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Vitamin D: a steroid hormone with progesterone-like activity

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Abstract. – OBJECTIVE: This review is aimed at demonstrating the progesterone-like activity exerted by the active form of vitamin D, or calcitriol (1,25(OH)2D). To achieve this outcome, we compared the effects *in vivo* and *in vitro* exerted by progesterone and vitamin D, with a special focus on the female reproductive system and pregnancy.

MATERIALS AND METHODS: This is a literature review of the most important articles published in English on vitamin D as a hormone, mainly found by MEDLINE. Furthermore, a section of our review contains some unpublished data, concerning the analysis *in silico* of the similarities between the steric structure of progesterone and calcitriol, based on the availability of the experimental structures of progesterone and vitamin D3 receptors in complex with their physiological ligands in the RCSB Protein Data Bank.

RESULTS: Vitamin D was shown to exert many physiological activities during the very early stages of gestation in perfect synchrony with progesterone. Both the molecules mutually help and reinforce the activity exerted by each one. A little bit later than progesterone is released, vitamin D secretion rises, but only if pregnancy occurs. Calcitriol contributes to prepare the endometrium to be receptive. Moreover, it supports the implantation process and the course of pregnancy through different but similar pathways to those used by progesterone, giving rise to a significant synergy of action. It is increasingly evident that vitamin D gives an essential support from the luteal phase onwards.

CONCLUSIONS: Based on the evidence displayed in this review we may define appropriately vitamin D as a steroid hormone with progesterone-like activity.

Key Words:
Calcitriol, CYP24A1, CYP27A1, CYP27B1, Embryo implantation, Inflammation, Osteopontin, Pregnancy, Progesterone, Progesterone receptor, Vitamin D, Vitamin D receptor.

Abbreviations

1,25(OH)2D = 1α,25-dihydroxyvitamin D; 1α-OHase: 1α-hydroxylase; CL = corpus luteum; CRF = corticotropin-releasing factor; CSF = colony-stimulating factor; CYP24A1 = Cytochrome P450 family 24 subfamily A member 1; CYP27A1 = Cytochrome P450 Family 27 Subfamily A Member 1; CYP27B1 = Cytochrome P450 Family 27 Subfamily B Member 1; DBP = vitamin D Binding Protein; FGF = Fibroblast growth factor; IL = interleukin; TNF-α = tumour necrosis factor alpha; Treg cells = T regulatory cells; VDR = vitamin D receptor; VEGF = Vascular endothelial growth factor.

Introduction

A Story Coming from the Thirties

Between the end of sixties and the beginning of seventies some advanced researches and studies brought out to understand much better the activity of calcitriol or 1,25(OH)2D, two names referred to the active form of vitamin D (in our paper "vitamin D" will be used with the same meaning of them). They offered a wider picture of this very intriguing molecule, until then considered only an essential vitamin for bone growth and for fighting rickets. In that period vitamin D was defined, for the first time, as a new steroid hormone and its chemical structure was characterized[1,2]. In 1971 Norman et al[3] published a seminal paper where they stated that "It is possible that 1,25- dihydroxycholecalciferol should be reclassified as a steroid hormone. Certainly, its secretion by the kidney followed by its selective accumulation in the target intestinal mucosa where it exerts its characteristic physiological effect on calcium metabolism satisfies the minimal definitions of a hormone". Furthermore, in the same years, its nuclear receptor was identified[4,5]. Vitamin D

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started to be considered a hormone, even though many years had to pass before this concept became established in the scientific community, as stated in 1997 by the Nutritional Committee Report for both North America and Europe[6]. Despite this and other authoritative opinions and declarations[7], some scholars still do not accept this classification[8], but they are a minority. It is worth of note that a couple of decades before the sixties, some studies (1934) demonstrated the hormonal properties exerted by a group of molecules having the cyclopentanoperhydrophenanthrene ring, with seventeen-carbon tetracyclic hydrocarbon. Ovariectomized rats (Wistar strain) were injected with 100 mg of each compound dissolved in sesame oil and the treatment was able to induce estrus in these animals, showing that such effect is due to the presence of the classic steroid skeleton[9]. A marked activity was shown by ergosterol and its irradiation product calciferol (crystalline vitamin D), and still greater activity by neo-ergosterol[9]. Later, Feyel-Cabanes (1949) and Chambon (1951) highlighted that ergosterol and 7-dehydrocholesterol, administered at non-estrogenic nor progestogenic doses, act synergically with progesterone in sustaining the pregnancy[10] or obtaining implantation of ovariectomized rabbits[11]. These interesting results were then forgotten. The times were not yet ripe to go ahead in that direction of research.

