EARLY PREGNANCY DEVELOPMENT AND OBSTETRIC OUTCOME

Robbert H.F. van Oppenraaij

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Publication of this thesis was financially supported by:

Department of Obstetrics & Gynaecology, Erasmus MC Rotterdam
Erasmus University Rotterdam
Nederlandse Vereniging voor Obstetrie en Gynaecologie (NVOG)
BMA BV (Mosos)
Medical Dynamics
Ferring B.V.

Graphic design cover: Robbert van Oppenraaij

Lay out and printing: Off Page, Amsterdam

ISBN: 978-94-6182-526-1

Early Pregnancy Development And Obstetric Outcome

Jonge zwangerschapsontwikkeling en obstetrische uitkomsten

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 21 januari 2015 om 13:30 uur

door

Robbert Henricus Franciscus van Oppenraaij
geboren te Amsterdam

Promotiecommissie:

Promotor:	Prof.dr. E.A.P. Steegers
Overige leden:	Prof.dr. V.W.V. Jaddoe Prof.dr. S.A. Scherjon Prof.dr. F.J. van Kemenade
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GENERAL INTRODUCTION

1.1

INTRODUCTION AND AIM OF THE THESIS

INTRODUCTION

Normal placental development in first trimester of pregnancy is of great importance for normal growth and development of the fetus in later pregnancy. Complications and events in early pregnancy could result in adverse outcome in the current or subsequent pregnancy. Most of the early pregnancy complications, such as blood loss and miscarriage, and late pregnancy complications, such as preeclampsia and uterine growth restriction, find their origin in placental maldevelopment. Placental development consists of development of the villous tree, development of the villous vascularization and adjustment of the spiral arteries. The latter is beyond the scope of this thesis.

To understand abnormal development of the villous vascularization, it is necessary to take note of normal early placentation first. Most of the knowledge on implantation and early placentation of the blastocyst is derived from the Boyd Collection.[1] The blastocyst implants at day 6-7 after conception in the endometrium. At that point the blastocyst consist mostly of cells in the outer layer which will evolve in trophoblast, only a few cells in the inner mass will proceed to form the fetus. During the following days after implantation the cells in the outer layer of the trophoblast fuse, forming a large continuous layer, now called the syncytiotrophoblast, not interrupted by intracellular spaces. Whilst the inner layer of the trophoblast remain mononuclear and form the cytotrophoblast. The syncytiotrophoblast expands into the uterine decidualized epithelium. Lacunae appear in the syncytiotrophoblast transforming into the intervillous space by fusion. Around 4 weeks gestational age (GA) the pillars of syncytiotrophoblast, from the chorionic plate to the basal plate, are invaded by the cytotrophoblast forming the anchoring villi. Primary villi grow and protrude into the lacunae from the sides of these anchoring villi. Two days later mesenchymal cells derived from the extraembryonic mesenchyme layer of the primary chorionic plate start to invade the villi, transforming them into secondary villi.[2] Between 3^{+4} and 3^{+6} weeks GA vasculogenesis, de novo vessel formation, starts.[3] Vasculogenesis is achieved by differentiation of these pluripotent mesenchymal cells into haemangiogenic stem cells which primarily forms cords.

During this stage two important processes take place: maturation and margination.[4] The primitive hemangiogenic cords will maturate into luminized vessels and will develop from centrally to periphally located vessels (margination). The appearance of the first capillaries within the villi marks the development of tertiar villi, or mesenchymal villi. The trophoblastic plugs in the uterine spiral arteries dissolve gradually between week 9 and 12 GA, resulting in a maternal intervillous circulation and establishing the materno-fetal exchange of gasses and nutrition at the very early vasculo-syncitial membrane.[5,6] Fetal and maternal blood will be separated by the vasculosyncitial membrane, which constitutes of syncytiotrophoblast, cytotrophoblast, basal lamina and endothelium.

In the last decade Lisman et al. investigated vasculogenesis and angiogenesis in normal and abnormal early pregnancies.[7] In first trimester non-viable pregnancies (after fetal death or empty sac pregnancies) the process of vasculogenesis was less evolved as compared to viable first trimester pregnancies terminated because of social reasons. In the non-viable pregnancies the maturation from hemangiogenic cords to vessels as well as the margination of centrally located vessels towards peripherally located vessels

was less developed as compared to viable pregnancies. The authors concluded that, as vasculogenesis was observed even in empty sac and molar pregnancies, vasculogenesis being the initiation of chorionic villous vascularization is fundamental, regardless of the viability of the pregnancy.[8,9] But for further development of the vascularization modulation directly or indirectly by embryonic signaling would be necessary.

A difference in the degree of vascularization between the viable and nonviable pregnancies could be used in clinical practice. A histological vascularization scoring system proved to be a simple tool to assess the chorionic villous vascularization and was helpful to distinguish between normal and abnormal embryonic development.[10]

In viable second trimester pregnancies complicated by aneuploidy Lisman et al. observed less peripheral vessels and more hemangiogenetic cords as compared to euploid pregnancies resulting in a less developed vasculo-syncitial membrane.[11] A possible explanation for the intrauterine growth restriction observed in those pregnancies was thereby provided.

Lastly, Lisman et al. investigated whether a 3 dimensional visualization of the chorionic villous vascularization using confocal laser scanning microscopy would be possible and useful to describe the spatial arrangement.[12] The 3 dimensional visualization proved to be feasible, the morphological and morphometric data was consistent and complementary with the 2 dimensional analysis. Furthermore these reconstructions provided more insight in the spatial arrangement between cords, and vessels and the development of the vasculosyncitial membrane.

These new insights in first trimester placental pathophysiology and new technical developments, like 3D virtual reality, inspired to study the relation between early pregnancy events and second and third trimester complications of pregnancy.[13,14] As a normal first trimester development is an essential basis for an uncomplicated second and third trimester development, early pregnancy events, either clinical or morphological, may be of prognostic value. The use of proper early pregnancy nomenclature is essential for these studies.

AIM OF THE THESIS

The following research objectives were defined:

1. To determine an unambiguous Dutch terminology to describe events in early pregnancy (Chapter 1.2)
2. To explore whether determining the due date by the last period is influenced by preference for certain dates, and whether this is associated with adverse obstetric and neonatal outcome (Chapter 2.1). To evaluate the effect of nonvisualized early pregnancy losses (biochemical pregnancy losses and failed pregnancies of unknown location) on the chance of live birth in the subsequent pregnancy (Chapter 2.2). To investigate whether events and complications in early pregnancy are associated with adverse obstetric and neonatal outcome (Chapter 2.3).
3. To investigate whether the use of a 3 dimensional Virtual Reality (VR) system to create enhanced 3 dimensional rendering images of 3 dimensional dataset created by a confocal laser scanning microscopy of early chorionic villous vascularization is consistent and

useful to perform morphometric measurements and to describe the spatial arrangement of the cords and vessels in relation to the vasculosyncytial membrane (Chapter 3.1). To determine the effect of smoking on the chorionic villous vascularization in first trimester of pregnancy using a 3 dimensional VR system (Chapter 3.2).

4. To study whether idiopathic second trimester fetal loss (Chapter 4.1), preeclampsia and intrauterine growth restriction (Chapter 4.2) are associated with an underdeveloped chorionic villous vascularization.

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1.2

DUTCH REVISION OF THE NOMENCLATURE FOR EARLY PREGNANCY EVENTS

Based upon:
De jonge zwangerschap: revisie van de Nederlands benamingen voor klinische en echoscopische bevindingen

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NTOG; 2008;152:20-4

ABSTRACT

The Dutch nomenclature used to describe clinical events in early pregnancy in the needs to be revised. Various terms, like "abortion" and "miscarriage", are used to describe the same phenomenon, which is confusing for both patients and doctors. In addition, the meaning of some old and poorly descriptive terms, like "missed abortion", has changed over time.

In accordance with the revision of the European nomenclature in the English language by the Special Interest Group for Early Pregnancy of the European Society for Human Reproduction and Embryology (ESHRE), a revision of the nomenclature in the Dutch language is needed as well.

An unambiguous Dutch terminology pertaining to early pregnancy, that corresponds to the English terminology is recommended; this includes the Dutch terms 'embryo' [embryo], 'foetus' [fetus], 'biochemische zwangerschap' [biochemical pregnancy], 'zwangerschap met onbekende lokalisatie' [pregnancy of unknown location], 'miskraam' [miscarriage], 'lege vruchtzak' [empty sac], 'gestopte hartactiviteit' [fetal loss], 'herhaalde miskraam' [recurrent miscarriage] and 'extra-uteriene zwangerschap' [ectopic pregnancy] and 'trofoblastziekte' [gestational trophoblastic disease], because these are based on well-defined clinical and ultrasonographic concepts.

INTRODUCTION

The Dutch nomenclature used to describe clinical early pregnancy events originates mostly from before the ultrasound era. Various terms are used for the same phenomenon and this may be confusing in practice, both for patients and physicians. In recent decades, the knowledge of the epidemiological and pathophysiological aspects of the early pregnancy is increased by the use of sensitive tests of human chorionic gonadotropin (hCG) and the introduciton of high-resolution transvaginal ultrasound. The Special Interest Group for Early Pregnancy (SIGEP) of the European Society for Human Reproduction and Embryology (ESHRE) presented a proposal to change in the European nomenclature.[1] This proposal was acquired by the Royal College of Obstetricians and Gynaecologists (RCOG Guideline No. 25: www.rcog.org.uk/resources/Public/pdf/green_top_25_management_epl.pdf).

In this article, we recommend a revision of the Dutch nomenclature based on current clinical and ultrasound findings (Table I). Before the manuscript was offered for publication, it was put to the members of the study group Early Pregnancy (werkgroep Jonge Zwangerschap) of the Dutch Society for Obstetrics and Gynaecology (Nederlandse Vereniging voor Obstetrie en Gynaecologie).

Gestational Age (zwangerschapsduur)

The distinction made between the embryonic and fetal period in pregnancy is classical. The embryonic period starts at conception and covers the first 8 weeks thereafter. In this period the organogenesis has been completed. Thereafter, the fetal period starts, during which only fetal growth takes place. The embryonic period is divided by embryologists into 23 internationally recognized morphological stages associated with the embryonic age, starting from the conception.[2]

Clinicians calculate the gestational age from the first day of the last menstrual period (gestation) or they use a more accurate method: the sonographic measurement of the crown-rump length (CRL), this length corresponds to a gestational age based on a theoretical ovulation date plus 2 weeks.[3]

Viable pregnancy

Some publications mention the 'egg' or 'ovum' when it comes to the period after conception. This is confusing because these terms are used interchangeably for both the oocyt and the embryo. In Dutch, the term 'vrucht' is confusing, because it sometimes refers to only the embryo and sometimes to the entire conception consisting of membranes, placenta and embryo.

Even the te use of the term 'embryo' may contribute to confusion. In reproductive medicine an embryo is synonymous with the group of cells that develops after fertilization of an oocyt, while anatomists traditionally use the term 'embryo' throughout organogenesis. Gynecologists and sonographers are already used to speak about 'fetal' heart activity and 'fetal' movements in that period. In the Dutch law an embryo has been defined as 'a cell or collection of cells with the ability to develop into a human being' and a fetus as 'an embryo in the human body'.[4]

Table I. Preferred Dutch and English terminology for early pregnancy events

NVOG 2008		ESHRE 2005
Preferred Dutch terminology	Dutch terms to avoid	Preferred English terminology
Eicel	Ei, Ovum	Oocyte
Embryo	Ovum	Embryo
Conceptus	Vrucht	Conception
Embryo	-	Embryo
Foetus	Embryo	Fetus
Zwangerschapsduur	Embryonale leeftijd	Gestational age
Zwangerschap met onbekende lokalisatie (ZOL)	Preklinische zwangerschap	Pregnancy of unknown location (PUL)
Persisterende ZOL		Persistent PUL
Extra-uteriene zwangerschap (EUG)	Ectopische zwangerschap, Buitenbaarmoederlijke zwangerschap	Ectopic pregnancy
Biochemische miskraam	Preklinische miskraam trofoblast in regressie	Biochemical pregnancy loss Failed PUL
Miskraam	(Spontane) abortus	Miscarriage
(In)complete miskraam	Abortus (in)completus	(In)complete miscarriage
Dreigende miskraam	Abortus imminens	Threatened miscarriage
Beginnende miskraam of miskraam in gang	Abortus incipiens	Inevitable abortion
Abortus provocatus Zwangerschapsafbreking	Abortus Zwangerschapsonderbreking	Termination of pregnancy
Vroege miskraam	Vroege abortus	Early miscarriage
Late miskraam	Late abortus	Late miscarriage
Niet vitale zwangerschap	Missed abortion	Nonviable pregnancy
Lege vruchtzak	Geresorbeerd embryo Windei	Empty sac
Gestopte hartactiviteit	Vruchtdood Missed abortion	Fetal loss
Retentietijd		Retention period
Herhaalde miskraam	Habituele abortus Recidiverende abortus	Recurrent miscarriage
Trofoblastziekte	Trofoblasttumor	Gestational trophoblastic disease
Complete mola (hydatidosa)	Klassieke mola	Complete gestational trophoblastic disease
Partiële mola (hydatidosa)	Incomplete mola	Partial gestational trophoblastic disease
Persisterende trofoblast	-	Persistent gestational trophoblastic disease
Choriocarcinoom	Chorionepithelioom	Choriocarcinoma
Placental site trophoblastic tumour		Placental site trophoblastic tumour

English terms to avoid	(Ultrasound) findings
Egg	-
Ovum	Fertilized oocyte that has begun cell division
	Gestational sac, placenta and embryo
	CRL < 10 mm (without fetal heart activity)
Embryo	Fetal heart activity or CRL > 10 mm
Embryonic age, Conceptual age, Menstrual age	Based on CRL measurement between 8-12 weeks or LMP date or adding two weeks to the number of completes weeks since fertilization
Preclinical pregnancy	Positive HCG and no identifiable pregnancy on ultrasound
	Persistent HCG levels and no identifiable pregnancy on ultrasound
	Viable or non-viable pregnancy outside the uterine cavity
Preclinical abortion Menstrual abortion	Falling HCG levels and no identifiable pregnancy on ultrasound
Spontaneous abortion	Until 20 weeks gestational age
(In)complete abortion	Until 20 weeks gestational age
Threatened abortion	Until 20 weeks gestational age
	Until 20 weeks gestational age
Medical abortion Legal abortion	Until 24 weeks gestational age
Early abortion	Before 12 Weeks gestational age
Late abortion	After 12 Weeks gestational age
Missed abortion	
Anembryonic pregnancy Blighted ovum	Gestational sac > 20 mm, without yolk sac and embryo.
Embryonic death Missed abortion	CRL > 6 mm without fetal heart activity
	Time period (weeks) between cessation of heart activity and detection of fetal loss
Habitual abortion Recurrent abortion	NVOG: two or more miscarriages not necessarily consecutive, ESHRE: three or more consecutive miscarriages
Hydatidiform mole	-
Molar pregnancy	Diploid (paternal)
Partial mole	Triploid (both paternal as maternal)
	Persistent HCG levels after curettage
	-

For pregnant women, the observation of cardiac activity as a first sign of new life, is such an important milestone that a change of the terminology used in the clinical use is justified. Although in the teratology it is important to distinguish between the embryonic and fetal period, we must accept that the current terminology is patient-oriented. Therefore, an internationally ultrasonographic definition for "fetus" has been made by using the term fetus after detection of heart activity or a CRL of at least 10 mm.[1]

Localization of pregnancy

Sensitive pregnancy tests and serum hCG assays detect pregnancy as early as the implantation and before the expected menstrual period, ie before sonographic detection is possible. If a pregnancy test is positive and sonographic detection of the localization of the pregnancy is not yet conclusive then the term a 'pregnancy of unknown location (PUL)' or 'zwangerschap met onbekende lokalisatie (ZOL)' should be used instead of preclinical pregnancy. If the location is not yet clear it may be both an intrauterine or an ectopic pregnancy and a viable or a non-viable pregnancy.[5]

A first indication of an intrauterine pregnancy is the appearance of a gestational sac. The gestational sac is visible from 4+2 weeks and then has a diameter of 2-3 mm. Characteristic is an echogenic area around the sac, which is caused by chorionic villi.[6] The viability is uncertain if the pregnancy ring has a diameter smaller than 20 mm and there are no signs that indicate the presence of a yolk sac or embryo. The viability is also uncertain if an embryo is visible with a crown-rump length less than 6 mm without cardiac activity. In the cited RCOG Guideline No. 25 it is recommended to repeat the ultrasound examination with an interval of at least 1 week.

Ectopic pregnancy

Although the term 'ectopische zwangerschap' is in agreement with the international nomenclature (ectopic pregnancy), in the Dutch language the term 'extra-uteriene graviditeit (EUG)' is preferable. The terms 'extra-uteriene graviditeit' and 'intra-uterine graviditeit' fit together after all and the abbreviation 'EUG' has become an integral part of our clinical jargon. Simultaneous occurrence of an intra- and extra-uterine pregnancy is called a "heterotopic" pregnancy.

