ORAL HEALTH IN PREGNANCY

Aura Heimonen

Academic dissertation

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Helsinki 2012
To all the young families with chronic diseases
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Dental examination
Periodontal examination
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ABSTRACT
INTRODUCTION: Infections have been shown to be associated with preterm birth (PTB). Infections have also been linked with other pregnancy outcomes, such as miscarriage (MC), pre-eclampsia, and gestational diabetes mellitus (GDM). Some previous studies have revealed that periodontal disease, a low-grade infection dominated by Gram-negative anaerobic and microaerophilic bacteria, is associated with an increased risk of PTB, as well as with MC and pre-eclampsia. However, the results have been inconclusive, and most studies have been conducted among women with a low socioeconomic status and a multi-ethnic background. Thus, this study was set up to investigate the association between oral health and pregnancy complications in a group of Finnish women. The study hypothesis was that pregnancy complications are reflected in women’s oral health and the markers of oral health differ between at-risk and healthy non-risk parturient women.

SUBJECTS AND METHODS: We examined 328 Finnish women with singleton births in this cross-sectional study. Within 2 days postpartum, the women were examined clinically with microbiological and saliva samples for biochemical analyses. The women completed a questionnaire about their health- and lifestyle-related behaviors and oral symptoms. Information about demographic factors, prenatal care, and medical and obstetrical history was obtained from medical records. Chronic diseases, medications, and the number of previous pregnancies, including adverse pregnancy outcomes, were recorded.

RESULTS: Dental health was uniformly good, and of the separate periodontal parameters, none predicted PTB. However, oral inflammatory burden index (OIBI), a combination of multiple oral infections, was significantly associated with PTB. Urgency-based dental treatment was associated with an increased risk of history of miscarriage (HMC), while preventive dental treatment was linked to a diminished risk of HMC. Self-reported poor oral health showed a positive association with MC. Salivary immunoglobulin A (sIgA) was associated with GDM and type 1 diabetes mellitus (T1DM) independent of C-reactive protein (CRP), but when T1DM women were excluded, sIgA lost its significance.
CONCLUSIONS: Combined effects of multiple oral infections were significantly associated with PTB among Finnish women with uniformly good dental health. Oral healthcare patterns affected birth outcomes and neglectful dental care patterns were associated with a higher probability of HMC. Women planning a pregnancy or who are already pregnant should be informed about the role of oral health in the course of pregnancy and the welfare of their fetus. Women should be referred to oral examination and necessary treatment and counselled for preventive oral self-care.
ABBREVIATIONS

A.a. Actinobacillus actinomycetemcomitans
ADA American Diabetes Association
AL Attachment loss
B.f. Bacteroides forsythus
BMI Body mass index
BOP Bleeding on probing
CAL Clinical attachment loss
CI Confidence interval
Cl Chloride
CPI Community Periodontal Index
CRP C-reactive protein
DM Diabetes mellitus
DMF Decayed, missing, filled
DMFS Decayed, missing, filled surfaces
ELISA Enzyme-linked immunosorbent assay
FIGO International Federation of Gynecology and Obstetrics
F.n. Fusobacterium nucleatum
GDM Gestational diabetes mellitus
HIV Human immunodeficiency virus
HMC History of miscarriage
H2PO4 Dihydrogen phosphate
HUCH Helsinki University Central Hospital
IADPSG International Association of Diabetes and Pregnancy Study Groups
IgA/G/M Immunoglobulin A/G/M
IL-1/6 Interleukin-1/6
LBW Low birth weight
MC Miscarriage
Na Sodium
NHMC No history of miscarriage
OD Optical density
OGTT Oral glucose tolerance test
OIBI Oral inflammatory burden index
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>OIS</td>
<td>Oral inflammatory score</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>P</td>
<td>Probability value</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PD</td>
<td>Probing depth</td>
</tr>
<tr>
<td><em>P</em>.g.</td>
<td><em>Porphyromonas gingivalis</em></td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>Prostaglandin E$_2$</td>
</tr>
<tr>
<td><em>P</em>.i.</td>
<td><em>Prevotella intermedia</em></td>
</tr>
<tr>
<td>PI</td>
<td>Plaque index</td>
</tr>
<tr>
<td>PLBW</td>
<td>Preterm low birth weight</td>
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<tr>
<td>PMN</td>
<td>Polymorphonuclear</td>
</tr>
<tr>
<td>PTB</td>
<td>Preterm birth</td>
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<tr>
<td>SGA</td>
<td>Small for gestational age</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences for Unix</td>
</tr>
<tr>
<td>sIgA/G/M</td>
<td>Salivary immunoglobulin A/G/M</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
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<td>WHO</td>
<td>World Health Organization</td>
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INTRODUCTION

Preterm birth (PTB), a birth before 37 weeks or 259 days of gestation (WHO 1977, Steer 2005), causes 75% of perinatal morbidity and mortality and is an important social and health problem with a significant economic burden. The prevalence of PTB varies greatly, being 12.3% of all births in the United States, 5-7% in Europe, and 5.1% in Finland (Goldenberg 2002, National Institute for Health and Welfare 2010, Klebanoff and Keim 2011). PTBs are more prevalent among women with a low socioeconomic status and low education, as well as those of black race. Miscarriage (MC), stillbirth, macrosomia, pre-eclampsia, and gestational diabetes (GDM) are the other main pregnancy complications.

The role of infection in adverse pregnancy outcomes has been widely investigated, linking both generalized and localized infections to PTB, MC, and pre-eclampsia (Friese 2003, Michels and Tiu 2007, Conde-Agudelo et al. 2008). Genital infections have been shown to be associated with pregnancy complications, and generalized infections, such as pneumonia, may expose woman to PTB (Goldenberg et al. 2008). Studies have also shown an association between periodontal disease and PTB (Offenbacher et al. 1996, Offenbacher et al. 2006, Rakoto-Alson et al. 2010, Baskaradoss et al. 2011). Inflamed periodontal tissues contain huge amounts of periodontal pathogens, endotoxins, and inflammatory mediators, and this bacterial load may affect the welfare of the fetus through bacteremia or result in local and systemic inflammatory and immune responses (Offenbacher et al. 1996).

Most of the previous studies have been conducted among multi-racial women with low socioeconomic status. Thus, we investigated the association between oral health and adverse pregnancy outcomes in a group of Finnish women with high education, high socioeconomic status, and easy access to health services. Our study hypothesis was that differences exist in the oral health status of women with risk pregnancy compared with healthy women with a no-risk pregnancy.
REVIEW OF THE LITERATURE

Pregnancy

Normal pregnancy lasts 38-42 weeks, concluding in labor in which uterine contractions lead to cervical dilation and finally to delivery of the infant. However, many pregnancies are complicated either due to pre-existing maternal disease or complications that begin or are first recognized during pregnancy. To prevent, detect, and treat these pregnancy problems, it is necessary to attend antenatal care. In Finland, almost all (99.8%) pregnant women attend antenatal care, and those who do not have an elevated risk of severe adverse pregnancy outcome (Raatikainen et al. 2007).

Some of the problems in pregnancy are evident early and with accurate treatment and follow-up these pregnancies may produce a healthy child, while others may have lifelong consequences or may even lead to loss of the baby. Pregnancy is usually divided into three parts: first, second, and third trimester. Each of these trimesters has their own special characteristics, and each trimester has typical pregnancy problems, e.g. MC is more prevalent in the first than in the second trimester (Michels and Tiu 2007).

During pregnancy the maternal body goes through anatomical and physiological changes to ensure the welfare of the growing fetus and prepare the mother for delivery. The changes in maternal hormone secretion result in metabolic, hemodynamic, and inflammatory alterations that support the fetus’ nourishment and development. In the first and second trimesters of pregnancy, fetal growth is very limited and nutrients are stored as fat deposits, whereas in the third trimester of pregnancy fetal growth is rapid and the transfer of nutrients through the placenta is increased (Herrera 2000). The mother also becomes insulin-resistant towards the end of pregnancy, and the availability of glucose is increased, facilitating continuous glucose transfer to the fetus (Kaaja and Pöyhönen-Alho 2006). Pregnancy is a hypervolemic state ensured by activation of the renin-angiotensin system, leading to a 50% increase in plasma volume (Kaaja and Greer 2005). The mother is immunosuppressed during pregnancy, enabling a genetically incompatible fetus to develop safely without danger of rejection by the mother. A thrombophilic state of pregnancy is essential for the hemostatic challenges of delivery (Kaaja and Greer 2005).
During pregnancy the placenta and ovaries produce increased amounts of both progesterone and estrogen. Estrogen maintains pregnancy, is responsible for fetal maturation, and is essential for regulating the production of progesterone (Albrecht and Pepe 1999). Progesterone, in turn, allows for the development of the endometrium and the prevention of contractions. Progesterone has a relaxation effect during pregnancy, and it can reduce myometrial estrogen sensitivity. At term, the myometrium becomes more sensitive to estrogen through the alteration in the expression rate of the progesterone and estrogen receptors (Kamel 2010).

The factors leading to the initiation of labor remain unclear. However, prostaglandins play a role, and prostaglandin E$_2$ can be used to induce labor (Williams et al. 2000). Oxytocin stimulates uterine contractions, and the number of oxytocin receptors in the uterus increases prior to labor (Williams et al. 2000). The concentration of estrogen increases in relation to progesterone, resulting in rising contractility. Spontaneous rupture of the membranes generally occurs during labor or at the beginning of labor, with contractions starting after a couple of hours. Most women have a vaginal delivery, but in Finland the rate of Cesarean sections has increased from 7.9% in 1975 to 17.1% in 2008, and slightly over half of these were non-elective Cesarean sections (Pallasmaa et al. 2008, National Institute for Health and Welfare 2010).

**Pregnancy complications**

**Preterm birth**

The definition for preterm birth according to the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (FIGO) is birth before 37 weeks or before 259 days of gestation (Table 1) (WHO 1977, Steer 2005). PTB is the most common perinatal problem in developed countries (Gibbs 2001, Goldenberg 2002) and is the greatest single risk factor for death in the first year of life (Kelly et al. 2006). In Europe, approximately 5-7% of all births are preterm, and in Finland the rate is approximately 5.1% (Goldenberg 2002, National Institute for Health and Welfare 2010). The survival rate of preterm infants has improved, leading to an increase in short-term morbidities such as respiratory distress syndrome (Goldenberg et al. 2000, Gibson 2007). The incomplete development of organs causes the biggest health problems in the case of PTB, and long-term disabilities include lung problems (immature lungs at birth), neurodevelopmental disturbance,

Of the PTBs, about 5% occur before 28 weeks' gestation and these infants are referred to as extremely premature, and 15% occur between 28 and 31 gestational weeks and are referred to as severely preterm infants. About 20% of the PTBs occur between 32 and 33 weeks and these are known as moderately premature infants, and a further 60-70% occur between 34 and 36 weeks and are known as near-term infants (Goldenberg et al. 2008). The rate of severe neonatal mortality and morbidity is high in the extreme prematurity group, as are severe disability and the risk for later behavioral, fine motor, and education difficulties (Wood et al. 2000). Of the PTBs, 20% are medically induced due to maternal or fetal complications, about 30% begin with premature rupture of the membranes, and almost 50% begin with spontaneous preterm labor (Pararas et al. 2006).

There are many known risk factors for PTB (Table 2). However, the underlying cause is generally not obvious. The most significant risk factor is a history of spontaneous preterm delivery (Goldenberg et al. 2000). Furthermore, multiple gestation is a common risk factor, and in Finland in 2006 altogether 44.6% of twins were born preterm (National Research and Development Centre for Welfare and Health 2008). Mercer et al. (1999) showed a 2.5-fold increased risk of spontaneous preterm delivery if there was a previous spontaneous preterm delivery, and the risk of PTB was inversely associated with gestational age of the previous PTB. The rate of PTB is unevenly distributed; among black women it is 16-18%, while among white women it is 5-9% (Goldenberg et al. 2008). Further, the risk for a very early PTB is several-fold higher in black women than in other racial or ethnic groups (Goldenberg et al. 2008). The disparity between black and white women is unexplained. Low socioeconomic status and inadequate prenatal care have been shown to increase the risk for PTB (Goldenberg 2002, Goldenberg et al. 2008, Debiec et al. 2010). Debiec et al. (2010) established that women without prenatal care had an over 7-fold risk of PTB relative to those who attended 75-100% of the recommended prenatal care appointments.

