

Effect of Ovariectomy with and without Replacement Therapy on Calcium Metabolism in Female Albino Rats

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ABSTRACT

Osteoporosis is a chronic, progressive disease of the skeleton characterized by decreased bone mass due to a reduction in both organic and non organic components. Women are more likely to develop the disease, especially postmenopausal women. **The aim of the present work** was to assess the effect of ovariectomy with or without replacement therapy on calcium metabolism in female albino rats and the possibility of preventing progression of osteoporosis by means of hormonal supplementation and to compare the effects of different supplementations.

The present study was done on 90 adult female albino rats that were divided four weeks after bilateral ovariectomy (OVX) (except the control group) into nine equal groups as follow: Control group (non-ovariectomized), Ovariectomized group, Ovariectomized estrogen treated (30 µg/kg/day SC, 8 weeks) group, Ovariectomized progesterone treated (10 mg/kg/day SC, 8 weeks) group, Ovariectomized estrogen (25 µg/kg/day SC, 8 weeks) and progesterone (10mg/kg/day S.C., 8 weeks) treated group, Ovariectomized calcium treated (70 µL/day – calcium gluconate 0.24gm/ml- orally, 8 weeks) group, Ovariectomized calcitonin treated (10 IU/kg SC, 8 weeks) group, Ovariectomized calcitonin and calcium treated group, Ovariectomized vitamin D treated (0.5µg/kg orally, 8 weeks) group. Blood samples were collected, centrifuged and used for determination of calcium, phosphorous, parathormone and calcitonin serum levels. The left femur of each rat was removed, cleaned, weighted in air and in water and the bone density was determined. Fat-free dry femurs obtained by extracting the femurs for 48 h in 90% petroleum ether and dried in a forced-air oven at 90^o C until constant weight was obtained. The bone ash weight which contains the minerals was obtained after ashing at 600^oC for 24 h. On the basis of the obtained weights the percentage of organic and non-organic components were calculated.

The results obtained revealed that ovariectomy produced a significant reduction in bone mineral density (BMD), dry weight, ash content, femoral calcium content and organic matrix weight compared to the control group, indicating that there was a decrease in bone mass rather than a decrease in bone minerals. Serum calcium concentration did not show any significant change following OVX, serum calcitonin level decreased after OVX and increased with estrogen supplementation. The combination groups which were supplemented either with (estrogen and progesterone) or with (calcitonin and calcium) -with insignificant differences between them- increased BMD, dry weight, ash content, organic matrix weight and ash

calcium content. The percentage changes in BMD were 18% and 17.3% in these groups respectively as compared to the ovariectomized group. Calcitonin decreased serum calcium level, increased partially BMD and increased significantly bone dry weight, organic matrix weight, ash content and ash calcium of left femur, compared to OVX group. The calcium supplemented ovariectomized group had insignificant difference with the ovariectomized group in all femur parameters. When it was compared with the control group, there was a significant reduction in BMD. This finding could be explained that, calcium deposition in bone could be dependent on calcitonin level which was decreased by ovariectomy. So, calcium potentiates the effect of calcitonin on bone mass that ovariectomized calcitonin and calcium treated female group had better effect on bone mass than calcitonin therapy alone. The bone mass was greater by 16.31 % in vitamin D treated ovariectomized group, 15.96% in both estrogen and calcitonin treated ovariectomized groups and 15.6% in progesterone treated ovariectomized group when compared to OVX group. There was a significant higher serum phosphorus concentration in ovariectomized group compared to the control group, while other ovariectomized treated groups had serum phosphorus concentrations that were similar to those of the control group. There was an insignificant change in serum parathormone levels in the ovariectomized and the ovariectomized treated groups compared with the control group.

In conclusion, the present study showed that the combination therapy either with estrogen and progesterone or with calcitonin and calcium was found to be the most effective treatment regimen in OVX rats.

INTRODUCTION

Bone is a dynamic tissue, being formed and resorbed continually under the control of hormonal and physical factors. Remodeling of bone takes place throughout life, with osteoclasts resorbing old bone and osteoblasts creating new bone. These cells continuously renew the skeleton while maintaining its strength and density. Normally, in the adult skeleton, 3% of cortical bone and 25% of trabecular bone are remodeled each year. An imbalance between these processes may result in loss of bone mass⁽¹⁾.

Osteoporosis is characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The

major cause of that disease is cessation of ovarian function after menopause. Estrogen deficiency in mammals increases bone turnover and results in a reduction in bone mass due to an imbalance between bone resorption and bone formation. OVX rats have been widely used as an animal model for the study of the prevention and treatment of postmenopausal osteoporosis. There are many observed similarities between ovariectomy-induced bone loss in rats and postmenopausal bone loss in humans⁽²⁾.

Several pharmacological agents are available for prevention and treatment of osteoporosis, and they can be classified according to their mechanism of action. The two main classes of drugs used to treat osteoporosis are: the antiresorptive

agents that block bone resorption by inhibiting the activity of osteoclasts and the anabolic agents that stimulate bone formation by acting primarily on osteoblasts and the antiresorptive drugs include estrogens, bisphosphonates and calcitonin. In contrast to drugs that slow bone turnover, anabolic agents directly stimulate bone formation; these drugs include parathyroid hormone, growth hormone, and sodium fluoride⁽³⁾.

