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Myocardium and Coronary artery architectural changes in ovariectomized rabbit and its relation with serum estrogen level.

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ABSTRACT

Forty eight sexually matured adult female rabbits were divided into 6 groups, control, A, B, C, D and E. The experimental groups were overictomized and scarified at the time interval of zero, 7, 14, 30,60and90 days respectively. Blood samples were collected twice, one before the operation and the second one before scarifying. Blood samples were centrifuged and then, serum was obtained and tested for estrogen. Also cardiac tissue and coronary arteries were prepared and stained with hematoxylin and eosin (H&E) method. And slides were then examined. The results showed statistically significant difference in estrogen level, and was declining with time after bilateral overictomy , and was (29.45 ± 0.65) (pg/ml)in control, while it was lower in the other groups the difference finally group E showed widely spaced cardiac fibers with fat deposition replacing the losted cardiomyocytes with congested thick walled blood vessels.

The coronary artery in the control and group A showed normal endothelial cell distribution without degenerative changes with normal wall thickness and lumen. The endothelial cells in group B showed minor changes, the wall thickness increased. No major changes in the lumen, more epithelial cell distribution and irregular outline in group C when compared to group Atheroma formation and clear atherosclerotic changes.

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Introduction:

Premenopausal women were observed to have a lower incidence of cardiovascular disease compared with postmenopausal women or age matched men (1). This has been ascribed to the protective actions of endogenous estrogens on the cardiovascular system and, particularly, on the vascular endothelium (2). Also proposed that the actions of endogenous estrogens on the cardiovascular system could be mediated directly on the vessel walls or indirectly through the modulation of cardiovascular risk factors. (2**)** Oestrogen (E2) appears to be responsible for the gender differences in endothelial nitric oxide (NO) release **(3).** Estrogen deficiency was reported to be associated with increased cardiovascular risk and endothelial dysfunction (4). Women with premature ovarian failure (or premature menopause) presented with severe vascular endothelial dysfunction compared to age-matched women with normal ovarian function (5**).** The coronary capillary network in middle-aged women may be regulated by physiological angiogenesis via VEGF, and reduction in coronary vascular endothelial growth factor(VEGF) expression by estrogen deficiency could play a role as a molecular pathogenesis in the development of coronary heart disease in postmenopausal women(6).

The aim of our study was to find the effect of ovariectomy (OVX) on coronary and cardiac tissue in association to estrogen level.

Material and method

The study was designed using forty eight sexually matured adult female rabbits of the local breed. They were divided randomly in to 6 group control, group A ,B,C,D,E each group contained 8 rabbits and have OVX then were scarified at these intervals of zero,7days,14days,30days,60days, 90days. Blood samples were collected from the female rabbits twice one before the operation and the other before sacrificing .The blood was collected from ear vein and from lateral saphenous vein in the leg. Blood samples were placed in non-heparin tubes for half an hour at room temperature for the purpose of blood clotting. Then, tubes were placed in the centrifuge for 15 minutes and 3,000 rpm. Serum was obtained, which was withdrawn and placed in new tubes, they were placed in a freezer until use. And then were tested for estrogen. Also cardiac tissue including coronaries was collected. These samples were treated in the flowing steps Fixation , Decalcification, Dehydration, Clearing, Infiltration , embedding , Tissue sectioning with Rotary microtome , Tissue attachment , De-wax and hydration , Staining with Haematoxylin and Eosin and finally Mounted .The microscopic investigation of tissue sections involved descriptive histology. A light microscope (Motic microscope) was used to perform the microscopic investigations of this study. Microscopic photographs were taken using (Optica\ SN 212973\Italy) then Data were analysed statistically using a statistical Minitab program under SPSS and Microsoft Excel XP system. The data were presented in simple measure of mean ± SD (standard deviation), minimum and maximum values.

Results:

Results for biochemical tests showed highly statistically significant difference between the studied groups regarding estrogen level. There was highly statistically significant difference between control group and the experimental groups regarding oestrogen level. The level was declining by time after bilateral ovariectomy(Table 1).

