Abstract. – OBJECTIVE: To evaluate changes in pH and Flow Rate (FR) of the Unstimulated Whole Saliva (UWS) in a sample of pregnant women in different gestational periods.

PATIENTS AND METHODS: After collecting demographic data and medical histories, as well as conducting an oral examination, a sample of pregnant women were instructed on how to prepare prior to the sample collection. At a time between 11.00 and 12.00 a.m., they were subjected to salivary collection (spitting method, time 5 minutes); the measurement of FR was carried out using a professionally calibrated precision scale and the pH with a portable pH meter.

RESULTS: The average FR of the women’s detected sample (0.40 ± 0.20 ml/min) was lower than that of non-pregnant women (0.48 ± 0.15 ml/min) of the same age (p < 0.05). We observed an increase (p < 0.001) of FR in the first trimester (0.56 ± 0.20 ml/min) compared to second (0.34 ± 0.14 ml/min) and third (0.31 ± 0.14 ml/min) trimester. The salivary pH of pregnant women was lower than the one detected in the non-pregnant women’s sample (p < 0.0001).

CONCLUSIONS: Our study highlighted an increase in the FR in the first trimester compared to that detected in the second and third trimesters of pregnancy which vice versa was lower than the average FR in non-pregnant women. This data, combined with the decrease in salivary pH, supports the hypothesis that correlates the FR increase with the attempt to counter the decrease in pH due to gastric regurgitation frequent in the first trimester. Further studies are necessary to evaluate salivary FR and pH in pregnant women’s samples related to the emesis phenomenon.

Key Words: Human saliva, Salivary pH, Salivary flow rate, Pregnancy, Ptyalism.

Introduction

Saliva is the main factor that guarantees homeostasis of the oral cavity: any changes in its flow or composition affect the local and general health of the individual. In the female sex, conditions that can affect oral health include certain periods of life (puberty, menstrual cycle, pregnancy and menopause) characterized by physiological changes in the levels of steroid hormones, estrogens and progesterone; oral variations can also occur during hormone replacement therapies and while taking certain medications, such as oral contraceptives.

The hormonal variations in pregnant woman cause systemic changes (cardiovascular, hematological, respiratory, renal, gastrointestinal, endocrine and genitourinary modifications) connected to fetal growth.

Important changes also occur in the oral cavity. Tissue changes induced by hormonal variations play a cofactor role in the pathogenesis of periodontal lesions that always recognize bacterial plaque as the primary and determining cause of gravidarum gingivitis. The presence of steroid hormones in high quantities induces a series of modifications that make the gingival tissues less resistant to periodontal pathogenic bacteria causing a worsening of the oral conditions of women already affected by gingivitis and periodontitis.

In 1996, Offenbacher et al. were the first to hypothesize a relationship between periodontal disease and pre-term childbirth. Many other studies followed without being able to confirm or definitively deny this association due to conflicting results linked to numerous possible confounding factors. An increased saliva production is also reported in pregnant women. Sialorrhea, also called gravidarum ptyalism, is a rare manifestation that occurs in particular during the first trimester of gestation in 0.05-0.3% of women. Indeed, the real incidence of sialorrhea in pregnancy is un-
known: studies in literature show values varying from 0.08% of pregnant women in the USA\textsuperscript{20}, 0.3% in Japan\textsuperscript{21}, 26% in Canada\textsuperscript{22}, 35% in Turkey\textsuperscript{23}, 100% in Haiti\textsuperscript{24}. This condition of unknown etiology is usually associated with nausea and vomiting (emesis or hyperemesis) and generally it occurs between the fourth and eighth week of gestation, it persists throughout the course of the first trimester (during which there is a significant increase in chorionic gonadotropin, estrogen and progesterone), it regresses starting from the third or fourth month of pregnancy and only in rare cases it persists or even increases until the childbirth\textsuperscript{20,21,24,25}. Episodes of nausea and vomiting, characterizing the pregnancy emesis, are very frequent with an estimated occurrence between 50% and 70% of pregnant women; these problems occur mainly in the morning and in some rare cases (0.3-2%), defined as pregnancy hyperemesis, they lead to weight loss, insufficient nutritional intake, loss of fluids, electrolyte imbalance, metabolic disturbances (acidosis) with such severe complications as to require hospitalization\textsuperscript{20,21,24}. Thaxter Nesbeth et al\textsuperscript{20} and Suzuki et al\textsuperscript{21} relate the onset of gravidic sialorrhea to nausea and vomiting, tracing everything back to hormonal imbalances. A systematic review of the literature published in 2011 by Veenendaal et al\textsuperscript{25} highlighted correlations between sialorrhea associated with emesis or hyperemesis, the female gender of the fetus and the birth of preterm and underweight according to the children’s gestational age (SGA: small for gestational age)\textsuperscript{22}; other studies, on the contrary, showed the same correlations associated with the birth of males\textsuperscript{21,26}.