The aim of the present work was to study the effects of ovariectomy on calcium metabolism with or without elementary substitution and to compare the results with those of hormonal replacement therapy. The effects of estrogen and progesterone supplementation will, also, be differentiated.

MATERIALS & METHODS

Ninety adult female albino rats, weighing 200-250 g were used in the study. Rats were housed at room temperature with 12:12 hour light/dark cycles. Rats were randomly classified into the following groups:

1. Control group (C); in which the rats were left freely wondering throughout the period of the experiment.
2. Ovariectomized group (OVX); in which rats were subjected to bilateral ovariectomy under ether anesthesia 4 weeks before the beginning of the experiment⁽⁴⁾.
3. Ovariectomized estrogen treated group (OVX+E); in which rats were subcutaneously injected with estradiol benzoate at a dose level of 30 µg/kg body weight/day, for 8 weeks, started 4 weeks after OVX⁽⁵⁾.
4. Ovariectomized progesterone treated group (OVX+P); in which rats received progesterone at a dose level of 10 mg/kg body weight/day, subcutaneously daily for 8 weeks, after 4 weeks after OVX⁽⁵⁾.
5. Ovariectomized estrogen and progesterone treated group (OVX+E+P); in which rats received estrogen and progesterone at a dose level of 25 µg/kg body weight/day, subcutaneously and 10 mg/kg body weight/day, subcutaneously respectively, daily for 8 weeks started 4 weeks, after OVX⁽⁵⁾.
6. Ovariectomized calcium treated female group (OVX+Ca); each rat received calcium at a dose level of 70 µL/day –calcium gluconate 0.24gm/ml-, orally daily for 8 weeks, started after 4 weeks after OVX. This dose was equivalent to the human dose corrected for rats according to *Paget and Barnes (1964)*.
7. Ovariectomized calcitonin treated group (OVX +Cal); each rat received calcitonin hormone at a dose level of 10 IU/kg body weight/day, subcutaneously daily for 8 weeks, started 4 weeks after OVX⁽⁴⁾.
8. Ovariectomized calcitonin and calcium treated group (ovx+Cal+Ca); in which each rat received the previous doses of calcitonin and calcium together as groups 6 and 7.
9. Ovariectomized vitamin D treated group (OVX+VitD); in which each rat received vitamin D₃ at a

dose level of 0.5µg/Kg, orally daily for 8 weeks started after 4 weeks of being subjected to bilateral ovariectomy⁽⁷⁾.

METHODS

- 1- **Ovariectomy:** Except the control group all rats were subjected to bilateral ovariectomy under ether anesthesia⁽⁸⁾.
- 2- **Bone volume:** The Left femur of each rat was removed, cleaned of soft tissue, wrapped in saline-soaked gauze and stored at -70°C until analyzed for bone density and chemical composition. All femurs were first weighed in air (**W**), then reweighed while suspended in water (**WW**) at room temperature. The difference between the two weights is the volume of the bone⁽⁹⁾.
- 3- **Bone density (Archimedes' principle):** It is the standard method for determination of density (g/cm³) of bones from small animals. Density was calculated using the formula: **Density = W/W-WW X P** Where, **P** is the density of distilled water at a given temperature and **W-WW** is the difference in weight, which is equivalent to the bone volume⁽¹⁰⁾.
- 4- **Preparation of dried femurs:** The left femurs were first dried to constant weight at 100°C for 24 h and weighed again for the determination of dry weight (**DW**) which includes organic, minerals, and fat as the water content was only evaporated. On the basis of the obtained initial weights of femur; **W** and **DW**, the weight of water content in the femurs was calculated using the formula: $W_{\text{water}} = W - DW$, and the percentage of water content $= W_{\text{water}} \times 100 / W$ ⁽¹¹⁾.
- 5- **Preparation of Fat-free dry femurs:** Fat-free dry femurs were determined by the method of *Watkins and Southern (1992)*⁽¹²⁾. The left femurs were extracted for 48 h in 90% petroleum ether and were dried in a forced-air oven at 90° C until constant weight was obtained.
- 6- **Preparation of femurs ash:** The bone ash weight (**AW**) which contains only the minerals was obtained after ashing at 600 °C for 24 h.⁽¹³⁾ Mineral content was calculated as the ratio of ash weight to dry weight. The ash was dissolved in 1 N HCl⁽¹⁴⁾ to measure the calcium and phosphate concentrations that were determined spectrophotometrically⁽¹⁵⁾. On the basis of the obtained weights (**W**, **DW**, and **AW**), the following calculations were made⁽¹¹⁾:
 % of non organic component = $W_{\text{non-org. comp.}} \times 100 / W$
 % of organic components = $W_{\text{org. comp.}} \times 100 / W$
- 7- Determination of serum calcium and phosphates: according to the methods described by *Fiereck, (1976)*⁽¹⁶⁾ and *Tietz, (1976)*⁽¹⁷⁾, respectively.
- 8- **Determination of serum parahormone (PTH) level according to the method described by Kruger et al. (1995)**⁽¹⁸⁾.
- 9- **Determination of serum calcitonin level**⁽¹⁹⁾

Statistical Evaluation of the experimental data:

Data were expressed as the mean \pm SE. Statistical significance of difference between groups was evaluated by unpaired two-tailed Student's "t" test. Values of $p < 0.05$ were considered statistically significant (20).

