Asian Journal of Andrology (2015) 17, 165–167

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LETTER TO THE EDITOR

Male Infertility

Can Vitamin D supplementation be used as adjunctive treatment for oligozoospermia or asthenozoospermia accompanied with Vitamin D deficiency?

Wen-Jie Yan, Nan Yu, Tai-Lang Yin, Liu Liu, Jing Yang

Asian Journal of Andrology (2015) **17**, 165–167; doi: 10.4103/1008-682X.138190; published online: 29 August 2014

Dear Editor,

We read the paper by Hammoud *et al.*[1] in the issue of *Asian Journal of Andrology* with great interests. This paper revealed that sperm concentration, sperm progressive motility, sperm morphology, and total progressively motile sperm count were lower in men with 25(OH) D ≥ 50 ng ml^{-1} when compared with men with 20 ng ml^{-1} ≤ 25(OH) D < 50 ng ml^{-1}. Total sperm count and total progressive motile sperm count were lower in men with 25(OH) D < 20 ng ml^{-1} when compared to men with 20 ng ml^{-1} ≤ 25(OH) D < 50 ng ml^{-1}. Therefore, they draw the conclusion that serum Vitamin D (VD) levels at high and low levels can be negatively associated with semen parameters.

Recent studies indicate that a great variety of actions mediated by VD/vitamin D receptor (VDR), including regulating transcription of several genes involved in mitotic activity in spermatogonial nuclei, affecting sperm metabolism, controlling estrogen synthesis in gonads, increasing intracellular Ca^{2+} levels and activating different signaling pathways (extracellular signal-regulated kinases 1/2 [ERK1/2], AKT and glycogen synthase kinase-3 [GSK3]) in human sperm, can influence spermatogenesis and sperm maturation. Hence, we propose that VD supplement may be a novel therapeutic opportunity in the treatment of oligozoospermia and asthenozoospermia for those accompanied with VD deficiency.

Development of spermatozoa depends on a complex series of events that occur in the reproductive organs. Spermiogenesis is an orderly, strict process of cell division and differentiation. Following spermiogenesis, the spermatozoa are transported to epididymis where they are stored before ejaculation and become motile. It is only during transit through the epididymis that spermatozoa undergo maturation and acquire progressive motility and the ability to fertilize ova. Epididymis is also a place where spermatozoa are stored before ejaculation.[2] Many factors have been implicated in sperm production and maturation, including VD, which attracts more and more attention.

Vitamin D is synthesized mainly in the skin, where ultraviolet ray B radiation converts 7-dehydrocholesterol to Vitamin D_3. Then, Vitamin D_3 is metabolized by the hepatic 25-hydroxylases to become 25(OH) D_3. Finally, the renal 1α-hydroxylase converts 25(OH) D_3 to 1,25(OH) $_2D_3$, which is the most biologically active metabolite of VD (**Figure 1**).[3] The actions of 1, 25(OH) $_2D_3$ are mediated by binding to its high-affinity receptor, the VDR. Furthermore, the cellular response to VD is not only dependent on VDR, but also on presence and activity of VD metabolizing enzymes.

Previous studies suggested than 1,25(OH) $_2D_3$ plays important roles in reproductive functions. VD deficiency in male rats reduced sperm counts, and female rats inseminated with semen from VD deficient male rats have lower fertility rates.[4,5] Moreover, retardation of spermatogenesis due to disturbances in sertoli and leydig cell function in VD-deficient rats is reversible and can be corrected by supplementing VD.[6] VD acts through VDR, and the expression of VDR has been shown in the mature human spermatozoa.[7,8] Significant

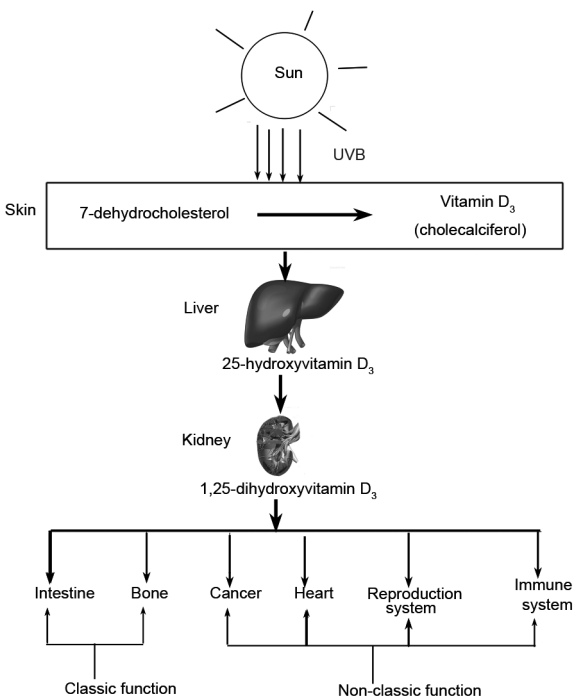

Figure 1: The metabolic pathway and function of Vitamin D. UVB: ultraviolet ray B.