The purpose of our observational study, after a literature review, was to evaluate the Flow Rate (FR) and the pH of the Unstimulated Whole Saliva (UWS) in a sample of pregnant women of different gestational age, oral-dental health status, geographical origin and socio-economic status.

**Patients and Methods**

After being authorized by our Ethical Committee (protocol 3401CL study n. CE 56/10), pregnant women living during the test in the Province of Novara (Italy) or in neighboring areas were enrolled after being informed of the purpose of our study and they signed the consent forms. An identification code consisting of letters and numbers was assigned to all subjects. The patients completed a medical history questionnaire in order to exclude high-risk pregnancies by a gynecologist, those with systemic diseases that could decrease saliva production, drug users, habitual consumers of alcoholic drinks and smokers.

The sample of women in different stages of gestation (first, second and third trimester) were subsequently subjected to oral examination to detect the presence of oral mucosal diseases, dental charting and periodontal health assessment. The examination of the oral cavity allowed the calculation of the DMFT score and the need for periodontal treatment using the CPITN (Community Periodontal Index of Treatment Needs) index\textsuperscript{27,28}. Later the enrolled subjects were submitted to a rigid protocol of behavioral norms, already validated in previous studies\textsuperscript{29-31}, in preparation for salivary collection. Patients were advised to keep a relaxed attitude and not to practice sports and/or physical efforts in the two days prior to the salivary collection\textsuperscript{21} After the sampling day, participants had to be free from symptoms of fever and/or cold; if they were hungry or thirsty, they could eat or drink water, but immediately afterwards they had to brush their teeth with a toothpaste provided by the examiners. During the last hour before the sample collection, they were not allowed to eat or drink.

The Unstimulated Whole Saliva (UWS) was collected for five minutes using the spitting method between 11.00 and 12.00 a.m. under controlled temperature (23.20°C) and humidity conditions (61.50%) in order to minimize variations induced by these variables. The undisturbed subject, sitting in a comfortable position, swallowed residual saliva present in the mouth before the beginning of the test. Then, with the head down and the mouth slightly open, saliva was allowed to drip from the lower lip into a pre-weighted, dry, deionized and sterilized plastic test tube (VACUTEST Kima® S.r.l. Arzegrande, Padua, Italy). No other conscious movements of the oral musculature or speaking were allowed during the salivary collection. In the last few seconds of the five minutes of the test, saliva accumulated in the mouth was spat out into the plastic test tube. In order to avoid salivary degradation, pH analysis was performed immediately after the sample collection using a portable pH meter (Hanna Instruments®, HI 9026, Woonsocket, RI, USA) which was calibrated daily on a regular basis\textsuperscript{29-31}. Then Flow Rate (F.R.) was measured by the weighting method (Precisa Balances, Series Bj; Dietikon, Switzerland) in order to allow a precise determination of the collected saliva\textsuperscript{22}. We
did not use calibrated and millimeter test tubes (volumetric method) in order to avoid incorrect data due to the presence of air bubbles or "salivary foam". The net weight of the collected saliva was obtained by subtracting the weight of the empty test tube (tare = T) measured at the beginning of each individual sampling from the total gross weight (L); finally, the salivary F.R. value per minutes was calculated according to the following formula: FR = (L-T) / 5 minutes. This calculation made it possible to obtain the F.R.'s value expressed in g/min with an uncertainty of ± 0.001 rpm. It is possible to convert the value obtained into mL/min considering the salivary density equal to 1 g/cm³ and thus obtaining g/min = mL/min. For the descriptive analysis of each sample mean, minimum and maximum value, standard deviation and relative standard deviation were calculated. To determine the existence of a relationship between the variables "first/second/third gestational trimester", "continent of origin", "DMFT", “CPTIN” and “FR” or “pH” values, one-factor analysis of variance value with minimum significance value p =0.05 was set.

