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Lipid Effects of Experimental Oophorectomy and Cardiovascular Risk Association

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ABSTRACT

In view of the controversy between hormonal replacement in postmenopausal women and decreased cardiovascular morbidity and mortality, and the effects of estrogens associated with progesterones, or progesterone alone, we developed a protocol to evaluate the repercussions of hormone therapy with progesterone in oophorectomized rabbits submitted to atherogenic diet, on the development of atherosclerotic lesions induced in animals. Forty New Zealand rabbits were oophorectomized and divided into three groups. After 90 days, when the animals were sacrificed and the lesions that developed in the aorta and coronary arteries were evaluated. The results showed an increase in serum cholesterol in the four groups of animals. In groups II, III and IV, there was an increase of approximately fifteen times the baseline values, with no significant differences between these groups observed in 5 time courses. Regarding triglyceride levels, we found that group III showed a significant increase in values in Time 4, but this difference was not verified in Time 5. When quantitatively analyzing the area occupied by the lesion, we found that there were no statistically significant differences between the groups submitted to the atherogenic diet, observing that the coronary arteries presented a lower number of compromised arteries.

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Abbreviations

CVD: Cardiovascular Disease
HE: Hematoxylin-Eosin
HRT: Hormone Replacement Therapy

Introduction

Prospective human assessment of the impact of hormone replacement therapy (HRT) on atherosclerotic lesions is virtually impossible, unless through angiographic studies [1]. Therefore, animal research has been of considerable interest as it provides clear evidence of the effects of steroids on the evolution of induced atherogenesis.

Experimental atherosclerosis has been carried out for several years and several animals are successfully used, highlighting non-human primates, pigs and rabbits [2].

The induction of atherosclerosis in rabbits represents a well-established experimental model, as it reveals many similarities with human atherosclerosis. In addition, an Israeli study recently found that the proteins of these animals are more similar to those of monkeys and other primates, such as humans, than with rodent proteins, thus allowing a closer approximation of the results with those verified in humans. The rabbits, through a diet rich in cholesterol, develop lesions similar to the human organism, characterized by accumulation of lipids in the intima of the aorta.

This experimental model has numerous advantages; among them, the: 1) evaluation of therapeutic efficacy is cited; 2) inhibition of atherosclerosis progression and even plaque regression; 3) the control of different variables that can influence the results such as genetic, environmental and sample size; 4) to induce the disease in a short period of time [3-8].

Complications of atherosclerosis, such as fissure, rupture and hemorrhages of plaques are very rare in lesions induced in rabbits. Thus, when searching for oophorectomized rabbits submitted to a hypercholesterolemic diet, it can be inferred, keeping the appropriate proportions, which occurs a condition very similar to that of postmenopausal women. It is evident, however, that non-human primates such as Cynomolgus monkey still represents the best model, because in addition to its susceptibility to atherosclerosis, it presents reproductive physiology similar to that of humans.

Therefore, Adams et al. studied oophorectomized monkeys, that is, in a state of frank estrogenic deficiency; both in serum cholesterol and in the extent of atherosclerotic plaque. Their results constituted indirect evidence of the effects of endogenous sex steroids on the extent of atherosclerosis [9].

In another study, Adams et al. observed direct evidence of the endogenous estrogen inhibitor effect on the progression of coronary artery atherogenesis; thus, they studied oophorectomized monkeys submitted to a hypercholesterolemic diet and divided them into three groups: the first, control, did not use any hormone; the second used the association estrogen and progesterone and the third received exclusively progesterone. After 30 months the animals were necropsied and the results showed that the monkeys of the two groups treated with hormones presented an identical inhibition of atherosclerosis (around 50% in relation to the control). Furthermore, they found that the antiatherogenic effects of hormonal therapy were independent of the plasma variation of total cholesterol [10].

According to Adams et al. the effects of replacement hormones on atherosclerosis could not stem from other risk variables, such as the second and third decades the risk of cardiovascular disease (CVD) is higher in men and remains twice as high until the fourth and fifth decade of life, when a more pronounced elevation occurs in women, achieving a similar incidence between the sexes during the sixth decade of life [10].

These data suggest that young women have a protective factor against CVD that is lost after the fifth decade of life. As the vast majority of women enter menopause at this age, it is postulated that the increased incidence of CVD can stem not only from the aging process, but from another additional factor - estrogen deficiency - which has gradually been taking place several years before menopause and becomes evident with the permanent interruption of menstrual cycles.

