

A Double Blind Randomised Controlled Trial Comparing Two Panty Liners with Different Surfaces with Respect to Microbial Colony per Square Centimeter

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ABSTRACT

Purpose: To compare microbial colonies per square centimeter on two different panty liner pads after 4-6 hour of vulvar contact. The secondary objective was to assess whether daily pad use induces dermatological changes in the vulva and to investigate its impact on the vulva in women with or without vaginal discharge or bacterial vaginosis.

Methods: A total of 250 healthy women aged 20-43 years participated in this study. Baseline vulvar and vaginal conditions were assessed through physical examinations, culture samples, and laboratory analyses. Participants were randomly assigned to one of the panty liner groups through internet-based random number generation. Even numbers were assigned to pad group 102 and odd numbers were assigned pad group 103. The panty liners were identical in appearance and neither the patients, nor the clinicians and microbiologist were aware of the technology until the study finished. After 4-6 hours of use, microbial cultures were obtained from the pads to determine colony counts, while dermatological evaluations of the vulva were conducted to assess any skin irritation or changes.

Results: The frequency of bacterial vaginosis, and percent of cases with pathogenic microbial species isolation were similar in the two groups at the time of randomization. The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively (280 ± 65 vs 275 ± 72 minutes $p < 0.58$). The-zinc coated group, coded as 102, had 60 (53.7%) patients without microbial growth, significantly lower than group 103 with non-coated regular panty liners ($n=44$, 37.6%, $p=0.02$). Number of colonies per square centimeter on zinc coated panty liners was significantly lower than the non-coated group (9324 ± 24046 vs 56663 ± 99618 colonies $p < 0.001$). Dermatological assessment of the vulva showed no notable difference between group and within group frequencies of vulvar erythema, and excoriation in either panty liner group.

Conclusion: The study confirmed that zinc-coated panty liners bear significantly less microbial colonies with 4-6 hours of use compared to non-coated panty liners. The use of panty liners lead to a non-significant decrease in vulvar erythema and excoriation after short term of use which should be re-evaluated for longer and repeated use.

Keywords: Daily pads, panty liner, vulvar irritation, bacterial vaginosis, hygiene products

INTRODUCTION

Daily pads, also known as panty liners, are thinner and narrower than standard menstrual pads, specifically designed for use during non-menstrual periods. They serve to absorb vaginal discharge, light vaginal bleeding, and, in some cases, small amounts of urine in women with urinary

incontinence. Despite their widespread use, daily pads have been associated with potential health concerns due to their tendency to trap heat and moisture against the skin, creating conditions that may predispose users to complications such as microbial overgrowth and skin irritation.¹ These risks are further heightened by the unique vulnerability of the vaginal mucosa, which lacks the robust barrier function of the skin,



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allowing pathogens to penetrate more easily, potentially leading to systemic exposure and adverse effects in the anogenital region.²

Research into the impact of panty liner use on vulvar and vaginal health has yielded varying findings. A study investigating the microbial flora of the labia revealed that it differs from the vaginal flora. Yet, no significant increase in clinically important microbial species was observed after six months of continuous use.³ Regarding dermatological effects, studies across different populations suggest that daily pads are generally well-tolerated. For instance, a 2011 study from China reported high vulvar skin tolerance to daily pads, consistent with findings from earlier studies in diverse populations.⁴

Zinc has started to be used in biological materials due to its antibacterial properties.⁵ The antibacterial properties of zinc also help biomaterial biocompatibility.⁶ The aim of the current study was to research any differences in microbial colony growth on zinc coated versus regular non-coated panty liners from the same company after 4-6 hours of vulvar skin and vaginal contact.

METHODS

Healthy, sexually active, consecutive women aged 20 to 43 years who visited Okan University Hospital *In Vitro* Fertilization Center between January and June 2021 were included in this study. The İstanbul Okan University Ethics Committee approval was obtained for the study (approval number: 128, date: 11.11.2020). All costs of the study was covered by the panty liner producer Hayat Chemistry Company, Turkey, and all participants were given one pack of free of charge panty liner at the end of the study. Participants were provided with both verbal and written information prior to their inclusion in the study, and their informed consent was obtained. Inclusion criteria were all consecutive women who started controlled ovarian hyperstimulation for in vitro fertilization (IVF) treatment, had no diagnosis of recurrent *in vitro* fertilization failure, and were free of immunosuppression. As exclusion criteria, patients with active vaginal bleeding and patients with zinc allergy were not included in the study.

The primary outcome of the study was difference in mean number of colonies between the two panty liners per square centimeter (cm²). Secondary outcome measures were number of women with vulvar erythema or excoriation.

