Department of Clinical Science and Education, Södersjukhuset
Karolinska Institutet, Stockholm, Sweden

PREGNANCIES WITH DECREASED FETAL MOVEMENTS: RISK FACTORS AND STRATEGIES FOR MITIGATION OF POOR NEONATAL OUTCOMES

Irene Sterpu

Stockholm 2020

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB,2020

ISBN 978-91-7831-940-4

PREGNANCIES WITH DECREASED FETAL MOVEMENTS: RISK FACTORS AND STRATEGIES FOR MITIGATION OF POOR NEONATAL OUTCOMES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

To be publicly defended in Aulan at Södersjukhuset, Stockholm Friday 11th of December 2020 at 9.00 am

By

Irene Sterpu

Principal Supervisor:
Associate professor Eva Wiberg-Itzel
Karolinska Institutet
Department of Clinical Science and Education
Division of Obstetrics and Gynecology

Co-supervisor(s):
Professor Helena Åkerud
Uppsala University
Department of Immunology, Genetics and Pathology
Division of Obstetrics and Gynecology

Professor Kristina Gemzell Danielsson
Karolinska Institutet
Department of Women's and Children's Health
Division of Neonatology, Obstetrics and Gynecology

Associate professor Ina Schuppe Koistinen
Karolinska Institutet
Department of Microbiology, Tumor and Cell Biology
Center for Translational Microbiome Research

Opponent:
Austin Ugwumadu
St George´s University of London
Department of St George Hospital
Division of Obstetrics and Gynecology

Examination Board:
Bo Jacobsson
University of Gothenburg
Department Institute of Clinical Science, Sahlgrenska Academy
Division of Obstetrics and Gynecology

Josefine Nasiell
Karolinska Institutet
Department of Clinical Science Danderyd Hospital
Division of Obstetrics and Gynecology

Baldvin Jonsson
Karolinska Institutet
Department of Women´s and Children´s Health
Division of Neonatology, Obstetrics and Gynecology

“Dream Believe Dare Do”

-Walt Disney

To my family, for always encouraging me to dream

ABSTRACT

Background and aims

The most feared complication of pregnancy is stillbirth. Globally there are 2.6 million stillbirths annually with more than 7000 deaths a day. Pregnancies with reduced fetal movements have a higher risk of stillbirth and growth restriction. In this thesis, we aimed to identify risk factors that are associated with poor neonatal outcome in the group of women with reduced fetal movements; to identify preventable stillbirths through an audit process; to investigate the intrauterine milieu and the existence of a placental microbiome in full-term pregnancies; to investigate if ultrasound and angiogenic markers can be used as predictors of the neonatal outcome in pregnancies with reduced fetal movement.

Methods

Study I was a retrospective cohort study were all women with pregnancies who attended health care for decreased fetal movements at Soder Hospital were included. A composite neonatal outcome was constructed and the risk factors for poor neonatal outcome were analyzed for this group.

Study II was a retrospective cohort study conducted as an audit by a multidisciplinary team. All stillbirths in Stockholm 2017 were included and the intention was to investigate the preventable deaths and standard of care.

Study III investigated the potential presence of a placental microbiome in full-term pregnancies in pregnancies with pre-labor cesarean deliveries and in vaginal deliveries.

Study IV was a pilot study in which it was investigated if the cerebroplacental ratio, the flow in the uterine artery and angiogenic factors could be used as predictors of poor neonatal outcome.

Results

There was an increased risk of having an Apgar ≤7 at 5' (RR 1.56, 95% CI 1.25-1.96), pH ≤ 7.10 (RR 1.34 CI 95% 1.12-1.61) and stillbirth (RR 5.53, CI 95% 2.81-10.85) in the RFM group compared with pregnancies without RFM.

30% of the stillbirth analyzed by the audit were assessed as preventable/possible preventable. The non-Swedish speaking women were overrepresented in this group.

When adding a Doppler examination to standard care for RFM there was a significant increase in the obstetrical intervention rate without improvement of the neonatal outcome. The predictive model for composite neonatal outcome based on additional Doppler angiogenic factors and parity had an AUC of 0.89 (95% CI 0.81–0.97).

Conclusion

The highest risk of having a poor neonatal outcome were the small for gestational babies (SGA) and the IVF pregnancies in the group of pregnancies with RFM. The audit process of stillbirth identified preventable deaths, delays in the health care system and cases with substandard care. This leads us to the conclusion that national audits can further improve the care for these patients and can possibly help us to reduce the rate of stillbirths. Non-Swedish women have a higher risk of stillbirth and those not speaking the language need individualized antenatal care. The angiogenic factors can be useful predictors of the neonatal outcome, but larger studies are needed. There is no evidence for a placental microbiome in human pregnancies at term.

LIST OF SCIENTIFIC PAPERS

I. **Risk factors for poor neonatal outcome in pregnancies with decreased fetal movements**

Sterpu I, Pilo C, Schuppe Koistinen I, Lindqvist PG, Gemzell-Danielsson K, Wiberg-Itzel E
Acta Obstet Gynecol Scand, 2020 Aug 99(8):1014-1021.

II. **Could a multidisciplinary regional audit identify avoidable factors and delays that contribute to stillbirth? A retrospective cohort study**

Sterpu I, Bolk J, Perers Öberg S, Hulthén Varli I, Wiberg-Itzel E
Manuscript accepted for publication by BMC Pregnancy and Childbirth

III. **No evidence for a placental microbiome in human pregnancies at term**

Sterpu I, Fransson E, W Hugerth L, Du J, Pereira M, Cheng L, Radu SA, Calderón-Pérez L, Zha Y, Angelidou P, Pennhag A, Boulund F, Scheynius A, Engstrand L,Wiberg-Itzell E, Schuppe-Koistinen I

American Journal of Obstet and Gynecol, 2020 Aug, epub ahead of print
https://doi.org/10.1016/j.ajog.2020.08.103

IV. **Predictive factors in pregnancies with reduced fetal movements-a pilot study**

Sterpu I, Pilo C, Lindqvist PG, Åkerud H., Wiberg-Itzel E
Manuscript submitted for publication

CONTENTS

List of abbreviations

AUC	Area Under Curve
ANC	Ante Natal Care
Ang	Angiopoietin
BMI	Body Mass Index
CI	Confidence Interval
CPR	Cerebroplacental Ratio
CTG	Cardiotocography
HIF	Hypoxia-Inducible Factors
IUGR	Intrauterine Growth Restriction
IUFD	Intrauterine Fetal Death
IVF	In Vitro Fertilization
LGA	Large for Gestational Age
MCA	Middle Cerebral Artery
MoM	Multiples of Median
OR	Odds Ratio
PlGF	Placental Growth Factor
RFM	Reduced Fetal Movements
ROC	Receiver Operating Characteristic
sEng	Soluble Endoglin
sFlt-1	Soluble Fms-like Tyrosine Kinase 1
SD	Standard Deviation
SGA	Small for Gestational Age
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

1 INTRODUCTION

According to WHO: “Stillbirths remain a neglected issue, invisible in policies and programs, underfinanced and in urgent need of attention and it is absent from the Millennium Development Goals and still missing in the Sustainable Development Goals”. Stillbirths take a large emotional and economic toll on individuals, families, medical personnel, and societies in its entirety.

In Sweden, even if the number of stillbirths is low compared to other countries, the stillbirth rate has been almost unchanged for the past two decades. The Lancet has recently run a series of articles on “stillbirths: ending preventable deaths” and suggested the following actions for high income countries as possible ways to reduce stillbirths: improvement in data collection, a better classification of the causes of death and improvement in standard care. The same studies concluded that the implementation of national perinatal mortality audits can be a way of reducing stillbirths’ rate (1-5).

It has been extensively discussed that pregnancies with reduced fetal movements have an increased risk of intrauterine growth restrictions and stillbirths. At the same time, we lack evidence that standard care which includes cardiotocography and ultrasound assessment of the amniotic fluid can identify the pregnancies with the highest risk of poor neonatal outcome.

The desired outcome for all pregnancies is a healthy mother and a healthy newborn. To achieve that it is crucial to be able to distinguish between risk pregnancies that need intensified monitoring and obstetric interventions and the normal pregnancies.

2 BACKGROUND

2.1 FETAL MOVEMENTS

2.1.1 Historical perspective

The first written description of fetal movements is found in the bible, where Rebecca said that the "children struggled within her" (Genesis 25:22). The interest and the fascination for what happens in the womb have been great during history. Hippocrates (460-370bc) suspected that fetal movements start at about 70-90 days after the conception. Leonardo da Vinci sketched the famous fetus in the womb.

A German obstetrician, Johann Ahlfeld, realized in 1869 that the maternal perception of fetal movements can be an indicator of fetal well-being (6). The Fells Institute conducted the first non-invasive studies of fetal behavior. The fetal heart rate was registered by stethoscope and the fetal movements were recorded for 2 hours a week during the whole pregnancy. Sontag and Wallance noted that fetal movements differ among fetuses, that maternal emotional stress and pre-eclampsia increases fetal movements and that fetuses reacted after vibratory stimulus was applied to the maternal abdomen (7).

The first ultrasound studies of fetal movements were reported by Reinold in 1971. Since then other methods of assessing fetal movements and fetal behavior have been tested: intrauterine fetal electroencephalogram (Lindsey 1942), fetal magnetoencephalography (Blum 1985), magnetic resonance imaging (Prayer 2006), functional magnetic resonance imaging (Gowland and Fulford 2004).

2.1.2 Why does the fetus move in the uterus?

During time three different theories on why the fetus moves have emerged and they are summarized by Christa Einspieler in her book "Fetal Behavior: a neurodevelopmental approach":

- *The epiphenomenal concept*: fetal movements represent an incidental epiphenomenon and have no adaptive significance.
- *The preparatory hypothesis*: the fetus moves to gain practice and experience needed for further development of motor coordination postnatally.

• *The functional hypothesis*: fetal behavior is functional and adaptive during the prenatal period. Fetal movements are necessary for normal muscular, skeletal, and neurological systems development.

Nowadays there is increasing evidence that the functional hypothesis is complementary to the preparatory theory and that they together best explain the function of embryonic and fetal movements. This hypothesis is sustained by studies by Pena and Shokeir that showed that the distribution of neurotransmitter receptors on the muscle fibers develop abnormally if the fetal movements are silenced pharmacologically or by disease (8).

2.1.3 Reduced fetal movements (RFM)

Previous studies have shown that decreased fetal movements are associated with pregnancy complications such as preterm birth, fetal growth restriction and stillbirth (9, 10).

Both medical professionals and mothers use monitoring of fetal movements as a reassurance of fetal well-being during pregnancy. Fetal movements differ during the pregnancy with a increase from week 16-20 to week 36, and a slight decline throughout the last month of pregnancy (11). The movements vary during normal pregnancies depending on the quantity of amniotic fluid, the position of the fetus, maternal medication and the wellbeing of the fetus (12).

When sonographically assessed, the fetal movements are classified into four groups: movements of the fetal trunk (hiccup, rotation, breathing), limbs, face, and head. The maternal perception of fetal movements can be different compared to the fetal movements visualized by the ultrasound. In one study, mothers perceived just 33-88% of the fetal movements seen om the ultrasound (13).

RFM are self-reported by the mothers, with guidance from midwifes and obstetricians. There is no gold standard method for counting and reporting RFM.

The maternal perception of fetal movements can be affected by stress and anxiety (14), medication and smoking (15), localization of placenta (16), maternal position (17), and the perception changes throughout the pregnancy.

It is crucial that the health care providers inform the pregnant woman about the importance for her to perceive and assess fetal movements. At the same time, we know that instructing women to monitor fetal movements can be associated with increased maternal anxiety.

Counting fetal movements can be a difficult task for the mothers, as there is no consensus in which method is the most accurate. There are two main methods that are commonly used- 1) "fixed time" and 2) "fixed number". The first one refers to fetal movements which was initially counted during 12 hours, but later modified to shorter and repeated periods of counting (18). The second method refers to measuring the time in which 10 fetal movements have been perceived and has been shown to have a higher compliance rate (19). However, a systematic review reported that the evidence was weak for fetal movement counting as a marker for fetal wellbeing (20).
The current guidelines from Australia, New Zealand and the Royal College of Obstetrics and Gynecology emphasize that it is the mother's perception of reduced fetal movements that is important.

Newer approaches, such as mindfetalness (focusing on the fetus movements 15 minutes a day with the women lying on the left side), that are trying to increase the women's awareness on fetal movements, have been evaluated in a cluster-randomized controlled study. And while the number of visits to healthcare due to RFM increased in the intervention group, there was no reduction in the number of newborns with low Apgar score. Surprisingly there was a decreased incidence in cesarean sections and small for gestational age (SGA) babies (21).

2.1.4 Management of reduced fetal movements (RFM)

The purpose with antenatal care is to identify risk pregnancies and to prevent, if possible, adverse maternal and fetal outcomes but to avoid over medicalization. As obstetricians, we try to intervene as little as possible, because most of the pregnancies with RFM are normal, but among them are also risk pregnancies.

There is no national consensus on the management of women with RFM, and these women are examined or the delivery induced according to local guidelines. The number of pregnant women seeking health care for decreased fetal movements has been increasing over the past years (at Soder Hospital there are around 2000 visits/year).

A Cochrane review from 2012 highlighted that there are no randomized control studies about the management of pregnancies with RFM and there is not enough evidence to decide which management would be the most appropriate (22). Data from Norway suggests that besides CTG which is used to assess the fetal well-being, ultrasound plays an important role by detecting

abnormalities in 11,6% of the cases with RFM and in most cases with ultrasound findings the abnormalities were important for the further management of the pregnancies (23).

A prospective cohort study on women undergoing elective cesarean section reported that fetuses with absence of movements on ultrasound prior to the delivery had lower pH, base excess (BE), O_2 and a higher CO_2 in the umbilical cord than women with active fetuses (24).

An international survey on 1714 women experiencing stillbirth after 28 weeks of gestation reported that 30,5 % of the women felt significantly less fetal movement and 8,5 % significantly more movements while 28% felt no change in fetal movements before the stillbirth (25).

