



HAL
open science

Does exposure to different menstrual products affect the vaginal environment?

Nicolas Tessandier, Ilkay Başak Uysal, Baptiste Elie, Christian Selinger, Sophie Grasset, Claire Bernat, Vanina Boué, Soraya Groc, Massilva Rahmoun, Bastien Reyné, et al.

► To cite this version:

Nicolas Tessandier, Ilkay Başak Uysal, Baptiste Elie, Christian Selinger, Sophie Grasset, et al.. Does exposure to different menstrual products affect the vaginal environment?. *Molecular Ecology*, 2023, *EVOLUTIONARY ECOLOGY OF HUMAN-ASSOCIATED MICROBES*, 32 (10), pp.2592-2601. 10.1111/mec.16678 . hal-03847887

HAL Id: hal-03847887

<https://hal.science/hal-03847887v1>

Submitted on 18 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 Does exposure to different menstrual products affect the
2 vaginal environment?

3 Nicolas Tessandier^{1,2,*}, Ilkay Başak Uysal^{2,#}, Baptiste Elie^{2,#}, Christian
4 Selinger², Claire Bernat², Vanina Boué², Sophie Grasset², Soraya Groc², Massilva
5 Rahmoun², Bastien Reyné², Noemi Bender³, Marine Bonneau⁴, Christelle Graf⁴,
6 Vincent Tribout⁵, Vincent Foulongne⁶, Jacques Ravel⁷, Tim Waterboer³,
7 Christophe Hirtz⁸, Ignacio G Bravo², Jacques Reynes⁹, Michel Segondy¹⁰,
8 Carmen Lia Murall^{2,11}, Nathalie Boule¹², Tsukushi Kamiya^{1,13}, Samuel
9 Alizon^{1,2,*}

10 ¹ Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS, INSERM,
11 Université PSL, Paris, France

12 ² MIVEGEC, CNRS, IRD, Université de Montpellier, France

13 ³ Molecular Diagnostics of Oncogenic Infections, Infection, Inflammation and Cancer Program,
14 German Cancer Research Center (DKFZ), Im Neuenheimer Feld, Heidelberg, Germany.

15 ⁴ Department of Obstetrics and Gynaecology, Centre Hospitalier Universitaire de Montpellier,
16 Montpellier, France

17 ⁵ Center for Free Information, Screening and Diagnosis (CeGIDD), Centre Hospitalier Universitaire de
18 Montpellier, Montpellier, France

19 ⁶ Pathogenesis and Control of Chronic Infections, Montpellier University, INSERM, EFS, Montpellier,
20 France.

21 ⁷ Institute for Genome Sciences, Department of Microbiology and Immunology, University of Maryland
22 School of Medicine, Baltimore, MD, USA

23 ⁸ LBPC/PPC- IRMB, CHU de Montpellier and Université de Montpellier, Montpellier, France

24 ⁹ Infectious Diseases Departement, University Hospital Montpellier; INSERM U1175/Institut de
25 Recherche et de Developement, Unité Mixte International, Montpellier, France.

26 ¹⁰ Pathogenesis and Control of Chronic Infections, University of Montpellier, INSERM, EFS, CHU
27 Montpellier, Montpellier, France.

28 ¹¹ Current address: Department of Biological Sciences, Université de Montréal, Montréal, Canada

29 ¹² Pathogenesis and Control of Chronic Infections, University of Montpellier, INSERM, EFS;
30 Departement of Pathology and Oncobiology, Laboratory of Solid Tumors, CHU Montpellier,
31 Montpellier, France.

32 ¹³ HRB Clinical Research Facility, National University of Ireland Galway, Ireland

33 # Equal contribution

34 * Corresponding authors: nicolas.tessandier@college-de-france.fr, samuel.alizon@cnrs.fr, Collège de
35 France, 11 place Marcelin Berthelot, 75005 Paris, France, phone: +33 144271667

36

Abstract

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

The vaginal ecosystem is a key component of women's health. It also represents an ideal system for ecologists to investigate the consequence of perturbations on species diversity and emerging properties between organisational levels. Here, we study how exposure to different types of menstrual products is linked to microbial, immunological, demographic, and behavioural measurements in a cohort of young adult women who reported using more often tampons ($n = 107$) or menstrual cups ($n = 31$). We first found that cup users were older and smoked less than tampon users. When analysing health indicators, we detected potential associations between cups use reporting and fungal genital infection. A multivariate analysis confirmed that, in our cohort, reporting using cups over tampons was associated with the higher odds ratio to report a fungal genital infection diagnosis by a medical doctor within the last 3 months. We did not detect significant differences between groups in terms of their bacterial vaginal microbiota composition and found marginal differences in the level of expression of 20 cytokines. However, a multivariate analysis of these biological data identified some level of clustering based on the type of menstrual product type preferred (cups or tampons). These results suggest that exposure to different types of menstrual products could influence menstrual health. Larger studies and studies with a more powered setting are needed to assess the robustness of these associations and identify causal mechanisms.

56

57

Keywords: vaginal microbiota; fungal infections; immunity; epidemiology; women's health

58 Introduction

59 There is a long history of considering organisms as ecosystems, and *vice versa* [1]. This
60 parallel has been particularly exploited to study within-host parasite dynamics [2]. With
61 the advent of metagenomics, there is a renewed interest in applying ecological theory to
62 understand microbiota dynamics [3, 4]. Beyond parasites or even the microbiota, the
63 importance of encompassing the diversity of these ecosystems, e.g. including immune
64 cells, is increasingly acknowledged, especially since the border between self and non-self
65 is tenuous in this context [5].

66 Some general questions pertaining to ecosystem functioning can be studied by con-
67 sidering an individual host as an ecosystem. For instance, it is challenging to clearly
68 define properties that emerge at a higher level of organisation in large-scale ecologi-
69 cal systems. However, the definition and measurement of individual health outcomes
70 (i.e., systems-level outcomes) is routine in clinical research. In contrast to free-living
71 ecosystems, individual hosts are also smaller, more numerous, and readily replicable,
72 making empirical ecosystem ecology research more accessible and reproducible [6].

