Hindawi Publishing Corporation
Advances in Andrology
Volume 2014, Article ID 808906, 10 pages
http://dx.doi.org/10.1155/2014/808906

Review Article

Functional Importance of 1α,25(OH)$_2$-Vitamin D$_3$ and the Identification of Its Nongenomic and Genomic Signaling Pathways in the Testis

Fátima Regina Mena Barreto Silva

Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Bairro Trindade, CP 5069, 88040-970 Florianópolis, SC, Brazil

Correspondence should be addressed to Fátima Regina Mena Barreto Silva; mena.barreto@ufsc.br

Received 11 December 2013; Accepted 1 May 2014; Published 29 May 2014

Academic Editor: Nafisa H. Balasinor

The 1α,25(OH)$_2$-vitamin D$_3$ (1,25-D$_3$) is known by its classic effects on Ca^{2+} metabolism and regulation of cellular proliferation and differentiation. The hormone 1,25-D$_3$ acts in the testis through nongenomic and genomic events being implicated in the success of spermatogenesis in rats and in human being. The aim of this review was to highlight the effect and intracellular pathways of 1,25-D$_3$ to modulate the spermatogenesis. The pivotal role of 1,25-D$_3$ in male reproduction is reinforced by the presence of VDR and 1α-hydroxylase in reproductive tract. Also, the marked expression of VDR and the VD metabolizing enzymes in human testis, ejaculatory tract, and mature spermatozoa implicates the 1,25-D$_3$ in spermatogenesis and maturation of human spermatozoa. Among genomic events, 1,25-D$_3$ influences the expression of calcium binding protein and stimulates aromatase gene expression through a nongenomic activation of the membrane-bound VDR receptor involving the PKA pathway in the testis. Also, 1,25-D$_3$ stimulates amino acid transport and exocytosis in testis by nongenomic events coupled to ionic currents triggered at plasma membrane. All together, the demonstration that 1,25-D$_3$ regulates both Sertoli cell and sperm function may be useful for the study and development of new therapeutic strategies for the male reproductive disorders.

1. General Aspects

The first scientific report associated with vitamin D deficiency and bone disease rickets was around 1645. However, the recognition of the rickets in patients with no sunshine exposition was only in the 20th century. In 1924, an additional key for vitamin D present in skin was the discovery that a precursor of vitamin D could be converted into vitamin D by exposure to sunlight or ultraviolet [1]. The hallmark era of vitamin D began in 1965–1970 since the chemical characterization of an active metabolite of vitamin D, 1α,25(OH)$_2$-vitamin D$_3$ (1,25-D$_3$), and its nuclear receptor (VDR) was reported [1, 2].

It is known that vitamin D$_3$ does not have any intrinsic biological activity [3]. After the knowledge that the precursor of vitamin D can be converted into vitamin D by exposure to ultraviolet light (UVB), it is now comprehensive that vitamin D is correctly named as vitamin only when sunlight exposure does not exist [4]. Nowadays, the biologically active form of vitamin D is known to be a steroid hormone and endocrine system of vitamin D is well accepted. The chemical characterization, the production of an active metabolite of 1,25-D$_3$, and its nuclear receptor occurred between 1965–1970 [5].

The interesting thing about vitamin D is that it undergoes obligatory metabolism prior to generation of its biological response [6]. The endogenous synthesis of 1,25-D$_3$ in the kidney represents the main source of 1,25-D$_3$ in the body. The UVB radiation starts the conversion of 7-dehydrocholesterol to cholecalciferol (provitamin D$_3$). So vitamin D$_3$ also can be endogenously produced by the animals or humans. However, in an inadequate sunlight access, the animal (or human) might largely depend on this vitamin from the diet or by individual oral intake of vitamin D$_3$ supplements [3, 7, 8].

After the cholecalciferol formation (inactive vitamin D_3), it undergoes two hidroxylations to form the compound 1,25-D_3 [2, 9]. In the liver, the production of 25(OH)D_3, the major form of vitamin D circulating in the blood compartment, is mainly mediated by the enzyme CYP2R1 encoded by the gene CYP27A1. So 25(OH)D_3, which enters in blood circulation and subsequently in the kidney (that functions as an endocrine gland), is hydroxylated by 1α-hydroxylase encoded by the gene CYP27B1 generating the hormone 1,25-D_3 and the candidate hormone 24R,25$(OH)_2D_3$, as well as 38 other vitamin D_3 metabolites [2, 3, 10]. So, both these dihydroxylates metabolites can be transported to distal organs by the plasma vitamin D binding protein (DBP), a serum glycoprotein-globulin produced by the liver [11]. The pool of DBP-bound vitamin D metabolites can be readily used or kept as a reservoir to be used during the reduced intake or production [12–14]. In target tissues, the active hormone 1,25-D_3 can bind and activate the vitamin D receptor (VDRnuc or VDRmem) and after that can be inactivated by CYP24A1 [2, 10, 15].

The effects of 1,25-D_3 are mediated through the 1,25-D_3 receptor (VDR) and VDR is expressed in over 38 tissues, which include brain and endocrine organs such as pancreas, pituitary, muscle, kidney, ovary, prostate, and testis [7, 16, 17]. As much in human as in animals models, it was demonstrated that 1,25-D_3 is a key regulatory factor of calcium homeostasis in both male and female. Although several studies suggest that 1,25-D_3 has a role in reproductive function, the association between hypospermatogenesis and a disturbance in vitamin D endocrine system is not clearly understood [18–20]. In this review, the physiological role and genomic and nongenomic effects of 1,25-D_3 on the testis and on spermatogenic process will be discussed.

2. 1,25-D_3 Receptor Distribution in the Testis of Rodents

Concerning the putative role of 1,25-D_3 in male reproduction, little information is available. The 1,25-D_3 classical receptor (VDR) is a member of the superfamily of nuclear receptors. It was discovered and identified in the intestine of chickens vitamin D-deficient, a chromatin-associated protein that binds the active hormone 1,25-D_3 [21]. It is distributed in more than 37 tissues able to generate genomic and/or rapid responses [22]. VDR is a DNA-binding transcription factor with a molecular weight of about 50 kDa [22]. The heterodimer of the 1α,25$(OH)_2D_3$-liganded VDR and unoccupied retinoid X receptor (VDR-RXR) recognize vitamin D responsive elements (VDREs) in the DNA sequence of vitamin D-regulated genes [23, 24].

Among 38 cell types and tissues, where the presence of VDR was detected, the testis of rodent and human beings is included. Several reports show the nuclear 1,25-D_3 receptors distribution in the testis of rodents [25–27]. Using whole testis, seminiferous tubules, and isolated testicular cells, a wide distribution of VDR mainly in Sertoli and in seminiferous tubules was characterized which are strongly related to cell proliferation and differentiation. The role of 1,25-D_3 in male reproduction was reinforced since a significant binding of the hormone was detected in certain processes during spermatogenesis and spermiogenesis, on sperma maturation, on epididymal fluid resorption, and on secretion and transport of spermatozoa, in rats [17, 28]. In addition, the spermatogenesis of 1,25-D_3-depleted rat is incomplete and exhibits impaired development, and degenerative changes in the seminiferous tubules are observed [27]. These are in agreement with a gonadal insufficiency that occurs in VDR null mutant mice which showed a decreased sperm count and motility and significant histological abnormalities in the testis [18].

