INVITED REVIEW

Vitamin D_3 Action within the Ovary – an Updated Review

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Short title: Vitamin D_3 in the ovary.

Summary

Vitamin D_3 is well-known as a major regulator of calcium and phosphorus homeostasis. A growing body of evidence highlights its crucial role in the regulation of reproductive processes in females. The role of vitamin D_3 in the female reproductive tract has been extensively investigated because its receptor is abundant in reproductive organs, including ovary. Importantly, besides expression of vitamin D_3 receptor, the ovary is an extrarenal site of vitamin D_3 metabolism. The influence of vitamin D_3 on follicular development and ovarian steroidogenesis has been investigated. Furthermore, vitamin D_3 deficiency has also been associated with polycystic ovary syndrome, premature ovarian failure and ovarian cancer. The objective of this review is to summarize our knowledge about the contribution of vitamin D_3 to physiological and pathological processes within the ovary.

Key words

Vitamin D_3 • Vitamin D_3 receptor • Ovary • Polycystic Ovary Syndrome • Premature Ovarian Failure

Vitamin D_3 (VD_3) deficiency is recognized as a global problem which increases the risk of many chronic diseases. The status of VD_3 in the organism depends on sun exposure, diet, intake of VD_3 supplements, lifestyle and genetic factors (DeLuca 2004). It is well-known that VD_3 is predominantly involved in the regulation of calcium and phosphorus homeostasis and crucial for bone mineralization. However, a growing body of literature indicates its pleiotropic actions within the organism including an influence on various physiological and pathological processes. The classical VD_3 target tissues are the intestine, kidneys and bones. Importantly, among the non-classical sites of VD_3 action are tissues of the female reproductive tract. VD_3 receptor (VDR) and VD_3 metabolic enzymes have been found in the ovary, uterus, fallopian tube, vagina and placenta of both human and animals, confirming the direct role of VD_3 in these organs (Lerchbaum and Obermayer-Pietsch 2012). In recent years there have been an increasing number of scientific papers suggesting a correlation between low VD_3 level and reduced fertility, metabolic and endocrine disorders, polycystic ovary syndrome (PCOS), premature ovarian failure (POF) and ovarian cancer (Muscogiuri *et al.* 2017). This review focuses on the influence of VD_3 on physiological processes within the ovary as well as its contribution to ovarian pathologies that is summarized in Figure 1.

VD_3 metabolism and mechanism of action

The main source of circulating VD_3 is endogenous synthesis in the skin following ultraviolet-B irradiation (UVB). Only small amount of VD_3 is derived from the diet (fatty fish, cod-liver oil, milk, eggs) or supplements (Bikle 2014). In keratinocytes, 7-dehydrocholesterol is converted to previtamin D_3 under UVB. Next, previtamin D_3 undergoes isomerization under the influence of the body's thermal energy and forms biologically inactive VD_3 (cholecalciferol). VD_3 is released from the keratinocyte membranes into the extracellular

space and hence into the blood, where it is transported with vitamin D binding proteins (VDBP). In the liver, hydroxylation to $25(OH)D_3$ (25-hydroxycalciferol, calcidiol) takes place in the presence of 25-hydroxylases (e.g. CYP2R1, CY24A1). A second hydroxylation in the kidneys involves 1α-hydroxylase (CYP27B1) action, resulting in biologically active VD_3, i.e. $1\alpha,25(OH)_2D_3$ (1α, 25-dihydroxycholecalciferol, calcitriol). Both calcidiol and calcitriol may be degraded as a result of further hydroxylation by CYP24A1(Christakos *et al.* 2016) (Figure 2). The concentration of calcitriol circulating in the blood is not a reliable indicator of the VD_3 level in the body because its content and metabolism are controlled by parathormone and depend on the concentration of calcium and phosphorus. Thus, calcidiol is considered the best indicator due to its long half-life and lack of mechanisms regulating its level (DeLuca 2004).