Table -1- Means ± SD of oestrogen levels of the control and the different experimental groups.

Groups	Oestrogen (pg/ml)		One-Way ANOVA	
	Mean ± SD	**Range**	**F**	**P-value**
Control (n=8)	29.45 ± 0.63	28.7 – 30.2	752.291	<0.001
Group A (OVX 7 days) (n = 8)	27.89 ± 0.38	27.3 – 28.3		
Group B (OVX 15 days) (n = 8)	26.74 ± 0.17	26.55 – 26.98		
Group C (OVX 30 days) (n=8)	24.54 ± 0.31	24.1 – 24.9		
Group D (OVX 60 days) (n=8)	23.89 ± 0.22	23.4 – 24.1		
Group E (OVX 90 days) (n=8)	18.21 ± 0.54	17.6 – 18.9		

Post hoc analysis using LSD test

Control Vs Group A	Control Vs Group B	Control Vs Group C	Control Vs Group D	Control Vs Group E
<0.001	<0.001	<0.001	<0.001	<0.001

The same result was obtained using both One-way ANVA and also in depth post hoc analysis using LSD test.

P > 0.05: Non significant; P < 0.05: Significant; P < 0.01: Highly significant

OVX = bilateral ovariectomy

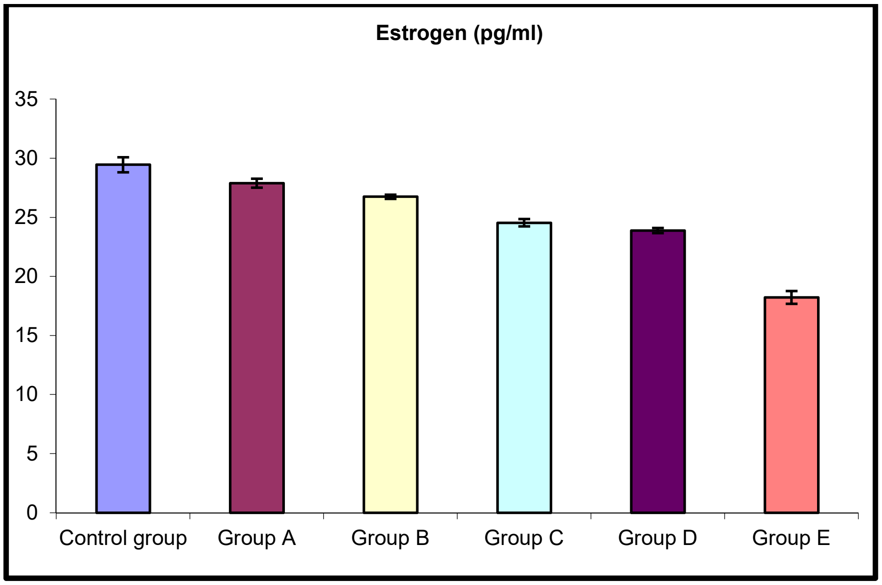


Fig (1): Bar graph of studied groups regarding oestrogen level

Histological changes

The control group showed normally distributed cardiac muscle fibers, longitudinal arranged **(Figure 2)** ***when*** compared with group A **Figure (3).** Only minimal congestion with no cellular infiltration was noticed**.** While in group B (Figure4**)** cardiac muscle fibres have wavy arrangement with more congestion in between fibers**.** In group C(**Figuer5)** cellular infiltration appears and spacing in between fibers was evidence with minor degenerative changes which became more clear in the group D(**Figure6)** haemorrhage areas were seen with widely separated cardiac muscle fibers and more degenerative changes finally, group E (**Figure7),** widely spaced cardiac muscle fibers with fat cell deposition replacing lost cardiomyocytes with congested thick walled blood vessels.

The coronary artery result was made as a comparison between all the experimental group. control group (figure 8) and group (A) shows normal endothelial cell distribution without degenerative changes, with normal wall thickness and lumen (figure 9) group (B) (figure10) clarify the endothelial cells with minor degenerative changes and increased wall thickness, no major change in the lumen, endothelial cell distribution and irregular outline (figure11) was found in group (C) . In group D (figure 12), the wall thickness increased in group (E) and shows irregular endothelial cell distribution, the coronary was much thicker when it was compared with the control group. Lumen narrowing and stenosis was very evident, and large atheroma formation with obvious atherosclerosis was pronounced (figure 13).