Nonviable pregnancy

In case of bleeding in the first trimester of pregnancy it is important to assess the viability of the pregnancy by ultrasound.[8] If the gestational sac is 20 mm or greater and heart activity, yolk sac and embryo are lacking, then it is called an 'empty sac' and 'lege vruchtzak' in Dutch. The embryo is not or not properly constructed, and not resorbed, as was previously thought. The term 'blighted ovum' or in Dutch 'windei' is better avoided because it is too much reminiscent to the poultry industry. If the crown-rump length is 6 mm or greater and heart activity is missing, then 'fetal loss' is certain, for the patient this situation is best translated as 'gestopte hartactiviteit'.

Both empty gestational sac and fetal loss are 'nonviable pregnancies', in Dutch 'niet vitale zwangerschap' - a miscarriage is inevitable. When it will occur, is uncertain, and is

depending on the hCG production. The term 'missed abortion' was introduced in 1878 by Duncan for very rare cases of retained miscarriage tissue for many months.[9] He described a number of patients in whom pregnancy symptoms diminished or completely disappeared, while the uterus failed to grow. It has always been unclear what the duration of the retention period should be to to speak about a 'missed abortion'. Especially, with current ultrasound in early pregnancy a nonviable pregnancy is not missed for months, so the term is confusing at least. We recommend the use of the term 'retention time' in combination with a nonviable pregnancy. The retention time is the number of weeks after cessation of heart in which a spontaneous miscarriage fails. This time is the difference between the actual gestational age and the gestational age where the heart activity stopped (based on the crown-rump length measured with absent cardiac activity).

Miscarriage

It is estimated that approximately 30% of all conceptions fail to implant and that approximately 30% fail to progress after implantation. This is mostly attributed to chromosomal and / or morphological abnormalities.[10] The term 'biochemical miscarriage' for such an early failure is a better description than 'preclinical miscarriage', 'trophoblast in regression' and 'menstrual abortion'. A biochemical miscarriage is the result of a positive urine or serum pregnancy test, without sonographic signs of intra-or extra-uterine pregnancy or residual tissue matching an incomplete miscarriage (see the cited RCOG Guideline No. 25).

The terms 'abortion' and 'miscarriage', in Dutch 'abortus' and 'miskraam', are used interchangeably, while the meaning is different. Patients associate the word 'abortion' specifically with a termination of pregnancy. Therefore, it is better to use the word 'miscarriage' for a expulsion of a nonviable pregnancy and to reserve the term 'termination of pregnancy', in Dutch 'abortus' or 'zwangerschapsafbreking', for termination of a pregnancy for social, maternal or fetal indications.[1]

In the Netherlands a miscarriage traditionally is defined as a spontaneous interruption of pregnancy before 16 weeks gestation. Internationally a miscarriage is confined to 20 or 24 weeks gestation. The historical background of these differences is formed by clinical observations. Before 16 weeks, the pregnancy is usually expelled in toto (the amniotic sac with the placenta and the fetus), whilst after 16 weeks usually the membranes rupture first, then the fetus is born, followed by the expulsion of the placenta.[11] It was also thought that the placentation was not yet completed before the 16th week.[12] We recommend to define miscarriage to 20 weeks, as is proposed by the Special Interest Group for Early Pregnancy of ESHRE.[13]

A distinction can be made between an early miscarriage before 12 weeks and a late miscarriage from 12 weeks onwards.[1] This distinction is useful because in early miscarriages chromosomal and / or morphological abnormalities are usually present, whereas in late miscarriages, maternal factors, such as coagulation disorders, are more prevalent.[14]

Recurrent miscarriage

There is an international debate regarding the definition for recurrent miscarriage, whether is should be at least two or three miscarriages and whether they should be consecutive or

not. In the Netherlands, recurrent miscarriage is defined as two or more miscarriages which do not necessarily have to be consecutive.[15] The term 'habitual abortion' is abandoned. 'Habitual' literally means 'to become a habit' and suggests that it is a permanent state of experiencing miscarriages.[16] This is incorrect because these women still have prognostic good chances of a live birth after several miscarriages.[17] It is therefore recommended to use the term 'recurrent miscarriage', in Dutch 'herhaalde miskraam'.[1]

Gestational trophoblastic disease

The term "gestational trophoblastic disease", in Dutch "trofoblastziekte", stands for, a group of diseases that originates in the trophoblast (placenta). The frequently used term "trophoblastic tumor" suggests a neoplasm or malignancy and is therefore easily be misinterpreted by patients. The gestational trophoblast diseases can histologically be divided into a "complete gestational trophoblastic disease" (a diploid karyotype), in Dutch 'complete mola (hydatidosa)', a "partial gestational trophoblastic disease" (a triploid karyotype), in Dutch 'partial mola (hydatidosa)', a 'choriocarcinoma' and a 'placental site trophoblastic tumor' (RCOG Guideline No. 38: www.rcog.org.uk/resources/Public/pdf/Gestational_Troph_Neoplasia_No38.pdf).

Transvaginal sonography, hCG assay, histological and immunohistochemical examination and karyotyping all play an important for the diagnosis. Clinical staging by means of a scoring systems is used for determining the optimal treatment option (www.oncoline.nl, by clicking on "Gynaecology" and "Persistent and choriocarcinoma trophoblast").

In Dutch 'complete mola' is a better term than the old-fashioned term 'klassieke mola' and 'partiële mola' is preferable to 'incomplete mola'. The Dutch word 'mola' is derived from the Latin word for 'mass' and 'hydatidosa' originates from the Greek 'hydatis', which 'drop of water' means.

A 'persistent gestational trophoblastic disease' is present if after treatment for a complete molar pregnancy the hCG concentration does not decreases spontaneously.[18] A 'choriocarcinoma' may occur after a gestational trophoblastic disease, but also following a term birth, ectopic pregnancy or a miscarriage.[19] For the 'placental site trophoblastic tumor' is no official Dutch word. Given the rare nature of this malignancy, such a Dutch designation is not missed in clinical practice.

CONCLUSION

The revision of the Dutch early pregnancy nomenclature is desirable for both, a clear and consistent clinical description that can be universally understood, and for research a clear classification of early pregnancy events is needed as well. The Dutch nomenclature is revised in accordance with the revision of the European nomenclature in the English language by the Special Interest Group for Early Pregnancy of the European Society for Human Reproduction and Embryology (ESHRE).

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2

EARLY PREGNANCY EVENTS AND OBSTETRIC OUTCOME

2.1

DETERMINANTS OF NUMBER-SPECIFIC RECALL ERROR OF LAST MENSTRUAL PERIOD; A RETROSPECTIVE COHORT STUDY

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BJOG 2014 doi:10.1111/1471-0528.12991

ABSTRACT

Objective

To assess the digit preference for last menstrual period (LMP) dates, associated determinants and impact on obstetric outcome.

Design

Retrospective cohort study

Setting

University medical centre (The Netherlands)

Population

Cohort of 24.665 LMP records and a subgroup of 4.630 cases with known crown-rump length (CRL) measurement and obstetric outcome.

Methods

Digit preference was determined by comparing the observed to expected counts of each day. Associated determinants were identified by multivariate regression analysis. Differences in obstetric outcome between LMP and CRL dating were analysed.

Main Outcome Measures

(Non)deprived neighbourhood, cycle irregularity, certainty of LMP date, maternal age, smoking, body mass index, parity and ultrasound investigator. Preterm and post-term delivery.

Results

LMP digit preference for the first (odds ratio (OR) 1.28, 95% confidence interval (CI) 1.20-1.36), fifth OR 1.10, 95% CI 1.03-1.17), 10th (OR 1.17, 95% CI 1.09-1.25), 15th (OR 1.31, 95% CI 1.23-1.40), 20th (OR 1.22, 95% CI 1.15-1.30), and 25th (OR 1.08, 95% CI 1.01-1.15) days of the month occurred more often than expected. Digit preference occurred more frequently in women living in a deprived neighbourhood (OR 1.21, 95% CI 1.06-1.39), with uncertain LMP (OR 2.03, 95% CI 1.63-2.52), or irregular cycle (OR 1.24, 95% CI 1.06-1.44). More post-term (≥42 weeks) deliveries (OR 1.27, 95% CI 1.05-1.54) were observed in LMP dating. This effect was larger in women with a digit preference (OR 1.56, 95% CI 1.03-2.37).

Conclusions

LMP digit preference occurs more often in women living in deprived neighbourhoods, with uncertain LMP or an irregular cycle. LMP-dated pregnancies are associated with more post-term pregnancies.

Keywords

Digit preference, Menstrual Period, Pregnancy dating

INTRODUCTION

Accurate assessment of gestational age is essential for optimal prenatal care, especially for the management in cases of pre-eclampsia, fetal growth restriction (FGR) and prematurity. The duration of pregnancy is calculated either by using the first day of the last menstrual period (LMP) or by measuring ultrasonographically the crown rump length (CRL) in the first trimester of pregnancy. The accuracy and reliability of both methods have been a matter of debate in many studies.[1,2] The LMP method depends on accurate LMP recall, regularity of the menstrual cycle, early bleeding, and factors that could influence ovulation timing, such as previous oral contraceptive use, a recent pregnancy or breastfeeding.[3-6] Ultrasound dating depends on the gestational age at the time of the ultrasound examination, the accuracy of the measurement and the quality of the ultrasound equipment; it is based on the assumption of uniform early fetal growth.[1,7,8] However, the uniformity of first-trimester fetal growth is a matter of debate as growth restriction in the first trimester of pregnancy has been described.[9,10] Because of these drawbacks, neither methods is suitable as the gold standard for the assessment of gestational age.[11]

In the USA, the estimated date of delivery in spontaneously conceived pregnancies is calculated by adding 280 days to the first day of the LMP and is then confirmed by, preferably, a first-trimester CRL measurement. The LMP-established date is preferred; when the discrepancy between the LMP and CRL method is larger than 7 days, the ultrasound-established date is used.[12] In the national UK and Dutch guidelines, it is recommend that all pregnancies should be dated by ultrasound (CRL) rather than by LMP.[13,14]

The LMP is known to be subject to recall error, which worsens with increasing time between LMP and recall, and has a tendency to overstate the duration of pregnancy.[4,6] Moreover, the LMP dates appear to be affected by digit preference. Although two studies have demonstrated an increased digit preference for the first, fifth, 10th, 15th, 20th, 25th and 28th days of the month,[4,5] little is known about the factors which influences digit preference. The aim of the current investigation was to assess the preference of certain dates of the LMP in our population, to determine which determinants are associated with LMP digit preference and to estimate the effect on pregnancy outcome.

METHODS

Data collection

We carried out a retrospective analysis using the ultrasound database Astraia (version 1.20.0_138) of the department of Obstetrics and Prenatal Medicine in the Erasmus MC, Rotterdam, the Netherlands. This dataset contains all obstetric ultrasound examinations performed at our hospital between January 2000 and December 2009.We included only cases with a reported LMP. Thus, cases with a known due date but without information about LMP and cases of artificial reproduction techniques in which the date of conception was reported instead of LMP were excluded from the analysis.

This database contains the following parameters: maternal age, parity, postal code, cycle regularity (yes/no/unknown), certainty of given LMP, <3 cycles after previous pregnancy

or cessation of contraception, conception (spontaneous or after ovulation induction/ intrauterine insemination (IUI)), body mass index (BMI; kg/ m^2), smoking (yes/ no), ultrasound investigator, date of ultrasound examination, CRL measurement between 15.5 and 60 mm (gestational age between 8^{+0} and 11^{+6} weeks) and the date of the CRL measurement. In cases in which more than one CRL measurement between 8^{+0} and 11^{+6} weeks was performed, the first CRL measurement was used for further analysis as the first measurement is usually used for determination of the due date. In the research period, the LMP-established due date was preferred unless the discrepancy between the LMP and CRL method was >7 days, when the ultrasound-established date was used.

As the Erasmus MC is a tertiary hospital centre, women were referred throughout pregnancy. Therefore, first-trimester ultrasound measurements were available in a part of the cohort.

Data analysis

Without digit preference, it can be expected that there will be no difference in the prevalence of the LMP date for every day of the month. LMP digit preference was determined by comparing the observed to expected counts of each day of a month, taken into account the number of months with <31 days. Chi-square tests were performed to determine whether digit preference was present in any of the subgroups: (non)deprived neighbourhood, cycle irregularity, certainty of LMP date, maternal age, smoking (yes/ no), cessation of smoking, BMI, parity and ultrasound investigator (consultant gynaecologist, resident or ultrasonographer). Using the Dutch index of deprivation 2007, together with participants' postal codes, we categorised women as residing within a deprived neighbourhood and women living outside of these areas. The deprivation index 2007 is a measure of neighbourhood deprivation designed by 'the Netherlands Ministry of Housing, Spatial Planning, and the Environment'.[15] This index aims to identify communities at risk based on five domains of deprivation, namely housing, employment, education, integration, and safety. Using the index, 83 of 4.878 Dutch postal codes have been identified as deprived, 23 of which are located in Rotterdam (28%).

Differences in subgroups were analysed using a logistic regression model adjusting for possible confounders. To illustrate the difference between groups in the patterns over the day of the month of the reported last menstruation, a Poisson model was estimated. In this model, the number of women who reported a specific day of the month was used as the response. To adjust for the fact that certain days occur less often than others (e.g not every month has a 31st day) the logarithm of the expected count was used as an offset variable. The interaction between the group and the day of the month (which we used as a categorical covariate) was used as explanatory variable. The antilogs of the parameters of this model were interpreted as the ratio between the observed dates of the month and the expected count. Obviously, there are a lot of parameters in this model [number of days of the month (28-31) times the number of subgroups]. Therefore, to increase the accuracy of the estimates, two forms of shrinkage were applied to reduce the variance of the estimates. First, the average (log) ratio for each day was slightly shrunken towards zero. Then, the estimates of the (log) ratios in the different subgroups were shrunken

towards this average for each day. The amount of shrinkage was chosen on the based of the Akaike information criterion, and so a balance between the fit of the model and the model complexity was obtained.[16]

The differences in clinical outcome were analysed using the chi-square test and, when appropriate, McNemar test. Differences are noted as the odds ratio (OR) and 95% confidence interval (95% CI). The statistical analysis was performed using SPSS for Windows, version 20.0.0 (SPSS Inc., Chicago, IL, USA). A probability value of P <0.05 was considered to be statistically significant in this study.

RESULTS

The database consisted of 32.188 pregnancies. Excluded were cases in which the LMP data were lacking (6.097) and those in which the duration of pregnancy was based on the conception date (1.426). This resulted in 24.665 cases with a reported LMP being available for further analysis. When corrected for the difference in length of the months, it is expected that the digits 1-28, 29, 30 and 31 will be reported in 3.28, 3.12, 3.04 and 1.93% of cases respectively. However, of these days the first (OR 1.28, 95% CI 1.20-1.36), fifth (OR 1.10, 95% CI 1.03-1.17), 10th (OR 1.17, 95% CI 1.09-1.25), 15th (OR 1.31, 95% CI 1.23-1.40), 20th (OR 1.22, 95% CI 1.15-1.30), and 25th (OR 1.08, 95% CI 1.01-1.15) days of the month were reported more frequently than expected. They were reported in 4.16, 3.81, 4.27, and 3.98% of cases,

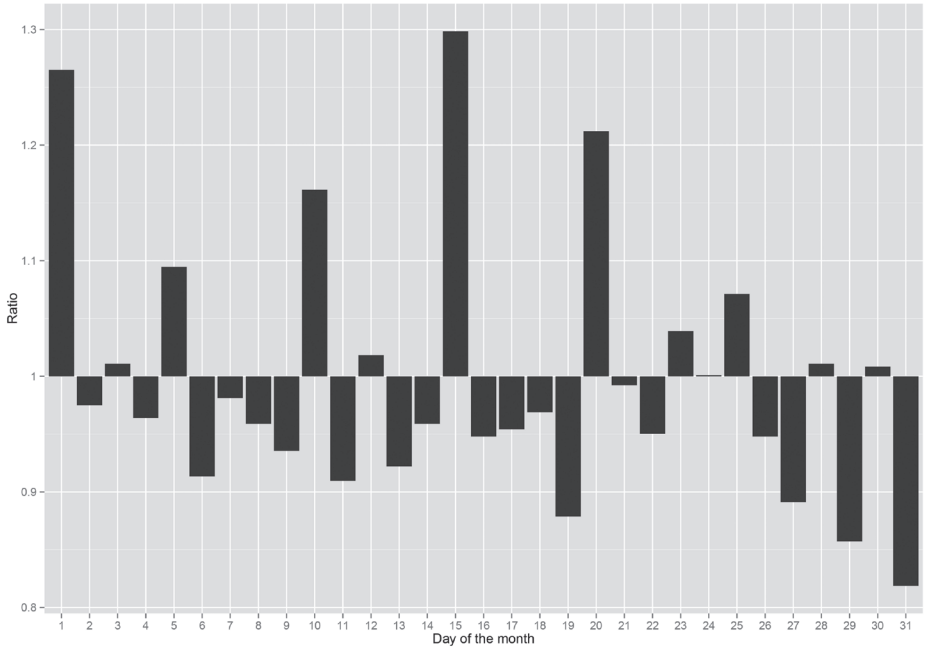

Figure 1. Digit preference for the date of the month of the last menstrual period (LMP). The preference is depicted by a ratio between the observed count of dates and the expected count of dates.

