

The effects of ovariectomy on the submandibular gland in young female adult rats

Ergin dişi sıçanlarda submandibular bez üzerine overektominin etkileri

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Summary

Aim: The salivary gland is one of the target tissues of estrogen. The aim of the study was to investigate the effects of ovariectomy on the stimulated salivary flow rate and morphology of the submandibular gland in sexually mature young rats.

Material and Methods: 72 Female Wistar albino rats of 15 weeks of age were used in this study. Submandibular ducts were cannulated intraorally with polyethylene tubes and stimulated salivary flow rates were determined at 1st, 2nd, 4th, 8th, 12th and 16th weeks after the ovariectomy. Histological sections of the submandibular glands were stained and assessed under a light microscope. The diameters of the acinar cells and parenchyma/stroma ratio were calculated using a software system.

Results: The stimulated submandibular flow rate was found to be significantly decreased at 1st, 2nd, 4th, 8th, 12th and 16th weeks after the ovariectomy. A decrease in the parenchymal structures and acinar cells, an increase in the interstitial connective tissue and fatty degeneration of the gland were observed.

Conclusion: Ovariectomy leads to a decrease in stimulated submandibular salivary flow rate. The changes in gland morphology may be responsible for the decrease in salivary flow rate in young adult rats.

Key Words: ovariectomy, stimulated saliva, rat submandibular gland.

Özet

Amaç:Tükürük bezi östrojenin hedef dokularından birisidir. Bu çalışmanın amacı cinsel yönden yetişkin genç sıçanlarda submandibular bezin morfolojisi ve uyarılmış tükürük akış hızının üzerine overektominin etkilerini incelemektir.

Yöntem ve Gereç: Bu çalışmada 15 haftalık 72 dişi Wistar albino sıçanı kullanılmıştır. Submandibular kanallar polietilen tüplerle intraoral olarak kanule edilmiştir ve overektomiden 1, 2, 4, 8, 12. ve 16. hafta sonra uyarılmış tükürük akış hızı tayin edilmiştir. Submandibular bezlerin histolojik kesitleri boyanmış ve ışık mikroskobu ile değerlendirilmiştir. Asiner hücrelerin çapları ve parankima/stroma oranı bilgisayar yazılımı kullanılarak hesaplanmıştır.

Bulgular: Uyarılmış submandibular akış hızının overektomiden 1, 2, 4, 8, 12 ve 16 hafta sonra belirgin derecede azalmış olduğu bulunmuştur. Parankimal yapılarda ve asiner hücrelerdeki azalma, intertisyel bağ dokusu ve bezlerin yağlı dejenerasyonunda artış gözlenmiştir.

Sonuç:Overektomi, submandibular tükürük akış hızında bir düşüşe neden olmaktadır. Bez morfolojisindeki değişiklikler genç yetişkin sıçanlarda tükürük akış hızındaki düşüşten sorumlu olabilir.

Anahtar Kelimeler: Overektomi, uyarılmış tükürük, sıçan submandibular bezi.

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Introduction

Hormonal changes in females may affect the physiology of the entire body including the oral cavity. Menstrual cycle (1, 2), pregnancy (3, 4), menopause (5, 6), hormone replacement therapy (7), and use of oral contraceptives (8) may induce short-term changes in salivary flow rates, buffering capacity, and biochemical composition.

Ovariectomy (OVX) was shown to cause morphological changes in various regions of the salivary glands in animals (9-13). The effects of OVX on the wet weights of the submandibular (SM) glands, which is asserted to have a correlation with the flow rate (14), is controversial (9, 13, 15,16).

The goal of the present study was to investigate the effects of OVX on the morphological characteristics and stimulated salivary flow rate of the SM gland in sexually mature young rats.

Material and Method

Experimental design:

72 Female Wistar albino rats, 14-16 weeks old, weighing between 240-260 g were used in this study. Animals were housed in groups of 6 animals per cage, and in a temperature ($21 \pm 1.0^\circ \text{C}$), humidity (30-70%) and light controlled room (12 hour/12 hour regular light/dark cycle). Rats were fed with standart rat chow and water *ad libitum*. The study was approved by the animal ethics committee of Ege University (Ref no:2010-2).

Estrus cycles were monitored by cytological examination of daily vaginal smears. Each animal's cycle was followed for a minimum of 15 days. Only rats showing at least two consecutive 4-day cycles were used in the experiment.