The Lancet's stillbirth series identified decreased fetal movements as one, among many others, of the key topics in trying to prevent the stillbirths (2).

2.2 STILLBIRTH

Despite efforts, the incidence of stillbirths is still high globally with an estimated number of 2,6 million stillbirths occurring every year. Half of them occur during the pregnancy.

According to statistics from the World Health Organization (2009), on the rate of stillbirth per country Sweden is ranking 12th out of the high-income countries, having the highest rate of stillbirth among the Scandinavian countries. The incidence of stillbirths in Sweden has shown only minor variations during the past 20 years with 400-450 stillbirths/year (26) (Figure 1).

However, the demography of Swedish population at reproductive age has changed over the years with an increase in the percentage of women with high BMI and older nulliparous (27). At the same time Sweden has higher rate of women born outside of Europe per capita compared with its Scandinavian neighbors (28).

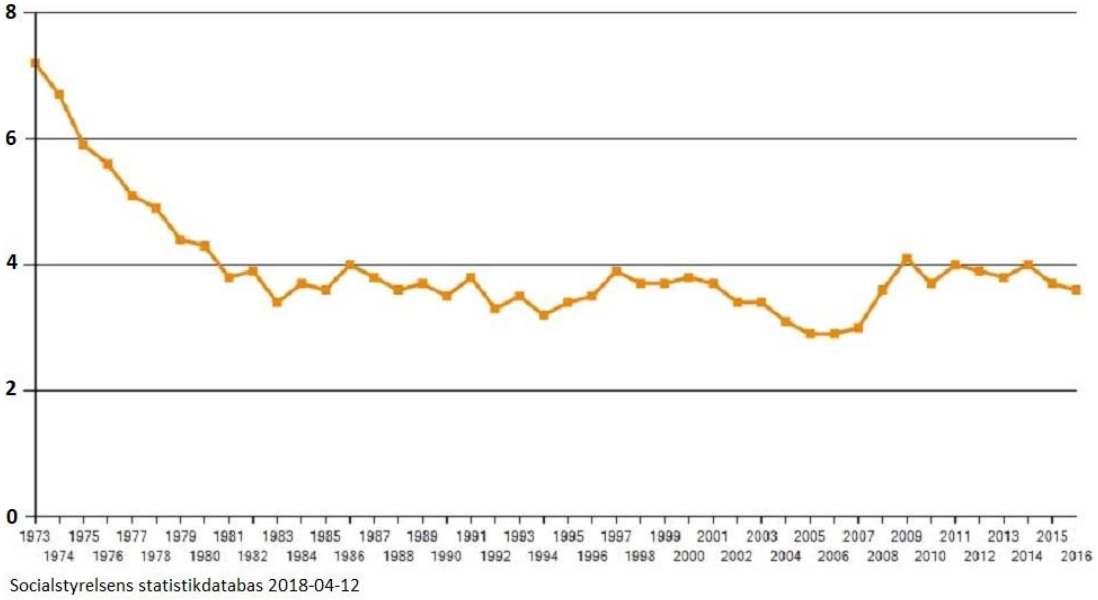


Figure1. Stillbirths/1000 live births

In Norway, in an attempt to lower the stillbirths' rates, the health care system did an intervention aiming at standardizing the information given to women with RFM and also to standardize the guidelines for management of RFM for obstetricians and midwives. Their recommended management of RFM included a non-stress test, an ultrasound to quantify fetal movements, measurement of the amniotic fluid volume and the fetal growth and anatomy, and the recommendation that women with RFM should be examined within 2 hours if no fetal movements were recorded, otherwise within 12 hours. Interestingly the reports of RFM, preterm births, fetal growth restriction and transfers to neonatal care did not increase during the intervention. The stillbirth rate decreased both in women with RFM and in the overall study population from 4.2% to 2.4% and respectively 3.0/1000 vs 2.0/1000 (23).

The AFFIRM study in the United Kingdom tried a similar approach by trying to increase the women's awareness on reduced fetal movements together with standardized medical management of the pregnancy. This was done in a large randomized stepped wedge cluster in 37 hospitals in UK and more than 400 000 pregnancies were included. The results of the study showed a non-significant trend towards fewer stillbirths in the intervention group and a statistically significant increase in the rates of cesarean sections and induction of labor (29).

Admission CTG has been shown to effectively reduce intrapartum stillbirth, both in low and high risk pregnancies. As compared to admission CTG, an in-house hospital care unit had 18-fold higher risk of stillbirth (0.09% vs 0.995%, OR 18.5 95% CI 3-111) (30).

2.2.1 Classification systems of stillbirths

One of the problems in reducing the number of stillbirths is the diversity of classification systems for cause of death in stillbirths, where more than 35 systems are used (31). Some of the classification systems focus on the fetal causes of death (32) while others focus on the maternal ones or on both (33, 34). At the same time, the classification systems used in high income countries require extensive diagnostics which are not available in a low-income setting.

The plethora of cause of death classification systems for stillbirth and the use of different definitions can make it difficult to compare stillbirths' rate between different countries.

2.2.2 The importance of audits

A perinatal audit is "systematic, critical analysis of the quality of perinatal care, including the procedures used for diagnosis and treatment, the use of resources and the resultant outcome and quality of life for women and their babies" (35).

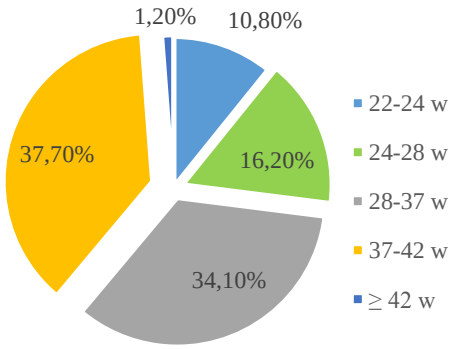
The stillbirths audit can be a tool to improve the obstetrical care, to identify preventable deaths and to evaluate the quality of antenatal and intrapartum care. Implementation of national perinatal mortality audits have been shown to reduce the perinatal mortality and identify elements of substandard care (36-38). The impact of an audit is dependent on the ability of implementing the recommendations from its conclusions.

With 75% of the stillbirths occurring in south Asia and sub-Saharan Africa, stillbirth is still a marker of inequity in the world.

2.2.3 What can we do to decrease the rate of stillbirth?

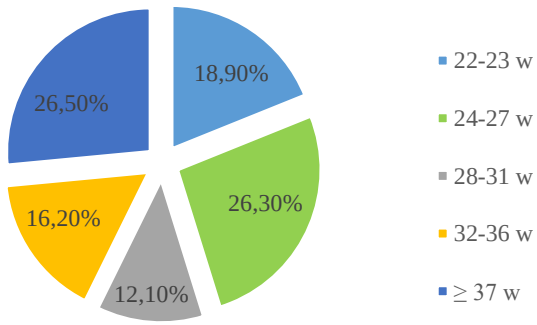
In high income countries like USA, Nya Zeeland and most of the European countries, 26% of all stillbirths occur in term pregnancies (fig.2a and b) where there is the possibility of obstetric interventions such as induction of labor (39-42). The solution to this problem is not so simple. In 2011, Denmark implemented a more proactive protocol with earlier induction of labor at 41+3 weeks of gestation compared to the previous routine of 42+0 weeks This change of praxis resulted in no differences in neonatal outcomes such as: stillbirths, neonatal death or low Apgar scores (43). It is possible that by moving the induction even earlier the results would be better. However, the induction rate would increase tremendously and the induction per se is associated with potential adverse outcomes (44).

Fig. 2a Distribution of stillbirth by gestational age Stockholm 2001-2015



Björk I, Pettersson K, Lindqvist PG. Stillbirth and factor V Leiden - A regional based prospective evaluation. Thromb Res. 2019

Fig.2b Distribution of neonatal deaths by gestational age Europeristat 2015



What seems to be important is to be able to identify the risk pregnancies and reserve the obstetrical interventions for these pregnancies.

The SGA babies are overrepresented in the stillbirth group compared to liveborn (45, 46). According to the Swedish National Board of Health and Welfare's "Stillbirths Report", in Sweden, there are up to 40% of SGA among stillbirths occurring < 37 weeks of gestation and 11% in the group ≥37 weeks of gestation (47). So, identifying the SGA babies should be a priority. However, previous studies, have shown that an ultrasound in the third trimester in low risk population has not improved the neonatal outcome (48, 49), but in a selected risk population it can be helpful.

A large South Swedish study performing routine 32-week ultrasound with a predetermined surveillance plan showed 4-fold lower risk of stillbirth/newborn death among identified SGA cases (50). The latest Cochrane assessment of late ultrasound showed 20-fold lower risk of stillbirth among those scanned (51).

2.3 PLACENTA

2.3.1 Role and importance

The placenta is a fascinating organ that ensures the normal development and growth of the fetus. It has multiple functions that are important for the normal development of the pregnancy such as: gas exchange, nutrients and waste transfer, secretion of hormones and fetal protection from infections by transfer of immunoglobulins (52).

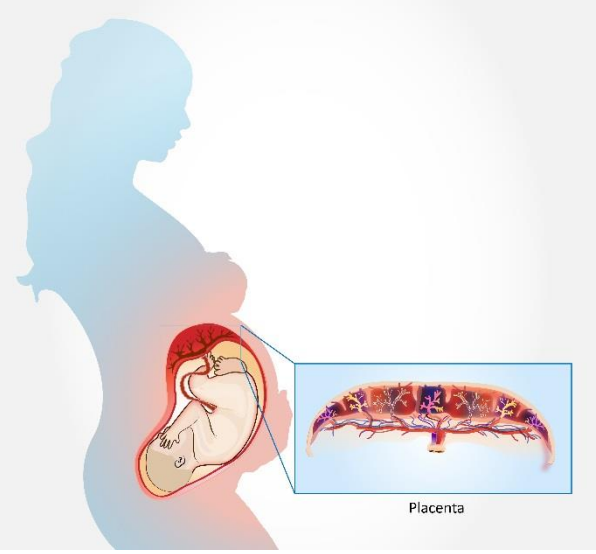


Fig.3 Placenta by Fuad Bahram

An inadequate functioning placenta can be associated with pregnancy and neonatal complications such as: SGA, preeclampsia, and stillbirth. Therefore, pathological examination of the placenta is of high importance especially in women with stillbirths.

Studies have shown that there are morphological changes in the placentas from pregnancies with RFM and adverse outcome compared with those without RFM. These placentas were smaller, less vascularized, had an abnormal endocrine function (53, 54) or had bigger area with signs of infarction, decreased villous vascularity and decreased trophoblast area compared with the controls (55).

2.4 ULTRASOUND

The first sonar studies of fetal movements were reported by Emil Reinold, an obstetrician from Vienna in 1971. He found that the first movements appear at a gestation of 9 weeks. Later studies with transvaginal probe showed that the earliest body movements occur at 7 weeks and 2 days (56).

Since then a lot of other parameters have been added to the ultrasound examination to assess the normal development and growth of the fetus. We know that a deterioration in the placental function will generate compensatory hemodynamic changes in the fetus with redistribution of the blood flow to the brain ("brain-sparing" effect) and other essential organs. This redistribution of the blood flow to the brain has been noticed for example in SGA fetuses and is associated with both perinatal and long-term neurodevelopmental adverse outcomes (57, 58). To assess these changes, it has been suggested the use of cerebroplacental ratio (CPR) as the ratio between the middle cerebral artery pulsatility index (MCA-PI) and the umbilical artery pulsatility index (UA-PI).

The uterine Doppler is a non-invasive way of assessing the maternal compartment of the placental function. One of studies regarding the use of uterine artery Doppler in the third trimester showed that it is at least as good predictor for the placental insufficiency as the uterine artery Doppler in the first trimester (59). This suggests that the uterine Doppler assessment has its place even later in the pregnancy.

Furthermore, studies from Prior et al showed that independent of fetal size the cerebroplacental ratio measured within 72 hours before a delivery could identify the fetuses needing obstetric interventions for intrapartum compromise (60). Consistent with these results, the study by Moralles-Rosello et al indicated that assessing the CPR- could be a feasible method of detecting placental insufficiency even in appropriate for gestation fetuses not reaching their potential (61). A large retrospective study with over 9000 pregnancies included showed that a low CPR within 2 weeks of delivery regardless of the fetal size was associated with intrapartum fetal compromise and admission to the neonatal unit (62). Another retrospective cohort study with 2812 patients shown that CPR evaluated in the third trimester is an independent predictor of stillbirth and perinatal mortality in a mixed risk population (63).

Triunfo et al reported that the Doppler evaluation of fetal vessels and maternal uterine artery at 37weeks in a group of low-risk pregnancies improved the prediction of adverse perinatal

outcome but not the prediction of small for gestation and fetal growth restriction (64). In contrast with these results a prospective cohort study by Rial-Castelo et al with 1030 low-risk pregnancies that performed fetal biometry and Doppler ultrasound examinations at 32-34 weeks did not find any improved predictive value of CPR and uterine Doppler over standard screening practice for impaired fetal growth (65). Notable is that the study site in Barcelona has a routine third trimester ultrasound screening.

A systematic review analyzing the performance of CPR found that 6 of 11 articles reported a significant association between low CPR and a lower mean birthweight of the fetus (66).

An Australian prospective observational study of low risk women at term showed that uterine artery PI is higher than 95th centile and the CPR is lower than 10th centile in pregnancies delivered with cesarean section for intrapartum fetal compromise and those with composite neonatal morbidity (67).

In a study by Khalil et al a CPR < 5th centile alone was significantly associated with an increased risk of perinatal mortality. However, this study did not find CPR MoM (multiples of median) to be a significant independent predictor of adverse neonatal outcome (68). These findings were in accordance with earlier studies by Nicolaides et al that showed that CPR alone at 35-37 weeks of gestation is a poor predictor of adverse perinatal outcome (69).

A recent meta-analysis by Heidweiller-Schreurs et al, including 128 studies involving 47748 women explored the prognostic accuracy of CPR in detecting adverse perinatal outcome. They summarized that CPR could add value to the uterine artery PI in predicting adverse perinatal outcome in singleton pregnancies. However, it is still unclear if this applies for all pregnancies (70).