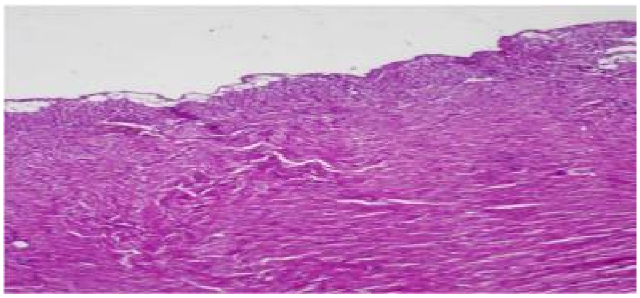


Figure (2) control group showing longitudinal arrangement of cardiac muscle fibers normally distributed (H&E.x 100).

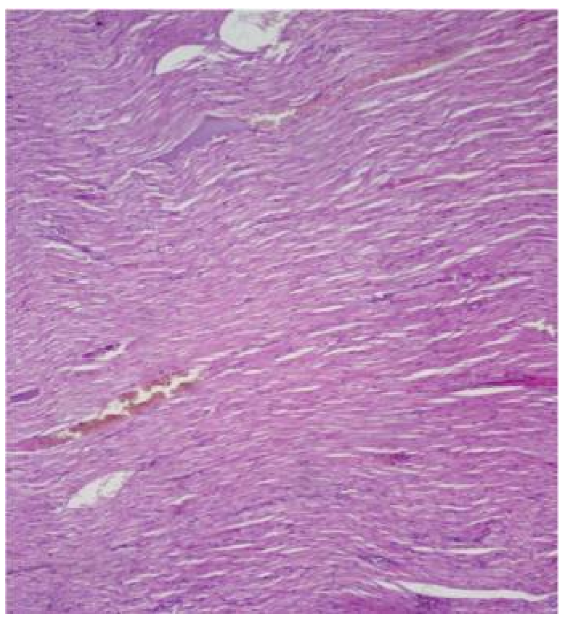


Figure (3) group A showing cardiac muscle arrangement longitudinal (H&E. X100) minimal congestion with no cellular infiltration

