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Effects of Ovariectomy and Estrogen Replacement on Rat Tongue Mucosa

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Objective: The aim of this study was to investigate the possible effect of sex steroid deficiency on the rat tongue mucosa as well as the effect of its replacement.
Methods: We chose 21 sexually virgin female Wistar albino rats in this study. We divided the model rats into 3 groups: Group-1:Control, Group-2: Sham-operated (bilaterally ovariectomized), and Group-3: Sham-operated, in addition we gave estrogen to Group-3. The tongue mucosa of ovariectomized rats was compared to that of control rats histologically. The same evaluation was also performed after hormone replacement therapy for the ovariectomized rats (Group-2,3).
Results: We found that, the ovariectomized animals presented reduced thickness of the tongue epithelium with an irregular keratinized surface. The tongue epithelium thickness was increased after hormone replacement therapy.
Conclusions: The findings demonstrate that in the rat, ovarian estrogens play an important role, and its deficiency possibly leads to the onset of tongue discomfort in ovariectomized rats.

Key Words: Ovariectomy, Estrogen, Tongue epithelium, Rat

Şıçanlarda Ovarektomi ve Östrojen Kullanımının Dil Mukozası Üzerine Olan Etkileri

Amaç: Çalışmada amacımız, östrojen eksikliği oluşturulan ve bu eksikliğin giderilmesiyle sıçan dil mukozasında meydana gelen değişikliklerin araştırılmasıdır.
Gereç ve Yöntem: Çalışmamızda 21 adet dişi erişkin Wistar albino cinsi sıçanlar kullanıldı. Sıçanlar, üç gruba ayrıldı. Grup-1: Kontrol grubu, Grup-2: Sham-operasyon(bilateral ovarektomi), Grup-3: Sham operasyon ve östrojen verilen grup idi. Kontrol grubu ile ovarektomi edilen grubun dil mukozası histolojik olarak karşılaştırıldı. Aynı karşılaştırılma işlemi, hormon kullanımından sonra ovarektomi gurubu ile de yapıldı (Grup-2, 3).
Bulgular: Ovarektomi edilen sıçanların dil epitel yapılarının düzensiz, keratinize ve kalınlığında azalma olduğu gözlemlenmesine karşın; hormon kullanımından sonra epitel kalınlığının artışı gözlemlenmiştir.
Sonuç: Bu bulgularımız, ovariumlardan salgılanan östrojenlerin önemli rolleri olduğunu ve eksikliklerinde dişilerde oral bozukluklara yol açabileceğini göstermektedir.

Anahtar Kelimeler: Ovarektomi, Östrojen, Dil epiteli, Sıçan.

Oral discomfort such as burning mouth syndrome,[1] oral dryness and dysgeusia are observed especially in postmenopausal women.[2] Half of the patients respond to hormone replacement therapy (HRT),[3] and the responders are known to have estrogen receptor on the oral mucosa.[3-5] These facts suggest that alteration of the sex steroid level may have an important role in the onset of oral discomfort in, at least, a substantial population of the postmenopausal women. However, few reports have dealt with the influence of the sex steroid on the oral mucosa,[6] and no direct evidence has been presented to date.

Estrogen replacement is frequently the treatment of choice for maintaining reproductive function and bone mineral density in post-menopausal women and amenorrheic adolescents. While estrogens effects on the reproductive system and bone are well established, less is known about how it affects other tissues.[7-9]
The lingual dorsal epithelium of adult mammals is generally composed of regularly ordered columns of cells with different degrees of keratinization, namely the anterior cell columns of the filiform papillae, the posterior cell columns of the filiform papillae and the interpapillar cell columns.[10-12] The epithelium of the posterior cell columns of the filiform papillae shows hair-like, hard keratinization in most of the mammals examined, and the epithelium of

the anterior cell columns of the filiform papillae shows the newborns skin – like soft keratinization. In rodents, the interpapillar epithelium shows very weak keratinization.