While there is no direct proof of this hypothesis, several lines of indirect evidence support this possibility:

1. Women with premature menopause have a higher risk of CVD [11].
2. Patients with late menarche have an increased risk of CVD, but not statistically significant [12].
3. Bilaterally oophorectomized women before menopause present a statistically significant increase in relative risk (2, 2) [13].

What would be the reasons that would justify the apparent premenopausal protection of estrogens? It is certainly a complex answer to be given. However, since most CVD results from atherosclerosis in large vessels, it is admitted that estrogens, among other actions, would act on lipoproteins creating an anti-atherogenic profile represented by increased levels of high density lipoprotein (HDL) - preventive against atherosclerosis - and by reducing rates of atherogenic lipoprotein, low density lipoprotein (LDL).

In fact, HDL-cholesterol (HDL-c) decreases in the pubertal phase of boys - in response to androgens - and in girls, in the face of reduced estrogens.

Inhibition of LDL-cholesterol oxidation

Estrogen at physiological levels in the blood has an important antioxidant action [14]. What's more, the steroid can regenerate other circulating antioxidants, such as tocophenols and beta-carotenes, preserving them within LDL-cholesterol particles. This antioxidant action of estrogens also preserves the vasodilatory function of endothelial agents [15].

Favorable impact on coagulation mechanism

Estrogens used in hormone replacement therapy increase fibrinolytic activity - a cardioprotective mechanism possibly mediated by nitric oxide and prostacyclin - reduce platelet aggregation and do not alter coagulation factors.

Direct effects on atherosclerotic injury

Few studies in humans have evaluated the impact of estrogeniotherapy on atherosclerotic lesions.

Aims

The present study aims to evaluate, in oophorectomized rabbits submitted to atherogenic diet, the effects of conjugated progestogens on:

1. Serum cholesterol and triglyceride levels.
2. Quantitative and qualitative alterations of atherosclerotic lesions in aorta and coronary arteries evaluated macroscopically and histologically.

Material and Methods

Experimental Material - Animals and Bioterium

The study group consists of adult New Zealand rabbits weighing between 2100 and 3500 grams.

Upon arrival at the bioterium, each animal was submitted to a general examination and weighing (Time 0) by a veterinary physician, thus avoiding the inclusion of animals with contagious diseases or that would compromise the evolution of the study. This test was performed weekly with the objective of detecting and treating some disease appropriately. With regard to the bioterium, it is equipped with an exhaust and ventilation system, allowing the ambient temperature to remain around 25°C. The sturdy wire cages were painted immediately before the animals arrived, which allowed their disinfection. Arranged side by side and on channels suitable for waste collection, thus avoiding accumulation inside, each cage has a ceramic container where the diet is packed, which is offered at will, and a hose with valved nozzle that allows the suction of water by the animals, as needed. In the bioterium, the animals remained under observation for a period of seven days for their adaptation.

Protocol Description

After the adaptation period, all rabbits were submitted to bilateral oophorectomy, and on the twenty-first postoperative day, they were randomly distributed into three groups of 10 animals said I, II, III and IV. Group I animals were submitted to normal diet, those of Group II were fed a hypercholesterolemic diet (0.5%) and placebo, those in Group III were fed a hypercholesterolemic diet (0.5%) associated with 5 mg/day of medriprogesterone acetate and those in Group IV were fed a hypercholesterolemic diet (0.5%) associated with 10 mg/day of medroxyprogesterone acetate, 0.625 mg/day - orally.

Oophorectomy

After anesthesia (Ketalar-50 mg/kg-Rompun 25 mg/kg), trichotomy and antisepsis of the region to be incised were performed. The animal was arranged on the surgical channel, having the paws attached to the extremities with the placement of a sterile fenestrated field, which was sutured to the skin, exposing only the incision area. The incision, made approximately four fingers below the costal, oblique, left, unilateral grid, 5-6 cm long.

After skin incision, and by planes of the muscular layer and parietal peritoneum, the abdominal cavity was opened with curved scissors or straight blunt. With ease, the animal's reproductive system was

located, and initially the left ovary and, later, the right ovary was pulled. Two tweezers were placed below the ovary in the parallel position, one below the other. With the simple cotton thread 3-0, several nodes were given between the upper tweezers and the lower part of the ovary, then sectioned above the point where the nodes were given, the ovary was removed and the tweezers were released. This procedure was repeated with the right ovary. Then we reviewed the cavity and sutured, by planes, to the skin. The muscle layer and the peritoneum sutured with 3-0 catgute thread, and the skin with 3-0 cotton thread. After oophorectomy, the animals were kept under observation for a minimum of 21 days, and the stitches were removed on the tenth postoperative day.