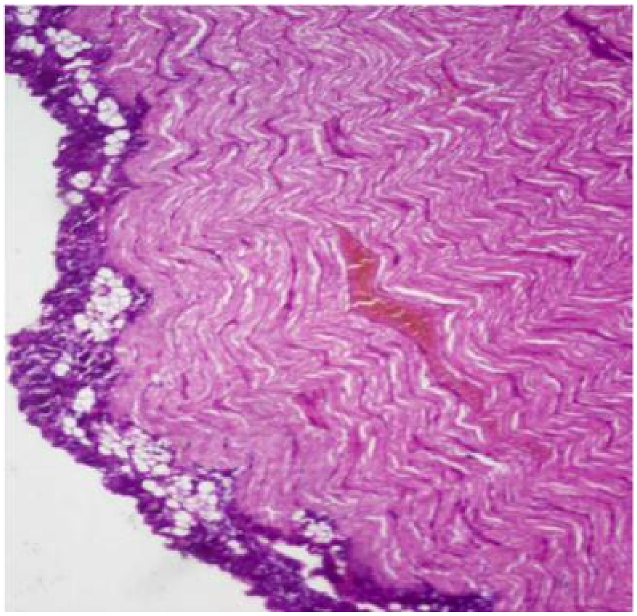
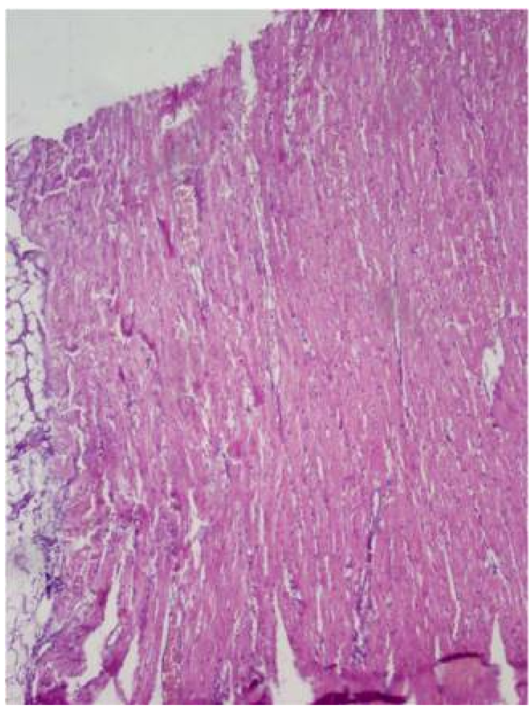
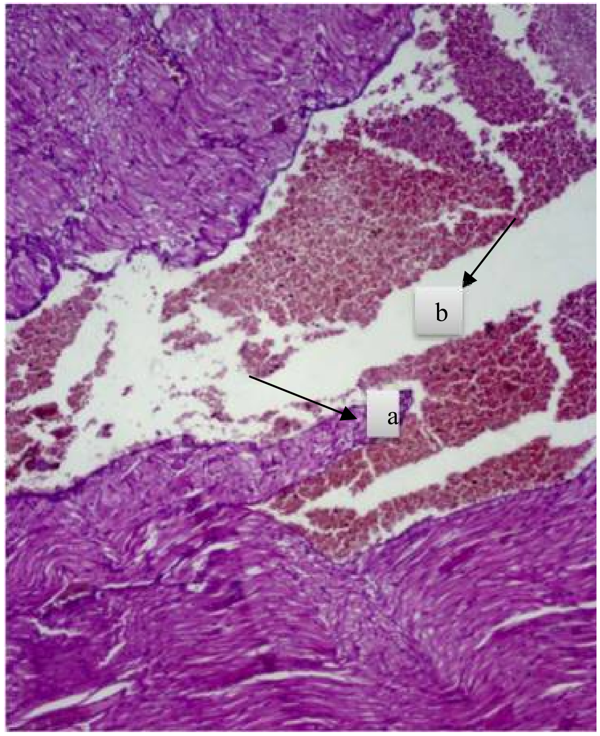


Figure (4) Group B section of rabbit cardiac muscle of (two weeks), showing longitudinal wavy arrangement of cardiac muscle fibers with more congestion. (H&E X100).



Figure(5) group C section of rabbit cardiac muscle of four weeks, showing longitudinal arrangement of cardiac muscle fibers with cellular infiltration & spacing in between fibers (H&E X100)



Figure(6): group (D) section of rabbit cardiac muscle of ovx 60 days(eight weeks) showing large hemorrhage areas(a)& widely separated cardiac muscle fibers degenerative changes (b). (H&E X100).