Jolly et al. (2000) revealed that women under 18 years of age were more likely to deliver preterm than older women. On the other hand, pregnant women aged 35-40 years were also at
increased risk of delivering before 32 weeks of gestation, and women aged >40 years had an even higher risk (Jolly et al. 2000, Joseph et al. 2005). Nutritional status affects the rate of PTB and studies have indicated that overweight women have a higher risk of delivering before 32 weeks of pregnancy (McDonald et al. 2010). In addition, women with a low body mass index (BMI) are at risk of delivering preterm, and the rate of PTB has been shown to be about 7% in women whose BMI is below 17 (Steer 2005). Maternal illnesses, such as hypertension, diabetes, and thyroid disease, are associated with increased risk of preterm delivery (Goldenberg et al. 2008, Stagnaro-Green 2009). The definition for cervical insufficiency is inability of the uterine cervix to retain pregnancy in the absence of contractions or labor. Smoking and use of alcohol during pregnancy have an unfavorable effect on birth outcome. Xiong et al. (2009) concluded that the benefit of early smoking cessation is clear, and McCovan et al. (2009) showed that severe adverse pregnancy outcomes may be reversed if smoking is stopped early in pregnancy. Alcohol use during pregnancy may also be a risk factor for preterm delivery (Parazzinin et al. 2003, Aliyu et al. 2010). The study of O’Leary et al. (2009) showed an increased likelihood of preterm delivery in women who ceased drinking alcohol before the second trimester, but drank alcohol at moderate or higher levels in the first trimester.

There is increasing evidence showing that infection and inflammation might be underlying causes of PTB (Goldenberg et al. 2000, Williams et al. 2000, Gibbs 2001, Goldenberg 2002, Wei et al. 2010), with up to 40% of PTBs occurring because of infection (Friese 2003). Intrauterine infections can result in spontaneous preterm delivery by stimulating uterine contractions or membrane rupture (Goldenberg et al. 2000). Histologic examinations of the fetal membranes have shown that infection is generally present in PTBs occurring before 30 weeks of gestation, but rarely in preterm deliveries at 34-36 weeks (Goldenberg et al. 2000). Localized infections of the genitourinary tract can lead to PTB, and bacterial vaginosis, an imbalance of the microbial ecosystem of the vagina, can increase the rate of PTB by 1.5- to 3-fold (Leitich et al. 2003, Guaschino et al. 2006). *Chlamydia trachomatis* and syphilis have also been associated with PTB in previous studies (Karinen et al. 2005, Tridapalli et al. 2007, Goldenberg et al. 2008). Systemic infections, such as pneumonia, may also be involved in PTB (Graves 2010). Moreover, evidence suggests that oral infections, mainly periodontal disease, are associated with PTB (Offenbacher et al. 1996). This low-grade infection results in local and systemic inflammatory and immune responses, which can be detected in serum.
### Table 1. Definitions of adverse pregnancy outcomes

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Preterm birth</td>
<td>Birth before 37 weeks or before 259 days of gestation</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>Birth weight less than 2500 g</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>Pregnancy loss before 22 weeks’ gestation</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>Fetal death at or after 22 weeks’ gestation</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>Glucose intolerance beginning or first diagnosed during pregnancy</td>
</tr>
<tr>
<td></td>
<td>Limiting values in oral glucose tolerance test are 0 h ≥ 5.3 mmol/L, 1 h ≥ 10.0 mmol/L, and 2 h ≥ 8.6 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Normalized blood glucose within 3 months after delivery</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>Birth weight &gt; 4000-4500 g at term or weight above the 90th percentile or &gt; 2 SD for gestational age</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>Hypertension (diastolic blood pressure ≥ 90 mmHg and systolic ≥ 140 mmHg)</td>
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<tr>
<td></td>
<td>and substantial proteinuria (≥ 300 mg 24 h) at or after 20 weeks’ gestation</td>
</tr>
<tr>
<td>Pregnancy-induced hypertension</td>
<td>Hypertension without proteinuria starting at 20 weeks or later</td>
</tr>
</tbody>
</table>

### Table 2. Risk factors of preterm birth

<table>
<thead>
<tr>
<th>Maternal factors</th>
<th>Obstetrical factors</th>
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</thead>
<tbody>
<tr>
<td>Young maternal age (&lt;18 years)</td>
<td>Old maternal age (&gt;40 years)</td>
</tr>
<tr>
<td>Increasing number of sexual partners (≥ 4)</td>
<td>Low education</td>
</tr>
<tr>
<td>Smoking &gt; 10 cigarettes per day</td>
<td>Alcohol use ≥ 3 units at once per day or 7 units/week</td>
</tr>
<tr>
<td>Poor condition of chronic disease (e.g. hypertension, diabetes)</td>
<td>Infection</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>Use of cocaine, amphetamine, cannabis</td>
</tr>
<tr>
<td></td>
<td>Multiple pregnancy</td>
</tr>
<tr>
<td></td>
<td>Pre-eclampsia</td>
</tr>
</tbody>
</table>

Modified from Käypä hoito –suositus, 2011
Low birth weight (LBW)

The definition of low birth weight is defined as weight less than 2500 g, very low birth weight of weight less than 1500 g, and extremely low birth weight as weight less than 1000 g (Michalowicz and Durand 2007). The worldwide prevalence of low birth weight (LBW) infants is 8-26% (WHO 2005). LBW is often a result of preterm birth and is defined as preterm low birth weight (PLBW). The term small for gestational age (SGA) is used if the baby weighs below the 10th percentile for gestational age (Williams et al. 2000, Mari and Hanif 2007). An infant can be SGA for either constitutional or pathological reasons (Mari and Hanif 2007). The underlying cause is maternal disease, such as chronic hypertension or diabetes mellitus (DM), which often leads to superimposed pre-eclampsia and placental insufficiency and the birth of an SGA baby (Mari and Hanif 2007).

Miscarriage

Miscarriage (MC), pregnancy loss before 22 weeks’ gestation, is the most common pregnancy complication (Rai and Regan 2006). Of clinically recognized pregnancies, 15% end in MC, while 30-50% of all conceptions miscarry (Rai and Regan 2006, Stephenson and Kutteh 2007). Sporadic MC affects 25-50% of women, while recurrent MC, the loss of three or more consecutive fetuses, affects about 1% of women (Rai and Regan 2006, Stephenson and Kutteh 2007). The rate of MC decreases as the pregnancy progresses (Michels and Tiu 2007). At least 50% of first trimester and about 24% of second trimester MCs occur because of chromosome abnormalities (Michels and Tiu 2007, Stephenson and Kutteh 2007). High maternal age is one of the risk factors for MCs (Nybo Andersen et al. 2000); when age exceeds 40 years, the clinical MC rate is as high as 45% (Stephenson and Kutteh 2007). Infections are linked to MCs, especially in developing countries (Michels and Tiu 2007). Bacterial vaginosis is a risk factor for late MC (Hay et al. 1994, Ugwumadu et al. 2003, Leitich and Kiss 2007), and other infections, such as Chlamydia trachomatis, may also lead to MC (Wilkowska-Trojniel et al. 2009). Other known risk factors are previous spontaneous abortion, multigravidity, smoking (Chatenoud et al. 1998), and fetal and maternal structural abnormalities. The risk factors for MC are summarized in Table 3.
Table 3. Risk factors for miscarriage

<table>
<thead>
<tr>
<th>Maternal factors</th>
<th>Obstetrical factors</th>
<th>Fetal factors</th>
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</thead>
<tbody>
<tr>
<td>Smoking &gt; 15 cigarettes per day</td>
<td>History of miscarriage</td>
<td>Chromosomal abnormalities</td>
</tr>
<tr>
<td>Heavy alcohol use</td>
<td>Uterine abnormalities (e.g. cervical incompetence)</td>
<td>Non-chromosomal structural abnormalities</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
<td>Developmental disorder of umbilical cord and placenta</td>
</tr>
<tr>
<td>Infections (e.g. chlamydia, toxoplasmosis)</td>
<td></td>
<td></td>
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<tr>
<td>Chronic diseases (e.g. hypertension, renal disease)</td>
<td></td>
<td></td>
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<tr>
<td>Old maternal age (&gt;35 years)</td>
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<td>Trombophilia?</td>
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Modified from Naistentaudit ja synnytys, 2011

Stillbirth

Stillbirth is defined as fetal death at or after 22 weeks of gestation. In Finland in 2010, the rate of stillbirths and deaths during the first week of life was 5.8 per 1000 births of all births (National Institute for Health and Welfare 2011). Stillbirth is more prevalent in developing than developed countries. African-American women have a 2-fold risk of stillbirth relative to Caucasian women. This may partly be due to reduced prenatal-care utilization among African-American women (Vintzileos et al. 2002). It has been hypothesized that differences in medical care explain low socioeconomic status as a risk factor for stillbirth (Stephansson et al. 2001). Other known risk factors for stillbirth are previous stillbirth, high maternal age, maternal obesity, postdate pregnancies, multiple pregnancies, diabetes mellitus, thrombophilia, and smoking (Fretts 2005, Smith and Fretts 2007, Flenady et al. 2011). Infection, congenital anomalies, abruptio placentae, and umbilical cord accidents may also cause stillbirth. However, in nearly 50% of stillbirths the cause remains unclear.
Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as glucose intolerance beginning or first diagnosed during pregnancy (Kjos and Buchanan 1999). The prevalence of GDM is 1.3-19.9% depending on diagnostic and screening criteria (Simmons 2011). In Finland, about 8.5% of pregnant women have GDM (Kaaja and Rönnemaa 2008). GDM is diagnosed by the oral 75 g glucose tolerance test (OGTT); the limiting values are 0 h ≥ 5.3 mmol/L, 1 h ≥ 10.0 mmol/L, and 2 h ≥ 8.6 mmol/L. According to the American Diabetes Association guidelines (ADA 2009), two or more of the venous plasma concentrations must be met or exceeded for a positive diagnosis. The International Association of Diabetes and Pregnancy Study Groups’ (IADPSG) diagnosis criteria for GDM requires that only one of the venous plasma concentrations must be met or exceeded; however, these values differ somewhat from the ADA values, being 0 h ≥ 5.1 mmol/L, 1 h ≥ 10.0 mmol/L, and 2 h ≥ 8.5 mmol/L (IADPSG 2010, O’Sullivan et al. 2011). GDM is associated with many maternal and neonatal complications such as macrosomia, pre-eclampsia, Cesarean section, and shoulder dystocia (Yogev and Visser 2009). In addition, exposure to elevated blood glucose concentrations during pregnancy is associated with increased risk for obesity, metabolic syndrome, and type 2 diabetes mellitus in adulthood (Simeoni and Barker 2009). However, if GDM is diagnosed and treated, these detrimental outcomes can be inhibited or at least relieved. The treatment of GDM includes dietary modification, blood-glucose monitoring, and medical therapy (Cheng et al. 2008). The risk factors for GDM comprise obesity, family history of diabetes, previous GDM, high maternal age, polycystic ovarian syndrome, and previous delivery of a macrosomic infant (Ben-Haroush et al. 2003, Haakova et al. 2003). GDM mothers also have more pre-pregnancy chronic hypertensive disease than non-GDM mothers, and hypertension is related to obesity (Yogev and Visser 2009, Fadl et al. 2010). GDM predicts development of maternal type 2 diabetes and metabolic syndrome in the future (Yogev and Visser 2009).

Macrosomia

Macrosomia, defined as birth weight > 4000-4500 g at term or above the 90th percentile or > 2 SD for gestational age, occurs in 45% of diabetic pregnancies (Weindling 2009, Yogev and Visser 2009, McGowan and McAuliffe 2010). In addition, women with high gestational weight gain are at increased risk of giving birth to a macrosomic infant and sustaining macrosomia-related maternal and neonatal morbidities (Hedderston et al. 2006, Stotland et al. 2006). The number of large infants has been on the rise during the last 2-3 decades, posing
problems in obstetrics. Fetal macrosomia increases the risk of maternal and fetal complications (Henriksen 2008). Maternal complications include prolonged labor, Cesarean section, and perineal injuries (Boulet et al. 2004, Stotland et al. 2004). Fetal short-term complications include shoulder dystocia, intrapartal hypoxia, asphyxia, and hypoglycemia (Lim et al. 2002), and even fetal death. Studies have also shown an association between macrosomia and such long-term health risks as overweight, diabetes, and metabolic syndrome (Harder et al. 2007, Voldner et al. 2008).

**Pre-eclampsia**
Pre-eclampsia complicates 2-8% of pregnancies, and it is the leading cause of maternal as well as perinatal mortality and morbidity (Steegers et al. 2010). Pre-eclampsia is defined as hypertension (diastolic blood pressure ≥ 90 mmHg and systolic blood pressure ≥ 140 mmHg) and substantial proteinuria (≥ 300 mg / 24 h) at or after 20 weeks of gestation (Steegers et al. 2010). The pathogenesis of pre-eclampsia is unknown, but the main hypothesis relies on defective placental angiogenesis causing disturbed placental function in early pregnancy (Steegers et al. 2010). Pre-eclampsia is also characterized by endothelial dysfunction.

**Pregnancy-induced hypertension**
Pregnancy-induced hypertension or gestational hypertension is defined as hypertension without proteinuria starting at 20 weeks or later. It is estimated that gestational hypertension complicates about 5-6% of pregnancies, and this may develop to pre-eclampsia later in pregnancy (Roberts et al. 2003, Magee et al. 2009). Studies have shown that mothers with overweight assessed by BMI early in pregnancy have a higher risk of pregnancy-induced hypertension as well as pre-eclampsia (Sibai et al. 1995, Sibai et al. 1997).

