

# Effects of Oral Environment Stabilization Procedures on *Streptococcus mutans* Counts in Pregnant Women

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The aim of this study was to determine the effect of oral environment stabilization (OES) on the counting of *Streptococcus mutans* in high-caries-risk pregnant women participants of a prevention program in a public teaching institution. The sample was composed of 30 pregnant women aged 18 to 43 years, who looked for treatment at the Preventive Dentistry Clinic of the Araraquara Dental School, UNESP. Saliva samples were collected before and after the OES procedures and were forwarded to the pathology for observation and quantification of *S. mutans* CFU. There was a decrease in the number of *S. mutans* CFU, which was significantly different ( $p < 0.0001$ ) between samples. Considering the age group, 70.0% were between 18 to 30 years old and 30.0% belonged to the 31-43-year-old age group. Data related to the pregnancy period showed that 73.4% were in the second trimester, 13.3% in the first and 13.3% in third trimester. OES showed to be an effective clinical procedure in diminishing the number of *S. mutans* CFU in the saliva of high-caries-risk pregnant women. This management is simple and effective, corresponding to the basic treatment needs of pregnant women that search dental care in this public service.

Key Words: *Streptococcus mutans*, pregnant women, disease prevention, oral environment stabilization.

## INTRODUCTION

The idea that dental caries is transmissible was first demonstrated by Keyes (1) in 1960, and the vertical mother-child relationship of this transmissibility has been emphasized through the years.

Mothers frequently have an intimate contact with their children during the first two years of their life. It is believed that it is in this period that *Streptococcus mutans* are initially transferred, in such a way that the maternal salivary levels guide the colonization and the disease extension in their children (2). According to Hanada (3), the reduction of microbial levels in highly infected mother's saliva can inhibit or retard their establishment in mouth of their children.

The principle of Oral Health Promotion shows, among other principles, that the dental caries must be prevented and health must be promoted aiming at the individual as a whole. One of the main and initial clinical procedures in this philosophy of treatment is the oral environment stabilization, a conjunct of techniques that control the quantity and quality of oral microbiota. Elimination of pathogen focuses and control of risk of caries disease progression are aimed by means of mass excavation of carious tissue, sealing of cavities with a temporary material and extraction of residual roots. This way, oral environment stabilization (OES) propitiates the establishment of a functional dental care plan, strengthening of dental structure, an intra-canalicular bacteriostatic action and the control of intra-personal

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transmissibility risk. In this context, the aim of this study was determine the number of colony forming units (CFU) of *S. mutans* in high-carries-risk pregnant women of a prevention program in a public teaching institution, before and after oral environment stabilization.

## MATERIAL AND METHODS

The present study was approved by the Research Ethics Committee of the Araraquara Dental School - UNESP (ADS-UNESP; Protocol number 56/04).

### Study Population

The study population comprised all pregnant women that received dental care between October 2006 and October 2008 at the Clinic of Preventive Dentistry of ASD-UNESP, a University Teaching Unit with cooperation with the Brazilian Public Health System - SUS. Among the 73 pregnant women enrolled in the study, 30 met all inclusion criteria for participation in the study: high caries risk; age over 18 years; no systemic diseases such as hypertension, diabetes or heart disease; no complications during pregnancy; giving birth after the end of the study.

### Material Collection and Transportation

Before the beginning of the dental treatment, saliva was collected (first collection) in a sterile, thread sealing, glass tube (Vidrolabor, São Paulo, SP, Brazil) during 1 min until a sufficient amount (1.0 mL) was obtained for laboratory analysis. Samples were collected and maintained on ice during all collection period and were transported to the Pathology Laboratory, not exceeding a 4-h period (considering the beginning of collection) (4). After 1 week from the end of environment stabilization, the second collection was realized, following the same steps of the first collection.