This was a double blind study with blocked randomization. The block size was determined to be the same for the number of people and there was no stratification based on any variables (e.g., age, body mass index). The two identical looking panty liner pads were prepared by the company with code 102 and 103 labeled on the pack without any additional information about the product. At the time of first visit on day 2-4 of the menstrual period patients were informed about the study and were assigned to each group with blocked randomization. Pre-prepared 250 code written closed envelopes were put in a closed box and mixed. Code 102 was written in 125 envelopes and 103 was written in another 125 sealed envelopes. The envelopes were taken from the box at the time of randomization and the panty liner pack was given according to the code

inside the envelope until the 250th patient was recruited. The flow chart of the study is presented in Figure 1.

The panty liners were outwardly identical and neither the patients, nor the clinicians or microbiologist were aware of the technology until the study was finished. The second visit of the patients for controlled ovarian hyperstimulation was done at the follicular phase of the cycle without any menstrual bleeding after 5-6 days of their first visit with planned weekends. The patients were instructed not to use panty liners or any sanitary products until the second visit when comprehensive clinical evaluation, including a physical examination of the vulva and a speculum-based assessment of the vagina was done. The evaluation focused on identifying dermatological conditions such as vulvar erythema, excoriation, and discharge. Biological samples were obtained from specific anatomical sites: cultures were collected from the interlabial space (between the labia minora and labia majora), while cervicovaginal samples and bacterial vaginosis specimens were retrieved from the upper lateral vaginal wall. These assessments aimed to establish baseline microbial profiles for participants prior to pad use. Patients were instructed to avoid sexual activity, douching, or other potential confounders before sample collection. Optimal conditions for processing swab samples (e.g., transport conditions, temperature control) were provided.

Swab samples were collected from the lateral wall of the vagina for Gram staining and applied to slides. These were evaluated for bacterial vaginosis using Nugent scoring at 100x magnification. In addition, a second swab was collected to assess for vaginal candidiasis. The samples were cultured on sabouraud dextrose agar (SDA) and incubated for 48 hours at 37 °C. When growth was observed, colonies were stained, and *Candida* was diagnosed upon detection of gram-positive blastospores. Species identification and antifungal susceptibility testing were performed using the VITEK 2 (BioMerieux) system with YST and AST-YS07 cards. Pathogenic isolates of vagina and vulva was defined by isolation of aerobic bacteria like *E. coli*, *Klebsiella* spp., *Streptococcus agalactiae*, *Enterococcus* spp. and *Candida* spp.

The patients were provided with coded panty liners and instructed to use the panty liner for 2-3 days between the second and third visit. On the day of third visit they used the last panty liner 4 to 6 hours prior to their ultrasound evaluation. This pad was evaluated for colony counts. At this third visit all patients continue to do their daily routines and after 4-6 hours of panty liner use microbial cultures were obtained from the pads to determine colony counts, while dermatological evaluations of the vulva were conducted to assess any skin irritation or changes. When the pads were retrieved a 1 cm² section from the area exhibiting the highest level of discharge or moisture was excised from the center of each pad using a sterile scalpel and placed in a Sabouraud broth tube. All samples were promptly transported to the laboratory for microbial analysis. After homogenization with a vortex mixer, 0.1 mL of the sample was cultured on SDA and incubated at 37 °C for 48 hours. Upon observing growth, colonies were stained, and *Candida* was diagnosed by detecting gram-positive blastospores. Colony counting was performed, and species identification and antifungal susceptibility testing were

