# Is Buccal Epithelium Under the Influence of Sex Hormones?

BUCCAL EPİTELDE SEKS HORMONLARININ ETKİSİ VAR MI?

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#### SUMMARY

Buccal smears were taken during the 45 cycles of 33 females on the days corresponding to follicular, ovulatory, luteal and premenstrual phases of menstrual cycle. Smears were evaluated according to maturation index. Serum progesterone values of luteal phase were determined to differentiate whether the cycle was ovulatory or anovulatory. The percentage of superficial cells of the ovulatory days of ovulatory cycles was found to be slightly higher than thet of the anovulatory cycles. More than one superficial cell peak was observed in a cycle of an anovulation detection.

Key Words: Buccal smear, Sex hormones, Ovulation detection

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The vaginal mucosa is one of the main targets of the ovarian sex hormones. The growth and maturation of the vainal epithelium are regulated by these steroids. Various degrees of maturation and differentiation of the vaginal epithelium are used as a measure of hormonal action.

Estrogen, a trophic hormone of the vaginal mucosa, causes proliferation, maturation and desquamation of vaginal epithelium. It has been postulated that the vesical mucosa, also undergoes similar cyclic changes and this effect of sex hormones could be detected in urine sediment (1). According to this opinion it might be expected that stratified squamous epithelium lining various body cavities could be influenced by the cyclic metabolic changes of women. To investigate this possible effect, we used buccal mucosa as a target tissue in this study.

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## ÖZET

Menstruel siklusun folliküler, ovulatuar, luteal ve premenstruel dönemlerine uyan günlerde 33 kadının 45 siklisu boyunca buccal yayma alındı. Yaymalar matürasyon indexine göre değerlendirildi. Luteal faz serum progesteron düzeyleri değerlendirilerek siklusların ovulatuar olup olmadıklarına karar verildi. Ovulatuar siklusların ovulatuar günlerindeki süperfisyal hücre oranının anovulatuar sikluslara göre daha yüksek olduğu görüldü. Anovulatuar siklusta birden daha fazla süperfisyal hücre piki saptandı. Buccal yaymanın ovulasyon saptanmasında anlamı tartışıldı.

Anahtar Kelimeler: Buccal yayma, seks hormonları, Ovulasyon saptanması

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## **MATERIALS AND METHODS**

From January 1987 to April 1988, 45 menstrual cycles of 33 females from hospital staff were evaluated. The women ranged in age from 19 to 34 years. Sixteen women were unmarried. The menstrual bleeding patterns were between normal ranged expect one oligomenorrheic women. There were no other detectable illness.

Women were informed to stop eating and theet brushing two hours before buccal examination. Ten or more buccal smears were taken and follicular ultrasonography were performed in each woman every other day beginning on the fifth day of menstruation. Optimum days of each phases of cycles (follicular, ovulatory, luteal, premenstrual) were determined by follicular ultrasonography according to follicular size. The day of maximum follicular size were accepted as ovulation day, and the last day before menstruation onsets were accepted as premenstrual day. The days between fifth day of menstruation and ovulation day and ovulation day and premenstrual day were accepted as follicular and luteal phases respectively.

Buccal mucosa were scraped by a wooden spatula with moderate pressure. The sample was deposited an a slide by passing the tip of the spatula and its slanted edge against the surface of the glass. Sliders were fixed in alcoholether (50%-50%) combination, and stained by Papanicolau's technique. All cytologic evaluation were made by the same cytopathologist without knowing any clinical information about the woman and the phase.

Three different counting containing 100 epithelial cells were made in each slide. Because of all other remaining cells were intermediate type, the mean value of superficial epithelial cells were recorded. Slides containing leukocyte infiltration more than 20 leucocytes in one high power field were accepted infective and discarded.

Midluteal blood samples of cycles were obtained and serum progesteron values were measured by RIA. Cycles that had progesteron level less than 5 ng/ml were accepted as anovulatory (2).

The mean superficial cell values of each phases were compared between ovulatory and non-ovulatory cycles using analysis of variance. p<0.05 is accepted as significant.

To investigate and compare the existence of estrogen receptors in the cytoplasms of vaginal and buccal epithelium, we used qeroxidase and antiperoxidase technique. The details of this technique were presented elsewhere (3).

#### **RESULTS**

Six slides were abandoned from evaluation due to intense infective appearance. Eleven cycles of remaining 39 cycles have been accepted anovulatory since their midluteal serum progesteron levels were below the threshold value.

The characteristics of the females and cycles were shown in Table 1. There is no significant difference between two groups in terms of age and parity.

The mean superficial cell values of each phases in both ovulatory and anovulatory cycles were shown in Table 2.

In ovulatory cycles the mean values of superficial cells of each phases were not significantly different from each other. In ovulatory cycles, the mean value of superficial cell of ovulatory phases was significantly higher than that of the other phases (p<0.05).

Cytoplasms of vaginal epithelium were highly positive for estrogen receptors while buccal epithelium showed slight estrogenic activity (Figure 1 and Figure 2).