RESULTS

1- Effect of ovariectomy on left femur parameters:

All left femur parameters in the OVX group had significantly lower values than those from the control except, % of water content which showed a significant higher level in the OVX group compared with the control group with insignificant difference of the ratio between the non organic to the organic component between the two groups. This is illustrated in table (1)

2- Effect of ovariectomy with estrogen supplementation on left femur parameters:

There were insignificant differences in all left femur parameters between the OVX+E group and the control group except the BMD which showed a significant lower value in the OVX+E group. There were significant higher values in all left femur parameters in the OVX+E group compared to the OVX group except % of water content which showed a significant lower value with insignificant difference in the ratio of non organic to organic component between the two groups. This is shown in table (1).

3- Effect of ovariectomy with progesterone supplementation on left femur parameters:

There were significant lower values of ash weight and BMD in the OVX+P group compared to the control group with insignificant difference in the other parameters between the two groups. There were significant higher values in all left femur parameters in the OVX+P group compared to the OVX group except % of water content and the ratio of non organic to organic component which showed significant lower values. This is illustrated in table (1).

4- Effect of ovariectomy with estrogen and progesterone supplementation on left femur parameters:

There were significant higher values in all left femur parameters in the OVX+E+P group compared to the OVX group except % of water content which showed a significant lower value with insignificant difference in the ratio of non organic to organic component between the two groups. This is illustrated in table (1).

5- Effect of ovariectomy with calcium supplementation on left femur parameters:

There were insignificant differences in all left femur parameters between the OVX+Ca group and the OVX group. All femurs of the OVX+Ca group had significantly lower values than those from the control except % of water content which showed a significant higher level in the OVX+Ca group compared with the control group with insignificant difference in the ratio of non organic to organic component

between the two groups. This is shown in table (2).

6- Effect of ovariectomy with calcitonin supplementation on left femur parameters.

There were insignificant differences in all left femur parameters between the OVX+Cal group and the control group except the BMD which showed a significant lower value in the OVX+Cal group. There were significant higher values in all left femur parameters in OVX+Cal group compared to the OVX group except % of water content which showed a significant lower value and insignificant difference in the ratio of non organic to organic component between the two groups. This is illustrated in table (2).

7- Effect of ovariectomy with calcitonin and calcium supplementation on left femur parameters:

There were significant higher values in all left femur parameters in the OVX+Ca+Cal group compared to the OVX group except % of water content which showed a significant lower value with insignificant difference in the ratio of non organic to organic component between the two groups. This is illustrated in table (2).

8- Effect of ovariectomy with vitamin D supplementation on left femur parameters:

There were significant higher values in all left femur parameters in OVX+vit D group compared to the OVX group except % of water content which showed a significant lower value with insignificant difference in the ratio of non organic to organic

component between the two groups. This is illustrated in table (3).

9- Changes in femur calcium and phosphorus content in different groups:

There were significant lower levels of femoral calcium content of the OVX, OVX+Ca and OVX+P groups compared to control group. Other ovariectomized treated groups had femoral calcium content levels that were similar to those of the control group. This is shown in table (4).

There was insignificant change in femoral phosphorus content in the ovariectomized and the ovariectomized treated groups compared with control group. This is illustrated in table (4).

10- Effects of treatment of different drug therapies on serum calcium, phosphorus, parathormone and calcitonin in different groups (Table 5):

There was insignificant change in serum calcium levels in OVX and the ovariectomized treated groups compared with the control group except, OVX+Cal group in comparison to all other groups.

There was significant higher serum phosphorus concentration in OVX group compared to control group, while, other ovariectomized treated groups had serum phosphorus concentrations that were similar to those of the control group.

There was insignificant change in serum parathormone levels in OVX and the ovariectomized treated groups compared with control group.

The OVX group and OVX+Ca group showed significant lower serum calcitonin level compared with the

control group. In all supplemented ovariectomized groups, there was a significant higher value in serum calcitonin level compared with the OVX group.

11- Effect of treatment with different drug therapies on BMD:

The ovariectomized group showed the lowest bone density between all groups compared with the control group. Ovariectomy resulted in 15.5% decrease in BMD of left femurs. The calcium supplemented ovariectomized group had insignificant difference with the ovariectomized group. When it is compared with the control group, there was a significant reduction by 14.37%. The highest increase BMD

was observed among the combination groups which supplemented either with (estrogen and progesterone) or with (calcitonin and calcium). The percentage changes were 18% and 17.3% respectively, as compared to OVX group. This is illustrated in figure (1).

12- Effect of treatment of different drug therapies on non organic (ash weight) components of the bone:

There were significant lower levels of ash weights of the OVX, OVX+Ca and OVX+P treated groups compared to the control group. Other ovariectomized treated groups had bone ash levels similar to those of the control group. This is shown in fig. (2).

Table (1): - Effect of ovariectomy, with estrogen, with progesterone and with estrogen and progesterone supplementation on left femur parameters (M±SE).

