Acta Derm Venereol 2004; 84: 277–284

Taylor & Francis
healthsciences

INVESTIGATIVE REPORT

# The Vulvar Skin Microenvironment: Influence of Different Panty Liners on Temperature, pH and Microflora

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**The aim of this study was to confirm findings that vapour-impermeable panty liners might impair skin climate, and to assess their impact on the skin microflora. Temperature, surface pH and aerobic microflora were measured on vulvar skin of 102 women. The mean skin temperature was 1.1°C higher when using a vapour-impermeable panty liner compared with not using one. Use of panty liners with vapour-permeable back sheets and acidic cores resulted in skin temperature, pH and microflora levels that were very close to those observed in persons not using liners. The temperature, pH and total number of microorganisms were significantly lower for users of vapour-permeable panty liners than for users of vapour-impermeable ones ($p<0.05$, $p<0.001$ and $p<0.001$, respectively). The microorganism densities were usually higher when using the vapour-impermeable panty liner, but mean differences were minor. The use of panty liners seems not to imply a microbial risk for normal, healthy women.** ***Key words: hygiene absorbent products; skin microflora; skin surface pH; skin temperature; vapour permeability; vulva.***

(Accepted January 28, 2004.)

Acta Derm Venereol 2004; 84: 277–284.

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An increasing number of women are using sanitary protection products (e.g. panty liners) between menstrual periods, in order to feel safe and clean. It has been assumed that more frequent use might have a negative influence, due to the fact that constantly covering the skin could affect both its temperature and humidity. In an earlier study we have shown that non-breathable panty liners (i.e. products with water vapour-impermeable back sheets) significantly increased vulvar skin temperature, humidity and pH, compared with the state when either no panty liners or panty liners with a breathable (water vapour-permeable) back sheet are worn (1). The impact on the skin microflora was not examined, however.

A study by Aly et al. (2) has shown that prolonged occlusion of forearm skin resulted in increased pH and increased bacterial growth. Physiological differences have been reported between forearm and vulvar skin, and the original higher hydration state of the vulvar skin may be an important factor (3, 4). It has been pointed out that for the microbiologist the vulva consists of several distinct ecotopes, where only the labia majora have been thoroughly studied (5). The microbial flora of the labia majora is characterized by a high density of microorganisms, and by the propinquity of the vaginal/urethral/rectal orifices (5, 6).

The use of breathable materials has been introduced for clothing (e.g. Gortex® fabrics used in rainwear) and nowadays also for absorbent hygiene products like diapers, menstrual pads and panty liners. Akin et al. (7) showed that disposable diapers with breathable back sheets resulted in reduced prevalence of *Candida* and diaper dermatitis. Schäfer et al. (8) concluded that hygiene products with vapour-permeable materials were able to reduce skin over-hydration.

In the present study we measured temperature and pH and aerobic microorganisms of interest, in a population of healthy women, on one occasion when not using panty liners and when using panty liners of two different constructions on two occasions. The purpose was to describe the vulvar skin microflora under different conditions and to assess if an expected rise in temperature, humidity and pH was sufficient to influence the microbial ecology.

## MATERIALS AND METHODS

### *Subjects*

In all, 102 healthy, Caucasian, female volunteers (mean age 36 years, range 20–45) with regular menstruation participated. The women were asked about genital hygiene habits, their use of contraceptives, earlier yeast infections, skin diseases and use of hygiene products. Subjects who had used antibiotics or vaginal medication less than 4 weeks prior to the study, or had current abnormal discharges, bleeding, itch or irritation in the vulvar area, were excluded. The study was approved by the local research ethics committee, and the subjects gave their written consent to inclusion.

### *Panty liners*

The usage of two different panty liners (products B and C) was evaluated and compared to absence of panty liners

 ISSN 0001-5555
DOI: 10.1080/00015550410029260

(group A). The panty liners were produced by the same company (SCA Hygiene Products, Sweden) and had identical design and composition with the following exception. Product B was a conventional-type panty liner with a non-breathable back sheet. Product C had a breathable back sheet (Exxaire XBF-110W, Tredegar Film Products, Kerkrade, the Netherlands, with a water vapour permeability of 8600 g/m$^2$ 24 h according to ASTM-F1249, American Society for Testing and Materials). Product C was acidified with a superabsorbent polymer (HySorb S7110, BASF, Germany). In these, 225 mg of polymer (pH 4.5) per product was embedded in a carrier layer of chemithermo-mechanical cellulose pulp (CTMP), leading to a higher product thickness of 2.3 mm compared with 1.4 mm for product B. The total amount of cellulose per product was 1.55 g in C vs 1.15 g in product B. The differences between the panty liners were unknown to the subjects, as well as to the research nurse performing the measurements.

