A Prospective, Randomized, Double-Blind Study of Vaginal Microflora and Epithelium in Women Using a Tampon with an Apertured Film Cover Compared with Those in Women Using a Commercial Tampon with a Cover of Nonwoven Fleece

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Healthy women with normal menstrual cycles were randomly assigned to use either a test tampon during cycle 1 and a reference tampon during cycle 2 or a reference tampon during cycle 1 and a test tampon during cycle 2. Tampons were identical except for their cover materials: apertured film for the test tampon and nonwoven fleece for the reference tampon. Product use was doubly blinded. Qualitative and quantitative analyses of vaginal cultures were done pre-, mid-, and postmenstrually for a broad panel of microorganisms, colposcopy was performed, and diary reports were collected; 101 of 105 enrolled subjects completed the study. Midmenstrual findings for a variety of organisms differed from pre- and postmenstrual observations whether subjects were using test or reference tampons. No statistically significant differences were noted in prevalence or colony counts at premenstrual versus mid- and postmenstrual visits for most microorganisms. Prevalences of Gardnerella and anaerobic gram-negative rods were significantly different between tampons at the premenstrual visit, when unusually low values were observed for the test and reference tampons, respectively. None of the changes or differences in microflora were considered to be clinically significant. It is noteworthy, however, that declines in the prevalence and abundance of Lactobacillus during the menstrual periods were less pronounced during the use of both test and reference tampons than those reported from previous studies. Colposcopy showed no abnormal findings with either tampon and no changes in vaginal or cervical epithelial integrity. Thus, all evidence from both microbiological and colposcopic evaluations indicates that the apertured film cover of the test tampon is as safe as the nonwoven cover of the reference tampon.

Commercial intravaginal menstrual tampons have been widely used by women since the 1930s. Billions of tampons are sold every year, with an estimated 50% to 70% of women in industrialized countries using them. Underlying public acceptance is the safety of tampons, which has been demonstrated repeatedly in studies involving microbiological analyses, gynecological examination, and subject evaluation (7, 13, 17). Tampons have been shown to not substantially alter the vaginal microflora, and studies of vaginal microflora at various times during the menstrual cycle have demonstrated that tampons have no significant effect on the normal changes that occur in prevalences and total colony counts of either aerobic or anaerobic organisms during menses (10, 14, 15, 16).

The regulatory requirements for the marketing of tampons vary worldwide. One of the most stringent regulatory bodies is the U.S. Food and Drug Administration (FDA), which classifies tampons as medical devices (class II, special controls) and requires preclinical and clinical function and safety studies in support not only of new tampon products but also of significant changes in the material or design of an already marketed tampon (5). Similar regulations exist in Canada and Australia. In other countries, including those in the European community, tampons are classified as commodities or consumer products, and clinical studies are not required for premarket approval. In Europe, the European General Product Safety Directive (4) and the European Code of Practice for Tampons (defined by the European Disposables and Nonwovens Association, an international trade association of absorbent hygiene product industries that, among other things, establishes standards for hygiene products) (3) are followed. Each manufacturer decides whether it is necessary to conduct a clinical trial to confirm the safety of significant changes in tampon materials or design (4).

Marketed tampons are currently made of rayon, cotton, or cotton-rayon fiber blends, and most tampons have an overwrap of lightweight nonwoven fleece. The degree of absorbency for each tampon, defined as grams of fluid absorbed using standardized laboratory test methods (3, 9), allows different tampon brands to be compared by both regulatory authorities and consumers. Although many tampon brands, levels of absorbency, and styles are now available, some manufacturers continue to further develop tampons in order to enhance comfort and ease of use.

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The o.b. tampon was first introduced in Germany in 1950, and a nonwoven fleece cover has been used in o.b. tampons since 1982. The normal-absorbency version (European Code of Practice for Tampons three-droplet category) of this tampon with an eight-groove design (the tampon that served as the reference tampon for this clinical trial) has been widely (billionfold) used since its introduction in 1992. Recently, a new tampon variant with an apertured film cover to permit fluid to flow into the core of the tampon while affording more comfortable insertion and removal, especially during light menstrual flow, was designed (Johnson & Johnson Consumer and Personal Products) (unpublished data). The current prospective, randomized clinical trial was undertaken to confirm the safety of this new tampon design with respect to vaginal microflora and vaginal and cervical epithelium. The data were also expected to improve our understanding of changes in vaginal microflora throughout the menstrual cycle.

