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Vitamin D in human reproduction: the more, the better? An evidence-based critical appraisal

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Abstract. – OBJECTIVE: Vitamin D is a fat-soluble secosteroid hormone that regulates calcium, magnesium, and phosphate homeostasis and plays a pivotal role as antiproliferative and immunomodulatory mediator. Considering the different sources of synthesis and dietary intake as well as the pleiotropic actions in extremely diverse (micro)environments of the body, the supplementation of this Vitamin should be carefully evaluated taking into account the several pathways that it regulates. In the current brief review, we aimed to summarize the available evidence about the topic, in order to suggest the best evidence-based supplementation strategy for human reproduction, avoiding the unuseful (and sometimes hazardous) empiric supplementation.

MATERIALS AND METHODS: Narrative overview, synthesizing the findings of literature retrieved from searches of computerized databases.

RESULTS: Accumulating evidence from in vitro fertilization (IVF) trials suggests that fertilization rate decreases significantly with increasing levels of 25OH-D in follicular fluid; in addition, Vitamin D levels in the follicular fluid are negatively correlated to the quality of embryos and the higher values of Vitamin D are associated with lower possibility to achieve pregnancy. Both low and high Vitamin D serum concentrations decrease not only spermatozoa count, but their progressive motility as well as increase morphological abnormalities. Finally, studies in animal models found that severe hypervitaminosis D can reduce the total skeletal calcium store in embryos and may compromise the postnatal survival.

CONCLUSIONS: Based on the retrieved data, we solicit to be extremely selective in deciding for Vitamin D supplementation, since its excess may play a detrimental role in fertility.

Key Words:
Vitamin D, *In vitro* fertilization, Ovary, Infertility, Sex steroid hormone receptors.

Introduction

Vitamin D is a fat-soluble secosteroid hormone that regulates calcium, magnesium, and phosphate homeostasis[1], and plays a pivotal role as antiproliferative and immunomodulatory mediator[2]. Despite the endogenous synthesis, it is possible to supplement Vitamin D with diet: in particular, Vitamin D3 (cholecalciferol) is mainly contained in sea fish fat and cod liver oil, while D2 (ergocalciferol) in plants and mushrooms.

Vitamin D3 is synthesized in the skin from 7-dehydrocholesterol, following the activity of ultraviolet B radiation[3]. It is metabolized within the body to the hormonally-active form known as $1,25(OH)_2D_3$ through two steps: cholecalciferol is hydroxylated to 25-hydroxycholecalciferol by 25-hydroxylase within the liver; after this first hydroxylation, Vitamin D is transported by vitamin D-binding protein (VDBP) into the bloodstream; subsequently, 25-hydroxycholecalciferol serves as a substrate for 1 α-hydroxylase (CYP27B1), forming the active $1,25(OH)_2D_3$ within the kidneys[4].

The blood level of 25-hydroxycholecalciferol reflects the amount of Vitamin D ingested with diet or synthetized in the skin, since hepatic 25-hydroxylase regulation by these parameters is negligible[5]. On the contrary, the activity of 1-α-hydroxylase in the kidney is tightly regulated: both parathyroid hormone and low blood levels of phosphate induce its synthesis and modulate the production of the active hormone[6].

Despite the impressive number of published studies, data are not still so robust to clearly elucidate all the pathways and effects of Vitamin D in human reproduction. Considering the different

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sources of synthesis and dietary intake as well as the pleiotropic actions in extremely diverse (micro)environments of the body, the supplementation of this Vitamin should be carefully evaluated taking into account the several pathways that it regulates. Based on these elements, in the current brief review we aimed to summarize the available evidence about the topic, in order to suggest the best evidence-based supplementation strategy for human reproduction, avoiding the unuseful (and sometimes hazardous) empiric supplementations.

Effects of Vitamin D on Hormonal and Metabolic Regulation: Master and Minions

Form the chemical point of view, Vitamin D is similar to steroid hormones, and both these compounds act at nuclear level. In particular, Vitamin D binds and activates the nuclear Vitamin D receptor (VDR), which is also a member of the steroid/thyroid/retinoid receptor superfamily[7]. Subsequently, VDR forms a heterodimer with retinoid-X receptor, binds to hormone response elements on DNA and finally drives a pleiotropic cascade of events[8-12]. Among these events, it is widely accepted that $1,25(OH)_2D_3$ has a key role in bone metabolism and calcium homeostasis[13], although it is also known to modulate cell proliferation, differentiation, cancer invasion, and angiogenesis[3,14-17].