Vitamin D and Steroid Hormones

In fairly recent years, the studies on calcitriol had a revival, even under the impulse of the discovery of areas, in developed countries, with deficiency and widespread rickets. Another cause for this renewed interest in the field came from the finding that many vitamin D receptors (VDR) in tissues and cells are not connected with its effects on bone, being linked to other, before unknown, benefits for health. Nowadays, vitamin D and its metabolites, from the structural point of view, are called secosteroids, i.e., compounds whose structure shows the above-cited steroid skeleton with seventeen-carbon tetracyclic hydrocarbon, that is not joined at the level of the bond located in position 9-10 of the ring B. Therefore, chemically they are like the mineralocorticoids, glucocorticoids, testosterone, progesterone, estrogens, chorionic gonadotropin. By now, both calcidiol and calcitriol are considered respectively pre-hormone and hormone. Indeed, they are synthetized endogenously in the skin by means of UVB stimulated photoconversion of 7-dehydrocholesterol and subsequent thermosensitive but noncatalytic isomerization to cholecalciferol. Then, cholecalciferol is hydroxylated by liver mitochondrial and microsomal 25-OHase CYP27A1. The resultant 25-hydroxycholecalciferol (calcidiol) is 1α-hydroxylated in the kidney by CYP27B1 (a mitochondrial 1α-OHase). This reaction yields the secosteroid 1α,25(OH)2D (calcitriol). Therefore, CYP27A1 and CYP27B1 are key enzymes involved in the synthesis of vitamin D, whereas the inactivating enzyme CYP24A1 determines the rate-limiting step for calcitriol catabolism[12]. In physiologic conditions at least the 80% of this vitamin is produced by the skin, whereas the remaining 20% or less is taken up from dietary sources. In areas rich in sunlight, this endogenous production provides enough quantity of vitamin D, at least in healthy young subjects. However, among people with darker skin, a severe and widespread deficiency may be found as seen in the US especially among blacks and Hispanics[13]. Moreover, subjects who are obese, have hypertension, low high-density lipoprotein cholesterol level, do not drink milk daily, are all significantly, independently associated with vitamin D deficiency[13].

Besides the chemical structure, there are other very convincing connections between vitamin D and the class of (steroid) hormones. These last ones are bound to transport proteins in plasma, however only the free molecule can spread into its target cells. These hormones are chemical messengers in a wide number of species and tissues to transmit signals. Obviously, the ability of steroid hormones to give rise to biological message depends on the presence of their receptors on their specific targets. Unlike the growth factors and peptide hormones which bind only to receptors on cell membrane, the fat-soluble steroid hormones can pass through the lipid bilayer surrounding the cell and interact also with their related receptors on the cell nucleus, inducing a genomic response. The receptor-hormone complex binds to DNA and triggers or represses the transcription of one or more genes[14]. Furthermore, steroid hormones bind to a variety of receptor types located near or associated with the plasma membrane[15-17]. In this way, they activate systems of second messengers, with rapid cellular responses that may range from seconds (e.g., opening of ion channels) to 10-60 min (e.g., activation of phosphatidylinositol-3'-kinase or endothelial nitric oxide synthase). Instead, the genomic responses commonly need from some hours to days before

becoming entirely manifest; they may be stopped by transcription or translation inhibitors[16]. Therefore, many steroid hormone receptors are in the cytoplasm and/or nucleus.