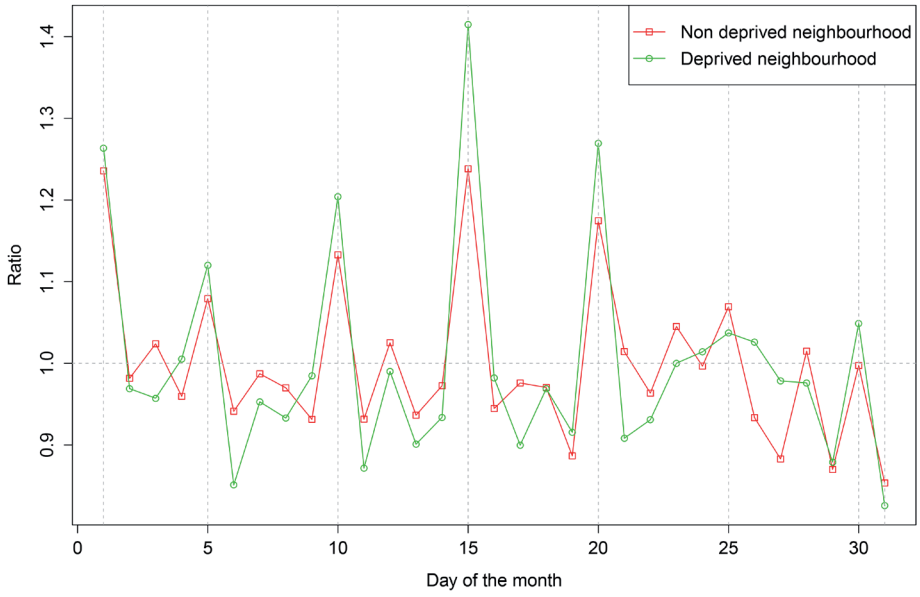

Figure 2A. A smoothed figure to illustrate the difference in digit preference for the date of the month of the last menstrual period (LMP) between women living in a deprived neighbourhood and women living in a non-deprived neighbourhood. The preference is depicted by a ratio between the observed count of dates and the expected count of dates.

respectively (Figure 1). The digit preference for these preferred LMP dates was most obvious for the first and 15th days of the month.

Furthermore, the sixth (OR 0.91, 95% CI 0.82-0.98), 11th (OR 0.91 (95% CI 0.84-0.98), 13th (OR 0.92, 95% CI 0.86-0.99), 19th (OR 0.88, 95% CI 0.81-0.94), 27th (OR 0.89, 95% CI 0.82-0.96), 29th (OR 0.85, 95% CI 0.78-0.91) and 31st (OR 0.81, CI 0.73-0.90) days of the month were recorded less frequently than expected. The least popular LMP dates were the 29th and 31st days of the month, observed 0.85 and 0.81 times less often than expected (2.65 versus 3.04% and 1.57 versus 1.93%). The remaining days of the month were recorded as expected.

A significantly larger LMP digit preference for women in deprived neighbourhoods ($p < 0.001$) was found using the chi-square test (Figure 2A). The same was true for women with an irregular menstrual cycle and for those with a reported uncertain LMP date ($p < 0.001$) (Figure 2B). No difference in digit preference was observed for parity, smoking, type of ultrasound investigator, maternal age and obesity.

Multivariate logistic regression also showed significant differences in digit preference for the first and 15th days of the month for women with a regular menstrual cycle and an uncertain LMP date, a certain LMP date but an irregular menstrual cycle and women of a deprived neighbourhood irrespective of certainty of LMP and cycle regularity (Table I). No differences were found for the type of ultrasound investigator, smoking, maternal age, obesity, cycle regularity with a certain LMP, a pregnancy within 3 cycles after cessation of conception or after previous pregnancy, and parity.

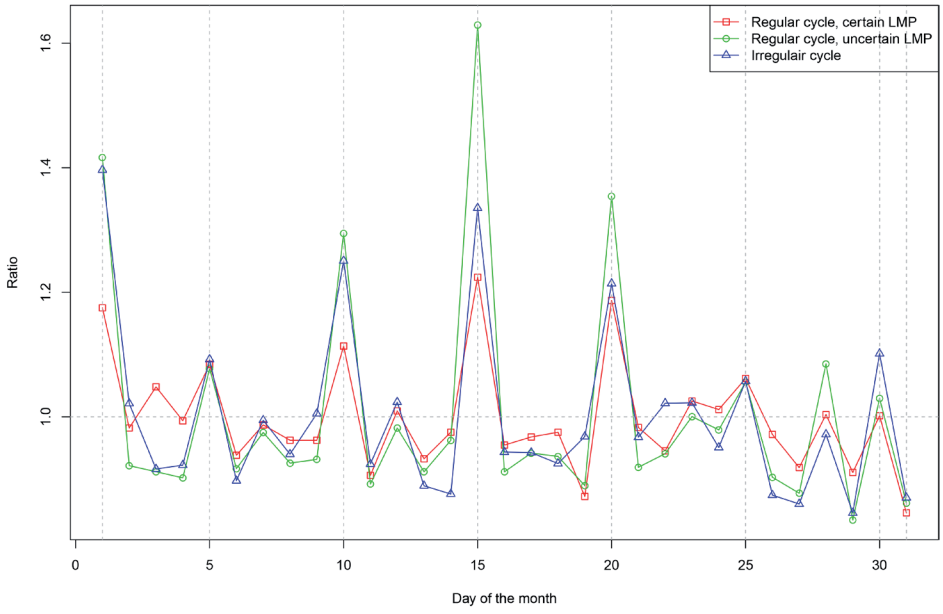

Figure 2B. A smoothed figure to illustrate the difference in digit preference for the date of the month of the last menstrual period (LMP) by certainty of LMP and the regularity of the menstrual cycle. The preference is depicted by a ratio between the observed count of dates and the expected count of dates.

Table I. Multivariate logistic regression on the association of determinants on the last menstrual period digit preference for the first and 15th days of the month.

		OR	95% CI	p-value
Investigator	Consultant gynaecologist	0.96	0.84-1.10	0.50
	Resident	1.04	0.90-1.21	0.59
	Ultrasonographer	1.03	0.87-1.21	0.73
Deprived Neighbourhood		1.21	1.06-1.39	<0.01
Cycle	Uncertain LMP	2.03	1.63-2.52	<0.01
	Irregular cycle	1.24	1.06-1.44	<0.01
	<3 cycles after contraception	1.11	0.87-1.39	0.40
	<3 cycles after pregnancy	1.21	0.86-1.67	0.26
	Unknown cycle	1.60	1.08-2.28	0.01
Multiparity		1.05	0.93-1.17	0.44
Smoking		1.09	0.93-1.18	0.29
	Cessation of smoking	0.90	0.66-1.17	0.45
Age (years)		0.99	0.98-1.00	0.18
BMI (kg/m^2)		1.00	0.99-1.01	0.99

CI, confidence interval; BMI, body mass index; LMP, last menstrual period; OR, odds ratio.

The recorded LMP occurred more often than expected on a Monday (OR 1.09, 95% CI 1.05-1.13), Thursday (OR 1.04, 95% CI 1.00-1.08) and Friday (OR 1.07, 95% CI 1.03-1.10). The recorded LMP occurred less frequently than expected on a Sunday (OR 0.88, 95% CI 0.84-0.91) and Tuesday (OR 0.94, 95% CI 0.91-0.97). On the remaining weekdays the recorded LMP occurred as often as expected (Wednesday (OR 1.00, 95% CI 0.97-1.04) and Saturday (OR 0.99, 95% CI 0.96-1.03)).

In 9.667 of the 24.665 cases, a first-trimester CRL measurement was available, the remainder of the cases (14.998) were seen after the first trimester. The due date in women with a preference for the first or 15th day of the month was 0.39 days later when determined by CRL relative to LMP. In comparison, in women with no preference for the first or 15th day of the month, the due date was 0.06 days later when determined by CRL relative to LMP ($p < 0.01$). In 4.630 of these 9.667 cases with available CRL measurements, the pregnancy outcome was known. After subtracting cases of miscarriage, termination of pregnancy and fetal death, 4.271 live births remained for further analysis. In these cases, pregnancy dating by LMP resulted in more post-term pregnancies (≥ 42 weeks) relative to CRL dating (OR 1.27, 95% CI 1.05-1.54). No differences were observed in post-term pregnancies of ≥ 41 weeks (OR 1.02, 95% CI 0.92-1.13) or in preterm pregnancies of < 37 (OR 1.00, 95% CI 0.86-1.17) and < 34 weeks (OR 0.94, 95% CI 0.72-1.24) in pregnancy dated by LMP or CRL.

More post-term pregnancies ≥ 42 weeks based on LMP were observed in the digit preference group relative to the non-digit preference group, whereas no difference was observed in post-term pregnancies of ≥ 42 weeks based on CRL dating (Table II). No differences were observed in birth weight, gestational age at birth, post-term pregnancies of ≥ 41 weeks, preterm pregnancies of < 37 and < 34 weeks in pregnancy dated by LMP or CRL for women with digit preference for the first and 15th days of the month relative to other days.

DISCUSSION

Main Findings

Of the 24.665 women in our database, the LMP was reported more frequently than expected on the first, fifth, 10th, 15th, 20th, and 25th days of the month, whereas the LMP date was reported less frequently than expected on the sixth, 11th, 13th, 19th, 27th, 29th and 31st days of the month. The preference occurred more frequently in women living in a deprived neighbourhood, in women with a regular menstrual cycle but an uncertain LMP, and in women with an irregular cycle. In women with a digit preference relative to women without a digit preference, the due date based on CRL was 0.33 days later than that based on LMP. More postterm (≥ 42 weeks) deliveries were observed in LMP-dated related to CRL-dated pregnancies. This association was stronger in women with a digit preference.

Strengths and limitations

The major strength of this cohort study was the large number of women included and the determinants available for analysis. To the best of our knowledge, this study has the

Table II. Digit preference and Obstetric outcome

	All days		Day 1 & 15		Other days		z-value or		
	N = 4.271	SEM/ %	N = 313	SEM/ %	N =3.958	SEM/ %	OR	95% CI	p-value
Birth weight (gram)	3415	20	3354	40	3420	21	-66	(-144, +12)	0.39
Pregnancy duration (LMP)	276.1	0.2	276.0	1	276.1	0.2	-0.1	(-2.2, +1.9)	0.97
Pregnancy duration (CRL)	276.2	0.2	275.4	0,9	276.3	0.2	-0.9	(-2.7, +0.9)	0.30
Postterm ≥42 weeks (LMP)	253	5.9%	27	8.6%	226	5.7%	OR 1.56	(1.03-2.37)	0.04
Postterm ≥41 weeks (LMP)	943	22.1%	75	24.0%	868	21.9%	OR 1.12	(0.86-1.47)	0.40
Preterm <37 weeks (LMP)	356	8.3%	26	8.3%	330	8.3%	OR 1.00	(0.66-1.51)	1.00
Preterm <34 weeks (LMP)	103	2.4%	11	3.5%	92	2.3%	OR 1.12	(0.81-2.89)	0.18
Postterm >42 weeks (CRL)	201	4.7%	15	4.8%	186	4.7%	OR 1.02	(0.60-1.75)	0.89
Postterm >41 weeks (CRL)	927	21.7%	68	21.7%	859	21.7%	OR 1.00	(0.76-1.32)	1.00
Preterm <37 weeks (CRL)	356	8.3%	24	7.7%	332	8.4%	OR 0.91	(0.59-1.40)	0.75
Preterm <34 weeks (CRL)	109	2.6%	13	4.2%	96	2.4%	OR 1.74	(0.97-3.15)	0.09

SEM, standard error of the mean; OR, odds ratio; CI, confidence interval; LMP, last menstrual period; CRP, crown-rump length

largest population to determine digit preference and its association with determinants and pregnancy outcome.

Some limitations to this study may apply. As our clinic is a tertiary referral hospital, data on both first-trimester ultrasound and obstetric outcome were not available in all women. Some women were referred by us to the midwife or secondary referral hospital after the first-trimester ultrasound and others were referred to our clinic for a consultation for antenatal ultrasound screening. However, a rather large group of 4.271 women remained for further analysis. Another limitation to the present study may be that data on ethnicity were not available.

Interpretation

In our large database, we confirmed findings of previous studies on LMP digit preference. Savitz et al.[4] observed a preference for days 1, 5, 15 and 20 of the month and not for days 10 and 25 of the month in 4.485 women. Waller et al.[5] found a preference for the 28th day of the month, as well as the first, fifth, 10th, 15th, 20th and 25th days of the month in 43.880 women. Comparing these previous studies with our present study, the most commonly reported day was the 15th of the month. It was observed 2.1 times[4], 1.7 times[5] and 1.3 times (present study) more frequently relative to the expected frequency. The above differences may be explained by factors such as the difference in size of the study population and in the years of data collection. The data in the study of Waller et al.[5] were collected in 1987, of Savitz et al.[4] in 1995-2001 and of the present study in 2000-2009. It may be that women nowadays are more aware of the importance of LMP and have a better recall of the date, or that electronic devices and phones may help to record these dates more precisely.

In women without digit preference, the difference between the due date based on CRL and on LMP was small (0.06 days); the difference was larger in women with digit preference (0.39 days). No other study has reported this finding. Consistent with other studies, in LMP-dated pregnancies relative to CRL-dated pregnancies, we observed an overestimation of post-term pregnancies of ≥ 42 weeks, but not for (severe) preterm, term and post-term (≥ 41 weeks) pregnancies.[4,7,17-19] We also found that, in women with preference for the first and 15th days of the month, this overestimation of post-term (≥42 weeks) deliveries was significantly higher than in women without a digit preference. The increase in post-term pregnancies in LMP-dated pregnancies is in concert with another study about recall of the LMP in 385 women with prospectively recorded LMP dates. Women with an inaccurate LMP recall tended to overestimate rather than underestimate the time since LMP.[6] It seems that women with an inaccurate recall of LMP tend to round the date down to one of the preferred dates and most likely to the first and 15th days of the month. This effect in our study was more apparent in women living in a deprived neighbourhood. Camarda et al.[20] discussed a complex composite link model, to determine the estimation of proportions of counts that were transferred to neighbouring digits. Although the model could be used to reclassify misreported digits and could be employed in future studies, it does not fit the purpose of the current study.

In a study embedded within The Generation R Study, a population-based cohort study of pregnant women between 2002 and 2006 in Rotterdam, Timmermans et al.,[21]

investigated obstetric outcome and perinatal risk factors differences between women living in deprived and non-deprived neighbourhoods using the same Dutch index of deprivation as the current study. Another study, using the same Dutch index of deprivation, reported on perinatal health inequalities using the Dutch National Perinatal Registry.[22] Both studies observed increased risks for pre-eclampsia, FGR, prematurity, lower 5-minutes Apgar score and perinatal mortality in women from deprived neighbourhoods.[21,22] The increased risks could be attributed to a greater accumulation of risk factors in these women. The pregnant women from a deprived neighbourhood were younger, had a lower education and income, more often a single status, a non-western ethnicity, an unplanned pregnancy and a late first booking relative to women from a non-deprived neighbourhood.[21,22] These factors can also contribute to the digit preference observed in women from a deprived neighbourhood.

CONCLUSIONS

We observed digit preference for LMP data in our population. This was especially the case for women living in a deprived neighbourhood and for women with an uncertain LMP or an irregular cycle. Pregnancy dating by LMP, relative to CRL dating, is associated with an increased number of post-term pregnancies, and the prevalence of post-term pregnancies is further increased in women with a digit preference. A discussion on the differences between LMP-based and CRL-based dating seem to end in favour of CRL dating.[11] Those who are in favour of LMP dating must realise that recall errors may be responsible for inaccurate pregnancy dating, with a preference for certain days of the week and month.

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2.2

NON-VISUALIZED PREGNANCY LOSSES ARE PROGNOSTICALLY IMPORTANT FOR UNEXPLAINED RECURRENT MISCARRIAGE

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ABSTRACT

Study question

Are non-visualized pregnancy losses (biochemical pregnancy loss and failed pregnancy of unknown location combined) in the reproductive history of women with unexplained recurrent miscarriage (RM) negatively associated with the chance of live birth in a subsequent pregnancy?

Summary answer

Non-visualized pregnancy losses contribute negatively to the chance for live birth: each non-visualized pregnancy loss confers a relative risk (RR) for live birth of 0.90 (95% CI 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage.

What is known already

The number of clinical miscarriages prior to referral is an important determinant for live birth in women with RM, whereas the significance of non-visualized pregnancy losses is unknown.

Study design, size, duration

A retrospective cohort study comprising 587 women with RM seen in a tertiary RM unit 2000–2010. Data on the outcome of the first pregnancy after referral were analysed for 499 women.