*Experimental design*

The whole study was carried out from February to the beginning of June 2001, and from August to December, with a break during the summer. The subjects were carefully instructed (orally and in writing) how to use the products and how to manage their hygiene. They were randomly assigned to the three different regimens, according to a pre-set random sequence so that all test variants were evenly distributed over the test period in order to avoid a possible bias due to climatic (and clothing) influence on the measurements. Hence, a third of the women started with each test regimen, all subjects tested all variants and in the calculations the subjects were their own controls (A vs B, A vs C and B vs C). The subjects were instructed to use only water for genital washing and not to have sexual intercourse on the evening before visiting the clinic for examination. All participants were provided with panties of the same type. Examinations were scheduled to take place between the 16th and 22nd day of the menstrual cycle. Three days before the visit, the subjects had to begin wearing the panty liner provided. Recommended usage was two to three liners during the day and one during the night, but slight deviations were permitted. No shaving of the genital area or use of any antibacterial soap was allowed during these 3 days. In the morning, after 3 days' use, the women had to apply a disposable thermistor probe between the panty liner and the skin before going to the clinic for measurements.

In the clinic, the subjects had to rest for 15 min, lying down. First the temperature was measured without removing the panties. Then the panties and the panty liners were removed, the panty liners were placed in plastic bags for microbial analyses and the pH was measured at the interior aspect of the upper part of the labium majus and in the perineum. The electrode was placed slightly off-centre in the perineum to avoid contamination of skin for the microbial sample. A sampling cylinder was then placed at the opposite site at the other labium majus, and the skin was gently 'scrubbed' with the detergent liquid for 1 min (see below, Vulvar skin microflora). Thereafter, the procedure was repeated on the skin at the perineal site.

During the visit the subjects were asked if they had felt any sensation of humidity, warmth, chafing, etc., and asked about any event that could have influence on the study. The research nurse observed the subjects' crotch anatomy, where a subject standing with straight legs and not having free passage for a paper sheet between her thighs, was recorded as having a narrow crotch. At each visit it was noted if the subjects' clothes were either loosely or closely fitting to the body. The vulvar skin was visually inspected. The measurements, including the 15-min resting period, took place in an ordinary examination room without air conditioning.

*Skin temperature*

The Craftemp® thermometer system was used. The disposable probe had a sensor head consisting of a heat-sensitive resistor. Accuracy was ±0.1 C, according to the manufacturer. The use of these measuring probes has been described by Karlsson et al. (9). The device was supplied by Astra Tech AB, Mölndal, Sweden, but is no longer available on the market. When measuring the temperature, the probe was fixed to the panty liner, or in group A to the panty, with a highly air-permeable self-adhesive fabric (Mefix®, Mölnlycke Health Care, Göteborg, Sweden). The sensor end of the disposable probe was positioned centrally on the panty liner so that it would record the temperature of the enclosed microenvironment between product/panty and vulvar skin. The other end of the probe was accessible outside the panty, so that the measuring device could be used without removing the panties or products. The temperature recorded in this way, in a state of equilibrium, should closely correspond to the pH and microflora measurements at the labium majus site.

*Skin surface pH*

The PH 900 pH meter (Courage+Khazaka, Cologne, Germany) was used with a Mettler-Toledo 304 flat electrode. The probe was cleansed with de-ionized water before each measurement, and the last drops were kept on the electrode to provide a wet state on the skin during measurement. A waiting period of approximately 30 s was maintained before the reading was recorded. The accuracy of the pH meter is 0.1 units. The instrument was calibrated each day with buffers of pH 4 and 7. Between subjects, the electrode was cleansed with alcohol.