The parallelism between the above picture and the behaviour of calcitriol, its receptor and the resulting downstream activity, is striking. In fact, as for steroid hormones, vitamin D and its metabolites circulate in plasma linked to a transport protein produced by the liver: vitamin D Binding Protein (DBP). Additionally, as for the classic steroid hormones, VDR is a transcription factor located both in the nucleus of cells and on the cell membrane. The complex between vitamin D and its nuclear receptor, once activated, binds to DNA and, in this way, modifies the expression of numerous genes (genomic response). On the other side, the interaction with the VDR located on the cell membrane induces the formation of second cellular messengers (such as cAMP, diacylglycerol, inositol triphosphate, arachidonic acid) or the phosphorylation of some cellular proteins. Therefore, calcitriol, as progesterone, estrogens, etc., exerts genomic and nongenomic actions. On the whole, this picture further confirms the hormonal nature of vitamin D[16].

Vitamin D deficiency causes osteopenia/osteoporosis, immune system disorders, metabolic syndrome (obesity and diabetes), cancer, kidney disease, neurological and cardiovascular diseases, pregnancy complications, and its administration proved to be useful in these pathologies. As well known, only cells having the specific receptor for a given hormone, respond to its stimuli; therefore, it is important to highlight that the distribution of VDR is almost ubiquitous, involving various organs and tissues. VDR was found in hematopoietic tissues, immune system, monocyte/macrophages, lymphocytes, skin, muscle, smooth muscle cells, myoblasts, heart cardiac muscle cells and atrial myocytes, pancreas β cells, mammary gland, adrenal gland medullary cells, prostate, brain hippocampus/selected neurons, cartilage chondrocytes, liver parenchymal cells, lung, Sertoli/seminiferus tubule, pituitary gland, thyroid, parathyroid, ovarian, myometrial and endometrial cells, placenta[18-22].

Progesterone and Vitamin D Structural Similarities

It was discovered that all the nuclear receptors of steroid hormones show common structure/function domains and are named as "nuclear receptors superfamily" including four classes with a shared evolutionary process. Progesterone and vitamin D receptors belong to two different classes of the above-mentioned superfamily[23]. This relationship has to be keep in mind, being in perfect agreement with the core of our review. In this context, we aimed at determining which are the similarities between the steric structure of progesterone and vitamin D, in consideration of their common phylogenesis, by using in silico methods.

The crystal structures of progesterone in complex with the ligand-binding domain (LBD) of the human progesterone receptor (PDB code 1A28)[24] and of vitamin D in complex with the LBD of the vitamin D nuclear receptor (VDR) (PDB code 1DB1)[25] have been reported in the RCSB Protein Data Bank. By structure-based pharmacophore model tool of LigandScout software[26], we highlighted the binding requirements for these two ligands that are schematically represented in Figure 1.

Progesterone showed the ability to bind its receptor establishing i) hydrogen bond interaction with Gln725 and ii) hydrophobic contacts through the methyl groups with Met909, Met759, Met756 and Trp755 (Figure 1A).

On the other hand, vitamin D interacts with its receptor establishing three different hydrogen bonds interactions: i) with Ser237 and Arg274 through the hydroxyl group in position 1, ii) with Tyr143 and Ser278 through the hydroxyl group in position 3, and iii) with His305 e His397 through the hydroxyl group in position 25. Furthermore, there are some hydrophobic contacts with the different hydrophobic residues present in the binding site (Figure 1B).

To obtain more insight on the structural similarity between progesterone and vitamin D, the two ligands have been aligned in their binding conformation creating a shared feature pharmacophore model by LigandScout software (Figure 2).

The alignment shows an interesting overlapping of vitamin D (stick grey) and progesterone (stick green) highlighting three convergent points that have been translated in three pharmacophore features describing chemical functionalities:

– one hydrophobic sphere (yellow), mapped by the methylene group in position 19 of vitamin D and the methyl group in position 19 of progesterone;
– another hydrophobic sphere overlapped by the methyl group in position 18 of vitamin D and the methyl group in position 18 of progesterone
– one hydrogen bond acceptor (red sphere) occupied by hydroxyl group in position 3 of vitamin D and the carbonyl group of progesterone.