**Oral health**

**Dental caries**
The presence of cariogenic bacteria, fermentable carbohydrates, and a susceptible host are needed for the development of dental caries (Keyes 1960). Cariogenic bacteria in dental biofilm produce organic acids during metabolism of fermentable carbohydrates (Loesche 1986), and these organic acids dissolve minerals in hard dental tissue. The main groups of bacteria needed in the caries process are mutans streptococci and lactobacilli (Featherstone 2008). Frequent consumption of fermentable carbohydrates increases the amount of these
bacteria (Marsh 1994). The progression of dental caries is a dynamic process since periods of demineralization and remineralization alternate (Kidd and Fejerskov 2004). Remineralization is achievable if fluoride, calcium, and phosphate are present in saliva, and it may completely arrest the progression of a lesion (Nyvad et al. 1999). Saliva has good buffering capacity, and salivary flow can clear bacteria from the tooth surface. Dental caries is a transmissible infectious disease, and the cariogenic bacteria mutans streptococci are usually transmitted to young children from their mothers (Alaluusua et al. 1996).

**Periodontal disease**
Gingiva, alveolar bone, periodontal ligament, and root cementum are the components of tooth-supporting tissues attaching the teeth to alveolar bone. The presence of a microbial biofilm around the gingival margin is needed for the development of gingivitis, and a local inflammatory reaction is achieved by bacterial infection, which in turn activates the innate immune system. This activation leads to an expression of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and prostaglandin E2 (PGE2) (Lee et al. 1995, Offenbacher et al. 1998, Hasegawa et al. 2003), and drives the destruction of connective tissue and alveolar bone. Furthermore, it may lead to production of C-reactive protein (CRP) from the liver (Pitiphat et al. 2006, Salzberg et al. 2006).

Gingivitis is reversible if plaque and calculus are removed, and this interception of gingivitis prevents its progression to periodontal disease. Periodontal disease is a chronic oral infection where a complex interplay of bacterial infection and host response are responsible for destruction of connective tissue and alveolar bone (Oliver et al. 1998). Periodontal disease is the most common cause of tooth loss worldwide (Darveau 2010). Periodontal microbiota from subgingival plaque sample is often analyzed in the case of progressive periodontal disease that does not respond to periodontal treatment. In addition, host response can be assessed from gingival crevicular fluid (Lamster 1997). The biofilm and the host inflammatory and immune responses vary among individuals, although the clinical presentation and diagnostic symptoms are similar (Offenbacher et al. 2007). Dental plaque forms naturally on teeth, and in the healthy state the bacterial composition remains relatively stable due to a dynamic balance of both synergistic and antagonistic microbial interactions. Synergistic microbial interactions include food chains between bacterial species and
metabolism of endogenous nutrients, while antagonistic microbial interactions include production of bacteriocins and bacteriocin-like substances. In disease, this homeostasis breaks down and imbalance of microflora exists (Marsh 1994). The commonly used clinical signs of periodontal condition and disease progression include plaque index (PI), bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL), and radiographic assessment of alveolar bone loss (Kaufman and Lamster 2000).

Periodontal microorganisms
Periodontal disease is the clinical result of a complex interaction between host and plaque bacteria, and it is polymicrobial, predominantly anaerobic infection. The pathogens involved in periodontal disease are Actinobacillus actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Prevotella intermedia (P.i.), Bacterioides forsythus (B.f.), and Fusobacterium nucleatum (F.n.) (Dahlen 1993). A.a. is strongly associated with progressing periodontitis (van Wilkelhoff et al. 1992). Periodontal microorganisms involved have certain characteristics that contribute to their ability to act as pathogens and participate in tissue destruction. First, microorganisms must have the capacity to colonize and survive on the ecosystem of the biofilm. Second, pathogens must have the ability to evade antibacterial host defense mechanisms that normally control infections though deletion of bacteria. And third, the microorganisms must have an ability to initiate tissue destruction directly through self-produced enzymes (American Academy of Periodontology 1999). For example, P.g. has been shown to produce enzymes (proteases, collagenase, fibrinolysin, phospholipase A) that could directly degrade surrounding tissues in the superficial layers of the periodontium (Birkedal-Hansen et al. 1988).

Saliva
Saliva is an important body fluid secreted by minor and major salivary glands. It has a role in lubricating oral tissues, participating in oral functions such as swallowing and speaking, and protecting oral tissues (Mandel 1987). This protection occurs through killing of microorganisms, antiviral activity, and inhibition or neutralization of harmful metabolic products by salivary components (Tenovuo 1998, Dawes 2008). There are three pairs of major salivary glands: parotid, submandibular, and sublingual, and their ducts open to the second maxillary molar, the side of the lingual frenulum, and the lingual sulcus, respectively. Most saliva (approximately 90% of total salivary volume) is derived from the major salivary glands.
(Dawes 2008, Greabu et al. 2009), while about 25% of whole saliva in unstimulated flow originates from the parotid, 60% from the submandibular, 7-8% from the sublingual, and 7-8% from the minor salivary glands. However, when the flow is stimulated, the parotid gland secretion increases by 10% (Dawes 2008). Furthermore, the time of day, diet, age, gender, many diseases, and medications may affect secretions from the different glands (Greabu et al. 2009). The minor salivary glands, numbering 600-1000, are located in the labial, buccal, lingual, and distopalatal parts of the oral mucosa (Eliasson and Carlén 2010). The secretion of saliva is divided into two stages: initial secretion and modification of saliva. The autonomic nervous system, both parasympathetic and sympathetic, controls the production of saliva. Parasympathetic stimuli increase the secretion of water and electrolytes, and sympathetic stimuli enhance the secretion of protein-rich saliva. Saliva is secreted by acinar cells as isotonic plasma ultra-filtrate. Saliva is then modified during passage through the ductal cell system by reabsorption of sodium (Na\(^+\)) and chloride (Cl\(^-\)), resulting in hypotonic secretion (Tenovuo 1997). The composition of saliva is about 99% water and ions such as Na\(^+\), Cl\(^-\), and dihydrogen phosphate (H\(_2\)PO\(_4\)-), and the ionic composition is derived from plasma. Organic components of saliva include urea, glucose, and proteins, such as amylase and glycoproteins, all of which have a role in protecting oral cavity tissues (Greabu et al. 2009).

Salivary antimicrobial systems are divided into immune and non-immune defense factors (Kirstilä et al. 1994). Saliva has a protective role in oral health and various roles in the digestive tract (Lima et al. 2010). Saliva contains a large amount of proteins; proline-rich proteins comprise nearly 70% and amylase most of the remainder of the total protein content of human parotid saliva. Many autoimmune, neurologic, and endocrinological diseases decrease salivary flow (Tscherp et al. 2010). The degree of hydration, body position, and drug use can affect salivary flow rates (Dawes 2008).

**Salivary immunoglobulins**

Salivary immunoglobulin A (sIgA) (> 85%) and immunoglobulin G (sIgG) are the major antibodies in saliva, forming 5-15% of total salivary proteins (van Nieuw Amerongen et al. 2004). Salivary IgA is synthesized by plasma cells in salivary glands, whereas most of the IgG in saliva is derived from serum via crevicular fluid, and thus, sIgG represents systemic immunity (Brandtzaeg 2007). However, a small part of sIgG may originate from glandular, gingival, or tonsillar plasma cells. Salivary IgA is secreted to interstitial fluid and taken up by
acinar and ductal cells of the salivary gland, which then secrete sIgA into saliva. Immunoglobulins have highly specific binding characteristics, and each immunoglobulin idiootype binds and agglutinates specific microbial species. When considering the entire population of salivary immunoglobulins, they bind the majority of microorganisms present in saliva. The main function of salivary immunoglobulins is the inhibition of bacterial adherence and colonization (van Nieuw Amerongen et al. 2004). Salivary IgA is considered to be part of the first line of defence against pathogens colonizing or invading mucosal surfaces, and it has been suggested to be a precise measure of local infection arising in oral mucosa (Seeman et al. 2004, Janket et al. 2010). Salivary IgA inhibits the adherence of bacteria to mucosal surfaces, ablates viruses from mucosal surfaces, and neutralizes toxins and enzymes (McNabb and Tomasi 1981, Seeman et al. 2004).

**Oral health and pregnancy**

**Female sex hormones**
Steroid sex hormones are derived from cholesterol, and they are known to affect bone integrity through metabolism of bone minerals (Mascarenhas et al. 2003). Estrogen and progesterone are the main female sex hormones produced by the ovaries and placenta, and they have an important role in physiological changes in women starting from puberty. Estrogen and progesterone function synergistically, controlling the menstrual cycle (Amar and Chung 1994), and they have important roles in both the maintenance of pregnancy and the initiation of labor. During pregnancy estrogen may reach levels 30 times higher and progesterone levels 10 times higher than during the menstrual cycle (Amar and Chung 1994, Mariotti 1994). Elevated levels of these hormones have a significant influence on different organ systems, including the periodontium (Amar and Chung 1994).

**Effect of female sex hormones on periodontal tissues**
Estrogen and progesterone receptors have been found in gingiva (Vittek et al. 1982, Kawahara and Shimazu 2003), and these hormones have been shown to increase vascular permeability and the amount of gingival crevicular fluid flow (Mealey and Moritz 2003). In addition, estrogen and progesterone may alter the immune system, and progesterone can stimulate the production of an inflammatory mediator PGE2 (Mealey and Moritz 2003). Estrogen receptors have also been found in periosteal fibroblasts (Aufdemorte and Sheridan 1981) as well as in periodontal ligament fibroblasts (Nanba et al. 1989); and thus, the sex hormones may directly
affect these periodontal tissues. In addition, both estrogen and progesterone have been demonstrated to have an impact on bone metabolism (Feldman et al. 1975, Erikssen et al. 1988).

Pregnancy does not cause gingivitis, but may worsen pre-existing disease (Laine 2002). The prevalence and severity of gingival inflammation have been shown to increase during pregnancy, with these changes disappearing postpartum (Löe and Silness 1963, Tilakaratne et al. 2000, Günsoy et al. 2008). Estrogen and progesterone affect cellular proliferation, differentiation, and growth of gingival fibroblasts (Mariotti 1994, Mealey and Mortiz 2003). Studies have also revealed that both estrogen and progesterone have a role in bone resorption and formation (Lobo et al. 1984, Kommm et al. 1988, Gallagher et al. 1991). Susceptibility to infections, including periodontal disease, increases during pregnancy, and the underlying mechanisms consist of alterations in the immune system (Brabin 1985), hormonal changes, limited T-cell activity (Taylor et al. 2002), decreased neutrophil chomotaxis and phagocytosis, and depressed antibody production (Zachariasen 1993). Periodontal bacteria P. i. and P. g. can use female sex hormones as a source of nutrients, and the amount of these bacteria is increased in the gingival crevicular fluid of pregnant women; this correlates positively with the severity of pregnancy gingivitis (Kornman and Loeshe 1980).

Studies have established that pregnant women have more gingival bleeding and inflammation than women postpartum; these changes are not associated with the amount of plaque (Löe and Silness 1963, Raber-Durlacher et al. 1994). The gingival inflammatory changes begin during the second month of pregnancy and increase in severity until the eighth month of pregnancy (Löe and Silness 1963, Löe 1965, Tilakaratne et al. 2000). Günsoy et al. (2008) showed that changes in bleeding on probing and periodontal pocket depth increased simultaneously without a relation to plaque between the first and second trimesters and then decreased during subsequent visits. Thus, these changes were reversible, indicating that pregnancy gingivitis does not predispose or proceed to periodontal disease.

**Dental caries during pregnancy**

No data indicate that dental caries incidence increases during pregnancy. Development of dental caries usually takes several years, and thus, the possible pregnancy-related increase in caries incidence is difficult to estimate. In a recent study by Vergnes et al. (2011), dental
Caries was not significantly associated with preterm birth or preterm premature rupture of membranes. Similarly, Durand et al. (2009) found no association between clinical dental caries and preterm birth, but low levels of lactobacilli in saliva were associated with preterm birth. However, a study by Agueda et al. (2008) linking periodontal disease and preterm birth reported a significant association between the presence of untreated caries and preterm birth.

**Oral health and adverse pregnancy outcomes**

**Periodontal disease and adverse pregnancy outcomes**

Oral health, mainly periodontal disease, has been connected to such systemic conditions as diabetes, cardiovascular disease, and PTB. Interestingly, pregnancy complications, such as pre-eclampsia and gestational diabetes, have also been associated with an increased risk for type 2 DM and cardiovascular diseases later in life (Kaaja and Greer 2005). Although the association between periodontal disease and adverse pregnancy outcome has been extensively investigated, the results have been inconclusive, with a number of studies finding an association (Jeffcoat et al. 2001, Radnai et al. 2004, Goepfert et al. 2004, Moreu et al. 2005, Offenbacher et al. 2006, Siqueira et al. 2007, Guimarães et al. 2010, Vogt et al. 2010, Rakoto-Alson et al. 2010, Baskaradoss et al. 2011), while others have not (Noack et al. 2005, Moore et al. 2005, Bassani et al. 2007, Vettore et al. 2008). Examples of studies of periodontal disease and adverse pregnancy outcomes are given in Table 4.