A study by Bligh et al tried to combine the cerebroplacental ratio and the blood test for PlGF (placental growth factor) and assess their screening performance for the detection of cesarean section for intrapartum fetal compromise and composite neonatal outcome. The study was conducted in a low risk population and women were enrolled from 36 weeks of gestation. There was no significant difference between the combined model with CPR and PlGF and its individual components (71).

2.5 ANGIOGENIC AND ANTIANGIOGENIC FACTORS

2.5.1 Definition

Angiogenesis, which is the formation of new blood vessels is a multifactorial and very complex process. This process can be affected in different situations like cancer, inflammatory diseases, hypoxia.

Angiogenesis allows hematopoietic cells to assure immune surveillance, dispose of waste, supply oxygen and nutrients to the cells. The process of angiogenesis is regulated by promoters and inhibitors and is very important in many biological processes as for example: reproduction and wound repair.

The most important regulator of angiogenesis is the vascular endothelial growth factor (VEGF) family. VEGF-A is essential for both vasculogenesis and angiogenesis. Homozygotes and even heterozygotes for the VEGF-A gene in mice die in the embryonic stage.

The endothelial cells have oxygen sensors for hypoxia-inducible factors(HIF) in order to be able to adjust their shape and blood flow when needed. Under hypoxia, HIF factors are activated and they generate an upregulation of angiogenic factors such as VEGF. When activated the VEGF exerts its biologic effect through interaction with cell surface receptors. VEGF is essential for maintaining vascular homeostasis and exerts its effect through two high-affinity receptor tyrosine kinases: VEGFR-1 (or sFlt-1) and VEGFR-2 (or KDR/Flk-1).

Several growths factors as: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and angiopoietin are needed for the blood vessels to function properly. Angiopoietin 1 (Ang 1) stimulates vessel maturation and promotes endothelial cell survival while angiopoietin 2 (Ang 2) antagonizes Ang 1 in vivo and acts by enhancing the decay of blood vessels.

Endoglin expression is also upregulated in hypoxic conditions. Endoglin which is a TGF-β super family coreceptor cooperates with VEGF to promote angiogenesis. Soluble endoglin (sEng) which is a soluble form of a receptor of TGF- β inhibits angiogenesis. Soluble fms-like tyrosine kinase 1 (sFlt-1) is another circulating receptor for VEGF and PlGF that suppresses angiogenesis by binding these proteins and preventing them from coming in contact with their receptors in the endothelial cells in the placenta (72-74).

2.5.2 Clinical use

Vasculogenesis and angiogenesis are very important for the normal evolution of the pregnancy. Furthermore, the balance between the angiogenetic and anti-angiogenetic factors is necessary for reproduction (75, 76).

Abnormalities in the maternofetal circulation, intrauterine growth restriction (IUGR), preeclampsia and other maternal diseases can affect the oxygen supply both before and during the delivery (77). Lately the use of angiogenic factors in obstetrics has increased and they are used as predictors in clinical praxis for example in preeclampsia.

The imbalance of these circulating factors has been noticed in preeclampsia, IUGR and in gestational hypertension. Nanjo et al showed even that the levels of circulating angiogenic and anti-angiogenic factors prior to delivery correlate to the severity of hypertensive disorders and to IUGR (78).

Studies in mice shown that HIF increases both in placenta and in the developing brain at gestational day 20 in cases of acute antepartum hypoxia (79). High levels of both HIF 1&2 (Hypoxia-inducible factors) were noticed in human placenta in conditions of chronic hypoxia as preeclampsia, IUGR and gestation at high altitude.

Research in the area has shown that a lower maternal plasma placental growth factor (PlGF), a higher soluble endoglin (sEng) and soluble VEGF receptor-1 (sVEGFR-1) were registered in pregnancies with subsequently stillbirths compared with normal pregnancies (80).

Recent data published by Chaiworapongsa et al have found that by testing PlGF, PlGF/sVEGFR-1, and PlGF/sEng in the maternal blood at 24-28 weeks of gestation it is possible to predict fetal death. According to that study, when the ratio calculated with the plasma concentrations of the angiogenic and antiangiogenic factors < 2.5th centile there was a 29-fold increase of stillbirth with a false positive rate of 3,5% (81). The unexplained stillbirths can be seen as an anti-angiogenic state where elevation in plasma sVEGFR-1 concentration was observed (82).

2.6 MICROBIOME

2.6.1 Definition

There are hundreds of trillions of microbes existing in symbiosis within the human body and they have a profound impact on modulating host function. The largest number of those microbes, containing bacteria, fungi, viruses, and protozoans reside in the gastrointestinal tract and have been shown to influence normal physiology across all body systems. During the past years, the concept of microbiome-gut-brain axis has emerged and the bidirectional communication between the central nerve system (CNS) and the gut has been investigated in numerous studies.

2.6.2 Importance

The microbiome influences the turnover of neurotransmitters in the CNS, stress reactivity, anxiety like behavior, the memory function (through brain derived neurotrophic factor), and modulation of the serotoninergic system (83). The gut microbiota's composition and biomass are affected by psychological stressors. The brain has an important role in modulating all the intestinal functions and can influence the normal habitat of the gut microbiota. Interestingly, it has been shown that the alternation in intestinal function induced by stress facilitates the expression of virulent bacteria (84).

New research shows the influence of gut microbiome on anxiety and depression (85, 86) and even on autism (87, 88). Animal studies have shown that the gut microbiome is very important for both the development and the maturation of CNS (89, 90).

Advanced culture-independent methods are revolutionising our insights into the microbial presence in the human body. Although the microbiome has been described as the first-line defence against colonisation by opportunistic pathogens, (91) it is still unclear how important the microbiome is for human health. The term *dysbiosis* is used to indicate an abnormal or unhealthy microbiota composition, yet is still poorly defined. Pregnancy is of particular importance since it is the crucial period to establish the microbiome in new-borns, with many important physiological changes in the expecting mother. Most microbiome research has currently focused on the (lower) gastrointestinal tract. However, there is increasing evidence that the microbiome (gut, vagina and other locations) plays an important role during pregnancy,

for example in pre-term birth but potentially also maternal complications such as pre-eclampsia, gestational diabetes and excessive weight gain (92).

The often asymptomatic vaginal *dysbiosis* (also called bacterial vaginosis) has been associated with an increased risk of sexually transmitted diseases, reduced fertility and potentially also with preterm labor and preterm delivery (93). The maternal microbiome is also seen as a major contributor to the microbiome in the infant through the birth process, and there is increasing evidence that this process starts even before birth (92, 94).

2.6.3 Is the womb sterile during pregnancy?

For the last century, it has been assumed that the intrauterine environment is sterile.

The microbial colonization starts early in life and it is very important for the physiological development of the human fetus. Researchers are still debating if the colonization starts during and after birth (*the sterile womb paradigm*) or already intrauterine (*in utero colonization hypothesis*).

The sterile womb paradigm is sustained by numerous studies. A study conducted by Harris and Brown showed no cultivable bacteria in the amniotic fluid of women delivered with C-section, when the duration of labor was less than 6 hours (95).

Fig. 4 By Ina Schuppe Koistinen
www.inasakvareller.se/

Further studies showed that in normal healthy pregnancies there are no bacteria in the amniotic fluid, placenta or in the meconium (96-100) and that all bacteria identified were from contaminations. Other studies using molecular techniques and next generation sequencing have suggested that the uterus and placenta have their own microbiome that contributes to the colonization of the fetus (101-103).

Perez-Munoz et al. reviewed in 2017 the published articles in the field and concluded that the assumptions of the sterile womb paradigm are still valid and most of the studies had positive finding due to contamination, flawed interpretation of findings and that the bacterial species identified by molecular techniques could not be verified by bacterial cultures (104). Since then more studies have been showing that there are no signs of a placental microbiome in uncomplicated pregnancies at term (100).

A study by Parnell et al from 2017 challenged this hypothesis by showing that the placenta harbors its unique microbiome and moreover that the microbiota has distinct profiles depending on the localization within the placenta and that the profiles are not altered by the mode of delivery (105). Furthermore, a recent study from Finland by Tapiainen et al has concluded that the microbiome of the first-pass meconium was not affected by perinatal factors, but it was affected by factors during pregnancy. This suggests that there is a transfer of microbes in utero and that the development of the gut microbiome starts in fetal life (106).

Presence of bacteria in the amniotic fluid or in the placenta have until now been associated with complications of pregnancy as spontaneous abortions, preterm labor (107), small for gestational age (108), neonatal sepsis (109), postpartum infections and stillbirth.

3 AIMS OF THE THESIS

The overall aim of this thesis was to identify risk factors for poor neonatal outcome and predictors that could optimize the neonatal outcomes in pregnancies with decreased fetal movements.

Specific aims:

Study I: To describe neonatal outcomes in pregnancies with reduced fetal movements and to identify risk factors for poor neonatal outcome in this cohort.

Study II: To investigate if a multidisciplinary audit could identify avoidable factors and delays that contribute to stillbirths.

Study III: To evaluate the potential existence of a microbiome in the placenta and the amniotic cavity in term pregnancies.

Study IV: To investigate if Doppler indices and angiogenic factors can be used as predictors of poor neonatal outcome in pregnancies with reduced fetal movements.

4 MATERIAL AND METHODS

A more detailed description of the methods is provided in the articles and manuscripts at the end of the thesis. All statistical analyses were performed in SPSS software version 25 (IBM Corp.). A brief overview of the papers and study design is provided in table 1.

Table 1. Brief overview of study designs

Study	**Study I**	**Study II**	**Study III**	**Study IV**
Type of study	Retrospective cohort study	Retrospective cohort study, audit	Cross-sectional study	Prospective cohort study
Setting	Soder Hospital	Stockholm	Soder Hospital	Soder Hospital
Study Population	All women with singleton pregnancies who attended health care at Soder Hospital for RFM between January 2016 and December 2017	All women delivering a stillbirth ≥22 weeks of gestation in Stockholm during 2017	Full-term pregnancies with elective cesarean section or vaginal deliveries during March to October 2017	Women with singleton pregnancies, of more than 34 weeks of gestation who attended health care for RFM between Maj 2016 and December 2017
Participants	3243	79	76	128
Exposures	RFM	Stillbirth	Elective cesarean section/vaginal deliveries	RFM

4.1 STUDY I

Study I is a retrospective cohort study on all pregnancies with reduced fetal movements at Soder Hospital between January 2016 and December 2017.

Inclusion criteria were singleton pregnancy, ≥ 22 weeks of gestation, who attended obstetrical care at Soder Hospital due to reduced fetal movements.

The primary outcome was:
A composite neonatal outcome where one or more of the following criteria were met: arterial pH in umbilical cord ≤7.1, 5 minutes Apgar ≤7, transfer to neonatal ward and stillbirth.
To compare the group with and without RFM, Chi-square and Mann-Whitney *U* test were used. A multivariable model was built with the risk factors that were significant in the univariable analysis.
The demographics of the women and new-born included in the study were presented as frequencies with percentages and medians with min-max values.
The Chi-square automatic interaction detection analysis was used to identify the risk factors associated with composite neonatal outcome in the group with RFM.

4.2 STUDY II

Study II is a retrospective cohort study were all stillbirths in Stockholm 2017 were included. An audit team of three obstetricians, one midwife and one neonatologist was assembled. All members of the team assessed all the stillbirth cases independently and then each case was discussed during the meetings.

Inclusion criteria: all stillbirth in Stockholm. With stillbirth defined as intrauterine fetal death at ≥ 22 weeks of gestation. All medical data regarding the antenatal care, the women health, pregnancy, and delivery were collected from the medical journals.

The primary outcomes of the study were:

- Preventable/non-preventable deaths
- The level of delay if there was any

The secondary outcomes were:

- Causes of death
- The standard of antenatal, intrapartum and postpartum care

4.3 STUDY III

Study III is a cross-sectional study and was conducted at Soder Hospital between March 2017 and October 2017.

Inclusion criteria:

Two groups of term births were included: 50 pre-labor elective cesarean sections and 26 vaginal deliveries. The women were Swedish or English speaking and above 18 years of age. All known fetal pathologies were excluded.

Primary outcome: placental microbiome

Secondary outcome: bacterial load in the amniotic fluid, vernix.

The general approach of the study was to compare the bacterial profiles of 50 term pre-labor cesarean deliveries and 26 term vaginal deliveries. For all 76 deliveries, the placenta (maternal side, middle, and fetal side sections), amniotic fluid, and vernix were collected. Saliva, a vaginal swab, and a rectal swab were also collected.
The bacterial load in all placental, amniotic fluid, and vernix samples, as well as technical/negative controls, were characterized through 16S rRNA gene qPCR. Targeted taxa-specific qPCR and 16S rRNA gene sequencing were additionally performed on maternal side placental samples. Bacterial culture was performed on all placental samples. The bacterial profiles of saliva, the vagina, and the rectum were characterized through shotgun metagenomic sequencing. Lastly, bacterial growth inhibition experiments were performed using *Escherichia coli* and placental samples.

4.4 STUDY IV

Study IV is a prospective cohort study conducted at Soder Hospital between May 2016 and December 2017.

Inclusion criteria: pregnant women seeking care due to RFM, Swedish or English speaking, singleton pregnancies, ≥ 34 weeks of gestation, no knowledge of fetal pathology.

The primary outcome was a composite neonatal outcome composed of one or more of the following criteria: arterial pH in umbilical cord ≤7.1, 5 minutes Apgar ≤7, transfer to neonatal ward, stillbirth and/or small for gestational age (SGA).

The women included in the study were offered after the standard care checkup an extra ultrasound examination of pregnancy in which Doppler parameters such as arteria uterine pulsatility index (PI), middle cerebral artery PI and umbilical cord artery PI were assessed.

The cerebroplacental ratio (CPR) was than calculated as a fraction between middle cerebral artery PI and umbilical artery PI.

