


# Influence Ovariectomy on Parathyroid Gland Function in Local Bitches

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## Abstract

**Background and Aims:** The present study was conducted to assess the effect of ovarian function on the parathyroid gland in local breed bitches in the center of Al-Diwaniyah city, Iran. **Materials and Methods:** The experiment was carried out on 21 local breed female bitches aged 18 to 30 months housed in the small animal housing of the College of Veterinary Medicine. The animals were divided into three groups of seven each: G1, animals in estrus phase, G2, animals in anestrus phase, and G3, the ovariectomized group. After expressed consent of the owners, ovariectomy was performed immediately after anesthetization. Blood samples were collected to analyze the levels of estrogen, PTH, calcitonin, serum calcium, ionized calcium and 1,25-dihydroxyvitamin D3. **Results:** Our results in respect of estrogen and PTH revealed a significant effect in G3 and G2 groups as compared with G1 group at  $p < 0.01$ . The concentration of calcitonin recorded insignificant decrease in G3, 5.35 pg/mL, when compared with G1 which was 6.98 pg/mL. The lowest serum calcium level was recorded in group G3, 8.29 mg/dL, and highest in the group G1, 11.21 mg/dL. The lowest concentration of 1,25-dihydroxyvitamin D3 was found in G3, 18.24 mg/dL, while the highest concentration was 39.56 mg/dL found in group G1. **Conclusion:** We conclude that decrease of estrogen levels leads to low calcium level, and then increase in the level of PTH in the blood. High level of calcium in the blood leads to increased secretion of calcitonin hormone from the thyroid gland, and calcitonin has no significant effect on the calcium regulation under normal conditions. A decrease in estrogen level leads to a low concentration of 1,25-dihydroxyvitamin D3 followed by decrease of the concentration of ionized calcium.

**Keywords:** Local Bitches, Parathyroid Gland, Ovariectomy

## 1. Introduction

Since dogs are pet animals, they are bred in large numbers, particularly in developing countries. However, many owners request removal of ovaries in the light of the undesirable behaviors of female dogs during estrus, their vulvar bloody secretions, and the diseases associated with reproduction<sup>[1-3]</sup>. The ovaries have an indirect relationship with parathyroid gland in the context of the effect of estrogen on the ossification of bone, metabolism of vitamin D into 1,25-dihydroxyvitamin D3 [1-25(OH)<sub>2</sub>D<sub>3</sub>], and absorption of calcium<sup>[4,5]</sup>. Parathyroid gland secretes parathyroid hormone (PTH) which acts to increase plasma calcium level and maintain ionized calcium concentration in the

extracellular fluid by direct effect on the bones where it increases bone resorption, and increases permeability of osteoclasts, osteocytes and osteoblasts to calcium ion ultimately leading to increase in the level of calcium in the body fluids<sup>[6,7]</sup>. These processes are stimulated by 1-25(OH)<sub>2</sub>D<sub>3</sub>. Additionally, PTH increases reabsorption of calcium from renal distal convoluted tubules and also has an indirect effect on the gastrointestinal absorption of calcium in the presence of 1-25(OH)<sub>2</sub>D<sub>3</sub><sup>[8]</sup>. Increase in the blood level of calcium ion leads to release the calcitonin hormone from the thyroid gland, which plays a role in decrease of calcium level in blood by direct inhibition of bone resorption and inhibition of permeability of osteoclasts, osteocytes and osteoblasts and increased excretion

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of calcium in the urine<sup>[7,9]</sup>. The 25-hydroxyvitamin D<sub>3</sub>, the inactive metabolite, is converted to 1,25-dihydroxyvitamin D<sub>3</sub> by the renal enzyme 1 $\alpha$ -hydroxylase<sup>[10-15]</sup>. The ovarian follicles release estrogens<sup>[16-18]</sup>. Estrogens stimulate calcium uptake and ossification of bone<sup>[4]</sup>. Estrogens act to increase the activity of 1 $\alpha$ -hydroxylase, the enzyme responsible for final synthesis of 1-25(OH)<sub>2</sub>D<sub>3</sub><sup>[5]</sup>. This in turn leads to increase of calcium ions in the blood by increasing absorption of calcium from intestine and reabsorption from kidney<sup>[19-22]</sup>. Therefore, an increase of calcium concentration in blood leads to decrease of release of PTH from parathyroid gland<sup>[23-25]</sup>.