Parameters \ Groups N=10	C	OVX	OVX+E	OVX+P	OVX+E+P
Bone density (mg/mm ³)	3.34±0.02	2.82±0.01*	3.27±0.01*°	3.26±0.02* °	3.33±0.01°
FFDW (mg)	401±1.7	384±3*	399±1.1°	397±1.09°	401±1.1°
Ash weight (mg)	259±1.4	244±1.3*	256±1.9°	254±1.6 *°	259±1.6 °
Organic matrix weight (mg)	160±2.3	148±2.3*	161±2.13°	162±1.5°	159±1.5 °
Ratio of non organic to organic	1.62±0.03	1.64±0.02	1.58±0.03	1.56±0.02°	1.62±0.02
% of non organic components	49.5±0.20	47.9±0.25*	49.13±0.35°	48.77±0.29	49.4±0.24°
% of organic components	30.5±0.4	29.1±0.4*	30.90±0.4 °	30.85±0.28°	30.30±0.2°
% of water content	19.8±0.2	22.8±0.5*	19.9±0.3°	20.11±0.34°	20.15±0.1°

*: Significant compared with control group °: Significant compared with OVX group

N: is the number of rats in each group

C: Control Group

OVX: Ovariectomized group E: Estrogen

P: Progesterone

Table (2): - Effect of ovariectomy, with calcium, with calcitonin, with calcium and calcitonin supplementation on left femur parameters (M±SE):

Parameters \ Groups N=10	C	OVX	OVX+Ca	OVX+Cal	OVX+Ca+Cal
Bone density (mg/mm ³)	3.34±0.02	2.82±0.01*	2.86±0.01*	3.27±0.02°	3.31±0.02°
Dry weight (mg)	420±1.5	392±2.5 *	394±2.4*	414±3.1°	417±2.6 °
FFDW (mg)	401±1.7	384±3*	383±3.2*	399±1.8°	400±1.5°
Ash weight (mg)	259±1.4	244±1.3*	245±1.5*	256±1.1 °	258±1.8 °
Organic matrix weight (mg)	160±2.3	148±2.3*	148±2.6*	159±2.3°	160±1.6°
Ratio of non organic to organic	1.62±0.03	1.64±0.02	1.65±0.03	1.60±0.02	1.61±0.02
% of non organic components	49.5±0.20	47.9±0.25*	47.9±0.26*	49.9±0.11°	48.9±0.22°
% of organic components	30.50.4	29.1±0.4*	28.9±0.5*	30.4±0.3°	30.34±0.2°
% of water content	19.8±0.2	22.8±0.5	23.1±0.5*	20.2±0.3°	20.4±0.2°

*: Significant compared with control group °: Significant compared with OVX group
 N: is the number of rats in each group C: Control Group
 OVX: Ovariectomized group
 E: Estrogen P: Progesterone Ca: Calcium Cal: calcitonin

Table (3):-Effect of ovariectomy with vitamin D supplementation on left femur parameters (M±SE):

Parameters \ Groups N=10	C	OVX	OVX+vit D
Bone density (mg/mm ³)	3.34±0.02	2.82±0.01*	3.28±0.01*°
Dry weight (mg)	420±1.5	392±2.5 *	415±1.8°
FFDW (mg)	401±1.7	384±3*	399±1.3°
Ash weight (mg)	259±1.4	244±1.3*	257±1.4°
Organic matrix weight (mg)	160±2.3	148±2.3*	158±1.3°
Ratio of non organic to organic	1.62±0.03	1.64±0.02	1.61±0.01
% of non organic components	49.5±0.20	47.9±0.25*	49±0.1 °
% of organic components	30.5±0.4	29.1±0.4*	30.3 ± 0.25°
% of water content	19.8±0.2	22.8±0.5*	20.4±0.24°

*: Significant compared with control group
 °: Significant compared with ovariectomized group
 N: is the number of rats in each group C: Control Group
 OVX: Ovariectomized group vit D: vitamin D

Table (4): - Changes in femur minerals content in relation to different therapies in normal and ovariectomized rats (M ± SE).

Parameters	Calcium (mg/g)	Phosphorus (mg/g)
Groups N=10		
Control group	68.92 ± 0.42	31.32 ± 0.41
Ovariectomized	65.4 ± 0.48*	32.35 ± 0.26
OVX + E+ P	68.77 ± 0.38°	31.25±0.39
OVX + Ca+Cal	68.75 ± 1.07°	32.52±0.46
OVX+ vit D	67.97 ± 0.56°	32.20±0.35
OVX + calcitonin	67.92 ± 0.36°	31.45±0.29
OVX + Estrogen	67.80 ± 0.37°	31.87 ± 0.49
OVX + progesterone	65.97 ± 0.51*	31.55 ± 0.31
OVX + calcium	65.05 ± 0.8*	32.3 2 ± 0.30

