

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

Şeyda Çalışkan<sup>1</sup>, Ömer Doğukan Saraç<sup>2</sup>, Canan Özcan<sup>3</sup>, Bertan Akar<sup>4</sup>

<sup>1</sup>Gölcük Necati Çelik State Hospital, Clinic of Medical Microbiology, Kocaeli, Turkey

<sup>2</sup>Kocaeli Private Akademi Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey

<sup>3</sup>Kocaeli City Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey

<sup>4</sup>Gölcük Private Medar Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey

## ABSTRACT

**Purpose:** The aim of the study is to compare microbial colonies per centimeter square of two different panty liner pad 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 sampling, and laboratory analyses. Participants were randomly assigned to one of the panty liner group according to internet based random number generator. Even numbers were assigned to pad group 102 and odd numbers were assigned pad group 103. The panty liners were identical in outlook 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 randomisation. The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively ( $m=280\pm65$ ,  $m=275\pm72$ ,  $p<0.58$ ). The zinc coated group coded as 102 had 60 (53.7%) patients without microbial growth which was significantly lower compared to the group 103 with non-coated regular panty liners ( $n=44$ , 37.6%,  $p=0.02$ ). Number of colonies per centimeter square in zinc coated panty liners were significantly lower than non-coated group ( $m=9324\pm24046$ ,  $m=56663\pm99618$ ,  $p<0.001$ ). Dermatological assessments of the vulva showed no notable between group and within group frequencies of vulvar erythema, and excoriation in either panty liner group.

**Conclusion:** The study confirms 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.<sup>1</sup> These risks are further heightened by the unique vulnerability of the vaginal mucosa, which lacks the robust barrier function of the skin,



**Address for Correspondence:** Ömer Doğukan Saraç, Kocaeli Private Akademi Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey

**E-mail:** omerdogukansarac@hotmail.com **ORCID ID:** orcid.org/0000-0002-9397-428X

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allowing skin allergens to penetrate more easily, potentially leading to systemic exposure and adverse effects in the anogenital region.<sup>2</sup>

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.<sup>3</sup> 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.<sup>4</sup>

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

## METHODS

A total of 250 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 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 mean number of colonies between the two panty liners per centimeter square. Secondary outcome measures were number of women with vulvar erythema or excoriation. Two hundred and sixty-eight women were eligible for the study and only women who rejected to use the pad (n=18) were excluded from the study leaving 250 cases for allocation.

