



The physiological role of Vitamin D in the female fertility in rats

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Abstract

This study was set up to assess the role of vitamin D on the female fertility using albino rats in order to get benefit from its use. For this study, fifty female albino rats obtained as weanling (21 days –old) weighing 30 g, were allocated randomly into 2 equal groups (First group was vitamin D-deficient rats which fed vitamin D deficient diet and the second group is vitamin D-replete rats which fed the same diet but received 2µg cholecalciferol per week in 0.1ml propylene glycol by a single intra peritoneal injection). A animals were maintained till become adult. The body weight was recorded from the age of 21 till the age of 4 months, the length and regularity of estrus cycle were monitored daily by vaginal smears and sera were used for determination of estrogen level. Tissue samples (ovaries and uteri) were used for histopathological examination. The obtained results showed a significant increase in the body weights of the replete females as compared with the deficient group. Moreover, the deficient females had longer days of estrus cycle. Estradiol level in the deficient females was lower than the replete females. Histopathological examination of the ovary showed abnormal development of the follicles and uterine hypoplasia was recorded in the deficient females as compared with the replete one. Vitamin Dare capable of inducing useful effects on the reproductive systems of female rats.

Keywords: Vitamin D, estrus cycle, estradiol, fertility, female rats.

Introduction

Vitamin D. considered as a hormone rather than vitamin, where it is essential for maintaining normal blood calcium and phosphorus The identification of its chemical structure showed that it was actually a steroid hormone not a vitamin¹.

1α, 25-dihydroxycholecalciferol is considered the active form of vitamin D, which produced its biological activities through genomic and non-genomic responses. The classic genomic responses generated by regulating gene transcription through binding with vitamin D receptors. The rapid or non-genotropic responses were through stimulation of different signal transduction events².

Other functions of vitamin D are modulation of cell differentiation, immune function, inhibition of cell growth, and control of hormonal systems³. In addition, it is also essential for normal gonadal function⁴, where, several studies revealed that vitamin D had a role in the functions of the male and female reproductive system⁵.

It was reported that vitamin D receptors (VDR) were expressed in parathyroid gland, intestine, skeleton, ovary and testis⁶ and in rodent's male and female reproductive tissues⁷.

Infertility considered one of the important problems, where it was influenced by many environmental, behavioral, genotoxic

and genetic factors⁸. Both male and female fertility could be reduced by the deficiency of vitamin D⁹.

The deficiency of vitamin D in female is indicated by a decrease in fertility ratio¹⁰, fetus size with retardation of neonatal growth¹¹. Moreover, impaired folliculogenesis and uterine hypoplasia were detected in VDR null-mutant mice¹². Furthermore, irregular and longer estrus cycle was reported in 1α-hydroxylase mutant mice¹³.

It was found that estrogen level was significantly decreased in VDR null-mutant mice which attributed that to the decreased in the activity of P450 aromatase and suppression of CYP19 gene expression⁷.

So, the aim of this work was studying the physiologic effect of vitamin D on the fertility of female through comparing the effect of deficiency and supplementation via recording body weights, estrus cycle observation for its length and regularity, determination of serum estrogen level. Histological examination of the ovary and the uterus.

Materials and methods

The procedures in this work were approved by the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Animals and diets: 50 female weanling albino rats weighing 30 g with average age of 21 days old, were obtained from the

Laboratory Animal Unit in Faculty of Veterinary Medicine, Zagazig University. The rats were divided into two equal groups (n=25); the first group is vitamin D-deficient rats which fed vitamin D deficient diet and the second group is vitamin D-replete rats which fed the same diet but received 2µg cholecalciferol (Memphis Company for pharmaceuticals and Chemical Industries, El-Amirya, Cairo, Egypt) per week in 0.1ml propylene glycol by a single intra peritoneal injection^{9,14}. All the female rats were kept in stainless steel cages and subjected to 12h of daily light. The first group was prevented from all sources of fluorescent light and sunlight. It was provided by incandescent lighting. The animals were supplied the diet and water¹⁵ (Table-1).

In addition, the animals received fat-soluble vitamins in cottonseed oil, three times per week that contains β-carotene (pro vitamin A) 70µg; menadione 105µg and 875µg of α-tocopherol weekly. The diet was adjusted to contain 0.47% calcium and 0.3% phosphorus¹⁶.