Which may be identical with parakeratinization; however, in many mammals other than rodents, this area shows no evidence of keratinization.[13]

In this study, we tried to clarify the effect of sex steroid deficiency on rat tongue mucosa as well as the effect of its replacement. First, tongue mucosa of ovariectomized rats was compared to that of control rats histologically. The underlying mechanisms were then investigated from the histological point of view.

MATERIALS AND METHODS

200-220g in weight, 21 sexually virgin female Wistar albino rats were obtained Department of Medical Science Application and Research Centre of Dicle University (DÜSAM). The animals were housed in individual cages and received a standard diet for rodents and tap water ad libitum. The room temperature and humidity were maintained at 22° C and 60 %, respectively. The light cycle was fixed at 12h.Twenty-one of these animals were divided into three groups randomly, an Control Group (Group1), Sham-operated Group (Group 2) and Sham-operated & an hormone replacement therapy (HRT) Group (Group3).

Control group, the animals of this group did not receive ovariectomy nor did they receive estrogen treatment. Animals in group control were given an intraperitoneal injection with 0.1 ml of carrier alone-1:3 mixture of 100% ethanol and 0.9 % saline W/V (Boots CO., Australia) each day for 3 days**.** Carrier A injection was given in order to create the stress environment as in the two other animal groups.

After 1 week acclimatization in an animal house, each group (14 animals) underwent an operation. The animals were anesthetized with intraperitoneal injection of Ketamine HCl (50mg/kg body wt.Parke-Davis) and Xylazine 2 %(100mg/kg body wt.Rompun-Bayer).In the sham-operated group(group2), and sham-operated & HRT group(group3) bilateral ovariectomy were performed by the dorsal approach as described previously.[14]

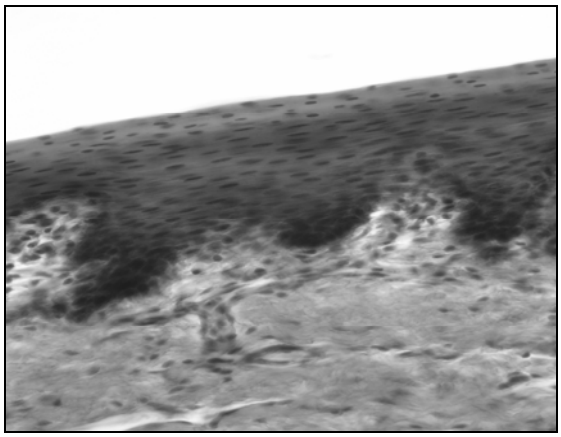
Then, Group2 rats were subcutaneously injected with equivalent amount 0.1 ml of a 1:3 mixture of benzyl alcohol and peanut oil, each day for 3 days. Animals in group 3 rats were injected with 0.1 ml carrier containing 1.0μg 17 β Oestradiol (1, 3, 5[10]-estratriene-3,17β-diol, Sigma).

At the time of excision 2 months after ovariectomy, the animals were sacrificed with overdose anesthesia, and the tongue from each animal was removed from the front part of the circumvallated papillae, cut at midline, and immediately fixed with 4% buffered formalin solution. After fixation, the specimens were dehydrated by a graded alcohol series and embedded in paraffin with the midline contacting the bottom. Five-micrometer sections were cut from midline, and the cutting directions were adjusted perpendicular to the surface of the tongue. The paraffin sections were stained with Hematoxylin-Eosin, and Hematoxylin-Van Giesson. The stained sections were later evaluated with light microscopy, and photographed by Nikon Eclipse-400 examination microscope.

RESULTS

In our study we observed, in the control group the mucous membrane is smooth on the lower surface of the tongue. The tongue's is lined with nonkeratinized stratified squamous epithelium. The lamina propria has papillae; similar to those in the dermis of the skin (Figure 1). The tongue's dorsal surface is irregular, covered anteriorly by a great number of small eminences called papillae (Figure 2).