MATERIALS AND METHODS

Study design. This double-blind, randomized, two-way crossover trial was conducted at three centers in Germany according to FDA regulations and the International Conference on Harmonisation Guidelines for Good Clinical Practice (4a). All subjects were fully informed and provided written consent for participation prior to enrollment. The protocol was reviewed and approved by the Ethik-Kommision bei der Landesartzkammer Hessen, Institutional Ethics Committee, Frankfurt, Germany.

Subjects. Study participants were healthy women, 18 to 45 years of age, with normal menstrual cycles (21 to 35 days) who regularly used o.b. normal-absorbency tampons (or other brands of similar absorbency [three droplets according to the European Code of Practice for Tampons!]) (3) for at least 4 days of their menses. At a screening visit, subjects underwent a gynecological examination, including vaginal and cervical colposcopy, and the vaginal pH was determined. Vaginal samples were collected to perform Gram staining (12) and microbiological cultures, and a Papanicolaou (Pap) smear was done unless results from a previous 2 months; treatment for or suspicion of ever having had toxic shock syndrome, systemic corticosteroids or current treatment for infection; regular urinary incontinence; history of genital herpes, erosion, inflammation, infection, and tampon fiber retention. A final physical examination was conducted at the cycle 2 postmenstrual visit. Throughout the study, subjects were instructed to contact the investigator if they experienced any adverse reactions, especially pelvic pain, perineal or vaginal itching, burning or abnormal discharge, or symptoms of toxic shock syndrome.

Subjects were asked to make every effort to use the study tampons correctly for two consecutive menstrual periods. If complete data were not available for any one cycle and/or the subject was unable to complete a cycle for personal or medical reasons, the investigators were allowed to roll over this cycle to cycle 3 of the study (cycle 3 using the appropriate tampon according to the randomization schedule. If any data were missing for two cycles or if the subject was not compliant with study tampon use for two cycles, the subject was withdrawn from the study.

Microbiology analyses. At screening, to determine the presence of vaginosis, vaginal swabs were obtained for Gram staining and quantitative culture. The standardized method for the interpretation of Gram-stained vaginal smears developed previously by Nugent et al. (12) was used; a score of 7 to 10 confirmed the diagnosis of bacterial vaginosis. At the pre-, mid-, and postmenstrual visits, microorganisms were identified (Lactobacillus species, Staphylococcus aureus, Escherichia coli, group B Streptococcus, Gardnerella vaginalis, anaerobic gram-negative rods, Enterococcus species, Candida albicans, and Candida species), mean colony counts (CFU/ml) per vaginal secretion were obtained, and the prevalence of each microorganism was calculated. Vaginal smears were obtained using two sterile dacron-tipped swabs rolled against the lateral wall of the vagina until saturation. The capacity of these swabs was previously determined by Hillier et al. (6) to be 0.1 ml by measuring the weight of vaginal fluid in 10 volunteers. The swabs were placed in a BBL Port-A-Cul transport device (Becton Dickinson, Heidelberg, Germany) and were submitted to the Laboratory for Gynecologic Research, Department of Obstetrics and Gynecology, Heidelberg, Germany, on the same day. The two swabs (containing 0.2 ml of vaginal secretions) were inoculated into 1.8 ml Hanks’ medium (1:10 dilution). Serial dilutions of 1:10 from 10−1 to 10−4 were prepared in sterile saline solution. One hundred milliliters of each solution was plated onto a series of different agar media; Columbia blood agar (Becton Dickinson) with 5% sheep blood for growth of S. aureus, Enterococcus species, and group B Streptococcus; MacConkey agar (Oxoid, Basingstoke, Hampshire, England) for E. coli; Rogosa agar (Becton Dickinson) for Lactobacillus species; Schaller K/V agar (Becton Dickinson) for anaerobic gram-negative rods; Chromagar (Mast Diagnostics, Bootle, Merseyside, United Kingdom) for Candida species; and Gardnerella vaginalis agar (Heipha, Heidelberg, Germany) for G. vaginalis. Isolates were identified using criteria established previously by Murray et al. (11). Colony counts were determined by an enumeration of the mean number of colonies of each species multiplied by the respective dilutions.