73 The vaginal ecosystem is an ideal study system to understand how within-host inter-
74 actions shape host health. First, the vaginal microbiota is a key component of women’s
75 health throughout their lives [7], modulating the risk of diseases such as fungal genital
76 infections, urinary tract infections, and sexually transmitted infections [8], such as hu-
77 man immunodeficiency virus [9]. Contrary to its gut counterpart, the vaginal microbiota
78 is characterised by relatively few, clearly-defined, community state types (CST), most
79 of which are dominated by one species of *Lactobacillus* bacteria [10]. Second, the mi-
80 crobes in the vaginal environment, including bacteria, fungi, and viruses, are involved in
81 a tight cross-talk with the local innate and adaptive immune response, the components
82 of which are known to be adapted to this highly variable environment [11]. In partic-
83 ular, the interaction between the immune response and sex hormones throughout the
84 menstrual cycle is well documented [12]. Finally, the vaginal ecosystem is perturbed by
85 factors including menses, sexual activities, and antibiotic treatments [13]. Longitudinal
86 follow-ups demonstrate that such perturbation can lead to shifts from one CST to an-
87 other [14]. Perturbations that jeopardise the balance between immunity, hormones, and

88 the vaginal microbiota could also hinder the efficiency of the immune system to control
89 bacteria, fungal, and viral pathogens [13].

90 Here, we explore whether exposure to a particular type of menstrual product, namely
91 menstrual cups or tampons, is associated with differences in the vaginal environment.
92 To this end, we analyse the vaginal microbiota, immune response (i.e., cytokines and
93 antibodies), and, more generally, the emerging property of this ecosystem, which revolves
94 around menstrual health [15].

95 Among the variety of products used during menses, menstrual cups are perceived as
96 a safe, practical, economical, and ecologically friendly alternative to tampons and san-
97 itary pads [16]. The majority of women using them report wanting to continue to use
98 them, both in high and low-income settings, showing a good level of acceptability [17,
99 16]. Nevertheless, there have been some reported cases of toxic shock syndrome, renal
100 colic, and allergies linked with menstrual cup usage [16]. For example, higher levels of
101 *Staphylococcus aureus* growth have been reported in menstrual cups compared to tam-
102 pons [18]. In 2019, a systematic review identified 12 clinical trials and 23 observational
103 studies comparing this type of menstrual product to others [16]. In the vast majority of
104 existing studies, the outcome studied was the practicality of the cups implementation,
105 e.g. acceptability or risk of leakage. Therefore, we know little about the potential mi-
106 crobiological and immunological implications of cup usage compared to other menstrual
107 hygiene products.

108 In the present study, we analysed biological, demographic, and behavioural longitu-
109 dinal data from 138 women. We compared two populations of women defined by the type
110 of menstrual product they reported using most often (tampons or menstrual cups) and
111 analysed microbiological, immunological, and clinical data while statistically accounting
112 for demographic and behavioural differences. Using statistical modelling, we identified
113 profile differences depending on the type of menstrual product used.

114 **Materials and methods**

115 **Cohort description and data curation**

116 Women included in the present study were enrolled in the PAPCLEAR longitudinal
117 clinical study [19], which followed 149 women between 2016 and 2020 to study human

118 papillomavirus (HPV) infections [19]. The women enrolled were between 18 and 25
119 years old, lived in the area of Montpellier (France), and reported at least one new
120 partner within the last 12 months. Their status for HPV and other genital infections,
121 immunological responses (antibodies and cytokines), and behaviours were followed for
122 up to two years (see [20] for details about the study protocol).

123 We selected participants who reported using tampons or cups as menstrual products
124 and for whom detailed cytokine profiles, microbiota metabarcoding data, and antibody
125 data at the inclusion visit were available. We assigned tampon or menstrual cup cate-
126 gories when a participant reported using either type of menstrual product over 75% of
127 the time over the whole duration of the study (see Figure S1 for details on how the as-
128 signment was performed). Women using menstrual cups more frequently than tampons
129 are denoted ‘menstrual cups users’ for convenience throughout the manuscript. There
130 was no difference in follow-up duration between women using mostly cups or mostly tam-
131 pons (Table 1). Other analyses of demographic, behavioural, and biological data were
132 performed on the first visit of the participant (inclusion visit). Overall, our analysis
133 includes data from 138 women.

134 Since the PAPCLEAR study was designed to understand the ecology of HPVs and
135 their interaction with immune responses and vaginal microbiota, the data from this
136 cross-sectional study is not perfectly balanced in terms of exposure to each type of
137 menstrual product. However, given the current lack of studies, it greatly improves our
138 understanding of women’s menstrual health.

139 **Biological analyses**

140 HPV infections were assessed from cervical smears using the LiPA₂₅ genotyping assay,
141 which discriminates between 25 genotypes [21]. Further details on HPV detection and
142 prevalence in this cohort can be found elsewhere [20]. A ‘focal’ infection was defined as
143 an infection by the same HPV genotype at the first and second visits.

144 The microbiota metabarcoding was performed on 200 µL of vaginal swabs specimen
145 stored at -80° in Amies medium. DNA extraction was performed using the MagAt-
146 tract PowerMicrobiome DNA/RNA Kit (Qiagen). Next-generation sequencing of the
147 V3-V4 region of the 16S gene [22] was performed on an Illumina HiSeq 4000 platform

148 (150 bp paired-end mode) at the Genomic Resource Center at the University of Mary-
149 land School of Medicine. Taxonomic assignment was performed using the internal soft-
150 ware package SpeciateIT (<https://github.com/Ravel-Laboratory/speciateIT>) and
151 the community state type (CST) was determined using the VALENCIA software package
152 [23].

153 Antibodies were analysed using a multiplex Luminex assay [24], as already described
154 in the context of HPV infections [20]. Cytokine data were obtained using MesoScale
155 discovery technology from vaginal secretions collected using ophthalmic sponges placed
156 directly on the cervical os for approximately one minute, as described in a previous
157 study which analyses this data in the context of HPV infections [25]. We used the same
158 protocol to obtain values that were normalised per total protein concentration in the
159 sample.