Recently, the pivotal role of 1,25-D_3 in male reproduction was described using concomitant evaluation of VDR and 1α-hydroxylase expression in all organs of male mice reproductive tract. Epithelial cells of epididymis, seminal vesicle, coagulating gland, ductus deferens, preputial gland, and prostate were the prominent cell types that concomitantly expressed VDR and 1α-hydroxylase. And an interesting fact was that specific band of approximately 54 kDa was observed in all male mice reproductive organs but not in the testis. Instead, in this organ 3 distinct bands of about 45, 57, and 63 kDa were seen in all mice examined and 45 kDa band was also observed in prostate tissue. Furthermore, testis and prostate express a higher level of 1α-hydroxylase. In addition, for VDR, Sertoli cells were the prominent cell in testis that expressed very high levels of nuclear VDR. All other intratubular cells including primary spermatocytes and spermatogonia expressed varying degree of VDR in their nucleus and cytoplasm. Also Leydig cells showed strong nuclear staining [29]. From these finds, increased evidence provides a central role of 1,25-D_3 in an active and full spermatogenesis. However, the aim is to understand the mechanism of action 1,25-D_3 in the testis to coordinate the complex spermatogenesis during the sexual development in order to warrant the male reproductive function.

3. 1,25-D_3 Receptor Distribution in the Testis of Human Beings

In the last few years, studies on the effect and mechanism of action of 1,25-D_3 through VDR have focused on human male reproduction. In human testis, the specific high affinity and low binding capacity of VDR for 1,25-D_3 were demonstrated [16]. Studies from Nangia et al. [30] revealed six forms of the VDR in the nuclear matrix of human testis. And in human testis was demonstrated a protein of 57 and 52 kDa molecular weight compared with 57 and 37 kDa in the rat testis. However, if the difference in molecular weight proteins with the anti-VDR antibody within tissues or species may represent different isoforms, proteolytic cleavage of a larger VDR, or even a posttranslational modification, it is not solved yet.

The advances in human germ cells increased the comprehension about the role of 1,25-D_3 in male fertility. Corbett et al. [31], through a prospective study of sperm collected from ten fertile men, showed for the first time the VDR on

human sperm located predominantly on the head/nucleus of the sperm and midpiece. Thus, in an attempt to understand the gonadal insufficiencies with 1,25-D_3 deficiency and VDR, Aquila et al. [32] studied the localization of human VDR in normozoospermic samples and the role of 1,25-D_3/VDR in sperm survival and capacitation. Although the action of 1,25-D_3 depends on its concentration, the authors suggested that 1,25-D_3/VDR may have an important role in sperm survival and in the acquisition of fertilizing ability. Also it was shown that the human sperm expresses the VDR and that 1,25-D_3 is locally produced since 25(OH)D_3-1,α-hydroxylase was detected in sperm [33]. In addition, the effect of 1,25-D_3 through VDR increased intracellular Ca^{2+} levels, motility, and acrosin activity revealing an unexpected significance of this hormone in the acquisition of fertilizing ability. So taking in mind that the cellular response for 1,25-D_3 is complex (since it depends on VDR expression, the cellular uptake of circulating 1,25-D_3, and also presence and activity of VD metabolizing enzymes), BlombergJensen et al. [34] performed a comprehensive analysis of the expression of VDR, VD activating (CYP2R1, CYP27A1, and CYP27B1), and VD-inactivating (CYP24A1) enzymes in the testis, epididymis, seminal vesicle, prostate, and spermatozoa. From those results and based on the marked expression of VDR and the VD metabolizing enzymes in human testis, ejaculatory tract, and mature spermatozoa, the authors suggested that 1,25-D_3 is important for spermatogenesis and maturation of human spermatozoa.

Interesting results concerning the association of testicular failure with bone mineral density were recently published [19]. The authors investigated CYP2R1 expression in pathological testis samples and relate this to vitamin D metabolism in young testiculopathic patients. Surprisingly, in all testiculopathic patients, 25-hydroxyvitamin D levels were significantly lower and parathyroid hormone levels higher compared to controls groups and the patients showed osteopenia and osteoporosis despite normal testosterone levels.

Another interesting point in human spermatozoa was about the use of VD-inactivating enzyme as a marker of the quality of the semen. It was described that the VD-inactivating enzyme CYP24A1, titrating the cellular responsiveness to VD, is transcriptionally regulated by VD and has a distinct expression at the sperm annulus. So the CYP24A1 expression was investigated and pointed as a marker for VD metabolism in human spermatozoa. In addition, the CYP24A1 expression correlated positively with total count, the concentration, motility, and morphology of the sperm was associated with semen quality. Also, based on functional studies, 1,25-D_3 increased intracellular calcium and sperm motility in young healthy men but was unable to increase motility in subfertile patients. Then, the authors suggested that CYP24A1 expression at the annulus may serve as a novel marker of semen quality [35].

4. Genomic Effects of 1,25-D_3 in the Testis

It is well known that vitamin D undergoes obligatory metabolism in order to generate its biological response. Briefly, an enzymatic insertion of a 25-hydroxyl group gives 25-hydroxylvitamin D (25-OH-D) that occurs in the liver. And a second step of metabolite modification occurs in the kidney where 25-OH-D may be converted into either 1,25-D_3 or 24*R*,25-dihydroxyvitamin D (24,25-D_3) [15, 36, 37]. One of the most known genomic effects for the active steroid metabolite of vitamin D, 1,25-D_3, is the induction of *de novo* synthesis of a vitamin D-dependent calcium-binding protein (CaBP) in intestinal mucosa [38]. Regarding testicular genomic events, vitamin D_3 via its active metabolite 1,25-D_3 influences cell proliferation and cell differentiation and expresses calcium binding protein mediated by VDR in rat testis and in TM4 Sertoli cell line [25, 39–42]. In addition, 1,25-D_3 nuclear receptors have been demonstrated in Sertoli cells and this hormone is also critical for the maintenance of normal reproduction in male rats since vitamin D-deficient rats have reduced fertility compared with normal vitamin D-replete rats [17, 27, 28]. These finds were confirmed since that vitamin D receptor null mutant mice showed gonadal insufficiencies and decreased motility and sperm count with histological abnormality of the testis [18].

It is well-known that 1,25-D_3 regulates aromatase (CYP19A1) in humans in a tissue specific way [43–45] and it is implicated in estrogen and androgen production and metabolism. Also the association with serum testosterone and 25-hydroxyvitamin D serum levels or vitamin D supplementation and testosterone levels in men was well characterized [46, 47].

Aromatase converts irreversibly androgens into estrogens and is present in the endoplasmic reticulum of various tissues including the mammalian testis [48, 49]. The aromatase enzyme complex comprises two proteins: a specific cytochrome P450 (P450arom) encoded by the CYP19 gene and a ubiquitous NADPH cytochrome P450 reductase [50, 51]. In mouse, the Cyp19 gene is localized on chromosome 9, and three promoters that control specifically the aromatase gene expression were also described [52]. In rat, the Cyp19 gene is situated on chromosome 8 and up to now three promoters were described: the promoter PI.f in brain [53] and in testis [54], the promoter PII [55], which is the main one directing aromatase gene expression in gonads [56, 57], and the promoter PI.tr only used in testis [54]. Zanatta et al. [58] described that VDR transcripts were present in immature Sertoli cells, in adult testicular germ cells, and in somatic cells as much as 1,25-D_3 increased the amount of aromatase transcript, mainly in 30-day-old rats. The physiological role of 1,25-D_3 in Sertoli cells was corroborated due to the stimulation of aromatase gene expression by the agonist 1α,25(OH)$_2$ lumisterol$_3$ and the suppression of the 1,25-D_3 effect by the antagonists 1β,25(OH)$_2$ vitamin D_3 and (23S)-25-dehydro-1α (OH)-vitamin D_3-26,23-lactone. In a whole, these data suggested that besides a genomic effect of 1,25-D_3, the existence of nongenomic activation of the membrane-bound VDR receptor involves the PKA pathway.

5. Nongenomic Effects of 1,25-D_3 on Testis

The secosteroid hormone, 1,25-D_3, mediates classic transcriptional events [59] and also acts through a VDRmem for

the rapid stimulation of calcium uptake in isolated rat intestinal epithelial cells, as well as calcium transport in perfused chick duodenum [60, 61]. Herein, some studies were summarized concerning nongenomic evidence for 1,25-D_3 in the testis able to modify testicular behavior and spermatogenesis as well.

