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Original Article

# Expression of Vitamin D Receptor in Seminal Vesicles of Cholesterol Formula Mice

Tae-Hee Kim[1], Hae-Hyeog Lee[1], Jun-Mo Kim[2], Seung Do Choi[3], Arum Lee[4], Sun Yong Hwang[4], Mijin Kim[4], Yeon-Suk Kim[4], SeungRae Yeom[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Urology, Soonchunhyang University College of Medicine, Bucheon, [3]Department of Obstetrics and Gynecology, Soonchunhyang University College of Medicine, Cheonan, [4]Department of Interdisciplinary Program in Biomedical Science, Soonchunhyang University, Asan, Korea

**Objectives:** Genomic function of vitamin D receptor (VDR) indicates spermatogenesis that is important for in male reproductive organ authors evaluated the VDR expression in seminal vesicles with high cholesterol (HC) formula diet rat, because there is no report about relationship or difference in VDR in seminal vesicles between HC and control.
**Methods:** Male C57BL/6 mice aged 5 weeks were raised for 13 weeks. After one week of adaptation-period, they were fed different diet on normal AIN-93G diet, or HC diet containing 2% cholesterol for 12 weeks. The antibodies used were rabbit anti-VDR primary polyclonal.
**Results:** There was no significant difference in VDR reactivity in seminal vesicles, body weight of rat and weight of seminal vesicles between HC group and normal control group.
**Conclusion:** Our data give the no difference in expression of VDR of seminal vesicles rat between HC formula diet and normal AIN-93G diet. But we confirmed the VDR expression in seminal vesicles. **(J Menopausal Med 2015;21:89-92)**

**Key Words:** Cholesterol, Seminal vesicles, Vitamin D receptor

## Introduction

Food has been known in important role in the relation of disease development and fertility.

Nowadays, diet component is correlated with disease prevention and reproductive function.

Cholesterol is subunit in the lipid of 3 main nutrition components. Total lipid is composed of total cholesterol, triglyceride, phospholipids and free fatty acid. Cholesterol is a representative of lipid substance which is made by liver and absorbed by some foods.

Cholesterol is known as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), good and bad cholesterol. High cholesterol (HC) diet induces coronary heart disease and other diseases. Cholesterol has a main function in structure and permeability in every cell membrane and a main role of precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D.

Vitamin D (1,25-dihydroxy-vitamin D3 [1,25-(OH)2D3]) has a genomic role and a non genomic role. Non genomic pathway of classical function is a regulator of bone and mineral metabolism. Genomic function is vitamin D receptor (VDR) binding pathway which gives the effect of protein synthesis by nuclear transcription.

The seminal vesicles secrete liquid with semen. Seminal vesicle has not been evaluated about the function. Vitamin

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**Address for Correspondence:** Tae-Hee Kim, Department of Obstetrics and Gynecology, Soonchunhyang University Bucheon Hospital, 170 Jomaru-ro, Wonmi-gu, Bucheon 14584, Korea
Tel: +82-32-621-5380, Fax: +82-2-6008-6874, E-mail: heeobgy@schmc.ac.kr

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D and VDR is evaluated human testis, ejaculatory tract, and mature spermatozoa. But seminal vesicle has no data about the VDR expression.

VDR has been reported as increasing in $Ca^{2+}$ in human spermatozoa.

1,25(OH)2D3 modulated cholesterol efflux, phosphorylation on tyrosine and threonine which increase sperm survival and sperm motility.[1]

1,25(OH)2D3 directly in the seminal fluid induced sperm motility.[2] Location of VDR expression indicates the action of Vitamin D.[3] Genomic function of VDR indicates spermatogenesis that is important for in male reproductive organ.[3] The cholesterol content of mammalian sperm affects the development of acrosomal responsiveness.[4] Authors evaluate the VDR expression in seminal vesicle with HC formula diet rat, because there is no report about relationship or difference in VDR in seminal vesicle between HC and control.

## Material and Methods

### 1. Animals

Male C57BL/6 mice aged 5 weeks were raised for 13 weeks. After one week of adaptation-period, they were fed different diet on normal AIN-93G diet, or HC diet containing 2% cholesterol for 12 weeks. Both diets have the same calories. Twelve mice were grouped as normal control (NC) group who were fed AIN-93 diet, and 10 mice were grouped as HC group .

### 2. Tissue collection and preparation

Male mice were sacrificed after anesthesia by cervical dislocation and seminal vesicle was removed. Seminal vesicle was fixed in 10% formalin for 48 hours and embedded in paraffin blocks, and then cut into 5 $\mu$m thick sections for immunohistochemistry (IHC).

### 3. IHC localization of VDR

Five-micron tissue sections were collected on poly-L-lysine-coated slides (Sigma-Aldrich Corp., St. Louis, MO, USA). Each tissue section was deparaffinized in xylene and rehydrated through a graded ethanol series. Thereafter, slides were washed in phosphate buffered saline (PBS; pH 7.4) three times for 5 minutes. For antigen retrieval, slides were heated in a microwave oven at 600°C with 0.01 M sodium citrate buffer (pH 6.0) for 20 minutes. After 1 hour cooling at room temperature, the slides were washed three times for 5 minutes in PBS.