Participants/materials, setting, methods

The study was conducted in the RM Unit at Rigshospitalet, Copenhagen, Denmark. We included all women with unexplained RM, defined as ≥3 consecutive clinical miscarriages or non-visualized pregnancy losses following spontaneous conception or homologous insemination. The category 'non-visualized pregnancy losses' combines biochemical pregnancy loss (positive hCG, no ultrasound performed) and failed PUL (pregnancy of unknown location, positive hCG, but on ultrasound, no pregnancy location established). Demographics were collected, including BMI, age at first pregnancy after referral and outcome of pregnancies prior to referral. Using our own records and records from other Danish hospitals, we verified the outcome of the first pregnancy after referral. For each non-visualized pregnancy loss and miscarriage in the women's reproductive history, the RR for live birth in the first pregnancy after referral was determined by robust Poisson regression analysis, adjusting for risk factors for negative pregnancy outcome.

Main results and the role of chance

Non-visualized pregnancy losses constituted 37% of reported pregnancies prior to referral among women with RM. Each additional non-visualized pregnancy loss conferred an RR for live birth of 0.90 (95% CI 0.83; 0.97), which was not statistically significantly different from the corresponding RR of 0.87 (95% CI 0.80; 0.94) conferred by each clinical miscarriage. Among women with ≥2 clinical miscarriages, a reduced RR for live birth was also shown: 0.82 (95% CI 0.74; 0.92) for each clinical miscarriage and 0.89 (95% CI 0.80; 0.98) for each non-visualized pregnancy loss, respectively. Surgically treated ectopic pregnancies (EPs)

were significantly more common for women with primary RM and no confirmed clinical miscarriages, compared with women with primary RM and ≥1 clinical miscarriage (22 vs. 6%, difference 16% (95% CI 9.1%; 28.7%); RR for ectopic pregnancy was 4.0 (95% CI 1.92; 8.20).

Limitations, reasons for caution

RM was defined as ≥3 consecutive pregnancy losses before 12 weeks' gestation, and we included only women with unexplained RM after thorough evaluation. It is uncertain whether the findings apply to other definitions of RM and among women with known causes for their miscarriages.

Wider implications of the findings

To our knowledge, this is the first comprehensive investigation of prior non-visualized pregnancy losses and their prognostic significance for live birth in a subsequent pregnancy in women with unexplained RM. We show that a prior non-visualized pregnancy loss has a negative prognostic impact on subsequent live birth and is thus clinically significant.

INTRODUCTION

The term early pregnancy loss covers three different clinical scenarios: miscarriage, where transvaginal ultrasound (TVS) or histological findings document an intrauterine demise before 12 weeks' gestation; ectopic pregnancy (EP), where TVS or laparoscopy identifies a pregnancy outside the uterine cavity; and biochemical pregnancy loss, where there is a positive pregnancy test but no ultrasound has been performed. In the event where a woman has a positive pregnancy test and TVS is performed, but neither an intrauterine nor an ectopic pregnancy is seen, the pregnancy is classified as a 'pregnancy of unknown location' (PUL).[1] Following an initial classification of PUL, the possible final diagnoses are: an ongoing intrauterine pregnancy; an ectopic pregnancy; a failed PUL; or an intrauterine miscarriage.[1]

When dealing with an acute early pregnancy complication, the distinction between different types of early pregnancy loss is very important as it has implications for the prognosis, treatment and follow-up of patients.[1,2] In contrast, when considering the past reproductive history of a patient referred to a recurrent miscarriage (RM) unit, the importance of early pregnancy losses such as failed PULs and/or biochemical pregnancy losses, has not been well studied. We hypothesize that biochemical pregnancy losses and failed PULs share similar prognostic importance. Therefore, in addition to separate analyses for biochemical pregnancy losses and failed PULs, we group these two diagnoses together as 'non-visualized pregnancy losses' defined as a pregnancy loss initially confirmed by a positive hCG, but not visualized by TVS, if performed.

The definition of RM is controversial. A guideline from the European Society of Human Reproduction and Embryology (ESHRE), as well as the Royal College of Obstetricians and Gynaecologists (RCOG) define RM as three or more consecutive pregnancy losses.[3,4] However, The American Society for Reproductive Medicine (ASRM) Practice Committee defines recurrent pregnancy loss as two or more clinical miscarriages confirmed by ultrasound or histology, not necessarily consecutive.[5] Non-visualized pregnancy losses are thus not included in the ASRM Practice Committee definition, nor in other recent publications.[6]

Non-visualized pregnancy losses in women with RM are increasingly diagnosed because very early pregnancy testing is readily available.[7] Whether non-visualized pregnancy losses should be included in the definition criteria for RM is controversial. If they negatively affect the chance of a subsequent live birth, then non-visualized pregnancy losses are clinically relevant.

To investigate whether prior non-visualized pregnancy losses are clinically relevant, we collected data over 10 years on the outcome of the first pregnancy after referral to the Danish Recurrent Miscarriage Unit.

MATERIALS AND METHODS

A retrospective cohort study comprising 918 consecutive women seen in the Danish RM Unit at the Fertility Clinic, University Hospital Copenhagen, Rigshospitalet from January 2000 to January 2011, was performed. We included only women who we considered as having unexplained RM, i.e. who fulfilled the following criteria: at least three consecutive

pregnancy losses, including both clinical miscarriages and non-visualized pregnancy losses; age <40 years at referral; regular menstrual cycle with length 23-35 days (variation from cycle to cycle was ≤2-3 days); normal uterine evaluation by hysteroscopy, hysterosalpingogram or uterine hydrosonography; normal parental karyotypes; and negativity for the lupus anticoagulant. We excluded women who had conceived after IVF/ICSI or donor insemination prior to referral. Figure 1 gives an overview of study flow. In short, 331 women were excluded. Forty women (7%) were lost to follow-up and according to their records, 48 women (9%) did not conceive after referral. Outcome of first pregnancy after referral was registered for 499 women: 290 with primary RM (PRM) (58%) and 209 with secondary RM (SRM) (42%). Of these, 368 (74%) had experienced ≥2 clinical miscarriages and thus fulfilled the ASRM criteria for recurrent pregnancy loss as well as the ESHRE/RCOG criteria.

As is standard practice in this RM unit, at first consultation, all women had given a detailed written account of their reproductive history along with documentation on where, when and how their previous pregnancies had been managed. Treatment regimens in a subsequent pregnancy varied according to medical history, and included 'tender loving care' (TLC) with or without intravenous immunoglobulin (IVIg). Twenty-seven women received IVIg or placebo from 2008 to 2013 (NCT00722475). One hundred and ten women received IVIg in a non-randomised fashion, before 2008 or being ineligible for the trial. These women had had at least four early pregnancy losses or at least one unexplained late miscarriage and two early pregnancy losses.

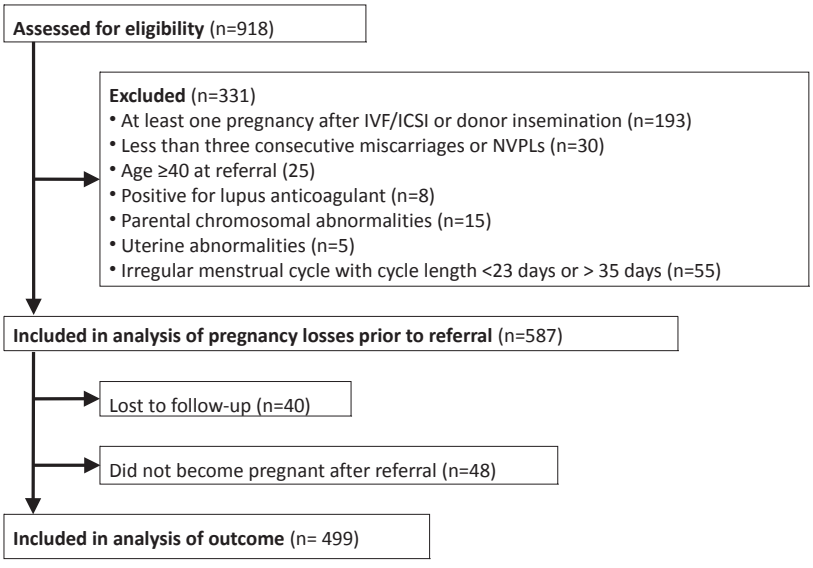

Figure 1. Inclusion of women in the cohort. All women were seen between January 2000 and January 2011. PRM, primary recurrent miscarriage; SRM, secondary recurrent miscarriage; NVPL, non-visualized pregnancy loss.

Patients were followed at the RM Unit until 16 weeks' gestation, after which the women were referred for continued monitoring at their local hospital. Information on outcome on first pregnancy after referral was obtained either from patient records or from the women themselves.

For this study, the women' information was entered in a Microsoft Office Access 2010 database by two of the authors (A.M.K. and O.B.C.). Double entry was avoided using the unique Danish identification number. Prior to statistical analysis, data quality was checked manually (A.M.K.).

We divided early pregnancy events into the following categories: miscarriage, where ultrasound or histology documented an intrauterine pregnancy loss before 12 weeks' gestation; EP, where a pregnancy loss was visualized outside the uterus by laparoscopy or TVS; failed PUL, where there had been a positive hCG, but no location was established by TVS; biochemical pregnancy loss, as a positive hCG, but no TVS performed. The two categories 'failed PUL' and 'biochemical pregnancy loss' were combined as 'non-visualized pregnancy losses'. As the study is retrospective, all diagnoses are final.

For 88% of the women in the cohort, we relied on self-reporting and records available at time of referral. However, as a data quality check, we obtained further details on reported non-visualized pregnancy losses for the 61 women born on the first to third of each month.

Statistics

As we have chosen to report our results as relative risk (RR) and the outcome 'live birth' was common (>10%), we used robust Poisson regression instead of standard logistic regression.[8] In the Poisson regression analysis we used non-visualized pregnancy losses as the independent variable and corrected for the risk factors PRM versus SRM; age at index pregnancy; the number of prior early and late miscarriages; EPs; and treatment. Equivalent analyses were performed with non-visualized pregnancy losses split into biochemical pregnancy losses and failed PULs. We also used miscarriage as independent variable equivalent to non-visualized pregnancy loss. As standard Poisson regression uses the log-link function, female age in years, early miscarriages, non-visualized pregnancy losses, biochemical pregnancy losses, failed PULs and EPs were modelled as linear variables on the logit scale. Testing for linearity showed no problems for any of the variables. Model control was performed. There were no signs of interaction for any of the variables and thus multiple regression analysis was deemed appropriate. For these analyses the statistical software package STATA 11 was used.

Fisher's exact test was used to test the hypothesis of equal proportions of ectopic pregnancies (EPs) between different groups of patients. *T*-test was used for comparison of gestational age between groups of pregnancy loss. For these analyses we used the statistical software package SAS 19.2.

RESULTS

Reproductive history

Of 2781 pregnancies reported at first consultation, 327 were births after Week 22 (12%). Of the 2454 pregnancy losses, there were 1426 miscarriages before Week 12 (58%), 578 were biochemical pregnancy losses (23%) and 334 (16%) were failed PULs. Thus non-visualized pregnancy losses constituted 37% of all pregnancy losses before referral in this group.

Additionally, there were 73 late miscarriages between Week 12 and 22 (3%) and 43 EPs (2%), (Figure 2). All EPs had been treated surgically.

Figure 3 shows the distribution of biochemical pregnancy losses, failed PULs and miscarriages by gestational age. The mean gestational age for biochemical pregnancy losses was 6.08 weeks (95% CI for the mean 5.96; 6.19) and for failed PULs 6.59 (95% CI 6.43; 6.75). The difference is 0.51 weeks (95% CI 0.33; 0.70). The mean gestational age for clinical miscarriages was 8.87 (95% CI 8.74; 9.01), significantly higher than for non-visualized pregnancy losses, mean difference 2.60 weeks (95% CI 2.44; 2.76).

As shown in figure 4, women with PRM and no clinical miscarriages had a statistically significantly higher frequency of surgically treated EPs than those with at least one clinical miscarriage (22 versus 6%, difference 16% (95% CI 9.1%; 28.7%);), corresponding to an RR for having had an EP of 4.0 (95% CI: 1.92; 8.20) in the former group. We did not confirm the finding for women with SRM.

The women for whom we attempted to obtain further details about prior non-visualized pregnancy losses reported a total of 123 non-visualized pregnancy losses, of which 77 (63%) were biochemical pregnancy losses and 46 (37%) were failed PULs. We were able to confirm the self-reported information in all cases except one; the woman reported a biochemical pregnancy loss, which actually was a miscarriage. Thus in 99% of cases, the self-reported information of non-visualized pregnancy losses was confirmed.

Importance of reproductive history on live birth

When analysing all 499 women in the cohort, the RR for live birth for each non-visualized pregnancy loss was 0.90 (95% CI 0.83; 0.97), and for each clinical miscarriage 0.87 (95% CI

Table I. Relative risk (95% CI) of live birth in the index pregnancy, unadjusted for treatment.

	All women	Women with ≥2 clinical miscarriages	Women receiving 'Tender loving care'
Variable	RR (95% CI)	RR (95% CI)	RR (95% CI)
Pregnancy losses	N=499	N=368	N=344
Age at index pregnancy after referral	0.98 (0.96;0.99)	0.98 (0.96;1.00)	0.97 (0.96;0.99)
Prior miscarriage	0.87 (0.80;0.94)	0.82 (0.74;0.92)	0.86 (0.78;0.96)
Prior non-visualized pregnancy loss[a]	0.90 (0.83;0.97)	0.89 (0.80;0.98)	0.90 (0.82;1.00)
Prior biochemical pregnancy loss	0.89 (0.82;0.97)	0.88 (0.79;0.98)	0.92 (0.83;1.02)
Prior failed pregnancy of unknown location	0.91 (0.82;1.02)	0.89 (0.77;1.04)	0.84 (0.71;0.99)
BMI	N=312	N=228	N=221
BMI ≤20	1.13 (0.90;1.41)	1.28 (1.00;1.63)	1.00 (0.77;1.31)
BMI 21-25	1.00 (reference)	1.00 (reference)	1.00 (reference)
BMI 26-29	1.02 (0.77;1.35)	1.00 (0.70;1.43)	0.85 (0.58;1.24)
BMI ≥30	1.04 (0.81;1.35)	1.06 (0.79;1.43)	0.97 (0.70;1.33)

[a]Non-visualized pregnancy loss: combines biochemical pregnancy losses and failed Pregnancies of unknown location.

0.8; 0.94). For biochemical pregnancy losses the RR for live birth was 0.89 (95% CI 0.82; 0.97) and for failed PULs 0.91 (95% CI 0.82; 1.02). We found no statistically significant difference between the RRs for live birth conferred by each non-visualized pregnancy loss and each miscarriage in any of the analyses.

For women with ≥2 clinical miscarriages, the RR for live birth was 0.89 (95% CI 0.80; 0.98) for non-visualized pregnancy loss and 0.82 (95% CI 0.74; 0.92) for clinical miscarriage and for biochemical pregnancy loss and failed PUL, the RR was 0.88 (95% CI 0.79; 0.98) and 0.89 (0.77; 1.04), respectively.

From Table I we noted that increasing age at first pregnancy after referral was a small, but consistently significant negative prognostic factor in almost all subgroups with RR for live birth ranging from 0.97 to 0.99 for each additional year.

When limiting the Poisson regression analysis to the 312 women (63%) for whom we had registered BMI, there was no significant change in RR for live birth and BMI in itself had no

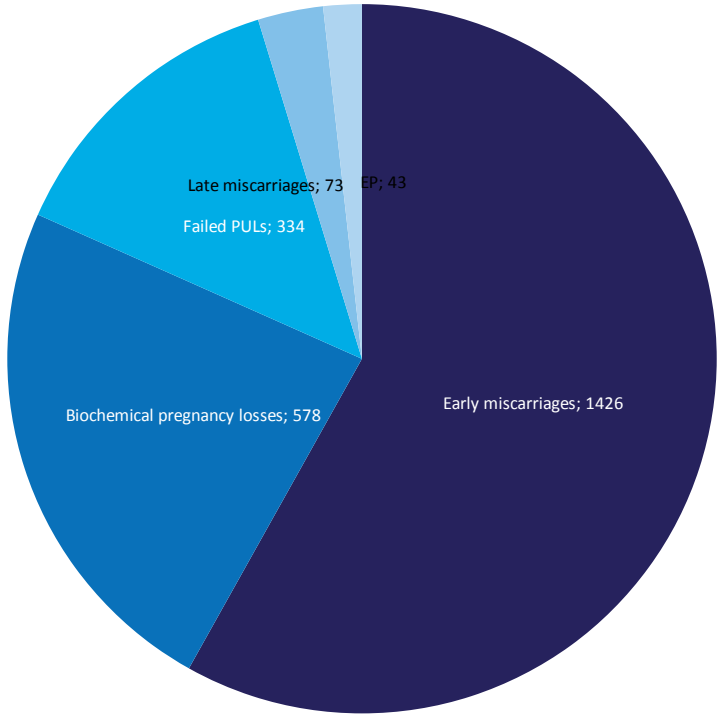

Figure 2. Number and type of pregnancy losses (reproductive history) reported by 587 RM women at first consultation. In addition, 269 births after 22 weeks' gestation were reported. EP: surgically treated ectopic pregnancy; late miscarriage: intrauterine pregnancy loss after 12 weeks' gestation; early miscarriage: histologically or ultrasonically confirmed intrauterine pregnancy loss before 12 weeks' gestation; biochemical pregnancy loss: positive hCG, no ultrasound performed; failed PUL: failed pregnancy of unknown location, positive hCG, but location not established by ultrasound.