*Vulvar skin microflora*

A modification of the Williamson-Kligman scrub technique (10) was used to collect samples from the two vulvar sites. Sterile stainless steel rings, 2.6 cm in inner diameter covering a 5.3 cm$^2$ area, were used. The subject was lying down with the pelvis in a raised position supported with pillow(s). The ring was placed halfway down on labium majus, by first setting the distal edge at slightly less than one ring diameter from the borderline between labia majus and minus, slanting the ring inwards while stretching the skin, so that the proximal ring-edge ends where labium majus meets labium minus. One millilitre of sterile 0.075 phosphate buffer (pH 7.9) containing 0.1% Triton X-100 was poured into the ring, the skin was rubbed gently with a blunt sterile glass rod for 1 min and the fluid was removed with a sterile Pasteur pipette and emptied into a sterile glass tube. For the perineal site the ring was placed in the middle between the posterior commissure and the anus, and the same procedure was repeated. In subjects where the perineum was shorter than the ring diameter, the ring was positioned with the lower edge just above the fold of the anus sphincter. The two samples together with the panty liner (in the plastic bag) were sent to the nearby laboratory within 2 h of sampling (storage in refrigerator). At the laboratory, the panty liner was dispersed and homogenized in 100 ml of saline in a Stomacher Labblender 400 (Seward Laboratory, London, UK) for 3 min. Tenfold serial dilutions were performed in phosphate-buffered saline; 0.1 ml of each dilution was plated onto non-selective and selective routine agar plates.

Table I. *Mean values (SEM) of temperature and pH; comparisons between regimens (n = 102)*

| | Regimen | | | Significance | | |
|---|---|---|---|---|---|---|
| | A | B | C | A vs B | A vs C | B vs C |
| Temperature (°C) | 34.6 (0.15) | 35.7 (0.10) | 35.3 (0.16) | *** | *** | * |
| pH (LM) | 5.2 (0.08) | 6.0 (0.10) | 5.3 (0.07) | *** | | *** |
| pH (PE) | 5.6 (0.07) | 6.0 (0.08) | 5.5 (0.07) | *** | | *** |

*$p \leq 0.05$; ***$p \leq 0.001$. A = no panty liner; B = non-breathable panty liner; C = breathable, acidic panty liner. LM = labium majus; PE = perineum.

Plates were incubated at 37°C and inspected after 1 and 2 days. The number of colony-forming units (CFU)/ml was determined. Using routine bacteriological techniques micro-organisms were categorized into: coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, enterococci, α-streptococci, group A, B, C or G streptococci, *Corynebacterium* spp., lactobacilli, *Escherichia coli*, other members of the Enterobacteriaceae, *Pseudomonas* spp., *Candida albicans*, and total number of aerobic microorganisms. The analytical data were expressed as logarithms for CFU per $cm^2$ of skin or per panty liner (100 ml dispersion).

### *Statistics*

For tests of significance between the three regimens (A, B, C), Student's t-test was used for temperature and pH, and Wilcoxon's sign rank test for microbial data (logged CFU, means counted on positive values). McNemar's test was used for subjective data (e.g. sensation of humidity) and to test co-variation of microorganisms (streptococci vs enterococci). Correlations were studied with Spearman rank coefficients.

## RESULTS

### *Skin microclimate*

The mean values and standard error of the means (SEM) for temperature and pH are presented in Table I. The mean skin temperature significantly increased by 1.1°C in the presence of non-breathable panty liner (B) compared with the absence of panty liner (A). The pH values at the labium majus and perineum sites were significantly higher by 0.8 and 0.4 units, respectively, for the same comparison (B vs A). For the regimen with an acidic panty liner with a breathable back sheet (C), the mean temperature was significantly increased by 0.7°C, and the mean pH increased by 0.1 units at the labium majus site and decreased by 0.1 units at the perineum, compared with absence of panty liner (A). The distributions of the individual temperatures of the women for the three regimens are shown in Fig. 1. The pH distributions are shown in Figs 2 and 3.

A significantly lower percentage of women (13% vs 34%; $p \leq 0.001$) reported a sensation of humidity for the breathable panty liner (C) than for the non-breathable panty liner (B), when asked during the examination. No significant differences were found as to the feeling of warmth or chafe. No visual reactions of the vulvar skin were observed on any occasion.