### Material Processing

Tubes containing saliva were vortexed for 30 (Vortex AP-56; Marconi Laboratory Equipment Inc., Piracicaba, SP, Brazil) to obtain a uniform suspension (homogenization). Next, the saliva was diluted in  $10^{-1}$  to  $10^{-4}$  decimal series in a 0.05 M phosphate buffer, pH 7.3 (dilution) (5). For *S. mutans* cultivation (material sowing), aliquots of 25  $\mu$ L from each dilution were

inoculated in duplicate in Petri dishes (Jprolab, São José dos Pinhais, PR, Brazil) containing Sucrose Bacitracin agar (SB<sub>20</sub>) (Acumedia; Neogen, Lansing, MI, USA) (6) and incubated for 48 h at 37°C in anaerobiosis, using the GasPak (BBL) (Voigt Global Distribution Inc., Lawrence, Kansas, USA) system. Colonies of *S. mutans* were counted with an electronic digital colony counter (Modelo CP-602; Phoenix, Araraquara, SP, Brazil).

### *S. mutans* Identification

The observation and counting of colonies (colonial morphology) with features belonging to *S. mutans* was performed under reflecting light with a stereomicroscope (Carl Zeiss, Jena, Germany), following the standards described for the SB<sub>20</sub> environment (6,7).

### Oral Environment Stabilization

Pregnant women identified as high-carries-risk individuals were subjected to the second saliva collection followed by an OES protocol that included: a) mass excavation: curettage of softened carious tissue with appropriate-sized dentin excavators, under relative isolation, followed by cavity washing with water-air spray, drying and sealing with conventional glass ionomer (Vidrion R) (SSWhite, Rio de Janeiro, RJ, Brazil); b) extraction: extraction of residual roots when no other intervention was possible for these teeth.

Data were analyzed statistically using the software InStat (GraphPad Software, Inc. La Jolla, CA, USA). The amount obtained from saliva samples before (s0) and after (s1) OES were tested regarding their normal distribution using the Kolmogorov-Smirnov test ( $p < 0.05$ ) and as their normality could not be verified, both groups were compared with the non-parametric Wilcoxon test ( $p < 0.05$ ).

## RESULTS

There was a decrease in *S. mutans* CFU counts, with a statistically significant difference ( $p < 0.0001$ ), between pregnant women's saliva samples before (s0) and after (s1) OES (Table 1).

Seventy percent of the pregnant women that looked for assistance at the "Dental Attention in Antenatal and Postnatal Program" supported by the discipline of Preventive and Community Dentistry of ASD-UNESP were aged 18 to 30 years, an age range

considered the most reproducible in women, mainly in primigravids. Thirty percent of the women belonged to the 31-43-year-old age group, when women usually had already had at least one child.

Considering the gestational trimester, 13.3% of pregnant women were in the first (from 1 to 3 months of gestation), 73.4% were in the second (from 4 to 6 months of gestation) and 13.3% were in the third (from 7 to 9 months of gestation).

## DISCUSSION

The Brazilian Public Health System (SUS), in special the oral health system, is suffering a period of transition of a strictly social model to a preventive model that promotes oral health. This later involves all the life cycles. As the main problem of the oral health in Brazil is dental caries, it is of great interest to public health and dentistry that high-caries-risk individuals or groups of them are identified, since the costs with prevention can be remarkably reduced, increasing the efficiency in preventive treatment plans (8).

Some of the etiologic factors of caries disease that

define an individual as being at high risk are the counts of cariogenic microorganisms, mainly *S. mutans*, and the potential of transmissibility from mothers to their children. Microbiological and molecular evaluations suggest that pregnant women are a high risk group for caries development (9).

This transmissibility occurs due to a large number of *S. mutans* in maternal saliva that get in contact with children before eruption of their teeth (10,11). Thus, mothers with high or low levels of *S. mutans* tend to have children with the same salivary levels of this microorganism (6,12). Studies have demonstrated that preventive care measures in pregnant women or mother with young children have resulted in reduction in *S. mutans* levels in the mothers and decrease in colonization of these microorganisms and caries development in their children (13). Berkowitz (14) believes that the main proposal should be avoiding, as earlier as possible, the transmission of *S. mutans* from mother, father or other individuals that get in contact with the child. It is recommended not only the determination of the level of infection in pregnant woman, but also oral care to reduce the number of cariogenic bacteria and the risk

of transmission together with information about these microorganisms transmission modes and caries prevention methods.

