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Influence of hormonal contraceptives on the oral microbiota

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Objectives: The aim of the present study was to test the hypothesis that use of oral hormonal contraceptives causes changes in the oral microbiota during the menstrual cycle.
Study design: Bacterial samples were collected weekly during a full menstrual cycle. Forty systemically and periodontally healthy women of childbearing age with a Community Periodontal Index of Treatment Needs (CPITN) score ≤ 1 using (n=20) or not using hormonal contraceptives (n=20) were studied. Seventy-three different species were analysed by checkerboard DNA-DNA hybridisation assay.
Results: Most prevalent in non-contraceptive users at the time of menstruation were *Fusobacterium periodonticum*, *Fusobacterium nucleatum nucleatum*, *Leptotrichia buccalis* and *Gardnerella vaginalis*. No differences in bacterial levels as an effect of age or an effect of menstrual cycle in non-contraceptive and contraceptive users were found. Trends of higher counts of only *G. vaginalis* were found at the time of menstruation in non-contraceptive users ($P<0.02$) compared with 3-week samples. No differences were found in comparison to other time points. At menstruation, 15.9% of the sites in women in the group not using oral hormonal contraceptives were positive (≥1.0 x 10^5 cells) for *Prevotella intermedia*, which changed to 16.1% at day 21 following the onset of menstruation. The corresponding values for *P. intermedia* in the group using oral hormonal contraceptives were 14.6% and 17.9% respectively.
Conclusions: No changes in subgingival bacteria as an effect of the use of hormonal contraceptives could be found. A high proportion of the women were identified with bacteria not commonly studied in subgingival samples. These results may not be similar in subjects who are not periodontally healthy.

■ Introduction

It is well established that hormonal variations which occur during puberty, menstruation, hormone replacement therapy, use of oral contraceptives or pregnancy affect the periodontium[1-3]. These clinical changes in the tissues of the periodontium appear during periods of hormonal fluctuation, as these tissues are targets for the actions of steroid hormones. The most common oral manifestation of elevated levels of steroid hormones is an increase of gingival inflammation with an accompanying increase in gingival exudate[4]. Oestrogen and progesterone modulate vascular responses and con-

nective tissue turnover in the periodontium[5]. The metabolism of the steroid hormones increases significantly in the presence of pre-existing, plaque-induced gingival inflammation[6].

Several reports have indicated that steroid hormones induce proliferation of specific periodontal microorganisms[7-9]. Due to such hormonal changes there might be a shift in the microbiota[7]. Other reports have found higher levels of gingival inflammation in women using oral contraceptives. It has been shown that the use of contraceptive preparations containing oestrogen and progesterone results in hormonal changes similar to those seen in pregnancy, associated with increased prevalence of gingivitis[10]. A 16-fold higher level of *Bacteroides* species (covering, according to the old nomenclature, also *Prevotella* and *Porphyromonas* sp.) was found in women taking oral contraceptives compared with women not using oral contraceptives in a control group[8]. A comparative study has confirmed that, in spite of similar plaque scores, long-term oral contraceptive users have more gingival inflammation than short-term oral contraceptive users. In addition, the extent of clinical attachment loss was significantly higher in the long-term users[11]. Contradictory results suggesting no differences in plaque levels, gingival inflammation and gingival crevicular fluid levels between women using oral contraceptives and controls without oral contraceptives have also been reported[12].

The complexity of the subgingival microbiota is well known[13]. It has been established that more than 500 bacterial species are present in periodontal sulci[14]. Previous studies on contraceptives and possible changes in the subgingival microbiota have only assessed a limited number of different species, such as *Prevotella intermedia*. To the authors' knowledge there are no data available assessing the changes of an expanded panel of bacteria from subgingival samples from women using or not using hormonal contraceptives.

The aim of the present study was to examine possible microbiological changes during the menstrual cycle in women of childbearing age who either use or do not use oral hormonal contraceptives. The null hypothesis to be tested was that there are no changes in the presence and levels of subgingival bacteria as an effect of the use of hormonal contraceptives and that the microbiological findings are similar to those found in women who were not using hormonal contraceptives.

■ Study design

The present study was approved by the Ethics Committee of the University of Berne, Switzerland, and conducted in 2007. Subjects were recruited among dental students and staff members at the School of Dental Medicine, University of Berne, and from the dental hygiene school in Berne. To avoid coercion, all subjects were recruited by letters of invitation.