* : Significant compared with control group

° : Significant compared with ovariectomized group

E: Estrogen P: Progesterone

Ca: Calcium Cal: calcitonin

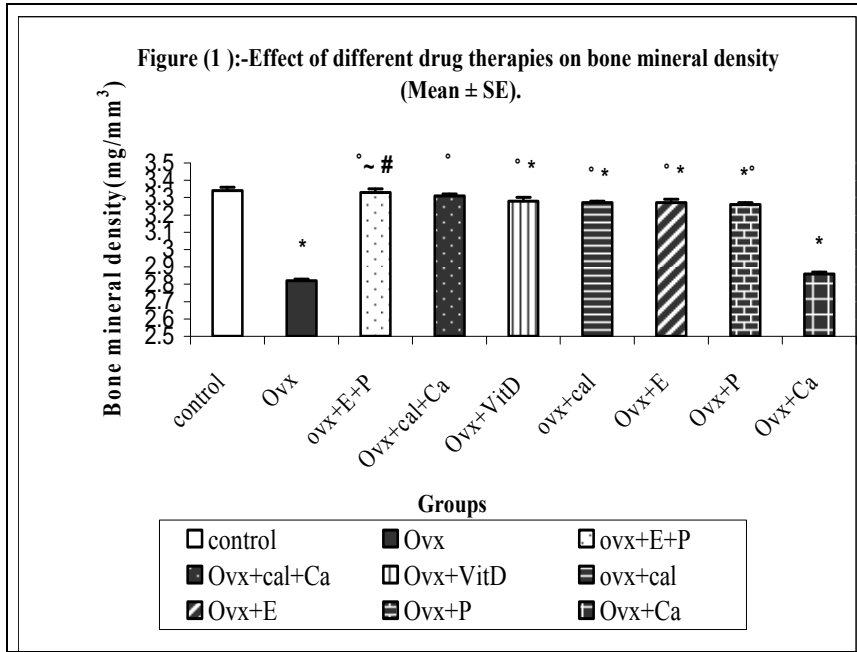
Table (5):- Effects of treatment of different drug therapies on biochemical parameters in OVX rats (M±SE):

Parameters	Calcium (mg/ dl)	Phosphorous (mg/ dl)	Parathormone (Pg/ml)	Calcitonin (Pg/ml)
Groups N=10				
Control group	10.2±0.15	7.4±0.08	2.4±0.09	20.2±0.4
Ovariectomized	10±0.10	9.7±0.14*	2.6±0.01	16.8±0.2*
OVX+E+ P	10±0.14	7.3±0.12°	2.5±0.1	21.2±0.5°
OVX+ Ca+Cal	10.1±0.13	7.4±0.1°	2.2±0.1	20.4±0.3°
OVX + vitamin D	10±0.12	7.6±0.1°	2.4±0.1	19.2±0.4°
OVX + calcitonin	9.21±0.05°*	7.7±0.1°	2.4±0.1	19±0.3°
OVX + Estrogen	10±0.10	7.5±0.16°	2.3±0.1	21.4±0.6°
OVX+ progesterone	9.9±0.21	7.2±0.13°	2.5±0.1	20.4±0.3°
OVX + calcium	10.4±0.18	7.7±0.06°	2.3±0.1	17.7±0.4*

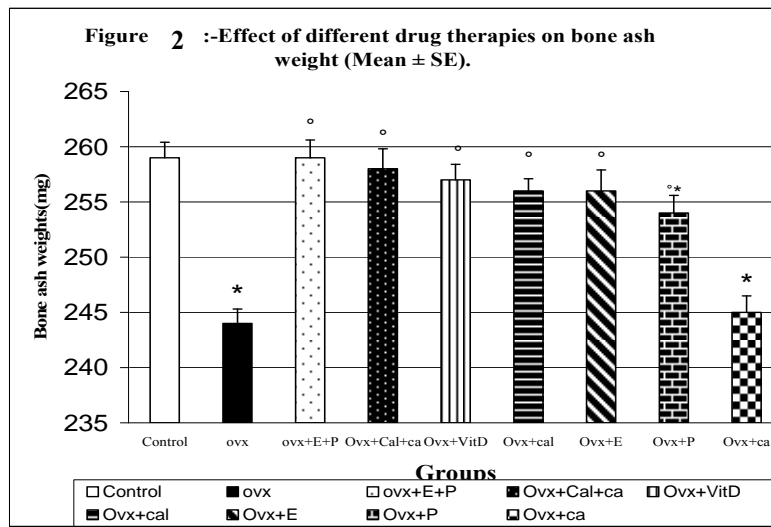
* : Significant compared with the control group

° : Significant compared with the ovariectomized group (OVX)

E: Estrogen P: Progesterone Ca: Calcium Cal: calcitonin



*: Significant compared with control group °: Significant compared with OVX group
 #: Significant compared with OVX+E group ~: Significant compared with OVX+E+P group
 OVX: ovariectomized E: estrogen P: progesterone Ca: calcium Cal: calcitonin



*: Significant compared with control group °: Significant compared with OVX group
 OVX: ovariectomized E: estrogen P: progesterone Ca: calcium Cal: calcitonin

DISCUSSION

Osteoporosis is a reduction in the amount of bone tissue per unit volume. Estrogen deficiency after menopause is an important cause of osteoporosis. For this reason, hormone replacement therapy is often recommended for osteoporosis prevention and treatment⁽²¹⁾.

The aim of the present work was to study the effect of OVX on calcium metabolism with or without elementary substitution and to compare the results with those of hormonal replacement therapy. The effects of estrogen and progesterone replacement were also differentiated. Therefore, in the present study OVX rats have been used as an animal model for the study of the prevention and treatment of postmenopausal osteoporosis.

The results obtained in the present study demonstrated that OVX induced a significant decrease in BMD and the femoral BMD of OVX rats was 15.5 % lower than that of control group and that finding is matched with that of **Kruger et al.**²² and **Hidaka et al.**²³ who concluded that ovariectomy resulted a significant reduction in BMD.