In the present study, 73.4% of pregnant women were between 4 and 6 months of gestation. It is important to note that, clinically, the second trimester is the most indicated for dental treatment, in which pregnant women do not have so much nausea and other physiological alterations, being the most stable trimester. On the other hand, in the first trimester, complaints about nausea are more frequent and the embryo is being implanted in the uterus walls, increasing the susceptibility of abortion

Table 1. Number of *S. mutans* CFU/mL in the saliva before (s0) and after (s1) oral environment stabilization in high-caries-risk pregnant women. Araraquara, SP, Brazil, 2008.

Women	CFU s0*	CFU s1*	Women	CFU s0*	CFU s1*
1	1.44 x 10 <sup>7</sup>	2.00 x 10 <sup>5</sup>	16	5.28 x 10 <sup>5</sup>	4.80 x 10 <sup>3</sup>
2	4.48 x 10 <sup>4</sup>	4.00 x 10 <sup>3</sup>	17	6.72 x 10 <sup>7</sup>	3.00 x 10 <sup>3</sup>
3	2.60 x 10 <sup>4</sup>	0.00	18	2.62 x 10 <sup>5</sup>	2.00 x 10 <sup>3</sup>
4	1.92 x 10 <sup>5</sup>	4.60 x 10 <sup>4</sup>	19	1.72 x 10 <sup>5</sup>	4.00 x 10 <sup>3</sup>
5	1.8 x 10 <sup>6</sup>	4.00 x 10 <sup>4</sup>	20	1.40 x 10 <sup>7</sup>	2.00 x 10 <sup>3</sup>
6	6.3 x 10 <sup>5</sup>	3.60 x 10 <sup>5</sup>	21	1.00 x 10 <sup>6</sup>	1.00 x 10 <sup>3</sup>
7	1.20 x 10 <sup>5</sup>	1.60 x 10 <sup>4</sup>	22	5.03 x 10 <sup>5</sup>	5.00 x 10 <sup>3</sup>
8	5.20 x 10 <sup>4</sup>	1.30 x 10 <sup>4</sup>	23	1.48 x 10 <sup>5</sup>	3.00 x 10 <sup>3</sup>
9	3.20 x 10 <sup>5</sup>	4.20 x 10 <sup>4</sup>	24	2.50 x 10 <sup>6</sup>	2.00 x 10 <sup>2</sup>
10	1.32 x 10 <sup>6</sup>	2.48 x 10 <sup>4</sup>	25	2.62 x 10 <sup>5</sup>	5.00 x 10 <sup>3</sup>
11	1.92 x 10 <sup>5</sup>	3.20 x 10 <sup>3</sup>	26	5.02 x 10 <sup>5</sup>	3.30 x 10 <sup>3</sup>
12	2.62 x 10 <sup>5</sup>	4.00 x 10 <sup>3</sup>	27	6.80 x 10 <sup>5</sup>	3.50 x 10 <sup>4</sup>
13	4.92 x 10 <sup>6</sup>	4.00 x 10 <sup>2</sup>	28	1.30 x 10 <sup>5</sup>	4.50 x 10 <sup>3</sup>
14	1.00 x 10 <sup>6</sup>	7.70 x 10 <sup>5</sup>	29	2.72 x 10 <sup>6</sup>	3.40 x 10 <sup>3</sup>
15	1.80 x 10 <sup>5</sup>	7.60 x 10 <sup>4</sup>	30	6.80 x 10 <sup>5</sup>	2.10 x 10 <sup>3</sup>

\*Statistically significant, Wilcoxon test (p<0.0001).

and bad embryo formations. In the third trimester, it is common the reduction of blood circulation and respiratory difficulty, due to the gravid uterus weigh, which causes great discomfort to the pregnant woman. It does not mean, however, that the dental treatment cannot be performed in the first and third trimesters. It must be considered the risk of gestation and the well-being of pregnant woman in order to plan the treatment need.

