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Estrogen as primary factor in vaginal healing in rats

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Abstract. – OBJECTIVE: Although the effect of estrogens on wound healing is already known, its complex mechanism is not fully understood in literature. The aim of this study is to investigate the effect of estrogen on vaginal healing after surgical intervention performed in the age group with low estrogen level and in an adult group with high estrogen level.

MATERIALS AND METHODS: Seven young and seven adult female Wistar Albino rats were procured. For control group, one animal was chosen each from the young (Group I) and adult groups (Group II), and their vaginal tissue was removed. An incision was made to the posterior vaginal wall under anesthesia and sutured with 5-0 polyglactin in all the rats. On the seventh postoperative day, the posterior vaginal wall was excised. A semi-quantitative method was used to evaluate the histological processes and structures during wound healing.

RESULTS: Although there were no evident differences in the evaluation of histological scoring system, the presence and distribution of new vascularization and fibroblasts showed that vaginal mucosal healing was more intense in adult rats.

CONCLUSIONS: The effect of estrogens on vaginal mucosal healing has been discussed in several experimental studies and literature information has been presented; it has been concluded that it would be beneficial to consider the positive effect of vaginoplasty procedures.

Key Words:
Estrogen, Wound healing, Feminizing genitoplasty, Disorders of sex development.

Introduction

Wound healing is a complex mechanism and consists of a few stages: inflammation-proliferation and remodeling[1]. The effect of estrogens on wound healing has been known for a long time and despite more than seventy years of animal experimentation and extensive knowledge[2], its mechanism is not fully understood. Recently, estrogen has been reported to affect every stage of wound healing[3].

Researchers have generally focused on the negative effects of the absence of estrogen, especially skin thickness, mucosal, or pelvic organ prolapse in postmoneauposal period[4].

This study aims to investigate the effect of estrogen on vaginal healing after surgical intervention performed in the young rat group with low estrogen levels and in an adult group of rats with high estrogen levels without any endocrinological intervention.

Materials and Methods

The legal ethical permission was given by the Animal Ethical Committee of the Gazi University and all procedures were carried out in accordance with the approved guidelines (G.U.ET-20.019).

Seven pups (age: 25 days and weight: 30-40 g) and seven adult female Wistar Albino rats or does (age: 8-10 weeks, weight: 200-220 g) were purchased from Gazi University Center for Experimental Research (GUDAM).

Before the procedure, anesthesia was inducted with intramuscular administration of Xylocaine® (Astrazeneca, Sweden) (5 mg/kg) and ketamine (45 mg/kg). Scarification was performed with cardiac blood extraction under deep anesthesia.

For the control group, one rat was taken each from the (young) pup and (grown-up) adult groups, and their vaginal tissue was removed under anesthesia and kept in 10% formalin saline; their blood samples were taken for estrogen, and then, the rats were sacrificed.

The remaining 12 subjects were divided into two groups. The young rat group (Group I) and

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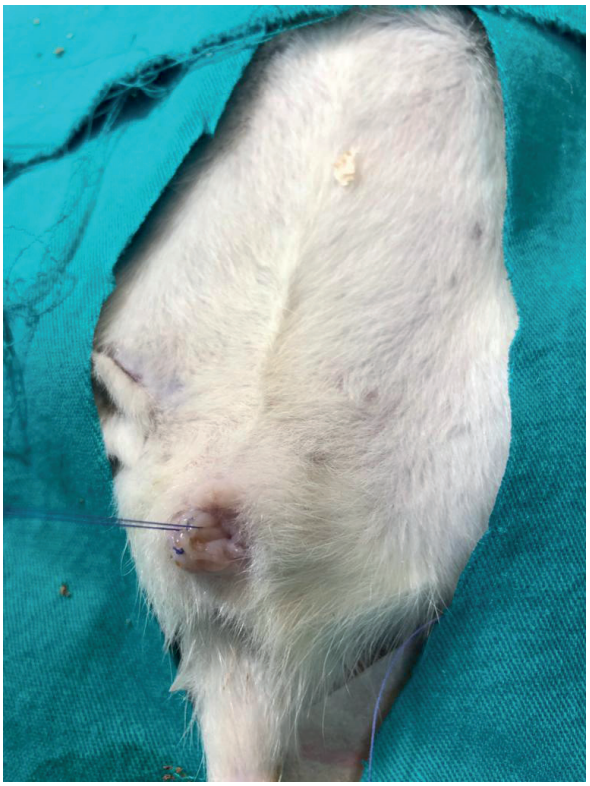