Peripheral blood of the women included in the study was collected and centrifugated within 30 minutes from collection and then stored at −80°C until use. Angiopoietin1(LXSAHM-01), Angiopoietin-2, PIGF and VEGFR1/Flt1 (LXSAHM-03) and VEGF were analyzed.

Plasma levels were measured using the multiplex human immunoassay kit from R&D Systems/ Biotechne. The procedure of Luminex analysis was follow according to manufacturer's instruction.

Four predictive models were then constructed: one based on standard care, standard care and CPR, angiogenic factors and angiogenic factors and CPR.

5. RESULTS

5.1 STUDY I

The flowchart of the pregnancies included in the study is presented in the figure 5.

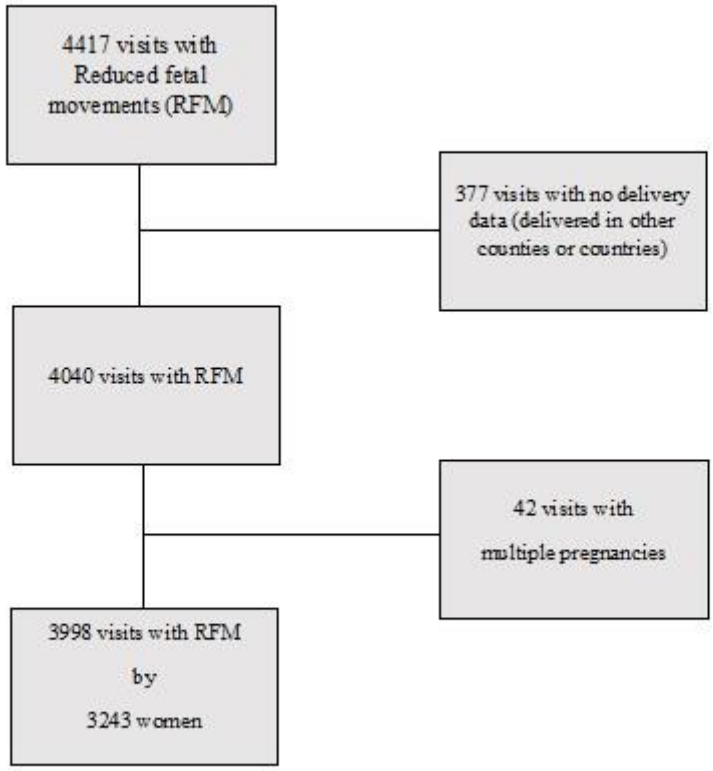


Fig 5. Flowchart RFM Soder Hospital 2016-2017

From January 2016 to December 2017 there were 3243 women who seek obstetrical care due to RFM at Soder Hospital. During the same period, there were 11944 singleton deliveries without RFM. Figure 6 shows the number of patients seeking care for RFM and the number of deliveries each month during the study period.

When comparing the two groups with and without RFM the women with RFM were younger (31 vs 33 years, $p<0.01$), more likely to smoke 3 months before the pregnancy (13.4% vs. 9.2, $p<0.01$), more likely to be nulliparous (57.1% vs. 44.6%, $p<0.01$) and have a history of psychiatric disorders (18.2% vs. 12.6%, $p<0.01$).

Regarding the composite neonatal outcome there were no statically significant differences between the two groups. Still when analyzing each parameter constructing the neonatal composite outcome there was an increased risk for the newborns from pregnancies with RFM of having a low Apgar (RR 1.56, 95% CI 1.25-1.96), low arterial pH at birth (RR 1.34, 95% CI 1.12-1.61) and stillbirth (RR 5.53, 95% CI 2.81-10.85).

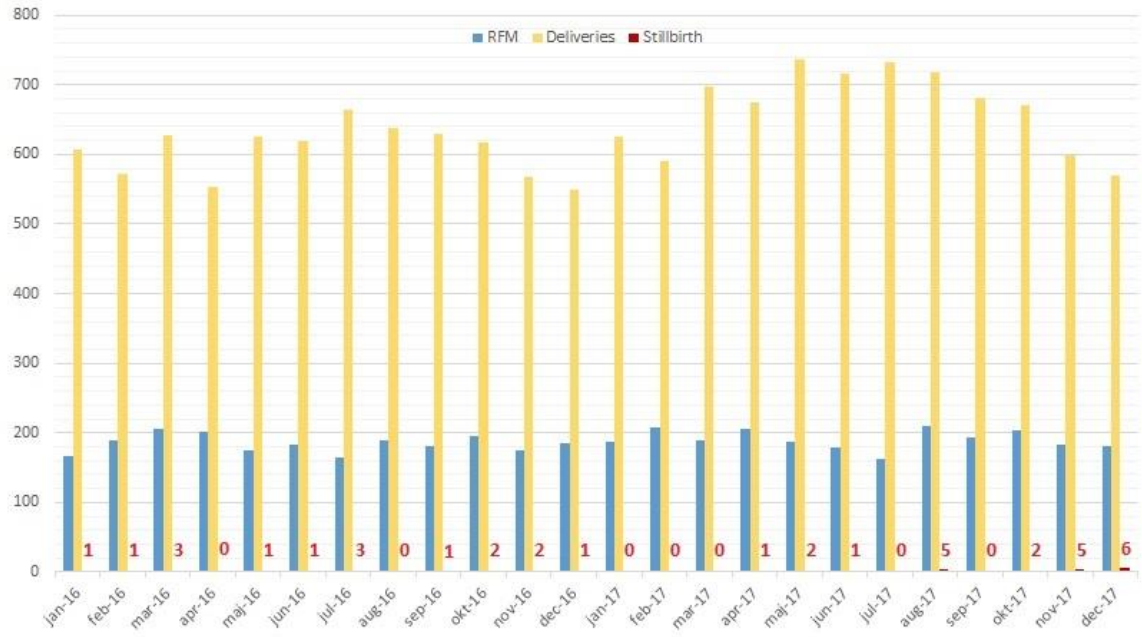


Fig.6 Women with RFM, deliveries and stillbirths at Soder Hospital 2016-2017

When comparing the group with recurrent RFM and the one with single episodes there were no statistically significant differences in the parameters that constitute the composite neonatal outcome, however the women with recurrent RFM were more likely to be nulliparous (62.6% vs. 55.9%, *p=0.04*) and have IVF pregnancies (9.1% vs. 6.6%, *p=0.04*).

There was an increased percentage of inductions of labor in the RFM group compared with the controls (23.5% vs. 16.5%, *p<0.01*).

The results from the Chi-square automatic interaction detection analysis showed that the highest risk for having the composite neonatal outcome was in the group with SGA fetus (18.4%), followed by the group with IVF (12.8%).

5.2 STUDY II

The rate of stillbirth in Stockholm has varied between 2.8-4.0/1000 livebirths for the past decade. When comparing the stillbirths group (n=79) with the liveborn group (n=28584) In Stockholm 2017 there are no statistical significant differences in age, pregnancy complications, smoking, IVF. In the group of stillbirths, the women of African origin (19.2% vs. 6.3%), the women with at least one previous miscarriage (33.3% vs 23.9%) and the SGA babies (40.5% vs. 3.9%) are overrepresented.

One of the primary outcomes was preventability of the stillbirth and 30.4 % of 79 stillbirths were assessed as possibly preventable (6/35 early stillbirths and 18/44 in the late stillbirth

group). There was a statistically significant difference between the group of Swedish speaking and non-Swedish speaking women regarding the preventability of stillbirth (24% vs 56%).

In 17% of the cases a patient delay was registered and in 15 % of the cases a delay due to the health care system. 28% of the cases were assessed as substandard antenatal care and 18% as substandard postnatal care.

The most frequent cause of death was IUGR/placenta insufficiency (20.8%) and 60.8% of the cases had more than one diagnosis that was associated with the stillbirth.

5.3 STUDY III

There were no statistical significant differences in the demographics of the women (n=76) and infants (n=77) included in the study when looking at the group of pre-labor cesarean section deliveries vs vaginal deliveries except regarding parity.

When analyzing the 16S rRNA gene by quantitative amplification and sequencing, the microbial content of the placenta was not higher than the background signals. A median of 57% of each sample were categorized as laboratory contaminants (figure 7).

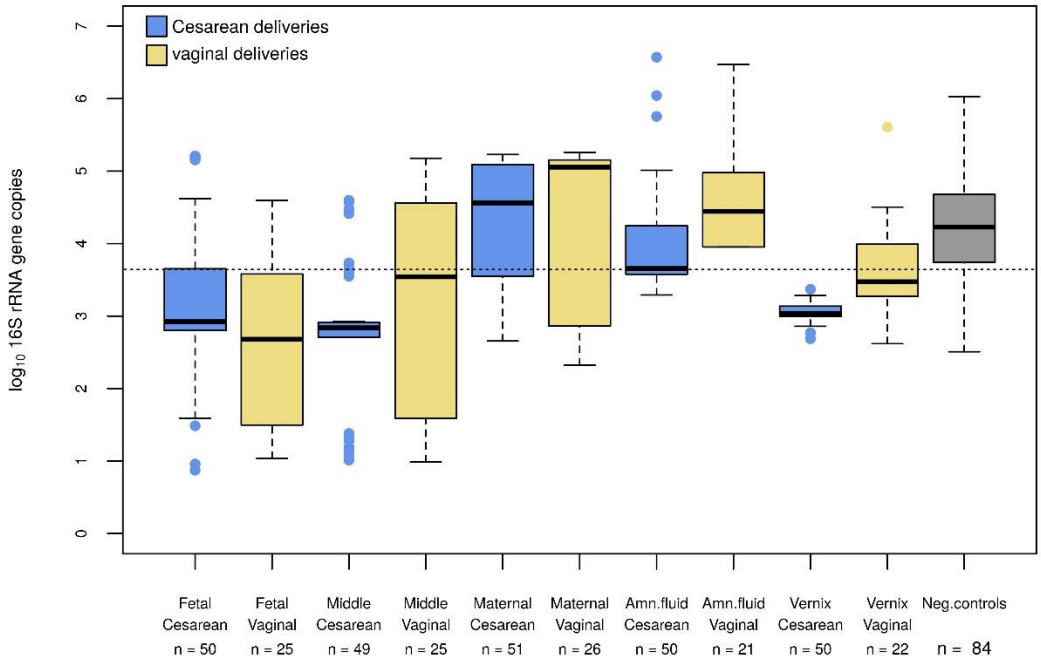


Fig.7 Amniotic fluid and placental tissue may contain bacterial DNA, at concentrations undistinguishable from negative controls

The maternal side of the placenta had the highest gene counts in both groups and was therefore chosen to validate the findings above by targeted qPCR for 10 specific bacterial taxa that could be expected to be found in the placenta based on previous published work. There were no statistically significant differences between the groups.

In the culture experiments, there were significantly more bacterial cultures observed in placental tissues from vaginal deliveries compared to cesarean section deliveries (table 2).

Table 2. Bacterial growth of placenta tissues according to delivery mode

	Delivery mode						
CFU range	**C-section (N=152)**			**Vaginal delivery (N=78)**			**C-section *versus* vaginal delivery**
	Maternal (n=51) n (%)	Middle (n=50) n (%)	Fetal (n=51) n (%)	Maternal (n=26) n (%)	Middle (n=26) n (%)	Fetal (n=26) n (%)	p-value
0	28 (54.9)	29 (58.0)	30 (58.8)	8 (30.8)	9 (34.6)	4 (15.4)	<0.001
1 – 5	17 (33.4)	13 (26.0)	10 (19.6)	7 (26.9)	5 (19.2)	5 (19.2)	0.787
6 – 30	4 (7.8)	3 (6.0)	8 (15.7)	3 (11.5)	6 (23.1)	10 (38.5)	0.996
>30	2 (3.9)	5 (10.0)	3 (5.9)	8 (30.8)	6 (23.1)	7 (26.9)	<0.001

Data expressed as absolute and relative values for total plate count n (%). CFU: Colony-forming Units. *p*-value for differences between delivery groups at the level of CFU range. The significance level was set at $p<0.05$. Fisher's Exact test was used for comparison.

The key findings of the study were:

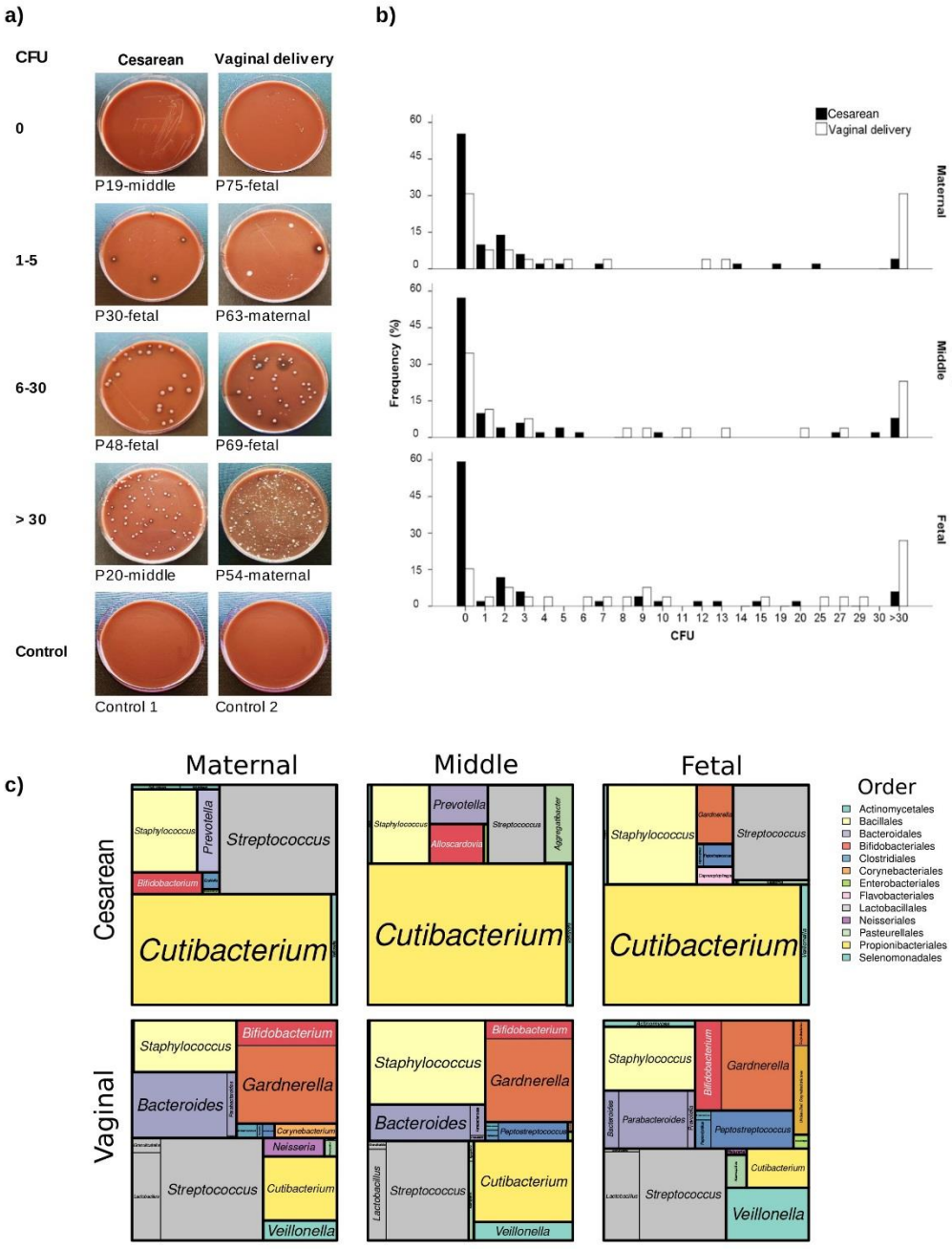
1) Bacterial signals in placental tissues, amniotic fluid, and the vernix did not exceed that of negative extraction controls.