Systemically and periodontally healthy premenopausal women using hormonal contraceptives were assigned to the test group. Systemically and periodontally healthy women using no hormonal contraceptives with a normal cycle were assigned to the control group. Exclusion criteria were pregnancy, an irregular cycle or use of antibiotics. Each group consisted of 20 participants. The study duration was 6 weeks, to cover one full menstrual cycle. The first visit corresponded to ±2 days of the first day of menstruation. Bacterial plaque samples from the mesiobuccal aspect of first molars were collected using sterile endodontic paper points (size 55; Dentsply Maillefer, Ballaigues, Switzerland). At each weekly visit, microbial plaque samples were taken by one examiner from the mesiobuccal aspect of each first molar. Prior to bacterial sampling any visible supragingival plaque was removed. The paper point was inserted into the periodontal sulcus and kept in place for 20 seconds. The paper point was placed in a dry vial, transported to the microbiology laboratory and frozen immediately at -20°C. Only at the first visit and immediately after bacterial sampling was the Community Periodontal Index of Treatment Needs (CPITN) assessed for periodontal conditions. Only subjects with no evidence of periodontitis (CPITN scores ≤ 1) were enrolled.

■ Microbiological processing

Samples were processed, in detail, as described elsewhere using the checkerboard DNA-DNA hybridisation assay[15-17]. A total of 73 species were included in the analysis (Table 1). These species included 36 species that have previously been studied extensively.

Table 1 Bacterial species and subspecies included in the DNA-DNA checkerboard kit.

Species panel 1	Collection*	Species panel 2	Collection*
1a. *Aggregatibacter actinomycetemcomitans* (a)	ATCC 29523	1. *Actinomyces neuii*	GUH 550898
1b. *Aggregatibacter actinomycetemcomitans* (Y4)	ATCC 43718	2. *Aerococcus christensenii*	GUH 070938
2. *Actinomyces israelii*	ATCC 12102	3. *Anaerococcus vaginalis*	GUH 290486
3. *Actinomyces naeslundii* (type I + II)	ATCC 43146	4. *Atopobium parvulum*	GUH 160323
4. *Actinomyces odontolyticum*	ATCC 17929	5. *Atopobium vaginae*	GUH 010535
5. *Campylobacter gracilis*	ATCC 33236	6. *Bacteroides ureolyticus*	GUH 080189
6. *Campylobacter rectus*	ATCC 33238	7. *Bifidobacterium biavatii*	GUH 071026
7. *Campylobacter showae*	ATCC 51146	8. *Bifidobacterium bifidum*	GUH 070962
8. *Capnocytophaga gingivalis*	ATCC 33612	9. *Bifidobacterium breve*	GUH 080484
9. *Capnocytophaga ochraceae*	ATCC 33596	10. *Bifidobacterium longum*	GUH 180689
10. *Capnocytophaga sputigena*	ATCC 33612	11. *Corynebacterium aurimucosum*	GUH 450453
11. *Eikenella corrodens*	ATCC 23834	12. *Corynebacterium. nigricans*	GUH 071035
12. *Eubacterium saburreum*	ATCC 33271	13. *Dialister* sp.	GUH071045
13a. *Fusobacterium nucleatum nucleatum*	ATCC 25586	14a. *Enterococcus faecalis*	GUH170812
13b. *Fusobacterium nucleatum polymorphum*	ATCC 10953	14b. *Enterococcus faecalis*	ATCC 29212
13c. *Fusobacterium nucleatum naviforme*	ATCC 49256	15. *Escherichia coli*	GUH 070903
14. *Fusobacterium periodonticum*	ATCC 33693	16. *Gardnerella vaginalis*	GUH 080585
15. *Lactobacillus acidophilus*	ATCC 11975	17. *Haemophilus influenzae*	ATCC 49247
16. *Leptotrichia buccalis*	ATCC 14201	18. *Helicobacter pylori*	ATCC 43504
17. *Parvimonas micra*	ATCC 19696	19. *Lactobacillus crispatus*	GUH 160342
18. *Neisseria mucosa*	ATCC 33270	20. *Lactobacillus gasseri*	GUH 170856
19. *Prevotella intermedia*	ATCC 25611	21. *Lactobacillus iners*	GUH 160334
20. *Prevotella melaninogenica*	ATCC 25845	22. *Lactobacillus jensenii*	GUH 160339
21. *Prevotella nigrescens*	ATCC 33563	23. *Lactobacillus vaginalis*	GUH 080585 0780928
22. *Porphyromonas gingivalis*	ATCC 33277	24. *Mobiluncus curtisii*	GUH 070927
23. *Propionibacterium acnes* (type I+II)	ATCC11827/28	25. *Mobiluncus mulieris*	GUH 070926
24. *Selenomonas noxia*	ATCC 43541	26. *Peptoniphilus* sp.	GUH 550970
25. *Staphylococcus aureus*	ATCC 25923	27. *Porphyromonas endodontalis*	ATCC35406
26. *Streptococcus anginosus*	ATCC 33397	28. *Peptostreptococcus anaerobius*	GUH 160362
27. *Streptococcus constellatus*	ATCC 27823 (M32b)	29. *Prevotella bivia*	GUH 450429
28. *Streptococcus gordonii*	ATCC 10558	30. *Prevotella disiens*	GUH 190184
29. *Streptococcus intermedius*	ATCC 27335	31. *Proteus mirabilis*	GUH 070918
30. *Streptococcus mitis*	ATCC 49456	32. *Pseudomonas aeruginosa*	ATCC 33467
31. *Streptococcus oralis*	ATCC 35037	33a. *Staphylococcus aureus* (yellow)	GUH 070921
32. *Streptococcus sanguinis*	ATCC 10556	33b. *Staphylococcus aureus* (white)	GUH 070922
33. *Streptococcus mutans*	ATCC 25175	33c. *Staphylococcus epidermidis*	GUH 130381
34. *Tannerella forsythia*	ATCC 43037 (338)	34. *Staphylococcus haemolyticus*	GUH071047
35. *Treponema denticola*	ATCC 35405	35. *Streptococcus agalactiae*	GUH 230282
36. *Treponema socranskii*	D40DR2	36. *Varibaculum cambriense*	GUH 070917
37. *Veillonella parvula*	ATCC 10790		