### *Vulvar skin microflora*

The results of the microbial analyses are shown in Table II. There were a significantly higher number of total microorganisms when wearing the non-breathable panty liner (B) at both labium majus ($p \leq 0.001$) and perineum ($p \leq 0.05$), compared with the absence of panty liner (A). A similar tendency is shown for 6 of the 10 microbial groups. For the intestinal bacteria

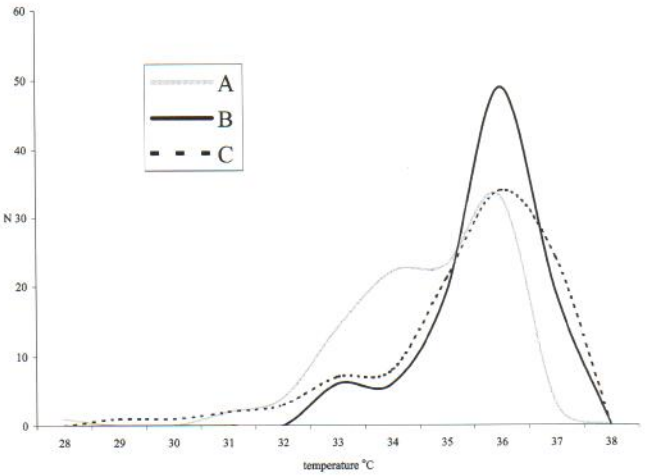


*Fig. 1.* The vulvar skin temperature. Frequency of subjects (N) within intervals of 1.0°C. A = no panty liner; B = non-breathable panty liner; C = breathable, acidic panty liner.

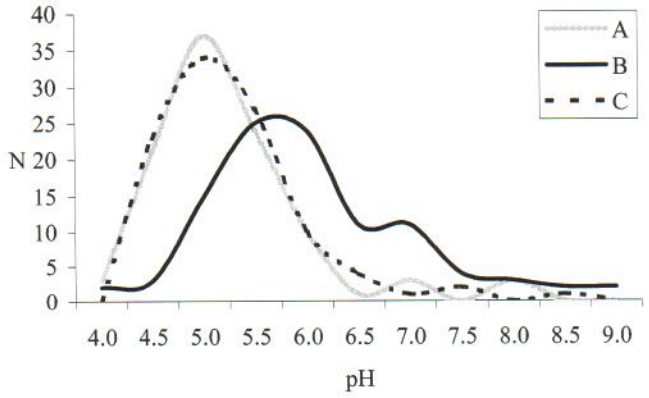


*Fig. 2.* The pH of inner aspects of labium majus. Frequency of subjects (N) within intervals of 0.5 pH units. A = no panty liner; B = non-breathable panty liner; C = breathable, acidic panty liner.

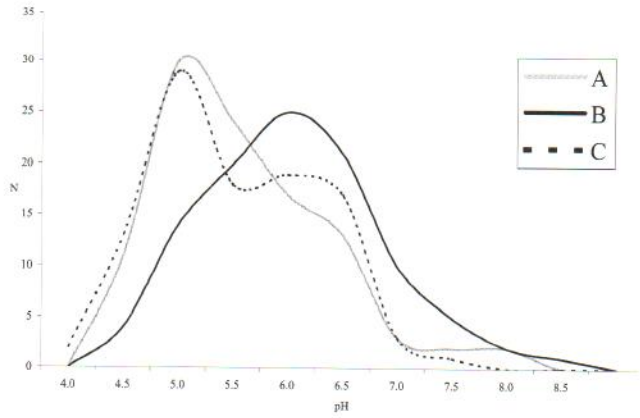


*Fig. 3.* The pH of the perineum. Frequency of subjects (N) within intervals of 0.5 pH units. A = no panty liner; B = non-breathable panty liner; C = breathable, acidic panty liner.

enterococci and *E. coli* the carrier rate was higher for the regimen with non-breathable panty liner (B) than without panty liner (A), especially at the perineum (46% and 66% vs 34% and 47%). No significant difference was found for *S. aureus* or *C. albicans*. For all species the number of microorganisms was lower in the regimen with the breathable, acidic panty liner (C) than for the non-breathable liner (B). In seven instances the differences were significantly lower. Fig. 4 shows the frequency of subjects at different microbial densities in the panty liners. In Table III the microbial density differences between regimens B and C found at the vulvar skin are compared to those found in the panty liners. In most instances the difference found in the panty liner was significantly higher than for the corresponding difference found on the skin.