Figure 1. A vertical incision was made to the vaginal posterior wall under anesthesia and sutured with 5–0 polyglactin.

the adult rat group (Group II) each consisted of six rats.

The vaginal introitus of pups were identified by the presence of a closed thick connective tissue that acts as a barrier and protects the vagina. In the rats of both groups, a vertical incision was made to the vaginal posterior wall under anesthesia and sutured with 5-0 polyglactin (Figure 1). After the procedure, these animals were nursed in the center. On the seventh postoperative day, all rats of both groups were anesthetized with the same procedure. The posterior vaginal wall, which was previously incised and sutured, was excised and sacrificed. Tissue samples were stored at 10% formalin saline.

Specimens of 4-5 μm thick were taken for histological examination, and histological staining was performed with hematoxylin and eosin. A semiquantitative method was used to evaluate the following histological processes and structures: re-epithelization, polymorphonuclear leukocytes (PMNL), fibroblasts, and new vessels. Histological sections were evaluated according to the following scales: 0, 1, 2, 3, 4 (Table I)[3].

Ethical Statement

The legal ethical permission was given by Animal Ethical Committee of the Gazi University and all procedures were carried out in accordance with the approved guidelines (G.U.ET-20.019).

Statistical Analysis

For each parameter, mean values ± SEM were calculated. Data from the semiquantitative evaluation were compared by using the nonparametric Mann-Whitney U test by IBM SPSS Statistics Subscription 1.0.0.1347 for Mac (Armonk, NY. USA). A value of $p < 0.05$ was considered significant.

Results

Histological Examinations

In the control group (pups and adults), a full-thickness squamous epithelium was present. Inflammation and new vessel formation were not observed. Horizontally extending spindle-shaped fibroblasts were available.

In Group I, re-epithelization was completed in all cases. Keratinization and crust were noticeable in only one subject. In other cases, the epithelium was nonkeratinized. Inflammation was observed in all cases. PMNL was observed in the epithelium in only one subject, while mild PMNL was observed in the stroma in three subjects. In half of the subjects, neovascularization was formed, mild intensity in the granulation tissue was observed in one subject under the epithelial area, and no new vessel formation was observed in one subject. Spindle fibroblasts were observed both in the horizontal and vertical planes in half of the subjects and only in the horizontal plane in the other half.

In Group II, re-epithelization was completed in all subjects. Half of the subjects had keratinization. Inflammation was present in five of the subjects and observed in the epithelium only in one subject and in the stroma in rest of the subjects. New vessels were observed in five of the six subjects, and these vessels were located in six epithelial areas in two of the subjects and in the granulation tissue in three subjects. No neovascularization was observed in one subject. In one of the subjects, bulging (new) fibroblasts were observed, while in the other four subjects, spindle-shaped fibroblasts were observed both in the horizontal and vertical planes and in one subject in the horizontal plane (Table II).

Blood estrogen level was resulted in < 5 pg/mL in pups and 9.82 pg/mL in adult rats.

Table I. Scales used in the semi-quantitative evaluation of histological sections.

Scale	Epithelization	PMNL	Fibroblasts	New vessels
0	Thickness of cut edges	Absent	Absent	Absent
1	Migration of cells (< 50%)	Mild ST	Mild ST	Mild SCT
2	Migration of cells (≥ 50%)	Mild DL/GT	Mild GT	Mild GT
3	Bridging the excision	Moderate DL/GT	Moderate DL/GT	Moderate GT
4	Keratinization	Marked DL/GT	Marked DL/GT	Marked GT

ST, surrounding tissue, i.e., tissue out of GT; DL, demarcation line; SCT, subcutaneous tissue; GT, granulation tissue; PMNL, polymorphonuclear leukocytes.