## DISCUSSION

Cytologic examination of cellular material from the female genital tract is an important diagnostic proce-

Table 1. The characteristics of cycles in ovulatory and anovulatory females

	Ovulatory	Anovulatory
Number of Females	20	10
Number of Cycles	28	11
Mean Parity (SD)	1.42±0.79	0.79±0.57 (NS)
Mean Age (SE)	27.811.68	24.56l1.40 (NS)
Mean Progesteron		
(ng/ml)	9.88+3.56	2.53±1.35

(NS: Not significant)

Table 2. The mean superficial cell numbers of each phases in ovulatory and anovulatory cycles (±SEM)

Ovulatory (TI-20)	Anovulatory (n-11)
10.1 ±4.0	9.014.3
13.2±5.0*	9.4±4.2
9.6+5.0	8.5±3.9
8.0+5.2	10.9±8.0
	13.2±5.0* 9.6+5.0

(\*p<0.05)

dure in obstetrics and gynecology. One of the important applications of vaginal cytology is evaluation of the endocrine status, since the morphology of the vaginal epithelium relects stimulation by the ovarian hormones.

The vaginal mucosa is one of the main targets of the ovarian sex hormones, mainly estrogen, which regulate the growth, maturation and differentiation of the vaginal epithelium, are used as a measure of hormonal action. Superficial cells derive from the superficial layer of the mature epithelium. When stimulated by an adequate amount of estrogens, vaginal epithelium achieves full maturation and a large number of superficial karyopicnetic cells appear in the smear (4).

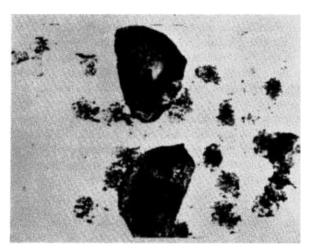


Figure 1. Strong estrogenic activity in the cells of vaginal epithelium

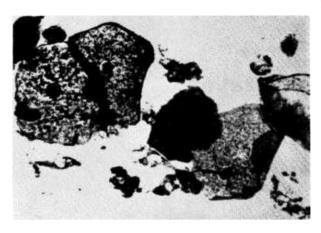


Figure 2. Weak estrogenic activity in the cytoplasm of buccal epithelial cells.

On the other hand, it was reported that the multilayered inner epithelium of bladder has some cyclic changes with vaginal mucosa (1). This old and peculiar finding called our notice to other multilayered epithelium of body cavities whether may show similar changes in accordance with menstrual cycle.

In a preliminary study of us, we had found some parallelism of cyclic cellular changes of buccal smear with vaginal smears (5). The main difference was the percentage of superfiial cells, which were somewhat lower in buccal smears. But the relative number of these cells were higher in presumptive ovulatory days of the cycle than that of the other days.

These findings prompted us to investigate the phenomenon more attentively. To determine the anovulatory cycles, we obtained serum progesterone levels as a marker of ovulation, since a progesterone level greater than 5 ng/ml is considered to be consistent with ovulatory cycles (2).

Our results showed a slight increase of the number of superficial cells in ovulatory days of ovulatory cycles while there was more than one peak of superficial cell count in anovulatory cycles.

Studies of Riley and associates indicate that the ovulatory smear was obtained on the first day of the thermal rise in 29% ovulatory cycles and on the day proceeding the thermal shift in 40% of cases. These investigators regarded vaginal smear method as a practical means of detecting time of ovulation. These outhors established correlation between vaginal smear changes and basal body temperature curve and the value of combining the two methods (6).

Studies correlating variation in vaginal cytology with serum gonadotrophins and progesterone have shown that the karyopicnetic index (KPI) of vaginal cells increased gradually to midcycle and reached a peak on the day following the LH surge. There after, there was a steady decline of the KPI to the end of

the menstrual cycle. The peak of KPI of the vaginal cells coincides with ovulation (4).

It was known that there is a great individual variability of tissue response to hormonal stimulation (7). For this reason, it is not reliable to decide ovulation only with the results of cytology. Confirmatory evidence of ovulation should be obtained from endometrial biopsy (4). For correct timing of the biopsy, serial smears may be helpful, the biopsy should be taken after the estrogenic smear is succeeded by progestational smear.

The effects of estrogen hormone are regulated via cellular receptors. These receptors were identified in many cells of the body including vaginal epithelium at which estrogen has specific biologic effects (Figure 1). We could not find any report that indicates the existence of estrogen receptors in the epithelial cells. We detected weak estrogenic activity in buccal epithelial tissue with our staining technique (Figure 2).

In fact, some effects of estrogens, such as inhibition of bone resorption and some changes in vascular permeability, can not be explained on the basis of reeptors (8). Therefore secondary mechanisms which are effected from cyclic hormonal changes may be responsible to cellular maturation of both vaginal and buccal epithelium.

In our opinion, some cyclic changes has been occurred in epithelial tissue of oral mucosa. Whether these changes can be applicable to clinical practice or has any contribution to the effects of sex hormones must be elucidated by other investigations.

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