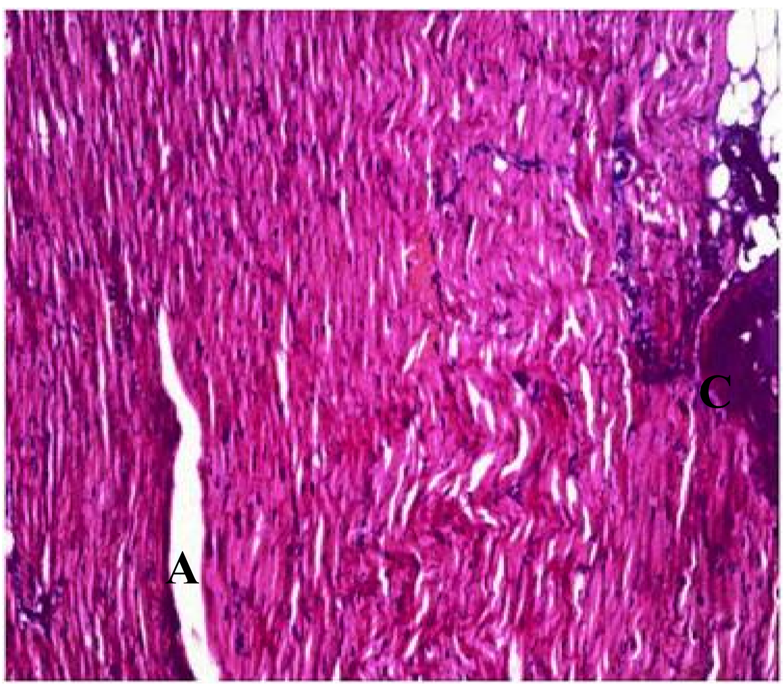


Figure (7) group E OVX. 90 days section of rabbit cardiac muscle of (twelve weeks), showing widely spaced cardiac muscle fibers (a) Notice fat cell deposition replacing lost cardiomyocytes(b) with dilated congested thick walled blood vessel (c). (H&E X100)

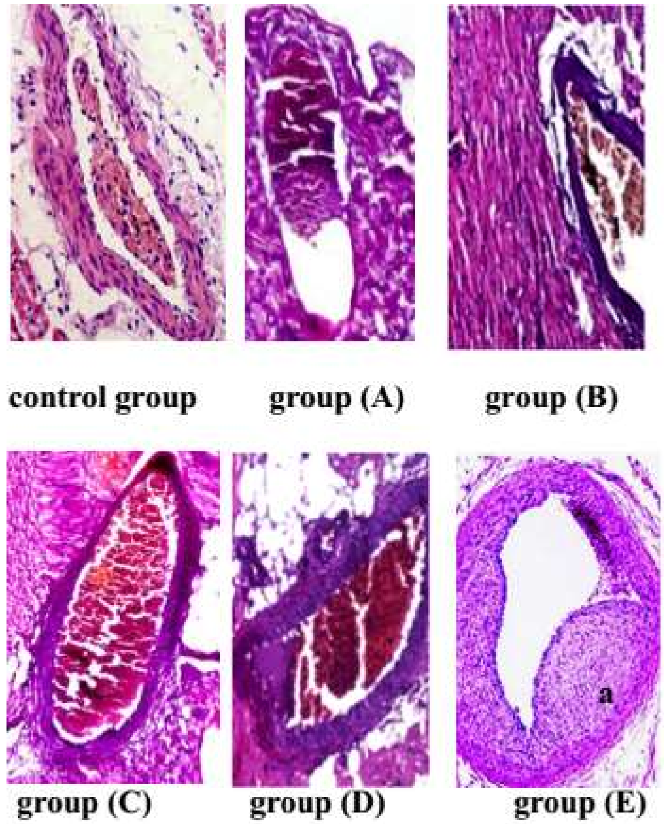


Figure (8): Section of coronary artery changes in the control group and all experimental groups to compare the degenerative changes, wall thickness and lumen stenosis. In groups (A, B &C) minor changes are seen. In Group (D) fat cell infiltration around the vessel, wall is thicker and narrow lumen. Group (E) shows atheroma (a) in the vessel wall, with much thicker and narrower lumen. (H&E. stain X100).

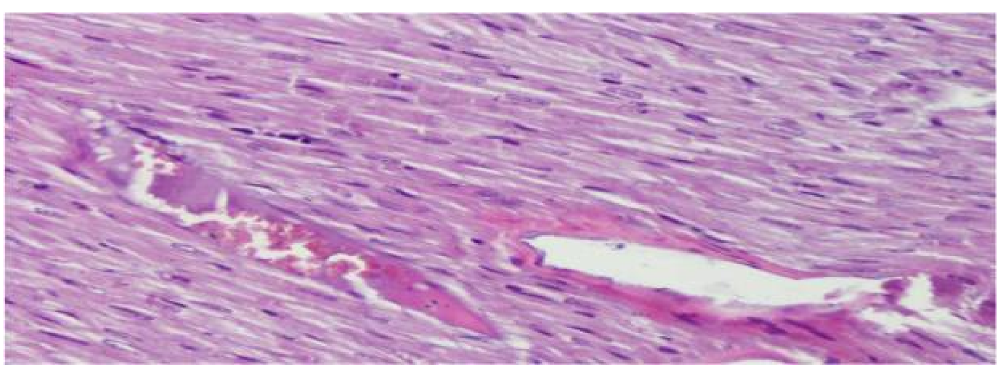


Figure (9): Group A OVX 7days: Show thin walled coronary with mild congestion surrounded by normally distributed myocardial tissue.(H&E 200X)