2) The 16S rRNA gene profiles of the maternal side of the placenta in women who had not taken antibiotics in pregnancy were largely dominated by contaminants (87% of amplicon sequence variants were deemed to be contaminants; most of the remaining sequence data were from the genera *Massilia and Escherichia*).

3) Bacterial culture yielded skin and vaginal bacteria, and was influenced by mode of delivery. Specifically, when there was bacterial growth from a cesarean-delivered placenta, the recovered bacteria (*Propionibacterium, Streptococcus and Staphylococcus*) were consistent with skin contamination during the surgery (figure 8).

4) The bacterial growth inhibition assay demonstrated that placental tissues had a modest inhibitory effect on bacterial growth, particularly if the tissue had a high bacterial load as determined by qPCR.

Fig.8 Bacteria grown from placentas are predominantly typical skin and vaginal taxa



(a) GC agar plates showing the bacterial growth from placental tissues after 48 hours. The placenta cultures are represented in ranges according to the number of colony-forming units (CFUs). "P" stands for participant. **(b)** Histogram showing frequency (%) distributions of CFUs according to the placental sample type and the delivery mode. Chi-squared tests (Fisher's exact tests) were performed with significance level at $p = 0.05$. The comparison was between vaginal delivery (white bars) and cesarean delivery (black bars) in each CFU-range group. **(c)** Treemaps showing the relative proportion of taxa that grew in culture by location in the tissue and mode of deliverv.

5.4 STUDY IV

Between January 2016 and December 2017, 3243 women attended health care for RFM at Soder Hospital. 128 women participated in the study and an extra Doppler examination was performed. The women included in the study were more likely to be multipara (51.6% vs 42,3%, $p<0.04$), to attend health care for RFM multiple times (32% vs 16.6%, $p <0.01$) compared with the controls (RFM with standard care). It was more common for the women in the study group to be induced (36.9% vs 23.1%, $p<0.01$), but there were no statistically significant differences in the delivery mode. There were no stillbirths in the study group and there were no statistically significant differences in the composite neonatal outcome.

Within the study group there was a higher rate of obstetrical interventions such as follow-ups, hospitalization, or induction of labor when the extra Doppler was performed compared with standard care (28% vs 5.4%, $p<0.01$). There were no differences in the MoM CPR, MoM UtA and MoM MCA between the group with and without composite neonatal outcome.

In 62 women of the study group the angiogenic factors (Angiopoietin-1, PlGF and VEGF) and the antiangiogenic factors /Angiopoietin-2 and sFlt1) were analyzed. The median value of these factors did not differ between women with and without composite neonatal outcome.

The predictive model constructed with the angiogenic, antiangiogenic factors and parity had a good predictive value with an AUC for the ROC curve of 0.89 (CI 95% 0.81-0.97).

6. DISCUSSION

6.1 STUDY I

The main results of the study are presented in figure 9. 8.5% of the cases had a composite neonatal outcome in the group with RFM. The group of SGA babies and IVF pregnancies had the highest risk of poor neonatal outcome with 18.4% and 12.8% respectively.

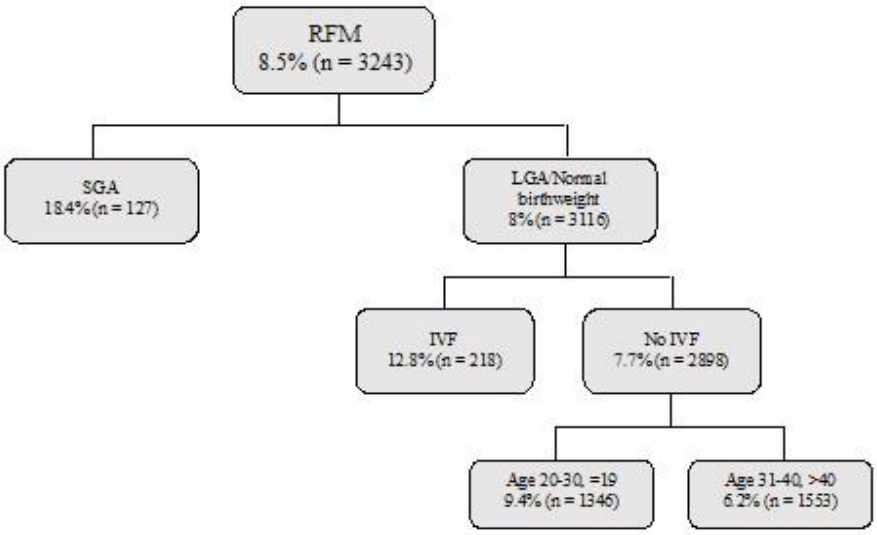


Fig.9 Tree classification of the risk factors for composite neonatal outcome in the RFM group.

The rate of presentation for RFM was 22% during the study period which is higher than the previous reported rates in other studies 8-17% (23, 110, 111), proving once again that RFM is a very common symptom during pregnancy.

Recurrent visits were 17.3% of the presentations with RFM which is lower compared to other studies that have reported up to 46% (112). In accordance with a previous study (112) there were no statistical significant differences in the neonatal outcome between women who presented for RFM once or multiple times. However other studies have reported an increased percentage of SGA babies in the group with recurrent RFM (111). Significant RFMs are likely to be late in the sequence of events that precedes fetal death: e.g. most severe IUGR babies move normally on scan. A pre-terminal event, usually just before CTG abnormalities,

will not happen multiple times. So, either recurrent RFMs is extremely serious or it is almost nothing.

When looking at the IVF pregnancies within the RFM group, there were statistically significant more nulliparous (76.4% vs 55.7%, p<0.01) and they sought care for RFM multiple times more frequent than the non-IVF group (22.3% vs 16.9%, p=0.04). Even more interesting is the neonatal outcome where the IVF group has a higher rate of SGA babies, neonatal ward and of poor neonatal outcome (table 3).

Table 3. Neonatal outcomes for the group with reduced fetal movements and IVF (n=229) and the group with RFM and no IVF (n=3007).

Poor Neonatal Outcome Score	Pregnancies with IVF N = 227	Pregnancies with no IVF n = 3007	p-value*
Apgar** ≤7 at 5 min (%)	8 (3.5)	94 (3.1)	0.69
Arterial pH**≤ 7.10 (%)	13 (6.6)	137 (5.8)	0.63
Neonatal ward transfer (%)	15 (6.6)	77 (2.6)	<0.01
Stillbirth (%)	2 (0.9)	15 (0.5)	0.34
SGA (%)	16 (7.0)	108 (3.6)	0.02
Composite outcome (%)	30 (13.1)	241 (8.0))	0.01

* significant if <0.05
**Not available on all neonates

Identifying the pregnancies with SGA in the RFM group could help us find the pregnancies with the highest risk of poor neonatal outcome. Previous studies have shown that a third trimester ultrasound in low-risk pregnancies may detect more SGA even if it does not affect the neonatal outcome (48, 49). However, a third trimester ultrasound for estimating the fetal growth in RFM population can be beneficial.

6.2 STUDY II

The main results of the multidisciplinary audit showed that there are 30% of the stillbirths in Stockholm that were assessed as possibly/probably preventable. The non-Swedish speaking women were overrepresented in this group. However, there were no statistically significant

differences regarding the preventability of stillbirth when just looking at the country of birth of the mother. Also, when assessing the level of delay in non-Swedish speaking women there was more often a patient related delay (37% vs 11%). There were even differences in the delays at the healthcare level, but not as high (19% vs 14%). These inequalities in the care were noticed even in other settings, like for example Italy where the preventable deaths occurred more frequently in non-Italian women (113).

This can be due to difficulties in understanding the information given at the ANC, difficulties in understanding how the healthcare system works and how to navigate a completely new healthcare system, especially when every contact with healthcare personnel is based on telephone contact. Previously published data from Sweden showed that women who have lived less than 5 years in Sweden have an increased risk of stillbirth (114). One could speculate that by providing adequate translation, an understanding of the healthcare system and culture doulas the quality of care can be improved for this group of patients.

The interventions that could have prevented the stillbirths were for example: earlier induction of labor, cesarean delivery, more frequent ultrasound, or extra clinical follow-ups.

There are over 35 classification systems in use internationally. In this study, we used the Stockholm stillbirth classification system (33). Compared with other systems there was a smaller percentage of cases that were classified as being of unknown cause (7.6% in our study compared with approximately 20% or 30% in other studies) (31, 115).

When looking at the protocol of the investigation for stillbirth there were no statistically significant difference between Swedish speaking and non-Swedish speaking women regarding incomplete investigation (6% vs 21%, p=0.28), placental examination (100% for both groups), karyotype (87% vs 100%, p=0.19) and autopsy (70% vs 50%, p=0.27). This confirms what has been shown in previous studies that all parents are extremely interested to find out the causes of death and in planning how to prevent it from happening again in the next pregnancy (116).

This kind of information on variables such as Swedish speaking or not is unfortunately difficult to assess when analyzing large register data sets. This is one among many others reason why national audits can be a useful tool in the fight to reduce stillbirths.

6.3 STUDY III

The main results of the study were that bacterial DNA from the placenta did not exceed the levels of the negative controls and that cultures from placental tissue yielded skin and vaginal bacteria depending on the delivery mode. Moreover, the placental tissue had an inhibitory effect on the bacterial growth. These findings confirmed that there is not a placental microbiome in normal healthy pregnancies.

There are contradictory results published regarding the existence of a placental microbiome (100, 102). The main difficulties in interpreting/analyzing the results of this type of studies are that the modern DNA-based methods are extremely sensitive and that there are traces of DNA everywhere: water, air, lab reagents. Moreover, there are differences in the population included in the studies (preterm and complicated pregnancies), the duration from delivery to sampling the placenta, that can partially explain the differences in results between studies.

The intrauterine environment is equipped with anti-inflammatory and anti-microbial characteristics at different levels: placenta, amniotic fluid, vernix, and fetal membranes. The physiology of the intrauterine environment and the fact that it is possible to breed germ-free animals seem to sustain our conclusions.

The conclusion of the study was that the evidence of bacterial presence in the human placenta at term was insufficient to conclude the existence of a placental microbiome. Although sporadic bacteria were found in the placenta of some subjects, these bacteria do not represent a placenta microbiome per se.

6.4 STUDY IV

Additional Doppler examination increased the rate of obstetrical interventions in the RFM group since the obstetricians in charge of the case were not blinded to the results and had to intervene according to the local guidelines.
Previous studies have shown that in midwifery-led practices in high-income countries a combination of low intervention rates, low cost and best outcomes has been noted (117).
In the group with additional Doppler there were no statistically differences in the CPR MoM between the group with and without composite neonatal outcome and no stillbirths.
Several research groups (Prior et al, Khalil et al., Dall'Asta et al., Kumar et al.), have recently addressed the issue of the identification of subclinical impairment of the placental function in apparently normally grown neonates by evaluating the CPR. These studies concluded that the

identification of the normally grown fetuses at risk for adverse events antepartum/intrapartum still represents an unresolved issue. These conditions are likely to account for the majority of the so far unexplained causes of stillbirth.

Research on angiogenic and antiangiogenic factors have shown that an imbalance between the angiogenic and antiangiogenic factors has been associated with placental insufficiency and poor neonatal outcome. In our study there were no statistical differences in the median values of the factors analyzed between the groups with and without composite neonatal outcomes.

When constructing a prediction model with angiogenic, antiangiogenic factors and parity the AUC of ROC was 0.89 which suggests that these factors may have a place in identifying the risk pregnancies for composite neonatal outcome within RFM group. The results should be interpreted cautiously since the numbers in the study are quite small and there is a risk of overfitting the model.

7. METHOLOGICAL CONSIDERATIONS

7.1 INTERNAL VALIDITY-SYSTEMATIC ERROR

Internal validity refers to how well the studies were conducted and how confident the researcher can be with the findings in the study.

7.1.1 Selection bias

Selection bias is one of the systematic errors that can affect the internal validity of the studies. It refers to an error in selecting the participants in the study that leads to differences in the relationship between exposure and outcome between those included in the study and the non-participants (118).

In study I all women with RFM at Soder Hospital were included over a two years' period. There were less than 10% of the visits for RFM that were delivered in other counties or countries and delivery data with the outcome was not available. The composite neonatal outcome was created due to the clinical significance and due to low numbers of cases in each of the categories.