Reproductive Medical Center, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China.
Correspondence: Dr. J Yang (dryangqing@hotmail.com)
Received: 07 May 2014; Revised: 15 June 2014; Accepted: 16 July 2014

gonadal insufficiency is demonstrated in VDR knock-out mice, with a decrease of sperm count and motility and histological abnormalities of the testis.[9] These results indicate that VD may play an important role in spermatogenesis and sperm maturation.

$1,25(OH)_2D_3$, the active form of VD, is a key regulator of calcium homeostasis and bone mineralization. VD affects calcium homeostasis by regulating intestinal absorption, urinary excretion, and secretion of PTH. What's more, calcium is essential for spermatogenesis, sperm motility, hyperactivation, and acrosome reaction.[10] Simultaneously, VD is vital for the synthesis of VD-dependent calcium transporters, the calcium pump, calbindin and calmodulin, which are all important for sperm function.[11,12]

$1,25(OH)_2D_3$ plays a pivotal role not only in systemic Ca^{2+} homeostasis but also in the intracellular Ca^{2+} homeostasis of various tissues.[13] Recently, it's reported that $1,25(OH)_2D_3$ is able to increase intracellular Ca^{2+}, although not in a dose-dependent manner.[14] And it has been confirmed that internal sperm Ca^{2+} stores provide sufficient Ca^{2+} for the induction of a hyperactive motility.[15] Hence, it may be safely said that $1,25(OH)_2D_3$ could influence the sperm motility by regulating intracellular Ca^{2+} content in human sperm. Sperm motility was enhanced upon 0.01 nmol l^{-1} and 0.1 nmol l^{-1} $1,25(OH)_2D_3$ and a significant dose-dependent effect from 0.01, 0.1, to 1 nmol l^{-1} $1,25(OH)_2D_3$ on increased acrosin activity was observed.[14]

Although VD function is associated closely with the control of calcium metabolism to a large extent, it is still proposed to have a variety of other biological roles, including influencing the cell cycle control.[16] The VDR, which is essential for VD-mediated events, can be found in both the cytoplasm and nucleus of VD target cells, comprising spermatogonia, spermatids and ejaculated spermatozoa in humans.[8] Especially, the nuclear expression of VDR in spermatogonia suggests a genomic action, where VDR forms a heterodimer with the retinoid receptor, binds to VD response elements and regulates transcription of several genes involved in mitotic activity, differentiation and apoptosis.[16] $1,25(OH)_2D_3$ could contribute to spermatogenesis by up-regulating certain specific genes in sertoli cells. Of these genes, the regulator of cellular cholesterol homeostasis Abca1 was expressed mainly in sertoli cells and influenced male fertility.[17] In fact, it has been reported that VDR is a transcription factor and $1,25(OH)_2D_3$/VDR was involved both in the early phases of the functional maturation of ejaculated sperm and sperm survival.[8] In addition, VDR is closely related to the nuclear matrix, and VD has a role in stabilizing chromosomal structure and preventing the induction of DNA double-strand breaks.[18,19] Interestingly, it has been proposed that the sperm nuclear matrix is crucial in the regulation of DNA fragmentation and degradation.[20] It can be speculated that $1,25(OH)_2D_3$/VDR acts as a protective genomic factor in the sperm nucleus. In addition to its genomic action, VD can also induce a nongenomic action, which is mediated through membrane bound or cytoplasmic VDR and alter cellular activity through second messengers.[16]

Sperm functions are associated with different signal transduction pathways. ERK1/2, AKT and GSK3 have been demonstrated to be involved in sperm activities.[21,22] In recent times, it has been further confirmed that $1,25(OH)_2D_3$ is able to activate different signaling pathways (ERK1/2, AKT and GSK3) through VDR. The result suggests that $1,25(OH)_2D_3$ is involved in sperm biological functions, comprising contributing to the sperm motility.[14]