The neutral nonmetabolizable amino acid transport model previously introduced [62], thereby distinguishing protein synthesis from alternative amino acid accumulation pathways, has been useful for identification of substances at plasma membrane and/or nuclear effect. The transport of neutral amino acids involves three major systems. Among them, the alanine-preferring (A) systems, specific for N-methylaminoisobutyric acid, are sodium, pH, and energy dependent. The hormonal effects on amino acid uptake appear to be restricted to system "A" [63]. This system is characterized to be composed of an inorganic component (electrochemical gradient) and an organic component (plasma membrane carriers) which are essential for cell survival [64, 65]. In the testis, the neutral amino acid transport, "A" system, was identified to be regulated by follicle-stimulating hormone, retinol, triiodothyronine, 1,25-D_3, and thyroxine [65–73]. So using this electrochemical amino acid "A" system as a tool, we provided evidence for rapid responses of 1,25-D_3 in immature rat testis involving voltage-dependent calcium channels (VDCC) and Ca^{2+}-dependent K^+ channels activities at plasma membrane to regulate neutral amino acid accumulation [65]. However, the involvement of VDRmem in amino acid uptake in the testis remains obscure and further computational studies on Sertoli cells are needed to better understand the role of this putative plasma membrane receptor for 1,25-D_3.

The involvement of voltage-gated chloride channels activity as an electric shunt that couples to H^+-ATPase-driven loading of secretory vesicles that are crucial for the onset of exocytosis is now well known. The steroid, 1,25-D_3, activates chloride channels and it seems to be needed for exocytosis in bone cells [74]. In particular, the secretory activities of Sertoli cells are critical to spermatogenesis and this cell expresses a variety of ionic channels involved in cellular secretion [75–79]. Furthermore, the hormonal regulation of fluid secretion by Sertoli cells is important for the development of spermatogenesis wave and involves the modulation of ionic channel activities [65, 73, 80–82]. Taking in mind that the secretory activities are highly dependent on ionic channel functions and critical for the spermatogenesis success [75], the role of 1,25-D_3 on exocytosis was investigated. Surprisingly, the rapid response to 1,25-D_3 in the mouse immature Sertoli cell line TM4 was mediated by chloride channel activation and culminated in cell secretion. Additionally, chloride currents were potentiated by the nongenomic VDR agonist 1α,25$(OH)_2$ lumisterol D_3 (JN), while 1,25-D_3 potentiation of channels was suppressed by nongenomic VDR antagonist 1β,25$(OH)_2$-vitamin D_3 (HL). In particular, the expression of outwardly rectifying ClC-3 channels in TM4 cells, which is sensitive to the specific blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and has outward rectifying characteristics, was detected at relatively high levels, among the ClC-7 members of the ClCn gene family investigated. Additionally, the agonists/antagonists of PKC and PKA increased and abolished the chloride currents in TM4 cells, respectively [83]. So we demonstrated for the first time that nongenomic 1,25-D_3 potentiation of chloride currents is coupled to exocytosis in TM4 Sertoli cells and pointed a functional role for 1,25-D_3 in male fertility via stimulation of secretory activities in the testis.

The plasma membrane effects and rapid responses describe for 1,25-D_3 include the opening of voltage-dependent calcium [84] and chloride channels [85] as also demonstrated for the testis [65, 83]. Zanatta et al. [86] reported that 1,25-D_3 induced calcium uptake in rat testis through a nongenomic mechanism of action mediated, at least in part, by PKA, PKC, and MEK. The ionic influence on the stimulatory effect of 1,25-D_3 in calcium uptake was characterized by ATP- and Ca^{2+}-dependent K^+ channels and Ca^{2+}-dependent chloride channels participation in the hormone mechanism of action. Beyond the calcium from the stocks involved, the effect of 1,25-D_3 on calcium uptake may also result from Na^+/K^+-ATPase pump inhibition. Also, in this study, the action of 1,25-D_3 in an enzyme associated with the plasma membrane of Sertoli cells was demonstrated for the first time. Gamma-glutamyl transpeptidase (GGTP; EC 2.3.2.2) has been detected in several tissues and also in the testis [87]. GGTP located at plasma membrane in high concentrations in cells involved in secretory activity [88] and in the testis participates in transferrin secretion from Sertoli cells [89]. This enzyme catalyzes the transfer of gamma-glutamyl peptides either to other peptides or to L-amino acids and may play a role in the synthesis of specific proteins known to be secreted by Sertoli cells [89, 90].

Particularly, in Sertoli cells, the exposition of 1,25-D_3 or the agonist of VDRmem (JN) produced similar stimulatory effect on calcium uptake suggesting that 1,25-D_3 action occurs via a putative membrane receptor. From these data, the participation of VDCC, PKA, PKC, and ERK activation as well as the influence of the Na^+/K^+-ATPase inhibition on Na^+/Ca^{2+} exchanger activation in reverse mode and consequently induction on calcium uptake into the cells were triggered in Sertoli cells after the treatment with 1,25-D_3, reinforcing once more the hormonal effect at plasma membrane [91, 92].

A recent report correlates the mechanism of action of 1,25-D_3 in whole testis and in Sertoli cells from immature rats on calcium uptake. It was characterized that the rapid responses to 1,25-D_3 on calcium uptake in rat Sertoli cells are mediated by VDCCs, PKC, ERK1/2, and p38 MAPK pathways. In addition, the VDCC activities are mandatory for a full stimulatory effect of 1,25-D_3 as much in whole testis as in Sertoli cells. Potassium and chloride channels also are strongly involved in this rapid response coordinated by 1,25-D_3. Some interesting finds were about the participation of PKC and ERK1/2 upstream activity in p38 MAPK activation that strongly pointed to a possible intracellular cross-talk between rapid calcium uptake and genomic events. Additionally, in Sertoli cells, the comparative effect of colchicine and ClC-3 channel blocker on calcium uptake provides evidence

for a secretory activity triggered by 1,25-D_3. These data highlight that 1,25-D_3 activates p38 MAPK and reorganizes microtubules, involving calcium, PKC, and ERK1/2 as upstream regulators and that extracellular calcium has a central role to rapidly start hormone-induced gene transcription and/or the secretory activity of immature Sertoli cell [93].

Although the calcium increase mediated by 1,25-D_3 in human male gamete has been reported, the role of this ion is unclear [33]. 1,25-D_3 increases intracellular calcium and sperm motility and induces the acrosome reaction in mature spermatozoa and the serum levels of the hormone are associated with sperm motility. In addition, 1,25-D_3 increases intracellular calcium through VDR and sperm motility in young healthy men but was unable to increase motility in subfertile patients [35, 94].

The role of calcium on mechanism of action of 1,25-D_3 in the testis has increased evidences that this secosteroid triggers rapid responses in testicular cells in some significant physiological events. Vimentin is an abundant protein in the intermediate filaments in the cytoplasm of Sertoli cells from immature and adult rat testis [95]. Vimentin is a target for the action of FSH, testosterone, thyroid hormones, and also 1,25-D_3 [96–99]. In the testis, the increase of vimentin phosphorylation is mediated by the activation of PKA, PKC, and MAPK but is independent of protein synthesis. Furthermore, the intra- and extracellular calcium are involved in rapid responses of 1,25-D_3. In addition, there is growing evidence that 1,25-D_3 serves as a primal regulator of ionic channels and also for PKA, PKC, and MAPK as well as aromatase expression in testis and Sertoli cells [73, 82, 92]. However, whether the action triggered by hormone at plasma membrane is independent to mediate rapid responses (exocytosis, enzymes activities) or cross-talk pathways can also culminate in genomic activities remains unclear.