In this view, since early 80's it was demonstrated that both decidua and placenta produce some active Vitamin D metabolites, including $1,25(OH)_2D_3$[18]. In addition, the presence of 1-α-hydroxylase and VDR receptors was shown in the ovary (particularly in granulosa cells)[19], endometrium[20], pituitary gland[21] and placenta[22], suggesting that Vitamin D can orchestrate several regulatory pathways in human reproduction[23,24]. 1-α-hydroxylase also seems to play a pivotal role for acrosome reaction in spermatozoa, in a paracrine/autocrine fashion, allowing the increase of intracellular concentration of Ca^{+2} ions driven by $1,25(OH)_2D_3$[25]. Interestingly, the influence of Vitamin D on human reproduction seems to be tightly connected not only to calcium homeostasis, but also to its paramount importance as direct regulator of the aromatase gene expression: indeed, accumulating evidence suggests that VDR-bound $1,25(OH)_2D_3$ acts as a transcription factor to regulate the expression of the CYP19 gene, which is widely known to encode aromatase, the key enzyme for estrogen production[26]. In addition, $1,25(OH)_2D_3$ induces the production of Vitamin D-24-hydroxylase (CYP24A1), which catalyzes its conversion to inactive metabolites[27,28]. Underlining these elements, it was recently shown that VDR expression in both human myometrium and endometrium significantly changes throughout the menstrual cycle, suggesting a hormonal-dependent regulation[29]. Furthermore, recent data showed that Vitamin D is able to stimulate the production of progesterone, estrogen, estrone and insulin-like growth factor binding protein 1 (IGFBP-1) in cultured human ovarian cells; interestingly, Vitamin D and insulin synergistically inhibit IGFBP-1 production, although Vitamin D alone stimulates IGFBP-1 production in the same cells[19]. These events clearly suggest that a cross-talk between hormonal and metabolic pathways occurs. In partial agreement with these data, Vitamin D deficiency was also found to be associated with insulin resistance through the modulation of insulin receptor expression[30]; furthermore, Vitamin D seems to increase insulin sensitivity[31]. Probably, the effects on estrogen homeostasis may be caused by Vitamin D regulatory mechanism on aromatase expression. Nevertheless, scholars showed that Vitamin D causes only poor changes on steroidogenic acute regulatory protein (StAR), 3-β-hydroxysteroid dehydrogenase (3-βHSD) and aromatase mRNA expression[19], so the scenario seems very far to reach a final shape. $1,25(OH)_2D_3$ seems to increase estrogen and progesterone production also in human placenta[22,32]. In addition, it has been shown to regulate human chorionic gonadotropin (hCG) expression and secretion in cultured syncytiotrophoblasts, and to stimulate estradiol and progesterone secretion from trophoblasts in a dose-dependent fashion[33]. Last but not least, Vitamin D can induce the transcription of HOXA10[34] in the endometrium, decidua and placenta. HOXA10 is known to play a pivotal role in orchestrating embryo implantation[35] and the development of female tract organogenesis[36], together with WNT/β-catenin[37]. Interestingly, treatment with Vitamin D increases mRNA and protein expression of HOXA10[34]. In the placental microenvironment, VDR modulates the transfer of calcium between trophoblast and the endometrial decidua, helping to avoid uterine contraction and, subsequently, reduces the risk of preterm delivery[38]. The presence of VDR was also demonstrated in testicles and spermatozoa[39]. In particular, $1,25(OH)_2D_3$ is able to increase intracellular Ca^{+2} concentration and the activity of acrosin in spermatozoa in an autocrine/paracrine fashion[25], accounting for the essential acrosome reactions during fertilization of the oocyte. Corroborating the hypothesis of autocrine/paracrine activity, it was found that CYP27B1, which is necessary to form active $1,25(OH)_2D_3$, is directly