Sperm metabolism is very important for the function of sperm. In Aquila study, it was observed that $1,25(OH)_2D_3$ affects sperm metabolism by reducing triglycerides content concomitantly with the increase of the lipase activity in sperm. In the metabolism of neutral lipids, the rate-limiting step lies at the level of the lipase, which catalyzes the hydrolysis of triglyceride. The increased lipid metabolism could meet the energetic demands for sperm capacitation.[14]

The relation between VD and sexual hormones is another interesting research focus. A study, including 2299 participants, revealed that VD had a positive correlation with serum androgen levels in men.[23] The researchers found that men with sufficient VD levels had significantly higher levels of testosterone and free androgen index and significantly lower levels of sex hormone binding globulin when compared to VD insufficient and VD deficiency. Meanwhile, the role of VD in the regulation of estrogen synthesis in male gonads has been investigated. The activity of aromatase is a key factor in estrogen synthesis. VDR null mutant mice showed gonadal insufficiencies, including decreased sperm count, and decreased motility with histological abnormality of the testis in the male. In these mice, it was observed that the aromatase activity was low in testis and epididymis; the gene expression of aromatase was also down regulated in these organs; however, the gene expression of estrogen receptor was normal.[9] These results showed that VD/VDR was essential for estrogen biosynthesis. In addition, estrogen receptor has been found in human sperm.[21] Estrogen is important for reabsorption of water during transit of sperm from the testis to epididymis, and low estrogen results in altered osmolarity, which can lead to dysfunction of spermatozoa.[24] In VDR null mutant mice, gonadal insufficiency was corrected and sperm motility was significantly increased by supplementation of estradiol.[9] However, some studies proved that serum VD level was not correlated to reproductive hormone values in human.[1,25] Therefore, until now, there is no consistent conclusion about the relation between VD and sexual hormones.

As mentioned above, there is a close relationship between VD metabolism and sperm development. However, it is wrong that higher VD levels must be good for spermatogenesis. In a study made by Hammoud *et al.*[1] men with higher VD levels (above 50 ng ml^{-1}) exhibited lower percent normal sperm head, percent progressive motile sperm, sperm concentration and total progressive motile sperm count after correcting for age, body mass index, season, alcohol intake and smoking. This phenomenon was also found in the analysis of Ramlau-Hansen *et al.*[25] The mechanisms may be explained by the effect of VD on intracellular calcium, sperm motility and acrosin reaction.[14] High VD levels can induce alterations in the systemic or local calcium and zinc levels, both known to play a role in spermatogenesis.[26,27]

In conclusion, VDR and VD metabolizing enzymes are widely expressed in human male germ cells and reproductive tract. VD/VDR system plays an important role in sperm physiology through its effects on affecting sperm metabolism, controlling estrogen synthesis in gonads, increasing intracellular Ca^{2+} levels and activating different signaling pathways (ERK1/2, AKT and GSK3) in human sperm. In addition, VD/VDR influences spermatogenesis and sperm function through both genomic and nongenomic pathways (**Figure 2**). Several studies, including clinical trials have proved that VD insufficient or deficiency could cause adverse effects on spermatogenesis and related to poor sperm parameters closely. So oligozoospermia or asthenozoospermia accompanied with VD deficiency may be treated by VD supplementation, which can improve the count as well as motility of the sperm. But further studies, particularly large sample of clinical trials, about the optimum dose and populations suited to the therapy are needed.

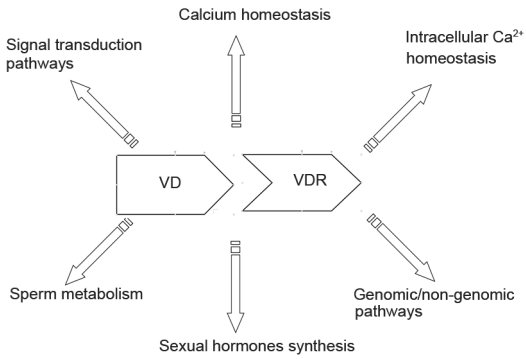

Figure 2: VD/VDR influences spermatogenesis and sperm function through several aspects. VD: vitamin D; VDR: vitamin D receptor.

AUTHOR CONTRIBUTIONS

WJY and JY conceived and designed the study. NY and TLY participated in the design of the study. LL assisted with the revising of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interest.

ACKNOWLEDGMENTS

This study was partially supported by the National Natural Science Foundation of China (No. 81370767).

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