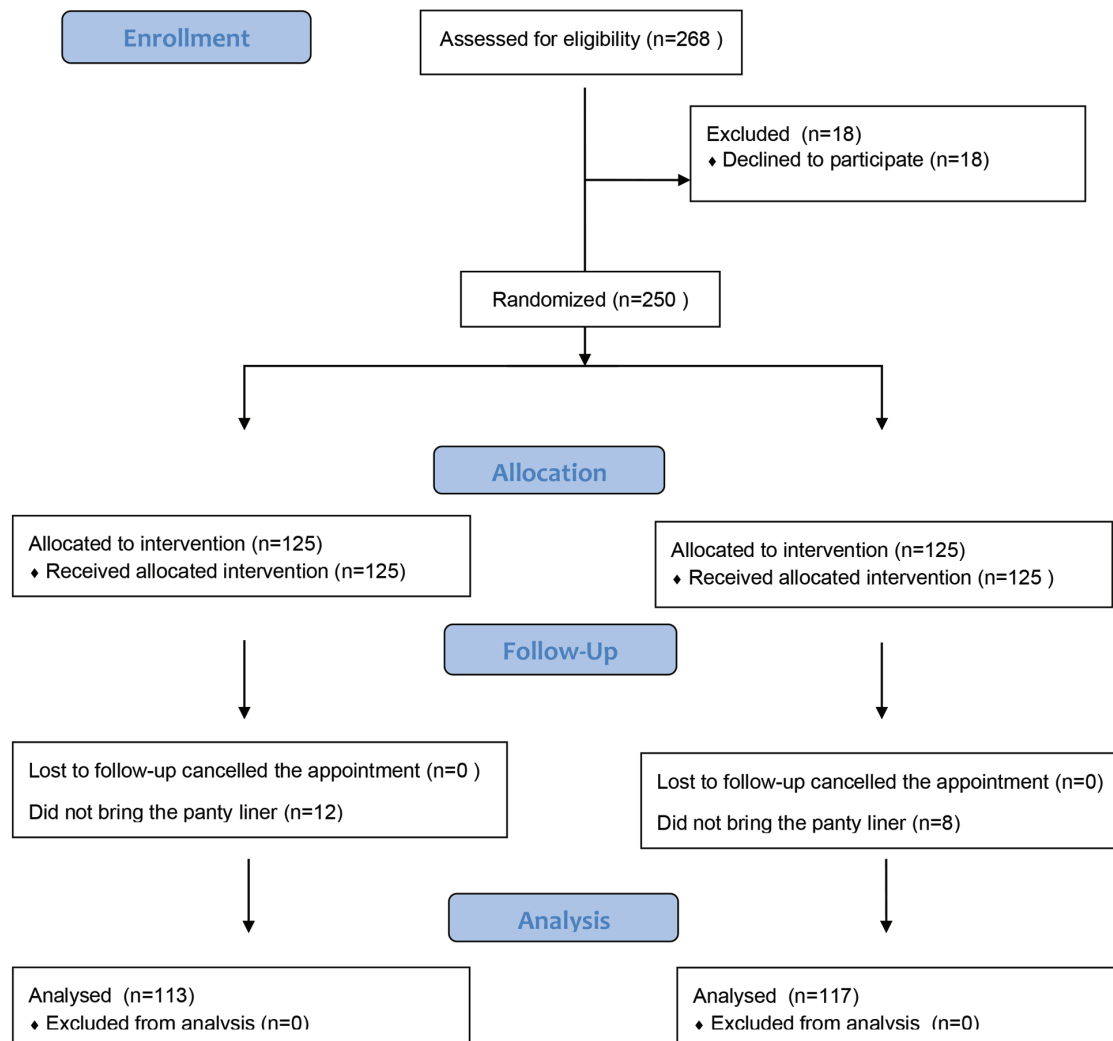


Figure 1. Consort flow diagram

carried out using the VITEK 2 (BioMerieux) system with YST and AST-YS07 cards. Whenever microbial cultures of all cases were evaluated and reported, the codes of the panty liner 102 was revealed to be zinc coated group and 103 was revealed to be non-coated regular panty liner group.

Statistical Analysis

The statistical analysis was done using SPSS, version 21 (IBM Inc., Armonk, NY, USA). Continuous variables are presented as means \pm standard deviation and categorical data are presented as counts and percentages. Comparison of the categorical data frequency between the two groups was done using chi square test. Comparison of categorical data frequency within the group prior to and after the use of panty liner was done using McNemar test. The comparison of continuous data between the groups was done using independent samples t test. A probability (p) less than 0.05 was considered to be statistically significant for all comparisons.

RESULTS

A total of 268 women were eligible for the study. Women who refused to use the pad ($n=18$) were excluded from the study leaving 250 cases for allocation. After allocation, 12 patients with code 102 did not bring their panty liners or did not comply with the use and were excluded from the analysis while eight patients with code 103 were excluded due to the same reasons. As a result 113 patients with code 102 and 117 patients with code 103 were analyzed.

