Abstract: The purpose of this feasibility study was to investigate the correlation of a salivary occult blood test (SOBT) with traditional periodontal measures to assess the feasibility of the SOBT as a measure of periodontal inflammation in a population of women during pregnancy. Considering the limitations of the previous SOBT studies, this study evaluated correlation of the Perioscreen Sunstar SOBT with traditional measures from a full mouth periodontal examination. Data were collected 3 times during pregnancy (12-14, 24-28, and 36 weeks) from women participating in an ongoing study of pregnancy and inflammation. Descriptive statistics and correlations were generated for SOBT scores with periodontal measures. Preliminary data were analyzed from 7 women with 3 visits, 7 with 2, and 9 with 1 visit. For these 44 visits’ data, the mean percent of sites with bleeding on probing (BOP) for SOBT scores = 0, 2, and 5 was 58% ± 18%, 68% ± 14%, and 72% ± 19%, respectively. Correlations for percent of sites with BOP and continuous SOBT score was 0.301, P-value = 0.0469 and dichotomous SOBT was 0.32, P-value = 0.0339. Results for feasibility, measured as recruitment of participants, acceptance of protocols, distribution of periodontal inflammation and preliminary correlations, support SOBT as a correlated marker of periodontal inflammation in this population of pregnant women.

Keywords: saliva; inflammation; periodontal diseases.

Introduction
The search for faster, easier, and less invasive tests for periodontal diseases continues (1). For our ongoing research of periodontal inflammation during pregnancy, we need a faster, easier, and less invasive test to detect periodontal inflammation. We are testing for oral occult blood as a marker of periodontal inflammation in an ongoing study of inflammation in a population of pregnant women. We chose this marker in part because the “tendency for the gingiva to bleed” was the “decisive criterion” of the Gingival Index (GI) (2). Also, the population of the original GI study was pregnant women (3) and there is a higher prevalence of periodontal inflammation in women during pregnancy compared to non-pregnant women (4,5). Therefore the aim of this study was to investigate
the correlation of a salivary occult blood test (SOBT) with traditional measures from a full mouth periodontal examination (FMPE) in the detection of periodontal inflammation, with the intent to assess the feasibility of the SOBT as a measure of periodontal inflammation in a population of women during pregnancy. Feasibility was assessed by recruitment, acceptance of the FMPE and SOBT, and assessment of correlation between the SOBT and FMPE periodontal measures in this sample of pregnant women (6).

Materials and Methods
All women who enrolled in an ongoing and Institutional Review Board approved study of pregnancy were invited to participate in this ancillary study. The women were 18-45 years of age, in good health and 10-14 weeks pregnant. Major exclusion criteria were pre-existing parathyroid disease or uncontrolled thyroid disease, multiple fetuses (e.g., twins, triplets), pre-existing sickle cell disease (not trait only), sarcoidosis, Crohn’s disease, or ulcerative colitis. The SOBT and FMPE measures were recorded in a dental clinical setting at 3 study visits during their pregnancy – at 12-14 weeks gestation, at 24-28 weeks, and at 36 weeks.

For the SOBT protocol, the women used a soft-bristle toothbrush (Colgate Classic, Colgate, New York, NY, USA) with instructions to wet the toothbrush and gently brush for 30 s, followed by a single water rinse. The women then rinsed with 3 mL distilled water for 10 s and expectorated into a 50 mL centrifuge tube. The tail of the Perioscreen (Sunstar, Osaka, Japan) strip was placed into the tube and the color change was scored after 5 min. The color change is due to the presence of human hemoglobin in the specimen. The immunochromatographic strip works by hemoglobin binding first with an anti-human hemoglobin monoclonal antibody-antigen complex moves upward on the strip by capillary action and binds to another anti-human hemoglobin monoclonal antibody in the result area of the strip. The strip color was compared against the manufacturer’s reference and assigned a score.

The FMPE included probed pocket depth (PD), attachment loss (AL), and bleeding on probing (BOP) at six periodontal sites per tooth, excluding third molars. FMPE was performed by a trained and calibrated registered dental hygienist blinded to the SOBT score. The PD was recorded using the UNC 15 probe and the distance from the free gingival margin to cemento-enamel junction was measured using the Michigan O Probe with Williams markings. Data were recorded chairside and double-entered into research electronic data capture (REDCap) (7). Descriptive and relational statistics were performed for the SOBT scores with BOP sites as both continuous and categorical measures. Statistical analyses were performed using SAS/STAT Version 9.2, (SAS Institute Inc., Cary, NC, USA).

Results
At the close of the feasibility study period, 23 of the 160 women enrolled to the pregnancy study participated in the FMPE component giving a rate of 14% (23/160). Data were available from all 3 visits for 7 of the 23 women, from 2 visits for 7 women and 9 women had 1 set of FMPE and SOBT data; thus data from a total of 44 visits were collected. Fourteen women had missing teeth; 5 women missing 4 teeth, 4 missing 2, and 5 missing 1 tooth. One woman had 2 posterior teeth which could not be scored because of gagging.

Results for the 23 women at their 12-14 weeks of pregnancy visit showed a trend for a higher mean proportion of periodontal sites having 3-5 mm probing depths with higher SOBT score (Fig. 1). Likewise, results for the 23 women at their 12-14 weeks of pregnancy visit showed a trend for a higher proportion of sites having BOP with a higher SOBT score (Fig. 2).