expressed in human male reproductive tract[40,41]. In addition, Vitamin D has been shown to stimulate calcium uptake through a nuclear receptor activity in Sertoli cells[42], whose secretory activities are ion channel-dependent. Despite accumulating evidence already suggested a clear role for $1,25(OH)_2D_3$ and VDR in the abovementioned pathways, the risk of adverse effects of Vitamin D supplementation is still surrounded by lights and shadows. In this regards, new data tried to shed light on this "grey zone". In particular, it was shown that transcriptional and translational regulation of progesterone biosynthesis-related genes in porcine granulosa cells is significantly altered by $1,25(OH)_2D_3$; in addition, the same study[43] found that progesterone concentration was decreased in response to $1,25(OH)_2D_3$. Progesterone is an essential hormone in reproduction[44,45], and it has been successfully used as prophylaxis in the prevention of spontaneous miscarriage[46-49] and preterm labour[50] thanks to its immunomodulatory properties at the maternal-fetal interface[51]. Considering these elements, it is possible to hypothesize that Vitamin D excess may cause a reduction of this important hormone and consequently play a severe detrimental role during early pregnancy.

It was found that *in vitro* calcitriol treatment in human prostate cancer cell line up-regulated Anti-Müllerian Hormone (AMH) mRNA expression levels[52] and that functional Vitamin D response element (VDRE) was found in the promoter region of human AMH gene[53]. Nevertheless, recent evidence from large and well-designed human-based cross-sectional analysis clearly demonstrates that Vitamin D deficiency is highly unlikely to have a detrimental effect on ovarian reserve[54]. Last but not least, the expression of the parathyroid hormone-related protein (PTH-rP) gene is repressed by $1,25(OH)_2D_3$[55-62]; considering that PTH-rP has a potent vasorelaxant activity in human endometrium[63,64], its reduction caused by Vitamin D supplementation may consequently alter the necessary remodeling of spiral artery and the correct placental vascular framework development during early pregnancy. In the following section we try to analyze the reflection of these strong pieces of evidence on the clinical practice.

Clinical Evidence about Vitamin D in Reproduction: a Critical Appraisal

The most important data about Vitamin D levels and human reproduction came from *in vitro* fertilization (IVF) trials. In particular, on one hand it was shown that fertilization rate decreases significantly with increasing levels of 25OH-D in the follicular fluid; on the other hand, the implantation rate did not significantly increase in the same cohort[65]. Considering that level of 25OH-D in follicular fluid is reflective of body stores of Vitamin D, this evidence suggests that Vitamin D deficiency does not play a pivotal role in the outcome of IVF and, furthermore, that 25OH-D level in follicular fluid could not be used as an independent predictor of clinical pregnancy.

In partial confirmation of these results, it was found that Vitamin D levels in the follicular fluid are negatively correlated to the quality of embryos and that higher values of Vitamin D are associated with lower possibility to achieve pregnancy; in addition, women with overt hypervitaminosis D had poor IVF outcomes[66]. Notably, in the same work, higher levels of Vitamin D corresponded with lower levels of glucose in follicular fluid, corroborating the cross-link between Vitamin D and glucose metabolism (as previously mentioned). In this abnormal condition, altered glucose concentration in the follicular fluid may be detrimental for oocyte maturation and growth of granulosa and cumulus cells, affecting directly the oocyte competence[67]. Furthermore, $1,25(OH)_2D_3$ seems to alter AMH sensitivity in granulosa cells obtained from women who underwent oocyte retrieval for IVF: according to a recent data analysis, cumulus granulosa cells cultured with $1,25(OH)_2D_3$ show a drastic and significant decrease (32%) in AMH Receptor-II mRNA levels[68]. These data are in partial agreement with previous researches that showed how Vitamin D down-regulated AMH gene and up-regulated follicle-stimulating hormone receptor gene expression in hen's ovaries[69,70]. Probably, a strong confounding factor which may play a detrimental role in data interpretation about this point is body mass index (BMI). Although it is widely accepted that higher BMI is an independent risk factor for infertility, it was also recently found that the body weight of the women with follicular fluid 25(OH) D deficiency measured in single follicles was significantly higher, regardless of the etiology of infertility[71]. Nevertheless, the literature overview about this point is still controversial: as example, others advocated a beneficial role of replete follicular Vitamin D levels for IVF outcomes[72]. These apparently contradictory results may depend (at least in part) not only on Vitamin D concentration in follicular fluid, but also on the expression of VDBP. Corroborating this hypothesis, it was found that decreased expression of VDBP in