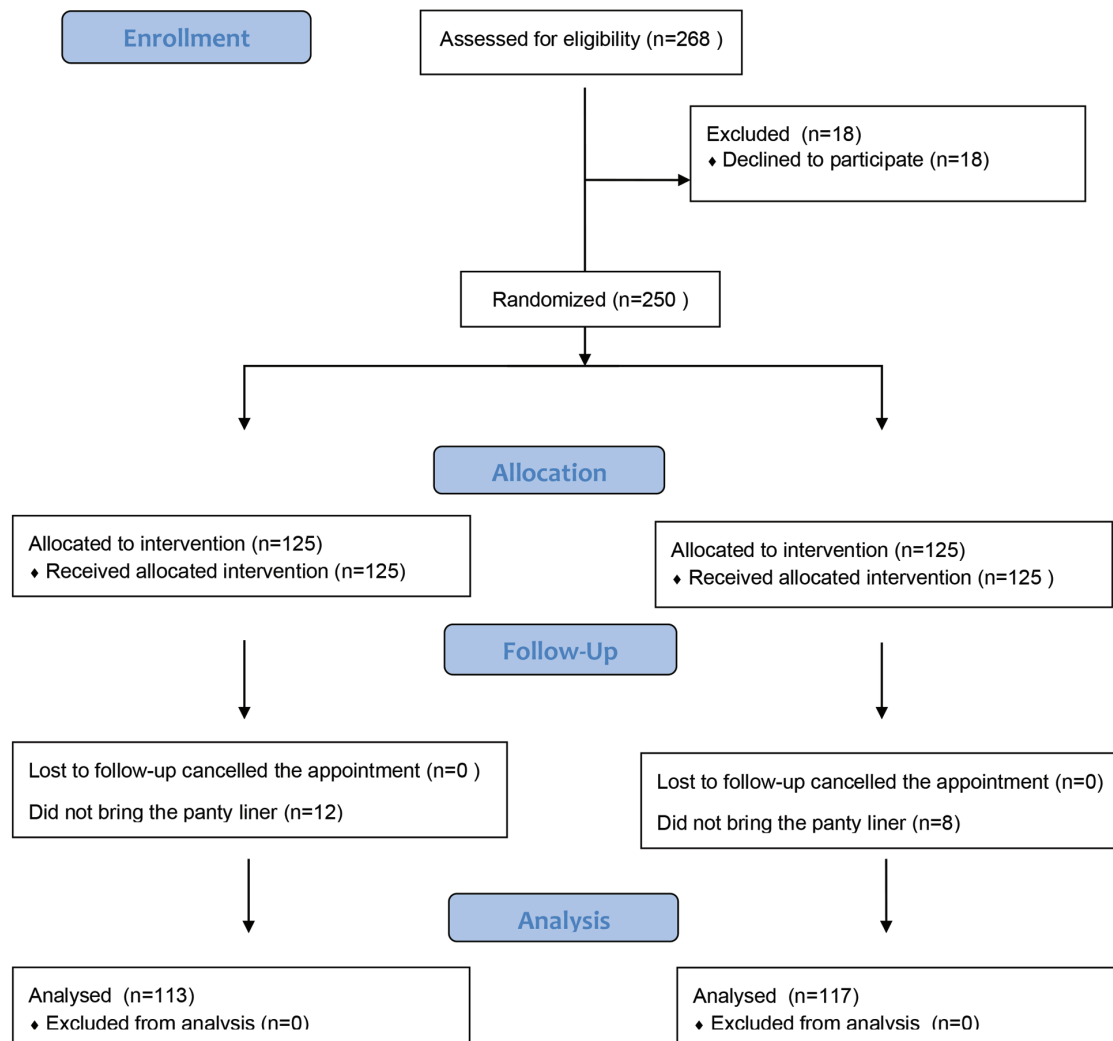
The study was a double blind study with blocked randomisation. The block size was determined to be the same for the number of people and there wasn't an stratification based on any variables (e.g., age, body mass index). The two identical looking panty liner pads were prepared by the company with code 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 closed envelopes. The envelopes were driven from the box at the time of randomization, the panty liner pack was given according to the code inside the envelope until the 250<sup>th</sup> patient was recruited. The flow chart of the study is presented in Figure 1. After allocation 12 patients with code 102 did not bring their panty liners or did not comply with the use and excluded from the analysis while 8 patients with code 103 were excluded due to same reasons. As a result 113 patients with code 102 and 117 patients with code 103 were analysed.

The panty liners were identical in outlook and neither the patients, nor the clinicians and microbiologist were aware of the technology until the study was finished. 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 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. Additionally, 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 *Escherichia 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 which was evaluated for colony count evaluations. 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 cm<sup>2</sup> 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



**Figure 1.** Consort flow diagram

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 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-zinc coated regular panty liner group.

### Statistical Analysis

The statistical analysis of the study was done using IBM<sup>®</sup> SPSS<sup>®</sup> Statistics version 21. Continuous variables were presented as means  $\pm$  standard deviation of the mean and categorical data was presented as numbers 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's test. The comparison of continuous data between the groups was done using independent samples t test. Probability (p) less than 0.05 was considered to be statistically significant for all comparisons.

### RESULTS

The demographical data given in Table 1. The frequency of vaginal discharge, foul odor in the perineum, vulvar itching, erythema on the vulva, excoriation on the vulva were similar in Zinc coated panty liner group (n=113) vs. non-coated group (n=117). The frequency of bacterial vaginosis in 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 not statistically different from the 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.