The concept of the ability of periodontal pathogens and their virulence factors to disseminate and induce both local and systemic inflammatory responses in the host originates from the year 1891, when Miller published the theory of “focal infection”. He suggested that focal oral infection was responsible for many localized and systemic diseases such as tonsillitis, pneumonia, endocarditis, and septicemia. However, because there was no scientific evidence supporting this theory, it was put aside for ten decades. In the early 1990s Collins et al. (1994) hypothesized that oral infection, such as periodontal disease, could act as a source of bacteria and inflammatory mediators that could spread systemically to the fetal-placental unit via the blood circulation and induce pregnancy complications. Collins et al. (1994) used the golden hamster model to examine the effects of P.g. infection on TNF-α and PGE₂ inflammatory mediator production and pregnancy outcome. A significant association was observed between increasing levels of both TNF-α and PGE₂ and fetal growth retardation as well as embryolethality. Infections caused by periodontal pathogens were suggested to elicit adverse pregnancy outcomes and the degree of PGE₂ and TNF-α was associated with the severity of
fetal effect. Periodontal disease was thus proposed to be a potential independent risk factor for adverse pregnancy outcomes.

The study by Offenbacher et al. (1996) was the first to investigate the link between periodontal disease and PTB in humans. The odd ratios were high: 7.9 for all PLBW cases and 7.5 for primiparous PLBW cases. Offenbacher and colleagues suggested that periodontal infection may serve as a reservoir of bacteria, endotoxins such as lipopolysaccharides, and inflammatory mediators such as PGE₂ and TNF-α. Periodontal disease may affect the welfare of the fetus by activating the innate immune system, leading to an increased expression of prostaglandins and inflammatory cytokines. C-reactive protein may be the mediator in this process, and evidence has emerged that pregnant women with periodontal disease have elevated CRP levels early in pregnancy (Horton et al. 2008). The levels of IL-6 and -8 as well as of TNF-α in maternal serum are associated with PLBW. Hasegawa et al. (2003) examined the association between periodontal condition and threatened preterm labor, which often results in PTB. They found that women with threatened preterm labor had worsened periodontal conditions and elevated serum IL-8 and IL-1β levels compared with control women. Dasanayake et al. (2001) also showed that second trimester levels of serum antibody against \( P.g. \) are related to LBW.

Periodontal pathogens have also been detected in the fetal-placental unit and have been demonstrated to colonize directly with the placenta and cause localized inflammatory response, resulting in PTB. Katz et al. (2009) used immunocytochemistry methods to identify the presence of \( P.g. \) antigens in placental tissues. They detected an increase in immunostaining intensity of the tissues sectioned from women with chorioamnionitis relative to those experiencing full-term pregnancy. These results suggested that \( P.g. \) may commonly colonize placental tissues and that the presence of this organism may contribute to the preterm delivery. Keelán et al. (2010) revealed that periodontal pathogens are capable in eliciting an inflammatory response in human decidual cells.

In the study by Madianos et al. (2001), umbilical cord blood samples from 351 infants were analyzed. Significantly higher levels of specific IgM against oral pathogens in PTB infants were found than in full-term infants. Because maternal IgM does not pass through the placental barriers, these results suggest a direct intrauterus fetal exposition to these bacteria,
which may have led to premature birth. Boggess et al. (2005) analyzed the umbilical cord blood samples of 640 infants and measured CRP, IL-1β, TNF-α, PGE₂, 8-isoprostan, and IgM levels against the periodontal pathogens Pn, P.i., Fusobacterium nucleatum, Peptostreptococcus micros, and Campylobacter rectus. The risk of prematurity was higher when IgM was detected against at least one periodontal pathogen and even higher when high levels of inflammatory mediators were measured.

Microorganisms can access the amniotic cavity by ascending from the vagina and the cervix, by hematogenous dissemination through the placenta, by accidental introduction during invasive procedures, and by reverse spreading through the fallopian tubes (Goldenberg et al. 2008). The microorganism reaching decidua may stimulate a local inflammatory reaction and the production of pro-inflammatory cytokines. In addition, microorganisms may cross intact fetal membranes and reach the amniotic cavity, where they can elicit the production of inflammatory mediators. Bearfield et al. (2002) showed by polymerase chain reaction (PCR) Streptococcus sp. and Fusobacterium nucleatum from amnionic fluid and these microorganisms were positively associated with adverse pregnancy outcomes.

Several studies have examined the effect of periodontal treatment on preterm birth. Michalowicz et al. (2006) studied 823 women, 413 of whom had scaling and root planing before 21 weeks of gestation and 410 postpartum. No significant differences were found between the two groups in birth weight or in the rate of delivery of infants small for gestational age. Treatment of periodontal disease during pregnancy was safe and improved the periodontal condition, but did not significantly alter the rate of PTB, LBW, or fetal growth restriction. In their other study, Michalowicz et al. (2009) observed no significant difference between women receiving scaling and root planing before 21 weeks of gestation or after delivery. This non-surgical periodontal treatment did not reduce systemic serum markers of inflammation. A randomized controlled trial by Newnham et al. (2009) resulted in similar findings, as they noted no differences in PTB, birth weight, pre-eclampsia, or other obstetric outcomes between women receiving periodontal treatment around 20 weeks of gestation, including mechanical removal of oral biofilms and oral hygiene instruction and motivation, and women not receiving this treatment. Likewise, in the study of Oliveira (2011), non-surgical periodontal treatment during the second trimester of gestation did not reduce the risk for PTB, LBW, or PLBW. However, several studies have also described the opposite results,
linking periodontal treatment during pregnancy to more favorable pregnancy outcome. A randomized controlled trial by López et al. (2002) showed that periodontal treatment before 28 weeks of gestation significantly reduced the rates of PLBW compared with periodontal treatment after delivery. Tarrannum and Faizuddin (2007) compared 100 women who received non-surgical periodontal therapy during pregnancy with 100 women in the control group who received periodontal therapy postpartum. Their results showed that the prevalence of PTB and LBW was higher in the women treated postpartum. The prevalence of PTB was 53.5% in the women in the treatment group and 76.4% in the control group. In addition, the prevalence of LBW was higher in the control group (53.9% vs. 26.3%). These results were supported by the findings of Gomes-Filho et al. (2010), who showed that successful periodontal treatment in pregnant women with periodontal disease is a protective factor promoting the birth of normal birth weight children. In addition, the study of Sant’Ana et al. (2011) revealed that periodontal treatment during pregnancy is associated with a decreased risk of developing adverse pregnancy outcomes. Jeffcoat et al. (2011) showed that scaling and root planing together with oral hygiene instruction was associated with a decreased incidence of spontaneous PTB. Interestingly, in this study, 97.7% of the women who had unsuccessful periodontal treatment had not seen a dentist for tooth cleaning.

The meta-analysis of randomized trials by Polyzos et al. (2009) showed that treatment with scaling and/or root planing during pregnancy significantly reduces the rate of PTB and may reduce the rate of LBW infants. However, after a new meta-analysis of more recent studies, Polyzos et al. (2010) concluded that treatment of periodontal disease with scaling and root planing cannot be considered an efficient way of reducing the incidence of PTB. However, a meta-analysis of randomized trials (George et al. 2011) concluded that periodontal treatment during pregnancy may reduce PTB and LBW incidence.

A prospective study by Offenbacher et al. (2006) showed a PTB incidence of 11.2% among periodontally healthy women compared with 28.6% in women with moderate to severe periodontal disease. Very preterm delivery was 1.8% among women without periodontal disease progression compared with 6.4% among women with disease progression. Guimarães et al. (2010) observed an association between maternal periodontal disease and preterm as well as extreme preterm birth. The study by Goepfert et al. (2004) found an association between severe periodontal disease and spontaneous PTB at less than 32 weeks of gestation.
Moreu et al. (2005) observed that periodontal disease is a significant risk factor for LBW, but not for preterm delivery. Radnai et al. (2004) studied 85 women postpartum and found a significant association between PTB and early localized periodontal disease. Also the average weight of the infants was lower in the periodontal disease group than in the control group. Moreu et al. (2005) noted an association between LBW and percentage of periodontal pockets with a depth > 3 mm, suggesting that periodontal disease is a significant risk factor for LBW. However, their study did not show any association between periodontal disease and preterm delivery. Vogt et al. (2010) examined the association of periodontal disease and adverse perinatal outcomes in a group of Brazilian low-risk pregnant women. In this cohort, periodontal disease was a risk factor for PTB, LBW, and premature rupture of membranes.

Studies observing no association between periodontal disease and adverse pregnancy outcome include a case-control study by Moore et al. (2005), who examined 154 women postpartum. No differences were found in oral hygiene, bleeding on probing, or loss of attachment. Similarly, Vettore et al. (2008) investigated the relationship between periodontal disease and PLBW by examining 15 measures of periodontal disease. The extent of periodontal disease did not increase the risk of PLBW. Bassani et al. (2007) conducted a case-control study among 915 women, and periodontal health did not explain negative LBW, PLBW, or intra-uterine growth restriction.

Many of the studies examining oral health and pregnancy complications were conducted among mothers of low socioeconomic status. Baskaradoss et al. (2011) conducted a case-control study among 300 women, 100 of whom had undergone spontaneous preterm delivery and 200 had delivered full-term. The majority of the women were from a low social class and their knowledge of oral health care was poor. A risk of nearly 3-fold for preterm delivery was observed in mothers with periodontal disease. A prospective cohort study by Mobeen et al. (2008) evaluated the relationship between periodontal disease and birth outcomes in a community setting in Pakistan among women, 33% of whom had no education. This study showed that periodontal disease was extremely common, and when the severity of periodontal disease increased, both the stillbirth and neonatal mortality rates rose.

Only 27% of women in a case-control study by Khader et al. (2009) had an education level higher than high school. In this cohort, extent and severity of periodontal diseases were
significantly associated with PLBW delivery. However, when Noack et al. (2005) investigated 59 pregnant German women, 83% of whom had middle or high socioeconomic status, they found no significant differences between women with high risk for PLBW infants and control women in any of the periodontal parameters examined. A retrospective cohort study examined 23,441 women of a middle or upper income group who were enrolled in a national insurance plan and who have delivered live births from singleton pregnancies in the United States (Albert et al. 2011). Women who received preventive dental care had significantly better birth outcomes than those who received no treatment. In addition, Pitiphat et al. (2008) examined the effect of periodontal disease on birth outcomes in an insured cohort of middle-income women. Their results suggested that periodontal disease is an independent risk factor for preterm delivery and/or small for gestational age infants.

**Oral health and miscarriage**

Only a few studies have examined the association between oral health and MC. Moore et al. (2004) showed a relationship between poor periodontal health and late MC, but no association between maternal periodontal disease in the first trimester of pregnancy and PTB or LBW. Farrell et al. (2006) demonstrated a weak relationship between poor periodontal health and late MC in women who never smoked.

**Oral health and pre-eclampsia**

Chronic subclinical infections increase maternal cytokine levels, which may affect vascular endothelial function and render pregnant women more prone to the development of pre-eclampsia. Studies have found an association between periodontal disease and pre-eclampsia. Contreras et al. (2006) reported that chronic periodontal disease and the presence of *P. g.*, *T. f.*, and *Eikenella corrodens* were significantly associated with pre-eclampsia. In addition, Nabet et al. (2010) showed an association between generalized periodontitis and induced PTB for pre-eclampsia, but not with spontaneous PTB or preterm premature rupture of membranes or other causes of PTB. Ruma et al. (2008) examined the relationship between maternal periodontal disease, maternal systemic inflammation, and the development of pre-eclampsia. Women with periodontal disease and systemic inflammation early in pregnancy had an increased risk for development of pre-eclampsia. Further, the study by Barak et al. (2007) showed a significant presence of periopathogenic microorganisms or their products in placentas of women with pre-eclampsia compared with healthy pregnant women. A meta-