Animals were randomly divided into two groups; ovariectomized (n=36) and sham operated (SHAM, n=36) rats. Each rat was anesthetized with intraperitoneal injection of Ketamine (75 mg/kg) (Eczacıbası İlaç Sanayi, İstanbul, Turkey) and Xylazine (8 mg/kg) (Bayer, İstanbul, Turkey). Bilateral incisions were performed approximately 1.5 cm below the ends of the limbs. OVX was performed by ligation and excision of the ovaries along the upper horns. In the sham operation, the ovaries were exposed as above and gently manipulated, but not excised.

Following the OVX and sham operation, salivary flow rates were determined at 1st, 2nd, 4th, 8th, 12th and 16th weeks. In order to minimize the diurnal effects on saliva secretions, the saliva samples were accumulated at 8:00 am. Rats were fasted for 12 h before the experiments, but given free access to water.

Rats were fixed in the supine position under Ketamine (75 mg/kg) and Xylazine (8 mg/kg) anaesthesia. Polyethylene cannulae (Intramedic PE-10, Becton & Dickinson, USA) were inserted into the oral openings of SM ducts. In order to stimulate salivary flow, pilocarpine hydrochloride (Sigma Chemical Co., St. Louis, Mo., USA) was dissolved in isotonic saline and administrated intraperitoneally at a single dose of 8 mg/kg of body weight. The samples were collected for ten minutes into micropipettes connected to each cannula to measure the volume. For a complete surgical removal of the gland, a 1 cm incision was made below the mandible. The SM glands were attentively isolated from the sublingual glands and were weighed. The wounds were sutured and closed. Output of saliva was determined gravimetrically and reported as $\mu\text{l/g}$ gland wet weight/min.

Histological Protocol:

The submandibular glands were fixed for 24 h in fixative, and processed for paraffin embedding. After routine processing, paraffin sections of each tissue sample of 5 μm thickness were obtained using a microtome (Leica RM 2145) and stained with routine Haematoxylin and Eosin (H&E) and assessed under a light microscope (Leica DM microscopy 6000B Stuttgart, Germany). Morphometric analysis were performed on SM glands taken from 6 individual rats, and the mean of 25 randomly-selected areas from each slide were determined. The diameters of acinar cells and parenchyma/stroma ratio were calculated using a commercially available software system (Leica Qwin plus V 3.5.0 Leica Microsystems, Stereo and Macroscopy Systems CH 9435 Heerbrugg, Switzerland).

Statistical Analysis:

Because the distribution of the data indicated a nonparametric approach, the Mann-Whitney U-Wilcoxon rank sum W test was used for analysing the independent data groups. The results for unpaired data were compared using Kruskal-Wallis one-way ANOVA by ranks. When the tests were significantly different, (when overall p was 0.05), Dunn's multiple posthoc comparison test was performed. Data are expressed as mean \pm SD. Probability value of less than 0.05 was considered statistically significant.

Results

OVX increased the body weight ($p < 0.05$). Significant age-related gains in the body weight were also observed in the SHAM and OVX groups ($\chi^2 = 14.765$, $p < 0.05$; $\chi^2 = 16.958$, $p < 0.01$). But, OVX did not significantly affect the wet weight of the salivary gland ($p > 0.05$) (Table-1).

Table 1. Body weights (g) and wet weights (mg) of the SM glands in the SHAM and OVX groups at 1st, 2nd, 4th, 8th, 12th, 16th weeks after surgery.

Weeks	Body Weight		Wet Weight	
	SHAM	OVX	SHAM	OVX
1	258 ± 2.4	252 ± 3.1	360.2 ± 5.1	357.8 ± 7.4
2	263 ± 1.8	272 ± 2.2**	356.5 ± 8.0	355.2 ± 8.7
4	277 ± 3.0	280 ± 2.6*	354.3 ± 9.2	354.9 ± 6.1
8	282 ± 2.7	288 ± 3.3*	359.2 ± 6.7	358.3 ± 5.2
12	286 ± 1.9	295 ± 2.0**	356.5 ± 7.9	360.5 ± 4.2
16	290 ± 2.5	320 ± 3.2**	358.8 ± 7.7	361.1 ± 5.0

Data are mean ± SD of six rats.