Statistical Analysis
To assess the existence of statistically significant differences between pregnant and not pregnant women the Student's t-test with the two-tailed method, with minimum p-value <0.05, was used.

Results
The final sample consisted of 51 pregnant women, including 28 European, 10 African, 8 Asian and 5 South American subjects.

The medium age of the sample was 30.69 years old (D.S. ± 5.37). The oral examination revealed an average DMFT score of 4.53 ± 3.20 (with values between 0 and 14); the CPITN index showed a code 0 in 11.75% (N = 6), a code 1 in 33.35% (N = 17), a code 2 in 31.45% (N = 16), a code 3 in 21.55% (N = 11) and a code 4 in 1.90% (N = 1) of the surveyed women. The values obtained were analyzed in order to evaluate the oral health status, the possible correlations between FR / pH and trimester of pregnancy, FR/pH and continent of origin, FR / pH and DMFT, FR / pH and CPITN. The values of FR and pH obtained were also compared with those of a sample of 50 non-pregnant women, medium age 25.17 (D.S. 7.42) years old, investigated in a previous study. The CPITN and DMFT indices didn’t show statistically significant differences related to the gestational age, the continent of origin, the FR and the pH of the UWS in pregnant women.

Our study showed an average FR of pregnant women (0.40 ± 0.20 ml/min) lower than that of non-pregnant women (0.48 ± 0.15 ml/min) investigated in the cited previous study with a statistically significant difference (0.08 ± 0.05 ml/min, p <0.05). A statistically significant increase (p <0.001) in saliva production was observed in the first trimester (0.56 ± 0.20 ml/min), while in the following six months of gestation we found a decrease in salivary production. Salivary flow rate was 0.34 ± 0.14 ml/min in the second trimester with a decrease of 0.22 ± 0.06 ml/min (39.28% lower from the first) and it was 0.31 ± 0.14 ml/min in the third trimester with a decrease of 0.25 ± 0.06 ml/min (44.64% lower from the first).

Regarding the salivary pH, the mean value observed in our sample of pregnant women was 6.34 ± 0.40, lower than that of the non-pregnant women sample (7.01 ± 0.30), with a statistically highly significant difference of 0.67 ± 0.09 (p <0.0001). There were no statistically significant differences in FR and pH with respect to the continent of origin.

Discussion
The review of the literature highlights a lack of homogeneity of FR and salivary pH values in pregnant women attributable to the choice of non-homogeneous, non-comparable or reduced samples, different sampling times and methods (drooling, spitting, swallowing) and different methods of FR and pH measurement.

All these factors make our results not comparable with those published by other Authors (Table I) who collected the SWS (stimulated saliva) nor by others who collected the U.W.S.; in some cases the measurement of FR was carried out according to the volumetric method, while in other studies the method for salivary collection was not specified.

In other studies, the sample collection time was not specified, so the influence of the circadian rhythm on salivary production was not taken into consideration. Moreover, in other studies the sample was small and the trimester of gestation of pregnant women at the time of collection was not specified. The results found in our research (Table II) highlight a statistically
Changes in salivary flow rate and pH in pregnancy

significant increase in salivary FR in women in the first trimester of pregnancy (0.56 ± 0.20 ml/min) compared to the average value of non-pregnant women.