The biological effect of $1\alpha,25(OH)_2D_3$ on target cells is mediated by VDR that belongs to the superfamily of ligand-activated steroids receptor and acts as a transcriptional factor. VDR is composed of a short N-terminal domain, a highly conserved DNA binding domain, a hinge region and an α-helical C-terminal ligand binding domain (Christakos *et al.* 2016). Calcitriol binding to the ligand binding domain induces heterodimerization of VDR with the 9-*cis*-retinoic acid receptor (RXR). The VDR-RXR complex is translocated to the nucleus and binds to the VD_3 response element (VDRE) regulating the expression of target genes. The activation/inhibition of transcription requires also the recruitment of wide range of co-regulators (Keane *et al.* 2017). Studies on the structure of VDR have shown the presence of two overlapping ligand binding sites in the C-terminal domain. They were defined as the genomic pocket (VDR-GP) and the alternative pocket (VDR-AP). The first of these initiates the genomic response, while the second one can cause both genomic and non-genomic effects (Mizwicki *et al.* 2004). The final signaling pathway triggered after ligand (calcitriol or its synthetic analogues) binding to VDR also depends on VDR localization in the cell. The receptor has been found in the cytoplasm/nucleus and mitochondria as well as in cell

membrane cavities, i.e. caveolae. VDR located in caveolae triggers a rapid cell response by activating receptors associated with G proteins, phosphatases, kinases and ion channels (Keane *et al.* 2017). Recently it has been demonstrated that $1\alpha,25(OH)_2D_3$ can act by interaction with the MARRS (Membrane-Associated Rapid Response Steroid) protein that occurs in caveolae together with VDR. This type of VD_3 receptor is also known as GRP58 (Glucose Responsive Protein, 58 kDa), ERp57 or ERp60 (Endoplasmic Reticulum Protein 57/60 kDa) and Pdia3 (Protein Disulfide Isomerase Family A, Member 3) (Hii and Ferrante 2016) (Figure 2).

The role of VD_3 in the regulation of folliculogenesis

A growing body of literature suggests that VD_3 plays an important role in the regulation of ovarian processes that determine female fertility. Female reproductive potential is expressed as the number of primary follicles in the ovary at birth, known as the ovarian reserve. It decreases during postnatal life as a result of the recruitment of primary follicles to the growth (Monniaux *et al.* 2014). This process is controlled by growth factors and hormones, among which the most important is anti-Müllerian hormone (AMH). It is produced by granulosa cells of preantral and early antral follicles and inhibits initial recruitment of follicles, maintaining the ovarian reserve (Visser *et al.* 2006). Recently the influence of VD_3 on AMH concentration, and thereby on the ovarian reserve, has been extensively discussed. Studies conducted on a group of premenopausal women with regular menstrual cycles showed a positive correlation between the plasma concentration of $25(OH)D_3$ and AMH. In addition, a decrease in the level of both hormones was observed in the winter and this effect was reversed after VD_3 administration (Merhi *et al.* 2012). The effect of VD_3 on AMH level is probably due to the presence of the VDRE sequence in the AMH gene promoter as found in

prostate cells (Malloy *et al.* 2009). Furthermore, Merhi *et al.* (2014) has observed that $25(OH)D_3$ deficiency in follicular fluid correlated with an increased expression of the transcript for AMH type II receptor (AMHR-II) in human granulosa cells. VD_3 has also been shown to reduce the phosphorylation of the Smad 1/5/8 protein that contributes to signal transduction from AMHR-II. Thus, VD_3 may increase the synthesis of AMH but also modulate its effect on follicular cells by regulating intracellular signaling pathways (Irani and Merhi 2014). Despite the lack of literature data indicating the direct role of VD_3 in maintaining ovarian reserve, the effect exerted on AMH suggests a synergistic action between both hormones.