160 **Statistical analyses**

161 To study the difference between the main characteristics of our two populations of in-
162 terest (Table 1 and Table 2), we used χ^2 -test or Kruskal-Wallis rank sum tests when
163 applicable.

164 We then performed multivariate analyses using generalised linear models (GLMs)
165 with a binomial distribution for the response variable for the models shown in Figure
166 1A, Table 3, and Supplementary Tables S4 to S8. In the first series of models, the
167 response variable was the type of menstrual product (cups or tampons). In the second
168 series, the response variable was being diagnosed or not with a fungal genital infection
169 within the last three months by a medical doctor.

170 We identified 15 covariates of interest (Table S1). Given the exploratory nature of the
171 study, we built models with all the possible combinations of covariates as explanatory
172 variables. We then selected the best models using the Akaike Information Criterion
173 corrected for small sample sizes (AICc). We used a lowest AICc +5 interval to identify
174 the most probable best models [26]. For each model, we computed the odds ratios
175 associated with each predictor along with a 95% confidence interval.

176 For clustering, factor analysis of mixed data was used when combining both factor
177 and numeric data (Figure 3B and S2), and multiple correspondence analysis was used

178 when analysing categorical data (Figures S2 and S4) [27]. Non-overlapping 95% con-
179 fidence ellipses indicate a statistically significant difference between cups and tampons
180 users. The covariates used for the socio-economic analysis using a multiple correspon-
181 dence analysis are listed in Table S2.

182 Statistical analyses were performed in R 4.1.3 [28] with the with the `kruskal.test`
183 function for Kruskal-Wallis rank sum tests (in Tables 1 and 2 for numeric data), `chisq.test`
184 for χ^2 test (in Table 1 and Figure 2B for proportional data), `wilcox.test` function
185 for Mann-Whitney test (Figure 2C) and the `glmulti` function for binomial regressions
186 [29] (in Figure 1A and Table 3). The factor analysis of mixed data and the multiple
187 correspondence analysis (in Figures 1B, 3B, S2, S3, and S4) were processed and repre-
188 sented using the `FactorMineR` and the `FactoExtra` ([https://github.com/kassambara/](https://github.com/kassambara/factoextra)
189 `factoextra`) packages [27].

190 Results

191 The 138 women included in the analysis were aged from 18 to 25 years old and primarily
192 university students (119/138, 86%). We stratified the population according to the most
193 frequent type of menstrual product used, *i.e.* either tampons, $n = 107$, or menstrual cups,
194 $n = 31$. Analysis of the main demographic characteristics (shown in Table 1) using
195 a generalised linear model (GLM) selection approach identified significant differences
196 between these two populations in terms of age and smoking status: cups users were
197 older and reported smoking less than tampon users (Figure 1A and Table S3). On the
198 other hand, a multiple correspondence analysis on 12 covariates associated with socio-
199 economic status and listed in Table S2 did not show any difference between the two
200 populations (Figure 1B).

201 We then explored associations between the type of menstrual product used and
202 microbiological covariates. First, we analysed vaginal microbiota diversity using 16S
203 metabarcoding data (Figure 2A). We found no significant difference in community state
204 types (CST) composition (Figure 2B), although there were some qualitative differences.
205 For instance, among our menstrual cup users, we found no occurrence of CST II, which
206 is dominated by *L. gasseri*, but slightly more CST I and V, which are dominated by
207 *L. crispatus* and *L. jensenii* [10]. We also did not find any significant differences in micro-
208 biota diversity, assessed using Shannon diversity index, between women using menstrual
209 cups or tampons (Figure 2C).

210 We then analysed six health-related covariates, most of which correspond to infections
211 (Table 2). The only significant difference was that women using cups also reported more
212 often having been diagnosed with fungal genital infections within the last 3 months. To
213 further investigate this association, we used our GLM selection method to identify the
214 covariates associated with this fungal genital infection. We found that reporting using
215 cups is the only predictor with a significant odds ratio (OR of 4.73, 95% CI: 1.44-16.1)
216 associated with the risk of reporting a fungal genital infection (the number of lifetime
217 partners is also present in the model, but not significant). Similar models for the other
218 five health-related covariates are shown in Appendix (Tables S4 to S8).

219 To investigate the potential effect of menstrual cups on the local immune response,

220 we analysed cytokine and chemokine relative concentrations in cervical samples. Among
221 the 20 analytes measured, IL-10, MIP-1 α and TNF- α appeared to be significantly lower
222 in women using cups (IL-10: $p = 0.012$, MIP-1 α : $p = 0.049$ and TNF- α : $p = 0.036$),
223 although these associations did not withstand correction for multiple testing comparisons
224 ($p = 0.24$, $p = 0.32$, and $p = 0.32$ respectively) (Figure 3A).

225 Finally, we performed a profile analysis using a factor analysis of mixed data approach
226 involving CST data, Shannon diversity index, and cytokine and chemokine relative con-
227 centrations. Women who use tampons and women using menstrual cups fall into two
228 largely non-overlapping clusters (Figure 3B and Supplementary Figure S2), suggest-
229 ing that the type of menstrual product used could be associated with a different im-
230 munological and microbial environment. Conversely, a similar multiple correspondence
231 analysis approach using blood seropositivity status for immunoglobulins G (IgG) and
232 immunoglobulins M (IgMs) of several sexually transmitted infections, including HPVs,
233 detected no clustering (Supplementary Figure S3), hinting that the women in these two
234 groups have similar exposure risks to sexually transmitted infections.

235 Discussion

236 The vaginal ecosystem is an essential part of women’s health and several ‘perturbations’
237 such as menses, sexual intercourse, or drug treatment can cause pronounced shifts [7].
238 In this study, we analysed whether exposure to different types of menstrual products,
239 namely menstrual cups or tampons, could be associated with differences in health indi-
240 cators or, more generally, in the vaginal environment.