*ATCC, American Type Culture Collection; D, sample from Forsyth Institute, MA, USA; GUH, Ghent University Hospital Collection, Ghent, Belgium.

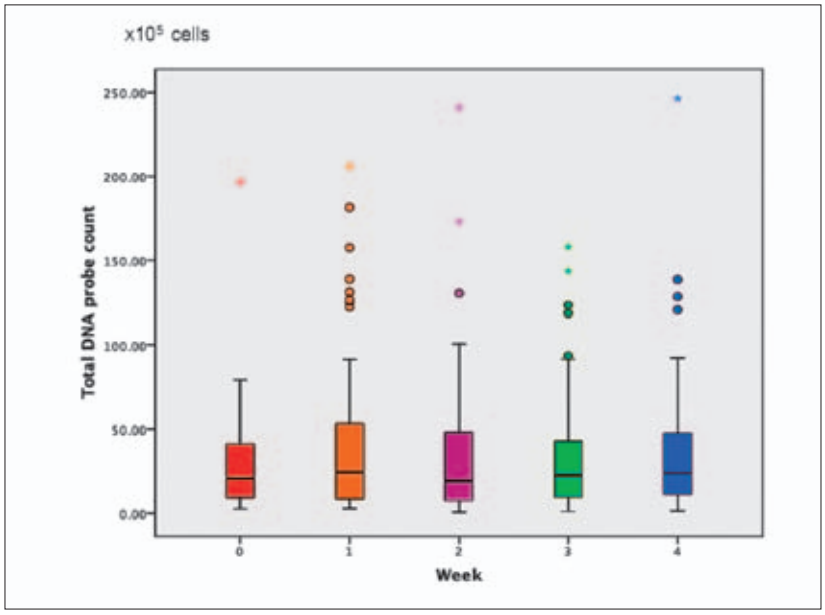

Fig 1 Boxplot diagram for women using hormonal contraceptives. Total bacterial load based on assessed species in panel 1. Week 0, time of menstruation; week 1, 7 days after menstruation; week 2, 14 days after menstruation; week 3, 21 days after menstruation; and week 4, beginning of new menstruation (asterisk indicates extreme outlier; circle indicates outlier).

