Correlation Between Cytological Characteristics of the Nasal Epithelium and the Menstrual Cycle

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Background: There has been considerable controversy concerning the effect of hormones on the nasal epithelium and, in particular, their association with the female reproductive state.

Objective: To investigate the relationship between cytological characteristics of the nasal epithelium and phase of the menstrual cycle.

Design and Subjects: Nasal smears were obtained from 15 women during the menstrual, follicular, and luteal phases, and the abundance of different cell types at each phase was compared with the abundance of equivalent cell types in vaginal smears during the follicular and luteal phases; the nasal smears were also compared with nasal smears from 20 postmenopausal women and 20 prepubertal girls. Epithelial cell counts were conducted by an observer blind to the origin of the samples.

Results: A significant correlation was found between the cytological characteristics of the nasal and vaginal smears and stage of the cycle, with cornified cells predominating during the follicular phase (median, 54%; range, 24%-65%) and rounded or spindle-shaped epithelial cells predominating during the luteal phase (median, 56%; range, 34%-73%). Cornified cells predominated in the nasal smears from the postmenopausal women (median, 71%; range, 60%-77%) and the prepubertal girls (median, 77%; range, 67%-81%) at all times tested.

Conclusion: Cell turnover in the nasal epithelium may be related to hormonal state, and investigation of the mechanisms underlying such change should help in identifying possible functional consequences and in treating nasal symptoms associated with the female reproductive cycle.


THERE HAS BEEN a long history of interest and controversy concerning the effect of hormones on the nasal epithelium.1,2 In women, a range of nasal symptoms has been described in association with hormonal changes occurring during the reproductive cycle. Observations include the appearance or increase in nasal complaints, such as congestion or rhinitis, among women taking the contraceptive pill3-7 and during pregnancy8-13 and histochemical changes in the nasal epithelium of menopausal women.14

With regard to the menstrual cycle, indications of hormone-related changes include morphological alterations in the sustentacular cells in the olfactory epithelium in monkeys15 and women16 and changes in nasal patency17,18 epistaxis,19 mucociliary transport time,2 and sensitivity to histamine.20 However, some investigators have failed to find a correlation between congestion and phase of the cycle, a correlation between fluctuations in olfactory sensitivity and hormonal changes in cycling women, or—despite reports21 that the nasal epithelium is responsive to several hormones—evidence of receptors for estradiol or progesterone in the nasal epithelium.24

Given these varied and sometimes contradictory reports, the present study investigates if a relationship exists between cytological characteristics of the nasal epithelium in women and phase of the menstrual cycle. Cytological studies have been rather few in this field compared with studies of other approaches, and the demonstration and description of such a relationship could prove useful in understanding nasal symptoms associated with the female reproductive cycle and the association between cell turnover and hormonal state more generally.

METHODS

The study proposal and test procedures were reviewed and approved by the ethics committees of the Faculty of Medicine, National University of Mexico, and the Military Central Hospital. Subjects or, in the case of the girls, their parents were informed about the purpose of the study.
study before obtaining their consent to participate. All subjects were residents of Mexico City, and all were healthy non-smokers who had not been using the contraceptive pill for at least 6 months before the study. The menstrual cycle was defined as the time between the first day of one menses and the first day of the next, with the first day of menstrual bleeding taken as day 1. The menstrual phase was defined as days 1 to 5, the follicular or estrogenic phase as days 6 to 14, and the luteal or progestogenic phase as days 15 to 28 or beyond.

STUDY GROUPS

Menstruating Women

Fifteen regularly menstruating women, aged 20 to 43 years (mean, 26.2 years; SD, 5.5 years), whose cycle length ranged from 28 to 30 days (mean, 29 days; SD, 0.3 days) were recruited. Nasal smears were obtained from the subjects once during the menstrual phase, and nasal and vaginal smears were obtained once during the follicular phase and once during the luteal phase.

Postmenopausal Women

Twenty postmenopausal women, aged 58 to 80 years (mean, 69.7 years; SD, 9.6 years), who had had their last menstrual bleeding at least 6 months before the study (mean, 14.2 years; SD, 5.1 years) had nasal smears obtained twice at time points selected to correspond to the time of testing of the menstruating women during the follicular and luteal phases of the cycle.

Prepubertal Girls

Twenty girls, aged 6 to 12 years (mean, 8.8 years; SD, 2.9 years), who had not yet started menstruating were tested as done for the postmenopausal women.

TESTING

This was performed by one of us (E.N.-P.) between 10 AM and 1 PM at the Military Central Hospital. Subjects were seated in a comfortable chair, and a cotton-tipped applicator (Curity Co; Mexico City, Mexico) was gently introduced as deeply as possible into each nostril and rotated in a standardized manner to obtain a sufficient number of shed cells. The applicator was wiped across a glass slide; the smear was allowed to dry, was stained using the Papanicolaou method, was again dried, and was covered with resin and a coverglass. In the cycling women, the vaginal smears obtained during the follicular and luteal phases for comparison with the nasal smears were stained in the same way.