Table II. *Positive samples (%; n = 102) and mean number of microorganisms for positive samples in labium majus (LM) and perineum (PE) in log CFU/cm² skin, and in panty liner (PL) in log CFU/product*

| | | Regimen | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | B | | C | | Significance | | |
| | Site | % | Mean (SEM) | % | Mean (SEM) | % | Mean (SEM) | A vs B | A vs C | B vs C |
| *S. aureus* | LM | 12 | 2.85 (0.37) | 10 | 3.50 (0.38) | 11 | 3.01 (0.36) | | | |
| | PE | 13 | 2.90 (0.40) | 15 | 3.24 (0.22) | 9 | 2.95 (0.37) | | | |
| | PL | | | 13 | 5.24 (0.30) | 13 | 4.17 (0.28) | | | |
| Coagulase-negative staphylococci | LM | 99 | 4.38 (0.06) | 100 | 4.83 (0.04) | 98 | 4.48 (0.05) | *** | | *** |
| | PE | 97 | 4.20 (0.07) | 98 | 4.29 (0.07) | 97 | 3.95 (0.08) | | * | *** |
| | PL | | | 99 | 6.09 (0.09) | 100 | 5.21 (0.07) | | | *** |
| Group B streptococci | LM | 28 | 2.60 (0.22) | 33 | 2.50 (0.20) | 34 | 2.38 (0.17) | | | |
| | PE | 38 | 3.45 (0.13) | 35 | 3.29 (0.19) | 38 | 2.94 (0.18) | | | |
| | PL | | | 29 | 4.40 (0.21) | 20 | 4.29 (0.23) | | | * |
| α-Streptococci | LM | 29 | 3.17 (0.16) | 29 | 3.05 (0.17) | 26 | 2.89 (0.20) | | | |
| | PE | 32 | 3.60 (0.16) | 37 | 3.70 (0.17) | 31 | 3.63 (0.16) | | | |
| | PL | | | 27 | 5.42 (0.21) | 19 | 4.50 (0.31) | | | * |
| *Corynebacterium* spp. | LM | 91 | 3.11 (0.11) | 94 | 3.49 (0.10) | 92 | 3.13 (0.10) | ** | | ** |
| | PE | 92 | 3.55 (0.09) | 93 | 3.64 (0.09) | 91 | 3.47 (0.08) | | | |
| | PL | | | 92 | 5.26 (0.11) | 85 | 4.47 (0.08) | | | *** |
| Lactobacilli | LM | 99 | 3.58 (0.10) | 96 | 3.93 (0.10) | 96 | 3.65 (0.08) | * | | * |
| | PE | 97 | 4.15 (0.09) | 99 | 4.33 (0.08) | 97 | 4.00 (0.08) | * | | *** |
| | PL | | | 93 | 5.98 (0.11) | 86 | 5.27 (0.10) | | | *** |
| Enterococci | LM | 30 | 2.66 (0.17) | 34 | 2.89 (0.16) | 33 | 2.67 (0.17) | | | |
| | PE | 34 | 2.99 (0.18) | 46 | 3.48 (0.14) | 44 | 3.27 (0.13) | * | * | |
| | PL | | | 35 | 5.10 (0.15) | 22 | 4.23 (0.20) | | | *** |
| *Escherichia coli* | LM | 13 | 1.72 (0.23) | 19 | 1.63 (0.20) | 18 | 1.49 (0.26) | | | |
| | PE | 47 | 1.73 (0.13) | 66 | 1.80 (0.12) | 49 | 1.84 (0.13) | ** | | * |
| | PL | | | 13 | 3.96 (0.25) | 7 | 3.51 (0.18) | | | |
| Other Enterobacteriaceae | LM | 4 | 1.05 (0.23) | 7 | 1.62 (0.42) | 7 | 1.06 (0.31) | * | | |
| | PE | 15 | 1.59 (0.25) | 11 | 1.59 (0.25) | 10 | 1.76 (0.28) | | | |
| | PL | | | 4 | 4.54 (0.50) | 2 | 3.89 (0.59) | | | |
| *Candida albicans* | LM | 12 | 1.27 (0.31) | 8 | 1.73 (0.33) | 5 | 1.82 (0.49) | | | |
| | PE | 15 | 1.88 (0.23) | 11 | 1.92 (0.23) | 15 | 2.13 (0.15) | | | |
| | PL | | | 10 | 4.23 (0.27) | 6 | 4.71 (0.37) | | | |
| Total microorganisms | LM | 100 | 4.95 (0.05) | 100 | 5.22 (0.04) | 100 | 4.99 (0.04) | *** | | *** |
| | PE | 100 | 4.99 (0.06) | 100 | 5.16 (0.05) | 100 | 4.87 (0.05) | * | | *** |
| | PL | | | 100 | 6.92 (0.09) | 100 | 6.05 (0.08) | | | *** |

A = no panty liner; B = non-breathable panty liner; C = breathable, acidic panty liner.
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