The effect of VD_3 on folliculogenesis has been demonstrated for the first time in studies conducted on *Vdr*- and *Cyp27b1*-knockout mice. They displayed increased ovarian interstitial tissue, weakened follicular development and lack of corpus luteum suggesting ovulatory disorders (Kinuta *et al.* 2000, Panda *et al.* 2001). The influence of VD_3 on follicular development *in vitro* has been studied on primates by Xu *et al*. (2016). They isolated preantral follicles and cultured them to the antral stage with addition of a low (25 pg/ml) or high (100 pg/ml) concentration of $1\alpha,25(OH)_2D_3$. The low dose had a positive effect on oocyte growth, survival and development of preantral follicles, suggested by the authors as being due to an increased sensitivity to follicle-stimulating hormone (FSH). However, after reaching the antral stage, the higher dose of $1\alpha,25(OH)_2D_3$ was more effective and promoted follicular growth (Xu *et al*. 2016). These results show that VD_3 affects both the early and late stages of folliculogenesis, and that its effect is dose-dependent.

The growth and development of ovarian follicles are associated with the proliferation and differentiation of granulosa cells. Yao *et al.* (2017) demonstrated an effect of VD_3 on the proliferation of goat granulosa cells by regulation of oxidative stress and changes in the

expression of genes regulated cell cycle. The influence of VD_3 on granulosa cell proliferation has also been observed in hens (Wojtusik and Johnson 2012).

The role of VD_3 in the regulation of steroidogenesis

Besides production of germ cells, the ovary synthesizes steroid hormones including progesterone, androgens and estrogens. Studies so far indicate that VD_3 regulates the expression and activity of steroidogenic enzymes and that the effect is tissue specific (Lundquist 2014). In human granulosa cells, there is an augmented expression and activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) as well as an increase in progesterone production (Merhi *et al.* 2014), consistent with the studies of Parikh *et al.* (2010). Studies on porcine granulosa cells *in vitro* revealed no effect of $1\alpha,25(OH)_2D_3$ on basal progesterone production, but noted its increase after insulin and FSH stimulation (Smolikova *et al.* 2013). In contrast, other studies on porcine granulosa cells showed a reduced progesterone synthesis following $1\alpha,25(OH)_2D_3$ treatment that was associated with decreased cholesterol side-chain cleavage enzyme (CYP11A1) mRNA and protein expression and increased 3β-HSD mRNA and protein expression (Hong *et al.* 2016). Results from experiments on the effect of VD_3 on ovarian estrogen synthesis are clearer. Studies on human (Parikh *et al.* 2010), porcine (Hong *et al.* 2017) and goat (Yao *et al.* 2017) granulosa cells revealed a stimulatory effect of $1\alpha,25(OH)_2D_3$ on estradiol and estrone production and on the expression of aromatase (CYP19A1), which converts androgens to estrogens. These results may be explained by the fact that the VDRE element is present in the promoter of the gene encoding CYP19A1 in human placental cells (Sun *et al.* 1998). The role of VD_3 in ovarian androgen synthesis has not yet been intensively studied. Parikh *et al.* (2010) found no effect of VD_3 on androgen production in humans. However, it has been observed that testosterone affects the

transcriptional activity of VDR in porcine granulosa cells by inhibiting the formation of VDR-RXR complexes (Herian *et al.* 2018). This information suggests that VD_3 is an important modulator of steroidogenesis in the human and mammalian ovary.

VD_3 and PCOS

PCOS is one of the most common endocrinopathies of women of reproductive age. It is characterized by ovulation disorders, irregular cycles, the presence of ovarian cysts, hyperandrogenism, abnormal level of gonadotropins and metabolic disturbances (hyperinsulinemia, insulin resistance, dyslipidemia) resulting in infertility. Recent studies show a reduced calcidiol level in women with PCOS suggesting a relationship between VD_3 deficiency and the occurrence of many PCOS symptoms (Dravecka *et al.* 2016, Shahrokhi *et al.* 2016). VD_3 deficiency is often associated with a disturbed calcium metabolism, which in women with PCOS may inhibit follicle maturation and ovulation. The diminished level of circulating VD_3 also reduces the activity and expression of CYP19A1, which disturbs conversion of androgens to estrogens. An increase in androgen concentration blocks follicular maturation before ovulation and leads to ovarian cyst appearance (Lorenzen et al. 2017).