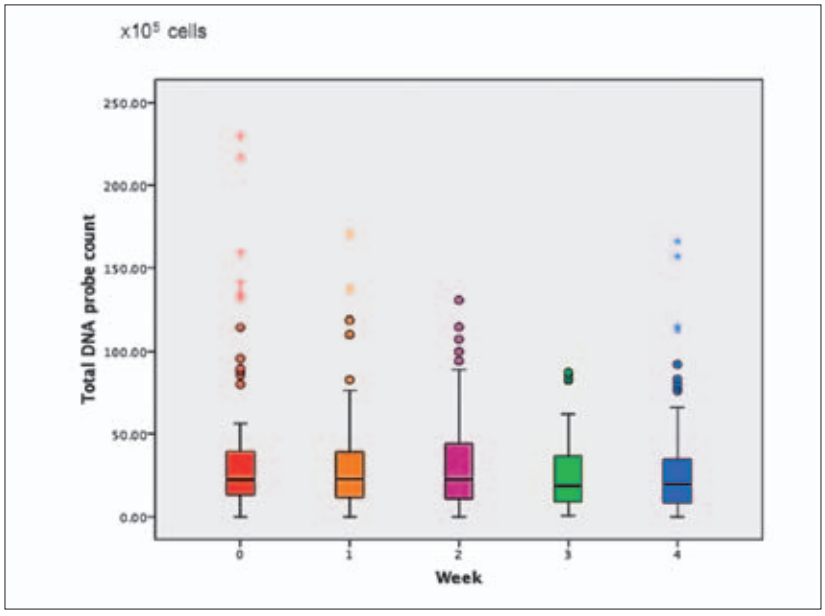

Fig 2 Boxplot diagram for women not using hormonal contraceptives (control). Total bacterial load based on assessed species in panel 1. Week 0, time of menstruation; week 1, 7 days after menstruation; week 2, 14 days after menstruation; week 3, 21 days after menstruation; and week 4, beginning of new menstruation (asterisk indicates extreme outlier; circle indicates outlier).

Given the objectives of the present study, a second panel of bacteria commonly assessed in studies of bacterial vaginosis was included. The new species in this panel were entered to the DNA-DNA checkerboard panel by the same methodology as described elsewhere for the first panel[15-17]. Chemiluminescent signals were detected using the Storm FluorImager (Molecular Dynamics, Sunnyvale, CA, USA) and digitised by the ImageQuantTM software program (version 2.00; GE Healthcare, Chalfont St Giles, UK), allowing comparison of the density of probes against the two standard-lanes (10^5 or 10^6 cells). Signals were converted to absolute counts by comparisons with these standards. Total bacterial load was defined as the sum of the bacterial load of each microorganism in the assay. Mean values of the four samples per subject were used as the subject-defined bacterial count for each microorganism. Both panels were tested for cross-reactivity at the detection level $>10^4$, with no evidence of cross-reactivity for any of the species and consistent with data published elsewhere[15].

Statistical methods

Descriptive statistical methods were used to assess the distributions of bacteria. Group comparisons were made with independent *t*-tests (equal variances not assumed) and by non-parametric Mann-Whitney U tests. Non-parametric Wilcoxon U test was performed on a site basis at the different time points. All data were analysed with the site as the unit of analysis. Statistical significance was assumed on the basis of a *P* value <0.001, thereby adjusting for multiple observations. The SPSS (Chicago, IL, USA) software program 16.0 for Mac was used for the analysis.

Results

The mean age of women using or not using hormonal contraceptives was 22.0 ± 4.1 and 28.4 ± 8.8 years respectively. The age ranges in subjects using or not using hormonal contraceptives were 17 to 32 years of age, and 16 to 44 years of age, respectively. This difference was statistically significant ($P<0.001$). No differences in clinical periodontal parameters were found between the two groups. No correlations between subject age and bacterial counts for any of the species studied were found.

Within 10 months from conclusion of the study none of the women were pregnant.

Microbiological data from 20 women using hormonal contraceptives and 20 women not using hormonal contraceptives were studied using bacterial samples from the first molars collected weekly over one full menstrual cycle. Statistical analysis by Kruskal Wallis analysis of variance (ANOVA) failed to demonstrate differences in bacterial counts for any species studied over time in both groups (*P* values between 0.19 and 0.90). This is illustrated in boxplot diagrams based on the sum of bacterial counts for species in panel 1 for women with and without hormonal contraceptives respectively (Figs 1 and 2). In consideration of the results from previous studies[7] assessing the microbiota during a menstrual cycle, the levels of *P. intermedia* are illustrated in boxplot diagrams for women with and without hormonal contraceptives respectively (Figs 3 and 4). On the day of menstruation, 15.9% of the women in the group not using oral hormonal contraceptives were positive (≥1.0x10^5 cells) for *P. intermedia*, which changed to 16.1% at day 21 following the onset of menstruation. In the group using oral hormonal contraceptives, the proportions of subjects positive for *P. intermedia* in subgingival samples were 14.6% and 17.9% respectively. No differences were found at the different time points between contraceptive users and non-users.