Diet Preparation

The diet administered high in cholesterol at the concentration of 0.5% was prepared as follows: each week were prepared around 40 kg of feed, amount that these animals consumed in 7 to 10 days. Pure cholesterol for this amount of feed (200 g) was weighed and then diluted in chloroform in a ratio of 1.0 g of cholesterol to 4 ml of chloroform. This solution, mixed in a magnetic stirrer for a few minutes until the complete dissolution of cholesterol was observed. At the same time that cholesterol was prepared, the feed was sifted and placed in a ventilated area of the bioterium. The cholesterol solution was placed in a high pressure spray, jet with pressure control and targeting, and vaporization of the cholesterol and chloroform solution was performed in the animal feed. With this the vaporization became quite homogeneous. The feed was then left in a ventilated environment for a minimum of 48 hours for complete evaporation and disappearance of chloroform odor. This ration was offered to the animals daily.

The preparation of the medication was done in a similar way. Before we added chloroform, the drug was dissolved in water and alcohol. We used a dose of 0.625 mg of conjugated estrogens per day, associated with approximately 20 g of diet, being administered until 9 a.m. next to the diet.

Blood Dosages

From the twenty-first postoperative day to the end of the study were 90 days. Blood samples were taken for total cholesterol and triglyceride dosages at the following times:
Time 1: On oophorectomy.
Time 2: 21st day after oophorectomy (start day of hypercholesterolemic diet).
Time 3: 51st day after oophorectomy (30 days of hypercholesterolemic diet).
Time 4: 81st day after oophorectomy (60 days of hypercholesterolemic diet).
Time 5: 110th day after oophorectomy, day of animal sacrifice (90 days of hypercholesterolemic diet).

The method used was as follows:

a) Cholesterol-specific automated colorimetric enzymatic method by hydrolysis of cholesterol esters in cholesterol.
b) Triglycerides - enzymatic colorimetric method automated by hydrolysis of triglycerides.

Animal Sacrifice

The animals were sacrificed on the 110th postoperative day. Anesthetized with Ketalar and Rompun (5 and 35 mg/kg, respectively), the animals were placed in the surgical channel and, after abdominal incision, the inferior vena cava was punctured with scalp No - 21, injecting an ampoule of Isosorbid Mononitrate 10 mg (Lab. Baldacci). Then, the dissection of the aortas was performed from the aortic arch to the bifurcation of the iliac arteries and hearts. The materials were fixed in formaldehyde solution (10%), buffered with phosphate ph=7.6 for a period of at least 24 hours.

Evaluation of Aortic Lesions

After fixation, the aortas were cored with Sudam III to visualize the atheromatous lesions. After the lesions were correlated, we quantified the injured area by computerized planimetry. The results were expressed as a percentage of injured area in relation to the total area of the aorta.

Small fragments of approximately 3 mm, of a transverse section of thoracic aorta removed from the first 2 cm, and of the abdominal aorta removed between 7 and 12 cm, were processed through the common methods of histology, embedded in paraffin, cut to the thickness of 5 micrometers and cored by hematoxylin-eosin (HE) and von Kossa method for calcium. This material was used for histopathological analysis of the intima and middle layers and determination of the height of the lesion.

We determined the height of the lesion through a morphometric evaluation in the most representative plate of the histological section, which presented higher height. We used a cariometric eyepiece (Zeiss) and the results were expressed in micrometers (mm).

Evaluation of Coronary Artery Lesions

The hearts after fixed were cross-sectioned into fragments of 3 mm thickness, and each heart was divided into six fragments (A, B, C, D, E and F) from the base to the tip, processed as the aortas and corados in HE. Coronary arteries were analyzed in two histological sections (B and D) prepared of fragments corresponding to the proximal and middle third of these arteries. With the help of the microscope, we did count the number of coronary arteries and the relationship between the number of vessels with injury and the total number of vessels evaluated, and the result was expressed as a percentage. We also analyzed, separately, the normal or altered aspect of the coronary arteries divided into two groups - large, peripheral, and small, deep.