**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

The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively ( $m=280\pm65$ ,  $m=275\pm72$ ,  $p<0.58$ ). The zinc coated group coded as 102 had 60 (53.7%) patients without microbial growth which was significantly lower compared to the group 103 with non-coated regular panty liners ( $n=44$ , 37.6%,  $p=0.02$ ). Number of colonies per centimeter square in zinc coated panty liners were significantly lower than non-coated group ( $m=9324\pm24046$ ,  $m=56663\pm99618$ ,  $p<0.001$ ). The microbial proliferation according to different panty liners are 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. Also, within group change in the frequency of vulvar erythema and excoriation was not statistically different. Vulvar skin findings before and after panty liner use is given in Table 4. There was no important harm or unintended effect in each group.

## DISCUSSION

In our study, we found that zinc coated panty liners bare less microbial colonies compared to the non-coated regular panty liners. While most studies in the literature<sup>2</sup> focus on isolating and analyzing individual pathogen species through separate cultures, our research 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.<sup>3</sup> on Chinese women, comparing two pads with non-woven and perforated surfaces, no significant difference was observed between the two groups. Similarly, in our 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.<sup>7</sup> mentioned 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.<sup>8</sup> demonstrated that breathable pads maintained vulvar microclimate stability better than traditional pads. Our double-blind study design address vaginal microclimate, vulvar findings and symptomatology and panty liner microbial colony forming unit count. This rigorous approach lends greater reliability to our findings when compared to similar studies.



**Table 3. Microbial proliferation according to different 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-microbial growth	60 (53.7%)	44 (37.6%)	0.02**
Colony count	9324±24046	56663±99618	<0.001***
*Not statistically significant, independent samples t-test, p>0.05 **chi-square test, statistically significant, p<0.05 ***Independent samples t-test, statistically significant, p<0.01			

**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, not significant, p>0.05 **McNemar's test, not significant, p>0.05			

Farage et al.<sup>2</sup> 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*, *Streptococcus* Gour A,B, D and *Streptococcus viridans* before and after six months of panty liner use. In our study, although no significant difference was observed in 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. It can be concluded that zinc has reduction effect on the number of colonies formed by the undesirable bacteria in the vaginal flora than 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<sup>9</sup> and also directly modulates the interaction between host cells and viral components.<sup>10</sup> 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.<sup>11</sup>

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.<sup>12,13</sup> 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 in *Candida albicans*.<sup>14</sup> Also Zinc was shown to cause

direct bacterial cell membrane disruption and also indirectly by inducing reactive oxygen species.<sup>15,16</sup> On the other hand extended exposure to ZnO was suspected to play a reversible role in aminoglycoside resistance and ampicillin and other b-lactam resistance in *E. coli* via modifying cell drug efflux systems, switching the bacteria to anaerobic respiration state and increasing ribosomal protein production.<sup>17,18</sup> In our study we did not study antibiotic resistance of bacteria as panty liner use was short lasting. Further studies may unravel if there is any increased number of antibiotic resistant microbial growth with zinc coated panty liners.

Giraldo et al.<sup>4</sup> 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. Additionally, our research assessed a single-day usage of 4-6 hours, whereas Giraldo et al.<sup>4</sup> study spanned a 75-day period. Kim et al.<sup>19</sup> investigated the presence of volatile organic compounds in pads used in Korea and found that these pads have no cancer and non-cancer risk. In our study, we did not conduct a research on this product produced in Turkey.

In a study conducted by Yadav et al.<sup>20</sup> in Nepal, high awareness and self-efficacy in menstrual hygiene management among female adolescents were noted. In our study, we found that women showed high compliance with the terms of use, although they were not evaluated 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 studied and were not specific to pathogen types.

## CONCLUSION

Research into the effects of daily panty liners used during non-menstrual periods-such as for vaginal discharge, spotting, and mild urinary incontinence-is crucial for enhancing patient comfort and quality of life. Studies examining these panty liners impacts on vulvar irritation, vaginal pH, microbiological outcomes, and potential triggers for discharge or itching provide valuable insights. Designing industrial 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.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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