First, using chick duodenal caveolae-enriched membrane fraction (CMF) isolated without the use of detergents to study 1,25-D_3 binding at plasma membrane evidenced the action of 1,25-D_3 on a putative VDRmem [14]. Also, as 1,25-D_3 is a secosteroid, the analogues are useful tools to be assayed in *ex vivo* and *in vitro* and also lately it has helped to understand the mechanism of action of 1,25-D_3 by *in silico* approach, since there is the possibility of the hormone binding at VDRmem or at VDRnuc to induce the alteration of cellular biological activity.

6. Structure/Function and Computational Analysis of 1,25-D_3 Binding in VDR-Genomic Pocket or Alternative Pocket

The flexibility of the secosteroid 1,25-D_3 can occur in three regions of the molecule: in the side chain with 360° rotation around the 5 carbon–carbon single bonds, in the broken B-ring with a 360° rotation around the 6,7 carbon–carbon single bond, and in the A-ring where a cyclohexane-like chair ↔ chair interconversion occurs which changes the orientation of 1α-hydroxyl and 3β-hydroxyl between the equatorial and axial orientations [14, 15]. The rotation around 6,7 single carbon bond permits generation of potential ligand shapes extending from the 6-s-*cis* (steroid like) to the 6-s-*trans* (extended). Several evidences reinforce that the preferred shape for VDRmem that triggers rapid responses is that represented by the 6-s-*cis* conformation and the preferred shape for VDRnuc is the 6-s-*trans* [13, 22, 100]. So in order to study the preferred shape of the ligands for nongenomic and genomic responses, a number of conformationally restricted analogs of 1,25-D_3 locked in the 6-s-*cis* or 6-s-*trans* conformation have been developed [100].

The effectiveness of these analogs to stimulate transcaltachia (a rapid response) or to induce osteocalcin expression in human osteoblast MB-63 cell (a genomic response) in comparison to 1,25-D_3 was measured [2, 100, 101]. The structure-function studies showed that the 6-s-*cis* analog, 1α,25-dihydroxylumisterol3 (JN), can efficiently induce transcaltachia in intestinal epithelium and also stimulate Ca^{2+} uptake in osteosarcoma cell line via VDRmem. However, this analog did not activate the genomic action, and it possessed only weak ability to bind to VDRnuc [100]. On the other hand, the 1β,25-dihydroxyvitamin D3 (HL) blocked rapid responses induced by 1,25-D_3 or JN and was recognized as a specific antagonist of the nongenomic action [102, 103]. Also the 1,25-D_3 genomic response was blocked by coincubation with the analog (23S)-25-dehydro-1α(OH)-vitamin D_3-26,23-lactone (MK), of which antagonistic action is caused by the inhibition of heterodimer formation between VDR and RXR and of VDR interaction with coactivator, steroid receptor coactivator 1 (SRC-1) [104]. Taking it in whole, 1,25-D_3 analog effects have demonstrated that the comparison of 6-s-*cis*- and 6-s-*trans*-locked analogs of 1,25-D_3 indicates that the 6-s-*cis* conformation is preferred for rapid nongenomic biological responses and that neither 6-s-*cis*- nor 6-s-*trans*-locked analogs are preferred for genomic biological responses [22, 100]. The interesting properties of some selected analogs of 1,25-D_3, after in-depth evaluation of biological activity based on their affinity for the VDRnuc and/or VDRmem, have supported to be very good tool to investigate a genomic pocket (GP) and alternative pocket (AP) that mediate regulation of gene transcription and rapid responses for the hormone, in *in silico* studies.

Also in the testis, the action of 1,25-D_3 occurs by interaction with both a well characterized VDRnuc to regulate gene transcription and with an as yet uncharacterized membrane-associated protein/receptor (VDRmem) to induce a variety of rapid, nongenotropic responses [58, 65, 83]. The studies of 1,25-D_3 at plasma membrane mediated by VDRmem started in whole testes, TM4 Sertoli cells line, primary culture of Sertoli cells, and germ cells carried out in *in vitro* studies [92].

Recently, the structure/function and computational analysis suggest that the VDRnuc or an isoform thereof can function as the membrane receptor propagating the rapid effects of 1,25-D_3 in Sertoli cells [105]. Following some data about 1,25-D_3/JN-induced and HL-antagonized, nongenomic responses as chloride channel opening coupled to exocytosis in TM4 Sertoli cells and TM4 Sertoli cells [72], 1,25-D_3/JN-induced and HL-antagonized, aromatase expression in testicular cells, mediated by rapid responses [58], and also because JN and HL are equipotent with 1,25-D_3 in stimulating

(JN) or antagonizing (HL) rapid responses but have 100-fold lower affinities for VDR, Menegaz et al. [105] hypothesized that a novel membrane receptor must exist and serve as the mediator of vitamin D sterol rapid responses. Furthermore, it is in accordance with previous studies that showed a nuclear VDR present in the caveolae-enriched membrane fraction from human, rodent, and chick tissues [14, 106]. So the studies carried out by Menegaz et al. [105] postulated that the VDR contains two overlapping ligand binding sites, a GP and an AP, that mediate regulation of gene transcription and rapid responses, respectively. The authors showed that the flexible VDR ligand docking calculations predict that the major blood metabolite, $25D_3$, and curcumin (CM) bind more selectively to the VDR-AP when compared with the 1,25-D_3. In VDR wild-type-transfected COS-1 cells and TM4 Sertoli cells, 1,25-D_3, $25D_3$, and CM each trigger voltage-gated outwardly rectifying chloride channel (ORCC) currents that can be blocked by the VDR antagonist $1\beta,25(OH)_2$-vitamin D_3 and the chloride channel antagonist (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid). In addition, VDR mutational analysis in transfected COS-1 cells demonstrates that the DNA-binding domain is not required, but the ligand binding and hinge domains of the VDR are required, for 1,25-D_3 and $25D_3$ to activate the ORCC. Through dose-response curves it was demonstrated that $25D_3$ and 1,25-D_3 are approximately equipotent in stimulating ORCC rapid responses, whereas 1,25-D_3 was 1000-fold more potent than $25D_3$ and CM in stimulating gene expression. The VDR-AP agonist effects of 1,25-D_3, $25D_3$, and low-dose CM are lost after pretreatment of TM4 cells with VDR small interfering RNA. Collectively, these results are consistent with an essential role for the VDR-AP in initiating the signalling required for rapid opening of ORCC. The fact that $25D_3$ is equipotent to 1,25-D_3 in opening ORCC suggests that reconsideration of the ability of $25D_3$ to generate biological responses *in vivo* may be in order. The demonstration that 1,25-D_3 regulates both Sertoli cell and sperm function may be useful for the study and development of new therapeutic strategies to the treatment of male reproductive disorders.