Statistical analyses. Because the study was a balanced, crossover trial, statistical analyses focused on the per-protocol population, defined as subjects who completed the study. The analysis of adverse events was performed on the intent-to-treat (ITT) population, which comprised all subjects who were randomly assigned. Statistical methods for categorical data were used to analyze the results of the microbiological tests (6, 21, 22). Prevalence was defined as the percentage of subjects carrying a microorganism at a single visit. Incidences were assessed by comparing the number and percentage of subjects with the organism present at the mid- or postmenstrual visit but not present at the premenstrual visit. The statistical significance of changes in numbers of subjects with microorganisms from the premenstrual visit to the mid- and postmenstrual visits was analyzed by
RESULTS

Demographic characteristics and subject disposition. A total of 105 women were enrolled in the study and randomized (ITT population) between 4 September and 2 October 2000; the last subject completed the study on 13 November 2000. Fifty-one women were randomly placed into the test-reference group and 54 women into the reference-test group. Nearly all enrollees in both groups were Caucasian, and age distributions were similar across treatment sequences (Table 1). There was little variation in menstrual flow, absorbency of tampons normally used, cycle length, and duration of flow across treatment sequences (Table 1).

In all, 101 of the 105 (96.2%) enrolled subjects completed the study (50 subjects in the test-reference group and 51 subjects in the reference-test group). Discontinuations were due to adverse events unrelated to the product (n = 2 for reference-test group) and noncompliance (n = 1 for each treatment group). Major protocol violations occurred in 7 subjects in the test-reference group and in 14 subjects in the reference-test group. Major protocol violations were questionable diary entries (n = 6); samples for microbiological culture being too old, showing no growth, or being lost (n = 5); visits not attended or out of the allowable time window (n = 5); tampon noncompliance (n = 3); and antibiotic comedication (n = 3). The per-protocol population included 43 subjects in the test-reference group and 40 subjects in the reference-test group. In both groups, 65% of patients reported using contraception (per-protocol population).

Microbiology results. Table 2 summarizes the prevalence and average colony counts for each tampon and indicates the statistical significance of comparisons within and between tampons.

Sequential changes in vaginal microflora during menstrual cycles. Midmenstrual findings for the vaginal microflora differed from those of pre- and postmenstrual observations for a variety of organisms whether subjects were using test or reference tampons. For midmenstrual versus premenstrual contrasts, prevalences and colony counts were generally lower for the Lactobacillus species and higher for S. aureus, E. coli, group B Streptococcus, and anaerobic gram-positive rods; several but not all contrasts reached statistical significance for within-tampon comparisons. Colony counts for G. vaginalis were higher...
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Colony count&lt;sup&gt;b&lt;/sup&gt; (mean log&lt;sub&gt;10&lt;/sub&gt; CFU/ml ± SD)</th>
<th>Prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Colony count&lt;sup&gt;b&lt;/sup&gt; (mean log&lt;sub&gt;10&lt;/sub&gt; CFU/ml ± SD)</th>
<th>Prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Colony count&lt;sup&gt;b&lt;/sup&gt; (mean log&lt;sub&gt;10&lt;/sub&gt; CFU/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> species</td>
<td>82 (98.8)</td>
<td>7.4 ± 1.38</td>
<td>74 (89.2)</td>
<td>7.1 ± 1.04</td>
<td>79 (95.2)</td>
<td>7.5 ± 1.16</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>9 (10.8)</td>
<td>3.9 ± 0.82</td>
<td>22 (26.5)</td>
<td>5.3 ± 1.75</td>
<td>11 (13.3)</td>
<td>4.0 ± 1.10</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>11 (13.3)</td>
<td>3.3 ± 1.04</td>
<td>43 (51.8&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>5.6 ± 2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24 (28.9&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>6.4 ± 2.03</td>
</tr>
<tr>
<td>Group B <em>Streptococcus</em></td>
<td>2 (2.4)</td>
<td>5.6 ± 0.76</td>
<td>17 (21.5&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>6.3 ± 1.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 (12.0&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>4.8 ± 2.11</td>
</tr>
<tr>
<td><em>G. vaginalis</em></td>
<td>3 (3.6&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>6.3 ± 1.06</td>
<td>13 (15.7&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>6.6 ± 1.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14 (16.9&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>6.6 ± 1.94</td>
</tr>
<tr>
<td>Anaerobic gram-negative rods</td>
<td>20 (24.1&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>3.4 ± 1.10</td>
<td>32 (38.6&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>4.3 ± 2.16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16 (19.3&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>3.8 ± 1.46</td>
</tr>
<tr>
<td><em>Enterococcus</em> species</td>
<td>12 (14.5)</td>
<td>4.1 ± 1.13</td>
<td>14 (16.9&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>4.7 ± 1.42&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13 (15.7&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>3.8 ± 1.13</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>9 (10.8)</td>
<td>3.9 ± 0.77</td>
<td>2 (2.4)</td>
<td>3.3 ± 0.48</td>
<td>2 (2.4)</td>
<td>1.8 ± 0.34</td>
</tr>
<tr>
<td><em>Candida</em> species</td>
<td>3 (3.6)</td>
<td>2.8 ± 1.00</td>
<td>12 (14.5&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>4.2 ± 1.52&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14 (16.9&lt;sup&gt;e&lt;/sup&gt;)</td>
<td>5.0 ± 1.23</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number of subjects at premenstrual visits is too small for statistical testing for *S. aureus* (reference tampon), group B *Streptococcus* (test and reference tampon), *G. vaginalis* (test tampon), anaerobic gram-negative rods (reference tampon), and *Candida* species (test and reference tampon).