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analysis of original research published between 1998 and 2010 revealed that maternal periodontal disease has strong associations with pre-eclampsia and prematurity (Matevosyan 2011). This meta-analysis found that higher rates of tobacco use (RR 3.02), bacterial vaginosis (RR 2.7), severe gingivitis (RR 2.47), higher probing depth (OR 2.35), clinical attachment level (OR = 2.76), bleeding on probing (RR 1.78), fetal tyrosine kinase (OR 1.6), and maternal CRP (OR 3.1) contributed to increased rates of pre-eclampsia (OR 1.68) and spontaneous preterm labor (RR 2.75).
Table 4. Examples of periodontal disease and adverse pregnancy outcomes studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of study</th>
<th>No. of studied women, characteristics of population</th>
<th>Definition of periodontal disease</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeffcoat et al. 2011, USA</td>
<td>Randomized, controlled clinical trial</td>
<td>322 African American 87.5%</td>
<td>≥ 3 tooth sites with an AL of ≥ 4 mm</td>
<td>Successful periodontal treatment associated with a decreased incidence of PTB.</td>
</tr>
<tr>
<td>Michalowicz et al. 2006, USA</td>
<td>Randomized, controlled clinical trial</td>
<td>823 White 29.0% Black 44.4% Hispanic 43.9%</td>
<td>≥ 4 teeth with PD ≥ 4 mm and CAL ≥ 2 mm and BOP ≥ 35% or more of tooth sites. Progression of periodontal disease: any increase in CAL ≥ 3 mm</td>
<td>Periodontal treatment did not significantly alter the rates of PTB, LBW, fetal growth restriction, or pre-eclampsia.</td>
</tr>
<tr>
<td>Agueda et al. 2008, Spain</td>
<td>Prospective study</td>
<td>1296 Caucasian 78.6% Black 13.8% Gipsy 16.7% Others 4.1%</td>
<td>Presence of ≥ 4 teeth with ≥ 1 site with PD of ≥ 4 mm and CAL ≥ 3 mm at the same site</td>
<td>A modest association between periodontal disease and PTB. No association between periodontal disease and LBW or PLBW.</td>
</tr>
<tr>
<td>Offenbacher et al. 2006, USA</td>
<td>Prospective study</td>
<td>1020 African American 46.2% White 47.7% Other 6.1%</td>
<td>Health: absence of PD ≥ 3 mm and no sites with AL &gt; 2 mm. Mild disease: between health and moderate-severe; Moderate-severe disease: ≥ 4 sites of ≥ 5 mm PB and ≥ 2 mm AL at ≥ 4 sites.</td>
<td>Periodontal disease increased the relative risk for PTB or spontaneous PTB. Periodontal disease progression during pregnancy may contribute to deliveries at less than 32 weeks of gestation.</td>
</tr>
<tr>
<td>Vettore et al. 2008, Brazil</td>
<td>Case-control study</td>
<td>542 White 32.2% Brown 47.6% Black 20.2%</td>
<td>15 different measures</td>
<td>Periodontal disease is not a risk factor for LBW, PTB, and PLBW.</td>
</tr>
<tr>
<td>Offenbacher et al. 1996, USA</td>
<td>Case-control study</td>
<td>124 White 29.8% Hispanic 8.9% Black 58.9% Asian 2.4%</td>
<td>CAL ≥ 3 mm at ≥ 60% of sites</td>
<td>Periodontal disease may be a significant risk factor for PLBW.</td>
</tr>
<tr>
<td>Noack et al. 2005, Germany</td>
<td>Case-control study</td>
<td>59 White 100%</td>
<td>Percentage of sites with CAL ≥ 3 mm</td>
<td>No association between periodontal status and PLBW.</td>
</tr>
</tbody>
</table>

AL: attachment loss  
PTB: preterm birth  
CAL: clinical attachment level  
BOP: bleeding on probing  
PD: probing depth  
PLBW: preterm low birth weight  
LBW: low birth weight
Oral health and gestational diabetes

Several studies have examined the association between periodontal disease and gestational diabetes. Xiong et al. (2009) demonstrated that periodontal disease was associated with an increased risk of GDM and that a dose-response relationship between an increased risk GDM and increasing severity of periodontal disease. In the study by Dasanayake et al. (2008), clinical and other periodontal disease-related parameters were collected at least 7 weeks before the diagnosis of GDM. No significant association was found between clinical periodontal disease and GDM. However, higher vaginal levels of *T. f.* were significantly associated with GDM. Guthmiller et al. (2001) investigated the association of type 1 diabetes (T1DM) with the periodontal status of pregnant women. Pregnant women with T1DM exhibited greater gingival inflammation and periodontal destruction than non-diabetic pregnant women. Likewise, Novak et al. (2006) concluded that women with GDM during pregnancy may be at increased risk for developing more severe periodontal disease than women without GDM. Chapper et al. (2005) showed that women with GDM and a history of pre-gestational obesity had significantly more gingivitis and periodontal attachment loss than women with normal pre-gestational BMI. In the study by Ruiz et al. (2011) Brazilian pregnant women with diabetes had a significantly higher gingival index, gingival margin location, probing depth, clinical attachment level, bleeding on probing, and tooth mobility index than non-diabetic pregnant women. The degree of periodontal disease was similar between GDM and T1DM.
HYPOTHESIS AND AIMS

General aim and hypothesis

The majority of previous studies on oral health and adverse pregnancy outcomes have been carried out among women with a low socioeconomic status and a multi-ethnic background. Thus, we set out to investigate the association between oral health and pregnancy complications in a group of Finnish women with homogeneous ethnicity and a high socioeconomic status. The study hypothesis was that pregnancy complications are reflected in the oral health of women, and markers of oral health differ between risk and non-risk subjects.

Specific aims were as follows:

1. To compare clinical and microbiological oral health parameters between preterm and full-term women (Study I).

2. To investigate dental care patterns, i.e. preventive and urgency-based dental treatment, in association with the history of miscarriage (Study II).

3. To examine whether the oral inflammatory burden index, which takes into account gingivitis, periodontitis, and oral mucositis, is associated with preterm birth (Study III).

4. To investigate the association of salivary immunoglobulin-A with diabetes and adverse pregnancy outcomes (Study IV).
SUBJECTS AND METHODS
This cross-sectional study was approved by the Ethics Committee of the Helsinki University Central Hospital (HUCH) (HUS 107/E6/2000, 25.10.2000), as well as the Institutional Review Board (TYH 3245 10.2.2003, T1020Y0003 1.12.2006), and registered in the clinical investigation register of HUCH at www.hus.fi (64.01.12.2006). The study was conducted according to the principles of the Declaration of Helsinki, and all participants provided written consent (Declaration of Helsinki 2000).

Subjects
The study sample included 482 women who gave birth at the Department of Gynecology and Obstetrics, HUCH, Finland, between September 2002 and May 2004. Women with problem pregnancies are referred to this tertiary referral hospital, leading to a high prevalence of women with adverse pregnancy outcomes. The women were randomly recruited in the labor ward postpartum and informed about the study. Primary exclusion criteria comprised illicit drug abuse and infection with hepatitis B, hepatitis C, or Human immunodeficiency virus (HIV). In addition, the study was restricted to singleton births. Cohort development in Studies I-IV is shown in Figure I.

Comparison groups and assessment of outcomes
Studies I and III
To investigate the association between preterm birth and oral health, the women were allocated into two groups according to whether they had delivered before (preterm birth) or at/after (full-term birth) 259 gestation days (37 weeks), consistent with the recommendations of the World Health Organization (WHO) and the International Federation of Gynaecology and Obstetrics (FIGO) (Steer 2005). Gestational age was determined based on the date of the last menstrual period and confirmed by ultrasound examination at 11-14 weeks of gestation. Among the 328 women, 77 were PTB, while 251 were full-term.
Study II
To evaluate the association between MC and oral health, the women were divided into two groups according to whether or not they had reported pregnancy loss before 24 weeks of gestation in one or more former pregnancies (HMC) (Rai and Regan, 2006); if no pregnancy loss was reported, the woman was considered to have no history of miscarriage (NHMC). Gestational age was determined, as in Studies I and III, based on the date of the last menstrual period and confirmed by ultrasound examination at 11-14 weeks of gestation. Among the 328 women, 74 had HMC and 254 had NHMC.

Study IV
To assess the association between oral health, diabetes, and adverse birth outcomes, the women were allocated into three groups according to whether the diagnosis was gestational diabetes, pre-existing diabetes, or no diabetes. Among the 328 women, 42 had gestational diabetes, 11 had pre-existing diabetes, and 275 were non-diabetic.

Clinical examination
A specially equipped dental unit was set up at the hospital. The dental examination of the women was performed by two dentists within 2 days postpartum. Both dentists were employees of the City of Helsinki Health Department, where monthly education meetings served as calibration sessions. If the mother was bedridden, a bedside examination was conducted using an otological light source. The two examiners were blinded to pregnancy complications. Details of the clinical examinations and analyses are provided in Tables 5 and 6.

Dental examination
Dental status, including caries, fillings, and missing teeth, was recorded. The World Health Organization's (WHO) decayed, missing, filled (DMF) tooth and surface indices (DMFS) were calculated based on the examination. If any initial (enamel) or dentin caries was detected, the tooth was defined as diseased. Third molars were separately recorded.
**Periodontal examination**

A calibrated probe was used to measure periodontal probing depth (PD), the distance from the gingival margin to the apical part of the gingival pocket, at six sites per tooth for all teeth. Gingival bleeding on probing (BOP) and visible dental plaque were recorded at four sites per tooth for all teeth. WHO CPI of Treatment Needs was also recorded according to Ainamo and Bay (1975). The severity of periodontal infection was assessed by the number of periodontal pockets with probing depth ≥4 mm and the number of bleeding gingival surfaces. Third molars were recorded separately for all periodontal parameters. The presence of pericoronitis was defined as bleeding on probing of the third molars.

**Mucosal examination**

Oral mucosa was systematically examined, and mucosal lesions, such as aphthous ulcers, lichen planus, amalgam tattoos, cheek bites, and furred tongue, were registered as yes/no.
Figure 1. Cohort development in Studies I-IV

482 women recruited

4 women dropped out for personal reasons

328 women examined

25 women delivered twins -> excluded

125 women – no clinical examination

STUDIES I & II
Of the 328 women examined

77 women with preterm birth
251 women with full-term birth

STUDY II
Of the 328 women examined

74 women with history of miscarriage
254 women without history of miscarriage

STUDY IV
Of the 328 women examined

275 women without diabetes
42 women with gestational diabetes
11 women with pre-existing diabetes
Table 5. Clinical examination and analyses

<table>
<thead>
<tr>
<th>Clinical examination</th>
<th>Subgingival plaque sampling</th>
<th>Salivary analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental caries</td>
<td>Presence of</td>
<td>Level of</td>
</tr>
<tr>
<td>• Tooth indices decayed, missing, filled (DMF)</td>
<td>• <em>Aggregatibacter actinomycetemcomitans</em></td>
<td>• Salivary immunoglobulin A</td>
</tr>
<tr>
<td>• Tooth surface indices decayed, missing, filled (DMFS)</td>
<td>• <em>Porphyromonas gingivalis</em></td>
<td>• Salivary immunoglobulin G</td>
</tr>
<tr>
<td>• Presence of initial or dentin caries</td>
<td>• <em>Prevotella intermedia</em></td>
<td>• Salivary immunoglobulin M</td>
</tr>
<tr>
<td>Periodontal health</td>
<td>• <em>Prevotella nigrescens</em></td>
<td>• Total protein</td>
</tr>
<tr>
<td>• Gingival bleeding on probing</td>
<td>• <em>Treponema denticola</em></td>
<td>• Albumin</td>
</tr>
<tr>
<td>• Visible dental plaque</td>
<td>• <em>Tannerella forsythia</em></td>
<td>• Polymorphonuclear elastase</td>
</tr>
<tr>
<td>• Periodontal pocket depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral mucosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Aphthous ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Lichen planus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Amalgam tattoos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Cheek bites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Furred tongue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subgingival plaque sampling and analyses

Supragingival plaque was removed with cotton swabs and the tooth surface was dried with cotton rolls before collecting subgingival dental plaque with sterile curettes from the periodontal pockets of first molars of the first and third jaw quadrants. If the first molar had been extracted, the sample was taken from the second premolar or molar from the same jaw quadrant. The samples were analyzed with polymerase chain reaction (PCR) for periodontal bacteria *A.a.*, *P.g.*, *P.i.*, *P.n.*, *T.d.*, and *T.f.* (Wahlfors et al. 1995, Meurman et al. 1997). This PCR method has been validated and is commonly used in our oral microbiology laboratory.
**Saliva sampling**

The unstimulated and stimulated salivary flow rates were measured. Unstimulated salivary flow was measured with the free flowing method and stimulated saliva by 5-min chewing of a 1-g piece of paraffin wax (Meurman and Rantonen 1994). Samples were then centrifuged for 4 min at 8000 g at 4°C and stored at -75°C to await further analyses.

**Salivary biochemical analyses**

The colorimetric Lowry method (Lowry et al. 1951) was used to determine total protein, and the immunoturbidometric Tina-Quant® kit (Roche, Basel, Switzerland) to analyze albumin. The enzyme-linked immunosorbent assay (ELISA) was applied to determine salivary immunoglobulin A, G, and M concentrations. All analyses were performed in duplicate, and standards and controls were used.

**Salivary elastase analysis**

Polymorphonuclear (PMN) elastase activity was analyzed using chromogenic substrate 1 mM succinyl-alanyl-alanyl-valine-p-nitroanilide (Sigma Chemicals, St. Louis, MO, USA). A spectrophotometer at 405 nm was used to detect the increase of optical density (OD) units before and after 1 h of incubation at 37°C. The difference in the OD values was used as the measure of elastase activity ($\Delta$OD$_{405}$/h).