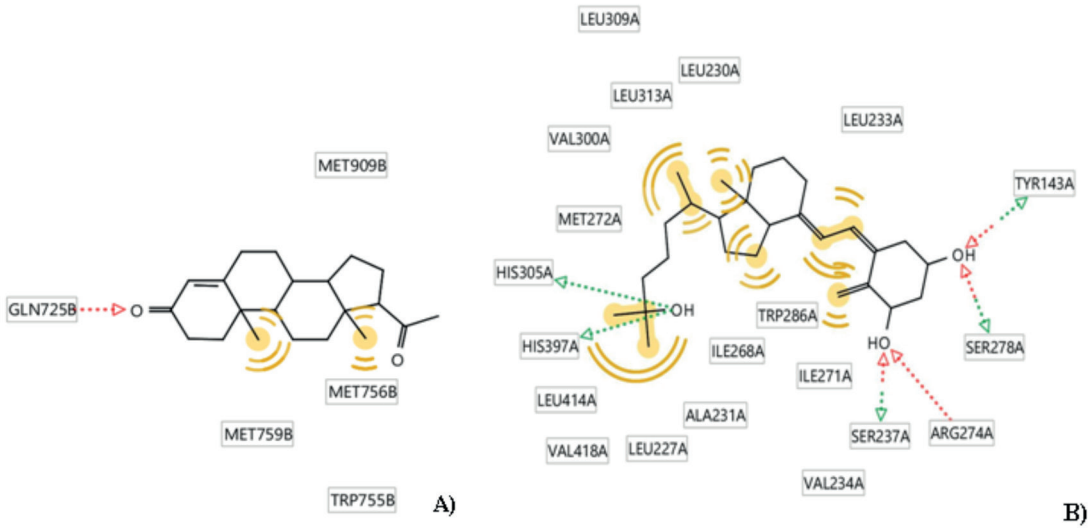

Figure 1. Interaction patterns of progesterone-LBD of progesterone receptor ***(A)*** and vitamin D-LBD of VDR ***(B)***. The labels refer to the amino acid residues involved in the interaction with the receptors: green and red arrows represent H-bond interactions, yellow areas highlight the groups involved in the hydrophobic contacts.

These information reveal that vitamin D contains some chemical functionalities useful for the binding to progesterone receptor.

In Figure 3 is represented a possible interaction of vitamin D with progesterone receptor obtained by superimposition of the two proteins 1A28 and 1DB1.

The results (Figure 3) showed that vitamin D could hypothetically interact with the progesterone receptor by establishing a H bond with Gln725 and hydrophobic interactions with the same hydrophobic amino acid residues involved in the interaction with progesterone. Clearly, this is only a hypothesis that has to be carefully tested in depth. However, this interaction, even if it is real, does not necessarily imply the induction of a physiological effect.

Progesterone Release in the Luteal Phase for Preparing the Scenario

The female reproductive system plays very complex roles, with the participation of a huge number of actors whose activity is finely regulated. In this process, progesterone and calcitriol play pivotal functions, and without their appropriate levels, reached in well-determined periods, gestation cannot occur. The menstrual cycle determines an essential timing for reproduction and it is divided into two main phases, the follicular and luteal phases, that must work in series, in an integrated manner, to allow pregnancy. During the follicular phase, also called proliferative phase, the follicles inside the ovaries start their development to prepare ovulation. This process occurs from the 1st to the 14th day of the menstrual cycle. The next luteal phase begins after ovulation and lasts normally from the 15th to the 28th day. It needs, on one hand, satisfactory secretions of progesterone by the corpus luteum (for this reason it is also termed secretory phase), and, on the other hand, a prompt endometrial response to its stimulus. In the luteal phase, the corpus luteum (CL), a transient endocrine gland which develops in this specific period, undergoes a prompt neovascularization. Such process is principally under the control of Vascular endothelial growth factor (VEGF) and Fibroblast growth factor (FGF), both upregulated in the luteinized granulosa cells and then absolute-

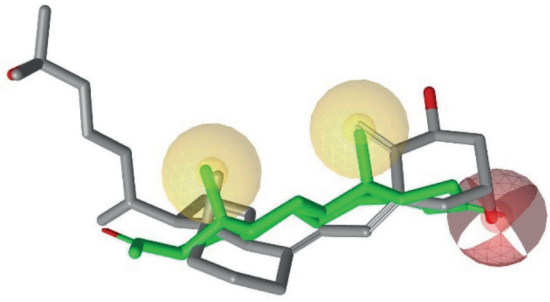

Figure 2. Shared-feature pharmacophore model. Progesterone and vitamin D are represented in green and grey stick respectively. Yellow spheres represent hydrophobic contacts while red sphere represents hydrogen bond acceptor.