Considering the conditions described above, we was aimed at intervening in these situations by establishing efficient, effective and fast preventive measures that could contribute to pain resolution with recovery of functionality, mainly during chewing, and to the reduction of *S. mutans* counts in the oral cavity of the studied group of pregnant women, preventing transmissibility to their children. In the same way as discussed in the present study, Rego et al. (15) and Signoretto et al. (16) stated that the control of patient's caries activity should be the initial step in a restorative treatment and therefore basic procedures, such as the OES, are necessary to reduce infection levels. These measures will help recovering the individual's oral health because oral flora will be balanced. However, successful management requires not only OES, but also remineralization of the tooth structure and restorative interventions when necessary. It is also extremely important that the individual recognizes the problem (17). The patients must be motivated and compliant, contributing to the success of all measures adopted, increasing their quality of life.

Mass excavation of carious lesions with the removal of infected and disorganized dentin and temporary sealing of the resulting cavities with glass ionomer cements not only contribute to OES, but also make possible the remineralization of dentin adjacent and subjacent to the temporary restoration, interrupting the metabolic cycle of remaining bacteria with the oral environment and arresting caries progression. It is known that the more fluoride-releasing restorative materials are used, the more fluoride reservoirs will exist in oral environment, which contributes to reduce *S. mutans* counts when compared with the use of non-fluoride-releasing materials (18).

Using the agar diffusion test, Da Silva et al. (19) evaluated the antimicrobial effect of glass ionomer cements against *S. mutans*, *S. sobrinus*, *L. acidophilus* and *A. viscosus*. The authors concluded that fresh and set Vidrion R presents *in vitro* antimicrobial activity and releases higher concentrations of fluoride, which

can influence the antibacterial activity observed. Silva et al. (20) also highlighted the action of Vidrion R and its importance in clinical studies that confirm *in vitro* findings to establish the real efficacy of these materials under different conditions present in the oral cavity. As mentioned in the literature (19,20), it was observed that Vidrion R, used for sealing of carious cavities after mass excavation, contribute for reducing the numbers of cariogenic microorganisms due to its antimicrobial properties. Moreover, it is a low-cost material that can be easily purchase by public health services.

In conclusion, the findings of this study reinforce that OES is effective in decreasing the number of *S. mutans* CFU present in the saliva of high-caries-risk pregnant women.

## RESUMO

O objetivo desta pesquisa foi determinar o efeito da adequação do meio bucal sobre a contagem de *Streptococcus mutans* em gestantes de alto risco à cárie, participantes de um programa de prevenção em uma instituição de ensino público, antes e após adequação do meio bucal. Amostras de saliva de 30 gestantes (18 a 43 anos) que procuraram atendimento na Clínica de Odontologia Preventiva da FOAr-UNESP foram coletadas antes e após procedimentos de adequação do meio bucal, e examinadas para observação e contagem das UFCs de *S. mutans*. Foi demonstrado que houve diminuição na quantidade das UFCs ( $p < 0,0001$ ) entre as amostras. Em relação à faixa etária, 70,0% das gestantes tinham entre 18 e 30 anos de idade e 30,0% pertenciam à faixa etária de 31 a 43 anos. Dados relativos ao período da gestação revelaram que 73,4% estavam no 2º trimestre e 13,3% estavam igualmente no 1º e 3º trimestres. A adequação do meio bucal se mostrou eficaz na diminuição das UFCs de *S. mutans* presentes na saliva de gestantes de alto risco de cárie. Este procedimento é simples e extremamente efetivo, atendendo às necessidades de tratamento básico de gestantes que procuram atendimento odontológico no serviço público de saúde.

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