Experimental design: Body weights: The female weanling rats in both groups were observed for their body weights from the beginning of experiment (at 21 days of age) nearly every month until the age of 4 months.

Estrus cycle analysis: The length and regularity of estrus cycle for a period of one month in 3 month-old females was monitored by daily vaginal smears¹⁷.

Serum Estradiol II level: Serum estradiol concentration was analyzed by electrochemiluminescence immunoassay (ECLIA) using Cobas e apparatus (Roche Diagnostics GmbH, Mannheim, Germany)^{18,19}.

Histopathological examination of the ovaries and uteri: The routine histopathological examination for samples from the ovary and uterus was performed²⁰.

Statistical Analysis: All data were analyzed using Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA)²¹. To estimate the difference between the replete and deficient group, Independent sample t-test was used according to (Students-t).

Data were presented as means and SEM and the differences among groups were considered significant at P< 0.05.

Results and discussion

It is obvious from Table-2 that there was no change in the body weights in both groups at the age of 22-50 days. The first group showed reduction in the body weight, which was more prominent at the age of 85days (112.84±2.8g) as compared with the replete females (129.36±1.1). At 120days, the difference in body weights was approximately about 32gm in replete females (159.72±4.5g) and (127.78±2.8g) in deficient females.

Table-1: Composition and nutrient level of the diet.

Ingredients				Amount %	
Corn gluten				14.8	
Soybean meal				6.8	
Ground yellow corn				70.9	
Cotton seed oil				6	
Calcium carbonate				1	
Dibasic calcium phosphate				0.3	
Ca and P-free salt mixture:				0.1	
Water soluble vitamin mixture				0.1	
Ca and P-free mixture (%)	NaCl 20.9	KCl 57.7	FeSO ₄ .7H ₂ O 3.22	CuSO ₄ .5H ₂ O 0.078	COCl ₂ .H ₂ O 0.004
NaF 0.113	ZnSO ₄ .7H ₂ O 0.44	MnSO ₄ .H ₂ O 0.04	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O 0.005	KI 0.01	MgSO ₄ 17.9
Water soluble vitamin mixture (%)	Riboflavin 0.5	Calcium pantothenate 2.8	Inositol 20	Vitamin B ₁₂ 0.002	Glucose monohydrate 73.7
Pyridoxine 0.5	Thiamin 0.5	Nicotinamide 2.0	Folic acid 0.02	Biotin 0.01	-

Table-2: The overall means of the body weights of vitamin D-deficient and replete female rats.

Groups age	First group (Deficient females)	Second group (Replete females)
22days	29.74±3.4 ^a	34.12±2.5 ^a
50days	62.72±6.7 ^a	76.08±5.6 ^a
85days	112.84±2.8 ^b	129.36±1.1 ^a
120days	127.78±2.8 ^b	159.72±4.5 ^a

^{a-b}Means with different superscripts in each row were significantly differ.

Table-3: The overall means of estrus cycle in vitamin D-deficient and replete female rats.

Parameters	First group (Deficient females)	Second group (Replete females)
Cycle length (days)	6.7±0.41 ^b	4.29±0.17 ^a
n. of cycles in 30 days	4.2±0.58 ^b	6.4±0.24 ^a
Percent of each phase		
Proestrus	4.64±1.7 ^b	15.28±0.8 ^a
Estrus	17.98±2.9 ^a	29.28±4.1 ^a
Metestrus	27.3±7.4 ^a	33.98±4.0 ^a
Diestrus	49.30±11.3 ^b	21.3±3.1 ^a

^{a-b}Means with different superscripts in each row were significantly differ.

The obtained results of estrus cycle analysis in Table-3) revealed that the length of estrus cycle were increased in the deficient group than in the replete one (6.7±0.41 and 4.29±0.17 days, respectively); the number of cycles in 30 days was decreased in the deficient group than that of the replete group (4.2±0.58 and 6.4±0.24 cycles, respectively). In addition, the percent of diestrus phase in the first group was increased as compared with the second group (49.31±1.3 and 21.3±3.1%, respectively).

Table-4: The overall means of serum estrogen levels in vitamin D-deficient and replete female rats.

Groups	Serum estradiol level (pg/ml)
First group (Deficient females)	8.57±2.03 ^b
Second group (Replete females)	28.62±2 ^a

^{a-b}Means with different superscripts within the column were significantly differ.