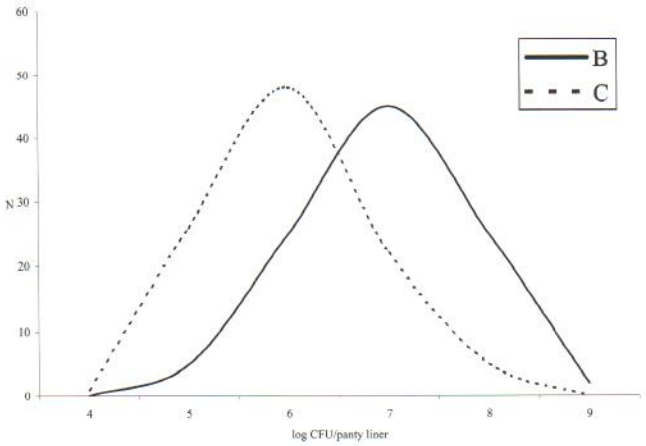


*Fig. 4.* Frequency of subjects (N) vs microorganism density in panty liners, intervals of one log unit CFU/panty liner. B = non-breathable panty liner; C = breathable, acidic panty liner.

### *Correlations*

Correlations according to the Spearman rank coefficient test are shown in Table IV for the primary result variables and a few selected co-factors. No correlation was found to temperature. pH correlated between the two sites (labium majus and perineum) and with the microflora level at the labium majus. The microflora at one vulvar site correlated strongly with the other site. We found no correlation between the result variables and tight or loose- fitting clothing. pH correlated with the crotch width, i.e. the subjects with a narrow crotch usually had a higher pH. A high correlation between the vulvar sites (data not shown in the table) was also found for α-streptococcci (r = 0.533), *C. albicans* (r = 0.738), *Corynebacterium* spp. (r = 0.301), enterococci (r = 0.582), lactobacilli (r = 0.336), *S. aureus* (r = 0.871), group B streptococci (r = 0.745), other members of Enterobacteriaceae than *E. coli* (r = 0.504), and was slightly lower for *E. coli* (r = 0.254) and CNS (r = 0.201). The r-values given above are Spearman rank coefficients calculated for group A.

A negative co-variation of enterococci with α-streptococci was found, i.e. they were hardly ever found simultaneously in one subject (labium majus: 29 subjects had α-streptococci only, 30 had enterococci only and 1 had both; perineum: 24 subjects had α-streptococci only, 26 had enterococci only and 9

Table III. *Microflora on skin vs microflora in panty liner (PL); differences between mean log densities in regimens B and C*

| | logB − logC (difference) | | | Significance | |
|---|---|---|---|---|---|
| | LM | PE | PL | LM vs PL | PE vs PL |
| *S. aureus* | 0.49 | 0.28 | 1.07 | | |
| CNS | 0.35 | 0.34 | 0.88 | *** | *** |
| Group B streptococci | 0.12 | 0.35 | 0.11 | | |
| α-Streptococci | 0.15 | 0.07 | 0.92 | * | * |
| *Corynebacterium* spp. | 0.36 | 0.17 | 0.80 | ** | *** |
| Lactobacilli | 0.28 | 0.33 | 0.71 | ** | * |
| Enterococci | 0.22 | 0.21 | 0.87 | * | ** |
| *Escherichia coli* | 0.14 | −0.04 | 0.45 | | * |
| Other Enterobacteriaceae | 0.55 | −0.17 | 0.65 | | |
| *Candida albicans* | −0.09 | −0.21 | −0.48 | | |
| Total microorganisms | 0.23 | 0.28 | 0.86 | *** | *** |

B = non-breathable panty liner; C = breathable, acidic panty liner; LM = labium majus; PE = perineum; CNS = coagulase-negative staphylococci. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$, [a]) No significant differences between LM and PE.

Table IV. *Spearman rank correlation between some result and co-factor variables; regimen A (no panty liner)*

| | Temperature | pH LM | pH PE | Microflora LM | Microflora PE | Age | Clothing | Crotch |
|---|---|---|---|---|---|---|---|---|
| Temperature | 1 | | | | | | | |
| pH LM | −0.061 | 1 | | | | | | |
| pH PE | −0.013 | 0.460** | 1 | | | | | |
| Microflora LM[a] | −0.033 | 0.290** | 0.398** | 1 | | | | |
| Microflora PE[b] | 0.011 | −0.025 | 0.131 | 0.484** | 1 | | | |
| Age | −0.049 | 0.109 | 0.267** | 0.053 | 0.091 | 1 | | |
| Clothing[c] | −0.061 | −0.060 | −0.099 | −0.051 | −0.010 | 0.098 | 1 | |
| Crotch[d] | 0.084 | 0.208* | 0.287** | 0.129 | 0.145 | 0.002 | 0.085 | 1 |

LM = labium majus, PE = perineum. $*p \leq 0.05$; $**p \leq 0.01$.
[a]total microorganisms at labium majus, [b]total microorganisms at perineum, [c]assessment of loose or tight clothing, [d]assessment of normal or narrow crotch.

had both – results from group A.). The probabilities that these distributions would occur by chance are $p \leq 0.001$ according to McNemar's test.