7. Conclusion

The spermatogenesis and steroidogenesis are the main functions of the testis and both are tightly regulated by endogenous and exogenous factors that can result in success of spermatogenesis or in male infertility. In contrast to steroids, the secosteroid 1,25-D_3 is able to achieve an interconversion to the 6-s-*cis* conformation referred to as steroid-like conformation with preferred binding at VDRmem and also display mobility to 6-s-*trans* conformation referred to as extended conformation with preferred binding at VDR-nuc. The hormonally active form of vitamin D, 1,25-D_3, is a pivotal hormone to maintain basic cellular processes through transcriptional events mediated by VDRnuc or rapid responses triggered by a putative plasma membrane receptor designated as VDRmem. A multitude of reports describe the testis as a target for 1,25-D_3 since both VDR and enzymes that metabolize vitamin D are present in rodents and in human. However, the upcoming studies should clarify the physiological relevance of autocrine and paracrine action of 1,25-D_3 and also the cross-talking between VDRnuc and VDRmem pathways to complete or keep an active ongoing spermatogenic wave and male fertility. Based on the existence of a VDR AP and VDR GP that mediate regulation of gene transcription (by 6-s-*trans* 1,25-D_3 form) and rapid responses (by 6-s-*cis* 1,25-D_3 form) on signalling cascades stimulated by 1,25-D_3, the *in silico* model may provide a useful platform for drug development through docking studies of new or known therapeutic drugs for the reproductive disorders based on the endocrine system of vitamin D. In addition, we have used electrophysiology modeling, molecular biology, and biochemistry to characterize strategic sites of action for drug target for further therapy in male infertility. Also, it is worthwhile to pay attention that some proteins known in the vitamin D metabolism or in the signalling cascade mediated by VDRmem or VDRnuc may be of clinical interest even for the male infertility diagnosis.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The studies carried out in the author's laboratory were supported by Conselho Nacional de Desenvolvimento Tecnológico (CNPq), Coordenação de Pessoal de Nível Superior (CAPES), CAPES/COFECUB (Project 554/07 and 133/07), Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC), and Financiadora de Estudo e Projetos (FINEP). The author gives thanks to the assistance of technicians of LAMEBs facilities (CCB/UFSC), Chirle Ferreira and Gilberto Domingos Marloch. Also she gives thanks to her first supervisor, Dr. Guillermo Federico Wassermann (Physiology Department at Federal University of Rio Grande do Sul, Porto Alegre, Brazil), who gave her the support to follow the scientific choice in her career life which has made her very fulfilled, and special thanks to the fine researchers Dr. Anthony W. Norman and Dr. Helen Henry (Biochemistry Department at University of California, Riverside, USA), who opened the doors for the world of vitamin D studies, in 2000. The author was very encouraged to hear their enthusiastic discussion in several opportunities. Also she dedicates this work in memory of Dr. Serge Carreau (Laboratory of Estrogen and Reproduction, University of CAEN—Basse Normandie, France), who made the difference in the scientific maturation of her research group and herself (from 2004 to 2013). She attributes the success of her findings to their teachings. Also she gives special thanks to Ph.D. students Ana Paula Zanatta and Renata Gonçalves for their dedication in the search and correction of references.

References

[1] A. W. Norman, *Encyclopedia of Hormones: Vitamin D*, Elsevier Science, New York, NY, USA, 2003.

[2] R. Bouillon, W. H. Okamura, and A. W. Norman, "Structure-function relationships in the vitamin D endocrine system," *Endocrine Reviews*, vol. 16, no. 2, pp. 200–256, 1995.

[3] A. W. Norman, "From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health," *American Journal of Clinical Nutrition*, vol. 88, no. 2, pp. 491S–499S, 2008.

[4] H. Goldblatt and K. N. Soames, "A study of rats on a normal diet irradiated daily by the mercury vapor quartz lamp or kept in darkness," *Biochemical Journal*, vol. 17, pp. 294–297, 1923.

[5] A. W. Norman, "Minireview: vitamin D receptor: new assignments for an already busy receptor," *Endocrinology*, vol. 147, no. 12, pp. 5542–5548, 2006.

[6] A. W. Norman and P. A. Roberts, "Steroid competition assay for determination of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D," *Methods in Enzymology*, vol. 67, pp. 473–478, 1980.

[7] R. Bouillon, G. Carmeliet, L. Verlinden et al., "Vitamin D and human health: lessons from vitamin D receptor null mice," *Endocrine Reviews*, vol. 29, no. 6, pp. 726–776, 2008.

[8] M. F. Holick, N. C. Binkley, H. A. Bischoff-Ferrari et al., "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 7, pp. 1911–1930, 2011.

[9] G. Ponchon, A. L. Kennan, and H. F. DeLuca, "'Activation' of vitamin D by the liver," *The Journal of Clinical Investigation*, vol. 48, no. 11, pp. 2032–2037, 1969.

[10] D. E. Prosser and G. Jones, "Enzymes involved in the activation and inactivation of vitamin D," *Trends in Biochemical Sciences*, vol. 29, no. 12, pp. 664–673, 2004.

[11] N. E. Cooke and J. G. Haddad, "Vitamin D binding protein (Gc-globulin)," *Endocrine Reviews*, vol. 10, no. 3, pp. 294–307, 1989.

[12] J. E. Bishop, E. D. Collins, W. H. Okamura, and A. W. Norman, "Profile of ligand specificity of the vitamin D binding protein for 1α,25- dihydroxyvitamin D_3 and its analogs," *Journal of Bone and Mineral Research*, vol. 9, no. 8, pp. 1277–1288, 1994.

[13] A. W. Norman, S. Ishizuka, and W. H. Okamura, "Ligands for the vitamin D endocrine system: different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 76, no. 1-5, pp. 49–59, 2001.

[14] A. W. Norman, C. J. Olivera, F. R. M. B. Silva, and J. E. Bishop, "A specific binding protein/receptor for 1α,25-dihydroxyvitamin D_3 is present in an intestinal caveolae membrane fraction," *Biochemical and Biophysical Research Communications*, vol. 298, no. 3, pp. 414–419, 2002.

[15] A. W. Norman and F. R. M. B. Silva, "Structure function studies: identification of vitamin D analogs for the ligand-binding domains of important proteins in the vitamin D-endocrine system," *Reviews in Endocrine and Metabolic Disorders*, vol. 2, no. 2, pp. 229–238, 2001.

[16] F. K. Habib, S. Q. Maddy, and K. J. Gelly, "Characterisation of receptors for 1,25-dihydroxyvitamin D_3 in the human testis," *Journal of Steroid Biochemistry*, vol. 35, no. 2, pp. 195–199, 1990.

[17] J. A. Johnson, J. P. Grande, P. C. Roche, and R. Kumar, "Immunohistochemical detection and distribution of the 1,25-dihydroxyvitamin D_3 receptor in rat reproductive tissues," *Histochemistry and Cell Biology*, vol. 105, pp. 7–15, 1996.

[18] K. Kinuta, H. Tanaka, T. Moriwake, K. Aya, S. Kato, and Y. Seino, "Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads," *Endocrinology*, vol. 141, no. 4, pp. 1317–1324, 2000.

[19] C. Foresta, G. Strapazzon, L. de Toni et al., "Bone mineral density and testicular failure: evidence for a role of vitamin D 25-hydroxylase in human testis," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 4, pp. E646–E652, 2011.

[20] C. Foresta, R. Selice, A. di Mambro, and G. Strapazzon, "Testiculopathy and vitamin D insufficiency," *The Lancet*, vol. 376, pp. 1301–1301, 2010.

[21] M. R. Haussler and A. W. Norman, "Chromosomal receptor for a vitamin D metabolite," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 62, no. 1, pp. 155–162, 1969.

[22] M. T. Mizwicki and A. W. Norman, "The vitamin D sterol-vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling," *Science Signaling*, vol. 2, no. 75, article re4, pp. 1–14, 2009.

[23] G. K. Whitfield, P. W. Jurutka, C. A. Haussler et al., "Nuclear vitamin D receptor: structure-function, molecular control of gene transcription, and novel bioactions," in *Vitamin D*, D. Feldman, J. W. Pike, and F. H. Glorieux, Eds., pp. 219–261, Elsevier Academic Press, Oxford, UK, 2nd edition, 2005.

[24] M. R. Haussler, P. W. Jurutka, M. Mizwicki, and A. W. Norman, "Vitamin D receptor (VDR)-mediated actions of 1α,25$(OH)_2$ vitamin D_3: genomic and non-genomic mechanisms," *Best Practice & Research: Clinical Endocrinology & Metabolism*, vol. 25, no. 4, pp. 543–559, 2011.

[25] J. Merke, W. Kreusser, B. Bier, and E. Ritz, "Demonstration and characterisation of a testicular receptor for 1,25-dihydroxycholecalciferol in the rat," *European Journal of Biochemistry*, vol. 130, no. 2, pp. 303–308, 1983.

[26] M. R. Walters, "1,25-dihydroxyvitamin D_3 receptors in the seminiferous tubules of the rat testis increase at puberty," *Endocrinology*, vol. 114, no. 6, pp. 2167–2174, 1984.