HISTOLOGICAL AND DATA ANALYSIS

Nasal and vaginal samples were analyzed and photographed using a microscope (Polivar) and bright field illumination at ×400 magnification. The analysis was performed by a technician experienced in such procedures, but blind to the origin of the slides (ie, the slides were coded in such a way that no information was available about subject group or phase of the cycle). The first 100 cells to be viewed on each slide were classified as follows: (1) young epithelial cells with a large clearly defined nucleus; (2) large, flat, and irregularly shaped cornified cells; or (3) rounded or spindle-shaped epithelial cells with a small but clearly defined nucleus (Figure).

Nonparametric Friedman 1-way analyses of variance for repeated measures, followed by post hoc Newman-Keuls pairwise comparisons in the case of significance, were used to compare the number of each cell type in nasal and vaginal smears across the menstrual cycle in the menstruating women and in nasal smears from the postmenopausal women and prepubertal girls. α = .05 Was the level of significance. The Spearman correlation coeffi-

Examples of the predominant cell types seen in vaginal smears from cycling women (1) during the follicular (B) and luteal (C) phases; and in nasal smears (2-4) from cycling women (2) during the menstrual (A), follicular (B), and luteal (C) phases and in postmenopausal women (3) and in prepubertal girls (4) at all times tested. A, Young epithelial cells. B, Cornified cells. C, Rounded epithelial cells. Bright field illumination was used (original magnification ×400).
Table 1. Relative Number of Cell Types in Nasal Smears From the Same Women Across the Menstrual Cycle, From Postmenopausal Women, and From Prepubertal Girls*

<table>
<thead>
<tr>
<th>Cytological Category</th>
<th>Cycling Women (n = 15)</th>
<th>Postmenopausal Women (n = 20)</th>
<th>Prepubertal Girls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Menstrual Phase</td>
<td>Follicular Phase</td>
<td>Luteal Phase</td>
</tr>
<tr>
<td>Young epithelial cells</td>
<td>40 (15-61)</td>
<td>8 (6-19)</td>
<td>22 (15-27)</td>
</tr>
<tr>
<td>Large, flat, cornified cells</td>
<td>11 (2-35)</td>
<td>54 (24-65)†</td>
<td>13 (5-18)</td>
</tr>
<tr>
<td>Rounded or spindled epithelial cells</td>
<td>28 (6-54)</td>
<td>19 (14-29)</td>
<td>56 (34-73)†</td>
</tr>
</tbody>
</table>

*aData are given as median (range) of cells. There were 100 cells per smear.
†These values are significantly higher (Friedman analyses of variance, P<.001).

Table 2. Relative Number of Cell Types in Nasal and Vaginal Smears From the Same 15 Women During the Follicular and Luteal Phases of the Menstrual Cycle*

<table>
<thead>
<tr>
<th>Cytological Category</th>
<th>Nasal Smear</th>
<th>Vaginal Smear</th>
<th>Nasal Smear</th>
<th>Vaginal Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular Phase</td>
<td></td>
<td>Luteal Phase</td>
<td></td>
</tr>
<tr>
<td>Young epithelial cells</td>
<td>8 (6-19)</td>
<td>9 (1-12)</td>
<td>22 (15-27)</td>
<td>3 (1-9)</td>
</tr>
<tr>
<td>Large, flat, cornified cells</td>
<td>54 (24-65)†</td>
<td>72 (56-81)†</td>
<td>13 (5-18)</td>
<td>10 (7-18)</td>
</tr>
<tr>
<td>Rounded or spindled epithelial cells</td>
<td>19 (14-29)</td>
<td>16 (8-31)</td>
<td>56 (34-73)†</td>
<td>76 (69-87)†</td>
</tr>
</tbody>
</table>

*aData are given as median (range) of cells. There were 100 cells per smear.
†These values are significantly higher (Friedman analyses of variance, P<.001).