The data from Table-4 revealed a significant decrease in serum estrogen levels in deficient female rats (8.57±2.03pg/ml) as compared with replete females (28.62±2pg/ml).

Discussion: Several reports indicated that vitamin D receptors were located in both testes and ovaries of young rats which clarify the physiological role played by this vitamin in the fertility, where, where, it was demonstrated that VDR is localized in the testis of immature rats²². Also, the immunofluorescence analysis of the testis of immature rats showed that Sertoli cells considered as a site for 1,25 dihydroxycholecalciferol action²³.

Concerning the ovary, VDR mRNA was expressed in the ovary of woman²⁴ and the ovary of pregnant mice²⁵.

It was reported that VDR expression is essential for the cell response to activated vitamin D²⁶.

Regarding body weights, there was a significant reduction in total body weight of the deficient female rats. These results are parallel to the previous reports of in male rats^{27,28} and in female rats¹¹.

It had been reported that vitamin D metabolites could directly affect metabolism in muscle via different pathways and its deficiency was associated with muscle weakness²⁹. Also, it had a stimulatory effect in the process of differentiation of pre adipocytes into adipocytes³⁰.

Furthermore, calcitriol increased insulin-like growth factor-1 receptors in osteoblasts, which in turn responsible for the physiologic effect of growth hormone³¹, in addition, it stimulate the synthesis of calmodulin, troponin C, actin and mitochondrial membrane proteins in the skeletal muscle³².

Regarding estrus cycle analysis in the deficient female rats, the results revealed irregular and longer estrus cycle together with increased time of diestrus, while normal and regular estrus cycle is exhibited by the replete female rats. These results are in agree with that of Sun et al.¹³. Moreover, it had been found that expression of VDR in the endometrium of non-pregnant mice in all stages of estrus cycle could explain the effect of vitamin D in occurrence of estrus³³.

In the same respect it was revealed that the deficiency of vitamin D₃ before onset of puberty caused prolonged estrous cycles that represented by prolonged diestrus and reduced frequency of proestrus and estrus³⁴.

Also, it was stated that Vitamin D might has a role in regulating ovulation in women³⁵.

Therefore, the longer and irregular estrus cycle may be the result of reduction in estrogen level produced by decreased activity of P450 aromatase.

Concerning estrogen level in Table-4, it revealed a decrease in serum estrogen level in deficient females and increase in its level in replete females. This agree with the results of Kinuta et al.⁷ and that of Sun et al.¹⁵ in female mice. Indeed, the explanation of these results is supported by the fact that aromatase gene (CYP19) encodes P450 aromatase which is the key enzyme in estrogen biosynthesis and control its level³⁶. That is to say, the decrease in estrogen concentration during vitamin D deficiency attributed to diminish P450 aromatase activity that resulted from decreased CYP19 gene expression.

Histopathological examination of the ovaries and uteri in the deficient females showed abnormal development of the follicles and corpus luteum formation as shown in Figure-1 and uterine hypoplasia as shown in Figure-2. The results of

histopathological examination of the ovaries and uteri in the deficient females are consistent with the previous study in VDR null-mutant female mice^{12,7,13}.

Altered hypothalamic-pituitary-ovarian axis function was in large part related to these defects¹³. In addition, estrogen deficiency could impair folliculogenesis and cause uterine hypoplasia as estrogen supplementation was found to increase uterine weight¹². Furthermore, the deficiency of aromatase gene produced ill developed uterus and ovary in mice³⁷. In addition, vitamin D showed a positive effect on endometrial thickness with higher pregnancy rats³⁸.

Therefore, we can say that estrogen deficiency, resulted from decreased CYP19 gene expression and decreased P450 aromatase activity in both gonads, is responsible for gonadal abnormalities.