[27] J. Merke, U. Hugel, and E. Ritz, "Nuclear testicular 1,25-dihydroxyvitamin D_3 receptors in Sertoli cells and seminiferous tubules of adult rodents," *Biochemical and Biophysical Research Communications*, vol. 127, no. 1, pp. 303–309, 1985.

[28] G. Schleicher, T. H. Privette, and W. E. Stumpf, "Distribution of soltriol [1,25$(OH)_2$-vitamin D_3] binding sites in male sex organs of the mouse: an autoradiographic study," *Journal of Histochemistry and Cytochemistry*, vol. 37, no. 7, pp. 1083–1086, 1989.

[29] A. R. Mahmoudi, A. H. Zarnani, and M. Jeddi-Tehrani, "Distribution of vitamin D receptor and 1α-hydroxylase in male mouse reproductive tract," *Reproductive Sciences*, vol. 20, no. 4, pp. 426–436, 2013.

[30] A. K. Nangia, J. L. Butcher, B. R. Konety, B. N. Vietmeier, and R. H. Getzenberg, "Association of vitamin D receptors with the nuclear matrix of human and rat genitourinary tissues," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 66, no. 4, pp. 241–246, 1998.

[31] S. T. Corbett, O. Hill, and A. K. Nangia, "Vitamin D receptor found in human sperm," *Urology*, vol. 68, no. 6, pp. 1345–1349, 2006.

[32] S. Aquila, C. Guido, I. Perrotta, S. Tripepi, A. Nastro, and S. Andò, "Human sperm anatomy: ultrastructural localization of 1α,25-dihydroxyvitamin D_3 receptor and its possible role in the human male gamete," *Journal of Anatomy*, vol. 213, no. 5, pp. 555–564, 2008.

[33] S. Aquila, C. Guido, E. Middea et al., "Human male gamete endocrinology: 1alpha, 25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) regulates different aspects of human sperm biology and metabolism," *Reproductive Biology and Endocrinology*, vol. 7, article 140, pp. 1–13, 2009.

[34] M. Blomberg Jensen, J. E. Nielsen, A. Jørgensen et al., "Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract," *Human Reproduction*, vol. 25, no. 5, pp. 1303–1311, 2010.

[35] M. Blomberg-Jensen, A. Jørgensen, J. E. Nielsen et al., "Expression of the vitamin D metabolizing enzyme CYP24A1 at the annulus of human spermatozoa may serve as a novel marker of semen quality," *International Journal of Andrology*, vol. 35, no. 4, pp. 499–510, 2012.

[36] A. W. Norman, *Vitamin D: The Calcium Homeostatic Steroid Hormone*, Academic Press, New York, NY, USA, 1979.

[37] W. H. Okamura, M. M. Midland, M. W. Hammond et al., "Chemistry and conformation of vitamin D molecules," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 53, no. 1–6, pp. 603–613, 1995.

[38] E. J. Friedlander, H. L. Henry, and A. W. Norman, "Studies on the mode of action of calciferol. Effects of dietary calcium and phosphorus on the relationship between the 25-hydroxyvitamin D_3-1α-hydroxylase and production of chick intestinal calcium binding protein," *The Journal of Biological Chemistry*, vol. 252, no. 23, pp. 8677–8683, 1977.

[39] M. R. Walters, D. L. Cuneo, and A. P. Jamison, "Possible significance of new target tissues for 1,25-dihydroxyvitamin D_3," *Journal of Steroid Biochemistry*, vol. 19, no. 1, pp. 913–920, 1983.

[40] M. R. Walters, B. C. Osmundsen, R. M. Carter, P. C. Riggle, and J. R. Jeter, "Accumulating evidence for a physiological role for 1,25-dihydroxyvitamin D_3 in new tragets: testis and heart," in *Vitamin D: Chemical, Biochemical, and Clinical Update*, A. W. Norman, K. Schaefer, H. G. Grigoleit, and D. V. Herrath, Eds., pp. 137–142, de Gruyter, New York, NY, USA, 1985.

[41] V. L. Akerstrom and M. R. Walters, "Physiological effects of 1,25-dihydroxyvitamin D_3 in TM4 sertoli cell line," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 262, no. 6, pp. E884–E890, 1992.

[42] N. Inpanbutr, J. D. Reiswig, W. L. Bacon, R. D. Slemons, and A. M. Iacopino, "Effect of vitamin D on testicular CaBP28K expression and serum testosterone in chickens," *Biology of Reproduction*, vol. 54, pp. 242–248, 1996.

[43] S. E. Bulun, I. M. Rosenthal, A. M. Brodie et al., "Use of tissue-specific promoters in the regulation of aromatase cytochrome P450 gene expression in human testicular and ovarian sex cord tumors, as well as in normal fetal and adult gonads," *Journal of Clinical Endocrinology and Metabolism*, vol. 77, no. 6, pp. 1616–1621, 1993.

[44] A. V. Krishnan, S. Swami, L. Peng, J. Wang, J. Moreno, and D. Feldman, "Tissue-selective regulation of aromatase expression by calcitriol: implications for breast cancer therapy," *Endocrinology*, vol. 151, no. 1, pp. 32–42, 2010.

[45] J. Lundqvist, M. Norlin, and K. Wikvall, "1α,25-dihydroxyvitamin D_3 exerts tissue-specific effects on estrogen and androgen metabolism," *Biochimica et Biophysica Acta*, vol. 1811, no. 4, pp. 263–270, 2011.

[46] E. Wehr, S. Pilz, B. O. Boehm, W. März, and B. Obermayer-Pietsch, "Association of vitamin D status with serum androgen levels in men," *Clinical Endocrinology*, vol. 73, no. 2, pp. 243–248, 2010.

[47] S. Pilz, S. Frisch, H. Koertke et al., "Effect of vitamin D supplementation on testosterone levels in men," *Hormone and Metabolic Research*, vol. 43, no. 3, pp. 223–225, 2011.

[48] S. Carreau, "Leydig cell aromatase: from gene to physiological role," in *The Leydig Cell in Health and Disease*, A. H. Payne and M. P. Hardy, Eds., pp. 189–195, Human Press, Totowa, NJ, USA, 2007.

[49] S. Carreau, H. Bouraïma-Lelong, C. Bois, L. Zanatta, F. R. M. B. Silva, and C. Delalande, "Aromatase, estrogens and testicular germ cell development," *Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry*, vol. 11, no. 1, pp. 33–39, 2011.

[50] S. Carreau and R. A. Hess, "Oestrogens and spermatogenesis," *Philosophical Transactions of the Royal Society B*, vol. 365, no. 1546, pp. 1517–1535, 2010.

[51] S. Carreau, C. Bois, L. Zanatta, F. R. M. B. Silva, H. Bouraima-Lelong, and C. Delalande, "Estrogen signaling in testicular cells," *Life Sciences*, vol. 89, no. 15-16, pp. 584–587, 2011.

[52] K. Golovine, M. Schwerin, and J. Vanselow, "Three different promoters control expression of the aromatase cytochrome P450 gene (Cyp19) in mouse gonads and brain," *Biology of Reproduction*, vol. 68, no. 3, pp. 978–984, 2003.

[53] N. Yamada-Mouri, S. Hirata, and J. Kato, "Existence and expression of the untranslated first exon of aromatase mRNA in the rat brain," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 58, no. 2, pp. 163–166, 1996.

[54] D. Silandre, C. Delalande, P. Durand, and S. Carreau, "Three promoters PII, PI.f, and PI.tr direct the expression of aromatase (cyp19) gene in male rat germ cells," *Journal of Molecular Endocrinology*, vol. 39, no. 1-2, pp. 169–181, 2007.

