Effect of ovariectomy and of estrogen treatment on the adrenal gland and body weight in rats

Berna G. Saruhan, *PhD,* ***Nurullah Ozdemir,*** *PhD.*

ABSTRACT

Objectives: To determine the effect of estrogen on adrenal gland histology, and to ascertain whether or not estrogen regulates body and adrenal gland wet weight gain in the ovariectomized rat model. Two experiments investigated the effects of ovariectomy and estrogen replacement on the body and adrenal gland weight.

Methods: We used 36 female Wistar Albino rats in this study. The study took placed in the Department of Medical Science Application and Research Center of Dicle University, Diyarbakir, Turkey, in 2002. Group 1 (control group) received no ovariectomy; all animals in the other groups were bilaterally ovariectomized and kept for 60 days. We designated group 2 and 3 as sham-operated group and bilaterally ovariectomized then in addition, group 3 received estradiol. We then cut the paraffin sections, which we obtained by routine histologic methods, into 5 μm and stained them with hematoxylin-eosin. We later evaluated the stained sections under a light microscope.

Results: The body weight was higher in the ovariectomized rats compared to the control groups. Ovariectomy did not result in significant changes in the wet weight of the adrenal gland. Furthermore, body weight increased after estrogen therapy, and the wet weight of the adrenal gland increased in the estrogen group. It was obtained in the sham-operated group that ovariectomy resulted in a decrease in the activity of the adrenal cortex. In the sham-operated and estrogen group, we observed a prominent capsule, expanded zona glomerulosa cells, regular parallel columns in zona fasciculata and there was an increased vascularization in the medulla, after the estrogen treatment.

Conclusion: We determined that bilateral ovariectomy can result in a decrease in the activity of the adrenal cortex. In contrast, estradiol injection can cause a significant increase in the activity of the adrenal cortex and medulla.

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The adrenal cortex is a major source of steroid hormones.[1] The adrenal cortex of adult mice is divided into morphologically and functionally distinct layers or zones.[2] In addition to this zonal organization, the adrenal cortex is arranged in radial cords of clonally related cells that extend from the zona fasciculata.[3] The adult adrenal cortex undergoes continual renewal, stem cells near the junction of the zona glomerulosa and zona fasciculata give rise to daughter cells, which displaced centripetally, where they undergo apoptosis.[3-4]

We examined the effect of gonadotropins and ovarian hormones on adrenal estrogens in female rats in relation to the ovarian function. The estrone level gradually decreased with adrenal development

From the Departments of Histology and Embryology (Saruhan), Departments of Pharmacology and Toxicology (Ozdemir), Veterinary Medicine Faculty, University of Dicle, Diyarbakir, *Turkey.*

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Address correspondence and reprint request to: Dr. Berna G. Saruhan, Departments of Histology and Embryology, Veterinary Medicine Faculty, University of Dicle, Diyarbakir, *Turkey.* Tel. +90 (412) 2488011 / 2488627. E-mail: bsaruhan@dicle.edu.tr

after ovariectomy carried out at 21 days of age. " Estrogen replacement is frequently the treatment of choice for maintaining reproductive function and bone mineral density in post-menopausal women and amenorrheic adolescents. While estrogen's effects on the reproductive system and bone are well established, less is known about how it affects other tissues.[5]

In females, sympathetic activity varies with changes in reproductive status but whether there is alteration of the expression of proteins, which are critical to the function of sympathetic neurons is yet undetermined. The 17 beta hydroxysteroid dehydrogenases (17 HSDs) have a central role in the regulation of the biological activity of sex steroid hormones. There is increasing evidence that in addition to their importance in gonads, these hormones also have substantial metabolic roles in a variety of peripheral tissues.[6] The identification of the estrogen receptor in the laboratory provided a mechanism to describe the target site specificity of estrogen action in uterus, vagina, pituitary gland and breast cancer.[7] In another experimental study, Zaki et al[8] determined that ovariectomy resulted in a decrease in the activity of the adrenal cortex. The effects of steroid hormones on the target tissues are exerted through their binding to a specific receptor. Therefore, the first step in understanding the mechanisms by which steroid hormones affect adrenal steroidogenesis is to demonstrate the presence of their receptors. Estrogen receptors (ER) have been found in the rat adrenal gland, however, we found no studies regarding ER in the ovine adrenal gland.[9]

We evaluated the effect of adrenal steroids upon implantation by examining the efficacy of oestradiol-17 beta on the initiation of implantation in ovariectomized, ovariectomized plus adrenalectomized or hypophysectomized pregnant rats treated with progesterone. More estrogen was required in ovariectomized animals to obtain results equivalent to those obtained with the other animal models.[10] We carried out an investigation on albino rats in order to study the effects of estradiol on the adrenocortical activity in ovariectomized female rats.