The present study demonstrated that the dry weight, ash content and organic matrix weight of left femurs of OVX group were significantly lower than that of the control group, indicating that there was a decrease in bone mass rather than a decrease in bone minerals. Similar finding was observed by **Reddy and Lakshmana**²⁴. That finding could explain that osteoporosis induced by

ovariectomy is characterized by decreased bone mass with preservation of normal ratio of mineral to matrix.

The results obtained in the present study, also, demonstrated that serum calcium concentration did not show any significant change following ovariectomy. Similar finding was observed by **Zhang et al.**²⁵ who reported that serum calcium level in OVX rats was insignificantly changed at 4 weeks, and decreased significantly at 18 weeks following ovariectomy by increased urinary calcium excretion. This could be explained by the fact that homeostatic mechanisms were able to maintain serum calcium level for four weeks despite ovariectomy⁽²⁶⁾. The homeostatic mechanisms which maintained serum calcium level after ovariectomy may be explained by the finding that estrogen deficiency increases the sensitivity of bone to PTH and other resorption-inducing agents and this further enhances the resorption defect⁽²⁷⁾.

The obtained data demonstrated that ovariectomy induced a significant increase in serum phosphate concentration. Similar finding was observed by **Gopala Krishnan et al.**²⁸ who, also, reported that serum levels of phosphate were elevated significantly in ovariectomized rats. This could be explained by **Dick et al.**²⁹ who reported that estrogen inhibits selectively sodium phosphate cotransport by direct interaction with estrogen receptors in proximal tubular cells and hence, proximal phosphate reabsorption.

The present study demonstrated that serum calcitonin level decreased after OVX and increased with estrogen supplementation. Similar findings were observed by **Filipović et al.**^{30&31} who reported that estrogen deficiency after OVX reduced the synthesis and release of calcitonin, while chronic treatment with estrogen had a positive effect on calcitonin secretory activity in thyroid C cells of OVX rats.

In the present study, the highest increase in BMD was observed among the combination groups which were supplemented either with estrogen and progesterone or with calcitonin and calcium. This may suggest that the antiresorptive action of estrogen and progesterone could be mediated apart by an increase in calcitonin levels.

The results obtained in the present study demonstrated that estrogen and progesterone together give complete improvement in BMD but either estrogen or progesterone therapy alone give partial improvement. These findings are in agreement with that of **Moskowitz**³², indicating that, estrogen and progesterone in balance enhance each other. This enhancement could be explained by the results of **Pei et al.**³³ who evaluated whether the capacity for proliferation and differentiation of progesterone-dependent osteoprogenitors in the female rat skeleton is related to the level of progesterone receptors and/or the level of circulating estrogen

The results obtained in the present study demonstrated that estrogen increased partially BMD. This finding is in agreement with that of **Wang et al.**³⁴ who found that

estrogen increased the bone density of the OVX rat femurs by up-regulating the expression of osteoprotegerin.

The results obtained in the present study demonstrated that progesterone partially increased bone mass. These findings are in agreement with those of **Suliman et al.**³⁵ who reported that in ovariectomized rat supplemented with progesterone, bone mass was protected by high level of progesterone present. This finding however, was in disagreement with that of **Roux et al.**³⁶ who found that dydrogesterone did not prevent bone loss due to ovariectomy in rats.

The results obtained in the present study demonstrated that plasma parathormone showed insignificant change in the ovariectomized and the ovariectomized treated groups compared with the control group. These results are matched with those of **Vincent et al.**³⁷ who reported that estrogen treatment or withdrawal had no significant effect on either basal or stimulated PTH secretion.

Calcitonin replacement in the present work decreased serum calcium, increased partially BMD and increased significantly bone dry weight, organic matrix weight, ash content and ash calcium of left femurs as compared to OVX group. These findings are in agreement with those of **Zaidi et al.**³⁸ who reported that calcitonin lowers serum calcium concentrations by decreasing bone resorption and increasing urinary calcium excretion and, also, those of **Zake et al.**³⁹ reported who that after treatment of OVX rats with calcitonin, bone density gave partial improvement and bone ash headed

towards normal ranges in comparison with group of OVX rats without calcitonin supplementation.

Kavuncu et al.,⁴ reported that calcitonin treatment resulted in an increase in spinal BMD and the effect of CT on bone structure in vivo appears to be dependent on the rate of bone turnover at the time of treatment. Treatment of OVX rats immediately following the operation with calcitonin for 6 weeks prevented the development of osteoporosis. Calcitonin induced-effect may be explained probably through its binding to osteoclasts receptors⁽³⁸⁾. This binding leads to loss of the ruffled border, cessation of motility and inhibition of osteoclastic secretion of proteolytic enzymes and the proton pump. Chronic administration of calcitonin reduces the number of osteoclasts⁽⁴⁰⁾. It is a potent inhibitor of osteoclastic bone resorption and has been reported to exert analgesic effects, properties which have led to its use as a treatment for postmenopausal osteoporosis⁽⁴¹⁾.

Despite a fall in serum calcium in OVX Calcitonin treated group, there was no effect of ovariectomy on PTH. These data suggest that the decrease in ionized calcium was insufficient to produce a response from the parathyroid gland. In a previous study by **Mason and Morris**⁴² who studied the effect of hypocalcemia on PTH secretion in ovariectomized rats found that serum levels of PTH were only increased when blood ionized calcium levels fell below 5.2 mg/dl.