One of the metabolic symptoms occurring in 60-80% of women with PCOS is insulin resistance. VD_3 has been shown to increase insulin synthesis and secretion, and expression of its receptor. In addition, it increases cell sensitivity to insulin by inhibiting the production of pro-inflammatory cytokines (Sung *et al.* 2012). The direct effect of VD_3 on insulin secretion and consequently on glucose metabolism is mediated *via* VDR present in β cells of the pancreas. Importantly, VDRE sequence was found in the promoter of the gene coding insulin (Sung *et al.* 2012). It is also believed that the indirect effect of VD_3 on insulin sensitivity depends on the regulation of intracellular calcium level, which is necessary for proper cell

signaling in the insulin-dependent tissues (muscle and fat) (Pittas *et al.* 2007). Insulin resistance results in elevated glucose concentrations, which may in turn modify proteins, lipids and nucleic acids in a non-enzymatic way leading to the formation of Advanced Glycation End-products (AGEs). An increased plasma concentration of AGEs as well as their accumulation in granulosa and theca cells of ovarian follicles has been observed in PCOS. These compounds bind to their soluble receptor (sRAGE) and induce the formation of reactive oxygen species and cytokines with pro-inflammatory properties. The involvement of AGEs and their receptors in the pathogenesis of PCOS is mainly associated with the disturbance of follicular growth. This effect can be attenuated by $1\alpha,25(OH)_2D_3$, which has anti-inflammatory properties (Merhi 2019).

VD_3 and POF

POF is defined as the loss of ovarian function before the age of 40 years. It is characterized by premature depletion of the ovarian reserve due to autoimmune damage or genetic predisposition. Typical POF symptoms include amenorrhea, high gonadotropins and low estradiol levels, as well as decreased AMH concentration in the plasma (Knauff *et al.* 2009). Keeping in mind the effect of VD_3 on the synthesis of AMH as an ovarian reserve marker, VD_3 contribution to the POF etiology seems to be possible. Research conducted on a population of women with POF showed the negative correlation between VD_3 deficiency and FSH level (Kebapcilar *et al.* 2013). On the other hand, study that confirmed the characteristic hormonal profile (high FSH and LH level, low estradiol level) in patients with POF, has also shown no changes in $25(OH)D_3$ concentration (Ersoy *et al.* 2015). The above mentioned results do not allow to unequivocally confirm the role of VD_3 in the pathogenesis of POF. However, it is suggested that VD_3 deficiency may decrease the AMH level, which in turn

leads to increased FSH concentration and the of POF as a consequence (Ersoy *et* occurrence *al.* 2015).

VD_3 and ovarian cancer

Epidemiological studies have shown that the occurrence of ovarian cancer is inversely correlated with exposure to UVB radiation, which is necessary for the synthesis of VD_3 in the skin, thus suggesting its involvement in the pathogenesis of ovarian cancer (Guo *et al.* 2018). Analysis of plasma calcidiol concentrations among ovarian cancer patients showed that this was significantly lower (under 20 ng/ml) than in the control group. In addition, a group of patients with calcidiol level below 10 ng/ml had a statistically lower survival rate. From the results, it was noticed that VD_3 deficiency may have greater impact on patients with more aggressive cancers (Colonese *et al.* 2015).

It is believed that VDR gene polymorphisms increase the risk of ovarian cancer. The most common is the single FokI nucleotide polymorphism located at the 5 'end, which leads to the synthesis of VDR protein with a longer amino acid sequence. Further identified polymorphisms - BsmI, ApaI and TaqI - are located at the 3 'end and do not affect the synthesis of functional VDR protein but regulate the stability of VDR mRNA (Guo *et al.* 2018).