<sup>b</sup> Prevalences are given as the absolute number of subjects and as a percentage of the per-protocol population.

<sup>c</sup> Mean colony counts expressed as log<sub>10</sub> CFU/ml of vaginal secretion.

<sup>d</sup> P < 0.05 for difference within test or reference tampons compared to each corresponding premenstrual time point.

<sup>e</sup> P < 0.05 for difference between test and reference tampons at the premenstrual time point.

### Summary of tampon use

Subjects wearing each tampon during the study were asked to use tampons for 5 h after use of either test or reference tampons (data not shown). The number of tampons used per cycle was recorded for each subject. The average time that a single tampon was used was approximately 5 h, and the maximum time that a single tampon was used was approximately 10 h. Within the study population, 80% of the women used between 11 and 19 tampons per cycle, while the remaining 20% used more than 19 tampons. The mean number of tampons used per subject was 15 ± 3. The number of tampons used per subject was not significantly different between tampons.

### Incidence

New occurrences at the midmenstrual visit not present at the premenstrual visit were noted for *S. aureus* in the test tampon, *G. vaginalis* in the test tampon, and *C. albicans* in the reference tampon. The number of subjects with *E. coli* was unusual for the reference tampon. Incidence was unusual for *E. coli* in the reference tampon and for *C. albicans* in the test tampon.

### Effect of tampon type

No statistically significant differences between test and reference tampons were noted for any of the microorganisms assessed. Differences between tampons with regard to vaginal microflora were noted for both tampons at midmenstrual visits than at premenstrual visits. The menstrual cycle appeared to have little effect on prevalence. Examination of the vaginal microflora is continued in the table.
TABLE 3. Summary of tampon use per subject (per-protocol population)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cycle</th>
<th>Test-reference (n = 43)</th>
<th>Reference-test (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average time of single tampon use in h:min (mean ± SD)</td>
<td>1</td>
<td>4.53 ± 1.03</td>
<td>5.01 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.57 ± 0.59</td>
<td>4.57 ± 0.54</td>
</tr>
<tr>
<td>Maximum total amt of time of tampon use per cycle in h:min (mean ± SD)</td>
<td>1</td>
<td>96:01 ± 21:10</td>
<td>98:13 ± 24:22</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>92:16 ± 19:24</td>
<td>97:09 ± 22:26</td>
</tr>
<tr>
<td>No. of tampons used/cycle (mean ± SD)</td>
<td>1</td>
<td>18.70 ± 4.67</td>
<td>18.90 ± 5.09</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.10 ± 4.12</td>
<td>19.30 ± 4.98</td>
</tr>
</tbody>
</table>

test-reference group and 14 of 54 (25.9%) reported 17 adverse events in the reference-test group. No adverse event was judged by the investigators to be related to a study tampon. The majority of adverse events were of mild or moderate intensity, with only one reported to be of severe intensity. No serious adverse events were reported. The most common adverse event was headache observed in four subjects (7.8%) in the test-reference group and in six subjects (11.1%) in the reference-test group. All other adverse events (including migraine, influenza-like symptoms, abdominal pain, spotting between menses, rhinitis, cystitis, and infection) were reported in only one or two individuals per group (1.8% to 3.9%). Two subjects were withdrawn from the study due to the use of prescribed antibiotic treatment for a common cold and cystitis; neither condition was considered to be related to the experimental product. Only minor changes of no clinical relevance in blood pressure, heart rate, and oral temperature were noted throughout the study in both the ITT and the per-protocol populations. No between-tampon differences in vital signs were apparent.