[55] M. Young and M. J. Mcphaul, "A steroidogenic factor-1-binding site and cyclic adenosine 3′,5′- monophosphate response element-like elements are required for the activity of the rat aromatase promoter in rat Leydig tumor cell lines," *Endocrinology*, vol. 139, no. 12, pp. 5082–5093, 1998.

[56] M. Lanzino, S. Catalano, C. Genissel et al., "Aromatase messenger RNA is derived from the proximal promoter of the aromatase gene in Leydig, Sertoli, and germ cells of the rat testis," *Biology of Reproduction*, vol. 64, no. 5, pp. 1439–1443, 2001.

[57] V. Pezzi, R. Sirianni, A. Chimento et al., "Differential expression of steroidogenic factor-1/adrenal 4 binding protein and liver receptor homolog-1 (LRH-1)/fetoprotein transcription factor in the rat testis: LRH-1 as a potential regulator of testicular aromatase expression," *Endocrinology*, vol. 145, no. 5, pp. 2186–2196, 2004.

[58] L. Zanatta, H. Bouraïma-Lelong, C. Delalande, F. R. M. B. Silva, and S. Carreau, "Regulation of aromatase expression by 1α,25$(OH)_2$ vitamin D_3 in rat testicular cells," *Reproduction, Fertility and Development*, vol. 23, no. 5, pp. 725–735, 2011.

[59] V. G. Pardo, R. Boland, and A. R. de Boland, "1α,25$(OH)_2$-vitamin D_3 stimulates intestinal cell p38 MAPK activity and increases c-Fos expression," *International Journal of Biochemistry & Cell Biology*, vol. 38, no. 7, pp. 1181–1190, 2006.

[60] I. Nemere, Y. Yoshimoto, and A. W. Norman, "Calcium transport in perfused duodena from normal chicks: enhancement within fourteen minutes of exposure to 1,25-dihydroxyvitamin D_3," *Endocrinology*, vol. 115, no. 4, pp. 1476–1483, 1984.

[61] J. C. Fleet, "Rapid, membrane-initiated actions of 1,25 dihydroxyvitamin D: what are they and what do they mean?" *Journal of Nutrition*, vol. 134, no. 12, pp. 3215–3218, 2004.

[62] H. N. Christensen, A. J. Aspen, and E. G. Rice, "Metabolism in the rat of three amino acids lacking alpha-hydrogen," *The Journal of Biological Chemistry*, vol. 220, no. 1, pp. 287–294, 1956.

[63] G. G. Guidotti, A. F. Borghetti, and G. Gazzola, "The regulation of amino acid transport in animal cells," *Biochimica et Biophysica Acta*, vol. 515, no. 4, pp. 329–366, 1978.

[64] F. R. M. B. Silva and G. F. Wassermann, "Kinetics of FSH stimulation of methylaminoisobutyric acid uptake in Sertoli cells in culture from testes of 15 day-old rats," *Medical Science Research*, vol. 27, no. 9, pp. 627–630, 1999.

[65] D. Menegaz, A. Rosso, C. Royer, L. D. Leite, A. R. S. Santos, and F. R. M. B. Silva, "Role of 1α,25$(OH)_2$ vitamin D_3 on α-[1-14C]MeAIB accumulation in immature rat testis," *Steroids*, vol. 74, no. 2, pp. 264–269, 2009.

[66] A. da Cruz Curte and G. F. Wassermann, "Identification of amino acid transport systems stimulated by FSH in rat testes," *Journal of Endocrinology*, vol. 106, no. 3, pp. 291–294, 1985.

[67] F. R. M. B. Silva, L. Renck, and G. F. Wassermann, "Retinol stimulates amino acid transport to Sertoli cell by a mechanism unrelated to protein synthesis," *Medical Science Research*, vol. 23, no. 3, pp. 155–156, 1995.

[68] F. R. M. B. Silva, L. D. Leite, K. P. Barreto, C. D'Agostini, and A. Zamoner, "Effect of 3,5,3′-triiodo-L-thyronine on amino acid accumulation and membrane potential in Sertoli cells of the rat testis," *Life Sciences*, vol. 69, no. 8, pp. 977–986, 2001.

[69] F. R. M. B. Silva, L. D. Leite, and G. F. Wassermann, "Rapid signal transduction in Sertoli cells," *European Journal of Endocrinology*, vol. 147, no. 3, pp. 425–433, 2002.

[70] K. C. Volpato, D. Menegaz, L. D. Leite, K. P. Barreto, E. de Vilhena Garcia, and F. R. M. B. Silva, "Involvement of K^+ channels and calcium-dependent pathways in the action of T3 on amino acid accumulation and membrane potential in Sertoli cells of immature rat testis," *Life Sciences*, vol. 74, no. 10, pp. 1277–1288, 2004.

[71] D. Menegaz, A. Zamoner, C. Royer, L. D. Leite, Z. A. Bortolotto, and F. R. M. B. Silva, "Rapid responses to thyroxine in the testis: active protein synthesis-independent pathway," *Molecular and Cellular Endocrinology*, vol. 246, no. 1-2, pp. 128–134, 2006.

[72] D. Menegaz, A. Barrientos-Duran, A. Kline et al., "1α,25$(OH)_2$-vitamin D_3 stimulation of secretion via chloride channel activation in Sertoli cells," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 119, no. 3–5, pp. 127–134, 2010.

[73] A. P. Zanatta, L. Zanatta, R. Goncalves, A. Zamoner, and F. R. M. B. Silva, "Integrin participates in the effect of thyrone on plasma membrane in immature rat testis," *Biochimica et Biophysica Acta*, vol. 1830, pp. 2629–2637, 2013.

[74] P. Biswas and L. P. Zanello, "1α,25$(OH)_2$ vitamin D_3 induction of ATP secretion in osteoblasts," *Journal of Bone and Mineral Research*, vol. 24, no. 8, pp. 1450–1460, 2009.

[75] L. D. Russell and M. D. Griswold, *The Sertoli Cell*, Cache River Press, Clearwater, Fla, USA, 1993.

[76] N. Lalevee, F. Pluciennik, and M. Joffre, "Voltage-dependent calcium current with properties of T-type current in Sertoli cells from immature rat testis in primary cultures," *Biology of Reproduction*, vol. 56, pp. 680–687, 1997.

[77] A. Jungwirth, T. Weiger, S. K. Singh, M. Paulmichl, and J. Frick, "Follicle-stimulating hormone activates a cAMP-dependent chloride conductance in TM4 Sertoli cells," *Biochemical and Biophysical Research Communications*, vol. 233, pp. 203–206, 1997.

[78] F. R. Boockfor, R. A. Morris, D. C. DeSimone, D. M. Hunt, and K. B. Walsh, "Sertoli cell expression of the cystic fibrosis transmembrane conductance regulator," *American Journal of Physiology—Cell Physiology*, vol. 274, no. 4, pp. C922–C930, 1998.

[79] N. Lalevée and M. Joffre, "Inhibition by cAMP of calcium-activated chloride currents in cultured Sertoli cells from immature testis," *Journal of Membrane Biology*, vol. 169, no. 3, pp. 167–174, 1999.

[80] C. Auzanneau, C. Norez, S. Noël, C. Jougla, F. Becq, and C. Vandebrouck, "Pharmacological profile of inhibition of the chloride channels activated by extracellular acid in cultured rat Sertoli cells," *Reproduction Nutrition Development*, vol. 46, no. 3, pp. 241–255, 2006.

[81] C. Auzanneau, C. Norez, F. Antigny et al., "Transient receptor potential vanilloid 1 (TRPV1) channels in cultured rat Sertoli cells regulate an acid sensing chloride channel," *Biochemical Pharmacology*, vol. 75, no. 2, pp. 476–483, 2008.

[82] A. P. Zanatta, L. Zanatta, R. Goncalves, A. Zamoner, and F. R. M. B. Silva, "Rapid responses to reverse T3 hormone in immature rat Sertoli cells: calcium uptake and exocytosis mediated by integrin," *PLoS ONE*, vol. 8, no. 10, Article ID e77176, 2013.