Discussion

The effect of estrogen on wound healing, which is a complex mechanism, has been investigated by performing ovariectomy and/or by giving estrogen externally. Experimental studies have been conducted on the effectiveness of estrogen in different subjects such as postmenopausal, mucosal, or pelvic organ prolapse[6]. However, an experimental study on the effect of estrogen on vaginal mucosal healing without ovarian intervention in the preadolescent and adult period has not been found in the English literature.

It has been shown in studies[7] that estrogen deficiency disrupts coagulation and hemostasis, thus playing an important role in wound healing, and the presence of "estrogen-mediated signaling transduction" in the entire inflammatory process.

Estrogen plays an important role in wound healing by taking part in re-epithelialization, granulation, proteolysis, and inflammatory response in skin cells. In postmenopausal women, the process has been shown to be corrected by replacement. In addition, topical estrogen application *in vivo* and *in vitro* is known to affect skin healing[4].

Two types of estrogen resets, α and β, have been identified on the skin. Estradiol affinities are similar. However, the tissue specifications are different, type β is more common in the skin[8]. Although both receptors are located in the lower urinary tract, bladder, and vagina, type α is mostly found in this region. Alpha estrogen is found in all vaginal layers (epithelium, lamina propria, and muscularis)[9]. Studies[8] show that α receptor plays an important role in cancer of the reproductive organs.

Alpha estrogen receptor has an important role in female reproductive phenotype maturation. It is also known to trigger cell proliferation in the uterus. Alpha rectifiers regulate mitosis and differentiation with estrogen stimulation. In female mice, in the absence of estrogen receptors, the uterus has been shown to remain hypoplastic. Again, in these mice, vaginal tissue has been observed to be immature and non-cornified[9].

The vaginal layer consists of healthy cells in reproductive aged women. Estrogen promotes the growth and development of these cells so that the vaginal epithelium remains thick and moist, and the vagina remains flexible and elastic[10].

In laboratory studies, it was shown that vaginal epithelial thickness, smooth muscle tissue, and vaginal blood flow decreased in rabbits with ovaries removed. After menopause, vaginal fragility increases, mucosal stratification decreases, and sensitization of the epithelium to abrasion increases. Estrogen replacement corrects this situation[11].

Rahn et al[12] reported that preoperative vaginal estrogen application increases the synthesis of mature collagen in the postmenopausal period,

Table II. Demographic and histological evaluation of groups (mean values ± SEM).

	Blood estrogen level	Age	Reepithelization	PMNL	Fibroblasts	New vessels
Group I	< 5 pg/mL	25 days	3.25 ± 0.250	1.75 ± 0.250	3.50 ± 0.289	1.25 ± 0.479
Group II	9.82 pg/mL	8-10 weeks	3.50 ± 0.224	1.50 ± 0.342	3.00 ± 0.258	1.33 ± 0.333
			p = 0.453	p= 0.693	p= 0.223	p= 0.908

PMNL: polymorphonuclear leukocytes.

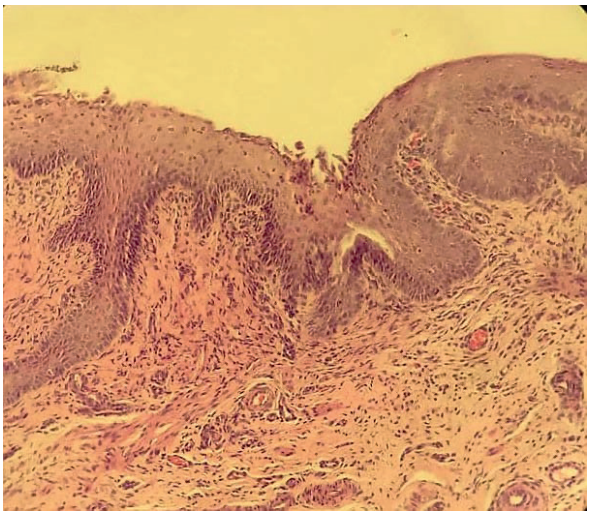


Figure 2. There are spindle-shaped fibroblasts lying in the horizontal plane in a adult rat. (Score 4)(with Hematoxylin-Eosin, Magnification x 200).