241 Menstrual cups are gaining in popularity as an environmentally sustainable and af-
242 fordable type of menstrual product, but the data surrounding their use are limited,
243 especially outside low-income countries [16]. Therefore, we first analysed the profiles
244 of the two populations of women defined based on the type of menstrual product they
245 reported using most. In terms of demographic covariates, we found that the main factors
246 that were associated with preferential use of cups over tampons were age (cup users were
247 older) and smoking status (tampon users smoked more). In terms of socio-economic sta-
248 tus, a multiple correspondence analysis did not suggest any differences between the two
249 populations, which could be due to the fact that the majority of the cohort member was

250 university students. A similar multiple correspondence analysis based on serological data
251 for a variety of sexually transmitted infections also did not find any difference, further
252 suggesting that these two cohorts are comparable in terms of their general lifestyle.

253 On the microbiological side, we did not find a significant difference between the
254 bacterial community compositions of the vaginal environment depending on the type of
255 menstrual product used. A recent preprint [30] studied vaginal microbiota in a large
256 cohort of 3,345 women aged 18 to 98 with a median age of 32 years old. Their results
257 show that women who reported using menstrual cups are more often associated with a
258 *Lactobacillus crispatus* cluster (which also includes *L. jensenii*, and could be described
259 as encompassing both CST-I and CST-V); which is consistent with the trend detected
260 in our dataset.

261 Moving to health-related factors, we detected a strong association between reporting
262 using menstrual cups and a recent diagnosis of a fungal genital infection. None of
263 the other five health-related variables that we analysed, such as reporting urinary tract
264 infections or the diagnosis of chlamydia infections, were significantly different. To further
265 investigate this trend, we used a generalised linear model selection approach. In almost
266 all the best models, reporting using cups more than tampons was significantly associated
267 with a higher risk of reporting fungal genital infections. It is noteworthy that the number
268 of partners was present in most of the best models (98.75% of the time), although never
269 significant.

270 Finally, we investigated the host component of the vaginal environment by analysing
271 local cytokine and chemokine concentrations. Three cytokines or chemokines were found
272 to exhibit potentially lower concentrations in women using cups (IL-10, MIP-1 α , and
273 TNF- α), although significance did not withstand correction by multiple hypothesis test-
274 ing. Furthermore, the joint analysis of microbiota and immunological data suggests that
275 women tend to segregate into two clusters based on the type of menstrual product they
276 use most suggesting general profile differences in terms of their vaginal environment.

277 Our study has several limitations, the strongest being the relatively small sample size
278 of the cohort used ($n = 138$), which was originally designed to study HPV infections.
279 This may hinder our ability to detect moderate or subtle changes induced by menstrual
280 cups in the vaginal environment. For example, we included the use of lubricants in the

281 analysis, but more detailed studies could also include details on contraception methods or
282 probiotic use. Another limitation of the study lies in its cross-sectional nature. Further
283 longitudinal analyses would be helpful to establish long-term potential impacts of the
284 use of menstrual cups on the local environment, for example, on the vaginal microbiota
285 composition, which can be highly variable over time for some women [31]. In particular,
286 prospective or retrospective cohort studies, where participants are stratified based on
287 the type of menstrual products used, could be a powerful means to study the occurrence
288 of a particular negative outcome, i.e. here fungal infections.

289 Regarding these infections, we cannot provide any further details regarding the fungal
290 species or their abundance with the current data. A quantitative polymerase chain
291 reaction approach targeting the ITS locus coupled with a metagenomics approach could
292 provide a more accurate picture, especially thanks to existing gene catalogues for the
293 vaginal microbiota, such as VIRGO which already includes more than 10,000 fungal
294 genes [32].

295 The existing literature on the usage of menstrual cups focuses on availability and ac-
296 ceptability, yet its health implications remain understudied. A recent systematic review
297 reported nine cases of urinary tract complaints [16], which we did not detect as being
298 significantly more frequent among menstrual cups users than for tampons users. Con-
299 versely, the primary adverse health outcome associated with menstrual cup use in our
300 study, i.e. fungal infections, was not included in the systematic review. Our results are
301 consistent with another recent study, which does not show adverse effects of menstrual
302 cups on the vaginal microbiota, and even potentially an increased proportion of some
303 *Lactobacillus*-dominated communities, which are considered beneficial for maintaining
304 the vaginal health [30]. These findings can help inform public health policies regarding
305 the use of menstrual cups and underline the need for larger, balanced cohort studies.

306 Health is partly the result of dynamic interactions between hosts and their microbes
307 that have a long coevolutionary history. The human vaginal microbiota is an ideal
308 study system for studying these questions for several reasons. First, its composition is a
309 known health moderator — *Lactobacillus*-dominated communities tend to decrease the
310 risk of acquisition of sexually transmitted infections [10]. Second, the composition of
311 the vaginal microbiota can vary over time as a result of menstrual cycles or age, as well

312 as a variety of ‘perturbations’ including sexual intercourse and chemical interventions
313 [14]. These two points are not unique to the vaginal microbiota but contrarily to other
314 microbiota, especially in the gut, the diversity is limited (both between and within
315 women) and the variability better understood. From an evolutionary point of view,
316 human vaginal mircobiota are unique for the high prevalence of *Lactobacillus*-dominance:
317 Lactobacilli are rarely dominant in other mammals, including non-human primates [33].
318 This human specificity obviously complicates experimental studies using animal model
319 systems. However, three-dimensional cell culture models now offer new opportunities to
320 manipulative experiments, for instance to test how the microbiota composition affects the
321 interaction between a parasite and host cells [34]. As illustrated by our study, integrating
322 knowledge across scales, i.e. going from the microbiological and immunological variables
323 to individual health is data-intensive and challenging as it requires multidisciplinary
324 knowledge from biological to public health. Nonetheless, it is a necessary challenge to
325 bridge the gap between molecular mechanisms and health outcomes.

326 **Acknowledgements**

327 The authors thank all participants of the PAPCLEAR study and clinical staff and nurses
328 for their help.

329 **Disclosure of Interests**

330 The authors declare the following financial interests/personal relationships which may be
331 considered as potential competing interests: TW serves on advisory boards for MesoScale
332 discovery (Merck) Sharp & Dohme.