Table I. Literature review.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sample</th>
<th>Age</th>
<th>Time</th>
<th>Type of saliva</th>
<th>Collection and analysis</th>
<th>FR</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laine et al³⁸</td>
<td>8 pregnant women</td>
<td>29-41 years old</td>
<td>08:00-11:00</td>
<td>S.W.S.</td>
<td>Collection: drooling (5 minutes) Analysis: FR: method not specified pH: pHmeter</td>
<td>0.4 ± 0.2 ml/min</td>
<td>7.31 ± 0.20</td>
</tr>
<tr>
<td>Kivelä et al³⁴</td>
<td>9 pregnant women (third trimester)</td>
<td>25-39 years old</td>
<td>08:30-13:00</td>
<td>S.W.S.</td>
<td>Collection: swallowing and spitting (6 minutes) Analysis: FR: method not specified pH: litmus papers</td>
<td>2.0 ± 0.1 ml/min</td>
<td>5.82 ± 2.35</td>
</tr>
<tr>
<td>Rockenbach et al³⁵</td>
<td>22 pregnant women (V-I trimester  of pregnancy)</td>
<td>27.9 years old</td>
<td>07:30-10:30</td>
<td>U.W.S.</td>
<td>Collection: method not specified (5 minutes) Analysis: FR: volumetric method pH: pHmeter after centrifugation</td>
<td>0.59 ml/min</td>
<td>6.7</td>
</tr>
<tr>
<td>Saluja et al³⁶</td>
<td>30 pregnant women (VI-IX month of pregnancy)</td>
<td>Age not specified</td>
<td>11:00-14:00</td>
<td>S.W.S.</td>
<td>Collection: swallowing (5 minutes) Analysis: FR: volumetric method pH: pHmeter</td>
<td>1.5 ml/min</td>
<td>6.41</td>
</tr>
<tr>
<td>Martinez-Pabón et al³⁷</td>
<td>35 pregnant women (I trimester: 1; II trimester: 7; III trimester: 2)</td>
<td>16-42 years old (medium age 25)</td>
<td>Time not specified</td>
<td>S.W.S.</td>
<td>Collection: method not specified (5 minutes) Analysis: FR: volumetric method pH: pHmeter</td>
<td>1.5 ml/min</td>
<td>7.5</td>
</tr>
<tr>
<td>Karnik et al²</td>
<td>30 pregnant women (I trimester: 1; II trimester: 7; III trimester: 22)</td>
<td>24.57 years old (medium age)</td>
<td>09:00-11:00</td>
<td>U.W.S.</td>
<td>Collection: method not specified (5 minutes) Analysis: FR: volumetric method pH: pHmeter after centrifugation</td>
<td>0.24 ml/min</td>
<td>6.56 ± 0.35</td>
</tr>
</tbody>
</table>

Table II. pH and FR results.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women</th>
<th>Non pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow Rate</strong></td>
<td>I Trimester</td>
<td>II Trimester</td>
</tr>
<tr>
<td><strong>milliliter/minute</strong></td>
<td>0.56 ± 0.20 ml/min</td>
<td>0.34 ± 0.14 ml/min</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>MIN: 6.21 ± 0.49</td>
<td>MIN: 5.16</td>
</tr>
</tbody>
</table>
The F.R. values decrease in the second and third trimester of pregnancy and these are lower than the average value of non-pregnant women. Veenendaal et al\textsuperscript{25} stated that sialorrhea or pregnancy ptyalism is a recurrent symptom in pregnant women\textsuperscript{25}, but in our study we only observed it in the data of the first trimester of pregnancy.

In two studies\textsuperscript{2,35}, after collection, the saliva was centrifuged and only subsequently the pH was measured, without taking into account the rapid degradation of the saliva.

In our study salivary pH is significantly lower than that found in non-pregnant women.

Our result is similar to what is reported in the literature which relates episodes of vomiting and gastric reflux observed in pregnancy with changes within the oral environment resulting in a decrease in salivary pH significantly lower than that found in non-pregnant women\textsuperscript{2,34,35}.

Finally, the average DMFT score of the women included in our study was better (4.53 ± 3.20) than that reported in the study conducted by Vasiliauskienė\textsuperscript{39} (12.06 ± 0.11) and also by Karnik et al\textsuperscript{2}, (7.97) indicating a lower incidence of caries.

**Conclusions**

Our research showed an increase in salivary FR in the first trimester of pregnancy, while in the second and third one we found lower values compared to a sample of non-pregnant women.

The changes in the salivary flow of the first trimester can be explained by the attempt to buffer the acidity secondary to the emetic phenomena responsible for the statistically significant decrease of pH\textsuperscript{5,40}; those of the second and third trimester of pregnancy may be associated with water retention that occurs during these periods of gestation\textsuperscript{41}.

The control of the pregnancy emesis could, therefore, also be an important factor for the maintenance of oral homeostasis\textsuperscript{20}. If emesis is important for frequency and duration, it is recommended to advise patients to maintain correct oral hygiene and to carry out repeated rinses with basic solutions containing sodium bicarbonate, which can neutralize acidity and potentially reduce tooth erosion and mucosal irritation\textsuperscript{42,43}.

**Conflict of Interest**

The authors declare that they have no competing or conflicting interests. Each author certifies that he or she has no commercial associations that might pose a conflict-of-interest connection with the submitted article.

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**Informed Consent**

Written informed consent was obtained from all subjects.

**Ethical Approval**

The study was approved by our Intercompany Ethical Committee (protocol 3401CL study n. CE 56/10).

**Authors’ Contribution**

The Authors declare that they have no conflict of interests.

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