The prevalence of positive counts of bacteria at levels >1.0x10^5 cells are presented for the 11 species with the highest positive levels in women not using or using hormonal contraceptives (Table 2). It is noticeable that the four bacterial species most commonly found at positive counts included *Fusobacterium periodonticum*, *Fusobacterium nucleatum nucleatum*, *Escherichia coli* and *Gardnerella vaginalis*.

Analysis of the microbiological data by Mann-Whitney U tests for bacterial levels at the time of menstruation and the following weekly assessments failed to demonstrate group differences in bacterial load over time for any of the 73 species. A significant difference was found for *E. coli* and *G. vaginalis* between counts at baseline and at day 21 after menstruation, and there were higher counts at the time of menstruation in non-contraceptive users (*P*=0.02). At the other weekly counts after menstru-

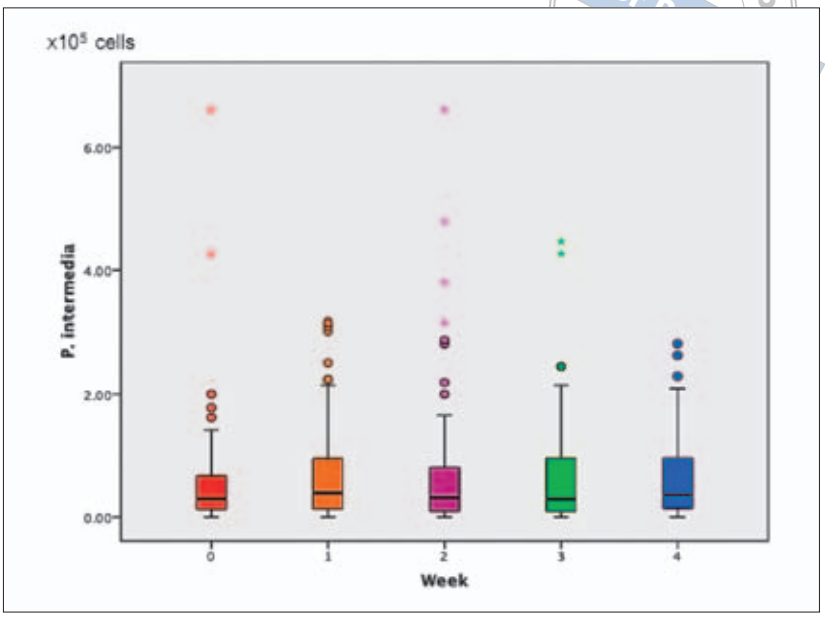

Fig 3 Boxplot diagram demonstrating the distribution of *Prevotella intermedia* in women using hormonal contraceptives. Week 0, time of menstruation; week 1, 7 days after menstruation; week 2, 14 days after menstruation; week 3, 21 days after menstruation; and week 4, beginning of new menstruation (asterisk indicates extreme outlier; circle indicates outlier).