Immunohistochemistry

Immunohistochemical reactions were performed to determine smooth muscle cells using monoclonal antibodies to smooth muscle actin (Dako, Code.-M851), and macrophages, by specific monoclonal antibody for macrophages of rabbit RAM-11, (Dako, Cód.-M633). As a secondary antibody, we used anti-immunoglobulin antibody of biotinilated mouse and streptoavidin peroxidase (Dako, code-K675); diaminobinzidine (DAB-Sigma Aldrich) and hydrogen peroxide (Merck).

Statistical Analysis

The variables were represented in tables by mean, standard deviation, median, minimum and maximum values.
Nonparametric tests were used due to the nature of the data.
The comparison between the parameters measured in the four groups (Groups I, II, III and IV) was performed using the Kruskal-Wallis Test (H). In the parameters in which the comparison was made between Groups II, III and IV, the Mann-Withney Test (U) was used.
The parameters measured twice in the same animal were compared by the Wilcoxon Test (z). In the variables in which more than two measurements were made (Time 1, 2, 3, 4 and 5), they were compared by the Friedman Test (X2).
The analysis of histopathological variables was performed using Fischer's exact test, comparing Groups II, III and IV.

The significance level of 0.05 (α=5%). Descriptive levels (P) lower than this value were considered significant.

Results

Weight of Rabbits

The animals were weighed at the beginning of the experiment (Time 1) (Table 1, Figure 1) and at the end (Time 5) (Table 2, Figure 2), at the time of animal sacrifice.

The initial weight was similar in all groups, as well as the final weight. We observed a significant increase in all groups in relation to the initial weight (Table 3).

Table 1: Initial weight of animals

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	2.694,44 + 276,64	2.725,00	2.100,00	3.000,00
II	2.830,00 ± 377,27	2.800,00	2.400,00	3.500,00
III	2.895,00 ± 304,09	2.825,00	2.600,00	3.500,00
IV	2.987,50 ± 188,51	2.950,00	2.700,00	3.300,00

H = 5,42 P = 0,1436

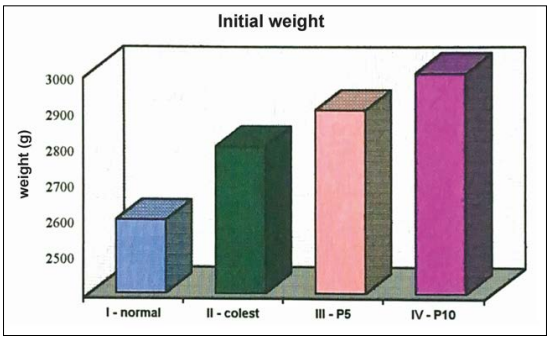

Figure 1: Distribution of groups in terms of initial weight

Table 2: Final weight (Time 5) of animals (g)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	3.490,00 ± 268,53	3.450,00	3.000,00	3.900,00
II	3.430,00 ± 275,08	3.450,00	3.000,00	3.900,00
III	3.370,00 ± 316,40	3.200,00	3.100,00	4.100,00
IV	3.456,25 ± 252,75	3.525,00	3.000,00	3.800,00

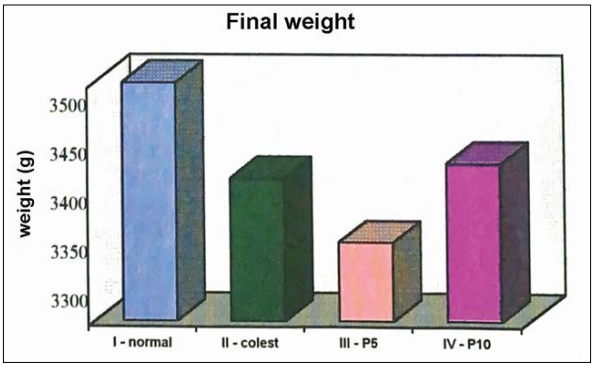

Figure 2: Distribution of groups in terms of final weight

Table 3: Comparison between initial weight (g) x Time 5 in each group

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Group	Comparison	
I	z = 2,66	P = 0,0077*
II	z = 2,70	P = 0,0069*
P5	z = 2,80	P = 0,0051*
P10	z = 2,52	P = 0,0117*

Cholesterol Levels

In the blood samples collected at various times (1 and 5), serum cholesterol was dosed. We observed that at the time of the first collection, Group I (normal) presented a slightly higher cholesterol value, although with a level within the normal range (Table 4). We can verify, however, that in Time II (pre-diet), and the cholesterol level did not differ between the groups (Table 5). In Time 3 (after one month of the diet), the cholesterol level increased, and there was a greater increase in the P5 Group (Table 5). In Time 4 (2 months of diet), as well as in Time 5 (3 months of diet) cholesterol remained equally high between groups (Tables 6 and 7). The statistical comparison of the variation between the dosages at various times in each group is shown in Table 8.