The results obtained in the present study demonstrated that calcium supplementation alone did not produce any increase in bone density

in ovariectomized rats. This could suggest that calcium deposition in bone could be dependent on calcitonin level which was decreased by ovariectomy. **Ku et al.**,⁴³ reported that estrogen deficiency decreases intestinal calcium absorption and the positive effect of calcium does in menopause is more related to building peak bone mass before the onset of menopause and doesn't affect the quantity of bone lost during the initial years of menopause.

In the present study, it was found that calcium potentiated the effect of calcitonin on bone mass that ovariectomized calcitonin and calcium treated female group had better effect on bone mass than calcitonin therapy alone. These findings are in agreement with **Nieves et al.**⁴⁴ who reported that additional calcium to standard antiresorptive therapy can benefit bone mass.

The results obtained in the present study demonstrated that active vitamin D₃ increased bone density in ovariectomized rats. This finding is in agreement with **Shibata et al.**⁴⁵ who reported that administration of vitamin D₃ inhibited tibial and vertebral bone loss because of estrogen deficiency in OVX rats.

The role of vitamin D metabolites in the treatment of osteoporosis is still controversial. Some clinical studies have suggested that therapy of patients with osteoporosis with active vitamin D metabolites increases BMD in the spine and forearm and reduces vertebral fractures⁽⁴⁶⁾. However, other studies have provided negative results⁽⁴⁷⁾. Studies in rats have shown clearly that active vitamin D metabolites can fully prevent estrogen

deficiency-induced bone loss by suppression of bone resorption⁽⁴⁸⁾.

The effect of 1, 25 (OH)₂ D₃ on bone is an indirect one, mediated by stimulation of calcium absorption from the intestine with a resultant suppression of PTH⁽⁴⁹⁾. However, it also may have direct antiresorptive effects independent of PTH suppression. Experiments using parathyroidectomized rats infused with human PTH-related peptide 1-34 as a model of hypercalcemia of malignancy have shown that 1, 25 (OH)₂ D₃ lowers hypercalcemia and bone resorption in a dose-dependent manner in that model, strongly implying an additional direct antiresorptive action of calcitriol on bone cells⁽⁵⁰⁾.

Vitamin D₃ acts as a transcriptional regulator of bone matrix proteins, it induces the expression of osteocalcin and suppresses synthesis of type I collagen. Research has shown that vitamin D₃ may increase aromatase activity within bone itself, enhancing the local conversion of androgens to estrogens in both men and women. This process does not increase plasma levels of estrogen, but rather limits its production and concentration to bone⁽⁵¹⁾.

In conclusion, the present study provides various therapeutic modalities for management of bone changes following deprivation from sex hormones. It was found that the combination therapeutic regimen as (estrogen with progesterone) is the most effective and protective modality. Calcitonin with calcium is an alternative modality if hormone

replacement therapy is contraindicated.

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تأثير أستئصال المبيضين مع العلاج التعويضي و بدونه علي التمثيل الغذائي للكالسيوم في أنث الفئران البيضاء

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هشاشة العظام تعبير يطلق على نقص غير طبيعي واضح في كثافة العظام (أي كمية العظام العضوية والغير عضوية) وهو أكثر أمراض العظام انتشارا وتزداد حدة هشاشة العظام مع تقدم العمر بصفه عامة ولدى النساء بعد سن الأياس بصفة خاصة.

أن الغرض من إجراء هذا البحث هو التعرف على تأثير العلاج الهرموني التعويضي (الاستروجين أو البروجيستيرون أو الأثنين معا) و فيتامين د و الكالسيوم والكالسيوم على مستوى الكالسيوم والفسفور وبعض الهرمونات المؤثرة عليهما في الدم وحاله العظام التي عليها وذلك في إنث الفئران البيضاء بعد إجراء عملية استئصال المبيضين لهم. أجري هذا البحث على عدد "٩٠" من أنث الفئران البيضاء حيث قسمت الي "٩" مجموعات (١٠ فئران في كل مجموعة) علي النحو التالي :-

المجموعة الأولى : المجموعة الضابطة لم تخضع لأستئصال المبيضين أو أي علاج تعويضي. المجموعات التجريبية و التي اشتملت على ٨٠ فارة خضعوا جميعا الي استئصال المبيضين وبعد ٤ أسابيع من استئصال المبيضين قسموا إلى ٨ مجموعات متساوية وعولجوا علي النحو التالي:

المجموعة الثانية : المجموعة المستأصلة المبيضين والغير خاضعة لأي علاج تعويضي.

المجموعة الثالثة: تمت معالجتها بهرمون الاستروجين بواسطة الحقن تحت الجلد (٣٠ ميكروجرام /كيلوجرام من وزن الفارة) يوميا لمدة ٨ اسابيع.

المجموعة الرابعة: تمت معالجتها بهرمون البروجيستيرون بواسطة الحقن تحت الجلد (١٠ ملليجرام/ كيلوجرام من وزن الفارة) يوميا لمدة ٨ أسابيع.

المجموعة الخامسة: تمت معالجتها بهرموني الاستروجين (٢٥ ميكروجرام/كجم) و البروجيستيرون (١٠ ملليجرام/ كجم) بالحقن تحت الجلد كما في المجموعة الثالثة والرابعة .

المجموعة السادسة: تمت معالجتها بالكالسيوم عن طريق الفم (٧٠ ميكروليتر/ فارة) (كالسيوم جلوكونات ٢٤ و ٠ جم/مل) (يوميا لمدة ٨ اسابيع).