**CRP analyses**

Serum samples were taken from the women in the first trimester of pregnancy, and serum CRP levels were analyzed from these samples. An immunofluorometric CRP kit was used (Innotrac Diag, Turku, Finland). The sensitivity of the assay is 0.05 mg/L, and its assay range is 0.05-50 mg/L.
Table 6. Methods used to analyze plaque, saliva, and serum samples

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontal pathogen</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>Total protein</td>
<td>Colorimetric Lowry method</td>
</tr>
<tr>
<td>Albumin</td>
<td>Immunoturbidometric assay</td>
</tr>
<tr>
<td>Salivary immunoglobulin A, G, M</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Polymorphonuclear elastase</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Immunofluorometric assay</td>
</tr>
</tbody>
</table>

Maternal characteristics
Questionnaires were used to obtain information about dental care history and health habits, oral symptoms, smoking and alcohol use, and general health- and lifestyle-related behavior of the women. Medical records were used for information about demographic factors, prenatal care, and medical and obstetric history. Chronic diseases and medications were recorded and validated against the medical records (validity 97.4%). Pre-pregnancy body mass index (BMI) and weight gain between the first and third trimesters were calculated. The number of previous pregnancies, including spontaneous miscarriages, PTB, and stillbirths, was also recorded from the medical records, as were complications such as infections and pre-eclampsia. Gestational diabetes was categorized using the simplified White's classification (Study I) (White 1978) and based on the combination of American Diabetes Association (ADA) and International Association of Diabetes and Pregnancy Study Groups (IADPSG) definitions (Study IV) (IADPSG 2010, O'Sullivan et al. 2011). Pre-existing diabetes was determined according to the ADA guidelines (American Diabetes Association 2009).
Statistical analyses

Statistical Package for Social Sciences for Unix, SPSS, Chicago, IL, USA (SPSS) was used to conduct the univariate analyses. Continuous variables were tested with either $t$-tests after evaluating normality or with non-parametric tests if the data did not fit a normal distribution. Categorical data were tested using chi-squared tests or Fisher’s exact test. Multivariate analyses were conducted by the logistic regression method using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Significance levels were set at $p = 0.05$ (two-tailed) and confidence intervals at 95%. Detailed information on the statistical analyses is given in the original publications.
RESULTS

Demographic data (Studies I-IV)

The mean age of the women was 31.1 ± 5.0 years. Of the women, 69.5% were highly educated, 22.3% had moderate education, and only 6.4% had no professional education. Most of the mothers did not smoke (74.1%) or consume alcohol (73.2%) at all during pregnancy. The mean BMI was 23.4 ± 4.2 kg/m², which is within the normal range, and weight gain during pregnancy was 13.4 ± 5.1 kg. Of the women, 18.8% had diagnosed diseases and 10.7% had infection during pregnancy, the most common being gynecological infection (6.1%). The prevalence of recorded medications was 30.8%, and these were mostly antimicrobial drugs (12.3%), which can be considered a proxy of infections. Of the women, 50.0% had given birth for the first time. Vaginal delivery was the most common route of delivery (87.8%), while 7.0% had an elective Cesarean section and 5.2% had an emergency Cesarean section. More details of the medical and obstetrical data are presented in Table 7. Of the 328 women, 77 had preterm and 251 full-term births (Studies I and III), and 74 had a history of miscarriage, while 254 had no history of miscarriage (Study II). Likewise, 42 women had gestational diabetes, 11 had diabetes, and 275 had no diabetes diagnosis (Study IV).
Table 7. General characteristics and medical and obstetrical data of the women in Studies I and III

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All N = 328</th>
<th>Preterm N = 77</th>
<th>Full-term N = 251</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>31.1 (5.0)</td>
<td>30.9 (5.4)</td>
<td>31.2 (4.9)</td>
<td>0.62</td>
</tr>
<tr>
<td>Education ≥ college, N (%)</td>
<td>228 (69.5)</td>
<td>53 (68.8)</td>
<td>175 (69.7)</td>
<td>0.89</td>
</tr>
<tr>
<td>Smoking, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During pregnancy</td>
<td>36 (11.0)</td>
<td>11 (14.3)</td>
<td>25 (10.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Before pregnancy</td>
<td>49 (14.9)</td>
<td>7 (9.1)</td>
<td>42 (16.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Not at all</td>
<td>243 (74.1)</td>
<td>59 (76.6)</td>
<td>184 (73.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>Alcohol consumption during pregnancy, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td>240 (73.2)</td>
<td>61 (79.2)</td>
<td>179 (71.3)</td>
<td>0.19</td>
</tr>
<tr>
<td>Less than once a month</td>
<td>64 (19.5)</td>
<td>9 (11.7)</td>
<td>55 (21.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>24 (7.3)</td>
<td>7 (9.1)</td>
<td>17 (6.8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Prepregnancy Body Mass Index, kg/m², mean (SD)</td>
<td>23.4 (4.2)</td>
<td>23.5 (4.6)</td>
<td>23.4 (4.1)</td>
<td>0.82</td>
</tr>
<tr>
<td>Maternal weight gain, kg, mean (SD)</td>
<td>13.4 (5.1)</td>
<td>11.7 (4.8)</td>
<td>13.9 (5.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Primiparity, N (%)</td>
<td>164 (50.0)</td>
<td>50 (64.9)</td>
<td>114 (45.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maternal diseases, N (%)</td>
<td>60 (18.8)</td>
<td>17 (23.6)</td>
<td>43 (17.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>Asthma</td>
<td>17 (5.3)</td>
<td>7 (9.6)</td>
<td>10 (4.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11 (3.4)</td>
<td>4 (5.2)</td>
<td>7 (2.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>Infection during pregnancy, N (%)</td>
<td>35 (10.7)</td>
<td>11 (14.3)</td>
<td>24 (9.6)</td>
<td>0.29</td>
</tr>
<tr>
<td>Medication, N (%)</td>
<td>101 (30.8)</td>
<td>38 (49.4)</td>
<td>63 (25.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial drugs</td>
<td>38 (12.3)</td>
<td>18 (24.3)</td>
<td>20 (8.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hospitalization caused by preterm contractions, N (%)</td>
<td>13 (4.0)</td>
<td>8 (10.4)</td>
<td>5 (2.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pre-eclampsia, N (%)</td>
<td>30 (9.1)</td>
<td>8 (10.4)</td>
<td>22 (8.8)</td>
<td>0.66</td>
</tr>
<tr>
<td>Cesarean section, N (%)</td>
<td>23 (7.0)</td>
<td>10 (13.0)</td>
<td>13 (5.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Emergency Cesarean section, N (%)</td>
<td>17 (5.2)</td>
<td>11 (14.3)</td>
<td>6 (2.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Due to missing values, not all categories have N = 328.
Dental health and preterm birth (Studies I, III)

Dental health was uniformly good, and preterm and full-term women had complete dentitions (28.9 ± 2.7 vs. 28.7 ± 1.7). Preterm women had more dental caries (93.5%) than full-term women (85.3%) (p = 0.06), although not significantly more, when assessed as initial and dentin caries in the mouth. No significant difference was observed in DMFS between the groups. The results of the oral health parameters are given in Table 8.

Table 8. Oral health parameters of Studies I and III

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preterm</th>
<th>Full-term</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teeth, mean (SD)</td>
<td>28.9 (2.7)</td>
<td>28.7 (1.7)</td>
<td>0.75</td>
</tr>
<tr>
<td>DMFS, mean (SD)</td>
<td>19.2 (17.7)</td>
<td>19.2 (14.4)</td>
<td>0.97</td>
</tr>
<tr>
<td>Prevalence of caries, N (%)</td>
<td>72 (93.5)</td>
<td>214 (85.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>Plaque-covered surfaces, mean (SD)</td>
<td>20.4 (20.0)</td>
<td>18.2 (18.1)</td>
<td>0.35</td>
</tr>
<tr>
<td>Number of gingival bleeding surfaces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quartile 1 (up to 10 surfaces)</td>
<td>11 (14.7)</td>
<td>62 (25.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>quartile 2 (10&lt; bleeding surface ≤ 22)</td>
<td>27 (36.0)</td>
<td>64 (26.3)</td>
<td>0.11</td>
</tr>
<tr>
<td>quartile 3 (22&lt; bleeding surfaces≤ 33)</td>
<td>18 (24.0)</td>
<td>50 (20.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>quartile 4 (33)</td>
<td>19 (25.3)</td>
<td>67 (27.6)</td>
<td>0.70</td>
</tr>
<tr>
<td>Number of &gt; 4 mm periodontal pockets, mean (SD)</td>
<td>1.8 (5.9)</td>
<td>1.8 (5.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>Oral inflammatory burden, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPI-based OIBI</td>
<td>1.62 (0.55)</td>
<td>1.33 (0.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Data-driven OIS</td>
<td>5.47 (2.86)</td>
<td>4.35 (2.25)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

SD: Standard deviation  
DMFS: Decayed, missing, filled surfaces  
CPI: Community periodontal index  
OIBI: Oral inflammatory burden index  
OIS: Oral inflammation score
Periodontal health and preterm birth (Studies I, III)
None of the individual periodontal parameters showed significant differences between preterm and full-term women. Periodontal disease was not a major problem in this study cohort, as 75% had less than one site of periodontal pockets ≥ 4 mm, and only five women had periodontal pocket depth ≥ 6 mm. When examining the role of periodontal disease and smoking by three-way cross-tabulation, neither appeared to be major risk factors for PTB. Because no significant differences were found in any of the separate periodontal parameters, the decision to examine the combined effects of many oral infections was made. Thus, oral inflammatory burden index (OIBI) (Study III) was constructed based on clinical findings (gingival bleeding on probing, probing depth, presence of dental calculus, and potential mucositis). Preterm women had significantly higher OIBI and oral inflammation score (OIS) values than full-term women (OIBI 1.62 ± 0.55 vs. 1.33 ± 0.66; p = 0.0004; OIS 5.47 ± 2.86 vs. 4.35 ± 2.25; p = 0.003).

Periodontal bacteria and preterm birth (Study I)
No significant differences were found in the prevalence of any periodontal bacteria (A.a., P.g., P.i., P.n., T.d., and T.f.) analyzed.

Predictors of preterm birth in the regression model (Studies I, III)
Periodontal disease assessed as probing depth of periodontal pockets did not predict preterm birth in the regression model in Study I. Low weight gain (<13 kg), antimicrobial treatment, and primiparity were the significant predictors in the model. In Study III, the multivariate regression model for the preterm birth OIBI was significantly associated with PTB (OR = 1.85, 95% CI: 1.10-3.10, p = 0.02). Primiparity (OR = 3.72, 95% CI: 1.84-7.52, p = 0.0003), antimicrobial treatment (OR = 4.37, 95% CI: 1.93-9.90, p = 0.0004), and low weight gain (OR = 1.97, 95% CI: 1.09-3.57, p = 0.03) were other significant covariates. OIS was significantly associated with PTB without adjusting for weight gain (p = 0.03), but when weight gain was added to the final model, OIS lost its significance (p = 0.16).

Oral health and history of miscarriage (Study II)
The use of urgency-based dental care within 12 months was more prevalent among women with a history of miscarriage (HMC) than in women with no history of miscarriage (NHMC)
(21.1% vs. 13.1%; p = 0.09). In addition, the HMC and NHMC groups were significantly different in terms of age (p < 0.05), antimicrobial treatment (p < 0.05), and infertility treatment (p < 0.05). When studying HMC, urgency-based dental treatment showed a significant association with HMC (OR = 2.54, 95% CI: 1.21-5.37, p = 0.01) and preventive dental treatment showed a marginally significant inverse association (OR = 0.53, 95% CI: 0.26-1.06, p = 0.07). Self-assessed moderate or poor oral health also revealed an increased likelihood of HMC relative to those who reported good or very good oral health (OR = 1.60, 95% CI: 0.88-2.90, p = 0.12).

**Association of salivary immunoglobulin A with diabetes and diabetic sequelae (Study IV)**

When comparing control, gestational diabetes, and type 1 diabetes (pre-existing diabetes) women, the GDM and control women were significantly different regarding BMI (p < 0.001), primiparity (p < 0.01), and serum CRP (p < 0.001), while control and T1DM women were significantly different in terms of unstimulated salivary flow (p < 0.01), salivary IgA (p < 0.01), and sIgA per mg salivary protein (p < 0.05). Salivary IgA was significantly associated with all diabetes in pregnancy, including GDM and T1DM, independent of CRP (OR = 1.83, 95% CI: 1.08-3.11, p = 0.02). Furthermore, sIgA normalized by total protein was significantly associated with all diabetes (OR = 1.02, 95% CI: 1.00-1.04, p = 0.03). However, after excluding women with T1DM, sIgA lost its significant association with gestational diabetes (OR = 1.42, 95% CI: 0.80-2.52, p = 0.23), suggesting that sIgA may be associated with the immunity-related aspect in diabetes. When age, smoking, and primiparity were adjusted, sIgA was highly predictive of T1DM (OR = 4.65, 95% CI: 1.61-13.40, p < 0.01). Salivary IgA in all diabetes was significantly associated with macrosomia (OR = 2.34; 95% CI 1.23-4.43; p = 0.01). This association also existed when examining women with GDM only (OR = 2.40; 95% CI 1.22-4.74; p = 0.01).
DISCUSSION
We investigated the association of oral health with adverse pregnancy outcome in a cohort of Finnish women where many of the confounding factors were already excluded based on the study setting. These excluded factors comprised low education, low socioeconomic status, and ethnicity. The difficulty in assessing previous studies on this topic arises from the different clinical criteria used to define periodontal disease, the different methods used to define oral health, the variation in sample sizes, and the different composition of subjects with regard to race and socioeconomic status.