The anti-tumor mechanism of VD_3 action involves the inhibition of cell proliferation by affecting the cell cycle regulatory proteins (p21, p27, cyclins). In addition, cell cycle inhibition in the G2/M phases and induction of ovarian cancer cell death by increasing mRNA and GADD45α protein expression were reported. Further research indicates that VD_3 inhibits cancer angiogenesis and metastasis. It has also been reported that it affects glucose and fatty acids metabolism in cancer cells (Guo *et al.* 2018). Previous studies confirm that

administration of VD_3 or its analogues is not an effective method of ovarian cancer treatment. On the other hand, proper VD_3 supplementation may reduce the risk of illness (Guo *et al.* 2018).

Conclusions

Numerous epidemiological data and results of animal studies confirm that VD_3 plays a key role in supporting ovarian function. It has a positive effect on folliculogenesis and maintenance of the ovarian reserve, and also stimulates steroidogenesis. There is growing concern that the global problem with VD_3 deficiency among women contributes to reproductive complications. Therefore, VD_3 supplementation seems to be a great opportunity for the treatment and insertion of ovarian pathologies. In conclusion, monitoring of plasma calcidiol level should become a preventive diagnostic for ensuring female health.

Acknowledgments

The websites https://biorender.com (BioRender) and https://smart.servier.com (SMART Sevier Medical Art) and were applied to prepare Figure 1 and Figure 2, respectively.

Conflict of Interest

There is no conflict of interest to declare.

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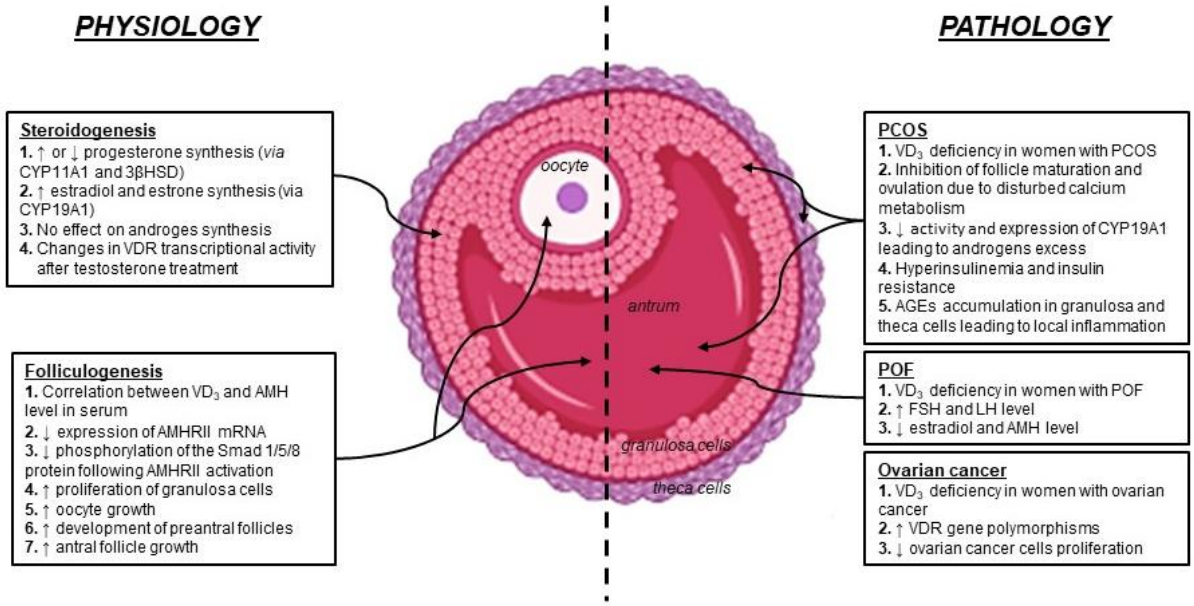
Legend to Figures