The aim of this study has been to assess the effect of ovarian function on the role of the parathyroid gland in the local breed bitches from Al-Diwaniyah city of Iraq.

## 2. Materials and Methods

Twenty-one local breed bitches, 18 to 30 months old, housed in the small animal house in the Veterinary Medicine College were used. All bitches were acclimated to the laboratory conditions of temperature and photoperiod for one month when all animals were vaccinated against endemic diseases, and immunized against parasites. The animals were divided into three groups of seven bitches each, as follows: group 1, bitches in estrus phase; group 2, bitches in anestrus phase; and group 3, ovariectomized. Ovariectomy was performed after written consent of the owners. General anesthesia of i.v. injection of a combination of ketamine (Alfasan, BV Woerden, Holland) at 5.5 mg/kg BW, and diazepam (Roche, Switzerland) at 0.28 mg/kg BW, was practiced<sup>[26]</sup>. Ovariectomy was performed immediately after anesthetization. The wound was closed and antibiotics were applied for the wound to heal. The ovariectomized animals were left for one month. At the end of one month 3 mL of the blood was collected from the jugular vein in a hypodermal syringe from all 21 animals. The blood was allowed to clot and centrifuged at 3,000 rpm for 10 min to obtain the serum which was stored at -20°C until used for hormonal assay in the laboratories of College of Biotechnology, by using ELISA apparatus (DNM-9602, Germany). The antibody included Canine Estrogen ELISA Kit for estrogen; Dog Parathyroid Hormone ELISA Kit for parathyroid hormone and Dog Calcitonin ELISA Kit for calcitonin; serum calcium was determined using Calcium Microplate Assay Kit, ionized calcium was determined using Canine Free Calcium (F-cal) ELISA Kit and 1,25-dihydroxyvitamin D<sub>3</sub> was

determined using 1,25-Dihydroxyvitamin D<sub>3</sub> (DHVD3) ELISA Kit.

The results are presented as the means and standard errors. The results were analyzed by using computerized SPSS system version twenty four (P<0.01)<sup>[27]</sup>.

## 3. Results and Discussion

### 3.1 Hormonal Study

The mean of estrogen level in G1, G2, and G3 were 29.31, 4.55 and 3.32 pg/mL, respectively. Thus, G3 and G2 recorded the lowest level whereas G1 recorded the highest level. The mean level of parathyroid hormone (PTH) was 24.33, 43.45 and 56.74 pg/mL in G1, G2 and G3 groups, respectively. Thus, the highest concentration was recorded in G3 and G2 groups whereas G1 recorded the lowest level. The mean level of calcitonin was 6.98, 6.02 and 5.35 pg/mL in the three experimental groups, respectively, which recorded a statistically insignificant difference between groups (Table 1). Our results in respect of estrogen and PTH revealed a significant effect in G3 and G2 as compared to G1 at p<0.01.

Our results are consistent with Mandigers and Nell<sup>[28]</sup> who found that very low level of estrogen results from the removal of the ovaries. Our results agree with Carrillo-López *et al.*<sup>[29]</sup> who explained that decrease of estrogen level leads to low calcium level, which in turn would cause increase in the level of PTH. The results of van Abel *et al.*<sup>[5]</sup> showed that estrogen acts to increase the effectiveness of 1 $\alpha$ -hydroxylase. In the presence of this enzyme, 25-hydroxyvitamin D<sub>3</sub> is metabolized into 1,25-dihydroxyvitamin D<sub>3</sub>, and the latter is important in the absorption of calcium from the intestine and reabsorption

**Table 1.** Serum hormone levels in the bitches

Hormone	Group		
	G1	G2	G3
Estrogen (pg/mL)	29.34 ± 0.861 <sup>a</sup>	4.55 ± 0.081 <sup>b</sup>	3.32 ± 0.009 <sup>b</sup>
PTH (pg/mL)	24.33 ± 0.702 <sup>a</sup>	43.45 ± 0.773 <sup>b</sup>	56.74 ± 0.568 <sup>b</sup>
Calcitonin (pg/mL)	6.98 ± 0.035 <sup>a</sup>	6.02 ± 0.032	5.35 ± 0.037