DISCUSSION

This large, randomized, double-blind, crossover study is, to our knowledge, the most rigorous clinical safety study of a new tampon to date and is among the most intensive studies of vaginal microflora changes in relation to menses and tampon use ever done. Assessments included colposcopy, diary reports, and microbiological analyses of specimens collected at three time points (pre-, mid-, and postmenstrual) for a broad panel of potentially clinically significant vaginal microorganisms (seven species or types of bacteria as well as Candida). No formal evaluation of the effectiveness of blinding was conducted, but it is highly unlikely that unblinding biased the results for several reasons: (i) randomization was performed by individuals in locations separate from the clinics where subjects were examined, (ii) products looked similar and were packaged identically, (iii) subjects did not have both products at the same time, and (iv) personnel at the microbiology laboratory had no access to the randomization code.

Qualitative and quantitative changes in specific vaginal microflora from pre- to mid- to postmenstrual visits in this study generally were similar between cycles of use of the two different tampons and similar to those reported from previous studies of tampon and other catamenial product use (1, 2, 14–16, 19, 20, 23). Thus, there were generally numerical if not statistically significant increases in the prevalence and/or colony counts of anaerobic gram-negative rods, as well as G. vaginalis, group B Streptococcus, E. coli, and S. aureus, from the pre- to midmenstrual visits, and these values returned or started to return to the premenstrual values by the postmenstrual visit. These observations are complicated by the inexplicably lower prevalence of G. vaginalis at the premenstrual visit before the period of test tampon use and of anaerobic gram-negative rods before the period of reference tampon use. Changes in Enterococcus species and C. albicans throughout the menstrual cycles were generally qualitatively similar but less pronounced and were virtually identical during the cycles of use of the two different tampons. For all microorganisms assessed, neither the changes during the menstrual cycles nor the differences between cycles of the use of the two tampons were of any clinical significance.

The results for Lactobacillus species in the current study also showed no differences between the cycles of use of the two different tampons but are worthy of special note because (i) lactobacilli are the predominant microbes in the healthy vagina and are considered to be responsible for preventing the overgrowth of potentially pathogenic microbes and (ii) the results here differed from those reported in previous studies (2, 16). Eschenbach et al. (2) found that the proportion of women with semiquantitatively high levels of Lactobacillus increased from 70% on days 1 to 5 of the cycle to 92% on days 7 to 12. Onderdonk et al. (16) observed that in vaginal swabs from women using tampons during their menstrual periods, Lactobacillus prevalence and colony counts increased from day 2 to day 4 to day 21: from 78% to 84% to 91% and from 6.21 to 7.08 to 7.91 log10 CFU/g, respectively. In the present study, only approximately 10% of the women lost Lactobacillus during menses (while using either the test or reference tampons), and among women who retained Lactobacillus, there was an average reduction in colony counts of only about a half log. Although the reductions in prevalence were statistically significant during both periods of tampon use and the reduction in colony counts was statistically significant during the period of reference tampon use, these changes were not clinically significant and were much less than those reported in previous studies (2, 16). The differences between our findings and those of previous studies may reflect differences in study populations (e.g., between women in the United States and women in Germany), laboratory methods, or the tampons used in the study. Further studies would be required to evaluate these possibilities.

In the present study, visual pelvic exams found no signs of vaginal or cervical irritation or ulcerations either during or after use of either the test or the reference tampon. Colposcopy after the use of either tampon showed no evidence of microulcerations.

Finally, the analysis of diary records demonstrated that both test and reference tampons were well tolerated. Most reported adverse events were of mild or moderate intensity and were not related to the study tampon. No serious adverse events were reported. The average tampon wear times were similar in both
treatment sequences and were comparable to that reported in another recent study of a new tampon (18).

In conclusion, the findings of this rigorously designed study conducted using a large sample of subjects demonstrate that the new tampon with an apertured film cover has no adverse effects on the vaginal microflora or on the vaginal and cervical epithelium and is as safe and well tolerated as the current commercially marketed tampon with a nonwoven fleece cover, a tampon with a long, extensive history of safe use.

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