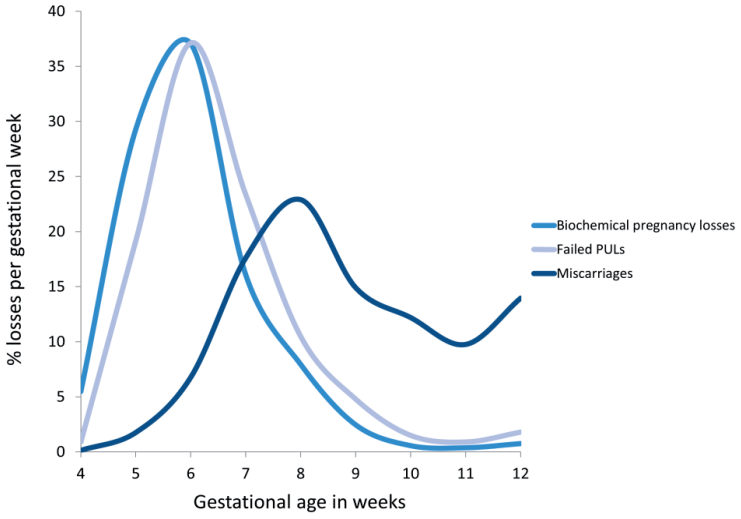

Figure 3. Percentages of biochemical pregnancy losses, failed PULs and miscarriages according to gestational age. PULS, pregnancy of unknown location.

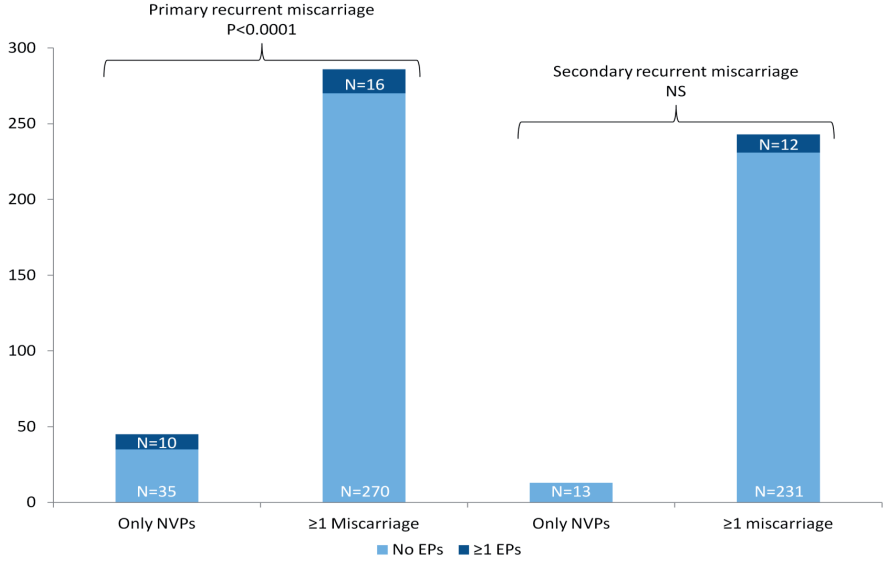

Figure 4. Frequency of a history of surgically treated ectopic pregnancies (EPs) according to presence or absence of confirmed miscarriages in the history, among 587 women; 331 with primary and 256 with secondary recurrent miscarriage. EP: surgically treated ectopic pregnancy; miscarriage; histologically or ultrasonically confirmed intrauterine pregnancy loss before 12 weeks' gestation; NVPLs: non-visualized pregnancy losses; biochemical pregnancy losses and failed pregnancies of unknown location combined.

significant effect on outcome, neither as a continuous variable nor as a grouped variable (BMI<20; 20-24; 25-29; ≥30; with BMI 20-24 as reference) (see Table I).

We analysed the 290 PRM and 209 SRM women separately and the RR did not change significantly, but as expected, the confidence intervals widened due to smaller numbers in each subgroup (Table III).

Treatment

In an analysis of RR for live birth by non-visualized pregnancy loss, clinical miscarriage and biochemical pregnancy loss and failed PULs among the 344 women who received only TLC we found comparable results as for the total group of 499 patients, as can be seen in Table I.

Ninety-eight women received IVIg in addition to TLC and had an RR for live birth of 1.27 (95% CI 1.07; 1.52) compared to TLC alone, and the RR of IVIg for live birth for patients with PRM was 1.39 (1.10; 1.76). Adjustment for treatment did not significantly alter the effect of non-visualized pregnancy losses, miscarriages, biochemical pregnancy losses and failed PULs on the RR for live birth (see Table II).

Table II. Relative risk (95% CI) of live birth in the index pregnancy, adjusted for treatment.

	All women		Women with ≥2 clinical miscarriages	
Variable		RR (95% CI)		RR (95% CI)
All women	N		N	
Age at first pregnancy after referral	499	0.98 (0.96;0.99)	368	0.98 (0.96;1.00)
Prior miscarriage		0.86 (0.79;0.93)		0.81 (0.72;0.91)
Prior non-visualized pregnancy loss[a]		0.89 (0.82;0.96)		0.88 (0.79;0.97)
Prior biochemical pregnancy loss		0.88 (0.81;0.97)		0.88 (0.79;0.98)
Prior failed pregnancy of unknown location		0.90 (0.80;1.00)		0.87 (0.74;1.02)
'Tender loving care'	344	1 (reference)	243	1.00 (reference)
'Tender loving care' and IvIg alone	98	1.27 (1.07;1.52)	77	1.36 (1.11;1.67)
'Tender loving care', IvIg and other	12[b]	1.17 (0.73;1.87)	10[c]	1.43 (0.99;2.07)
Other	45[d]	0.87 (0.62;1.21)	34[e]	0.90 (0.60;1.33)
Primary recurrent miscarriage				
Age at first pregnancy after referral	290	0.98 (0.96;1.00)	208	0.99 (0.97;1.01)
Prior miscarriage		0.88 (0.78;0.99)		0.79 (0.64;0.97)
Prior non-visualized pregnancy loss		0.91 (0.82;1.02)		0.88 (0.75;1.04)
Prior biochemical pregnancy loss		0.90 (0.79;1.02)		0.84 (0.70;1.02)
Prior failed pregnancy of unknown location		0.96 (0.86;1.08)		0.95 (0.78;1.17)
'Tender loving care'	224	1 (reference)	159	1.00 (reference)
IvIg alone	45	1.39 (1.10;1.76)	34	1.50 (1.15;1.96)
IvIg and other	10[f]	1.40 (0.93;2.12)	8[g]	1.76 (1.35;2.31)
Other	11[h]	0.83 (0.43;1.60)	7[i]	0.89 (0.39;2.03)

Table II. Relative risk (95% CI) of live birth in the index pregnancy, adjusted for treatment. (*Continued*)

	All women		Women with ≥2 clinical miscarriages	
Variable		RR (95% CI)		RR (95% CI)
Secundary recurrent miscarriage				
Age at first pregnancy after referral	209	0.97 (0.95;1.00)	160	0.97 (0.94;1.01)
Prior miscarriage		0.84 (0.75;0.94)		0.82 (0.72;0.94)
Prior non-visualized pregnancy loss		0.86 (0.76;0.97)		0.86 (0.75;0.99)
Prior biochemical pregnancy loss		0.89 (0.79;1.01)		0.91 (0.79;1.04)
Prior failed pregnancy of unknown location		0.79 (0.65;0.97)		0.77 (0.57;1.04)
'Tender loving care'	166	1 (reference)	84	1.00 (reference)
IvIg alone	53	1.15 (0.89;1.48)	43	1.18 (0.87;1.60)
IvIg and other	2[j]	0 (0.00;0.00)	2[k]	0.00 (0.00;0.00)
Other	34[l]	0.85 (0.57;1.28)	31[m]	0.84 (0.53;1.33)

[a]Non-visualized pregnancy loss: combines biochemical pregnancy losses and failed pregnancy of unknown location (PUL).
[b]Includes: IvIg+progesterone (n=3); IvIg+heparin (n=1); IvIg+donor lymphocytes (n=1); IvIg+prednisone (n=6); and IvIg+prednisone+progesterone (n=1).
[c]Includes: IvIg+lymphocytes (n=1); IvIg+prednisolone (n=5); IvIg+progesterone (n=3); and IvIg+progesterone+prednisolone (n=1).
[d]Includes: heparin (n=5), donor lymphocytes (n=4), prednisolone (n=2), progesterone (n=7) and participants in double blinded randomized controlled trial of IvIg versus placebo (n=27).
[e]Includes: donor lymphocytes (n=4); progesterone (n=5); heparin (n=4); and participants in double blinded randomized controlled trial of IvIg versus placebo (n=25)
[f]Includes: IvIg+donor lymphocytes (n=1); IvIg+heparin (n=1); IvIg+prednisone (n=6); IvIg+prednisone+progesterone (n=1); and IvIg+progesterone (n=1).
[g]Includes: IvIg+donor lymphocytes (n=1); IvIg+prednisolone (n=5); IvIg+progesterone (n=1); and IvIg+progesterone+prednisolone (n=1).
[h]Includes: donor lymphocytes (n=4); prednisone (n=2); heparin (n=3) and progesterone (n=2);
[i]Includes: donor lymphocytes (n=4); progesterone (n=1); and heparin (n=2).
[j]Includes: IvIg+progesterone (n=2).
[k]Includes: IvIg+progesterone (n=2).
[l]Includes: heparin (n=2); progesterone (n=5); and participants in double blinded randomized controlled trial of IvIg versus placebo (n=27).
[·]Includes: progesterone (n=4); heparin (n=2); and participants in double blinded randomized controlled trial of IvIg versus placebo (n=25).

DISCUSSION

Non-visualized pregnancy losses represent a significant proportion of the pregnancy losses experienced by women referred to the Danish RM clinic. We have demonstrated that non-visualized pregnancy losses and miscarriages both have a negative prognostic influence on the chance for live birth in the first pregnancy after referral among women with unexplained RM. The number of clinical miscarriages before referral has been reported to be an important determinant for RM women' prognosis for live birth.[9,10] To our knowledge, this is the first investigation of the prevalence and prognostic significance of non-visualized pregnancy losses in women with RM.

Table III. Relative risk (95% CI) of live birth in the index pregnancy per primary or secondary RM women.

	All women	Women with ≥2 miscarriages	Women receiving 'Tender loving care'
Variable	RR (95% CI)	RR (95% CI)	RR (95% CI)
Primary recurrent miscarriage	N=290	N=208	N=224
Age at index pregnancy after referral	0.98 (0.96;1.00)	0.99 (0.97;1.01)	0.98 (0.95;1.00)
Prior miscarriage	0.91 (0.81;1.02)	0.82 (0.68;1.00)	0.87 (0.75;1.01)
Prior non-visualized pregnancy loss[a]	0.94 (0.85;1.04)	0.91 (0.78;1.06)	0.93 (0.82;1.05)
Prior biochemical pregnancy loss	0.91 (0.81;1.03)	0.86 (0.72;1.03)	0.94 (0.83;1.06)
Prior failed pregnancy of unknown location	1.00 (0.90;1.12)	1.00 (0.83;1.20)	0.86 (0.70;1.04)
Secundary recurrent miscarriage	N=209	N=160	N=120
Age at index pregnancy after referral	0.97 (0.94;0.99)	0.97 (0.94;1.00)	0.97 (0.94;1.00)
Prior miscarriage	0.83 (0.75;0.93)	0.82 (0.72;0.93)	0.85 (0.73;0.99)
Prior non-visualized pregnancy loss[a]	0.86 (0.77;0.96)	0.86 (0.75;0.98)	0.87 (0.74;1.02)
Prior biochemical pregnancy loss	0.89 (0.79;1.00)	0.89 (0.78;1.02)	0.89 (0.76;1.05)
Prior failed pregnancy of unknown location	0.80 (0.66;0.98)	0.78 (0.58;1.04)	0.81 (0.60;1.09)

[a]Non-visualized pregnancy loss: combines biochemical pregnancy losses and failed pregnancy of unknown location (PUL).

Presently, non-visualized pregnancy losses in the history of women with RM are largely ignored by gynaecologists and general practitioners. The finding that non-visualized pregnancy losses and early miscarriages have a similar negative effect on RR for live birth is thus very important. At least for women with RM, our findings support the assumption that the majority of failed PULs are early intrauterine miscarriages.[2]

When interpreting our results it is important to note that the definition of RM applied in the study was three or more consecutive early pregnancy losses. Even though our calculations were based on a linear model on the logit scale, we are unable to project the results to sporadic pregnancy losses or other definitions of RM, e.g. two consecutive or non-consecutive early pregnancy losses. We did show a statistically significant reduction in relative risk for live birth also for women who fulfil the ASRM definition of recurrent pregnancy loss, i.e. ≥2 clinical miscarriages. The findings in this study should prompt further inquiry into an evidence-based definition of RM.

As the cohort only included women with unexplained RM, our results may not apply to other groups of patients, such as patients with chromosomal abnormalities, irregular menstrual cycles and patients with RM after IVF/ICSI.

To our knowledge, there are no data regarding the cost-effectiveness of RM evaluation or treatment if non-visualized pregnancy losses are included in the definition, although this is already clinical practice in several European countries such as Great Britain and Denmark. As resources in clinical care are limited, this is would be a logical next step by health care economists and relevant policy makers.

Gestational age

The exclusion of non-visualized pregnancy losses in RM definitions is probably based on reports that a transiently positive pregnancy test at the time of menstrual period is a common finding in normal women.[7] It is therefore noteworthy that the mean gestational age at time of diagnosis of both biochemical pregnancy losses and failed PULs in this study was ~ 6 weeks.

The gestational age for non-visualized pregnancy losses is based on last menstrual period and may therefore be unreliable. However, as all women in the cohort had regular menstrual cycles with a variation of no more than 2-3 days for each individual woman, we assume that the estimate of gestational age in prior pregnancies is reasonably accurate. This is substantiated by our data validation where the consistency between patient files and information given at first consultation was 99%.

Life style factors

High BMI has been reported to be prognostically negative in RM.[11,12] In these studies the authors do not distinguish between women with regular and irregular menstrual cycles. In our study, we did not demonstrate a negative impact of high BMI on the chance of live birth. Increased maternal age decreases the RR for live birth in the first pregnancy after referral. The RR described in this study is for each additional year, and as such aligns well with previously published studies.[9,10]

Aetiology of non-visualized pregnancy losses

It is probable that some of the non-visualized pregnancy losses are due to chromosome anomalies, as documented by several studies and reviewed by Macklon et al.[7,13-15] On the other hand, more of the non-visualized pregnancy losses would have been classified as miscarriages if the women had been monitored as extensively in their first pregnancies as they are in the Danish RM Unit, where all patients are followed with ultrasound from 6 weeks' gestation.

We found that the frequency of surgically treated EPs in PRM women's reproductive history was significantly higher if they had no clinical miscarriages in their reproductive history compared with those with at least one confirmed intrauterine pregnancy loss. Therefore, we propose that at least some of their non-visualized pregnancy losses may be spontaneously resolved EPs.

CONCLUSIONS

To our knowledge, this is the first study to report on the occurrence and impact of non-visualized pregnancy losses in women with unexplained RM after spontaneous conception and ≥3 consecutive pregnancy losses.

We demonstrate that non-visualized pregnancy losses are frequent. Each additional non-visualized pregnancy loss decreases the RR for live birth with ~ 10%, which is the same impact conferred by a clinical miscarriage. For women with at least two clinical miscarriages the RR for live birth is decreased with almost 10% by each non-visualized pregnancy loss

and by almost 20% for each clinical miscarriage. The data and results support the inclusion of non-visualized pregnancy losses in definitions of RM. Further studies are needed to confirm or refute our findings.

ACKNOWLEDGEMENTS

We thank biostatistician Helle Højmark Eriksen, Department of Hospital Affairs, Aalborg University Hospital, Aalborg, Denmark for statistical analysis in STATA.

AUTHORS' ROLES

A.M.K. collected data, entered data in a database, did quality control, performed statistical analyses in SAS 9.2 and wrote the paper. R.H.v.O., S.Q, R.G.F., M.S. and M.G. contributed to interpretation of data and critically revised the manuscript. O.B.C. initiated the study, participated in data collection and contributed to interpretation of data and critically revised the manuscript. All co-authors approved the final manuscript before submission.

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2.3.1

PREDICTING ADVERSE OBSTETRIC OUTCOME AFTER EARLY PREGNANCY EVENTS AND COMPLICATIONS; A REVIEW

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Human Reproduction Update 2009;15:409-421

ABSTRACT

Background

The aim was to evaluate the impact of early pregnancy events and complications as predictors of adverse obstetric outcome.

Methods

We conducted a literature review on the impact of first trimester complications in previous and index pregnancies using Medline and Cochrane databases covering the period 1980-2008.