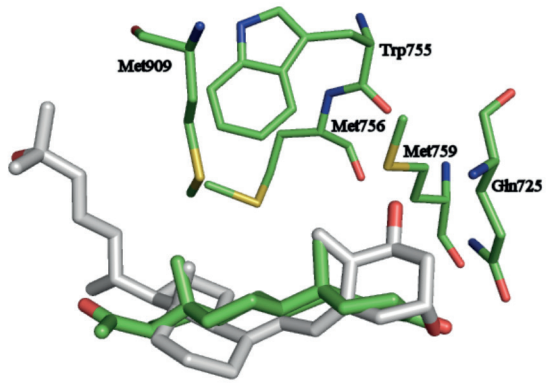

Figure 3. Superimposition of the crystal structures 1A28 and 1DB1. Amino acid residues present in the progesterone receptor, involved in the interaction with the ligand, are represented by green stick. Progesterone is represented by green stick while vitamin D is represented by grey stick.

ly necessary for the embryo implantation[27,28]. CL produces several different hormones; among them, progesterone is the most important since its activity is necessary and sufficient to hinder the proliferation of endometrium and promote its receptive state for blastocyst implantation to maintain early pregnancy. Progesterone is produced by the luteal cells in a pulsatile way with levels that may vary up to sevenfold in a range of a few hours. Its synthesis depends on the availability of appropriate levels of circulating cholesterol as substrate and on low stimulation by luteinizing hormone (LH), produced by gonadotropic cells of the anterior pituitary gland. Therefore, progesterone prepares the environment conducive to pregnancy success and it is categorically necessary, during the secretory phase, to induce the endometrium receptivity to the embryo implantation, even if moderate concentrations are sufficient[29]. In this scenario, vitamin D plays the role of second main actor and its function comes to light from several examples that we present below. Overall, it modulates the innate and adaptive immune system, controls cell proliferation and functions as a regulator of various and important metabolic processes, with effects on the implant, the production of cytokines and the immune response to infections[30-33].

Activities in Common Between Vitamin D and Progesterone in the inception of Pregnancy

Vitamin D

The metabolism of 1,25(OH)2D in pregnancy differs drastically from what normally occurs in the non-pregnant state. Increased circulating levels of vitamin D were detected through human pregnancy, independently of calcium homeostasis[34-36]. In addition, the above-cited presence of VDR on ovaries, uterus, endometrium and placenta should be emphasized. It is noteworthy that the number of VDR increases continuously during the first and second trimester of pregnancy, meaning that this vitamin plays a key physiological role in pregnancy. Especially in the first months of gestation adequate levels of vitamin D are even more essential, affecting not only the well-being of the mother but also that one of the fetus. VDR was detected in the endometrium throughout all the phases of the menstrual cycle and also later, namely in proliferative phase endometrial stromal cells, in secretory phase endometrial stromal cells and in early pregnant decidual cells[37]. The levels of 1α-OHase mRNA were comparable in endometrial stromal cells without any influence due to the phase of the cycle. Instead, such concentrations were found significantly higher in decidual cells[37]. As a side note, we observe that vitamin D, detected in the seminal fluid[38], could also function as a trigger molecule to provide additional levels to further support certain processes underlying a successful onset of pregnancy. It was observed that, in response to IL-1β secreted by the blastocyst, the dendritic cells of the endometrium and the macrophages produce 1α-OHase (the enzyme responsible for the last step of the synthesis process) and, consequently, calcitriol[37]. On the other hand, the stimulation of the endometrial 1α-OHase expression due to TNF is not as potent as that one achieved using very low concentrations of IL-1 β[37]. This evidence means that between the two main pro-inflammatory cytokines, at least in this case, the central role is played by IL-1 β, whereas TNF exerts a secondary function, despite its strong effects in many contexts. Thus, there is not only an increased calcitriol production in the first stages of pregnancy, but also a wide array of new synthetized receptors in the endometrium, ready for binding.