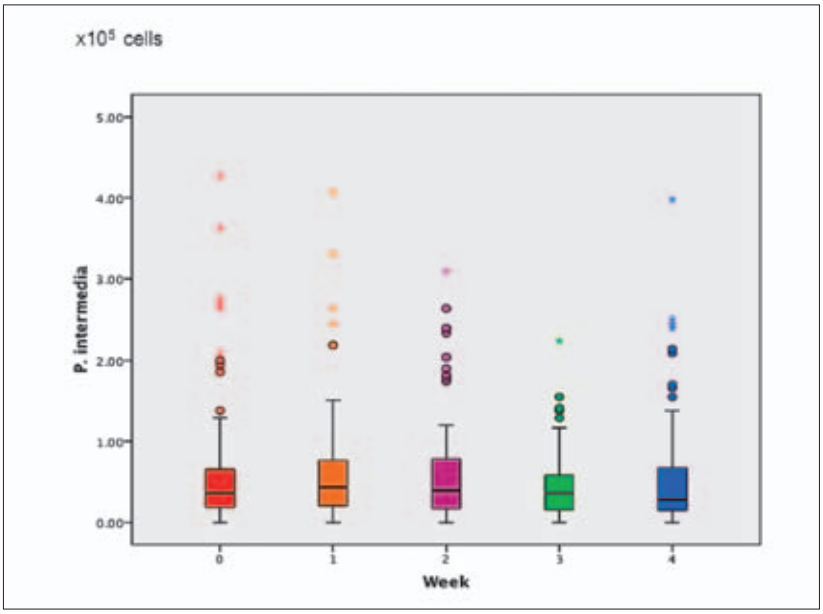

Fig 4 Boxplot diagram demonstrating the distribution of *Prevotella intermedia* in women not using hormonal contraceptives. Week 0, time of menstruation; week 1, 7 days after menstruation; week 2, 14 days after menstruation; week 3, 21 days after menstruation; and week 4, beginning of new menstruation (asterisk indicates extreme outlier; circle indicates outlier).

Table 2 Distribution of subjects with positive counts (≥1.0x10^5 cells) for the 12 most commonly identified species in non-contraceptive users at the time of menstruation and 3 weeks later.

Species	Non contraceptive users			Contraceptive users		
	Menstruation	3 weeks	*P* value	Menstruation	3 weeks	*P* value
Fusobacterium periodonticum	64.9	60.8	0.95	58.0	57.1	0.56
Fusobacterium nucleatum nucleatum	63.6	60.9	0.91	51.1	47.6	0.71
Leptotrichia buccalis	61.4	63.2	0.82	49.9	48.8	0.94
Gardnerella vaginalis	50.5	40.8	0.63	28.4	31.0	0.29
Veillonella parvula	48.9	51.4	0.54	40.9	46.0	0.34
Escherichia coli	35.4	37.5	0.59	27.3	39.3	0.64
Haemophilus influenzae	31.8	30.3	0.89	29.5	35.7	0.44
Fusobacterium nucleatum polymorphum	28.4	33.3	0.41	30.7	39.3	0.91
Bacteroides ureolyticus	24.1	26.1	0.53	28.4	40.3	0.23
Parvimonas micra	22.8	20.5	0.82	8.0	20.2	0.38
Prevotella intermedia	15.9	16.1	0.87	14.6	17.9	0.70

ation, no group differences in individual bacterial loads were found for any of the species studied. The distribution of *G. vaginalis* is illustrated in a boxplot diagram for women using and not using hormonal contraceptives respectively (Fig 5). It is noticeable that there are several outliers and extreme outliers of *G. vaginalis*.

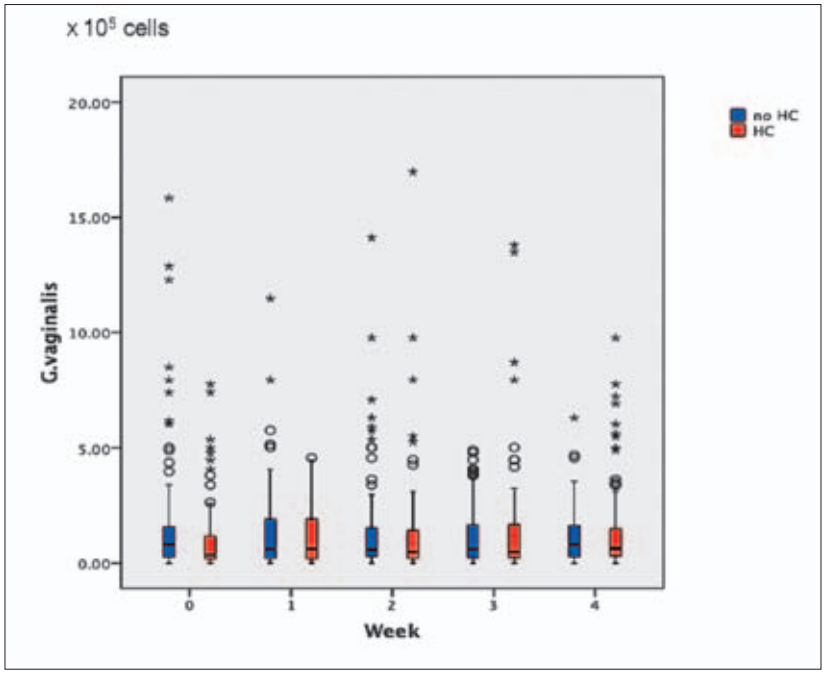

Fig 5 Boxplot diagram demonstrating the distribution of *Gardnerella vaginalis* in women not using (no HC), or using hormonal contraceptives (HC). Week 0, time of menstruation; week 1, 7 days after menstruation; week 2, 14 days after menstruation; week 3, 21 days after menstruation; and week 4, beginning of new menstruation (asterisk indicates extreme outlier; circle indicates outlier).