In study II which is a retrospective cohort study all women with stillbirth in Stockholm during 2017 were included. All cases were including in the analysis.

There was no loss to follow up in studies III and IV. In study III all eligible women for participating in the study were invited. There were only two patients of 78 invited who refused to participate due to fear of discomfort when taking the samples.

The inclusion rate in study IV was very low and that is mainly due to patients not being invited to participate. We estimate that around 10% or less of the patients were asked to participate. This was due to high workload of the medical personnel and the possibility of having extra ultrasound examinations just once a day during office hours. In this study, only Swedish and English-speaking women were included in the study due to the lack of possibilities of translating the informative material. This can contribute to selection bias as previous studies have shown that women from Africa for example have a worse neonatal outcome.

7.1.2 Information bias

Another type of systematic error is the information bias which occurs during data collection. The most common type of information bias is misclassification where for example exposed participants are classified as non-exposed or participants with the outcome are classified as not having the outcome (118).

In a retrospective cohort studies, such as study I and II, there are some limitations that are specific for this type of research. Whenever conducting register-based studies the researcher relies entirely on the data quality existing in the register. For all studies, the statistical analysis and regressions were based on complete cases. There were no multiple imputations for the missing values.

Study II was a retrospective study, but also a multidisciplinary audit. The information in all the medical journal was assessed and interpreted by each member of the audit team individually and after that discussed at the meetings. There was consensus on the evaluation of the cases at all, except two cases.

Studies III and IV were prospective studies were researcher went through all the medical journals of all women included in the studies. This can minimize the risk of misclassification, missing values, or information bias. One of the problems inherent with prospective studies is the lost to follow up, which was not topical for our two studies.

7.1.3 Confounding

A confounder is a variable that influences both the exposure and the outcome. When planning a study, it is important to choose which confounders we adjust for, otherwise they can introduce bias. Even so, there is still a risk of residual confounding which can be known and discussed or unknown. In the latter case, there is no way of knowing their impact on the exposure or on the outcome.

The decision on the variable included in each study was made after discussion between clinicians and depending on variable availability in medical journals**.**

In study I in the multivariable regression analyzes we controlled for possible confounders and interactions. In study I the final model was constructed by initially analyzing the known risk factors for having the composite outcome: high BMI, advanced maternal age, smoking, parity, pregnancy complications, IVF. After analyzing the association for each individual

factor, a multivariable model was constructed with only the statistically significant variables from the univariable analysis. When analyzing the interactions between the significant variables an interaction between IVF and parity was noted and was accounted for in the new multivariable model. IVF is interesting in study I since its association with stillbirth is generally thought to be small and we have in the regression model adjusted for maternal age. We have not found described in the literature a clear association between IVF with RFM and stillbirth. There is a known association between IVF and low birth weight, though the etiology is still unclear. In our dataset, the finding with increased risk was not related to high age, parity, or SGA. It may therefore be a yet unknown confounding variable.

A direct acyclic graph (DAG) can be used to display the relationship between different variables and /or the outcome and to identify possible causal pathways. The DAG methodology can be used to help select the variables included in the study and to construct a model where bias is reduced. A DAG was constructed for study I using the free online toll DAGitty (119) (figure 10).

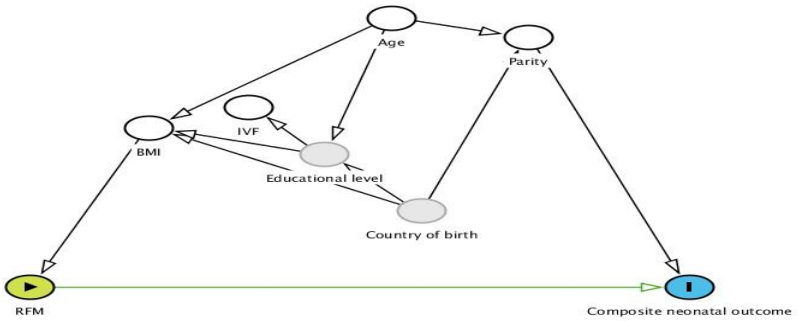


Fig.10 A visualization of possible confounders on Study I

7.2 POWER CALCULATION

In study I all patients with RFM at Soder Hospital during a two years' period were included. In study II all stillbirths in Stockholm 2017 were included. In study III the aim of the study was to describe the placental microbiome in full-term pregnancies and the number of cases

included was based both on previous published articles and on a pragmatic calculation on what is feasible. When study IV was designed, and the inclusion began there were no studies describing the predictors which we wanted to investigate in the group of patients with RFM. Because of that it was not possible to assume about expected differences between the groups.

7.3 RANDOM ERRORS

This type of errors is unpredictable and cannot be replicated if the experiments are repeated. To express the precision of the results of the experiment p-value and CI are used. With a small sample size, the CI will be wider and there is a risk of not being able to prove the real differences between the groups.

Study I has a large sample size and the random errors are not a problem. Study II has a small population of stillbirths that we are comparing with all deliveries in Stockholm and all stillbirths in Sweden (which are large samples). But when analyzing only the group of stillbirths in Stockholm, the results should be interpreted cautiously.

Random errors can be avoided by increasing the sample size. Study IV has a small number of participants and the results would be more accurate if the sample size was increased. However, at the time of the study it was not possible to increase the number of ultrasound examinations and this is a pilot study meant to generate the hypothesis for future studies.

7.4. CROSS-VALIDATION

Cross-validation is a technique used to prevent the problem of overfitting a model. To tackle this problem the dataset is usual split in test subsets so we can test that the prediction model will perform well on new data.

In study IV we investigated possible ultrasound and angiogenic factors predictors of the neonatal outcome. In this study, there are no problems with confounders since we are not looking at a causality relation. There is a possible risk of overfitting the model in this study due to the small number of cases/outcomes included in the study. Of the same reason, it was not possible to do a cross-validation of the dataset. This study was designed as a pilot study that will generate a hypothesis for future studies.

7.5 EXTERNAL VALIDITY AND GENERALIZABILITY

External validity refers to how applicable the results of the studies are in different populations; how generalizable the findings are.

Study I includes pregnancies seeking care for RFM in an urban setting, at a secondary hospital with almost 8000 deliveries a year. The demographic of this population can be slightly different from the rest of Sweden, but the result can be applied in similar settings in Sweden or Western Europe.

Study II is a small study were all stillbirths in Stockholm for one year were included. It is very difficult to include large numbers of patients due to the rarity of the outcome. In order to get more information about the generalizability of the results, a comparison with the whole group of stillbirths in Sweden and of all the livebirths in Stockholm during the same period was made.

Study III included a small number of full-term normal pregnancies from similar setting as Study I. In this study the presence of microbiome in the placenta was investigated. The study describes the intrauterine environment regarding the microbiome and the results can be extrapolated to full-term uncomplicated pregnancies in general.

Study IV is a prospective pilot study of women who seek care for RFM aiming to investigate if ultrasound and angiogenic markers can be helpful in predicting the fetal wellbeing in these pregnancies. The results can be applied in similar obstetric settings and for comparable populations.

In study III and IV only English and Swedish speaking women who could understand the oral and written consent and information were included in the studies. This can of course affect the generalizability of the studies.

8. FUTURE RESEARCH

Even if one could consider that the incidence of stillbirth is low in Sweden, every single lost child is a personal tragedy. Despite increased awareness on fetal movements in the media and among health care providers, the incidence has remained almost constant during the last three decades. We still need to find new methods of identifying the risk pregnancies in the group of RFM.

Thoughts on future projects:

1. To investigate the neonatal and maternal outcome of pregnancies where the labor is induced due to reduced fetal movements.
2. To investigate the molecular mechanism of stillbirths, including the microbiome of women and new-borns in stillbirths.
3. A prospective study to investigate the pregnancy outcome of non-Swedish speaking women with support of coulture doulas.

9. ETHICAL CONSIDERATIONS

9.1 General considerations

One of the aims of the research was to assess the efficacy of our present methods of investigating this group of women (with RFM) and to study if there are other diagnostic methods than the ones in use. Another aim of the study is to try and improve the standard, outcome, and knowledge for the patients with decreased fetal movements.

Ethical approval has been obtained for all 4 studies from the Regional Board of Ethics in Stockholm. Study III-IV were prospective studies. If the participants met the inclusion criteria and there were no contraindications or exclusion criteria, the women gave their written informed consent to participate in the studies. All participants in these studies received both oral and written information about the studies. Any of the participants could at any time decide to withdraw from the studies without affecting the treatment of women or infant.

Study I and II are retrospective register-based studies were all study participants are given a serial number and during the analysis phase the individuals cannot be identified. In all the studies, all data regarding the patients was recorded and stored appropriately and according with established legislation and guidelines. Files were password-protected and was accessible only to the researchers involved in the studies.

In study IV an additional ultrasound was done and blood, urine and saliva were sampled from the study participants. We assessed that no harm was caused to the participants beside the discomfort of the blood sample. In study III all the samples were collected during the cesareans section or during the vaginal delivery at the time when performing the standard procedures

All biological material was anonymized and impossible to trace to the patient's identity when handling and analyzing the samples in the laboratory. The materials were stored in accordance with the laws for biobanks in Sweden. Sample donors could at any time withdraw their consent and demand the collected samples are destroyed.

9.2 The goodness and no harm principles

When designing and performing the studies we always tried to apply the goodness principle and principle of no harm. We assessed that the gain for the study population is greater than the discomfort of collecting the samples. No direct benefit can be predicted for the women included in the study. Earnings are more long term in nature. We aimed to gain not only more information about this heterogenous group but also hopefully even identify new methods of predicting the risk population for stillbirths.

There are no conflicts of interests for the researchers involved in these studies.

10. SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Intrauterin fosterdöd (IUFD) är en av de mest allvarliga graviditetskomplikationerna. Varje år dör drygt 400 foster intrauterint vid en graviditetslängd ≥ 22 veckor. Globalt dör ca 2,65 miljoner barn intrauterint (vid graviditetslängd över 28 veckor). I Sverige är incidensen av intrauterint döda foster/1000 födda barn 3,8-4,4. Trots ökad uppmärksamhet i media samt ökade insatser inom mödrahälsovården har incidensen varit i stort sett oförändrad i Sverige de senaste 25 åren.

Bakomliggande orsakerna till IUFD kan vara infektion, moderkaks- och navelsträngs-komplikationer, missbildningar och kromosomavvikelser, dålig tillväxt av fostret samt sjukdomar hos modern. Men vissa gånger är IUFD dock helt oförklarligt.

Fosterrörelser är en viktig parameter för utvärdering av fostrets välmående. Frekvensen och kvinnans känsla av fosterrörelser varierar under graviditeten med en successiv ökning från graviditetsvecka 16 till 36, för att sedan minska något den senaste månaden innan förlossningen. Fosterrörelser varierar beroende på fostervattenmängd, fostrets bjudning, mammans medicinering och hälsotillstånd. Det finns inget facit för hur många fosterrörelser en blivande mamma faktiskt ska känna under graviditeten, vad som är normalt och vad som är onormalt. Tidigare studier visar dock att minskade fosterrörelser har ett samband med graviditetskomplikationer så som IUFD.

Minskade fosterrörelser är idag ett hett debatterat ämne. Sedan 2011 har många artiklar publicerats och mycket information presenterats i svenska dagstidningar samt sociala media. På Södersjukhuset i Stockholm söker ungefär 2500 kvinnor per år då de upplever att fostret rör sig mindre intrauterint. Svårigheten i den obstetriska vardagen är att kliniskt handlägga denna stora patientgrupp. Erfarenheten säger att de flesta av dessa graviditeter är normala, och målet är en så liten interventionsgrad som möjligt. Samtidigt döljer sig riskgraviditeterna bland dessa fall. En Cochran-översikt från 2012 som studerade handläggningen av kvinnor som söker för minskade fosterrörelser konstaterade att det inte fanns tillräcklig information från de randomiserade studier som är gjorda om den bästa handläggningen av dessa graviditeter.

I denna avhandling ingår fyra studier. I första delstudien var målet att identifiera riskfaktorer associerade med dåligt neonatalt utfall i gruppen av gravida som sökte vård för minskade fosterrörelser på Södersjukhuset under 2016–2017. Studien visade att i gruppen med

minskade fosterrörelser, hade barn som är små för gestationsåldern den högsta risken för dåligt neonatalt utfall (ca 18,4%), följd av IVF graviditeter (12,4%).

Den andra delstudien var en multidisciplinär regional audit angående alla intrauterina fosterdödsfall i Stockholm 2017. Audit gruppen gjorde en bedömning av alla fall. Totalt 30 % av IUFD klassades som möjliga att förhindra. I 15% av fallen bedömdes att det var förseningar i handläggningen som var vårdrelaterad. En eventuellt förebyggbar IUFD var vanligare bland icke-svenskspråkiga kvinnor (p=0.03).

I 20% av IUFD finns histologiska tecken på infektion vid undersökning av placenta, utan att vi alltid kan bevisa en infektion med odlingar. Tanken med den tredje studien var att kartlägga om det finns ett mikrobiom intrauterint under en normal fullgången graviditet. 50 kvinnor som genomgick planerat kejsarsnitt innan värkarbetets start och 26 kvinnor som födde vaginalt inkluderades i studien. Prover från placentan, fostervattnet, fosterfettet analyserades. Vi hittade inga tecken på ett mikrobiom i normala graviditeter.

I den fjärde delstudien studerades faktorer som kunde predicera neonatalt utfall för graviditeter där kvinnorna upplevde minskade fosterrörelser. Kvinnor som sökte vård för detta på Södersjukhuset under 2016–2017 erbjöds att delta i studien och att genomgå ett extra ultraljud av fostret där man undersökte flödet i navelsträngen och flödet i en artär i barnets huvud. I samband med undersökningen togs också ett blodprov på kvinnan för att analysera syrebristrelaterade faktorer i blodet. Att göra en extra ultraljudundersökning ledde till ett signifikant högre antal obstetriska interventioner såsom igångsättning av förlossningen, inläggning på antenatal-avdelning eller extra kontroller. En bra prediktiv modell har erhållits när syrebristmarkörerna har använts.