Using blood cells of women with a history of recurrent pregnancy losses (RPL) Vitamin D was reported to hinder *in vitro* the proliferation of T helper 1 (Th1) cells and to decrease the production of their cytokines, such as IFN-γ, IL-2 and TNF-α. On the other hand, vitamin D stimulated the secretion of T helper 2 (Th2) cytokines; among them, IL-10 is the most important anti-inflammatory cytokine released under the effect of calcitriol[39]. Furthermore, *in vitro* vitamin D significantly decreased in a dose-dependent manner

the cytotoxicity of natural killer (NK) cells[39] and it was able to suppress in the same cells the secretion of proinflammatory cytokines, such as IFN-γ and TNF-α, and to increase IL-10 release[39], as well as CSF2, IL-1, IL-6 too, thus modulating the inflammation that normally develops in the initial phase of pregnancy[40].

Accordingly to that, vitamin D significantly lowers the frequency of T helper 17 (Th17) cells in addition to reducing the Th17/Treg ratio in peripheral blood of patients with recurrent miscarriage compared with the control group[41]. The same modulatory effect of inflammation was seen in the induction and upregulation of the suppressive activity of T regulatory (Treg) cells in skin-draining lymph nodes[42].

Vitamin D by binding to VDR on the endometrium, upregulates the target genes, such as calbindin and osteopontin, also called secreted phosphoprotein one (SPP1)[37]. Development of receptivity requires changes in endometrial gene expression; endometrial expression of calbindin mRNA was shown to be highly regulated during implantation. Indeed, calbindin participates to the regulation of endometrial receptivity acting as calcium binding protein: it is well known that the function of reproductive organs and its musculature is depending on the influx of extracellular calcium or the release of intracellular stores[43]. Osteopontin is an adhesion protein involved in implantation and decidualization[44]. Its action has a direct influence as signal transduction at the uterine-placental interface throughout pregnancy, including the regulation of immune cells behavior and cytokine production. Both calbindin and osteopontin are essential for the embryo implantation and for the development of the placenta. Their effects on the endometrium favor the success of pregnancy[37,45-47]. Therefore, 1,25(OH)2D is reputed to exert an autocrine/paracrine action aimed at regulating both acquired and innate immune responses at the fetal-maternal interface, as confirmed by the presence of the VDR in the endometrium. This hypothesis, in general terms, was born about twenty years ago and is based on the widespread co-expression of VDR and 1α-OHase in many tissues where vitamin D plays various physiological functions[48].

Notably, vitamin D in human cumulus granulosa cells significantly increases the expression of 3β-HSD, necessary for conversion from pregnenolone to progesterone[49] and in this way it significantly increases the production of such hormone by granulosa cells. Moreover, vitamin D directly stimulates the production of progesterone in cultures of human ovarian cells[50]. It also provokes the synthesis or blocks the release of key molecules that have, respectively, beneficial (such as activin A, CRF, VEGF)[51-53] or harmful effects (such as estrogens, besides the already cited TNFα and other inflammatory cytokines)[53-56] in the period between the luteal phase and the 12th week of pregnancy (decidualization and so on).

Vitamin D production and VDR expression promote an increase in the level of two target genes in both cycling and early pregnant endometrium, CYP24 and the already cited osteopontin. CYP24, the rate-limiting step for calcitriol catabolism, is one of the most potent 1,25(OH)2D-responding genes and its protein product is responsible for the hydroxylation reaction that deactivates the vitamin D, by a negative feedback mechanism[57,58].

Progesterone

Progesterone receptor, as VDR, is expressed in the glandular epithelium and stroma of the human endometrium during the menstrual cycle[59]. In experiments with human and mouse T cells progesterone was seen to induce the expression of the VDR gene, which is one of the major genes responding to progesterone. It was able to elicit the VDR mRNA expression in human Treg, Th1, and Th17 cells with various cytokine conditions, and in spleen CD4+ T cells and uterus from pregnant or progestin-injected mice[60]. The specificity of this effect is demonstrated by its suppression due to the receptor antagonist of progesterone, RU486[60]. Therefore, progesterone promotes VDR gene expression in T cells in heterogeneous conditions and different species. The expression of VDR due to progesterone rises the sensitivity of T cells to calcitriol and facilitates a more efficient regulation of T cells by calcitriol with an increase of Tregs and suppression of effector T cells which exert a potential pro-inflammatory activity[60]. Progesterone-induced VDR in T cells allows to prevent adverse immune responses, causing complications during gestation[60]. Of note, if VDR gene is knocked down with siRNA, the inhibition of T cell differentiation into effector T cells caused by progesterone and calcitriol significantly decreases[60].