Data are Means ± SE; Different letters denote significance of the difference (p<0.01).

of calcium at the kidney; the level of calcium in turn affects the PTH level in the blood<sup>[30-33]</sup>. Our results are in agreement with de Barboza *et al.*<sup>[20]</sup> that low level of estrogen affects absorption of calcium from the intestine, and with Jorde<sup>[23]</sup> who found that estrogen facilitates reabsorption of calcium at the kidney. The lack of significance with regard to estrogen level between G3 and G2, is consistent with the observation that the main source of estrogen is ovary<sup>[34]</sup>. The calcitonin level did not differ significantly between groups. This result is in agreement with the finding that high levels of calcium in the blood leads to increased secretion of the calcitonin from the 'C' cells of thyroid gland, and calcitonin has no profound effect on the calcium regulation under normal condition<sup>[35]</sup>.

### 3.2 Biochemical Study

The lowest concentration of calcium was found in G3 and G2, where they were 8.29 and 9.12 mg/dL, respectively, when compared to G1 which was 11.21 mg/dL. Thus, G3 and G2 recorded a significant difference ( $p < 0.01$ ) in comparison with group G1. Our results are in agreement with reports which explained that lower levels of estrogens lead to decrease of calcium which has an important role in the absorption of calcium from the intestine and absorption at the kidney<sup>[20,22]</sup>.

The results of ionized calcium in this study showed that the lowest mean level of ionized calcium were 3.42 and 4.21 mg/dL in G3 and G2, respectively, and the highest mean concentration was 6.01 mg/dL of ionized calcium in G1. It was observed that there was a significant difference ( $p < 0.01$ ) in ionized calcium concentration of G3 and G2 when compared to G1 (Table 2). The results are in accordance with the finding that decrease of estrogen leads to decrease of 1,25-dihydroxyvitamin D3 which in turn leads to decrease in concentration of ionized calcium<sup>[29]</sup>. The results are consistent with the report that decrease of absorption of calcium from intestine results from low level of 1,25-dihydroxyvitamin D3.

The lowest concentrations of 1,25-dihydroxyvitamin D3 were 18.24 and 20.73 mg/dL in G3 and G2, respectively, while the highest concentration was 39.56 mg/dL in G1. The G3 and G2 groups recorded a significant difference ( $p < 0.01$ ) from group G1 (Table 2). The results concur with the reports which showed that estrogen levels increase the effectiveness of 1- $\alpha$ -hydroxylase enzyme, which converts 25-hydroxyvitamin D3 into 1,25-dihydroxyvitamin D3 in renal tissue<sup>[5,31-33,37]</sup>. Our results agree with the finding that a decrease in concentration of 1,25-dihydroxyvitamin D3 resulted from the low level of estrogen<sup>[38,39]</sup>.

## 4. Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. We were explaining the ethical state of animals in our study in details in the materials and method section and included the references that we were depending on.

## 5. Disclosure of Potential Conflicts of Interest and Current Submission

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed journal.

## 6. Authorship and Conflict of Interest

All authors listed have contributed sufficiently to the study, deserving to be included as authors, and all those who are qualified to be authors are listed in the author

**Table 2.** Biochemical analysis in experimental groups.

Parameter	Experimental Groups		
	G1	G2	G3
Serum calcium (mg/dL)	11.21 $\pm$ 0.021 <sup>a</sup>	9.12 $\pm$ 0.067 <sup>b</sup>	8.29 $\pm$ 0.067 <sup>b</sup>
Ionized calcium (mg/dL)	6.01 $\pm$ 0.008 <sup>a</sup>	4.21 $\pm$ 0.002 <sup>b</sup>	3.42 $\pm$ 0.008 <sup>b</sup>
1,25-dihydroxyvitamin D3 (pg/mL)	39.56 $\pm$ 0.072 <sup>a</sup>	20.78 $\pm$ 0.198 <sup>b</sup>	18.24 $\pm$ 0.125 <sup>b</sup>

Values are Means  $\pm$  SE; Different letters denote significance of the difference ( $p < 0.01$ ).

byline. To the best of our knowledge, no conflict of interest, financial or otherwise, exists.

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