11. ACKNOWLEDGEMENTS

I would like to express my gratitude to all who helped and encouraged me during my journey. Although the list below is far for being complete, I would like to especially thank the following:

Eva Wiberg Itzel, my main supervisor for guidance, for introducing me to scientific research, for always finding a way forward with our projects. For inspiring me all the time and for being such an enthusiastic person. You manage to juggle a hundred balls in the air at the same time and still wake up at 5 in the morning with very innovative ideas. To our past and future journeys!

Kristina Gemzell Danielsson, my co-supervisor for your openness, kindness and professionality and for always sharing your knowledge so generously. You are possibly the fastest email responder I know!

Ina Schuppe Koinstinen, my co-supervisor for her expertise, for her German way of being direct and effective, for always finding time to email me "interesting to read" articles. For reminding me to celebrate even the small victories along the way. I will groove in the park with you anytime! And thank you for being the "Godmother" of the thesis.

Helena Åkerud, my co-supervisor for providing knowledgeable answers whenever I needed them, for asking the right questions during the writing process and for making time for me in your busy, busy schedule.

Marion Ek, my colleague and mentor for always being positive and finding the time to check on my progress.

Per Lindqvist, my colleague and co-author for excellent comments and sound advices, for his scientific expertise and improving the manuscripts. Do not relax, because I will come up with future projects to call or e-mail about!

Christina Pilo, co-author and former colleague for her enthusiasm, hard work and dedication both at the Ultrasound Department and in the Stockholm Stillbirths Group. Thank you for all the songs at the disputation parties along the years and for proofreading my texts.

Tove Wallström, my colleague and friend. You are a role model as an obstetrician, as a researcher, as a friend. I still wonder how you have time for everything! Thank you for all the kind words and encouragement, and for caring.

Fuad Bahran, director of the research centre at Södersjukhuset**,** for always providing me with lab expertise and material and for nice discussions on research and life in general. I still need a map to find the research freezer in the hospital's catacombs.

Carina Ursing thank you for the beautiful painting on the cover. And for not giving up until it felt perfect. You are a true artist!

Hans Pettersson the most patient statistician at KI SÖS. Thank you for helping me to navigate the troubled waters of statistics. It is not over yet… I will continue to come by with more questions and fika.

Jenny Bolk, co-author and colleague. Thank you for all your immediate and constructive comments, for all the ideas and the patience and for helping me with the framework.

Hanna Åmark and **Anja Marklund** for helping me to not get lost during the painful process of applying for public defense of the thesis and giving me helpful advice each step of the way.

Stockholm stillbirth group for all the work in assessing and gathering data on all stillbirths in Stockholm since 1998.

Sebastian Brusell-Gidlöf, Corinne Pedroletti and Ingegerd Lantz head and former heads of the Department of Obstetrics and Gynecology at Södersjukhuset for creating a scientific working environment.

Henrik Graner, Philip Gottlieb and Ann Kristin Rönnberg my clinical supervisors during my trainee years, for all the things I have learned from you.

My colleagues at the department of Obstetrics and Gynecology, Södersjukhuset. You are the best! Thank you for your understanding and encouraging words during the years I have worked here. Thank you for sharing good and difficult times together. Special thanks to the ultrasound department **Bahrim Alemdar, Katarina Ernstson, Emma Frövenholt Hertting, Lotta Levin, Julia Savchenko** for helping me with the ultrasound examinations sometimes even during your lunch breaks.

Center for Translational Microbiome Research, a multicultural lab with lots of expertise and wonderful people. Special thanks to **Lars Engstrand, Emma Fransson, Marcela Pereira, Luisa Hugerth, Marica Hamsten** for introducing me to the world of microbiome and for patiently answering all my questions.

Thank you to all women who have participated in these studies. These studies would not have been possible without your contribution!

My **friends** (you know who you are), thank you for making this journey a lot more fun, spicing it with delicious dinners and lunches. I feel rich to have you all in my life!

My parents, **Didina** and **Nicolae Pădeanu** for always believing in me, trusting me to make my own choices and supporting me no matter what. Vă iubesc!

Bogdan, thank you for understanding me even when I do not understand myself, for keeping me earthly when the research tends to drift me away, for giving me wings and inspiring me to dream when I get buried in work and for always putting the FAMILY first.

Thank you also for unfailing technical support. The computer software seems to hold a grudge against me ☺.

My children **Luca** and **Luna**, for your warmth, fun and unconditional love. "I love you for all that you are, all that you have been and all that you are yet to be" and hope to always be a part of your lives.

The studies in this thesis were supported by:

Södersjukhuset-Karolinska Institutet Research local funding

The National Board of Health and Welfare – Study II

12. REFERENCES

1. Flenady V, Wojcieszek AM, Middleton P, Ellwood D, Erwich JJ, Coory M, et al. Stillbirths: recall to action in high-income countries. Lancet (London, England). 2016;387(10019):691-702.

2. Flenady V, Middleton P, Smith GC, Duke W, Erwich JJ, Khong TY, et al. Stillbirths: the way forward in high-income countries. Lancet (London, England). 2011;377(9778):1703-17.

3. Lawn JE, Blencowe H, Waiswa P, Amouzou A, Mathers C, Hogan D, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet (London, England). 2016;387(10018):587-603.

4. de Bernis L, Kinney MV, Stones W, Ten Hoope-Bender P, Vivio D, Leisher SH, et al. Stillbirths: ending preventable deaths by 2030. Lancet (London, England). 2016;387(10019):703-16.

5. Heazell AEP, Siassakos D, Blencowe H, Burden C, Bhutta ZA, Cacciatore J, et al. Stillbirths: economic and psychosocial consequences. Lancet (London, England). 2016;387(10018):604-16.

6. F A. Onduration of pregnancy. Monatscschr Geburtsh Gynäkol. 1869;19:155-65.

7. Sontag LW WR. Preliminary rapport of the Fels Fund study of fetal activity. Am J Dis Child. 1934;48:1050-7.

8. Shenker L, Reed K, Anderson C, Hauck L, Spark R. Syndrome of camptodactyly, ankyloses, facial anomalies, and pulmonary hypoplasia (Pena-Shokeir syndrome): obstetric and ultrasound aspects. American journal of obstetrics and gynecology. 1985;152(3):303-7.

9. Dutton PJ, Warrander LK, Roberts SA, Bernatavicius G, Byrd LM, Gaze D, et al. Predictors of poor perinatal outcome following maternal perception of reduced fetal movements--a prospective cohort study. PloS one. 2012;7(7):e39784.

10. Stacey T, Thompson JM, Mitchell EA, Ekeroma A, Zuccollo J, McCowan LM. Maternal perception of fetal activity and late stillbirth risk: findings from the Auckland Stillbirth Study. Birth (Berkeley, Calif). 2011;38(4):311-6.

11. Sadovsky E, Laufer N, Allen JW. The incidence of different types of fetal movements during pregnancy. British journal of obstetrics and gynaecology. 1979;86(1):10-4.

12. Sorokin Y, Dierker LJ, Jr. Fetal movement. Clinical obstetrics and gynecology. 1982;25(4):719-34.

13. Hijazi ZR, East CE. Factors affecting maternal perception of fetal movement. Obstetrical & gynecological survey. 2009;64(7):489-97; quiz 99.

14. Sjostrom K, Valentin L, Thelin T, Marsal K. Maternal anxiety in late pregnancy: effect on fetal movements and fetal heart rate. Early human development. 2002;67(1-2):87-100.

15. Coppens M, Vindla S, James DK, Sahota DS. Computerized analysis of acute and chronic changes in fetal heart rate variation and fetal activity in association with maternal smoking. American journal of obstetrics and gynecology. 2001;185(2):421-6.

16. Tuffnell DJ, Cartmill RS, Lilford RJ. Fetal movements; factors affecting their perception. European journal of obstetrics, gynecology, and reproductive biology. 1991;39(3):165-7.

17. Cito G, Luisi S, Mezzesimi A, Cavicchioli C, Calonaci G, Petraglia F. Maternal position during non-stress test and fetal heart rate patterns. Acta obstetricia et gynecologica Scandinavica. 2005;84(4):335-8.

18. Sadovsky E, Yaffe H. Daily fetal movement recording and fetal prognosis. Obstetrics and gynecology. 1973;41(6):845-50.

19. Pearson JF. Fetal movement recording: a guide to fetal well-being. Nursing times. 1979;75(38):1639-41.

20. Mangesi L, Hofmeyr GJ, Smith V, Smyth RM. Fetal movement counting for assessment of fetal wellbeing. The Cochrane database of systematic reviews. 2015(10):Cd004909.

21. Akselsson A, Lindgren H, Georgsson S, Pettersson K, Steineck G, Skokic V, et al. Mindfetalness to increase women's awareness of fetal movements and pregnancy outcomes: a cluster-randomised controlled trial including 39 865 women. Bjog. 2020;127(7):829-37.

22. Hofmeyr GJ, Novikova N. Management of reported decreased fetal movements for improving pregnancy outcomes. The Cochrane database of systematic reviews. 2012(4):Cd009148.

23. Tveit JV, Saastad E, Stray-Pedersen B, Bordahl PE, Flenady V, Fretts R, et al. Reduction of late stillbirth with the introduction of fetal movement information and guidelines - a clinical quality improvement. BMC pregnancy and childbirth. 2009;9:32.

24. Vintzileos AM, Fleming AD, Scorza WE, Wolf EJ, Balducci J, Campbell WA, et al. Relationship between fetal biophysical activities and umbilical cord blood gas values. American journal of obstetrics and gynecology. 1991;165(3):707-13.

25. Warland J, O'Brien LM, Heazell AE, Mitchell EA. An international internet survey of the experiences of 1,714 mothers with a late stillbirth: the STARS cohort study. BMC pregnancy and childbirth. 2015;15:172.

26. Stockholm:Socialstyrelsen. Statistikdatabas för graviditeter, förlossningar och nyfödda april 2018 [updated april 2018. Available from: http://www.socialstyrelsen.se/statistik/statistikdatabas/graviditeter/ forlossningarochnyfodda.

27. quality.2003 SNBoHaWTSMBRasoca. The Swedish Medical Birth Register acessed 10/01/2020 [Available from: https://www.socialstyrelsen.se/en/statistics-and-data/registers/register-information/the-swedish-medical-birth-register/.

28. Silje Vatne Pettersen LO. Immigrants in Norway, Sweden and Denmark. Scandinavian comparative statistics and integration Samfunnsspeilet. 2013;5.

29. Heazell AEP, Weir CJ, Stock SJE, Calderwood CJ, Burley SC, Froen JF, et al. Can promoting awareness of fetal movements and focusing interventions reduce fetal mortality? A stepped-wedge cluster randomised trial (AFFIRM). BMJ open. 2017;7(8):e014813.

30. Parts L, Holzmann M, Norman M, Lindqvist PG. Admission cardiotocography: A hospital based validation study. European journal of obstetrics, gynecology, and reproductive biology. 2018;229:26-31.

31. Flenady V, Frøen JF, Pinar H, Torabi R, Saastad E, Guyon G, et al. An evaluation of classification systems for stillbirth. BMC pregnancy and childbirth. 2009;9(1):24.

32. Wigglesworth JS. Monitoring perinatal mortality. A pathophysiological approach. Lancet (London, England). 1980;2(8196):684-6.

33. Varli IH, Petersson K, Bottinga R, Bremme K, Hofsjo A, Holm M, et al. The Stockholm classification of stillbirth. Acta obstetricia et gynecologica Scandinavica. 2008;87(11):1202-12.

34. Lawn JE, Yakoob MY, Haws RA, Soomro T, Darmstadt GL, Bhutta ZA. 3.2 million stillbirths: epidemiology and overview of the evidence review. BMC pregnancy and childbirth. 2009;9 Suppl 1(Suppl 1):S2.

35. Dunn P MG. A Report Produced for the European Association of Perinatal Medicine: European Association of Perinatal Medicine; 1996.

36. Dahl LB, Berge LN, Dramsdahl H, Vermeer A, Huurnink A, Kaaresen PI, et al. Antenatal, neonatal and post neonatal deaths evaluated by medical audit. A population-based study in northern Norway - 1976 to 1997. Acta obstetricia et gynecologica Scandinavica. 2000;79(12):1075-82.

37. Bergsjø P, Bakketeig LS, Langhoff-Roos J. The development of perinatal audit: 20 years' experience. Acta obstetricia et gynecologica Scandinavica. 2003;82(9):780-8.

38. Eskes M, Waelput AJM, Erwich J, Brouwers HAA, Ravelli ACJ, Achterberg PW, et al. Term perinatal mortality audit in the Netherlands 2010-2012: a population-based cohort study. BMJ open. 2014;4(10):e005652.

39. Zeitlin J, Mohangoo A, Cuttini M, Alexander S, Barros H, Blondel B, et al. The European Perinatal Health Report: comparing the health and care of pregnant women and newborn babies in Europe. J Epidemiol Community Health. 2009;63(9):681-2.

40. MacDorman MF, Kirmeyer SE, Wilson EC. Fetal and perinatal mortality, United States, 2006. Natl Vital Stat Rep. 2012;60(8):1-22.

41. Ministry of Health W. Fetal and Infant Deaths 2008 and 2009 2012 [Available from: https://www.health.govt.nz/publication/fetal-and-infant-deaths-2008-and-2009.

42. Björk I, Pettersson K, Lindqvist PG. Stillbirth and factor V Leiden - A regional based prospective evaluation. Thromb Res. 2019;176:120-4.

43. Rydahl E, Declercq E, Juhl M, Maimburg RD. Routine induction in late-term pregnancies: follow-up of a Danish induction of labour paradigm. BMJ open. 2019;9(12):e032815.

44. Heimstad R, Romundstad PR, Eik-Nes SH, Salvesen KÅ. Outcomes of Pregnancy Beyond 37 Weeks of Gestation. Obstetrics & Gynecology. 2006;108(3):500-8.

45. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr. 1996;85(7):843-8.