Furthermore, progesterone promoted Th2 cells formation and the release of their anti-inflammatory cytokines[61] and regulated the differentiation of human naive cord blood fetal T cells into Treg cells[62]. It also suppressed Th1 and Th17-related genes and enhanced Th2-related genes in the lymphocytes obtained from pregnant cows[63]. It is

remarkable that progesterone inhibited IFN-γ and IL-17 release in dose-dependent manner in both pregnant and non-pregnant cows[63]. In addition, progesterone neutralized the cytotoxicity of NK cells by hindering their degranulation and perforin release[64]. Moreover, in mononuclear cells isolated from human placental blood progesterone was able to antagonize the effects of lipopolysaccharide (LPS) significantly reducing TNF-α, IL-1β, IL-6, IL-8, and MIP-1α release. Instead, the levels of IL-10 grew in a remarkable way when LPS was added in vitro with progesterone[65]. As calcitriol, progesterone stimulates the synthesis or suppresses the release of pivotal molecules exerting, respectively, helpful (such as activin A, CRF, VEGF) or detrimental effects (such as estrogens or TNF)[64,66-69] in the period between the luteal phase and the 12th week of pregnancy. Finally, progesterone induced the release of osteopontin in human decidua[70] and calbindin in the uterus of Egyptian buffalo[46]. These effects are briefly depicted in Figure 4.

Vitamin D effect to promote Treg activity in presence of progesterone appears strongly reduced when VDR gene is knocked down[60]. Of note, progesterone induced a greater sensitivity in T cell toward calcitriol to up-regulate the Treg-associated molecules (FoxP3, CD8, LAP-TGF-β1).

The efficacy of Treg cells in immune suppression is higher when they are produced under the combined effect of calcitriol and progesterone than when there is only one of these effector molecules[60]. All these data demonstrate that progesterone potentiates the anti-inflammatory effects of vitamin D, and *vice versa*.

Studies on Vitamin D in Pregnancy and Assisted reproductive technology (ART)

The relevance of vitamin D activity for pregnancy, from the luteal phases onwards, is confirmed by the findings of several studies. Insufficient levels of vitamin D have been related to the pathogenesis and the abnormal development of luteal phase. Vitamin D deficiency is prevalent in pregnant women with a major risk of miscarriage and recurrent pregnancy losses. Furthermore, the treatment with vitamin D or its precursor was found effective in women looking for gestation and in expectant mothers[71]. Several investigations confirm this picture. A study analyzed 1072 wo-