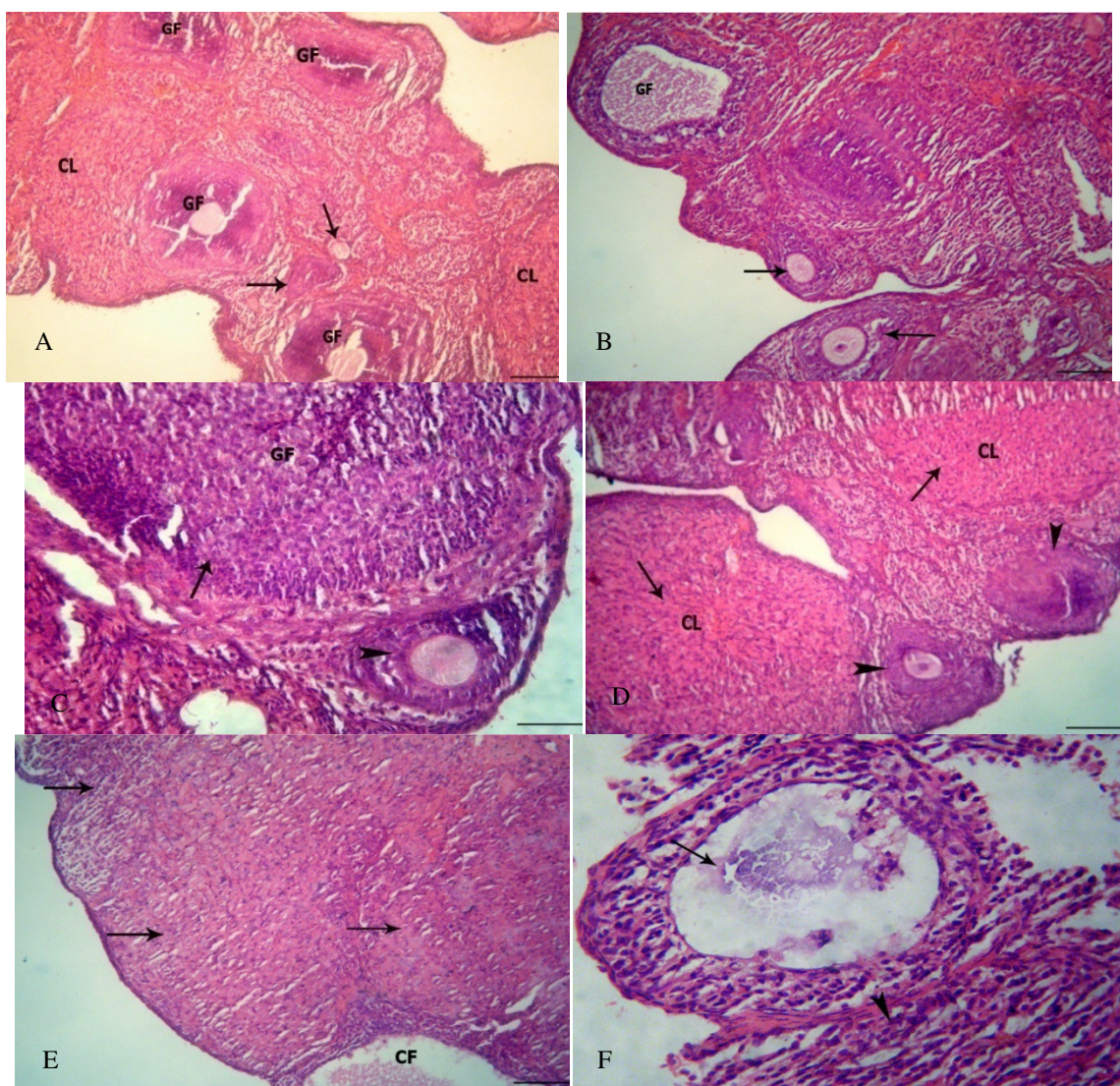


Figure-1: Ovary tissue sections of female rats: (a,b,c): vitamin D replete group shows numerous healthy primary and few preantral follicles (arrows), Graffian (GF) and corpora lutea “CL” (a), a higher magnification for preantral follicles “arrows” (b) and Graffian (arrow; GF) preantral follicles “arrowhead” (c). (d,e,f):vit-D deficient group shows corpora lutea (arrows; CL) and few preantral follicles “arrowheads” (d,e) and follicles with pyknoticgranulosa cells and dilated antrum (f). HE (Bar = 50 µm).