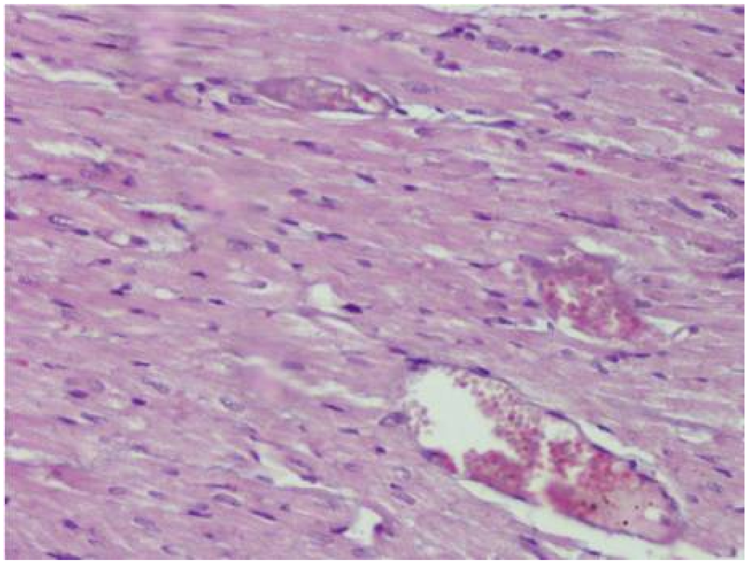


Figure (10) Group B OVX 14 days: More congestion is seen with more or less normal architecture as group A and control. (H&E 200X)

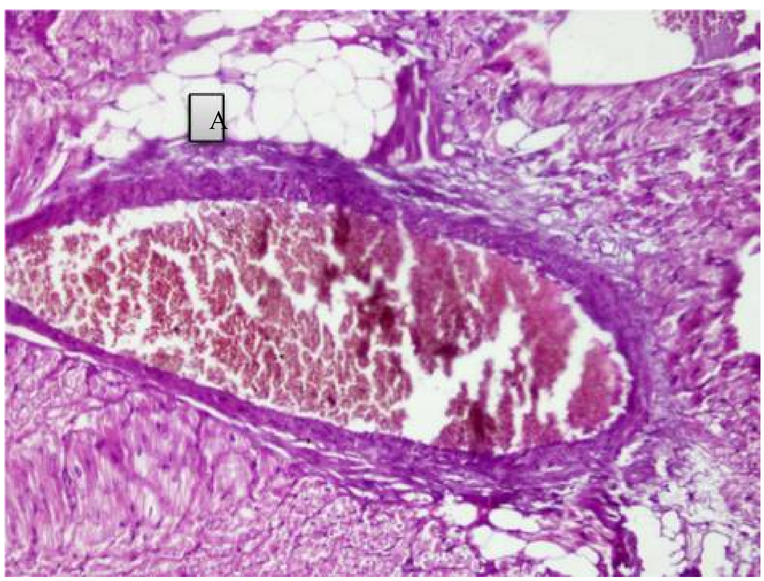


Figure (11) Group C OVX 30 days: The coronary wall showed thickness and More congestion also fatty cell infiltration (A) around with destruction of the cardiac muscle and architecture when compare previous groups. (H&E 200X)