the follicular fluid is associated with improved IVF outcomes[73]. In addition, Firouzabadi et al[74] showed that Vitamin D levels in the follicular fluid were comparable in the pregnant as well non-pregnant women. This element suggests at least two key points: first of all, the poor efficacy of follicular fluid Vitamin D levels in predicting the pregnancy rate during IVF program; second, the dosage of Vitamin D before IVF program could be considered outdated, since the follicular fluid concentration is a reliable mirror of the whole body store of Vitamin D. Moreover, Vitamin D reserve is higher in women with endometriosis, a condition characterized in most of the cases by infertility[75]. Notably, a retrospective cohort study of 188 infertile women undergoing IVF showed a positive correlation between serum 25OH-D levels and IVF success rate among non-Hispanic white women; nevertheless, an opposite correlation was seen among Asian women, where pregnancy rates were higher in those with lower serum 25OH-D levels[76], confirming that serum concentration of Vitamin D is not reliable and robust to predict IVF outcomes. Considered altogether, the available evidence allows us to hypothesize that a correlation between the serum/follicular Vitamin D level and the pregnancy rate in IVF cycle does not occur. Probably, the real field in which the game is played should not be considered the ovary, but the endometrium[77]: some authors found that Vitamin D deficiency was not associated with the number of follicles and oocytes or with the morphology of the embryo in IVF procedures and, furthermore, that Vitamin D deficiency and insufficiency was associated with lower pregnancy rates in recipients of egg donation[78]. Adding these two pieces of evidence to the puzzle, it is possible to hypothesize that Vitamin D at physiologic levels has a beneficial role only on endometrial receptivity[79], but conversely an excess of this Vitamin plays a detrimental role in the ovarian homeostasis, disturbing the oocytes development and consequently embryo quality. From the molecular point of view, it is possible that the observed effects in clinical practice may derive from the Vitamin D action on hormonal homeostasis. Indeed, $1,25(OH)_2D_3$ can reduce significantly estrogen receptor (ER)-α, progesterone receptor (PR)-A and –B and steroid receptor coactivator (SRC) expression in human uterine leiomyoma cells[80], suggesting a clear role of this Vitamin as antagonist of sex steroid hormone receptors. This antagonistic effect on sex steroid hormone receptors was also confirmed in several oncological conditions, including endometrial[81,82], ovarian[28], breast[83,84] and other[85-88] cancers. Apart from female fertility, the probability of a negative effect of Vitamin D also on male fertility was suggested after a discovery of a molecular similarity of VDBP to antisperm antibodies[89]. In addition, several studies[90-92] found that both low (<50 nmol/L) and high (>125 nmol/L) Vitamin D serum concentrations decrease not only spermatozoa count but also their progressive movement as well as increase morphological abnormalities, even after adjustment for age, season, BMI, alcohol intake, and smoking. If excess of Vitamin D can be considered a serious danger for both male and female fertility, hypervitaminosis D seems to be equally dangerous also after conception: evidence from animal model suggests that the embryo is not protected against maternal hypervitaminosis D, but rather that $1,25(OH)_2D_3$ is transferred through the placental barrier where it reduces the total skeletal calcium store in embryos and may compromise postnatal survival[93-95].

Conclusions

To date, there are not specific guidelines regarding Vitamin D supplementation for women affected by endocrine disturbances and infertility. Our literature analysis leads us to solicit the necessity to be extremely selective in deciding for its supplementation, according to patient's condition. In particular, comorbidities[96-99] and/or specific period of life[100,101] which may influence the response to Vitamin D should carefully be evaluated, especially considering the effects on immune system[102,103]. In addition, the "empiric" supplementation of Vitamin D in healthy women seems not to be evidence-based and, probably, may play detrimental effects on fertility[104,105]. Confounding and opposite results have been obtained so far from IVF clinical studies: an overall analysis allows us to speculate that Vitamin D at physiologic levels has a beneficial role on endometrial receptivity, whereas an excess of this molecule plays a detrimental role on oocytes development and embryo quality, probably due to its anti-estrogenic effect (i.e. Vitamin D is able to reduce significantly ER-α, PR-A and —B and SRC expression in human uterine leiomyoma cells). According to this element, Vitamin D supplementation should be administered in selected populations and during specific moments of the ovarian cycle, in order to support specifically the

luteal phase. Finally, Vitamin D should be supplemented at appropriate dosage and, according to the most updated recommendations[106], only if serum concentration falls below 50 ng/ml (equivalent to 125 nmol/L).

Declaration of Interest

All authors have no proprietary, financial, professional, or other personal interest of any nature in any product, service, or company. The authors alone are responsible for the content and writing of the paper.

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