at ages of 3–5 years whose mothers had low levels of 25(OH)D in pregnancy. Although childhood eczema, respiratory infections, neurocognitive parameters and autism have all been related to maternal deficiency, a cause and effect relationship is not clear (Gale et al., 2008; Grant and Soles, 2009; Morales et al., 2012; Whitehouse et al., 2012). Collectively, the existing data imply that maternal vitamin D status may have long-lasting health implications for their progeny, and negative connotations of maternal vitamin D deficiency may extend well into the years beyond infancy. These concepts, yet again, underscore the need for appropriately designed future studies.

Vitamin D and lactation: human data

Human breast milk is a poor source of vitamin D (Hollis and Wagner, 2011; Holick et al., 2012). Therefore, maternal vitamin D sufficiency is thus particularly relevant in exclusively breastfed infants. Daily intake of 600 IU of vitamin D as per current recommendations (ACOG Committee Opinion, 2011; IOM, 2011) is suggested to be inadequate for either prevention or for the correction of documented vitamin D deficiency during the periods of pregnancy and lactation (Hollis and Wagner, 2004; Bodnar et al., 2007b). Additional supplementation with 1000 IU of vitamin D beyond the dose inclusive in the commonly utilized prenatal vitamins (400 IU) has been suggested to ensure that serum levels of 25(OH)D are maintained >30 ng/ml (Endocrine Society Clinical Guidelines, 2011; Holick et al., 2011). To satisfy the requirements of an infant who is exclusively breastfed, the mother requires 4000–6000 IU/day to transfer enough vitamin D into her milk if she chooses not to give a vitamin D supplement to the infant (Hollis and Wagner, 2004; Endocrine Society Clinical Guidelines, 2011).

Recommended daily vitamin D intake

Despite circulating 25(OH)D level being recognized to reliably reflect an individual's vitamin D status, a consensus regarding the *optimal* serum level for health maintenance is, however, lacking (Aloia, 2011). The revised guidelines issued by the Institute of Medicine (IOM) identify serum 25(OH)D threshold of 20 ng/ml (50 nmol/l), at and above which, adverse skeletal sequelae may be avoided (IOM, 2011; ACOG, 2011; Table II). The Endocrine Society of North America, however, maintains a higher 25(OH)D threshold of 30 ng/ml to differentiate between states of vitamin D sufficiency (>30 ng/ml) and insufficiency (≤30 ng/ml ≥20 ng/ml). Of particular concern is whether the IOM specified RDA for vitamin D is sufficient to maintain 25(OH)D levels >30 ng/ml in the *at-risk* populations such as those already deficient in vitamin D or during pregnancy and lactation (Lee et al., 2007; IOM, 2011; Holick et al., 2012). The Endocrine Society Clinical Practice Guidelines recommend higher daily allowance for vitamin D during pregnancy and lactation than proposed by the IOM (Table III).

Conclusions

In the recent years emerging data have suggested that vitamin D is not only critical for the maintenance of bone health and for calcium and phosphate homeostasis, but also imposes multisystem regulatory effects that modulate overall wellbeing and health (Holick et al., 2007). Herein, we have reviewed the existing literature that supports a plausible role for vitamin D in reproductive physiology. Summarizing the available data, we have attempted to convey the similarities as well as differences in data collected from studies carried out in rats, transgenic mice as well as in clinical settings. It is, however, noteworthy that

Table II Recommended daily allowance and tolerable upper limit for vitamin D as per guidelines issued by the IOM.

Age (years)	RDA vitamin D (IU/day)	Upper limit vitamin D (IU/day)
1–3	600	2500
4–8	600	3000
9–18	600	4000
19–50	600	4000
51–70	600	4000
71+	800	4000
Pregnancy	600	4000
Lactation	600	4000

Source: IOM (2011).

Table III Recommended daily allowance and tolerable upper limit for vitamin D in pregnancy and lactation as per the Endocrine Society Practice Guidelines.

Age	Daily requirement (IU/day)	Upper limit vitamin D (IU/day)
Pregnancy		
14–18	600–1000	4000
19–30	1500–2000	10000
31–50	1500–2000	10000
Lactation		
14–18	600–1000	4000
19–30	1500–2000	10000
31–50	1500–2000	10000

Source: Holick *et al*. (2011).

transgenic models of vitamin D deficiency (Vdr and cyp27b1 null) are not defined by a critical circulating level of vitamin D or its metabolites. Thus, the direct extrapolation of existing data from transgenic mice to humans must be treated with caution. Nevertheless, a growing body of literature suggests that an individual's vitamin D status may adversely impact reproductive functions. However, the dearth of prospective interventional studies and studies that define the mechanisms whereby vitamin D affects reproductive physiology underscores the dire need for appropriately designed intervention trials. Given its recognized safety, accessibility and ease of administration vitamin D supplementation could prove to be a cost effective strategy to improve public health.

Author's' roles

All authors contributed to the different components of the review paper. All authors have drafted and/or critically read and revised the manuscript for important intellectual content and have approved the final version of the manuscript for submission.

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Conflict of interest

None declared.

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