RESULTS

MENSTRUATING WOMEN

As seen in Table 1, distinctive differences were found in the relative numbers of the 3 cell types in the samples obtained from the nasal epithelium during each phase of the menstrual cycle. Although differences in the number of cell types were not statistically significant during the menstrual phase (Friedman test; $F_{2,14}=1.53$, $P=.22$), the follicular phase was characterized by significantly more large, flat, and irregularly shaped cornified cells ($F_{2,14}=8.8$, $P<.001$), and the luteal phase by significantly more rounded or spindled epithelial cells with a clearly defined nucleus ($F_{2,14}=17.8$, $P<.001$). Not surprisingly, this pattern was confirmed by the relative abundance of the 3 cell types recorded across the phases of the cycle. As shown in Table 1, young epithelial cells were more frequently seen in the menstrual phase ($F_{2,14}=5.09$, $P=.03$); large, flat, cornified cells in the follicular phase ($F_{2,14}=6.71$, $P<.001$); and rounded or spindled epithelial cells in the luteal phase ($F_{2,14}=17.8$, $P<.001$). As shown in Table 2, in the vaginal smears from the cycling women, large, flat, cornified cells also predominated during the follicular phase ($F_{2,14}=48.0$, $P<.001$) and rounded or spindled epithelial cells predominated during the luteal phase ($F_{2,14}=33.3$, $P<.001$). A comparison of numbers of the 3 cell types in the nasal and vaginal smears obtained during the follicular and luteal phases showed their relative abundance in the 2 sets of samples to be significantly correlated (Spearman $r=0.54$ [P<.01] and $r=0.40$ [P<.05] for the follicular and luteal phases, respectively).

POSTMENOPAUSAL WOMEN AND PREPUBERTAL GIRLS

Table 1 also shows the cytological characteristics of the nasal smears from the postmenopausal women and the prepubertal girls. At all times tested, large, flat, cornified cells were seen significantly more frequently than the other 2 cell types in both groups ($F_{2,10}=28.9$ and $F_{2,10}=38.1$, respectively; $P<.001$ for both), a profile similar to that seen during the follicular phase of the cycling women.

COMMENT

The findings of the present study are consistent with those of previous reports of a relationship between state of the nasal epithelium and phase of the menstrual cycle, although, because of the different nature of the evidence collected and diagnoses used, a direct comparison of the findings among reports is difficult. However, to our knowledge, this is the first study to show a similar pattern of change in cytological characteristics of nasal and vaginal smears across the menstrual cycle. Although vaginal smears were not obtained during menstruation, it is well-known that young epithelial cells are more commonly seen at this time than at other stages of the cycle, as was seen in the nasal smears in the present study.

Despite the small sample of cycling women (n=15) and the fact that they were only tested across one cycle, we have confidence in the findings for several reasons. Subjects were selected so as to ensure a relatively homogeneous population for what we thought to be the main relevant criteria: changes in the cytological characteristics of the vaginal smears between the follicular and luteal phases conformed to the classic...
and clinically routinely applied description, and nasal smears from the 2 noncycling control groups (postmenopausal women and prepubertal girls) showed relatively homogeneous and apparently unchanging cytological characteristics across testing. Moreover, the cytological profile of these groups corresponded to that of the follicular estrogen-dominated phase, as would be expected in the absence of luteinizing hormone–dependent ovulation, and a subsequent progesterone-dominated luteal phase.

However, it is still not clear whether such changes in the nasal epithelium are because of a direct hormonal effect. Doubt about such effects has been raised in the literature by failure to find a relationship between circulating levels of female sex hormones and nasal symptoms or changes in olfactory sensitivity across the menstrual cycle21,22 and failure to find receptors for female sex hormones in the nasal epithelium of cycling women.21 Nevertheless, the possibility of endocrine effects, even if indirect, is difficult to discount given, for example, the report7 that in the guinea pig, systemic administration of estrogen can increase the density of cholinergic muscarinic receptors in the nasal epithelium and that progesterone can decrease the density of α1-adrenergic receptors, and that the enhanced turnover of vomeronasal receptor cells seen in pregnant mice can be simulated by the peripheral administration of estradiol.23

The functional significance of these findings is also not yet clear, although they suggest several questions for future research. One is whether the cytological changes in the nasal epithelium reported herein include the olfactory epithelium and are related in any way to the persistently reported, but still debated, changes in olfactory sensitivity across the menstrual cycle.22,25 Another is whether cytological changes across the menstrual cycle occur in other tissues and, thus, what such changes might eventually reveal about mechanisms regulating cell turnover more generally. Given the relative ease of access to the nasal epithelium and the relative ease of directly applying substances to it and of obtaining biopsy specimens, it should be useful to investigate more closely factors influencing the marked and rapid changes in the cytological features reported herein.

In summary, the results of the present study indicate a close correlation between phase of the menstrual cycle and cytological characteristics of the nasal epithelium and the female reproductive tract, suggesting that cell turnover in the nasal epithelium is influenced by hormonal state. Further investigation of the mechanisms underlying such change should help in identifying possible functional consequences and in treating nasal symptoms commonly reported in relation to the female reproductive cycle.

Accepted for publication July 18, 2002.

This study was supported by grants DGAPA IN200197, DGAPA IN200999, and CONACYT 25193-N from Convenio Sanidad Militar-Programa Universitario de Investigación en Salud, Mexico City.

We thank Olga Donatti-Albarrán, BA, for technical support, Carolina Rojas Castañeda, BA, for bibliographic assistance, and the Hospital Central Militar for providing the facilities for conducting the tests.

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