333 **Contributions**

334 NT, CLM, NBo, and SA designed the study. NT, CLM, BE, BR, TK, IGB, and SA
335 designed the experiments. CB, VB, SGro, MR, and NBe performed experiments. CH,
336 JRa, and TW contributed reagents, materials, and analysis tools. CB, VB, SGra, SGro,
337 MR, MB, CG, VT, VF, CLM, JRe, IGB, MSe, NBo, and SA contributed to study design,
338 patient recruitment, and clinical data acquisition. NT, IBU, BE, BR, CS, TK, CLM,

339 TK and SA performed data analyses. NT, IBU, IGB, CLM, TK and SA wrote the initial
340 version of the manuscript. All authors approved the final version of the manuscript.

341 **Ethics**

342 The PAPCLEAR trial was promoted by the Centre Hospitalier Universitaire (CHU) de
343 Montpellier and has been approved by the Comité de Protection des Personnes (CPP)
344 Sud Méditerranée I on 11 May 2016 (CPP number 16 42, reference number ID RCB 2016-
345 A00712-49); by the Comité Consultatif sur le Traitement de l'Information en matière de
346 Recherche dans le domaine de la Santé on 12 July 2016 (reference number 16.504); and
347 by the Commission Nationale Informatique et Libertés on 16 December 2016 (reference
348 number MMS/ABD/AR1612278, decision number DR-2016-488). This trial was autho-
349 rised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé on 20
350 July 2016 (reference 20160072000007). The ClinicalTrials.gov identifier is NCT02946346.
351 All participants provided written informed consent.

352 **Funding**

353 This work was supported by the European Research Council (ERC) under the European
354 Union's Horizon 2020 research and innovation programme [grant agreement No 648963
355 to SA] and by the CUPS2 project from the Agence Nationale de la Recherche. NT is an
356 ANRS-MIE fellow. IBU is funded by the FHU INCH. TK is funded by the Fondation
357 pour la Recherche Médicale. The sponsors had no role in the study design; in the
358 collection, analysis, and interpretation of data; in the writing of the report; and in the
359 decision to submit the article for publication.

360 **Data availability**

361 Table 1, 2, 3, 4, Figures 1, 2, 3, Supplementary Tables S1, S2, S3, S4, S5, S6, S7, S8
362 and supplementary figures S1, S2, S3 have associated raw data. The data that support
363 the findings of this study are available from the corresponding author upon request, and
364 data are available in the Zenodo public repository (10.5281/zenodo.6913875).

References

- 365 1. Baalen Mv and Huneman P. Organisms as Ecosystems/Ecosystems as Organisms.
366 Biol Theory 2014; 9:357–60. DOI: [10.1007/s13752-014-0194-7](https://doi.org/10.1007/s13752-014-0194-7)
- 367 2. Smith VH and Holt RD. Resource competition and within-host disease dynamics.
368 Trends Ecol. Evol. 1996; 11:386–9. DOI: [10.1016/0169-5347\(96\)20067-9](https://doi.org/10.1016/0169-5347(96)20067-9)
- 369 3. Costello EK et al. The application of ecological theory toward an understanding of
370 the human microbiome. 2012 Jun. DOI: [10.1126/science.1224203](https://doi.org/10.1126/science.1224203)
- 371 4. Murall CL et al. Invasions of host-associated microbiome networks. *Networks of in-*
372 *vasion: A synthesis of concepts*. Vol. 56. Advances in ecological research. Academic
373 Press, 2017. DOI: [10.1016/bs.aecr.2016.11.002](https://doi.org/10.1016/bs.aecr.2016.11.002)
- 374 5. Pradeu T. Immunology and individuality. eLife 2019 Apr; 8. DOI: [10.7554/eLife.47384](https://doi.org/10.7554/eLife.47384)
- 375 6. Rynkiewicz EC, Pedersen AB, and Fenton A. An ecosystem approach to under-
376 standing and managing within-host parasite community dynamics. Trends in Par-
377 asitology 2015; 31:212–21
- 378 7. Smith SB and Ravel J. The vaginal microbiota, host defence and reproductive
379 physiology. J Physiol 2017; 595:451–63. DOI: [10.1113/JP271694](https://doi.org/10.1113/JP271694)
- 380 8. Wijgert JHHM van de. The vaginal microbiome and sexually transmitted infections
381 are interlinked: Consequences for treatment and prevention. PLOS Medicine 2017
382 Dec; 14. DOI: [10.1371/journal.pmed.1002478](https://doi.org/10.1371/journal.pmed.1002478)
- 383 9. McKinnon LR et al. The Evolving Facets of Bacterial Vaginosis: Implications for
384 HIV Transmission. AIDS Res Hum Retroviruses 2019; 35:219–28. DOI: [10.1089/
385 aid.2018.0304](https://doi.org/10.1089/aid.2018.0304)
- 386 10. Ravel J et al. Vaginal microbiome of reproductive-age women. Proc Nat Acad Sci
387 USA 2011; 108:4680–7. DOI: [10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
- 388 11. Zhou JZ, Way SS, and Chen K. Immunology of the Uterine and Vaginal Mucosae.
389 Trends Immunol 2018; 39:302–14. DOI: [10.1016/j.it.2018.01.007](https://doi.org/10.1016/j.it.2018.01.007)
- 390 12. Wira CR, Rodriguez-Garcia M, and Patel MV. The role of sex hormones in immune
391 protection of the female reproductive tract. Nat Rev Immunol 2015; 15:217–30.
392 DOI: [10.1038/nri3819](https://doi.org/10.1038/nri3819)
- 393 13. France M et al. Towards a deeper understanding of the vaginal microbiota. Nat
394 Microbiol 2022; 7:367–78. DOI: [10.1038/s41564-022-01083-2](https://doi.org/10.1038/s41564-022-01083-2)
- 395
- 396