* $p < 0.05$; ** $p < 0.01$; when compared to SHAM

The stimulate SM salivary flow rate significantly decreased 1 week after OVX ($p < 0.05$), and at the 2nd, 4th, 8th, 12th, 16th weeks when compared to the SHAM (2nd week: $p < 0.05$; 4th week: $p < 0.01$; 8th week: $p < 0.05$; 12th week: $p < 0.05$; 16th week: $p < 0.01$) (Table-2).

Differences between the flow rates with regard to the weeks were significant in the OVX group ($\chi^2 = 20.718$, $p < 0.001$). The significant differences were between the 1st and 12th weeks ($p < 0.05$) and the 1st and 16th weeks ($p < 0.01$). The decrease in flow rates at the 16th week was more than the decrease at the 12th week (12th week: -11%, 16th week: -15%). No significant differences associated with weeks were found in the SHAM group ($\chi^2 = 11.070$, $p > 0.05$) (Table-2).

Normal histological characteristics of the submandibular gland were observed in 1, 2, 4, 8, 12 and 16-week SHAM groups. Parenchyma of the gland showed regular serous and mucous acini with prismatic cells of secretory ducts and well-organized luminal structures (Figure- 1A).

Histology of submandibular glands in the 1, 2, 4, 8, 12 and 16-week ovariectomized groups revealed a time-dependent loss of normal histological appearance and reduction in parenchymal structures. Decrease in the amount of acinar cells in the parenchyma was accompanied by a marked increase in the number and mass of fat cells in the stroma. A prominent increase in the interstitial connective tissue of the stroma, and an irregular organization in the lumens of secretory ducts were observed (Fig 1B, C, D, E, F, G).

The mean diameters of the acinar cells in the SHAM and OVX groups at 1st week were determined as $24.05 \pm 1.71 \mu\text{m}$; $24.37 \pm 1.02 \mu\text{m}$, respectively, and did not change at the 2nd, 4th, 8th, 12th and 16th weeks ($p > 0.05$) (Table-3).

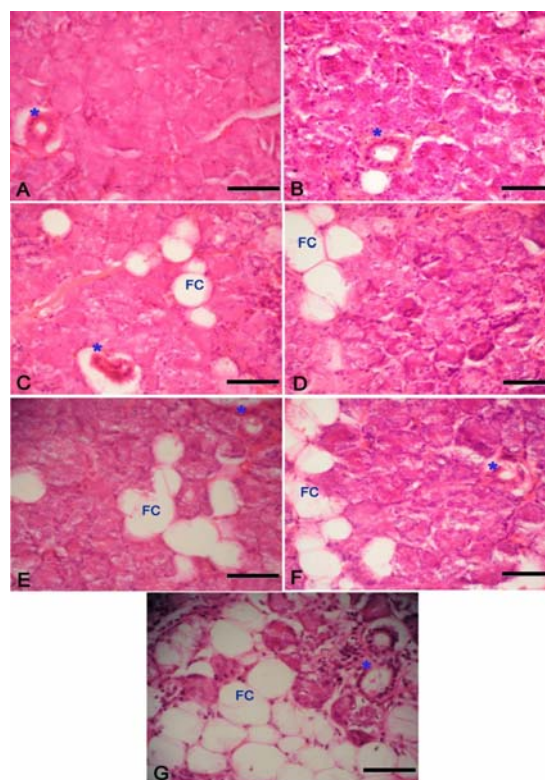


Figure-1. Histological appearance of the submandibular gland; (A) in the sham-operated group at 16 weeks after the surgery and in the ovariectomized rats at; (B) 1 week, (C) 2 weeks, (D) 4 weeks, (E) 8 weeks, (F) 12 weeks and (G) 16 weeks after the surgery. H.E. staining x 40. Bar: 100 μm . Asterixes denote secretory duct. FC; Fatty cell.

Table 2. Salivary flow rates ($\mu\text{l/g}$ gland wet weight/min) of the SHAM and OVX groups at 1st, 2nd, 4th, 8th, 12th, 16th weeks after surgery.

Weeks	Flow Rates	
	SHAM	OVX
1	55.02 ± 7.4	49.87 ± 5.1*
2	54.67 ± 6.1	48.97 ± 3.8*
4	54.85 ± 5.7	49.12 ± 3.8**
8	54.20 ± 9.8	48.25 ± 5.3*
12	51.30 ± 6.0	44.40 ± 6.3*†
16	51.65 ± 9.3	43.47 ± 5.5**††

Data are mean ± SD of six rats.