The main finding was that OIBI, a combination of multiple oral infections, was significantly associated with PTB, although none of the separate periodontal parameters predicted preterm birth. In addition, urgency-based dental treatment was associated with increased risk of HMC, while preventive dental treatment was related to a diminished risk of HMC. Self-reported poor oral health showed a positive association with miscarriage. Salivary immunoglobulin A was associated with GDM and T1DM independent of CRP, but when T1DM women were excluded, sIgA lost its significance.

Subjects and methods
Participants consisted of 482 women with a high education (69.5% were highly and 22.3% moderately educated), high socioeconomic status, and easy access to health services. Most of the previous studies concerning oral health and preterm birth have been conducted among individuals of multiracial backgrounds (Jeffcoat et al. 2001, Jarjoura et al. 2005, Moore et al. 2005, Offenbacher et al. 2006, Michalowicz et al. 2006, Siqueira et al. 2007, Vettore et al. 2008, Newnham et al. 2009, Jeffcoat et al. 2011) or with low socioeconomic status (Mitchell-Lewis et al. 2001, López et al. 2002, Budunelli et al. 2005, Jarjoura et al. 2005). These are factors known to be associated with preterm birth.

The clinical examination was carried out at the hospital in a specially equipped dental office. Because the dental office did not contain dental x-ray apparatus, we were unable to take radiographs and diagnose potential periapical lesions. This contributed to the construction of the oral inflammatory burden index. However, it is unlikely that many women had periapical lesions because no deep caries cavities were detected in the clinical examinations and none of the women reported any toothache.
Initially, 482 women participated in this cross-sectional study. The clinical examination took place within two days postpartum following the example of Offenbacher et al. (1996), who conducted clinical examinations within three days postpartum. However, for practical reasons, it was impossible to examine all women within two days postpartum, and this led to the exclusion of 125 women. In addition, we excluded 25 mothers who delivered twins, and four women dropped out for personal reasons. Our final study group thus consisted of 328 women, who had all been examined within two days postpartum and had had singleton births.

Because the dental examination was performed postpartum, no causal relationship between oral health and PTB can be concluded. Furthermore, because of the cross-sectional study design, we were unable to assess any longitudinal changes in the oral health parameters. However, our study design is not uncommon, as several previous studies have examined women postpartum (Offenbacher et al. 1996, Davenport et al. 2002, Radnai et al. 2004, Budunelli et al. 2005, Jarjoura et al. 2005, Noack et al. 2005, Guimarães et al. 2010).

Although we did not restrict the age of the women participating, our study cohort did not include very young mothers; all mothers were aged between 18 and 44 years. According to Jolly et al. (2000), women under 18 years of age are more likely to deliver preterm than older women. Our study cohort included women older than 35 years, although earlier studies have shown that pregnant women over 35 years have an elevated risk of delivering preterm (Jolly et al. 2000, Joseph et al. 2005). The mean age of our study cohort was 31.1 ± 5.0 years; thus, most of the women were younger than 35 years, and no significant differences were found in age between preterm and full-term women. Furthermore, the average age of primiparous women in the greater Helsinki area is 30.0 years, and 25.7% of parturient women are ≥ 35 years (National Institute for Health and Welfare 2010).

Of the women, 11.0% smoked during pregnancy, with the mean number of daily cigarettes being 5. This is somewhat less than the proportion in the Perinatal statistics for the Nordic countries, which reported that 14.7% of Finnish women smoked throughout their pregnancy in 2008 (National Institute for Health and Welfare 2010). Of the women in the present study, 73.2% did not use alcohol at all during pregnancy, and most of the women consuming alcohol reported drinking less than once a month. Approximately 3-5% of pregnant Finnish women drink more than 10 units/week (Halmesmäki 2009).
We defined preterm birth as birth before 259 gestation days (37 weeks) in accordance with WHO and FIGO (Steer 2005). Up to 10.1% of preterm babies may be misclassified if the gestational age is rounded up to gestational weeks (Balchin et al. 2004). At the maximum 7 days will be misclassified and for intrauterine development these 7 days at this late gestational age may make a significant difference.

Regarding oral health parameters, we measured periodontal probing depth, gingival bleeding on probing, and teeth with visible dental plaque. The severity of periodontal infection was assessed by the mean number of periodontal pockets with a probing depth ≥ 4 mm and the mean number of gingival bleeding surfaces. Although probing depth is a commonly accepted clinical measure of periodontal disease, it uses the gingival margin as a reference point and the position of the gingival margin may vary. Thus, using clinical attachment level is more precise because the cemento-enamel junction used as a reference point in clinical attachment level is stable (Xiong et al. 2007). Noack et al. (2005) used the percentage of sites with attachment loss ≥ 3 mm based on the American Academy of Periodontology consensus report (1999) to describe the severity of periodontal disease, and Rakoto-Alson et al. (2010) used the definition of clinical attachment loss ≥ 4 mm for at least three sites from different teeth. The study by Radnai et al. (2004) combined bleeding on probing and probing depth, as their definition for periodontal disease was at least one site of ≥ 4 mm probing depth and bleeding on probing for ≥ 50% of teeth. Newham and colleagues (2009) defined periodontal disease as the presence of periodontal pockets of ≥ 4 mm at ≥ 12 probing sites in fully erupted teeth, and, together with our study, represents the minority of studies that have not included clinical attachment level in the definition of periodontal disease. Some researchers have combined probing depth and clinical attachment level, as did Lopez et al. (2002), Siqueira et al. (2007), and Agueda et al. (2008), who defined periodontal infection as the presence of four or more teeth with one or more sites with probing depth ≥ 4 mm and with clinical attachment loss ≥ 3 mm at the same site. This variation in definition of periodontal disease leads to biases, as well as difficulties in comparing studies.

Oral health and preterm birth
Nearly all of the women in our cohort had complete dentitions, with a mean of 28.7 ± 2.0 teeth. Furthermore, none of the women had a history of tooth loss due to poor oral health. Examining a mouth with excellent oral health is challenging because the inflammatory burden
of separate oral health parameters is low. The impact of dental disease is also difficult to assess when the women have hardly any dental disease. Many of the previous studies have been performed among women with poor oral health and studies have had wide differences in oral health, as for example López et al. (2002) had inclusion criteria > 18 natural teeth, Tarannum and Faizuddin (2007) ≥ 20 completely erupted teeth, excluding third molars, and Newnham et al. (2009) ≥ 20 teeth.

We found no differences in any of the separate periodontal health parameters, i.e. plaque-covered surfaces, gingival bleeding, > 4 mm periodontal pockets, or prevalence of investigated periodontal bacteria. Contrary to our expectations, periodontal health status was good and periodontal disease challenge was low in our study cohort, as only five women had periodontal pocket depth ≥ 6 mm and 75% had less than one site of pockets ≥ 4 mm. However, many women had gingival bleeding, probably due to pregnancy gingivitis. Our findings (Study I) are in agreement with those of Vettore and colleagues (2008), who examined 15 criteria for periodontal disease based on periodontal pocket depth and clinical loss of attachment, and none of these factors appeared to be significant predictors for adverse birth outcomes. The study by Noack and colleagues (2005) examining German Caucasian women with middle or high socioeconomic status also did not find any significant differences in periodontal health parameters between women delivering preterm low birth weight infants and control women.

Because our study cohort had excellent oral health and separate oral health parameters have low inflammatory potential alone, the OIBI was constructed to overcome these deficiencies (Study III). This index utilized the Community Periodontal Index (CPI) (Ainamo et al. 1982) and takes into account the presence of gingivitis, potential mucositis, and periodontal disease; it was significantly associated with PTB in this study population. This supports the idea that periodontal disease alone might underestimate the total inflammatory burden from the oral cavity, and, together with probing depth and clinical attachment levels, studies should assess mucosal irritation from calculus deposits and gingival bleeding. In addition to OIBI, antimicrobial drug treatment during pregnancy, low weight gain, and primiparity were the strongest explanatory factors for PTB. These three explanatory factors are known to be risk factors for PTB, thus, this finding was expected. Together with OIBI, we created a data-driven OIS. This oral inflammation score included mathematically combined, independently
collected data on gingivitis, periodontitis, and oral mucositis from this cohort. The objective of this score was to validate CPI’s role as an inflammation burden index. OIS was significantly associated with preterm birth, but when weight gain was added to the model the association became non-significant. This lack of a significant association with PTB may be because of our small study sample.

The presence of periodontal bacteria was not significantly different between PTB and full-term groups in our study. Because the women had a low prevalence of deep periodontal pockets, it is understandable that no differences in the presence of periodontal bacteria between preterm and full-term women existed. However, some previous studies have detected higher levels of periodontal pathogens in PLBW mothers than in full-term and normal birth weight mothers (Offenbacher et al. 1998, Mitchell-Lewis et al. 2001, Hasegawa et al. 2003). The study by Mitchell-Lewis et al. (2001) found significantly higher levels of *Campylobacter rectus* and the study by Hasegawa et al. (2003) significantly higher levels of *Tannerella forsythia* in PTB mothers than in full-term mothers.

When we compared preterm and full-term women concerning dental caries, the prevalence of any initial (enamel) and dentin caries was higher in preterm women (93.5% vs. 85.3%, \( p = 0.06 \)). Agueda et al. (2005) found similar results, reporting a significant association between the presence of untreated caries and PTB in secondary analyses. However, dental caries is not expected to trigger any marked systemic inflammatory reactions.

Previous studies have suggested that in up to 50% of PTBs an infection may be in the background (Lockwood 2002). *Study III* showed that combining the effects of multiple oral infections may exceed the threshold for an effect on birth outcome, even though no evident periodontal disease existed. Increased bacterial load in oral tissues may affect the welfare of the fetus through bacteremia or result in local and systemic inflammatory and immune responses (Offenbacher et al. 1996).

Salivary IgA had a strong relationship with T1DM, an autoimmune disease, but it was not associated with GDM. T1DM has an autoimmune background and GDM can be considered to be mostly BMI-/obesity-related. In addition, GDM and T1DM have different progression, as GDM usually disappears after delivery, while T1DM is a permanent disease. Exaggerated
immune response may have a role in the association between salivary IgA and T1DM because previous studies have reported that humoral and mucosal immune dysregulation is a causative etiology for T1DM (Tenovuo et al. 1986, Ben-Aryeh et al. 1993, Belazi et al. 1998, Iughetti 1999).

Our study is one of the few to have examined the association between oral healthcare patterns and history of miscarriage. Moore et al. (2004) showed a relationship between poor periodontal health and late miscarriage, but no association between maternal periodontal disease in the first trimester of pregnancy and PTB or LBW. Farrell et al. (2006) established a weak relationship between poor periodontal health and late miscarriage in women who never smoked. Our study revealed that urgency-based dental treatment was positively associated with HMC and may be related to the pro-inflammatory response generated by oral infection. Poor oral health in HMC women may also be due to the neglect of health issues in general, including oral health, after miscarriage. Stephenson and Kutteh (2007) have shown that mothers who have had a miscarriage are often depressed, and this may lead to neglect of health issues.
SUMMARY AND CONCLUSIONS

In our cohort of ethnically and socioeconomically homogeneous Finnish women, periodontal health status was uniformly good. No association was found between any specific periodontal parameter and adverse pregnancy outcome. However, the combined effects of multiple oral infections, as expressed in the OIBI, showed a significant association with preterm birth. Data-driven oral inflammation score was also significantly associated with preterm birth, but became non-significant after adjusting for weight gain.

Oral healthcare patterns affected birth outcomes in this cohort. Poor dental care patterns were positively associated with history of miscarriage, while preventive dental care patterns were associated with a lower probability of history of miscarriage.

Salivary IgA was significantly associated with diabetes in pregnancy and macrosomia, but it became non-significant when type 1 diabetes was excluded from the analyses. Thus, sIgA may reflect autoimmunity of type 1 diabetes and the general immunity change in pregnancy.

The results of previous studies concerning oral health and adverse pregnancy outcomes have been inconclusive; while some studies have reported an association, others have found no association at all. Many studies with a positive association have been conducted among African-American women with poor oral health and low socioeconomic status, whereas studies conducted among Caucasian women with good oral health and high socioeconomic status have failed to show a relationship. When the oral health – adverse pregnancy outcome association is examined in a group of women with excellent oral health, the total burden of oral inflammation should be assessed instead of only studying separate oral health parameters.