Table 4: Serum cholesterol (mg/dl) of animals in Time 1 (pre-oophorectomy)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	131,20 ± 43,11	129,00	51,00	205,00
II	105,90 ± 67,75	72,50	42,00	227,00
III	88,80 ± 32,18	79,00	44,00	141,00
IV	90,25 ± 26,59	88,00	60,00	140,00

H = 4,63 P = 0,2005 (P5=P10=II)ǂI

Table 5: Serum cholesterol (mg/dl) of animals in Time 2 (pre-atherogenic diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	112,30 ± 34,54	114,00	56,00	168,00
II	96,90 ± 30,37	86,50	61,00	154,00
III	97,90 ± 35,61	86,50	57,00	171,00
IV	101,87 ± 23,63	106,00	72,00	140,00

H = 1,88P = 0,5969

Table 6. Serum cholesterol (mg/dl) of animals in Time 3 (30 days after start of diet)

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Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	111,70 ± 47,65	100,00	69,00	238,00
II	1.549,80 ± 642,88	1.427,50	579,00	2.631,00
III	2.099,60 ± 962,59	1.923,00	766,00	3.821,00
IV	1.417,25 ± 567,28	1.262,50	937,00	2.582,00

H = 23,29 P < 0,0001* IǂP10=IIǂP5)

Table 7: Serum cholesterol (mg/dl) of animals in Time 4 (60 days after start of diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	130,89 ± 52,58	132,00	54,00	204,00
II	1.302,30 ± 414,45	1.298,00	669,00	2.115,00
III	1.515,80 ± 375,27	1.429,50	1.020,00	2.031,00
IV	942,50 ǂ 172,59	954,50	655,00	1.255,00

H = 25,89 P < 0,0001* IǂP10=IIǂP5)

Table 8: Serum cholesterol (mg/dl) of animals at Time 5 (90 days after start of diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	185,00 ± 72,81	179,00	96,00	308,00
II	984,80 ± 293,37	889,00	730,00	1.522,00
III	1.462,90 ± 574,32	1.506,00	669,00	2.380,00
IV	1.321,75 ǂ 596,56	1.052,00	926,00	2.671,00

H = 25,18 P < 0,0001* Iǂ(II=P10=P5)

Triglyceride Levels

Triglycerides were determined in the various blood collections, observing that although Groups I and III started the experiment with higher level, but with values within normality (Table 9), at the time of the second collection (pre-diet), the values did not differ between the groups (Table 10).

One month after the beginning of the diet, triglyceride values did not differ between groups (Table 11). After 2 months, we verified an increase in the progesterone groups in relation to the other 2 groups (Table 12). However, at the time of the last collection, triglyceride values did not differ between groups (Table 13). The statistical analysis of the values in each collection within each group is shown in Table 14.

Table 9: Comparison of cholesterolemia in the Times 1 x 2 x 3 x 4 x 5 in each group

Group	Comparison	
I	X2 = 9,49	P = 0,0600
II	X2 = 31,68	P = 0,0001* (II = I) ǂ V ǂ (IV = III)
P5	X2 = 31,36	P = 0,0001* (I = II) ǂ (V = IV) ǂ III
P10	X2 = 25,60	P = 0,0001* (I = II) ǂ IV ǂ (V = III)

Table 10: Serum triglycerides (mg/dl) of animals in Time 1 (pre-oophorectomy

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	127,00 ± 57,30	115,50	68,00	245,00
II	71,90 ± 34,20	56,50	41,00	133,00
III	111,20 ± 28,80	106,00	67,00	150,00
IV	71,50 ǂ 29,38	74,00	32,00	120,00

H = 11,73 P = 0,0084* (P10=III)ǂ(P5=I)

Table 11: Serum triglycerides (mg/dl) of animals in Time 2 (pre-diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	107,50 ± 33,41	91,00	81,00	174,00
II	110,60 ± 30,43	106,00	73,00	151,00
III	93,70 ± 22,50	89,00	68,00	131,00
IV	115,37 ǂ 75,14	86,00	54,00	237,00