* $p < 0.05$; ** $p < 0.01$; when compared to SHAM

† $p < 0.05$; †† $p < 0.01$; when compared to OVX 1st week

Table 3. Parenchyma / stroma ratio at 1st, 2nd, 4th, 8th, 12th, 16th weeks after surgery.

Weeks	Parenchyma / Stroma Ratio	
	SHAM	OVX
1	8.50 ± 0.5	7.50 ± 0.5
2	8.42 ± 0.6	6.52 ± 0.4*
4	8.52 ± 0.4	6.02 ± 0.3*†
8	8.45 ± 0.5	3.6 ± 0.5**†
12	8.50 ± 0.4	3.57 ± 0.3**†
16	8.51 ± 0.5	1.58 ± 0.1**††

Data are mean ± SD

* $p < 0.05$, ** $p < 0.01$; when compared to SHAM

† $p < 0.05$, †† $p < 0.01$; when compared to OVX 1st week

Discussion

Stimulated salivary flow rate was determined to be significantly lower than SHAM, in every following week after OVX. Our results suggest that OVX permanently decreases the salivary flow rate and this decrease gradually continues even 12 and 16 weeks after the surgery.

Salivary glands contain sex hormone receptors (17), and the expression of estrogen receptors and the effects of steroid hormones can also be assessed in saliva samples and their salivary concentrations correlate to those in serum (18). Since no studies about the effects of OVX on flow rate have been reported so far, we compared our results with menopausal findings. Our findings were in agreement with the findings of the researchers suggesting that postmenopausal women have lower salivary flow rates (stimulated submandibular saliva and non-stimulated and poliorpin-stimulated whole saliva) than premenopausal women (5-7).

OVX increased the body weight gain significantly, but the wet weights of the salivary glands did not change. These findings are consistent with those of some early reports (13, 16).

Our findings in all ovariectomized groups revealed a time-dependent loss of normal histological appearance. There was a decrease in the amount of acinar cells with marked increase in the number of fat cells. A prominent increase in the interstitial connective tissue of the stroma, a reduction in the parenchymal structures and an irregular organization in the lumens of secretory ducts were observed. Morphological changes caused by OVX found in the earlier studies are the decrease of acini/field and percent of acini and the increase of the diameter of acinar cells (9, 12). Several studies have shown fatty degeneration in glands following OVX (10, 12, 13). All morphological alterations in the SM gland found in the present study may be responsible for the decrease in salivary flow rate after OVX.

12th and 16th weeks following OVX may be asserted as a critical term for the physiology and morphology of the SM gland since we found that the decrease in salivary

flow rate and the histological changes in SM gland revealed a time-dependent course, similar to the irreversible histological alterations detected in SM glands after 16 weeks following OVX in a study performed in mice (11).

It has been suggested that physiological aging leads to a decrease in salivary flow rate as a consequence of parenchymal atrophy, fatty degeneration and decreased volume of acini (19). No significant flow rate differences associated with weeks were found in the SHAM group. Our results may indicate that OVX alone affects the flow rate and morphology of the SM gland in young (adult) rats, probably due to the alteration of sex hormones.

Salivary hypofunction is associated with oral and pharyngeal disorders and requires early diagnosis and intervention. However, the flow rate value below 45% of normal level is defined as salivary hypofunction (20). Xerostomia or "dry mouth" is usually the clinical expression of decreased salivary secretion; however, it may occur without a low salivary flow rate (21). It has been proposed that patients who have a reduction in salivary flow greater than 50%, usually experience "Xerostomia" (22). As a result of our study, we can not suggest that small decreases (10-16%) in stimulated SM salivary flow rate cause important clinical problems, but may potentialize other xerostomic factors. A gradual loss of functional reserve capacity which in normal conditions are responsible for adequate organ functioning within minimum physiological limits, occurs in physiological aging (23), and women are more prone to oral complaints and reduced salivary flow rates (24, 25). Medicated women usually possess lower salivary flow rates when compared to men with the same condition (26). If a decrease in salivary flow rate related to sex hormone deficiency is added to these factors, salivary flow can decrease under the limits that cause various oral complaints and disorders such as pain, burning sensation, taste alterations as well as a predisposed oral environment for burning mouth syndrome, caries, and periodontal diseases which emerge frequently during this period (24, 25).

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