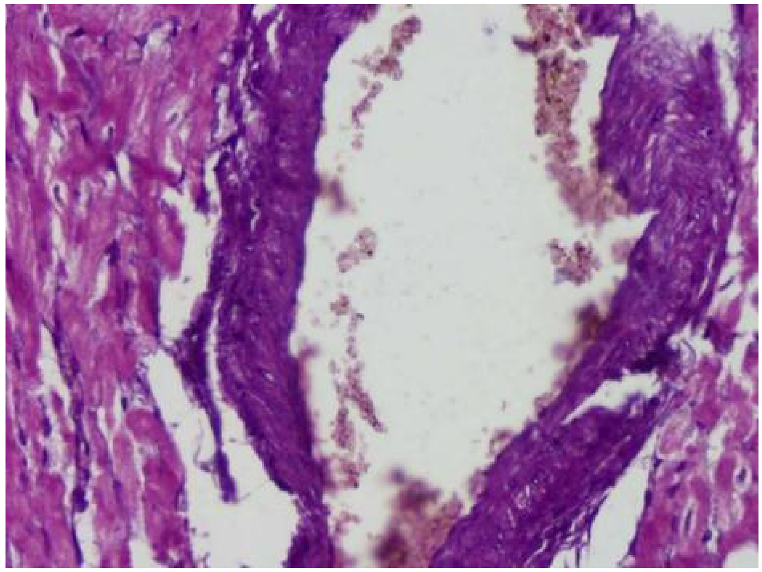


Figure (12) Group D OVX 60 days: The coronary wall shows much thickness and more congestion is seen. Separation of cardiac muscle around is evidence of degenerative changes (H&E 400X)

Figure (13) Group E OVX 90 days: The coronary wall shows much thickness and more congestion some fat cell (a) infiltration evidence of degenerative changes (H&E 200X)

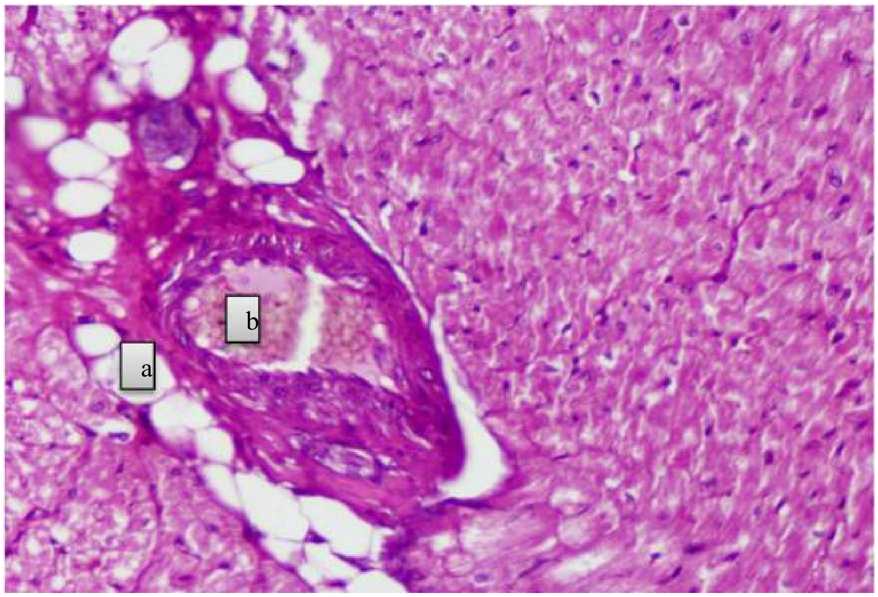


Figure (14) Group E OVX 90 days: The coronary wall is very thick and some fat cell (a) infiltration evidence of atheroma was formed with lumen occlusion, (b). (H&E.x200).

Discussion

Estrogen

Highly statistically significant difference was found between the studied groups regarding estrogen level. The level was declining by time after bilateral ovariectomy.(Mean ± SD) was (29.45 ± 0.63) (pg/ml) in control group while, it was lower in groups with bilateral ovariectomy and the difference significantly decreased till reach (18.21 ± 0.54) (pg/ml) after 90 days of the procedure in group E. Which normally due to ovariectomy which is the major source of estrogen. Almost all studies proved that estrogen level decreased significantly after bilateral ovariectomy and menopause like status for example a study made by ***park and his group (7)*** in their study, Serum level of 17b-estradiol revealed a significant decrease in the oophorectomy group.(Mean ± SD) (25.4± 5.1) (pg/ml) compared with the control.(Mean ± SD) (38.5± 7.6) (pg./ml) also the study of **Funsun Sumar(8)** and his colleagues, in Selcuk University/ turkey on ovariectomized rats normal Control level was, (Mean ± SD)was (62.21 ± 4.46) while in OVX group with normal diet was, (Mean ± SD) (10.90±1.43) (pg./ml).