The demographical data are given in Table 1. The frequency of vaginal discharge, foul odor in the perineum, vulvar itching, erythema on the vulva, and excoriation on the vulva were similar in the Zinc coated panty liner group ($n=113$) vs. non-coated group ($n=117$). The frequency of bacterial vaginosis in the Zinc coated group was 15.9% ($n=13$) which was not statistically different in non-coated group 17.9% ($n=21, p=0.6$). The percent of cases with pathogenic microbial species isolation in the vagina ($n=23, 28\%$) and vulva ($n=42, 51.2\%$) of the coated group was again not statistically different from the

Table 1. Demographic data

Demographic data	Zinc-coated group (n=113) n (%)	Non-coated group (n=117) n (%)
Age (mean)	33.1	32.3
Marital status	Never married: 25 (22.1) Currently married: 30 (26.5) Other: 58 (51.3)	Never married: 26 (22.2) Currently married: 31 (26.4) Other: 60 (51.2)
Cigarettes per day	0: 58 (51.3) 1-9: 30 (26.5) 10+: 25 (22.1)	0: 60 (51.2) 1-9: 31 (26.4) 10+: 26 (22.2)
Frequency of alcohol drinking	None: 31 (27.4) Less than weekly: 35 (30) Weekly or more: 47 (41.5)	None: 32 (27.3) Less than weekly: 36 (30.7) Weekly or more: 49 (41.8)
Hormonal contraception	Yes: 20 (17.6) No: 93 (82.3)	Yes: 22 (18.8) No: 95 (81.1)

Table 2. Baseline symptomatology and genital findings

Syptoms-findings	Zinc-coated group (n=113) n (%)	Non-coated group (n=117) n (%)	p*
Vaginal discharge	15 (18.3)	23 (19.7)	0.8
Foul odor in the perineum	18 (22)	25 (21.4)	0.9
Vulvar itching	7 (8.5)	10 (8.5)	0.9
Erythema on the vulva	8 (9.8)	12 (10.3)	0.9
Excoriation on the vulva	8 (9.8)	12 (10.3)	0.9
Bacterial vaginosis	13 (15.9)	21 (17.9)	0.6
Pathogen in the vagina	23 (28)	43 (36.8)	0.1
Pathogen in the vulva	42 (51.2)	60 (51.3)	0.9

*chi-square test, not significant, $p > 0.05$

vagina (n=43, 36.8%, $p=0.1$) and vulva (n=60, 51.3%, $p=0.9$) of the non-coated group. The baseline symptomatology and genital findings are given in Table 2.

The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively (280 ± 65 vs 275 ± 72 minutes, $p < 0.58$). In the zinc-coated group coded as (102) there were 60 (53.7%) patients without microbial growth. This proportion was significantly higher than in group 103 with non-coated regular panty liners (n=44, 37.6%, $p=0.02$). Furthermore, the number of colonies per cm^2 of the zinc-coated panty liners was significantly lower than in the non-coated group ($m=9324 \pm 24046$ vs 56663 ± 99618 , $p < 0.001$). The microbial proliferation in the two types of panty liners is given in Table 3.

The number of cases with vulvar erythema and excoriation was similar in the two groups before and after panty liner use. Furthermore, within group change in the frequency of vulvar erythema and excoriation was not significant. Vulvar skin findings before and after panty liner use is shown in Table 4. There were no adverse events or side effect in either group.

DISCUSSION

These results show that zinc-coated panty liners harbored less microbial colonies compared to the non-coated regular panty

liners. While most studies² focus on isolating and analyzing individual pathogen species through separate cultures, the present study prioritized assessing the total number of cultured microorganisms. Although this approach represents a limitation when compared to studies that provide detailed pathogen-level data, a key strength of our study lies in its dual focus on microbial counts and vulvar symptomatology and findings within a single research setting.

When daily pads were examined symptomatically in terms of side effect profiles such as edema, erythema, burning, stinging, and itching, in a study conducted by Xuemin et al.³ in Chinese women, comparing two pads with non-woven and perforated surfaces, no significant difference was observed between the two groups. Similarly, in the present study, there was no significant difference between and within the groups with and without a zinc-coated surface in terms of erythema, excoriation, and itching.

Basit et al.⁷ discussed traditional beliefs about hygienic products and economic problems in their study conducted in Bangladesh during the flood period. We did not mention financial access to the product in our study, but only 18 (0.6%) of the 268 women in the clinic refused to use pads.

Runeman et al.⁸ demonstrated that breathable pads maintained vulvar microclimate stability better than traditional pads. Our

Table 3. Microbial proliferation in the two types of panty liners

Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	p
Vulva pantyliner contact time	280±65	275±72	0.58*
Non-microbialgrowth	60 (53.7%)	44 (37.6%)	0.02**
Colonycount	9324±24046	56663±99618	<0.001***
*independent samples t-test **chi-square test ***Independent samples t-test			

Table 4. vulvar skin findings before and after panty liner use

Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	p*
Pre-existing vulvar erythema	11 (9.8%)	12 (10.3%)	0.9
Post-use vulvar erythema	7 (6.1%)	9 (7.7%)	0.6
Within group comparison (p)	0.12**	0.25**	
Pre-existing vulvar excoriation	11 (9.8%)	12 (10.3%)	0.9
Post-use vulvar excoriation	7 (6.1%)	9 (7.7%)	0.6
Within group comparison (p)	0.12**	0.25**	
*chi-square test **McNemar's test			

double-blind study design addressed vaginal microclimate, vulvar findings and symptomatology and panty liner microbial colony forming unit (CFU) count. This rigorous approach lends greater reliability to our findings when compared to similar studies.