- 397 14. Gajer P et al. Temporal dynamics of the human vaginal microbiota. *Science Trans-*
398 *lational Medicine* 2012; 4:132ra52. DOI: [10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)
- 399 15. Hennegan J et al. Menstrual health: a definition for policy, practice, and research.
400 *Sex. Reprod. Health Matters* 2021; 29. DOI: [10.1080/26410397.2021.1911618](https://doi.org/10.1080/26410397.2021.1911618)
- 401 16. Eijk AM van et al. Menstrual cup use, leakage, acceptability, safety, and availability:
402 a systematic review and meta-analysis. *Lancet Public Health* 2019; 4:e376–e393.
403 DOI: [10.1016/S2468-2667\(19\)30111-2](https://doi.org/10.1016/S2468-2667(19)30111-2)
- 404 17. North BB and Oldham MJ. Preclinical, clinical, and over-the-counter postmarket-
405 ing experience with a new vaginal cup: menstrual collection. *J Womens Health*
406 2011; 20:303–11. DOI: [10.1089/jwh.2009.1929](https://doi.org/10.1089/jwh.2009.1929)
- 407 18. Nonfoux L et al. Impact of Currently Marketed Tampons and Menstrual Cups on
408 *Staphylococcus aureus* Growth and Toxic Shock Syndrome Toxin 1 Production *In*
409 *Vitro*. *Appl. Environ. Microbiol.* 2018; 84. DOI: [10.1128/AEM.00351-18](https://doi.org/10.1128/AEM.00351-18)
- 410 19. Murall CL et al. Natural history, dynamics, and ecology of human papillomaviruses
411 in genital infections of young women: Protocol of the PAPCLEAR cohort study.
412 *BMJ Open* 2019; 9:25129. DOI: [10.1136/bmjopen-2018-025129](https://doi.org/10.1136/bmjopen-2018-025129)
- 413 20. Murall CL et al. HPV cervical infections and serological status in vaccinated and
414 unvaccinated women. *Vaccine* 2020; 38:8167–74. DOI: [10.1016/j.vaccine.2020.](https://doi.org/10.1016/j.vaccine.2020.10.078)
415 [10.078](https://doi.org/10.078)
- 416 21. Geraets DT et al. The original SPF10 LiPA25 algorithm is more sensitive and
417 suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J*
418 *Virol Meth* 2015; 215-216:22–9. DOI: [10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)
- 419 22. Frank JA et al. Critical Evaluation of Two Primers Commonly Used for Amplifi-
420 cation of Bacterial 16S rRNA Genes. *Appl Environ Microbiol* 2008; 74:2461. DOI:
421 [10.1128/AEM.02272-07](https://doi.org/10.1128/AEM.02272-07)
- 422 23. France MT et al. VALENCIA: a nearest centroid classification method for vaginal
423 microbial communities based on composition. *Microbiome* 2020; 8:166. DOI: [10.](https://doi.org/10.1186/s40168-020-00934-6)
424 [1186/s40168-020-00934-6](https://doi.org/1186/s40168-020-00934-6)
- 425 24. Waterboer T et al. Multiplex human papillomavirus serology based on in situ-
426 purified glutathione s-transferase fusion proteins. *Clin Chem* 2005; 51:1845–53
- 427 25. Selinger C et al. Cytokine response following perturbation of the cervicovaginal
428 milieu during HPV genital infection. *Immunol. Res.* 2021; 69:255–63. DOI: [10.](https://doi.org/10.1007/S12026-021-09196-2)
429 [1007/S12026-021-09196-2](https://doi.org/1007/S12026-021-09196-2)

- 430 26. Burnham K and Anderson D. Model Selection and Inference: A Practical Information-
431 Theoretic Approach. 2. 2002 :75
- 432 27. Lê S, Josse J, and Husson F. FactoMineR: An R Package for Multivariate Analysis.
433 Journal of Statistical Software 2008; 25:1–18. DOI: [10.18637/jss.v025.i01](https://doi.org/10.18637/jss.v025.i01)
- 434 28. R Core Team. R: A Language and Environment for Statistical Computing. R Foun-
435 dation for Statistical Computing. Vienna, Austria, 2021
- 436 29. Calcagno V and Mazancourt C de. glmulti: An R Package for Easy Automated
437 Model Selection with (Generalized) Linear Models. Journal of Statistical Software
438 2010; 34:1–29. DOI: [10.18637/jss.v034.i12](https://doi.org/10.18637/jss.v034.i12)
- 439 30. Lebeer S et al. Citizen-science map of the vaginal microbiome. ResearchSquare
440 2022; preprint. DOI: [10.21203/rs.3.rs-1350465/v1](https://doi.org/10.21203/rs.3.rs-1350465/v1)
- 441 31. Ravel J et al. Daily temporal dynamics of vaginal microbiota before, during and
442 after episodes of bacterial vaginosis. Microbiome 2013 Dec; 1:29
- 443 32. Ma B et al. A comprehensive non-redundant gene catalog reveals extensive within-
444 community intraspecies diversity in the human vagina. Nat Comm 2020; 11:940.
445 DOI: [10.1038/s41467-020-14677-3](https://doi.org/10.1038/s41467-020-14677-3)
- 446 33. Miller EA et al. Lactobacilli dominance and vaginal pH: Why is the human vaginal
447 microbiome unique? Frontiers in Microbiology 2016 Dec; 7:1936. DOI: [10.3389/
448 fmicb.2016.01936](https://doi.org/10.3389/fmicb.2016.01936)
- 449 34. Edwards VL et al. Three-dimensional models of the cervicovaginal epithelia to
450 study host-microbiome interactions and sexually transmitted infections. Pathogens
451 and Disease 2022 Aug. DOI: [10.1093/femspd/ftac026](https://doi.org/10.1093/femspd/ftac026)

Table 1: **Key characteristics of participants included in the study.** *n* indicates the number of individuals, p-value refers to the outcome of a Kruskal-Wallis Rank Sum Test (`kruskal.test` function in R), IQR is the interquartile range, SD the standard deviation.