*Corynebacterium* spp., CNS and lactobacilli correlated positively to pH and to each other (data not shown).

## DISCUSSION

The findings in an earlier, smaller (n = 12) study (1) were confirmed, that temperature and pH were higher when using non-breathable panty liners compared with no use. By introducing breathable and acidic materials, a reduction of the vulvar skin temperature and skin surface pH was achieved, compared with the use of conventional, non-breathable materials. That the breathable panty liner did not yield temperatures as low as in the previous study (1) (mean temperature 35.3 vs 34.5°C), might be due to the thicker cellulose core used as a carrier for the acidic buffering system. The temperature-lowering effect of the breathability is more evident considering that the thicker and more insulating C panty liner still has a 0.4°C lower mean temperature compared with B (Table I). As only healthy subjects with normal, regular menstruation cycles were included in this study, it can be expected that temperature and pH values normally vary within a few temperature degrees and pH units, as shown in Figs 1–3. In a similar study population, Wilhelm et al. (11) reported pH values at labium majus that were close to the results from our study.

The pH increase effect with non-breathable materials is probably primarily due to the increase in skin surface humidity, as measured in the previous study (1) and assessed by the wearers in this study. A drying process of vulvar skin resulting in lower vulvar pH was also reported by Elsner and Maibach (3). The equivocal use of the term skin pH has been pointed out by Rieger (12), who calls it the 'apparent pH' on the skin. The measurement at the skin surface with the pH electrode is the result of the water extractable ions. By definition pH is the negative logarithm of the concentration (more correctly activity) of hydrogen ions in an aqueous solution. It will therefore increase at higher humidity/dilution. The higher skin surface pH obtained is probably also due to better ion transportation to the deeper layers of epidermis at higher humidity, in accordance with the findings of Öhman & Vahlquist (13), who showed an increasing pH gradient when the skin is stripped layer by layer. Carefully interpreted, pH measurements will give information about environmental change on the skin. It is known that pH plays a decisive role in biochemical processes of the skin, in the skin defence as well as in the bacterial flora (14, 15). The higher pH found at the perineum site vs the labium majus, and in subjects with a narrower crotch (Table IV), is probably also due to a higher humidity. The effects of the microbial metabolism may also contribute to the pH changes observed.

The second goal of the study was to see if microclimate changes in the vulva influenced the skin microflora. Literature about microbiology in vulvar sites is scarce, and thus we found only three articles reporting original data (5, 6, 16). Our findings of the carrier rates for the enteric bacteria *E. coli* and enterococci agree with both Aly et al. (6) and with Elkins and Cox (16). As can be seen in Table II, the carrier rate and density of microorganisms increased for many species in women with the non-breathable panty liner, compared with the situation when no panty liner was used. The significant differences found for *E. coli* and enterococci might point to an increased risk of contamination or survival of intestinal bacteria. However, the microbial densities are low and coincide with numbers reported by others (5, 6), for similar study populations of healthy, premenopausal women. Furthermore, significant differences were not found for other potentially pathogenic species (*S. aureus*, group B streptococci, α-streptococci and *C. albicans*), which – together with the low density levels – indicates that the hygienic risk is still low.

The carrier rates found for *C. albicans* (5–15%) and *S. aureus* (9–15%) were lower than reported by Aly et al. (6), where the carrier rate for *S. aureus* was as high as 67%. The discrepancy may reflect hygienic differences in the study populations, as well as other lifestyle factors and differences in time and geographical location. Redondo-Lopez et al. (17) emphasize that the vaginal flora is part of a dynamically changing ecosystem and it will vary with the stage in the menstrual cycle. This may also be the reason for variations between different vulvar studies. In our study we selected days 16 to 22 in the menstrual cycle for examination and sampling, and it was not possible to determine any variation due to differences in these 7 days. The high carrier rate of lactobacilli (96–99%) in our study compared with only 40% in the study by Aly et al. (6) also accentuates the differences in study populations. The possibility should not be excluded that the high numbers of lactobacilli might reflect effective sampling, transport lasting less than 2 h and prompt culturing techniques, as well as the healthy status of the study subjects. The present study does not support the proposals made by several authors (17, 18) that the number of lactobacilli depresses the number of unwanted microorganisms.