Results

Clinically relevant associations of adverse outcome in the subsequent pregnancy with an OR >2.0 after complications in a previous pregnancy are the risk of perinatal death after a single previous miscarriage, the risk of very preterm delivery (VPTD) after two or more miscarriages, the risk of placenta previa, premature preterm rupture of membranes, VPTD and low birth weight (LBW) after recurrent miscarriage and the risk of VPTD after two or more termination of pregnancy (TOP). Clinically relevant associations of adverse obstetric outcome in the ongoing pregnancy with an OR >2.0 after complications in the index pregnancy are the risk of LBW and very low birth weight (VLBW) after a threatened miscarriage, the risk of pregnancy-induced hypertension, pre-eclampsia, placental abruption, preterm delivery (PTD), small for gestational age and low 5-min Apgar score after detection of an intrauterine haematoma, the risk of VPTD and intrauterine growth restriction after a crown-rump length discrepancy, the risk of VPTD, LBW and VLBW after a vanishing twin phenomenon and the risk of PTD, LBW and low 5-min Apgar score in a pregnancy complicated by severe hyperemesis gravidarum

Conclusions

Data from our literature review indicate, by finding significant associations, that specific early pregnancy events and complications are predictors for subsequent adverse obstetric and perinatal outcome. Though, some of these associations are based on limited or small uncontrolled studies. Larger population-based controlled studies are needed to confirm these findings. Nevertheless, identification of these risks will improve obstetric care.

INTRODUCTION

Early pregnancy events and complications are among the most common general medical complications in women during pregnancy. Most of these occur before 12 weeks of gestation and include (recurrent) miscarriage, vaginal bleeding, intrauterine haematoma (IUH), vanishing twin and hyperemesis gravidarum (HG). The presence of any complication can be extremely distressing for the women. For the clinician, it is important to interpret the symptoms and to understand the short- and long-term consequences of early pregnancy complications, especially for reassuring and supporting the couple at a difficult time.[1,2] In particular, it is pivotal to evaluate the likelihood of subsequent adverse obstetric outcome as secondary complication and to be vigilant in screening and intervening, if possible, to avoid or reduce the anticipated detrimental effects.

Data on the link between early pregnancy complications and obstetric outcome and their possible treatment or prevention are sparse and mainly limited to retrospective small series of many different pathologies or large series describing specific pathology. This has prompted the ESHRE Special Interest Group for Early Pregnancy (SIGEP) to perform a comprehensive review of first trimester pregnancy events and complications as a precursor or indicator of late pregnancy events and beyond. Given the paucity of good quality data, this paper rests on the best available evidence rather than on a formal systematic review.

METHODS

For this review, the following conditions were included and evaluated as early pregnancy complications (presenting <12 weeks of gestation), defined as adverse clinical events in the obstetric history: previous miscarriage, recurrent miscarriage (RM, three or more miscarriages), termination of pregnancy (TOP) or complications in the ongoing pregnancy: threatened miscarriage, intrauterine haematoma (IUH), crown-rump length (CRL) discrepancy, vanishing twin, hyperemesis gravidarum (HG) and pregnancy with an intrauterine device (IUD).

As late pregnancy sequelae, we have included: antepartum hemorrhage (APH), pregnancy-induced hypertension (PIH, blood pressure >140/90 mmHg measured twice, taken at least six h apart), pre-eclampsia (PE, as PIH and ≥300mg protein in 24 h urine sample), placental abruption, placenta previa, preterm premature rupture of membranes [PPROM, rupture of membranes <37 weeks gestational age (GA) and more then 24 hour before delivery], preterm delivery (PTD, <37 weeks GA), very preterm delivery (VPTD, <34 weeks GA), intrauterine growth restriction (IUGR, birthweight <5th percentile for GA, small for gestational age (SGA, birthweight <10th percentile for GA), and the impact on perinatal outcome including low birth weight (LBW, <2500 g), very low birth weight (VLBW, <1500 g), congenital malformations, low 5-min Apgar score (<7), intrauterine fetal death (>24 weeks GA) and perinatal death (within 30 days after delivery).

For each specific early pregnancy event and complication we performed a systematic literature search using Medline and the Cochrane Database, covering the period between 1980 and October 2008. Free text search terms and Medical Subject Headings (MeSH) terms for each specific early pregnancy complication were combined with each late pregnancy

sequel and perinatal outcome. Furthermore, we used, as an 'umbrella' approach, MeSH terms of each early pregnancy complication combined with *pregnancy outcome* and each late complication combined with *aetiology* or *risk factors*. Hereafter, reference lists of the retrieved publications were searched by hand. Excluded were studies in which (i) another language than English was used, (ii) an appropriate control group was missing (no control group present or non-comparable groups due to major differences in patient characteristics) and (iii) the early or late pregnancy complications were poorly defined or were merged.

For each study the odds ratio (OR), favoured for retrospective cohort and case-control studies and prospective studies with multivariate analysis, or relative risks (RR), used in prospective randomized controlled trials and cohort studies, with the associated 95% confidence interval (CI), were retrieved. In this review we did not apply formal meta-analysis as heterogeneity of data did not allow for this technique. Results were summarized in two tables. The definitions of the levels of evidence and grades of recommendation used in this review originate from the Oxford centre for evidence-based medicine (2001).The prognostic value of previous first trimester events for the subsequent index pregnancy is summarized in Table I. The prognostic value of first trimester complications for the same pregnancy is summarized in Table II.

Previous miscarriage

A sporadic miscarriage is defined as a single or maximum two episodes of spontaneous pregnancy loss before 20 weeks GA.[3] The overall incidence of clinical sporadic miscarriage is ~12%.[4] After a previous miscarriage, the risk of miscarriage in the subsequent pregnancy is increased to 16-20%.[5,6]

Possibly, the pathological mechanisms which can be attributed to miscarriage (like thrombophilia disorders, maternal immunologic and hormonal abnormalities, infection, incompetent cervix and uterine abnormalities), interpregnancy interval and treatment modality could explain some of the associations with adverse obstetric outcome.[7,8] However, it is informative for couples to know that a small study has found that the interval to the next pregnancy and the live birth rate in the next pregnancy does not depend on the treatment modality for the miscarriage.[9]

There is in the next pregnancy after a miscarriage an increased risk of PPROM, PTD, VPTD. This risk increases with the number of miscarriages and these women also have an increased risk of placental abruption, placenta previa and SGA (Table I, Addendum Table III and IV).

Obstetric outcome

Although a previous live birth will reduce the risk of developing PE in a subsequent pregnancy compared with nullipara, this association is not found between a single previous miscarriage and the risk of developing PE, neither in nullipara nor in multipara. [8,10-14] In only one small study, a protective effect is described of having had a single previous miscarriage among multiparas (OR 0.1, 95% CI 0.0-0.5) but not among nulliparas.[12] In a recent well-controlled study, Bhattacharya *et al.*[15] found no increased risk of PE (OR 1.1, 95% CI 0.9-1.3) after a single miscarriage, furthermore Thom *et al.*,[8] also did not find an increased risk (OR 1.0,

95% CI 0.8-1.3) in a larger but poorly controlled study. After two previous miscarriages, a higher incidence of severe, but not mild, PE (OR 1.5, 95% CI 1.3-1.8) is reported.[7] A single previous miscarriage is not associated with an increased risk for placental abruption and placenta previa,[8,15,16] but after two miscarriages the probability of placental abruption (OR 1.5, 95% CI 1.1-1.7) and placenta previa (OR 1.7, 95% CI 1.3-2.3) is increased.[7]

In a large population-based Swedish study of 601 883 cases, Buchmayer *et al.*[17] found an increased risk of PPROM (OR 1.9, 95% CI 1.5-2.6), PTD (OR 1.1, 95% CI 1.1-1.2) and VPTD (OR 1.5, 95% CI 1.2-2.7) in women presenting with a single previous miscarriage. In other smaller studies, the risk of PPROM was not found to be increased.[8,18-20] An increased risk of PTD has also been reported in other studies,[15, 20, 21-25] although not always reaching statistical significance.[8, 16, 19, 26-29] Several large studies have also reported comparable increased risks of VPTD,[20, 21, 25] whereas in smaller studies, only a slightly increased risk of VPTD was reported.[8, 15, 19, 28] In all studies, the risk of PPROM (OR 1.2, 95% CI 1.1-1.3), PTD (OR 1.6, 95% CI 1.3-1.9) and VPTD (OR 2.7, 95% CI 1.8-4.0) is increased more in women presenting with two or more miscarriages when compared with women with only a single previous miscarriage.[7, 17]

Perinatal outcome

Only a slight increase in the risk of SGA[8, 21, 22, 24, 30] and LBW[8, 15, 16, 21, 30] has been reported by several authors in women with a single previous miscarriage. Sheiner *et al.*[7] has reported no increased risk of LBW in a large population-based Israeli study of 7503 cases presenting with two or more previous miscarriages, whereas Basso *et al.*[21] has observed an increased risk of SGA (OR 1.4, 95% CI 1.2-1.6) and LBW (OR 1.9, 95% CI 1.5-2.4) in a large population-based Danish study of 5268 cases presenting with two or more miscarriages.

Although it has been suggested that some congenital anomalies were associated with previous miscarriages[30] and one study has reported an increased incidence of congenital malformations after two miscarriages,[16] this trend was not confirmed by a large study.[8]

A single previous miscarriage is not associated with a low 5-min Apgar score, but a recent retrospective study has found an increased risk of intrauterine fetal death (OR 1.9, 95% CI 1.1-3.6) and an increased risk of neonatal death (OR 2.2, 95% CI 1.1-4.8).[8, 15, 16] These increased risks were not found in a larger study of two or more miscarriages.[7]

Recurrent miscarriage

RM is conventionally defined as three or more consecutive miscarriages occurring before 20 weeks GA.[31] RM occurs in ~1% of fertile couples although higher incidences like 7.4% are observed.[4] The predicted risk for a subsequent miscarriage after RM ranges between 8 and 58% depending on the maternal age, the number of previous miscarriages and the karyotype of previous miscarriages.[31-33] In this review, we focused on idiopathic RM because different treatments of well-established causes of RM such as thrombophilia or diabetes could have a different impact on the rate of complications in subsequent pregnancy. Only one small study fulfilled these criteria,[34] while in all other studies, no differentiation in underlying causes of RM was made.

RM is associated with an increased risk for PE, placental abruption, placenta previa, PPROM, PTD, SGA, LBW and congenital anomalies (Table I and Addendum Table V).

Table I. Early pregnancy events and complications as risk factors for adverse obstetric outcome in the subsequent pregnancy.

	Previous miscarriage(s)			
Obstetric outcome	One	G	Two or more	G
Pregnancy-induced hypertension	ns	D	ns	D
Pre-eclampsia	ns	B	OR 1.5 (1.3-1.8) [7]	C
Placental abruption	ns	C	OR 1.5 (1.1-1.7) [7]	C
Placenta previa	ns	C	OR 1.7 (1.3-2.3) [7]	C
PPROM	OR 1.9 (1.5-2.6) [17]	B	OR 1.2 (1.1-1.3) [7]	B
Preterm delivery <37 weeks	OR 1.1 (1.1-1.2) [17]	B	OR 1.6 (1.3-1.9) [15]	B
Very preterm delivery <34 weeks	OR 1.5 (1.2-1.7) [17]	B	OR 2.7 (1.8-4.0) [15]	B
Perinatal outcome				
Intrauterine growth restriction <5th	no data	D	no data	D
Small for gestational age <10th	ns	B	OR 1.4 (1.2-1.6) [21]	C
Low birth weight <2500g	ns	B	OR 1.9 (1.5-2.4) [21]	C
Very low birth weight <1500g	no data	D	no data	D
Congenital malformation	ns	D	Inconclusive [8,30]	D
Low 5-min Apgar score	ns	C	ns	C
Intrauterine fetal death	OR 1.9 (1.1-3.6) [15]	C	no data	D
Perinatal death	OR 2.3 (1.1-4.8) [15]	C	ns	C

Data are reported as Odds Ratio (OR) or Relative Risk (RR) with 95% Confidence Interval (CI) of the best and largest studies; ns, not statistical significant; no data, no available data; PPROM, premature preterm rupture of membranes. G grade of recommendation: A, consistent level 1 studies; B, consistent level 2 or 3 studies or extrapolations from level 1 studies; C, level 4 studies or extrapolations from level 2 or 3 studies; D, level 5 evidence or troublingly inconsistent or inconclusive studies of any level.

Obstetric outcome

Although after two or more miscarriages, a higher risk of PE and placental abruption has been reported in a large study,[7] we did not find additional information about the same risks in women with RM or about any protective effect of RM for PE.[8] This might be due to the fact that studies on RM are smaller and have poor stratification and therefore possible bias in comparison to studies on two or more miscarriages.

RM is associated with an increased risk of placenta previa (RR 6.0, 95% CI 1.6-22.2) in subsequent ongoing pregnancies.[8] PPROM was associated with RM[20] and Thom *et al*.[8] has reported on the association between RM and PTD (RR 1.5, 95% CI 1.1-2.1) and VPTD (RR 2.4, 95% CI 1.4-7.3). Similar increased odds have been reported by others.[20, 22, 27, 28, 34] The risk of PTD is increased by 25% after four or more previous miscarriages.[20,27].

Perinatal outcome

The relationship between two or more miscarriages and SGA (OR 1.4, 95%CI 1.2-1.6) found in a large population-based Danish study has already been mentioned in the previous

Recurrent miscarriage		Termination of pregnancy			
Three or more	G	One	G	Two or more	G
no data	D	ns	D	no data	D
ns	C	ns	C	ns	C
ns	C	ns	D	ns	D
RR 6.0 (1.6-22.2) [8]	C	ns	C	ns	C
OR 2.6 (1.6-4.5) [20]	C	OR 1.4 (1.1-1.7) [45]	B	OR 1.9 (1.3-2.9) [44]	C
RR 1.5 (1.1-2.1) [8]	B	OR 1.2 (1.1-1.3) [25]	B	OR 1.9 (1.4-2.5) [25]	B
RR 2.4 (1.4-4.3) [8]	B	OR 1.5 (1.1-2.0) [47]	B	OR 2.6 (1.1-5.9) [47]	B
no data	D	no data	D	no data	D
ns	C	ns	B	ns	B
RR 2.0 (1.4-2.8) [8]	C	ns	B	ns	D
no data	D	OR 2.7 (1.1-7.1) [48]	D	OR 3.6 (2.3-5.5) [55]	D
RR 1.8 (1.1-3.0) [8]	C	ns	B	ns	D
ns	C	ns	C	ns	C
no data	D	ns	B	ns	C
ns	D	ns	B	ns	C

paragraph on previous miscarriage.[21] Other smaller studies have reported similar, although not statistically significant, increased risks of SGA in women with RM compared with.[8, 27, 34, 35]

In a large case-control study, an increased risk of LBW (RR 2.0, 95% CI 1.4-2.8) has been reported in women with RM[8] and a similar trend has been reported in smaller studies.[27,35] The finding that women presenting with RM had themselves been born with a decreased mean birthweight and an increased rate of preterm birth compared with controls suggest that the predisposition of PTD and LBW may be a genetically determined trait among women with RM.[36]

Women with a history of RM may have a higher risk to deliver a child with congenital malformations (RR 1.8, 95% CI 1.1-3.0) than normal.[8] No relationship has been found between RM and low 5-min Apgar score or intrauterine fetal death or perinatal death.[8, 34, 35]

Termination of pregnancy

Legal abortion is defined as a surgical or medical TOP before 24 weeks GA.[3] Worldwide every 12-39 women/ 1000 women have a TOP and 31 TOPs are conducted for every 100

live births.[37] TOP rates are lower in Western Europe than in Eastern Africa and Eastern Asia due to better access to family planning and contraception.[37] In developed countries, the commonly used procedure for first trimester TOP has been a vacuum aspiration. In the last decade, medical abortion with mifepristone and misoprostol has been increasingly used for pregnancies before 10 weeks gestation whereas for second trimester TOP, the use of surgical versus medical techniques has been highly variable in different countries and regions of a same country. In a most recent large long-term safety study no difference in risk of miscarriage, ectopic pregnancy, PTD and LBW has been observed between a previous TOP by medical technique or by vacuum aspiration, respectively.[38]

Analysing the adverse pregnancy outcomes following a TOP is difficult because of considerable variations in the type of procedure used, the GA at the time of TOP, the number of previous TOP and the choice of an adequate control group.[39] Furthermore TOP and adverse pregnancy outcome share health behavioral pregnancy risk factors such as smoking, substance abuse, unemployment and a poor socioeconomic status.[40, 41] Besides these confounders, the associations found between previous TOP and adverse obstetric outcome could be explained by a short interpregnancy interval, cervical damage due to cervix-dilatation, the surgical method used, GA at TOP, infection related to the procedure and tissue retention.[42, 43] Most studies evaluating the risk of adverse pregnancy outcome in women with a history of TOP are of small size, have mixed spontaneous miscarriage and voluntary TOP and insufficiently correct for selection bias.

Despite these methodological drawbacks it can be concluded that a history of TOP is associated with an increased risk for PPROM, PTD and VPTD. These risks depend on the number of TOP (Table I, Addendum Table VI and VII).