Methods. Thirty-six normally cycling female virgin Wistar albino rats, 200-220 g in weight, were obtained from the Department of Medical Science Application and Research Center of Dicle University, Diyarbakir, Turkey. They were housed in individual cages in a temperature controlled environment (22^{o}C) with a 12:12 hour light–dark cycle. All rats were fed with standard pellet food and ad libitum tap water. The rats were divided into 3 groups of 12. Group 1 (control), the animals of this group did not receive ovariectomy nor did they receive estrogen treatment. Animals in group 1 were given an intraperitoneal injection with 0.1 ml of carrier A alone- (1:3 mixture of 100% ethanol and 0.9% saline W/V [Boots Co., Australia]) each day for 3 days. The carrier A injection was given in order to create the stress environment as in the 2 other animal groups. Surgical ovariectomy was performed to group 2 and 3 rats.

Ovariectomy. All rats in group 2 and 3 were food–deprived prior to surgery. Then the rats were anesthetized by intraperitoneal injection of Ketamine (50 mg/kg body weight Parke-Davis) and xylazine HCL 2% (100 mg/kg body weight Rompun-Bayer).[11] Bilateral ovariectomies were carried out in 24 animals using a dorsal approach in a sterile surgical theater. After bilateral ovariectomy, the rats were allowed to recover for 6-8 weeks before being treated. Group 2 was designated as sham-operated group. Then, the rats were subcutaneously injected with equivalent amount 0.1 ml of a 1:3 mixture of benzyl alcohol and peanut oil, each day for 3 days (carrier B). Group 3 was designated as sham-operated and estrogen group. Animals in group 3 were given a subcutaneous injection with 0.1 ml carrier B containing 1.0 μg 17-β estradiol (1, 3, 5-10 estratriene –3, 17-β diol, sigma).

Intraperitoneal injection was used in the control group similar to that in previous studies. Furthermore, it is known that intraperitoneal injection has a faster impact on the body. However, in the 2 other groups, subcutaneous injection was used as the carrier B is an oily substance.[12,13] We weighed the animals at 3 different times; at the start of the study, after 2 months of ovariectomy and at the end of the estrogen treatment.

After approximately 2 months of ovariectomy, the animals were anesthetized and killed by cardiac exsanguination. The adrenal gland was excised, weighed, fixed in formalin fluid and embedded in paraffin. Five to-six micron serial sections were prepared and stained with hematoxylin-eosin. The stained sections were later evaluated with light microscopy.

Statistical analysis. The data are presented as mean ± SE. Statistics were calculated using Minitab. All results in the study were analyzed using the Fisher-Tukey test, with $p<0.05$ as the criterion for significance for all statistical comparisons.

Results. ***Adrenal gland histology.*** In the control group, the appearance of adrenal glands was normal. The examination of sections of adrenal gland showed it to be covered by a capsule consisting of dense collagenous connective tissues. The capsule sent thin septa to the interior of the

Figure 1 - In control, the appearance of adrenal gland was normal. The gland consisted of 2 concentric layers, the adrenal cortex and the adrenal medulla. (Hematoxylin and eosin × 20).

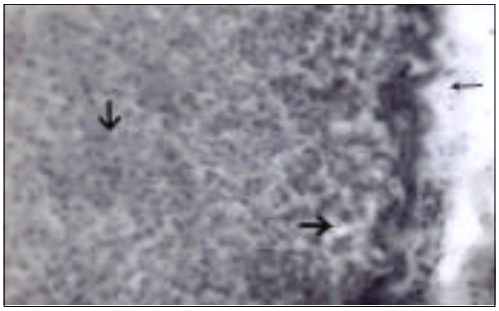

Figure 2 - In sham-operated group (group 2) the appearance of adrenal gland, a noticeable thin capsule, degenerated cortex cells and irregular parallel zona fasciculata cells were observed in light microscopy. (Hematoxylin and eosin × 20).

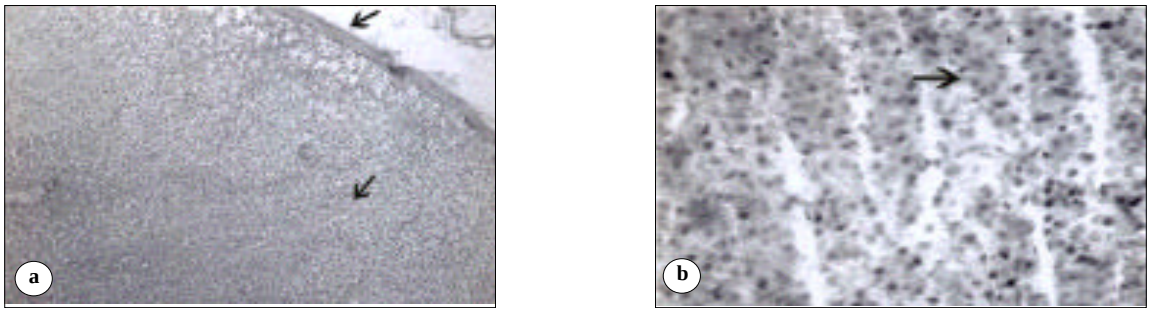

Figure 3 - In the sham-operated+estrogen group (group 3): **(a)** the appearance of adrenal gland, a prominent capsule, regular parallel columns in zona fasciculata were observed. (Hematoxylin and eosin × 20). **(b)** The appearance of adrenal gland, expanded zona glomerulosa cells were observed. (Hematoxylin and eosin × 40).