CNS, *Corynebacterium* spp. and lactobacilli were found in almost all subjects. The other species were less common and occurred irregularly, although the average carrier rate was relatively constant for the three regimens. The 'transient' species were seldom found in the same subject on all three occasions, possibly due to external factors like hygiene during toilet visits,

recent vulva washings, etc. It was seen that CNS, *Corynebacterium* spp. and lactobacilli co-varied with each other and with pH. This may indicate that when the conditions are good for bacterial growth, they are beneficial for all forms. For instance, a humid environment will definitely favour microbial growth. We also believe, as pointed out earlier, that an increase in pH depends on the higher humidity. That is why lactobacilli were also found to correlate positively with increased pH. As seen in Table IV, we studied the correlations against the number of total microorganisms found, but found no correlation with either temperature or type of clothing used. However, we could see a correlation between the microflora at the labium majus with the pH. This may indicate that the effect of temperature increase is less influential than increases in pH/humidity. Other correlations found were between the two vulvar sites (labium majus and perineum). The number of bacteria is higher at the perineum than at labium majus, for a majority of species. Also the carrier rate is higher at the perineum, especially so for *E. coli* and to a lesser degree for enterococci. Furthermore, several species grew to a certain density at the perineum before they occurred at the labium majus (not shown). This supports the rationale of hygienic measures to prevent microorganisms spreading from the anus via the perineum to the vulvo-genital area.

A negative co-variation was found between α-streptococci and enterococci, indicating an antagonistic behaviour, or competition for the same ecological niche (receptors, nutrients, etc.). Examples are given in the literature of species from both genera having bacteriocin-mediated antagonism against the other (19, 20).

As seen in Table III, the difference between the use of non-breathable panty liners and the use of breathable, acidic liners is less for the microflora found on the skin at the labium majus and perineum than for the microflora within the panty liners. This might be due to effects of different micro-environments and presumably to a relatively stronger pH effect inside the panty liner. The pH within the acidic panty liner results from a so-called superabsorbent (polyacrylic acid) buffering at pH 4.5. In comparison the materials in the conventional panty liner have a much lower buffering ability, and the pH is around 6. It has been shown that common skin bacteria growth is more reduced at pH below 5.0 (21). The yeast *C. albicans* is the only species that has a higher CFU density in the acidic panty liner than in the conventional panty liner. This is in agreement with the findings in an earlier study (22), where a reduction of pH from 6.0 to 4.5 did not influence the number of *Candida* cells. It could be questioned if the cellulose material that differs between panty liner B and C plays a role, but investigations have shown that the antimicrobial effects of different combinations of cellulose and acidic superabsorbent polymers have mainly been caused by the pH (data on file, SCA Hygiene Products). The influence of microorganisms that may be harboured by the panty liners from the beginning is negligible, as species are not the same as in vulvar skin flora, and total viable aerobic count is below $10^2$ CFU/g (*European Pharmacopoiea*, 3rd edn, 2.6.12).

The temperature and pH increases from covering the vulvar skin with non-breathable panty liners (B vs A) in Figs 1–3, whereas use of breathable panty liners did not cause the same increase (C vs A). There are numerous reports in the literature showing the effects of pH and occlusion-caused humidity on skin microflora and skin reactions (2, 7, 14, 22–25). We found that the use of non-breathable panty liners slightly increases the carrier rate and density level of microorganisms, but that the use of breathable, acidic panty liners keeps the levels very close to the normal, 'undisturbed' situation (Table II and Fig. 4).

## ACKNOWLEDGEMENTS

We are very grateful to Maria Brander who carried out the fieldwork virtually flawlessly, Britt-Louise Olsson for skilful laboratory assistance and Maria Flodén for every form of support. We also thank Nils Blomqvist and Lars Gustafsson for statistical guidance, and Stig E. Holm for advice on microbial matters. The microbial analyses were performed at the Bacteriology Laboratory, SU/Östra Hospital, Göteborg. SCA Hygiene Products AB supported the study financially and supplied all the products.

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