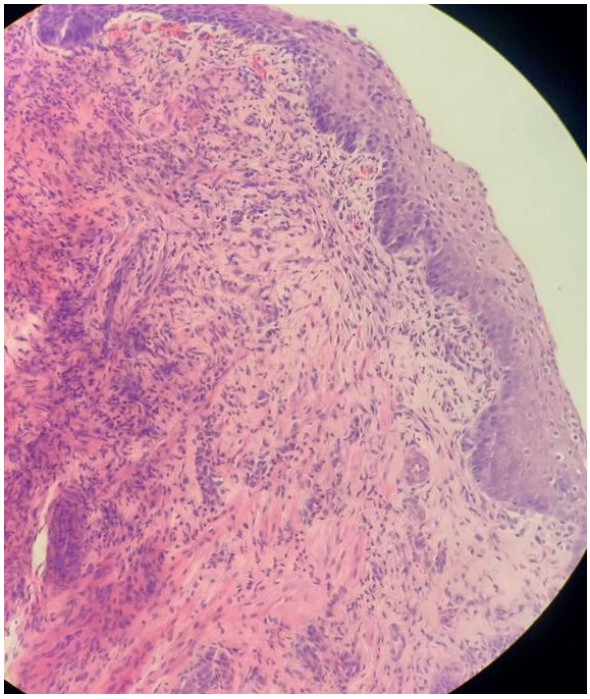


Figure 3. Reepithelialization was completed in the incision area, and the epithelium was bridged. Keratinization was noted in a adult rat. (Score 4) (with Hematoxylin-Eosin, Magnification x 200)

decreases degrading enzyme activity, and improves connective tissue integrity for surgical suture placement during surgical repair to increase the thickness of the vaginal wall.

In this study, although there are many factors affecting wound healing, it was aimed to determine the effect of low estrogen and normal estrogen levels on vaginal mucosal healing by considering the age groups of the subjects and by looking at their blood levels. Although there are no evident differences in the scoring of histological evaluation, the presence and distribution of neovascularization and fibroblasts show that vaginal mucosal healing is more intense in adult (Figures 2, 3). This situation was attributed to the low estrogen effect found in baby rats in the blood analysis. This result is consistent with the results of clinical and experimental studies on estrogen and wound healing.

There are certain limitations of this study: (1) difficulty in using a large number of subjects and (2) the inability to study the polymerase chain reaction, between hydroxyproline and collagen for technical reasons.

One of the issues of intense debate in individuals with disorders of sexual development is related to the timing of surgical correction[13]. Advocates of early surgical correction think that it can be performed as soon as the diagnosis is made[14]. Those who are prodelaying surgical correction think that the procedure should be performed under optimal conditions, since complications are common in early surgical procedures and correction is often required at advanced ages. Akbıyık et al[15] argued that it is necessary to wait for favorable conditions to perform the surgical correction in their series of 41 cases. One major advantage of estrogen is its support to the vaginal tissue and the surgical advantage it brings.

Conclusions

The effect of estrogens on vaginal mucosal healing has been discussed in the light of the experimental study outputs and literature information presented. Although it has been concluded that it would be beneficial to consider the positive effect of estrogen to vaginoplasty and surgical procedures, decisions on timing of surgical correction of human disorders of sexual development, may came essentially from experimental and observational data on humans and just secondly from animal experimental data.

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Conflicts of Interest
The authors declare no conflicts of interest.

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