H = 1,90P = 0,05928

Table 12: Serum triglycerides (mg/dl) of animals in Time 3 (30 days post-diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	86,40 ± 33,03	80,00	49,00	149,00
II	91,80 ± 31,10	96,00	43,00	135,00
III	93,70 ± 71,16	89,00	68,00	131,00
IV	133,50 ǂ 59,43	140,50	34,00	219,00

H = 4,63P = 0,2011

Table 13: Serum triglycerides (mg/dl) of animals in Time 4 (60 days post-diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	89,78 ± 33,81	86,00	55,00	172,00
II	66,60 ± 26,19	62,50	33,00	107,00
III	162,80 ± 53,12	165,00	65,00	217,00
IV	141,25 ǂ 32,00	147,50	98,00	183,00

H = 19,50 P = 0,0002* (I = II)ǂ(P5=P10)

Table 14. Serum triglycerides (mg/dl) of animals in Time 5 (90 days post-diet)

Group	Average ± Standard Deviation	Median	Minimum	Maximum
I	148,10 ± 40,82	164,00	90,00	197,00
II	130,20 ± 65,98	106,50	77,00	257,00
III	250,70 ± 157,18	217,50	50,00	557,00
IV	154,50 ǂ 36,74	163,00	95,00	198,00

H = 5,58 P = 0,1339

Area of Atherosclerotic Lesion in Aorta

The atherosclerotic lesion in the aorta was determined by computed planimetry of the fixed aortas, after staining with Sudam III. Macroscopically, in the groups that received an atherogenic diet, we observed intense lipid deposition, with predominance in the thoracic region of the aorta in relation to the abdominal region. There was no macroscopic difference between the groups that received a hypercholesterolemic diet, with great individual variability.

The total area of the aorta, the area of the corado lesion and the percentage of the total area of the aorta occupied by the lesion were determined. We found that the extent of the lesions in the aortas did not differ among the groups that received a hypercholesterolemic diet and developed atherosclerosis. Group 1 - normal diet - did not present lesion, being excluded from the statistical analysis.

Morphometry of Intimal Thickening of the Aorta

The intimal thickening observed after ingestion of a hypercholesterolemic diet was quantified in histological sections of two aortic fragments, removed from the site with major lesion of the thoracic and abdominal region. We determined the highest value of the intima, middle layer and the intima/mean ratio of the thoracic fragment.

Also in the abdominal fragment, the thicknesses of the intima layer, middle layer and the intima/middle ratio were determined. We did not observe in any of the differences between the groups. The differences between the thoracic and abdominal fragments, we found that the lesions in the thoracic fragments were higher than those of the abdominal fragments (Figure 3).

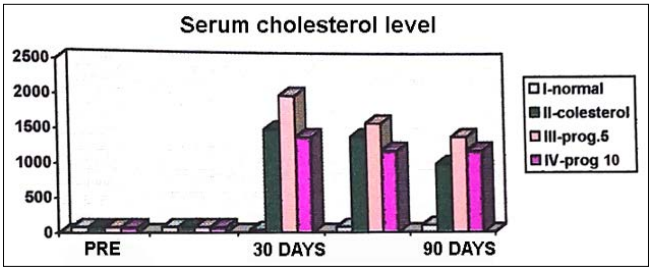

Figure 3: Serum cholesterol (mg/dl) of Groups I, II, III and IV at various times

Histopathological Analysis of Thoracic and Abdominal Fragments of the Aorta

Fragments of the thoracic and abdominal aorta were processed by histological techniques and HE and von Kossa staining was performed.

We observed in the sections cored by HE, large intimal thickening, with intense lipid infiltration in animals submitted to hypercholesterolemic diet. We observed a histological cut of rabbit aorta with normal diet with tenuous intimal layer and endothelial cells. We also verified in the aortas of hypercholesterolemic animals, proliferation of smooth muscle cells both in the intima and middle layer, as well as macrophages with lipids in the middle layer.

Histological sections of the aorta were analyzed for the presence of calcium, through special staining with von Kossa reaction. The analysis was performed only with the observation of presence or absence of positive reaction. We verified a positive reaction in the intimate and middle layer. We observed in the thoracic fragment, intense reaction in histological slides of all animals in Group 2 (cholesterol). In the groups that received progesterone, 40 (P5) and 71% (P10) of the animals showed calcium in the deep region of the intimate layer, values lower than that of the group without hormone. In the middle layer, we did not observe any difference between the groups. Analysis in the abdominal segment of the aorta showed no significant difference in both the intima and mean layer.