Endogenous peroxidases were blocked by treating the sections with 0.3% hydrogen peroxide (Fisher ChemAlert Guide, New Jersey, USA) for 30 minutes, followed by washing three times with PBS. The slides were then incubated in a humidified chamber with blocking buffer (5% bovine serum + 0.5% tween-20 in PBS) for 1 hour at room temperature. Primary antibody was applied in a moist chamber overnight at 4°C. Polyclonal antibodies were raised against VDR, estrogen receptor-beta (ER-$\beta$), and ezrin. The antibodies used were rabbit anti-VDR primary polyclonal antibody (ab3508, 1 : 2000 dilution, Abcam Inc., Cambridge, MA, USA), rabbit anti-ER-$\beta$, primary polyclonal antibody (ab3577, 1 : 500, Abcam Inc., Cambridge, MA, USA), and mouse anti-ezrin primary monoclonal antibody (ab4069, 1 : 100, Abcam Inc., Cambridge, MA, USA). After three additional rinsing steps with PBS for 5 minutes, biotinylated universal antibody (anti-rabbit/mouse IgG) was added for 1 hour, and the samples were treated with Vectastain avidin-biotin complex (ABC) kit for 30 minutes. After being washed with PBS, the sections were developed using diaminobenzidine (DAB; Vector Laboratories Inc., Burlingame, CA, USA) for 1 minute at room temperature. Counterstaining was performed with Mayer's hematoxylin (Merck, Darmstadt, Germany). All experiments were executed with control staining without the primary antibody to ensure that negative controls remained unstained.

**Table 1.** Body weight and seminal vesicle's weight

| | Normal control | High cholesterol |
|---|---|---|
| Initial BW (g) | 16.83 ± 0.72 | 17.00 ± 0.47 |
| Final BW (g) | 30.25 ± 2.60 | 31.30 ± 1.57 |
| Seminal vesicle weight (g) | 1.19 ± 0.59 | 1.23 ± 0.21 |

Values are expressed as mean ± SD
BW: body weight

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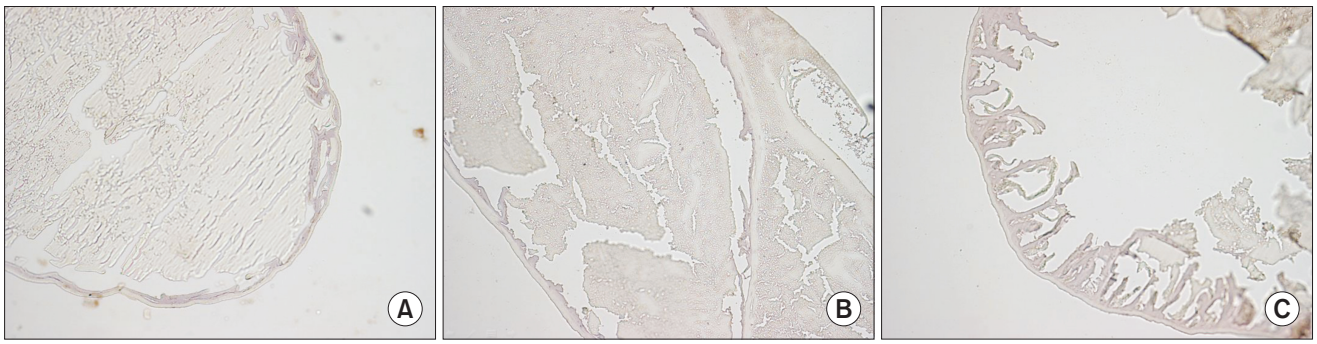


**Fig. 1.** Localization of vitamin D receptor in the seminal vesicle of each of groups as brown color. Immunohistochemistry was performed as described in the Material and Methods section. (A) normal control group (NC), (B, C) high cholesterol (HC) group, magnification: × 100. NC and diet on normal AIN-93G diet, HC diet on HC.

## Results

The body weight and seminal vesicle's weight are presented in Table 1. There are no significant differences between both groups.

The results of VDR have been described in Figure 1 as IHC. Epithelial cells of seminal vesicle showed reactivity in the nucleus with weak stain in cytoplasm (Fig. 1).

Overall, regardless of cholesterol concentration on diet, VDR express with weak stain in seminal vesicle. There was no significant difference of VDR reactivity between HC and NC in seminal vesicles. It seemed that HC diet dose not effect to VDR expression in seminal vesicle.

## Discussion

Our data give the no difference in expression of VDR of seminal vesicle rat between HC formula diet and normal AIN-93G diet. Authors had researched between VDR and menopausal female reproductive organ then confirmed VDR in menopausal mouse model.[5~7] And authors had considered between VDR and male reproductive organs so that we confirmed the VDR expression in seminal vesicle. Seminal vesicle has been known as container and secreting organ of liquid which mainly becomes semen. Seminal vesicle fluid results in alkaline PH in semen. PH is associated the semens motility in vagina after coitus.[8]

Seminal vesicle function has been not evaluated, but fluid content is important for semen function for fertility.

The dietary cholesterol is correlated with serum cholesterol level in male.[9] Chronic alcoholics induced higher serum FSH levels which decreased the total sperm count in semen.[10]

Dietary, social patterns are correlated with male reproductive hormone and semen quality.

Vitamin D is produced in skin, which has mainly role about bone and mineral metabolism. Nowadays, vitamin D deficiency is correlated with cardiovascular disease by VDR signaling.[11]

1,25(OH)2D3 decrease diabetes-derived macrophages Cholesterol Uptake which give the message to reduce the cardiovascular disease.[11] And It has a role to control the cholesterol uptake also. VDR play a role to regulate cholesterol and bile acid metabolism.[12] VDR have role in increasing serum total cholesterol concentration in mice. But gender and diet have more effect on serum lipid concentration and regulation of cholesterol than role of VDR.[13]

There was no difference in VDR expression in both groups. Authors had limitation about the small cases, only cholesterol formula diet, only seminar vesicle expression. Authors should evaluate other reproductive fertility organ in male and cholesterol formula diet based on the large case. Authors further evaluate the VDR analogue expression in seminal vesicle in HC formula diet. We should evaluate about the liquid component in semen from seminal vesicle under specific diet habit and VDR function.

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## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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