Non-parametric analysis by Wilcoxon rank test also failed to demonstrate differences in paired comparisons between bacterial counts in samples at the time of menstruation and any of the following weeks. This was found in both groups.

■ Discussion

In the present study there was no statistically evident influence of hormonal contraceptives on the subgingival microbiota. No cyclic pattern was found in either group during the whole observation period and no statistically significant difference in the mean total bacterial load was detected between the two groups.

Early reports suggested that oral contraceptives might enhance the extent and severity of gingivitis[10,18]. More recent studies have failed to demonstrate that the use of hormonal contraceptives enhances the extent or severity of gingivitis[12]. A contributing factor may be the modified formulation of the current generation of oral contraceptives. They contain only a fraction of the androgenic hormonal content of previous generations of oral contraceptives, and therefore may have only a negligible effect on the gingiva[12]. Other recent investigations have confirmed these findings[19,20].

Several authors have reported an increase in the proportion of *P. intermedia* in dental plaque in women using hormonal contraceptives and in pregnancy[6-8]. A significant increase in subgingival

levels of *P. intermedia* has been demonstrated in women at approximately 20 days following the beginning of use of oral hormonal contraceptives[18].

Furthermore, it has been established that maintaining low plaque levels during hormonal contraceptive usage or pregnancy can prevent inflammatory changes. The clinical data in the present study show that the subjects had good oral hygiene. No probing depths greater than 4 mm were present, plaque indices[21] were below 20% and bleeding on probing[22] was between 0% and 5%. Therefore, the impact of hormonal changes under good oral hygiene conditions could be assessed. The results demonstrate that natural hormonal changes during a menstrual cycle do not alter the subgingival microbiota within the levels of accuracy for detection and quantification by the hybridisation assay. Consistent with routine clinical findings, the microbiological data demonstrate low counts of the bacteria associated with periodontitis (i.e. *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola*) (results not shown). The positive counts of *Fusobacterium* species were within the limits of previous data for periodontally healthy subjects[13-15].

Serum C-reactive protein (CRP) levels have been studied extensively in studies of subjects with periodontitis[23]. Recent studies have identified that serum CRP levels are approximately twice as high among oral hormonal contraceptive users as among non-users and that this difference remains significant when adjusting for diet and phase of the menstrual cycle[24]. Elevated CRP related to combined oral contraceptive use may influence the rate of cardiovascular events in young women[25]. In the present study, serum CRP was not assessed. Future studies should consider the levels of serum CRP and serum oestrogen and progesterone levels in relation to changes in gingival inflammation during menstrual cycles of women using or not using hormonal contraceptives.

G. vaginalis is commonly associated with bacterial vaginosis and biofilm development[26]. There are no studies that have assessed the levels of *G. vaginalis* in subgingival bacterial samples. The present study demonstrated that *G. vaginalis* was present at very high levels compared with the levels of the other bacteria studied. It is of interest that there was a trend for lower levels of *G. vaginalis* in subjects using oral hormonal contraceptives. Similar differences in vaginal levels of *G. vaginalis* have been reported between non-contraceptive users and those using vaginal hormonal delivering devices[27]. Further studies are needed to assess the role of *G. vaginalis* in periodontal conditions.

In summary, the present study supported the null hypothesis and identified that no changes in the presence and levels of subgingival bacteria as an effect of the use of hormonal contraceptives could be found in subjects without evidence of periodontitis. Furthermore, the results demonstrated similar microbiological findings in women not using hormonal contraceptives. Unexpectedly, *G. vaginalis* was identified in the subgingival samples of many women, and levels remained stable over the menstrual cycle in subjects using or not using oral hormonal contraceptives.

■ Conclusions

Within the limitations of this case control study and within the levels of accuracy for detection and quantification by the hybridisation assay, the following conclusions can be drawn.

1. No cyclic variation in the subgingival microbiota of periodontally healthy women during a menstruation period was found.
2. No changes in subgingival bacteria of periodontally healthy women as an effect of the use of hormonal contraceptives were found.