	Tampon	Menstrual cup	p
n	107	31	
PAPCLEAR follow-up duration (days, median [IQR])	220.00 [116.50, 374.50]	270.00 [74.00, 437.50]	0.740
Lifetime number of partners (median [IQR])	8.00 [4.00, 15.00]	11.00 [4.00, 16.50]	0.396
Age (years, median [IQR])	21.00 [20.00, 23.00]	23.00 [22.00, 24.00]	0.021
Age at menarchy (years, median [IQR])	13.00 [12.00, 14.00]	13.00 [12.00, 14.00]	0.695
Age at sexual debut (years, median [IQR])	17 [15.00, 18.00]	16 [15.00, 17.00]	0.338
Body Mass Index (BMI, median [IQR])	21.15 [19.78, 23.40]	21.62 [20.56, 23.22]	0.483
Smoking (%)			0.129
No	64 (59.8)	24 (77.4)	
Occasionally	15 (14.0)	4 (12.9)	
Often	28 (26.2)	3 (9.7)	
Antibiotics (last two weeks) = Yes (%)	7 (6.5)	3 (9.7)	0.842
Lubricant (last two weeks) (%)	14 (13.1)	5 (16.1)	0.891
Intercourse with a regular partner (last two weeks) (%)	63 (58.9)	18 (58.1)	1.000
Intercourse with an occasional partner (last two weeks) (%)	15 (14.0)	4 (12.9)	1.000
Stress level (past week) (%)			0.168
0 (Min)	20 (18.7)	5 (16.1)	
1	45 (42.1)	13 (41.9)	
2	30 (28.0)	13 (41.9)	
3 (Max)	12 (11.2)	0 (0.0)	
Menses (last two weeks) (%) (Yes)	58 (54.2)	20 (64.5)	0.416
HPV vaccinated (%)	55 (51.4)	19 (61.3)	0.443
HPV positive (focal) (%)	49 (45.8)	10 (32.3)	0.256
HPV positive (multiple HPVs) (%)	33 (30.8)	9 (29.0)	1.000

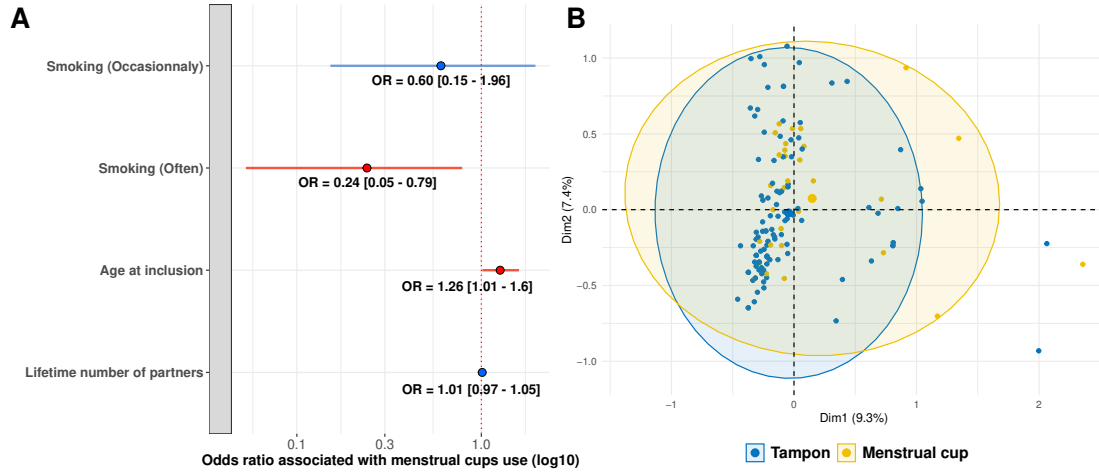


Figure 1: **Profile of menstrual cup users** A) Odds ratio of covariates associated with the use of menstrual cups. Results show the odds ratio (OR) of the factors selected in the best generalised linear model against menstrual cup as the response variable and using an Akaike Information Criterion corrected for small sample size (AICc). All covariates included in the model selection process are listed in Table S3. 95% confidence intervals are shown in brackets. B) Multiple Correspondence Analysis of socio-economic variables clustering participant using tampons or menstrual cups. Ellipses indicate 95% confidence intervals. Blue indicates tampon users, whereas yellow indicates women using menstrual cup.

Table 2: **Values of the main health metrics included in the study.** We show the number of occurrences and associated percentages (in parentheses) for each metric in the two study populations of size n . ‘p-value’ indicates the outcome of a t-test.

	Tampon	Menstrual cup	p-value
n	107	31	
Fungal genital infection (last 3 months) (%)	7 (6.5)	7 (22.6)	0.023
Genital infection (last 3 months) (%)	5 (4.7)	1 (3.2)	1.000
Chlamydia infection (last 3 months)(%)	4 (3.7)	1 (3.2)	1.000
Vaginosis (last 3 months)(%)	1 (0.9)	2 (6.5)	0.248
Urinary tract infection (last 3 months)(%)	12 (11.2)	6 (19.4)	0.378
HPV infection (focal) (%)	49 (45.8)	10 (32.3)	0.256

Table 3: Factors associated with the reporting of a fungal genital infection. Results show the odds ratios (OR) of the factors selected in the best generalised linear model with fungal genital infection as the response variable and using an Akaike Information Criterion corrected for small sample size (AICc). All covariates included in the model selection process are listed in Table 4. SE stands for standard ‘error’. CI 2.5% and CI 97.5% indicate the lower and upper bounds of the 95% confidence interval.

Response = Fungal genital infection	OR	OR SE	CI 2.5%	CI 97.5%	p.value
Menstrual cup	4.731	2.857	1.440	16.058	0.010 *
Lifetime number of partners	0.998	0.027	0.938	1.047	0.952

* : $p < 0.05$

Table 4: Frequency of occurrence of the covariates among the 80 best GLM models with fungal genital infection as a response variable (Model 2). The models were selected using AICc, assuming an AICc + 5 cutoff. The columns indicate the number and percentage of models that contain each covariate, as well as the percentage of models where the associated p-value is marginally significant (lower than 10%) or significant (lower than 5%).