In conclusion, the combined effects of multiple oral infections may exceed the threshold for an effect on birth outcome, even in women without evident oral health problems. Based on the findings of this study, all women planning a pregnancy and those who are already pregnant should be referred to oral examination and treatment. Pregnant women and those planning pregnancy should be counselled for preventive oral care.
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Aura Heimonen
REFERENCES


Albrecht ED, Pepe GJ. Central integrative role of oestrogen in modulating the communication between the placenta and fetus that results in primate fetal-placental development. Placenta 1999;20:129-139.


Declaration of Helsinki. Recommendations for doctors using human subjects in biomedical research. Adopted by the 18th World Medical Association Assembly in Helsinki, Finland, and amended by the 29th, 35th, 41st, 48th WMA General Assembly and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000.


Friese K. The role of infection in preterm labour. BJOG 2003;110:52-54.


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Salzberg TN, Overstreet BT, Rogers JD, Califano JV, Best AM, Schenkein HA. C-reactive protein levels in patients with aggressive periodontitis. J Periodontol 2006;77:933-939.


APPENDIX I

POTILASTIEDOTE

Tutkimuksen nimi: Äidin suun terveydentilan vaikutus raskauden kulkuun, sikiön hyvinvointiin sekä lisääntymisterveyden häiriöihin.

Viimeaikaisissa tutkimuksissa on todettu yhteys äidin infektion ja lapsen alhaisen syntymäpainon ja ennenaikaisen synnytyksen välillä. Äidin suun infektiot – etenkin hampaiden kiinnityskudossairaudet – voivat olla yksi lisätekijä alhaisen syntymäpainon aiheuttajana.


Tutkimusraportissa ei ole yksilöitääkseen tai potilasasiakirjoista. Tutkimusraportissa ei ole yksilöitävissä tutkimukseen osallistunutta henkilöä. Tutkimuksessa syntyvää, yksittäistä henkilöä koskeva tutkimusmateriaali hävitetään tai arkistoidaan henkilötietolain edellyttämällä tavalla.

Lisätietoja antaa tutkimushoitaja Anneli Sinkkonen, puh. 62560

Tutkija: Hammaslääkäri Aura Heimonen
Helsingin terveysvirasto
Keskinen terveyskeskus
puh. 310 50679
SUOSTUMUS

Tutkimuksen nimi: Äidin suun terveydentilan vaikutus raskauden kulkueen, sikiön hyvinvointiin sekä lisääntymisterveydenhäiriöihin.

Olen saanut suullisesti ja kirjallisesti selvityksen oikeustani, tutkimuksen tarkoituksesta ja siinä käytettävistä menetelmistä.

Suostun minulle tehtävään suun ja hampaiston tutkimukseen ja annan luvan käyttää äitiyskortin ja potilasasiakirjojen tietoja tutkimustarkoituksiin.

Helsinki _____/____ 2002

__________________________________________  ______________________________________
Tutkimushenkilön allekirjoitus ja nimenselvunnys  Suostumuksen vastaanottajan allekirjoitus ja nimenselvunnys

__________________________________________
Henkilötunnus

__________________________________________
Osoite
APPENDIX II

TUTKIMUS: Äidin suun terveydentilan vaikutus raskauden kulkuun, sikiön hyvinvointiin sekä lisääntymisterveydenhäiriöihin

Terveydenhoitajan täytettävä haastattelulomake

HAASTATTELIJAN TIEDOT
1. Haastattelijan nimi____________________________________________________________
2. Sairaalan nimi ja osoite
3. Sairaalan puhelinnumero
4. Haastattelu koskee
   1. tapausta
   2. verrokkia
5. Tutkimusnumero

ÄIDIN TIEDOT
6. Äidin sukunimi
7. Äidin etunimet
8. Äidin tämänhetkinen osoite
9. Äidin kotiosoite
10. Äidin puhelinnumero
11. Äidin kotikunta
12. Äidin henkilötunnus
13. Äidin ammatti
14. Lapsen isän ammatti
15. Lääkkeiden käyttö raskauden aikana
   särky- ja kuumelääkkeet
   antibiootit, sulfat
   lääkkeet gynekologisiin tulehdusiin
   ei kyllä ei tietoa
TIEDOT LAPSESTA

16. Lapsen sukupuoli 1. tyttö 2. poika 3. epäselvä
17. Lapsen tila syntymän jälkeen 1. elävänä syntynyt
2. kuolleena syntynyt
18. Mikä oli lapsen syntymäpaino__________g ei tietoa
    pituus __________cm ei tietoa
19. Montako Apgar pistettä lapsi sai 10 minuutin kuluttua synnytyksestä
    __________pistettä ei tietoa

TIEDOT SYNNYTYKSESTÄ

20. Äidin siviilisääty 1. naimisissa tai avoliitossa
    2. naimaton
    3. eronnut
    4. leski
21. Äidin ammattikoulutus 1. ei ammattikoulutusta
    2. ammattikoulu
    3. työpaikkakoulutus
    4. opistotasoinen koulutus
    5. korkeakoulututkinto
    6. muu, mikä ___________________
22. Kuinka kauan raskaus kesti________vko
23. Montako lasta syntyi
24. Lapsiveden määrä
25. Mikä oli painonne
    a) raskauden alussa __________kg ei tietoa
    b) ennen synnyttystä __________kg ei tietoa
26. Mikä on pituutenne
27. Mikä on Rh-veriryhmänne Rh+ Rh- ei tietoa
28. Kuinka monta kertaa kävitte äitiysneuvolassa raskauden aikana
    1. alle 6 kertaa
    2. yli 6 kertaa
    3. ei tietoa
29. Onko teillä ollut ennen tätä raskautta jokin seuraavista tiloista ja kuinka monta kertaa
    ei kyllä lkm ei tietoa
    keskenmeno
    kohdun ulkoinen raskas
    keskosynnytys
    kuolleena syntynyt
    lapsi
30. Monesko raskaus tämä on (luetaan mukaan kaikki raskaudet, keskenmenot, raskauden-
    keskeytykset, kohdun ulkoiset raskauden ja synnytykset) ja meneillään oleva raskaus
31. Monesko synnytys tämä on (mukaan lukien kaikki aikaisemmat synnytykset)

TIEDOT ÄIDIN SAIRAUKSISTA

32. Oliko teillä raskausajana tai synnytyksen jälkeen jokin seuraavista sairauksista tai oireista
   1. supistelujen vuoksi sairaalahoito
   2. infektiosta raskauden, synnytyksen ja/tai postpartaalivaiheen aikana
   3. laskimotulppa/keuhkoveritulppa raskauden, synnytyksen ja/tai postpartaalivaiheen aikana
   4. istukan tauti: äiti terve ennen 20 rv: hypertensiivinen raskaus, rr yli 140/90 mmhg 20 rv jälkeen
   5. rr nousu (rr yli 140/90 mmhg) ja proteinuria (pre-eklampsia)
   6. diabetes
   7. verenpainetauti
   8. munuaistauti
   9. oligohydramnion, afi alle 5
   10. polyhydramnion, afi yli 25
   11. apgar-pisteet 7 tai alle 7
   12. poikkeava vuoto (alatiesynn. yli 500 ml, sektio yli 1000 ml)
   13. napantuorakomplikaatio
   14. sikiön virheellinen tarjonta ja siitä johtuvat komplikaatiot
   15. kiireellinen/häätä sekto
   16. lapsivesi-infektiio
   17. ennenaikainen synnytys, ennen 37 rv
   18. äiti terve: kasvuhidastuma yksinään
   19. sikiön epämuodostuma
   20. vastasyntyneen infektiio
   21. synnyttysvaurio
   22. sikiön kuolema

TIEDOT JOISTAKIN ÄIDIN ELÄMÄNTAIVOISTA ODOTUSAIKAAN

33. Tupakoitteko
   1. kyllä
   2. ei
   3. aiemmin, olen lopettanut……..vuotta sitten

34. Jos kyllä, niin
   1. päivittäin, noin……..tupakkaa
   2. säänollisesti, mutta ei päivittäin
   3. satunnaisesti
35. Alkoholin käyttö raskauden aikana
1. ei lainkaan
2. harvemmin kuin kerran kuukaudessa
3. harvemmin kuin kerran viikossa
4. harvemmin kuin päivittäin
5. päivittäin

36. Huumeiden käyttö
1. ei lainkaan
2. olen käyttänyt huumeita

TUTKIMUS: Äidin suun terveydentilan vaikutus raskauden kulkuun, sikiön hyvinvointiin sekä lisääntymisterveyden häiriöihin.

HAMMAHSHOITOTOTTUMUKSIA KARTOITTAVA HAASTATTELULOMAKE

1. Koska kävitte viimeksi hammaslääkärissä
   1. alle ½ vuotta sitten
   2. ½-1 vuosi sitten
   3. 1-2 vuotta sitten
   4. yli kaksi vuotta sitten
   5. ei koskaan

2. Miten päädyitte hammaslääkärin hoitoon
   1. hammaslääkäri kutsui minut hoitoon
   2. terveydenhoitaja lähetti hoitoon
   3. otin itse yhteyttä hammashoitolaan
   4. lääkäri lähetti minut hammaslääkärin hoitoon

3. Mikä oli viimeinen hammashoitopaikkanne
   1. yksityishammaslääkäri
   2. terveyskeskushammaslääkäri
   3. työterveyshuollon hammaslääkäri

4. Onko viimeisen hoitosarjan yhteydessä
   1. tarkastettu hampaat
   2. paikattu hampaita
   3. hoidettu ensairauutta
   4. opetettu hampaiden puhdistamista tai muuta ehkäisevää hoitoa
   5. tehty proteettinen työ
   6. muita toimenpiteitä

5. Miten arvioitte hampaittenne kuntoa tällä hetkellä
   1. hyvä
   2. melko hyvä
   3. keskitasoinen
   4. melko huono
   5. huono
   6. en osaa sanoa
6. Kun puhdistat hampaasi, millä sen yleensä teet (voi olla useampia vaihtoehtoja)
   1. tavallisella hammasharjalla
   2. sähköhammasharjalla
   3. hammaslangalla ja/tai hampastikulla
   4. muu, mikä

7. Onko teillä ollut mielestänne odotusaikana tavallista enemmän ienverenvuotoa
   1. kyllä
   2. ei
   3. en osaa sanoa

8. Kuinka usein harjaatte hampaanne
   1. ei koskaan
   2. harvemmin kuin joka toinen päivä
   3. joka toinen päivä
   4. kerran päivässä
   5. useammin kuin kerran päivässä

9. Suun tuntemukset
   Onko teillä esiintynyt suun alueella raskauden aikana
   1. kielen kirvelyä
   2. suun polletta
   3. limakalvokipua
   4. suun kuivumista

TIEDOT JOISTAKIN ÄIDIN ELINTAJOISTA ODOTUSAIKAN

10. Tupakoitteko
    1. kyllä
    2. ei
    3. aiemmin, olen lopettanut……..vuotta sitten

11. Jos kyllä, niin
    1. päivittäin, noin……..tupakkaa
    2. säännöllisesti, mutta ei päivittäin
    3. satunnaisesti

12. Alkoholin käyttö raskauden aikana
    1. ei lainkaan
    2. harvemmin kuin kerran kuukaudessa
    3. harvemmin kuin kerran viikossa
    4. harvemmin kuin päivittäin
    5. päivittäin
**TUTKIMUSLOMAKE**

Tutkimus: Äidin suun terveydentilan vaikutus raskauden kulkuun, sikiön hyvinvointiin sekä lisääntymisterveydenhäiriöihin

Nro________________ Pvm/tutkija___________________________________

<table>
<thead>
<tr>
<th>Potilaan nimi/adreematarra</th>
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</thead>
</table>

1. Aikaisempi hammashoito: säännöllistä ( ) terveyskeskuksessa ( )
   vain tarvittaessa ( ) yksityishammaslääkäri ( )
   hammasteknikko ( )

2. Plakkitutkimukset: pvm________ näytteenottohampaat_______
   - B. forsythus ______
   - P. gingivalis ______
   - P. intermedia ______
   - A. actinomyce._______

3. Status

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<tr>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
</table>

4. Kariesindeksit DMF_______ DMFS_______ DS_______ juurikaries_______

5. Proteesit: yläleuka_______________ (1= kyllä, 2 = ei)
   alaleuka______________________

6. Purentalihakset (1 = ok, 2 = muutos) ________________________________
7. Leukanievet: (1 = ok, 2 = rahina, 3 = naksuminen, 4 = purentahäiriö esim. ristipurenta, saksipurenta, syväpurenta, progenesis)

8. Syliittutkimukset:

   pvm

   leposylien eritys ml/min

   stimuloitu syljeneritys ml/min

   puskurikapasitetti (1 = ≤ 4, 2 = 4.5-5.5, 3 = ≥ 6)

9. Limakalvot: (1 = ok, 2 = muutos, kuten afta, katteinen kieli, oireeton stomatiittti,

   amalgamitauointi, 3 = lichen planus, 4 = proteesin aiheuttama muutos, 5 = muutos, jota varten

   lähetetty biopsiaan)

10. Paron status (taskut/mm, plakki/pinnat, ienverenvuoto/pinnat, ienveräymät):

    | CPI |
    |-----|
    |     |