Histological Analysis of Coronary Arteries

Two fragments of the heart, proximal and distal, were processed and analyzed for the incidence of altered coronary arteries. We found in the proximal fragment the smallest number of altered coronary arteries in the group that received progesterone 5 mg (48%) and greater reduction in the group with 10 mg (37%), significantly different values when compared to Group II (59%). This difference, however, was not observed in the distal fragment of the heart (Figure 4).

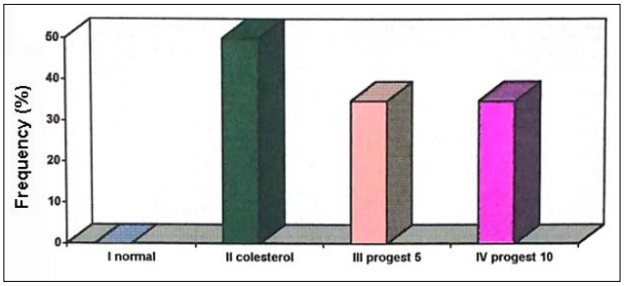

Figure 4: Frequency of altered arteries in the distal fragment of the heart

Figure 5 illustrates the total frequency of altered arteries in the two heart fragments. We observed that the peripheral coronary arteries, with a larger diameter, presented small intimal thickening, with macrophage cells eccentrically grouped in the vessel. More significant morphological changes were observed in small values located more deeply in the cardiac musculature.

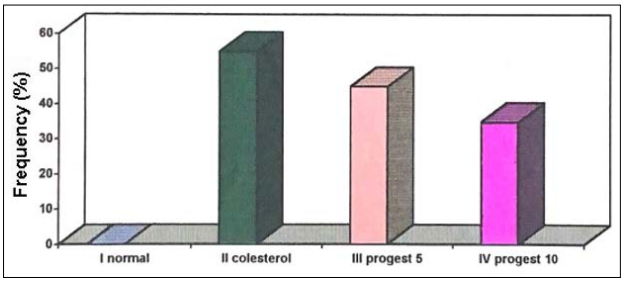

Figure 5: Frequency of altered arteries in the two fragments of the heart

Discussion

The results obtained show parallelism with other studies.
In addition to menopause, there are other risk factors for CVD that can associate with it, thus enhancing its installation; among them, smoking, sedentary lifestyle, obesity, hypertension, diabetes mellitus, are listed, with a 50% reduction in the relative risk of coronary disease of estrogen users [16].

The Lipid Research Clinics Follow-up Study, in turn, found in a study of 2,270 women over 8.5 years - a 63% drop in the relative risk of fatal CVD in estrogen users, including also the protective effect on former smokers [17].

Another study investigated for 15 years, 2,575 women who were 55 years old at the beginning of the study; in the last year of evaluation, 816 deaths were observed, 444 of which were due to CVD. The analysis of the results showed that estrogen therapy was responsible for reducing mortality between 40-60%. From the point of view of public health, only now CVD in postmenopausal women has received appropriate attention; in fact, research has now intensified more intensively in order to evaluate the consequences of early intervention with estrogen therapy through prospective randomized placebo-controlled studies.

Rare are autopsy studies, related to the age of installation of surgical menopause with the degree of coronary artery atherosclerosis. In one study, atherogenesis was found in women with surgical menopause when compared to those in normoestrogenic state [18].

In another study, when comparing bilaterally oophorectomized women with other women labeled "hyperestrogenic" - because they have breast cancer - atherosclerotic lesions in oophorectomized women were statistically much higher than in "hyperestrogenic" lesions.

Conclusions

We can conclude that the therapy with medroxyprogesterone acetate at doses of 5 and 10 mg per day in oophorectomized New Zealand rabbits submitted to the atherogenic diet, revealed at the end of 90 days:

1. Serum dosages: absence of changes in cholesterol and triglyceride levels.
2. Aorta:

- Absence of differences in the area and height of the lesions, in the intima and middle layers;
- Smaller number of aortas with calcium deposits.

3. Coronary arteries: smaller number of compromised arteries.

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None.

Conflicts of interest

No conflict of interest.

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