46. Monasta L, Giangreco M, Ancona E, Barbone F, Bet E, Boschian-Bailo P, et al. Retrospective study 2005-2015 of all cases of fetal death occurred at ≥23 gestational weeks, in Friusli Venezia Giulia, Italy. BMC pregnancy and childbirth. 2020;20(1):384.

47. Welfare SNBoHa. Dödfödda barn-En inventering och förslag på åtgärder 2018 [Available from: https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/ovrigt/2018-12-36.pdf.

48. Henrichs J, Verfaille V, Jellema P, Viester L, Pajkrt E, Wilschut J, et al. Effectiveness of routine third trimester ultrasonography to reduce adverse perinatal outcomes in low risk pregnancy (the IRIS study): nationwide, pragmatic, multicentre, stepped wedge cluster randomised trial. Bmj. 2019;367:l5517.

49. Skråstad RB, Eik-Nes SH, Sviggum O, Johansen OJ, Salvesen K, Romundstad PR, et al. A randomized controlled trial of third-trimester routine ultrasound in a non-selected population. Acta obstetricia et gynecologica Scandinavica. 2013;92(12):1353-60.

50. Lindqvist PG, Molin J. Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2005;25(3):258-64.

51. Bricker L, Medley N, Pratt JJ. Routine ultrasound in late pregnancy (after 24 weeks' gestation). The Cochrane database of systematic reviews. 2015;2015(6):Cd001451.

52. Griffiths SK, Campbell JP. Placental structure, function and drug transfer. Continuing Education in Anaesthesia Critical Care & Pain. 2014;15(2):84-9.

53. Higgins LE, Rey de Castro N, Addo N, Wareing M, Greenwood SL, Jones RL, et al. Placental Features of Late-Onset Adverse Pregnancy Outcome. PloS one. 2015;10(6):e0129117.

54. Winje BA, Roald B, Kristensen NP, Frøen JF. Placental pathology in pregnancies with maternally perceived decreased fetal movement--a population-based nested case-cohort study. PloS one. 2012;7(6):e39259.

55. Warrander LK, Batra G, Bernatavicius G, Greenwood SL, Dutton P, Jones RL, et al. Maternal perception of reduced fetal movements is associated with altered placental structure and function. PloS one. 2012;7(4):e34851.

56. Luchinger AB, Hadders-Algra M, van Kan CM, de Vries JI. Fetal onset of general movements. Pediatric research. 2008;63(2):191-5.

57. Cruz-Martinez R, Figueras F, Oros D, Padilla N, Meler E, Hernandez-Andrade E, et al. Cerebral blood perfusion and neurobehavioral performance in full-term small-for-gestational-age fetuses. American journal of obstetrics and gynecology. 2009;201(5):474.e1-7.

58. Meher S, Hernandez-Andrade E, Basheer SN, Lees C. Impact of cerebral redistribution on neurodevelopmental outcome in small-for-gestational-age or growth-restricted babies: a systematic review. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015;46(4):398-404.

59. Llurba E, Turan O, Kasdaglis T, Harman CR, Baschat AA. Emergence of late-onset placental dysfunction: relationship to the change in uterine artery blood flow resistance between the first and third trimesters. American journal of perinatology. 2013;30(6):505-12.

60. Prior T, Mullins E, Bennett P, Kumar S. Prediction of intrapartum fetal compromise using the cerebroumbilical ratio: a prospective observational study. American journal of obstetrics and gynecology. 2013;208(2):124.e1-6.

61. Morales-Rosello J, Khalil A, Morlando M, Papageorghiou A, Bhide A, Thilaganathan B. Changes in fetal Doppler indices as a marker of failure to reach growth potential at term. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;43(3):303-10.

62. Khalil AA, Morales-Rosello J, Morlando M, Hannan H, Bhide A, Papageorghiou A, et al. Is fetal cerebroplacental ratio an independent predictor of intrapartum fetal compromise and neonatal unit admission? American journal of obstetrics and gynecology. 2015;213(1):54.e1-10.

63. Khalil A, Morales-Rosello J, Townsend R, Morlando M, Papageorghiou A, Bhide A, et al. Value of third-trimester cerebroplacental ratio and uterine artery Doppler indices as predictors of stillbirth and perinatal loss. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016;47(1):74-80.

64. Triunfo S, Crispi F, Gratacos E, Figueras F. Prediction of delivery of small-for-gestational-age neonates and adverse perinatal outcome by fetoplacental Doppler at 37 weeks' gestation. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017;49(3):364-71.

65. Rial-Crestelo M, Martinez-Portilla RJ, Cancemi A, Caradeux J, Fernandez L, Peguero A, et al. Added value of cerebro-placental ratio and uterine artery Doppler at routine third trimester screening as a predictor of SGA and FGR in non-selected pregnancies. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2018:1-7.

66. Dunn L, Sherrell H, Kumar S. Review: Systematic review of the utility of the fetal cerebroplacental ratio measured at term for the prediction of adverse perinatal outcome. Placenta. 2017;54:68-75.

67. Bligh LN, Alsolai A, Greer RM, Kumar S. Screening for adverse perinatal outcomes: uterine artery Doppler, cerebroplacental ratio and estimated fetal weight in low-risk women at term. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2017:1-7.

68. Monaghan C, Binder J, Thilaganathan B, Morales-Rosello J, Khalil A. Perinatal Loss at Term: The Role of Uteroplacental and Fetal Doppler Assessment. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017.

69. Akolekar R, Syngelaki A, Gallo DM, Poon LC, Nicolaides KH. Umbilical and fetal middle cerebral artery Doppler at 35-37 weeks' gestation in the prediction of adverse perinatal outcome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015;46(1):82-92.

70. Vollgraff Heidweiller-Schreurs CA, De Boer MA, Heymans MW, Schoonmade LJ, Bossuyt PMM, Mol BWJ, et al. Prognostic accuracy of cerebroplacental ratio and middle cerebral artery Doppler for adverse perinatal outcome: systematic review and meta-analysis. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2018;51(3):313-22.

71. Bligh LN, Alsolai AA, Greer RM, Kumar S. Pre-labour screening for intrapartum fetal compromise in low risk pregnancies at term: cerebroplacental ratio and placental growth factor. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017.

72. Karumanchi SA, Epstein FH. Placental ischemia and soluble fms-like tyrosine kinase 1: cause or consequence of preeclampsia? Kidney international. 2007;71(10):959-61.

73. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. The New England journal of medicine. 2004;350(7):672-83.

74. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proceedings of the National Academy of Sciences of the United States of America. 1993;90(22):10705-9.

75. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature. 1996;380(6573):439-42.

76. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996;380(6573):435-9.

77. Smith GC, Crossley JA, Aitken DA, Jenkins N, Lyall F, Cameron AD, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. Obstetrics and gynecology. 2007;109(6):1316-24.

78. Nanjo S, Minami S, Mizoguchi M, Yamamoto M, Yahata T, Toujima S, et al. Levels of serum-circulating angiogenic factors within 1 week prior to delivery are closely related to conditions of pregnant women with pre-eclampsia, gestational hypertension, and/or fetal growth restriction. The journal of obstetrics and gynaecology research. 2017;43(12):1805-14.

79. Trollmann R, Strasser K, Keller S, Antoniou X, Grenacher B, Ogunshola OO, et al. Placental HIFs as markers of cerebral hypoxic distress in fetal mice. American journal of physiology Regulatory, integrative and comparative physiology. 2008;295(6):R1973-81.

80. Romero R, Chaiworapongsa T, Erez O, Tarca AL, Gervasi MT, Kusanovic JP, et al. An imbalance between angiogenic and anti-angiogenic factors precedes fetal death in a subset of patients: results of a longitudinal study. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2010;23(12):1384-99.

81. Chaiworapongsa T, Romero R, Erez O, Tarca AL, Conde-Agudelo A, Chaemsaithong P, et al. The prediction of fetal death with a simple maternal blood test at 20-24 weeks: a role for angiogenic index-1 (PlGF/sVEGFR-1 ratio). American journal of obstetrics and gynecology. 2017;217(6):682.e1-.e13.

82. Espinoza J, Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Nien JK, et al. Unexplained fetal death: another anti-angiogenic state. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2007;20(7):495-507.

83. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(7):3047-52.

84. Alverdy J, Holbrook C, Rocha F, Seiden L, Wu RL, Musch M, et al. Gut-derived sepsis occurs when the right pathogen with the right virulence genes meets the right host: evidence for in vivo virulence expression in Pseudomonas aeruginosa. Annals of surgery. 2000;232(4):480-9.

85. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. Trends in neurosciences. 2013;36(5):305-12.

86. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linlokken A, Wilson R, et al. Correlation between the human fecal microbiota and depression. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2014;26(8):1155-62.

87. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? BioEssays : news and reviews in molecular, cellular and developmental biology. 2014;36(10):933-9.

88. Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. Applied and environmental microbiology. 2004;70(11):6459-65.

89. Barbara G, Stanghellini V, Brandi G, Cremon C, Di Nardo G, De Giorgio R, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. The American journal of gastroenterology. 2005;100(11):2560-8.

90. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. Genes, brain, and behavior. 2014;13(1):69-86.

91. Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. The Journal of physiology. 2017;595(2):451-63.

92. Neu J. The microbiome during pregnancy and early postnatal life. Seminars in fetal & neonatal medicine. 2016;21(6):373-9.

93. van de Wijgert JH, Borgdorff H, Verhelst R, Crucitti T, Francis S, Verstraelen H, et al. The vaginal microbiota: what have we learned after a decade of molecular characterization? PloS one. 2014;9(8):e105998.

94. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. Maternal Microbiome and Pregnancy Outcomes That Impact Infant Health: A Review. Advances in neonatal care : official journal of the National Association of Neonatal Nurses. 2015;15(6):377-85.

95. Harris JW BJ. The bacterial content of the uterus at cesarean section. American Journal of Obstetrics & Gynecology. 1927;13(2):133-43.

96. Lewis JF, Johnson P, Miller P. Evaluation of amniotic fluid for aerobic and anaerobic bacteria. American journal of clinical pathology. 1976;65(1):58-63.

97. Evaldson GR, Malmborg AS, Nord CE. Premature rupture of the membranes and ascending infection. British journal of obstetrics and gynaecology. 1982;89(10):793-801.

98. Ovalle A, Martinez MA, Kakarieka E, Gomez R, Torres J, Fuentes A, et al. [Placental histopathology in premature rupture of membranes. Its relationship with microbiological findings, maternal, and neonatal outcome]. Revista medica de Chile. 1998;126(8):930-42.

99. Greig PC. The diagnosis of intrauterine infection in women with preterm premature rupture of the membranes (PPROM). Clinical obstetrics and gynecology. 1998;41(4):849-63.

100. de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, et al. Human placenta has no microbiome but can contain potential pathogens. Nature. 2019;572(7769):329-34.

101. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? Research in microbiology. 2008;159(3):187-93.

102. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Science translational medicine. 2014;6(237):237ra65.

103. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Scientific Reports. 2016;6:23129.

104. Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48.

105. Parnell LA, Briggs CM, Cao B, Delannoy-Bruno O, Schrieffer AE, Mysorekar IU. Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles. Sci Rep. 2017;7(1):11200.

106. Tapiainen T, Paalanne N, Tejesvi MV, Riikola MP, Korpela K, Pokka T, et al. Maternal influence on the fetal microbiome in a population-based study of the first-pass meconium. Pediatric research. 2018.

107. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PloS one. 2008;3(8):e3056.

108. DiGiulio DB, Gervasi MT, Romero R, Vaisbuch E, Mazaki-Tovi S, Kusanovic JP, et al. Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses. Journal of perinatal medicine. 2010;38(5):495-502.

109. Wang X, Buhimschi CS, Temoin S, Bhandari V, Han YW, Buhimschi IA. Comparative microbial analysis of paired amniotic fluid and cord blood from pregnancies complicated by preterm birth and early-onset neonatal sepsis. PloS one. 2013;8(2):e56131.

110. Binder J, Monaghan C, Thilaganathan B, Morales-Rosello J, Khalil A. Reduced fetal movements and cerebroplacental ratio: evidence for worsening fetal hypoxemia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2018;51(3):375-80.

111. Scala C, Bhide A, Familiari A, Pagani G, Khalil A, Papageorghiou A, et al. Number of episodes of reduced fetal movement at term: association with adverse perinatal outcome. American journal of obstetrics and gynecology. 2015;213(5):678.e1-6.

112. Bhatia M, Mitsi, V. , Court, L. , Thampi, P. , El-Nasharty, M. , Hesham, S. , Randall, W. , Davies, R. and Impey, L. The outcomes of pregnancies with reduced fetal movements: A retrospective cohort study. . Acta Obstet Gynecol Scand 2019;Accepted Author Manuscript.

113. Po G, Monari F, Zanni F, Grandi G, Lupi C, Facchinetti F. A regional audit system for stillbirth: a way to better understand the phenomenon. BMC pregnancy and childbirth. 2019;19(1):276.

114. Ekeus C, Cnattingius S, Essen B, Hjern A. Stillbirth among foreign-born women in Sweden. Eur J Public Health. 2011;21(6):788-92.

115. Vergani P, Cozzolino S, Pozzi E, Cuttin MS, Greco M, Ornaghi S, et al. Identifying the causes of stillbirth: a comparison of four classification systems. American journal of obstetrics and gynecology. 2008;199(3):319.e1-4.

116. Ellis A, Chebsey C, Storey C, Bradley S, Jackson S, Flenady V, et al. Systematic review to understand and improve care after stillbirth: a review of parents' and healthcare professionals' experiences. BMC pregnancy and childbirth. 2016;16(1):16.

117. Shaw D, Guise JM, Shah N, Gemzell-Danielsson K, Joseph KS, Levy B, et al. Drivers of maternity care in high-income countries: can health systems support woman-centred care? Lancet (London, England). 2016;388(10057):2282-95.

118. Tripepi G, Jager KJ, Dekker FW, Zoccali C. Selection Bias and Information Bias in Clinical Research. Nephron Clinical Practice. 2010;115(2):c94-c9.

119. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. Int J Epidemiol. 2016;45(6):1887-94.