المجموعه السابعة: تمت معالجتها بهرمون الكالسيوم بواسطة الحقن تحت الجلد (١٠ وحدات دولية/ كيلوجرام من وزن الفارة) يوميا لمدة ٨ اسابيع.

المجموعة الثامنة: تمت معالجتها بهرمون الكالسيوم و الكالسيوم معا بنفس الجرعات المستخدمة في المجموعة ٦ و ٧ سابقة الذكر.

المجموعة التاسعة: تمت معالجتها بفيتامين د عن طريق الفم (٠.٥ ميكروجرام /كيلوجرام من وزن الفارة) يوميا لمدة ٨ أسابيع

تم اخذ عينات الدم بعد ٨ أسابيع من المعالجة التعويضية وتم قياس نسبة الكالسيوم والفسفور وكلا من هرمون الكالسيوم و هرمون الغدة الجار درقية. كما تم استخلاص عظمة الفخذ اليسري ومعالجتها وتم حساب كثافة العظام وباقي متغيرات البحث الاخرى و التي تشمل وزن عظام الفخذ الجافة ، وزن المسحوق العظمي ومحتواه من الكالسيوم و الفوسفور في نهاية هذه الفترة .

أدي استئصال المبيضين الي حدوث نقص دال إحصائيا في كثافة العظام و المسحوق العظمي و محتوى الكالسيوم في عظمة الفخذ مقارنة بالمجموعة الضابطة. وعدم حدوث تغيير في مستوى

الكالسيوم في الدم في المجموعة المستأصلة المبيضين مع حدوث نقص دال إحصائيا في مستوى الكالسيوم الذي تحسن بعد المعالجة بالاستروجين.

أن المعالجة بالاستروجين أو البروجيستيرون منفردا اعطى تحسنا جزئي، بينما أعلى نسب التحسن في المحتوى المعدني للعظام ظهرت للمجموعات التعويضية المركبة والمعالجة بهرموني الأستروجين و البروجيستيرون أو المعالجة بالكالسيوم و الكالسيوم معا وكانت نسب التحسن ١٨% و ١٧.٣% علي التوالي مقارنة بالمجموعة المستأصلة المبيضين مع عدم وجود تغيير دال إحصائيا بينهما من ذلك يتضح أن كلا من هرموني الاستروجين و البروجيستيرون يعملان جزئيا على زيادة نسبة الكالسيوم في الدم.

أن الكالسيوم قد أدى الي نقص في مستوى الكالسيوم في الدم مع الزيادة الجزئية في الكثافة المعدنية للعظام وزيادة ذات دالة إحصائيا في الوزن الجاف للعظام و المحتوى العضوي و المعدني لها و كذلك محتواها من الكالسيوم في المجموعات المعالجة بالكالسيوم بالمقارنة بالمجموعة المستأصلة المبيضين و ذلك يختلف مع مجموعة التعويض بالكالسيوم فقد أظهرت نتائج البحث عدم وجود اي فروق ذات دالة إحصائية في المتغيرات قيد البحث مع مجموعة استئصال المبيضين و بالمقارنة بالمجموعة الضابطة فقد أظهرت وجود فروق ذات دالة إحصائية في المحتوى المعدني للعظام بنسبة ١٤.٣٧% لصالح المجموعة الضابطة و قد أدت المعالجة بالكالسيوم و الكالسيوم معا الي تحسن في المتغيرات قيد البحث عن استخدام كلا منهما منفردا.

تحسنت الكثافة المعدنية للعظام جزئيا بمقدار ١٦.٣١% في المجموعة المستأصلة المبيضين و المعالجة بفيتامين د. و بمقدار ١٥.٩٦% في المجموعة المستأصلة المبيضين و المعالجة بكلا من الكالسيوم او الاستروجين. و بمقدار ١٥.٦% في المجموعة المستأصلة المبيضين و المعالجة بالبروجيستيرون بالمقارنة بالمجموعة المستأصلة المبيضين.

قد أظهرت نتائج هذا البحث حدوث نقص دال إحصائيا في مستوى الكالسيوم في مجموعتي الفئران المستأصلة المبيضين و المعالجة بالكالسيوم مقارنة بالمجموعة الضابطة ووجود زيادة ذات دالة إحصائية في مستوى الكالسيوم في جميع المجموعات التعويضية بالمقارنة بالمجموعة المستأصلة المبيضين • و زيادة ذات دالة إحصائية في مستوى الفوسفور في الدم في المجموعة المستأصلة المبيضين مقارنة بالمجموعة الضابطة •

أن نتائج هذا البحث تلفت النظر الى أهمية استخدام المركبات التعويضية للوقاية من هشاشة العظام -التي تحدث فسيولوجيا او جراحيا نتيجة لاستئصال المبيضين او توقف عملهما- مثل استخدام الأستروجين و البروجيستيرون معا للذاتان يعتبران أحسن وسيلة تعويضية في علاج هشاشة العظام. وان استخدام الكالسيوم و الكالسيوم معا يصلح كبديل مناسب في علاج هشاشة العظام ما بعد سن الاياس في الحالات التي يتعذر معها استخدام الاستروجين و البروجيستيرون أو الحالات التي لديها موانع طبية تحول دون استخدامه.