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■ References

1. Silness J, Löe H. Periodontal disease in pregnancy. II Correlation between oral hygiene and periodontal condition. Acta Odont Scand 1964;22:121-135.
2. Löe H. Periodontal changes in pregnancy. J Periodontol 1965;36:209-217.
3. Krejci CB, Bissada NF. Women's health issues and their relationship to Periodontitis. J Am Dent Assoc 2002;133: 323-329.
4. Mariotti A. Sex steroid hormones and cell dynamics in the periodontium. Crit Rev Oral Biol Med 1994;5:27-53.
5. Soory M. Targets for steroid hormone mediated actions of periodontal pathogens, cytokines and therapeutic agents: some implications on tissue turnover in the periodontium. Curr Drug Targets 2000;1:309-325.
6. Sooriyamooorthy M, Gower DB. Phenytoin stimulation of 5α-reductase activity in inflamed gingival fibroblasts. Med Sci Res 1989;17:989-990.
7. Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. J Periodontal Res 1980;15:111-122.
8. Jensen J, Liljemark W, Bloomquist C. The effect of female sex hormones on subgingival plaque. J Periodontol 1981;52:599-602.
9. Raber-Durlacher JE, Van Steenberghen TJ, Van der Velden U, de Graaff J, Abraham-Inpijn L. Experimental gingivitis during pregnancy and post-partum: clinical, endocrinological, and microbiological aspects. J Clin Periodontol 1994;21:549-558.
10. Kalkwarf KL. Effect of oral contraceptive therapy on gingival inflammation in humans. J Periodontol 1978;49: 560-563.
11. Tilakaratne A, Soory M, Ranashanasinghe AW, Corea SM, Ekanayake SL, de Silva M. Effects of hormonal contraceptives on the periodontium, in a population of rural Sri-Lankan women. J Clin Periodontol 2000;27:753-757.
12. Preshaw PM, Knutsen MA, Mariotti A. Experimental gingivitis in women using oral contraceptives. J Dent Res 2001;80:2011-2015.
13. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontology 2000 2005;38:135-187.
14. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005;43:5721-5732.
15. Socransky SS, Haffajee AD, Smith C, Martin L, Haffajee JA, Uzel NG, Goodson JM. Use of checkerboard DNA-DNA hybridization to study complex microbial ecosystems. Oral Microbiol Immunol 2004;19:352-362.
16. Katsoulis J, Heitz-Mayfield LJ, Weibel M, Hirschi R, Lang NP, Persson GR. Impact of sample storage on detection of periodontal bacteria. Oral Microbiol Immunol 2005;20:128-130.
17. Katsoulis J, Lang NP, Persson GR. Proportional distribution of the red complex and its individual pathogens after sample storage using the checkerboard DNA-DNA hybridization technique. J Clin Periodontol 2005;32:628-633.
18. Pankhurst CL, Waite IM, Hicks KA, Allen Y, Harkness RD. The influence of oral contraceptive therapy on the periodontium: duration of drug therapy. J Periodontol 1981;52:617-620.
19. Taichman LS, Eklund SA. Oral contraceptives and periodontal diseases: rethinking the association based upon analysis of National Health and Nutrition Examination Survey data. J Periodontol 2005;76:1374-1385.
20. Klinger G, Eick S, Klinger G, Pfister W, Gräser T, Moore C, Oettel M. Influence of hormonal contraceptives on microbial flora of gingival sulcus. Contraception 1998;57:381-384.
21. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol 1972;43:38.
22. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-235.
23. Ioannidou E, Malekzadeh T, Dongari-Bagtzoglou A. Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. J Periodontol 2006;77: 1635-1642.
24. Darlene M, Dreona DM, Slavin JL, Phinney SD. Oral contraceptive use and increased plasma concentration of C-reactive protein. Life Sci 2003;73:1245-1252
25. Williams MJ, Williams SM, Milne BJ, Hancox RJ, Poulton R. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. Int J Obes Relat Metab Disord 2004;28: 998-1003
26. Swidsninski A, Mendling W, Loening-Baucke V, Swidsinski S, Dörffel Y, Scholze J et al. An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am J Obstet Gynecol 2008;198:97.e1-6. Epub 19 Nov 2007.
27. Watts DH, Rabe L, Krohn MA, Aura J, Hillier SL. The effects of three nonoxynol-9 preparations on vaginal flora and epithelium. J Infect Dis 1999;180:426-437.