Figure 1. Micrograph of the lower surface of the tongue from control rat. The tongue is lined with non keratinized stratified squamous epithelium (Hematoxylin-Eozin X400).



In the sham-operated group, the lower surface epithelium was remarkable keratinization and the microscopic papillae depth was decreased (Figure 3-4). It was determined that, the tongue epithelium was apparently smaller in the sham-operated animals than

that in the control animals (Figure 3-4). Sham-operated animals papillae filiformis were determined irregular structure (Figure5).

Figure 2. Micrograph of superior surface of the lingual epithelium from control rat. (Hematoxylin-Eosin X100)

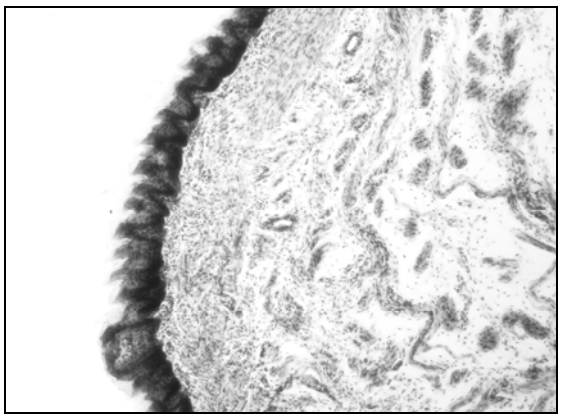
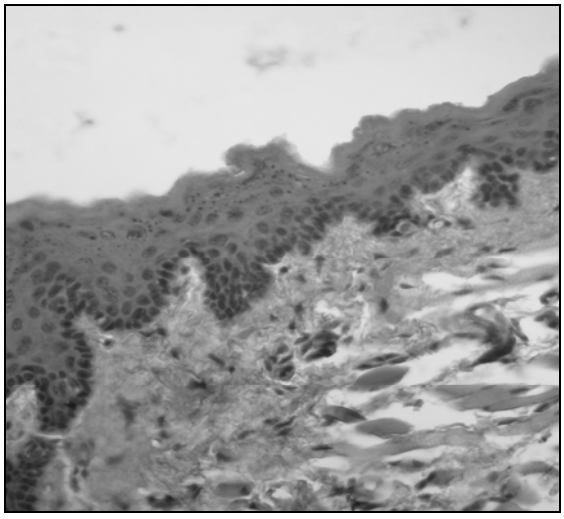


Figure 3. Micrograph of the lower surface of the tongue from a Sham-operated rat. The keratinized surface epithelium and irregular microscopic appearance of papillae were seen (Hematoxylin-Eosin X200).



On the other hand, effect of hormone replacement therapy on the mucosal thickness was investigated in this animal model. In the Sham-operated & HRT group, that filiform papillae were keratinized and regular structure (Figure 6). Also, while continuously keratinized the tongue epithelium and thickness was increased the lower surface epithelium (Figure 7). In the dorsum of the tongue, the epithelial thickness and papillae height increased in the sham-operated & HRT group (Figure 8).

Figure 4. Micrograph of the lower surface of the tongue from a sham-operated rat. The keratinized surface epithelium and the thickness of the tongue epithelium was decreased (Hematoxylen-Van Giesson X200).