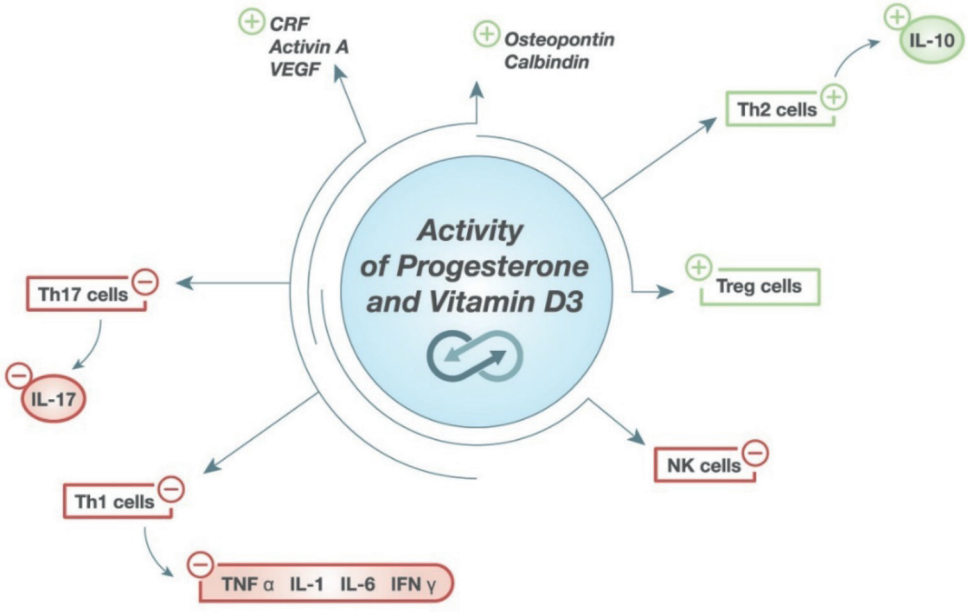

Figure 4. Progesterone and vitamin D share several biological activities, as an inducer of anti-inflammatory pathways (Th2 and Treg cells). In addition, they stimulate the release of corticotropin-releasing factor (CRF), activin A, vascular endothelial growth factor (VEGF), osteopontin, calbindin. Moreover, both progesterone and vitamin D inhibit the proinflammatory pathways (NK, Th1 and Th17 cells). All these effects contribute in supporting pregnancy since its inception. It is noteworthy that their action is synergic.

men attending an academic infertility centre: a high rate of hypovitaminosis D among childbearing women was demonstrated to be related with infertility[72]. In another work, the estimation of 25-hydroxy cholecalciferol (25-OHD) in patients was made before the treatment protocol for ICSI. The mean value of 25-OHD, number of oocytes, fertilized oocytes and endometrial thickness were significantly higher in pregnant women. A significant positive association of 25-OHD with clinical pregnancy and thickness of endometrium was observed. Deficiency of 25-OHD in females hinders the accomplishment of optimal endometrial thickness required for implantation of the embryo after ICSI. The improvement in vitamin D status positively affected the success results in ART[73]. Also, Ozkan et al[74] found that women with higher vitamin D level in their serum are significantly more likely to achieve clinical pregnancy following IVF-embryo transfer. The same group highlighted that another favourable parameter for ART outcome is the higher content of vitamin D in follicular fluids. As stated by Hollis and Wagner[75], there is growing evidence of the important role exerted by vitamin D supplementation during pregnancy to achieve satisfying circulating levels, associated to a lower risk of comorbidities of pregnancy and better outcomes. Concerning the safety in pregnant women, vitamin D supplementation was demonstrated to be completely safe at the dose of 4,000 IU/day for 24-28 weeks[76,77]. Finally, a recent systematic review[78] showed clearly an association between vitamin D levels in women undergoing ART and their reproductive outcomes. Vitamin D deficiency and insufficiency represent a crucial key point to consider in women following ART procedures.

Conclusions

Vitamin D and progesterone modulate numerous components of the immune system in the whole organism and at fetoplacental level too. Their action involves both the immune cells (macrophagic, dendritic, uterine natural killer, and lymphocytic cells) and non-immune cells (trophoblast cells), shifting the equilibrium toward a Th2 profile. Vitamin D shows several physiological activities during gestation in perfect synchrony with progesterone or also acting, in some cases, as a substitute. The molecules mutually help and reinforce the activity exerted by each one. Overall, the data so far known suggest that vitamin D, whose metabolism is strictly regulated, contributes to prepare the endometrium to be receptive. Moreover, it supports the implantation process and the course of pregnancy through different, but similar pathways to those used by progesterone, giving rise to a significant synergy of action[79]. A little bit later than progesterone is released, vitamin D enters the scene, and at endometrium level, its concentration strongly increases only if pregnancy occurs (i.e., after fertilization). It is increasingly evident that calcitriol gives an essential support from the luteal phase onwards, as glimpsed for the first time by the pioneering studies carried out by Feyel-Cabanes[10] and Chambon[11] between the thirties and the fifties of the past century. In conclusion, based on the evidence displayed in this review we may define appropriately vitamin D as a steroid hormone with progesterone-like activity.

Conflict of Interest

Vittorio Unfer and Sara De Grazia are employees at Lo.Li. Pharma, Rome, Italy. The other authors declare that they have no conflict of interests.

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