Table 1 - Comparison of the body weights.

Groups	At the start of experiment	After 2 months	Following 3 days of drug administration
Group 1 (n=12)	201.33 ± 0.45	213.5 ± 1.07	212.92 ± 0.96
Group 2 (n=12)	201.67 ± 0.48	235 ± 1.51*	235 ± 1.51
Group 3 (n=12)	201.83 ± 0.52	235.83 ± 1.49*	247.08 ± 0.96

Group 1 - control group, group 2 - sham-operated group, group 3 - sham-operated group + estrogen.
*Statistically significant difference between the 2 groups (group 2 and 3 after 2 month of ovariectomy) $p<0.05$.

Table 2 - Comparison of the weight adrenal gland.

Group	Control	Sham-opr	Sham-opr+estrogen
Adrenal gland weight	71.9 ± 4.6 g	69.8 ± 3.7 g	92.4 ± 4.8 g

sham-opr - sham-operated

gland as trabeculae. The gland consisted of 2 concentric layers; the adrenal cortex and the adrenal medulla (**Figure 1**).

It was determined in the sham-operated group that ovariectomy resulted in a decrease in the activity of the adrenal cortex. A noticeable thin capsule, degenerated cortex cells and the irregular parallel zona fasciculata cells were observed in light microscopy (**Figure 2**).

In the sham-operated and estrogen group, a prominent capsule, expanded zona glomerulosa cells, regular parallel columns in zona fasciculata and increased vascularization in the medulla were seen after the estrogen treatment (**Figure 3a & 3b**).

As a result, it was determined that bilateral ovariectomy can result in a decrease in the activity of the adrenal cortex. Estradiol injection can cause a significant increase in the activity of the adrenal cortex and medulla.

Body weight. Comparison of the means of group 1 (control group), group 2 and group 3 at the start of trial were found to be insignificant ($p>0.05$). Comparison of the live weight means of the control group with the other 2 groups after the second month (post ovariectomy) was found to be significant ($p<0.05$). Our results show that body weight significantly increased in ovariectomized rats. In this trial, paired t-test was performed to elucidate whether the administration of the drug was effective in those animals or not, and it was determined that administration of the drug was significantly effective on body weight ($p<0.05$). **Table 1** shows that subcutaneous administration of estradiol, which causes to increase body weight.

Adrenal gland weight. As indicated in **Table 2**, ovariectomy did not result in significant changes in the wet weight of the adrenal gland ($p>0.05$). On the other hand, it was increased in the sham-operated and estrogen group ($p<0.05$).

Discussion. The adrenal gland is actually 2 glands in one. The adrenal cortex, the outer portion of the adrenal gland, is responsible for most of the body's steroid production. The term corticosteroid is derived from this origin. Steroids have many functions, but their main action is in the form of salt retention, which in turn leads to regulation of the blood pressure as well as sex steroid production. The adrenal medulla is the central portion of the gland and is primarily responsible for the production of catecholamines (epinephrine, norepinephrine, and dopamine).[14]

Fekete et al[15] first demonstrated that gonadectomy can cause undifferentiated cells in the subcapsular region of the female mouse adrenal cortex, which transformed into sex steroid producing cells that are histologically and functionally similar to ovarian tissue. Subsequent studies established that gonadectomy–induced adrenocortical tumor formation occurs in both female and male mice, but is highly strain dependent.[11,16-18] Subcapsular, spindle cell tumors, have been reported in the adrenal glands of other gonadectomized rodents, such as rats, guinea pigs, hamsters and ferrents.[19] Zaki et al[8] determined that ovariectomy resulted in a decrease in the activity of the adrenal cortex. Estradiol injection can cause a significant increase in the activity of the adrenal cortex to values exceeding that of normal females. In this study, we determined in the control group that ovariectomy results in a decrease in the activity of the adrenal cortex.

Previous studies have shown that estrogen replacement increases voluntary physical activity independent of changes in food intake and body weight. While much work has been carried out to determine the effects of hormone replacement on reproductive organs in post-menopausal women, no study has examined ovariectomy effects on adrenal gland. Our study may be source for further research examining the relationship between estrogen and adrenal gland in post menopausal women.

Other experimental studies reported the results of endocrinological examinations in rats administered with anabolic-androgenic steroids and also physical changes.[20-21] In their pathological evaluation, the heart and adrenal gland were severely damaged. Although the net wet weight of the adrenal glands of rats was unchanged, vacuolar degeneration could be observed within the zona reticularis. The compact cells in the upper margin of the zona fasciculata tended to be dominant.[22]

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