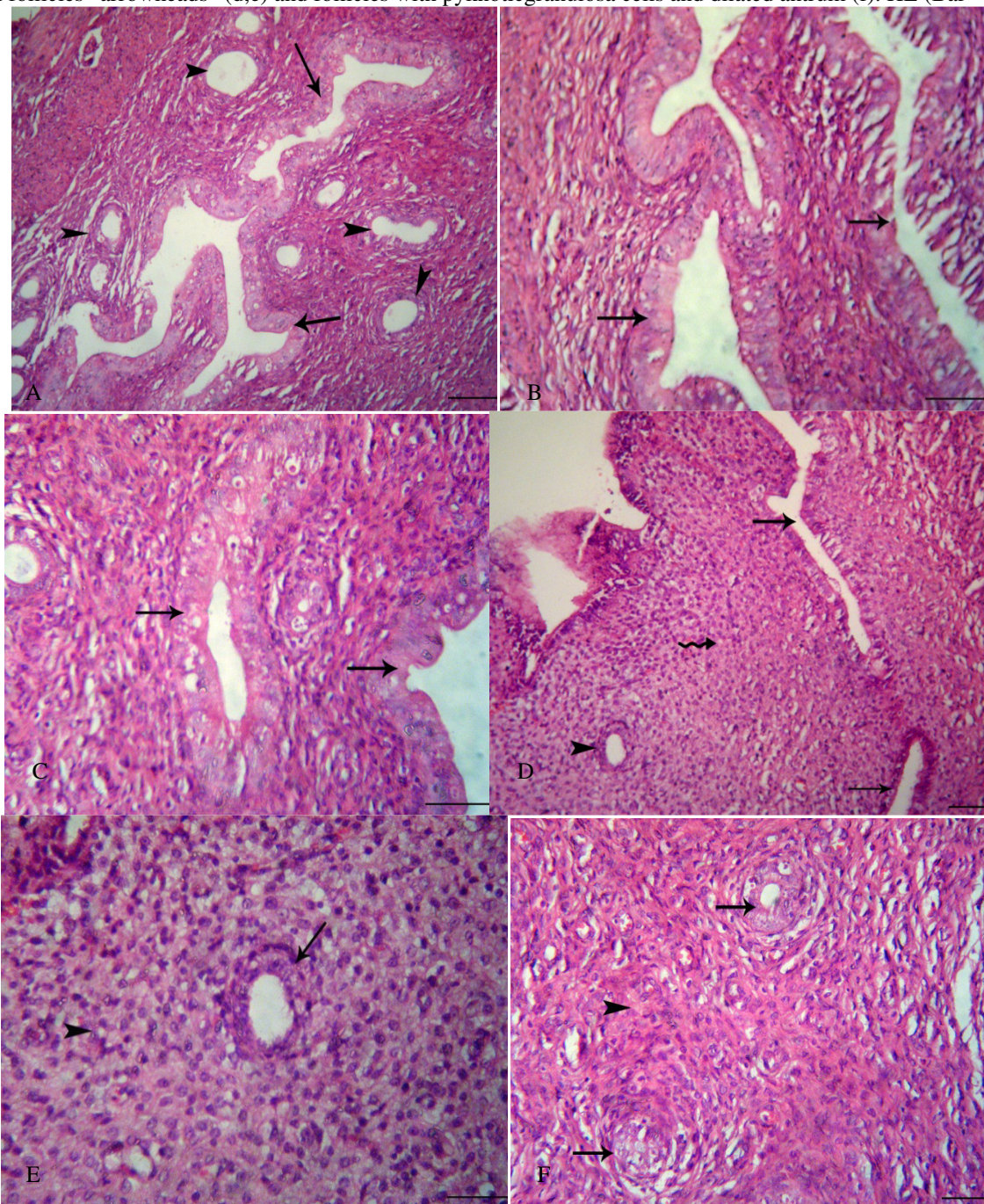


Figure-2: Uterus tissue sections of female rats (a, b ,c): vitamin D replete group shows numerous irregular tortuousand wide-lumen endometrium (arrows) and endometrial glands (arrowheads) with tall columnar epithelium. (d,e,f): :vitamin-D deficient group shows uterine hypoplasia with absence of the folding of luminal epithelium with narrow lumen (arrows), inactive glands (arrowhead) and increased the stromal cells “irregular arrow” (d) and a higher magnification to show the inactive glands (arrows) and increased the stromal cells “arrowheads” (e,f). HE (Bar = 50 µm)

Conclusion

Our results revealed that vitamin D is important for the female fertility and it has a positive effect on the female fertility as indicated by detection of the effect of deficiency and supplementation of vitamin D on some reproductive parameters in the female rats, where vitamin D deficiency caused a decrease in the body weights, estrogen level. Longer and irregular estrus cycle, abnormal development of the follicles in the ovary and uterine hypoplasia.

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