Cardiac tissue changes

The cardiac changes was not clear as in femur it seems it needs more time to appear more clear. Control rabbit group showed normally distributed cardiac muscle fibers longitudinal arranged the same in group A7D OVX with minimal congestion. While in group B14D OVX, cardiac muscle fibers were wavy arrangement with more congestion in between fibers in group C30D OVX, cellular infiltration appears and spacing in between fibers as evidence of minor degenerative changes which became more clear in the group D60D OVX, hemorrhage areas were seen with widely separated cardiac muscle fibers and more degenerative changes. Finally, in group E90D OVX, widely spaced cardiac muscle fibers with fat cell deposition replacing lost cardiomyocytes with congested was seen. A thick walled blood vessel also was clearly identified. No studies were found to compare the finding which was not very pathognomonic.

Coronary artery changes

The Control group Showed thin walled coronary with surrounding normally distributed myocardial tissue without any atherosclerotic. The difference in Group A 7D OVX, Showed thin walled coronary with mild congestion surrounding by normally distributed myocardial tissue, while, in Group B 14D OVX, the congestion was more evident but with more or less same normal architecture as group A and control. In group C30 D OVX, the coronary blood vessel wall showed thickness and more congestion also fatty cell infiltration around with destruction of the cardiac muscle distribution and architecture when compared previous groups which consider significant change after one month of OVX. In Group D 60D OVX, the coronary wall showed much thickness and more congestion is seen, separation of the cardiac muscle around was evidence of degenerative changes. The best picture of the effect appeared in Group E 90D OVX The coronary wall thickened was with sever congestion and fat cells infiltration was evidence of degenerative changes also the coronary wall was very thick and important changes was found with atheroma and lumen occlusion, also with fat cells infiltration around. When the present result were compared with the study of **Subrina**(6) study said that we used an experimental model of estrogen deficiency induced by ovariectomy (OVX) in middle-aged female rats, which can be expected to exhibit the same changes in coronary capillary network of the heart as observed in postmenopausal women. There was a marked reduction in arteriolar capillaries after OVX. Thus, OVX significantly diminished the proportion of arteriolar capillaries which is consistent with the present results. A study on the OVX rats by **Sokolis(9)** and his colleagues they were working on larger blood vessel, the carotid artery, to show the effect of OVX and the result was characterized by an increased opening angle, thickness, and area of the vessel wall and of its medial layer which was constant with the present study, but the unchanged lumen diameter may be because of that the carotid vessel is larger blood vessels. Also another study showed the atherosclerotic changes which was close to the result of the present study **Parker (10)** and his colleagues related the early atherosclerotic plaque in animals fed cholesterol for only 2 weeks consisted of an accumulation of small numbers of cells within the intima, these cells were elongated with irregular nuclei, and a few of them were moderately vacuolated, In both kinds of foam cells, the vacuoles contained amorphous as well as laminated, concentrically arranged dense material.

CONCLUSION

In conclusion, it was found that overictomy (OVX) in associated with estrogen level declining by time after bilateral ovariectomy. The histological change of cardiac changes were seen but after one month of OVX as separated cardiac muscle fibers and degenerative changes which became widely spaced cardiac muscle fibers was with fat cell deposition replacing the lost cardiomyocytes with congested thick walled blood vessels. Lastly, coronaries minor congestion was seen early in group A and B while the changes became more evident in group C and D, thickened wall with more congestion and fatty cellular infiltration around became more in Group E and the full atherosclerotic changes was seen in group E after 90 days The coronary wall was very thick atheroma was formed with lumen occlusion and fat cell infiltration around.

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