Model [2]: Fungal genital infection	Presence in best models (#)	Presence in best models (%)	p.value < 0.1	p.value < 0.05
Menstrual cup	79	98.75	98.75	98.75
Lifetime number of partners	79	98.75	0.00	0.00
Age at first visit	39	48.75	0.00	0.00
Intercourse with occasional partner (last two weeks)	16	20.00	0.00	0.00
HPV positive (multiple infections)	16	20.00	0.00	0.00
Vaccinated against HPV	16	20.00	0.00	0.00
Intercourse with regular partner (last two weeks)	16	20.00	0.00	0.00
Menses (last two weeks)	16	20.00	0.00	0.00
Used Lubricant (last two weeks)	16	20.00	0.00	0.00
Antibiotics (last two weeks)	16	20.00	0.00	0.00
HPV positive (focal)	16	20.00	0.00	0.00
Stress level (last week) (1, minimum)	2	2.50	0.00	0.00
Stress level (last week) (2)	2	2.50	0.00	0.00
Stress level (last week) (3, maximum)	2	2.50	0.00	0.00
Smoking (Occasionally)	2	2.50	0.00	0.00
Smoking (Often)	2	2.50	0.00	0.00

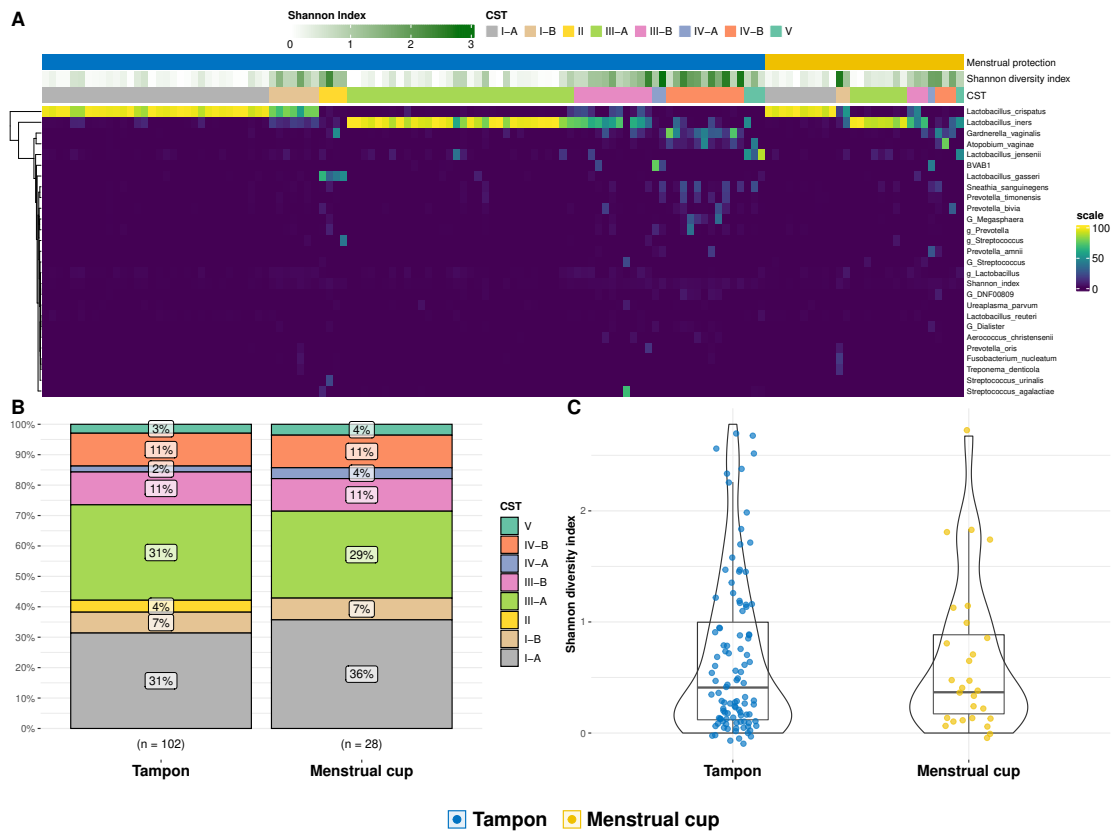


Figure 2: Metabarcoding differences between women using mainly menstrual cups or tampons. A) Abundance and diversity of the top 1% most abundant bacterial species found in participants, B) Community State Types (CST) distribution, C) Shannon diversity index. In A and B, colours show the type of menstrual product used (tampons in blue and cups in yellow).

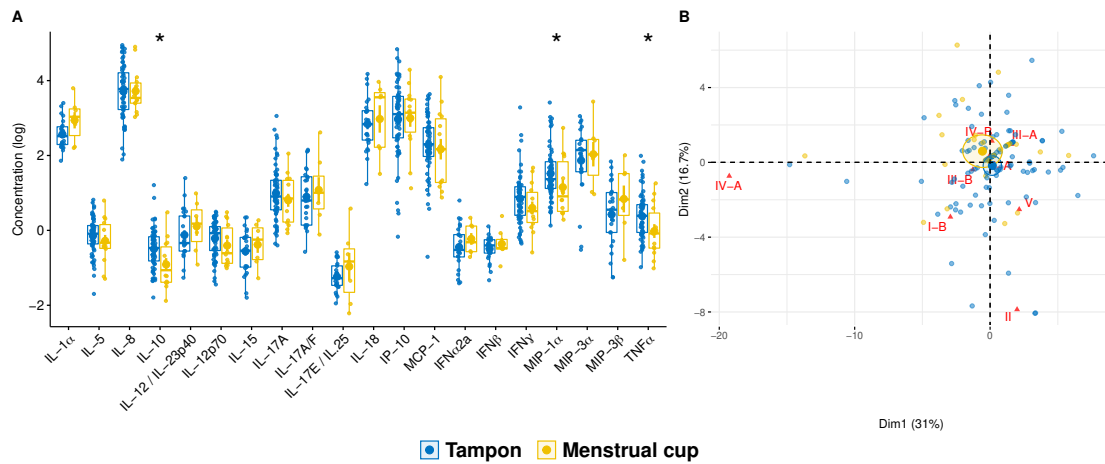


Figure 3: **Immunological differences between women using mainly menstrual cups or tampons.** A) Cytokines local concentrations (on a log scale), and B) Outcome of a multi-parametric clustering analysis using factor analysis of mixed data. In A, presence of a star indicates significance ($p < 0.05$). Ellipses indicate 95% confidence intervals. In A and B, colours show the type of menstrual product used (tampons in blue and cups in yellow). In B, the vaginal Community State Types (CSTs) are shown in red.