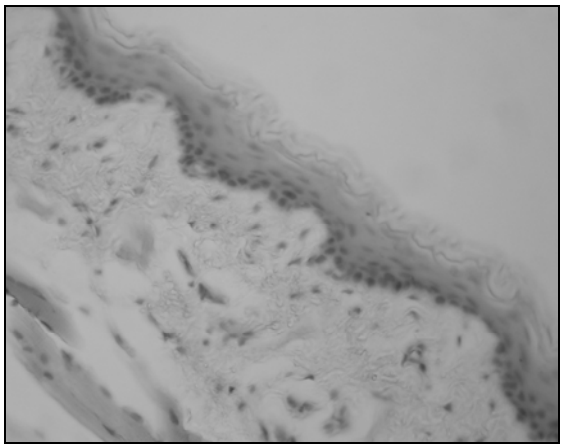
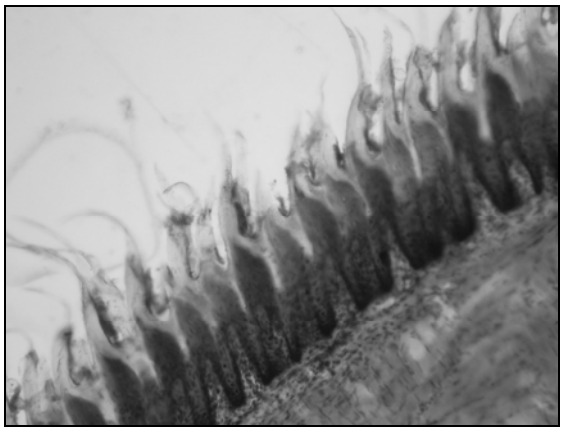


Figure 5. The appearance of papillae filiformis in the sham-operated group. The papillae filiformis were determined keratinization and irregular structure (Hematoxylin-Eosin X200).



DISCUSSION

To prove the role of a hormone in the pathogenesis of any disease, firstly, hormone must first exist in the target tissue. Second, the hormones specific receptor must reside in the target tissue. Third, the absence of the hormone in the target tissue should contribute to the onset of the disease. Finally, the replacement of the hormone should induce the response. It is known that sex steroids exist in saliva.[6] Since their levels change according to the systemic levels, the oral mucosa is affected by the sex steroid in saliva as well as the systemic levels. Also in the oral mucosa, the existence of sex steroid hormone receptor has been reported in postmenopausal women.[4,5] Adult ovariectomy caused loss of a number of secreted

protein products of the parotid and submandibular glands in the saliva of female rats.[15] Female rat submandibular gland contains almost four times the amount of oestrogen receptor as the parotid gland.

Figure 6. The appearance of papillae filiformis and tongue epithelium in the sham- operated & estrogen replacement therapy group. The filiform papillae regular keratinized structure and the thickness of tongue epithelium also increased after hormone replacement therapy (Hematoxylin-Eosin X200).

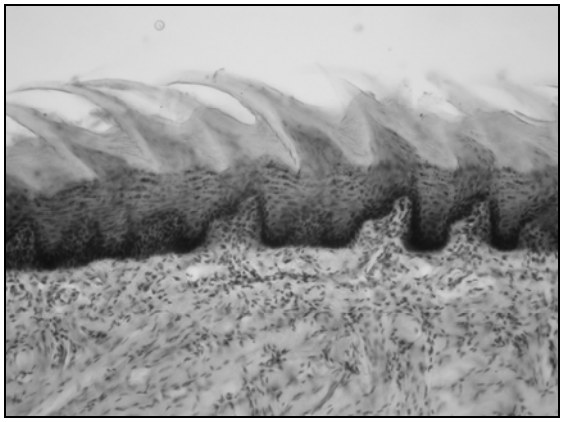
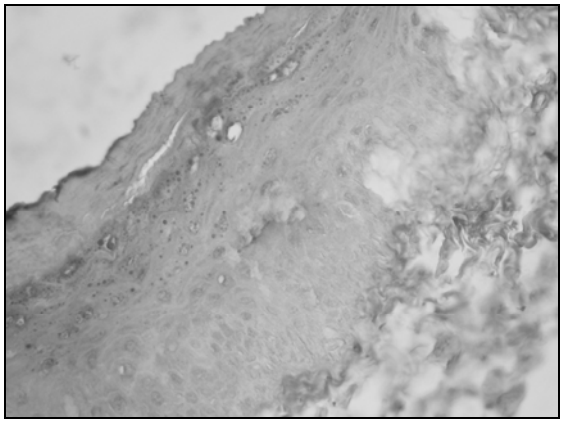