Obstetric outcome

After a history of TOP, no increased risk of PIH has been found.[11] In four small studies, a low risk of PE has been found after a single TOP, similar to the low risk observed in multipara,[11, 13, 14, 44] but a high risk of PE has been found in three small studies.[10, 12, 40] After two or more TOP, similar risks of PE have been found.[10, 11, 40, 44] In the EUROPE study, a history of TOP has been associated with placenta previa (OR 2.3, 95% CI 1.3-4.0) but not with placental abruption.[45] However, in a larger case-control study, no association was found between TOP and placenta previa, even after two or more TOP.[46]

In both the EUROPE and the EPIPAGE study, an increased risk of PPROM (OR 1.4, 95% CI 1.1-1.7 and OR 1.7, 95% CI 1.2-2.5) has been found.[45, 47] The risk of PPROM is increased more (OR 1.9, 95% CI 1.3-2.9) in women presenting with two or more TOP.[44] An increased risk for PTD (OR ranging 1.2-1.8) and VPTD (OR ranging 1.1-1.8) has been reported in subsequent large studies[22, 23, 24, 25, 28, 29, 42, 47, 48] and small studies.[19, 40, 45, 49] The rise in risk of PTD and VPTD is directly related to the number of previous TOP. In women with two or more TOP, the risk of PTD (OR ranging 1.2-2.5) and VPTD (OR ranging 1.4-2.9) is further increased.[19, 22, 25, 28, 29, 40, 42, 45, 47, 49, 50]

Perinatal outcome

In none of the studies was an association observed between one or more previous TOP and SGA, LBW, intrauterine fetal death, perinatal death, low 5-min Apgar score or congenital malformations.[13, 16, 22, 24, 40, 44, 47-54] Though two small and poor controlled studies found an association between one or more TOP and VLBW.[48, 55]

One study has reported only a higher risk of intrauterine fetal death in women whose TOP had been complicated by an infection.[56]

Threatened miscarriage

First trimester vaginal bleeding is the most common complication of pregnancy, occurring in 14-20% of ongoing pregnancies, whereas ~50% of these women will miscarry regardless of ultrasonographic evaluation of viability.[57-61] The risk of threatened miscarriage to proceed to full miscarriage depends on GA and is diminished to 2-14% after confirmation of viability.[59, 60, 62] An association between vaginal bleeding in the first trimester and an adverse perinatal outcome has been established for several decades.[63, 64] The majority of these older publications report on small retrospective and non-controlled studies and there are only a few state-of-the-art prospective studies available for analysis.[59, 60, 65, 66]

Vaginal bleeding during early pregnancy most often originates from the placenta. It is thought that bleeding between the chorionic membrane and the uterine wall can result in a spectrum of effects on pregnancy development and outcome. At one end, direct pressure and disruption of the placental bed can result in miscarriage. At the other end of the spectrum is placental abruption, placenta previa, PPROM, LBW, PTD and fetal death, where there is minimal or no disruption to uteroplacental development but a chronic inflammatory reaction within the decidua and placental membranes, with weakening and eventual rupture of the membranes or resulting in myometrial activity.[59]

Threatened miscarriage in the first trimester is associated with an increased risk of APH, placental abruption, placenta previa, PPROM, PTD, LBW and VLBW (Table II and Addendum Table VIII) and these risks are more increased in women presenting with heavy bleeding.

Obstetric outcome

First trimester vaginal bleeding gives a 2-fold increased risk of APH in the second and third trimester of pregnancy.[61, 67, 68] Women with first trimester bleeding are not at an increased risk of developing PIH and PE.[59-61, 69]

In a large prospective cohort study, threatened miscarriage was found to be a risk factor for placental abruption, after a light bleeding (OR 1.6, 95% CI 1.1-2.6) as well as after a heavy bleeding (OR 3.6, 95% CI 1.6-7.9).[60] A comparable increased risk of placental abruption (OR 2.8, 95% CI 2.0-3.7) was also observed in a large retrospective case-control study[66] and in another study.[61] The risk of placenta previa in threatened miscarriage is increased (OR 1.8, 95% CI 1.1-2.9) in a large retrospective study.[61] In a subgroup with heavy bleeding of a prospective study, the same risk is increased (OR 2.5, 95% CI 0.9-6.9), however not significantly.[60]

Women presenting with heavy bleeding are at higher risk of SGA (OR 2.6, 95% CI 1.2-5.6) [60] whereas women with light bleeding are not at increased risk.[60, 65, 68, 69] In almost

Table II. Early pregnancy events and complications as risk factors for adverse obstetric outcome in the ongoing pregnancy.

Obstetric outcome	Threatened miscarriage	G	Intrauterine haematoma	G
Antepartum haemorrhage	OR 1.8 (1.7-2.0) [61]	B	no data	D
Pregnancy-induced hypertension	OR 1.4 (1.1-1.8) [60]	B	RR 2.1 (1.5-2.9) [78]	C
Pre-eclampsia	OR 1.4 (1.1-1.8) [60]	B	RR 4.0 (2.4-6.7) [78]	C
Placental abruption	OR 1.6 (1.1-2.6) [60]	B	RR 5.6 (2.8-11.1) [78]	C
Placenta previa	ns	B	no data	D
PPROM	ns	B	ns	D
Preterm delivery <37 weeks	OR 1.3 (1.1-1.7) [60]	B	RR 2.3 (1.6-3.2) [78]	B
Very preterm delivery <34 weeks	OR 1.9 (1.6-2.2) [61]	B	no data	D
Perinatal outcome				
Intrauterine growth restriction <5th	no data	D	no data	D
Small for gestational age <10th	ns	B	RR 2.4 (1.4-4.1) [78]	B
Low birth weight <2500g	RR 2,3 (1,9-2,7) [64]	B	no data	D
Very low birth weight <1500g	RR 2.2 (1.3-3.5) [64]	B	no data	D
Congenital malformation	OR 1.4 (1.0-2.1) [65]	B	ns	C
Low 5-min Apgar score	ns	C	RR 2.6 (1.9-3.5) [78]	C
Intrauterine fetal death	ns	B	ns	D
Perinatal death	ns	C	ns	D

Data are reported as Odds Ratio (OR) or Relative Risk (RR) with 95% Confidence Interval (CI) of the best and largest studies; ns, not statistical significant; no data, no available data; PPROM, premature preterm rupture of membranes; CRL crown-rump length. G grade of recommendation: A, consistent level 1 studies; B, consistent level 2 or 3 studies or extrapolations from level 1 studies; C, level 4 studies or extrapolations from level 2 or 3 studies; D, level 5 evidence or troublingly inconsistent or inconclusive studies of any level.

all studies [59, 60, 68, 70, 71], a 1.9-3.7 fold increased risk of PPROM is observed, except in one.[61] An increased risk of PTD (RR ranging 1.3-3.1) and VPTD (RR ranging 1.6-5.3) is found in all studies after threatened miscarriage, in both normal and IVF pregnancies[29, 59-61, 64-73] The risk of PTD is increased more (OR 3.0, 95% CI 1.9-4.5) after heavy first trimester bleeding.[29, 60, 66]

Perinatal outcome

Overall, the mean birthweight after threatened miscarriage is lower than in controls.[59, 60, 68, 72] An increased risk of LBW (RR 2.3, 95% CI 1.9-2.7) and of VLBW (RR 2.2, 95% CI 1.3-3.5) was observed in a large prospective study[64] as well as in a large retrospective study[61]. Similar increased risks of LBW and VLBW have also been reported in other small studies although not always reaching statistical.[53, 65-68, 70, 72, 73]

Other perinatal outcomes like low 5-min Apgar score, intrauterine fetal deaths, perinatal deaths seem to be unaffected, but the risk of congenital malformation (OR 1.4, 95% CI 1.0-2.1) seems to be increased.[59, 61, 64, 65-68, 70, 72, 73]

CRL discrepancy	G	Vanishing twin	G	Hyperemesis gravidarum	G
no data	D	ns	C	no data	D
ns	C	ns	C	ns	C
no data	D	ns	C	ns	D
no data	D	ns	C	no data	D
no data	D	ns	C	no data	D
no data	D	no data	D	no data	D
ns	C	OR 1.6 (1.2-2.0) [111]	B	RR 3.0 (1.9-4.3) [122]	C
OR 2.0 (1.1-4.0) [96]	C	OR 3.0 (1.9-4.8) [111]	B	no data	D
OR 2.8 (1.9-4.3) [96]	C	no data	D	no data	D
OR 1.1 (1.0-1.2) [95]	B	OR 1.6 (1.1-2.3) [115]	B	RR 1.5 (1.0-2.2) [122]	B
OR 1.8 (1.2-2.3) [96]	C	OR 2.0 (1.5-2.6) [111]	B	RR 2.8 (1.7-4.3) [122]	B
no data	D	OR 3.0 (1.9-4.7) [111]	B	OR 1.4 (1.0-2.0) [125]	C
no data	D	ns	C	inconclusive [124,126]	D
no data	D	no data	D	RR 5.0 (2.6-9.6) [122]	C
no data	D	no data	D	ns	C
ns	C	OR 3.7 (1.5-8.9) [111]	D	ns	C

Intrauterine haematoma

IUHs are crescent-shaped echolucent areas between the chorionic membrane or placenta and the myometrium.[74, 75] In ~18-39% of the women presenting with threatened miscarriage, a subchorionic or retroplacental haematoma can be seen on ultrasound.[69, 76] Seventy per cent of women diagnosed with an IUH by ultrasound will experience vaginal bleeding.[77, 78] An acute haemorrhage is hyperechogenic to isoechogenic compared with the placenta, while resolving haematomas become hypoechogenic within one week and sonolucent within two weeks.[79] Persistence of a first trimester subchorionic haematoma does not affect the utero- and umbilico-placental circulation.

The risk of miscarriage is independent of vaginal bleeding, the size and the localization of the IUH; however, the risk is 2.4-fold higher when the haematoma is diagnosed before nine weeks gestation.[76, 80, 81] An IUH during early pregnancy most often originates from the placenta. The putative mechanisms of the association of an IUH and adverse obstetric outcome are similar as described in the threatened miscarriage chapter.

An IUH is associated with an increased risk for PIH, PE, placental abruption, PTD, SGA, fetal distress and intrauterine fetal death (Table II and Addendum Table IX).

Obstetric outcome

Women presenting with a first trimester IUH have a higher risk of PIH (RR 2.1, 95% CI 1.5-2.9), PE (RR 4.0, 95% CI 2.4-6.7), placental abruption (RR 5.6, 95% CI 2.8-11.1) and SGA (RR 2.4, 95% CI 1.4-4.1) in comparison with women without an IUH and vaginal bleeding.[78] An increased risk of placental abruption and SGA was also observed in other studies.[69, 77]

The risk of PPROM was not increased in women with an IUH.[69, 77] The risk of PTD, however, was increased (RR 2.3, 95% CI 1.6-3.2) in a prospective study comparing women with and without an IUH.[78] The increased PTD risk appeared independent of vaginal bleeding and was comparable with risks observed in other studies .[69, 76, 77, 82] From older studies it is known that there is no association between the observed incidence of premature delivery and the size of the haematoma.[83]

Perinatal outcome

IUH specific data on LBW or VLBW were unavailable. The risks of congenital anomalies and perinatal death were not significantly increased in these women.[77, 78] Fetal distress has been observed more frequently (RR 2.6, 95% CI 1.9-3.5) in these women as compared with a control group,[78] as well as intrauterine fetal death (OR 2.8, 95% CI 1.7-2.4).[77]

Crown-Rump Length discrepancy

The classical Robinson and Fleming study on CRL is still the main reference for the assessment of GA in early pregnancy.[84] Reevaluation of CRL curves based on high-resolution real-time ultrasound only demonstrated small systematic differences.[85] The predictive value of CRL measurements is illustrated by the fact that if an embryo has developed up to 5mm in length, subsequent loss of viability occurs in 7.2% and loss rates drop to 3.3% for embryos of 6-10mm and to 0.5% for embryos over 10mm.[86]

A CRL discrepancy exist when the observed CRL is smaller than expected on the basis of amenorrhea in women with a regular menstrual cycle or known date of ovulation.[87] A CRL discrepancy may be caused by variations in growth rate, sometimes occurring in the pre-implantation period and referred to as diapause.[88] CRL discrepancy is found in certain types of aneuploidy.[89, 90] The discrepancy is most commonly found in first trimester fetuses with trisomy 13 and 18, and triploidy by contrast to trisomy 21 and monosomy.[89, 91-94]

In ongoing euploid pregnancies a CRL discrepancy is associated with VPTD, IUGR, SGA, and LBW, in both singleton and twin pregnancies (Table II and Addendum Table X).

Obstetric outcome

We could not find data on the association between CRL discrepancy and PIH, PE, placenta previa, placental abruption and PPROM.

A CRL discrepancy of 2-6 days is related to an increased risk of SGA (OR 1.1, 95% CI 1.0-1.2),[95] IUGR (OR 2.8, 95% CI 1.9-4.3) and VPTD before 32 weeks (OR 2.0, 95% CI 1.1-4.0), but it was not related to PIH nor to PTD between 33-36 weeks.[96] Adverse pregnancy outcome has also been observed in cases presenting with a growth discrepancy of >14

days at the mid-second trimester ultrasound examination.[97] Furthermore in exactly dated pregnancies, conceived by assisted reproductive technology (ART), a strong association has been observed between fetal growth in first trimester and birthweight, suggesting that impairment of fetal growth starts in the first trimester.[95, 98, 99]

Perinatal outcome

A CRL discrepancy of 2-6 days smaller than expected is related to an increased risk of LBW (OR 1.7, 95% CI 1.2-2.3), but not to perinatal death.[96] No data are available on the relationship between CRL discrepancy and low 5-min Apgar score, intrauterine fetal death and congenital malformation

Intertwin disparities in fetal size

Intertwin disparity can be observed in both monochorionic and dichorionic twin pregnancies.[100] It has been a matter of debate whether the smaller or the larger fetus should be used to determine the gestational age.[101, 102] Although one study did not find an increased risk of adverse pregnancy outcomes in twins with first trimester CRL discordance,[103] other studies have observed an increased risk of congenital malformation, aneuploidy, PTD, SGA, IUGR, and intertwin birth weight discordance of > 20-25% when a cutoff value of > 85th-95th percentile was used.[101, 102, 104-106] No association has been found between CRL discordance and twin-to-twin transfusion syndrome in monochorionic twin pregnancies.[107]

Vanishing Twin

The disappearance of gestational sacs or embryos after documented fetal heart activity in multiple pregnancies is known as the vanishing twin phenomenon.[108] Among pregnancies with twin sacs or embryos, ~30% will result in singletons and <10% will result in no fetuses at all.[109-111]

Increased levels of pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), potentially influencing the results of first trimester screening, have been reported in vanishing twin ART pregnancies compared with spontaneous conceived pregnancies.[112] But, a more recently performed study did not find a difference in PAPP-A and β-HCG levels when vanishing twin pregnancies were compared with singleton ART population.[113]

Compared with uncomplicated control ART-pregnancies with the same number of viable fetuses, there is an increase in adverse obstetrical outcome after the vanishing twin/triplet phenomenon. This might be due to early implantation crowding, resulting in an unfavorable implantation site with uteroplacental insufficiency,[114] or vaginal blood loss being an independent risk factor.[68, 115]

Although the vanishing phenomenon occurs in both spontaneously conceived and in ARTs pregnancies, the vanishing twin phenomenon described in the following studies is performed in ART-population for both cases and controls. The vanishing twin phenomenon is associated with PTD, VPTD, SGA, LBW and VLBW (Table II and Addendum Table XI).

Obstetric outcome

An association with an increased risk of PE has been observed in a small study[116] on vanishing twins, but not confirmed by a larger study.[115] No difference is found for the risk of vaginal

bleeding, placenta previa and placental abruption, but an increased risk of SGA (OR 1.6, 95% CI 1.1-2.3) has been observed in comparison with singletons from a single gestation and the risk increased with increasing GA at the time of vanishing.[115] An association between vanishing twin with PTD has been observed by several authors.[109, 111, 116-118] An increased risk of PTD (OR 1.6, 95% CI 1.2-2.0) and VPTD before 32 weeks gestation (OR 3.0, 95% CI 1.9-4.8) has been found comparing survivors with singletons.[110] The increased risks are almost entirely due to vanishing twins that occurred after eight weeks gestation.[111]

Perinatal outcome

An increased risk of LBW (OR 2.0, 95% CI 1.5-2.6) and VLBW (OR 3.0, 95% CI 1.9-4.7)[111, 115] after a vanishing twin has also been reported by several other studies.[109, 117, 118]

No difference has been found in the incidence of congenital malformations and perinatal death.[111, 118] Though, in IVF/ ICSI pregnancies, an increased risk of cerebral palsy in those children resulting from pregnancies, where the number of embryos transferred was higher than the number of children born, has been reported.[119] This association has not been found in vanishing twin pregnancies.[111]

Hyperemesis gravidarum

HG is characterized by intractable nausea and vomiting leading to dehydration, electrolyte and metabolic disturbances, nutritional deficiency and weight loss. HG complicates 0.3-1.5% of all pregnancies, and in spite of extensive research during the last 4 decades the aetiology of HG remains unknown.[120] As a consequence therapy and patient care remains empirical and symptomatic.