[83] D. Menegaz, C. Royer, A. Rosso, A. Z. P. D. Souza, A. R. S. D. Santos, and F. R. M. B. Silva, "Rapid stimulatory effect of thyroxine on plasma membrane transport systems: calcium uptake and neutral amino acid accumulation in immature rat testis," *International Journal of Biochemistry & Cell Biology*, vol. 42, no. 6, pp. 1046–1051, 2010.

[84] L. P. Zanello and A. W. Norman, "Stimulation by 1α,25$(OH)_2$-vitamin D_3 of whole cell chloride currents in osteoblastic ROS 17/2.8 cells. A structure-function study," *The Journal of Biological Chemistry*, vol. 272, no. 36, pp. 22617–22622, 1997.

[85] Z. Xiaoyu, B. Payal, O. Melissa, and L. P. Zanello, "1α,25$(OH)_2$-vitamin D_3 membrane-initiated calcium signaling modulates exocytosis and cell survival," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 103, no. 3–5, pp. 457–461, 2007.

[86] L. Zanatta, A. Zamoner, R. Gonalves et al., "Effect of 1α,25-dihydroxyvitamin D_3 in plasma membrane targets in immature rat testis: ionic channels and gamma-glutamyl transpeptidase activity," *Archives of Biochemistry and Biophysics*, vol. 515, no. 1-2, pp. 46–53, 2011.

[87] C. Lu and A. Steinberger, "Gamma glutamyl transpeptidase activity in the developing rat testis. Enzyme localization in isolated cell types," *Biology of Reproduction*, vol. 17, no. 1, pp. 84–88, 1977.

[88] L. W. DeLap, S. S. Tate, and A. Meister, "γ-glutamyl transpeptidase of rat seminal vesicles; effect of orchidectomy and hormone administration on the transpeptidase in relation to its possible role in secretory activity," *Life Sciences*, vol. 16, no. 5, pp. 691–704, 1975.

[89] S. B. Meroni, D. F. Cánepa, E. H. Pellizzari, H. F. Schteingart, and S. B. Cigorraga, "Effects of purinergic agonists on aromatase and gammaglutamyl transpeptidase activities and on transferrin secretion in cultured Sertoli cells," *Journal of Endocrinology*, vol. 157, no. 2, pp. 275–283, 1998.

[90] G. G. Glenner, J. E. Folk, and P. J. McMillan, "Histochemical demonstration of a gamma-glutamyl transpeptidase-like activity," *Journal of Histochemistry and Cytochemistry*, vol. 10, pp. 481–489, 1962.

[91] L. Zanatta, A. Zamoner, R. Gonçalves et al., "1α,25-dihydroxyvitamin D_3 signaling pathways on calcium uptake

in 30-day-old rat sertoli cells," *Biochemistry*, vol. 50, no. 47, pp. 10284–10292, 2011.

[92] L. Zanatta, A. Zamoner, A. P. Zanatta et al., "Nongenomic and genomic effects of 1α,25$(OH)_2$ vitamin D_3 in rat testis," *Life Sciences*, vol. 89, no. 15-16, pp. 515–523, 2011.

[93] A. Rosso, M. Pansera, A. Zamoner et al., "1α,25$(OH)_2$-vitamin D_3 stimulates rapid plasma membrane calcium influx via MAPK activation in immature rat Sertoli cells," *Biochimie*, vol. 94, no. 1, pp. 146–154, 2012.

[94] M. Blomberg Jensen, P. J. Bjerrum, T. E. Jessen et al., "Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa," *Human Reproduction*, vol. 26, no. 6, pp. 1307–1317, 2011.

[95] J. Paranko, M. Kallajoki, L. J. Pelliniemi, V. P. Lehto, and I. Virtanen, "Transient coexpression of cytokeratin and vimentin in differentiating rat sertoli cells," *Developmental Biology*, vol. 117, no. 1, pp. 35–44, 1986.

[96] M. D. Show, M. D. Anway, J. S. Folmer, and B. R. Zirkin, "Reduced intratesticular testosterone concentration alters the polymerization state of the Sertoli cell intermediate filament cytoskeleton by degradation of vimentin," *Endocrinology*, vol. 144, no. 12, pp. 5530–5536, 2003.

[97] W. A. Spruill, A. L. Steiner, L. L. Tres, and A. L. Kierszenbaum, "Follicle-stimulating hormone-dependent phosphorylation of vimentin in cultures of rat Sertoli cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 80, no. 4, pp. 993–997, 1983.

[98] A. Zamoner, P. F. Corbelini, C. Funchal, D. Menegaz, F. R. M. B. Silva, and R. Pessoa-Pureur, "Involvement of calcium-dependent mechanisms in T3-induced phosphorylation of vimentin of immature rat testis," *Life Sciences*, vol. 77, no. 26, pp. 3321–3335, 2005.

[99] A. Zamoner, P. Pierozan, L. F. Vidal et al., "Vimentin phosphorylation as a target of cell signaling mechanisms induced by 1α,25-dihydroxyvitamin v in immature rat testes," *Steroids*, vol. 73, no. 14, pp. 1400–1408, 2008.

[100] A. W. Norman, W. H. Okamura, M. W. Hammond et al., "Comparison of 6-s-cis and 6-s-trans locked analogs of 1α, 25$(OH)_2$-vitamin D_3 indicates that the 6-s-cis conformation is preferred for rapid nongenomic biological responses and that neither 6-s-cis- nor 6-s-trans-locked analogs are preferred for genomic biological responses," *Molecular Endocrinology*, vol. 11, no. 10, pp. 1518–1531, 1997.

[101] L.-X. Zhou, I. Nemere, and A. W. Norman, "1,25-dihydroxyvitamin D_3 analog structure-function assessment of the rapid stimulation of intestinal calcium absorption (transcaltachia)," *Journal of Bone and Mineral Research*, vol. 7, no. 4, pp. 457–463, 1992.

[102] A. W. Norman, I. Nemere, K. R. Muralidharan, and W. H. Okamura, "1β,25$(OH)_2$-vitamin D_3 is an antagonist of 1α,25$(OH)_2$-vitamin D_3 stimulated transcaltachia (the rapid hormonal stimulation of intestinal calcium transport)," *Biochemical and Biophysical Research Communications*, vol. 189, no. 3, pp. 1450–1456, 1992.

[103] A. W. Norman, R. Bouillon, M. C. Farach-Carson et al., "Demonstration that 1β,25-dihydroxyvitamin D_3 is an antagonist of the nongenomic but not genomic biological responses and biological profile of the three A-ring diastereomers of 1α,25-dihydroxyvitamin D_3," *The Journal of Biological Chemistry*, vol. 268, no. 27, pp. 20022–20030, 1993.

[104] C. M. Bula, J. E. Bishop, S. Ishizuka, and A. W. Norman, "25-dehydro-1α-hydroxyvitamin D_3-26,23S-lactone antagonizes the nuclear vitamin D receptor by mediating a unique noncovalent conformational change," *Molecular Endocrinology*, vol. 14, no. 11, pp. 1788–1796, 2000.

[105] D. Menegaz, M. T. Mizwicki, A. Barrientos-Duran, N. Chen, H. L. Henry, and A. W. Norman, "Vitamin d receptor (VDR) regulation of voltage-gated chloride channels by ligands preferring a VDR-alternative pocket (VDR-AP)," *Molecular Endocrinology*, vol. 25, no. 8, pp. 1289–1300, 2011.

[106] J. A. Huhtakangas, C. J. Olivera, J. E. Bishop, L. P. Zanello, and A. W. Norman, "The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1α,25$(OH)_2$-vitamin D_3 in vivo and in vitro," *Molecular Endocrinology*, vol. 18, no. 11, pp. 2660–2671, 2004.