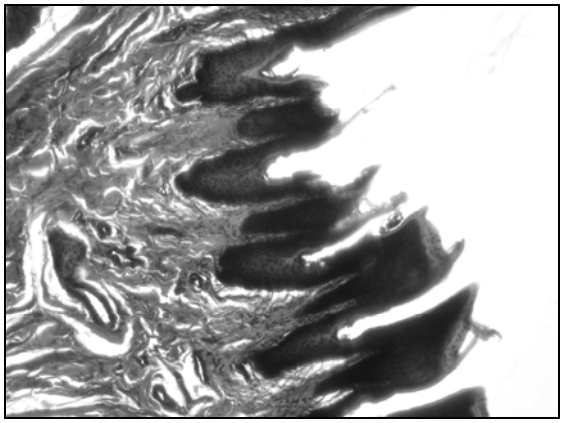
Figure 7. Micrograph of the lower surface of the tongue from a sham-operated & Estrogen group. The thickness of tongue epithelium increased after hormone replacement therapy and continuous keratinization.(Hematoxylen-Van Giesson X400).



Campbell et al. (1990) have proposed that the presence of oestrogen receptor on salivary gland cells may be responsible for promoting gender differences in the composition of saliva in response to the female reproductive cycle.[16] Accordingly, we attempted to clarify the effect of a sex steroid hormone deficiency as well as the effect of replacement in the tongue epithelium of ovariectomized rats.

The results of this study suggested that estrogen deficiency might cause a mucosal change in tissue level. The thickness change was a focus of this study, and it was investigated until 2 months after ovariectomy. Clinically, the histological change in oral mucosa has not been well studied in postmenopausal women. However, in the genitourinary system, the thickness of the mucosa was affected by the levels of sex steroid.[17,18] Both the oral and vaginal mucosa are similar in some respects, both presenting pluristratified epithelium.[2] In our previous suggested that, the length of uterinal and vaginal epithelium were decreased in the bilaterally ovariectomised group. After oestrogen treatment the length of uterinal and vaginal epithelium were increased.[19, 20] If a similar mechanism were present in the tongue mucosa, the level of sex steroids could alter the thickness of the tongue mucosa.

Figure 8. Micrograph of the superior surface of the lingual epithelium from sham-operated & estrogen replacement therapy group. Animals presented keratinized surface and the papillae length were increased. Also, the microscopic papillae was apparently to get deeper and irregular epithelial downgrowth (lete-peg) height was increased. (Hematoxylin-Eosin X200)



In this study, the thickness of the tongue epithelium was significantly smaller in ovariectomized rats and apparently keratinization. The underlying mechanism of this histological change; one possible explanation could be the proliferative activity of epithelial cells. Recent investigations demonstrated the interesting role of estrogen, which accelerates the synthesis of epidermal growth factor, in the uterus and prostate.[17,18] Also, Vittek et al. have reported that estrogen affects cellular proliferation, differentiation and keratinization of the gingival epithelium in the rat.[21]

The results of hormone replacement therapy in the present study showed that the replacement of estradiol could compensate for the change at least in some region of the tongue epithelium. Actually,

epithelial cell proliferation is a complex process which involves numerous growth factors and cytokines. However, there should be a mechanism which connects the level of sex steroids and the histological change of mucosal epithelium. The relationship between sex steroids and the mucosal thickness is not clear in humans. Even were there a change in mucosal thickness in humans, it would not necessarily involve a relationship between sex steroids and the onset of oral discomfort. However, the thin or histologically weak mucosa might exert some negative effect on the sensory nevre endings.

Also, Kazuhide et al. have suggested that, ovariectomized animals presented an irregular corneal (keratinized) surface and the epithelial thickness was apparently smaller in the ovariectomized animals than that in the control animals.[22]

The results of our animal experiments suggest the possible extrapolation of our hypothesis to human patients. Although there is a species difference in the histology and hormonal receptors between human and rat, our results suggest the direct effect of sex steroids in postmenopausal women. These finding indicated overectomy leads to an increase tongue epithelium disorders as well as unique ability to alters cell and surface histology.

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