Fig. 1. Vitamin D_3 contribution to physiological and pathological processes within the ovary. AGEs: advanced glycation end-products; AMH: anti-Müllerian hormone; AMHRII: AMH receptor type II; 3β-HSD: 3β-hydroxysteroid dehydrogenase; CYP11A1: cholesterol side-chain cleavage enzyme; CYP19A1: cytochrome P450 aromatase; FSH: follicle-stimulating hormone; GCs: granulosa cells; LH: luteinizing hormone; PCOS: polycystic ovary syndrome; POF: premature ovarian failure; TCs: theca cells; VD_3: vitamin D_3; VDR: vitamin D_3 receptor

Fig. 2. Overview of vitamin D_3 metabolism and intracellular action. CYP2R1: 25-hydroxylase present in the endoplasmic reticulum; CYP24A1: 24-hydroxylase; CYP27A1: 25-hydroxylase present in the mitochondria; CYP27B1: 1α-hydroxylase; MAARS: membrane-associated rapid response steroid protein; RXR: 9-*cis*-retinoic acid receptor; UVB: ultraviolet-B irradiation; VDBP: vitamin D_3 binding protein; VDR: vitamin D_3 receptor; VDRE: vitamin D response element

396 Fig. 1



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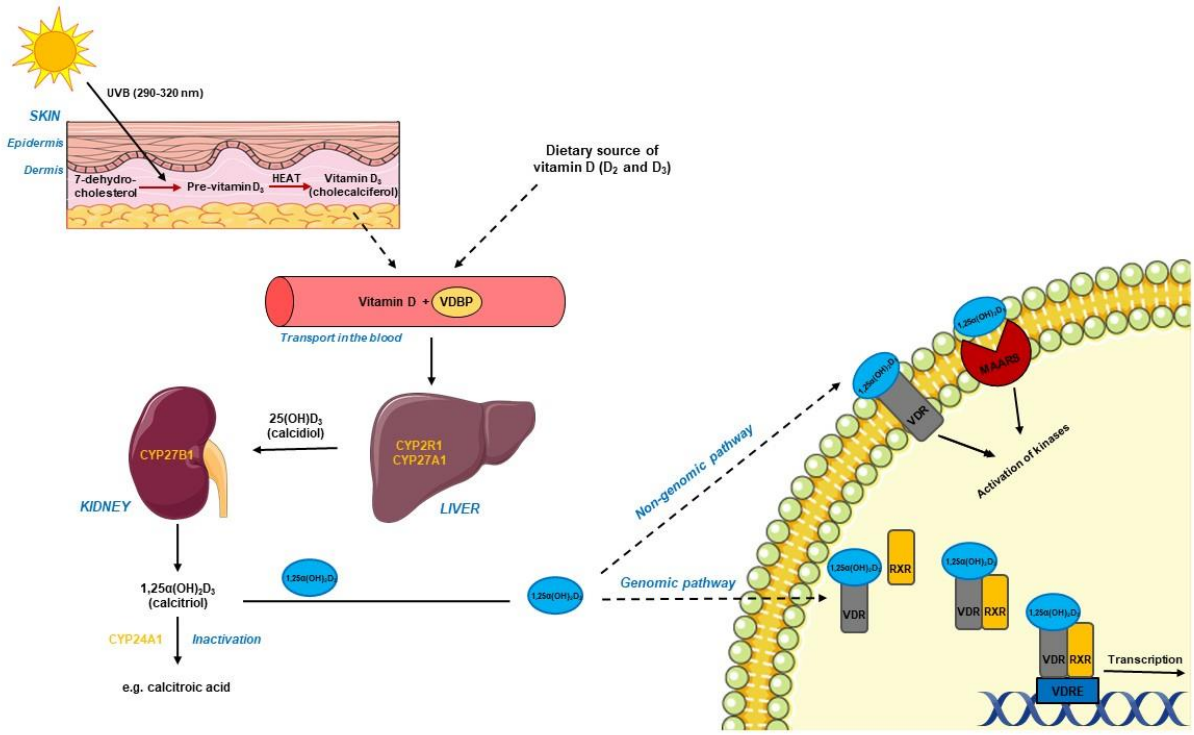
399 Fig. 2



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