For the data from the 44 visits, the percent of sites with BOP ranged from 33%-97%, mean 65% ± 17%. The SOBT results for these visits included 14 visits as “0” as negative or 0 µg/mL human hemoglobin, 20 visits as “2” as positive for 2 µg/mL, and 10 visits as “5” as positive for 5 µg/mL. The percent of sites with BOP for SOBT = 0 was 58% ± 18%, SOBT = 2 was 68% ± 14%, and for SOBT = 5 was 72% ± 19%. BOP was also analyzed as a total count of sites with BOP. For 44 visits, the sum of sites with BOP ranged from 48 to 150, mean 104 ± 26. The mean (±S.D.) sum of sites with BOP for SOBT = 0 was 92 ± 29, SOBT = 2 was 107 ± 21, and for SOBT = 5 was 113 ± 29.

Again using data from the 44 visits, the Pearson correlation coefficient for SOBT and BOP, with SOBT scores as a dichotomous variable (negative = score 0 and positive = 2 or 5) and BOP as the percent of total sites with BOP, was statistically significant (0.32, P-value 0.0339). For BOP as the sum of sites with BOP, the correlation with dichotomous SOBT was 0.294, P-value 0.0526. Likewise, when SOBT scores were used as a continuous variable, i.e. 0, 2, or 5, the Pearson correlation coefficient with the percent of sites with BOP was statistically significant (0.301, P-value 0.0469), and with BOP as the sum of sites with bleeding on probing was 0.280, P-value 0.0647.
Discussion

Overall findings from this feasibility study support continued use of these protocols to determine the correlation of Perioscreen SOBT measures with traditional periodontal measures from the FMPE in this population of women during pregnancy. Our outcomes of interest were recruitment, acceptance of the SOBT and FMPE protocols, and correlation of SOBT with BOP and pocket depth periodontal measures in this sample of pregnant women. With regard to recruitment, the response rate was lower than anticipated based upon our experience with another population of women during pregnancy (8). The major reason for women not participating was the additional 1 hour time required for the FMPE. In the previous study, we used a partial mouth periodontal examination (PMPE) of shorter duration. Also, the previous study visits were scheduled separately from the monthly obstetric visit whereas the current study visits were scheduled in tandem with obstetric visits. As for the acceptance of procedures, the protocol for the SOBT was well accepted by study coordinators and the participants. The FMPE was also well accepted; only one woman was not comfortable with probing of the distal surfaces of posterior teeth. The FMPE is fundamental to our study to provide traditional measures of periodontal status for comparison (9). Important to our study was the relatively wide range of periodontal inflammation indicated by the SOBT, BOP, and probed pocket depth measures in this sample of pregnant women.

Six previous studies have assessed the correlation of various oral occult blood tests with various periodontal measures (10-15). Comparison of our results with those of the published studies is challenging because of the differences amongst the oral occult detection methods, periodontal measures and study populations. Two studies from 1978 used Gindex (Janar Company, Grand Rapids, MI, USA) colorimetric test of hemoglobin in oral specimens compared with the GI for gingivitis using partial mouth designs (10,11). The authors concluded that Gindex was useful to discriminate different levels of inflammation in adult prison inmates with moderate levels of gingival disease, but not useful for children with mixed dentition or adult hygiene students, in part because of the low disease levels (10). A second clinical study found statistically significant correlations of Gindex hemoglobin testing of saliva with GI scores ($r = 0.79$), and also of crevicular fluid with GI scores ($r = 0.49$) (11). Another study used a fecal occult blood test (FOBT) to detect salivary occult blood with positive tests for 9.5% of adults having ≤30% periodontal sites with BOP, 66.7% of those with 31-50% sites BOP, and 85.7% of adults with >50% sites with BOP. Sensitivity and specificity of the FOBT for ≤30% sites BOP as negative, and >30% sites BOP as positive were 0.76 and 0.91, respectively (12). A fourth study of 221 pregnant women (smokers excluded) compared SOBT scores (Salivaster-Bld, Tokyo, Japan) to periodontal scores determined from the Community Periodontal Index of Treatment Needs (CPITN). SOBT was positively and significantly correlated with CPITN scores for periodontitis ($P < 0.001$) (13).

Two more recent studies used the same Perioscreen
SOBT as in this feasibility study. In a screening survey of dental students, 13.6% of the Japanese and 43.5% of Chinese dental students tested positive by the SOBT (14). And in 2011, a community-based sample of 1,998 adults aged 40-79 years and with ≥20 teeth were enrolled in a study comparing Perioscreen SOBT with periodontal status determined using the Third National Health and Nutrition Examination Survey protocol with PMPE (15,16). Healthy periodontal status was defined as <15% of teeth with BOP and 0% of teeth with probing depths ≥4mm. Poor periodontal status was defined as ≥15% of teeth with BOP or >0% of teeth with probing depths ≥4mm. Using those case definitions, the SOBT sensitivity was 0.72 and the specificity was 0.52 (15). Though further comparisons with the historical studies are not warranted because of differences in the periodontal measures (9), previous studies together this study’s results support further investigation of the Perioscreen SOBT as a correlative marker of periodontal inflammation.

In summary, the results of this feasibility study provide evidence of a positive correlation of the Perioscreen SOBT with traditional measures of periodontal inflammation from FMPE in this sample of pregnant women. A future study will determine the accuracy and reliability of this faster, easier and less invasive test as a marker of periodontal inflammation in our ongoing study of inflammation during pregnancy.

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