Farage et al.² conducted a study comparing daily pads with deodorant and a control group without deodorant in terms of aerobic bacteria cultures. They reported no statistically significant difference in positive cultures of undesirable microorganisms such as *Candida albicans*, non-*Candida* yeasts, *Candida* spp, *Gardnerella vaginalis*, *Staphylococcus aureus*, *coliforms*, *proteus*, *pseudomonas*, *streptococci* Groups A,B, D and *Streptococcus viridans* before and after six months of panty liner use. In the present study, although no significant difference was observed in incidence of bacterial vaginosis between the two pads tested, the total colony count of cultured bacteria from the panty liners was significantly lower for the zinc-coated group. This suggests that zinc has an inhibiting effect on the number of colonies formed by the undesirable bacteria in the vaginal flora compared with non-zinc coated regular panty liners.

Zinc can be classified as a form of immunotherapy and has effects on macrophage and neutrophil functions, natural killer cell/phagocytic activity, and various inflammatory cytokines.⁹ Zinc also directly modulates the interaction between host cells and viral components.¹⁰ Although the exact mechanism remains unclear, there are promising reports in the literature of zinc being used effectively in various topical and oral forms and concentrations for the treatment of cutaneous viral warts.¹¹

The zinc ion was found to have a more profound antibacterial effect on gram-positive bacteria such as *S. aureus* and *Staphylococcus epidermidis* compared to gram-negative bacteria.^{12,13} The proposed mechanism of action include binding of zinc to the membranes of microorganisms and increasing

the lag time and new microbial cell generation time, such as in *C. albicans*.¹⁴ Moreover, zinc has been shown to cause direct bacterial cell membrane disruption and indirectly through mediating the induction of reactive oxygen species (ROS).^{15,16} However, extended exposure to zinc oxide was suspected to play a reversible role in aminoglycoside resistance and ampicillin and other b-lactam resistance in *Escherichia coli* by modifying cell drug efflux systems, switching the bacteria to anaerobic respiration state and increasing ribosomal protein production.^{17,18} Antibiotic resistance of bacteria as panty liner use was short lasting. Further studies are needed to investigate if zinc coated panty liners may lead to an increased number of antibiotic resistant organisms.

Giraldo et al.⁴ examined the effects of breathable versus conventional daily pads and found no significant differences in vulvovaginal irritation or bacterial vaginosis. Their study included colposcopic examinations, while our study relied on speculum-assisted visualization of the vagina and cervix. In addition, the present study only considered a single-day use of 4-6 hours, whereas the study of Giraldo et al.⁴ extended to a 75-day period.

Kim et al.¹⁹ investigated the presence of volatile organic compounds in pads used in Korea and found that these pads have no cancer or non-cancer risk. Again, given the short term nature of the present study, this aspect was not investigated.

In a study conducted by Yadav et al.²⁰ in Nepal, high awareness and self-efficacy in menstrual hygiene management among female adolescents were noted. In our study, population women showed high compliance with the terms of use, although they were although they were in this respect.

The strength of our study is that it was double-blind. The weakness of our study is that microbiological cultures were not identified and therefore pathogen levels were unavailable and there was also no testing of antibiotic resistance.

CONCLUSION

Research into the effects of daily panty liners used during non-menstrual periods, such as for vaginal discharge, spotting, and mild urinary incontinence, has become important with increasing use and will be important for enhancing patient comfort and quality of life. Studies examining the impact of panty liners with different formulations and constructions on vulvar irritation, vaginal pH, microbiological outcomes, and potential triggers for discharge or itching will be important for safe product development. Designing commercial products based on such findings can significantly improve user comfort and deliver broader health benefits.

Ethics

Ethics Committee Approval: The İstanbul Okan University Ethics Committee approval was obtained for the study (approval number: 128, date: 11.11.2020).

Informed Consent: Participants were provided with both verbal and written information prior to their inclusion in the study, and their informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: Ş.Ç., C.Ö., B.A., Concept: Ş.Ç., Design: Ş.Ç., Data Collection or Processing: Ş.Ç., Ö.D.S., Analysis or Interpretation: Ş.Ç., Ö.D.S., Literature Search: Ö.D.S., Writing: Ş.Ç., Ö.D.S.

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