In women with nausea during pregnancy, the probability of miscarriage is decreased (OR 0.3, 95% CI 0.2-0.3) and this is directly linked with the severity of symptoms.[121] Also adverse obstetric outcome is linked with the severity of the symptoms. The adverse obstetric outcome is mostly limited to women with a poor maternal weight gain during pregnancy.[122] This suggests that deficient malnutrition and the lack of vitamins and oligo-elements could play a role in these associations.

Severe hyperemesis is associated with PTD, SGA, LBW, low 5 min Apgar score and possibly also with fetal congenital anomalies (Table II and Addendum Table XII).

Obstetric and perinatal outcome

There are many observations about an increased incidence of female neonates (53-66%) in pregnancies complicated by HG.[123]

Women with HG do not present with a higher risk to develop PIH and PE.[122, 124] Infants of mothers with HG have a higher PTD risk, as well as a higher risk for LBW and VLBW, and these neonates are more likely to be SGA.[122, 124, 125] The increased risks are probably due to the low pregnancy weight gain (<7kg during pregnancy) because it has been demonstrated that women with HG and >7kg weight gain during pregnancy do not have an increased risk of PTD, LBW, VLBW and SGA.[122, 126, 127] Other studies, evaluating the effect of only low maternal weight gain during pregnancy on pregnancy outcome, have also found an increased risk of PTD, LBW and VLBW.[128-130] In a large retrospective study on women with HG and a low

weight gain of <7 kg, an increased risk of PTD (RR 3.0, 95% CI 1.9-4.3), SGA (RR 1.5, 95% CI 1.0-2.2), LBW (RR 2.8, 95% CI 1.7-4.3) and low 5-min Apgar score (RR 5.0, 95% CI 2.6-9.6) has been found compared with normal pregnancies.[122]

There are only a few data on the increased risk of congenital anomalies in HG women.[126, 131-133] There is an increased risk of anomalies of the central nervous system and skeletal malformations most likely due to nutritional deficiencies in oligo-elements and vitamins such as folic acid and vitamin K. No difference has been found in the incidence of intrauterine fetal death and perinatal death.[122, 124, 125, 131, 134]

Pregnancy with an intrauterine device

IUDs are widely used as contraceptives. A cumulative pregnancy rate of <2% for a copper IUD and ~1% for a levonorgestel-releasing IUD has been reported.[135] Dislocation of the IUD is frequently observed in pregnant women with an IUD.[136]

Pregnancies developing with an IUD *in situ* are associated with an increased risk of early and late miscarriage and PTD.

Obstetric outcome

A few studies have found that ongoing pregnancies with an IUD left *in situ* have a 2-fold increase in the risk of early and late miscarriage (50-57%) and a 4-fold increase in risk of PTD (17-22%), compared to controls in which the IUD is removed in first trimester.[137, 138] It is advised to remove the IUD with visible strings, as following removal of the IUD, the chance of miscarriage (20-25%) and PTD (4-6%) is decreased.[137, 138] If the strings of the IUD are not visible, it can be considered to remove the IUD under ultrasound guidance or by hysteroscopy.[139, 140] These are observational studies and there are no RCTs comparing obstetric outcome in IUD *in situ* or after removal of an IUD and controls.

Intrauterine exposure to copper is not associated with any known teratogenic effect.[138, 141] Teratogenic effects of a levonorgestrel-releasing IUD have not been reported.

CONCLUSIONS

In this comprehensive review, we describe the impact of various specific first trimester events and complications for adverse effects on pregnancy outcome in second or third trimester of pregnancy. A few of these associations are based on large good-controlled population-based or prospective studies. Though many studies describing the impact of a single first trimester complication are small, retrospective series, have poor stratification bias and poor matching of cases and controls. Many of the controlled studies did not make adjustments for all known relevant confounders for adverse obstetric outcome, such as age, ART, economical status, education level, ethnicity, length, marital status, parity, previous obstetric outcome, prolonged infertility, smoking and maternal weight or did not stratify for other first trimester complications. More large controlled studies, using local National Birth Registries, are needed to confirm our findings. In particular larger studies concerning the risk of adverse late pregnancy outcome in women presenting with idiopathic RM, IUH and CRL discrepancy are needed.

Data from our literature review indicate a strong association between specific early pregnancy events and subsequent late obstetric complications in the subsequent or ongoing pregnancy. In particular, the risk of PTD and VPTD is increased after any of these first trimester complications. In all specific early pregnancy complications the increased risks of late obstetric complications are related to the severity and/or to the recurrence of the first trimester complication. Though some of the found associations, are small (between OR 1.0-2.0) and thus the clinical relevance of these association could be questionable.

Clinically relevant associations (grade of recommendation A, B or C) of adverse obstetric and perinatal outcome in the subsequent pregnancy with an OR >2.0 after complications in a previous pregnancy are the risk of perinatal death after a single previous miscarriage, the risk of VPTD after two or more miscarriages, the risk of placenta previa, PPROM, VPTD and LBW after RM and the risk of VPTD after two or more TOP. Clinically relevant associations (grade of recommendation A, B or C) of adverse obstetric and perinatal outcome in the ongoing pregnancy with an OR >2.0 after complications in the index pregnancy are the risk of LBW and VLBW after a threatened miscarriage, the risk of PIH, PE, placental abruption, PTD, SGA and low 5-min Apgar score after detection of an IUH, the risk of VPTD and IUGR after a CRL discrepancy, the risk of VPTD, LBW and VLBW after a vanishing twin phenomenon and the risk of PTD, LBW and low 5-min Apgar score in a pregnancy complicated by severe HG. The identification of these high risk groups should enable better management protocols and new therapeutic protocols to improve neonatal outcome.

ACKNOWLEDGEMENTS

We thank Prof. G.J. Bonsel PhD, Institute of Health Policy and Management (iBMG), Erasmus University Medical Center, The Netherlands, for his critical reading of the manuscript and his advice.

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2.3.2

OBSTETRIC OUTCOME AFTER EARLY PLACENTAL COMPLICATIONS

Based upon:
Obstetric outcome after early placental complications

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Current Opinion in Obstetric and Gynecology; 2010:452-457

ABSTRACT

Our analysis of the literature review indicates a link between early pregnancy complications involving the placenta and subsequent adverse obstetric and perinatal outcomes. Some of these associations are based on limited or small uncontrolled studies. Larger population-based prospective controlled studies have recently been published confirming most of these findings. The pathophysiology and aetiology of these early pregnancy complications and the effect on the utero-placental interface and thus on the subsequent pregnancy outcomes are depicted.

INTRODUCTION

In Chapter 2.3.1 a comprehensive overview of the existing literature about the associations between early pregnancy complications/ events and adverse obstetric outcome in the ongoing or subsequent pregnancies is described. Increased risks of adverse obstetric outcome after a first trimester complication were demonstrated and these risks were associated with the severity and/ or recurrence of the complications.

Most early pregnancy complications occur before 12 weeks of gestation and involve placentation and early placental development, There is increasing evidence showing that many failures of placentation are associated with an imbalance of free radicals, which will further affect placental development and function and may subsequently have an influence on both the fetus and its mother.[1,2] The placental syncytiotrophoblast is extremely sensitive to oxidative stress, partly because it is the outermost tissue of the conceptus and so exposed to the highest concentrations of oxygen coming from the mother, and partly because it contains surprisingly low concentrations of the principal antioxidant enzymes, particularly in early pregnancy. Endometrial glands are known to play a pivotal role in regulating placental development in many mammal species including humans.[1] These are not only as a source of nutrients but the epithelial cells and their secretions are strongly immunoreactive for a wide variety of growth factor and cytokines and could have important effects on the developing of the materno-fetal interface from a very early stage in pregnancy.[3] It is likely that placental-related complication such as preeclampsia stem from a defect in the early trophoblastic-decidual interaction leading to abnormal placentation and in particular abnormal development of the utero-placental circulation.

Limitations

The strength of the review outlined in Chapter 2.3.1 is that it is a complete overview of almost all important associations between early pregnancy complications and adverse obstetric outcome. One of the limitations is the lack of a proper meta-analysis of the primary data. Because of the paucity of systematic data, the heterogeneity of the data and the extent of the review, the paper rest on the best available evidence rather than on a formal systematic review. For some associations, for which many well-controlled large studies were available, we performed a meta-analysis using Mix 1.7 with a random effects model.[4,5] The odds ratios obtained by the meta-analysis were similar or would not differ greatly in comparison with the odds ratio used of the best available study, see Table I and II.[6,7] That is why we concluded, that the used figures of the best available studies are a good approximation of the true risk. Moreover, more recent large meta-analysis and controlled population-based prospective studies have confirmed previous data.

To clearly indicate the quality and strength of each finding we have provided the level of evidence and grade of recommendation for each study and association. Some associations were based on limited small and/ or poorly controlled studies (level of evidence 3B or higher); consequently these associations (recommendation grade D) have to be interpreted with caution.

Table I. Early pregnancy events and complications as risk factors for adverse obstetric outcome in the subsequent pregnancy.

	Previous miscarriage			
Obstetric outcome	One	N	Two or more	N
PIH	*1.2 (0.6-2.3)*	1	*2.2 (0.5-7.2)*	1
PE	*0.9 (0.8-1.1)*	7	*1.0 (0.9-1.1)*	3
Placental Abruption	*1.1 (0.8-1.7)*	2	**1.5 (1.1-1.7)**	1
Placenta Previa	*1.7 (0.9-3.2)*	2	**1.7 (1.3-2.3)**	1
PPROM	**1.3 (1.0-1.8)**	4	**1.6 (1.1-2.1)**	4
Preterm <37 weeks	**1.3 (1.2-1.4)**	13	**1.9 (1.7-2.2)**	11
Preterm <34 weeks	**1.5 (1.3-1.8)**	8	**2.7 (2.2-3.3)**	6
Perinatal outcome				
SGA	*1.0 (1.0-1.1)*	5	**1.3 (1.1-1.5)**	3
LBW <2500g	**1.2 (1.0-1.3)**	5	*1.5 (0.9-2.5)*	3
LBW <1500g	no data	0	no data	0
Cong. malformation	*1.3 (1.0-1.7)*	2	no data	0
5-min AS <7	*1.0 (1.0-1.2)*	2	*1.0 (0.8-1.4)*	1
Fetal death	*1.6 (0.9-2.8)*	1	no data	0
Perinatal death	*2.1 (1.0-3.9)*	1	*1.2 (0.9-1.4)*	1

Data are reported as Odds Ratio (OR) with 95% Confidence Interval (CI)

The Medline and Cochrane databases were used for this literature search. Thus the EMbase database was not used. With the use of Medline and by searching the reference list by hand, we believe that we found the most and the most important studies addressing each association. Smaller studies missed would not affect the outcome, as the paper rest on the best available evidence. Nevertheless, there is a small possibility that significant recently published studies, will affect the outcome.

Miscarriage and recurrent miscarriage

In the subsequent pregnancy, after one or more miscarriages, women are at increased risk for PPROM and (very) preterm delivery (PTD). The increased risks are small after a single previous miscarriage, but increase with the number of miscarriages. A meta-analysis concerning the association between miscarriage and PTD observed similar odds ratios as in our study.[8] After two or more previous miscarriages, but not after three or more miscarriages, increased risks of preeclampsia, placental abruption and SGA were found. These associations were based on few small studies, hence explaining the differences. The risk of placenta previa and LBW were increased after two previous miscarriages and increase with the number of miscarriages. The association of placenta previa was only investigated by a single study for each study group and the association of LBW could be well explained by the increased risk of preterm delivery.[9,10]

Recurrent miscarriage		Termination of pregnancy			
Three or more	N	One	N	Two or more	N
no data	0	*1.0 (0.6-1.8)*	1	no data	0
1.1 (0.6-2.0)	2	*0.9 (0.7-1.1)*	4	*0.6 (0.2-1.8)*	3
1.2 (0.4-3.1)	1	no data	0	no data	0
6.0 (1.6-22.0)	1	*1.0 (0.7-1.6)*	1	*1.4 (0.8-2.5)*	1
2.1 (1.5-2.9)	2	**1.3 (1.0-1.7)**	4	**1.8 (1.3-2.6)**	2
2.4 (1.8-3.4)	5	**1.3 (1.2-1.4)**	12	**1.6 (1.4-1.9)**	10
3.8 (1.6-9.0)	3	**1.5 (1.3-1.7)**	6	**2.1 (1.1-3.9)**	5
1.3 (0.9-1.7)	2	*1.0 (0.9-1.1)*	5	*1.1 (0.9-1.3)*	5
2.0 (1.4-2.7)	2	**1.2 (1.0-1.4)**	6	**1.5 (1.2-1.7)**	5
no data	0	**2.7 (1.1-7.1)**	1	no data	0
1.8 (1.1-3.0)	1	*1.1 (0.9-1.2)*	2	*1.3 (0.7-2.3)*	1
0.6 (0.3-1.6)	1	*1.1 (0.8-1.6)*	1	*0.8 (0.3-2.0)*	1
no data	0	*0.5 (0.2-1.1)*	2	no data	0
no data	0	*1.5 (0.4-6.1)*	2	*0.5 (0.1-3.8)*	1

Often the interpregnancy interval after a miscarriage is short. A short interval period between birth and the subsequent pregnancy is associated with preterm birth.[11] It is thought that pregnancy and lactation result in a poor maternal nutritional status. An inadequate time for the mother to recover results in depletion of maternal nutrients, vitamins and oligo-elements with increased risks of adverse obstetric outcome in the subsequent pregnancy.[12] However, Buchmayer et al. demonstrated that the increased risk of preterm delivery is not associated with a short interpregnancy interval after a previous miscarriage.[13] And Love et al. concluded that women with an interpregnancy interval after a miscarriage shorter than 6 months have even better reproductive outcome and lower complication rates in the subsequent pregnancy as compared with women with a longer interpregnancy interval.[14]

The predicted risk for a subsequent miscarriage after a recurrent miscarriage ranges between 8 and 58% depending on the maternal age, the number of previous miscarriages and the karyotype of previous miscarriages.[15,16] The risk of recurrence increases with the maternal age and number of successive losses. However, the overall prognosis is not better for couples with a prior live birth.[17] Recurrent miscarriage has been directly associated with parental chromosomal anomalies, maternal thrombophilic disorders and structural uterine anomalies and indirectly with maternal immune dysfunction and endocrine abnormalities.[17] However, as the majority of recurrent miscarriage cases following investigation are classified as idiopathic, that is no identifiable cause in either partner, it is generally accepted that

within the idiopathic group there is considerable heterogeneity and that it is unlikely one single pathological mechanism can be attributed to their recurrent miscarriage history. There continues to be a considerable debate about cause and association as the exact pathophysiological mechanisms of most known aetiologies have not been precisely elucidated. Current research is directed at theories on defects in nature's quality control related to implantation, trophoblast invasion and placentation, as well as factors which may be embryopathic and most women with recurrent pregnancy loss probably have several risk factors for miscarriage.

The treatment modality could affect the obstetric outcome in the subsequent pregnancy. As compared with expectant management, surgical evacuation of a miscarriage is associated with a higher risk of infection.[18] The MIST trial showed similar infection (2-3%) and subsequent pregnancy (79-86%) rates after expectant, medical or surgical management.[19,20] Unfortunately, no study has addressed the association between treatment modality and adverse obstetric outcome in the subsequent pregnancy and thus remains unknown. As the risk of preterm delivery increases with the number of miscarriages which in turn can partially be attributed to thrombophilia disorders, uterine abnormalities, maternal immunologic and hormonal abnormalities, it is possible that these factors are associated with this increased risk. Only a single small study looked at the underlying cause of recurrent miscarriage and adverse obstetric outcome, but the numbers were too small to permit correct analysis according to different subgroups.[21] As treatment modality, the underlying pathological mechanisms as risk factors for adverse obstetric outcome after a previous miscarriage need to be subject for further research.

Termination of pregnancy

Women are more at risk of PPROM and (very) preterm delivery in the subsequent pregnancy after a previous TOP. This was a dose-dependent relationship, as the risk increased after two or more TOPs. A meta-analysis concerning the association between TOP and PTD observed similar odds ratios as in our study.[8] Analyzing the adverse pregnancy outcomes following a TOP is difficult because of considerable variations in the type of procedure used (medical or surgical), the gestational age at the time of TOP, cervical damage leading to cervical incompetence, intra-uterine damage leading to adhesions, intra-uterine infection facilitated by cervical instrumentation or tissue retention and short interval pregnancy are possible factors associated with preterm delivery. Unfortunately, not all of these factors are adequately investigated. The question arises which of these factor(s) of TOP are responsible for this association and whether they are preventable risk factors. Also, TOP, repeat TOPs and adverse pregnancy outcome share common risk factors such as poor health behavioural pregnancy risk factors such as smoking, substance abuse, unemployment, poor socioeconomic status, single status and young, which could explain the increased risks.[22-24] However, all large included studies, which investigated the association between TOP and preterm delivery, controlled for these confounders.

Two studies demonstrated no risk difference of preterm delivery between a medical and surgical procedure.[25,26] Zhou et al. showed that women who had a TOP with reported complications did have a similar risk of preterm delivery as compared with women who had

a TOP without any complication.[27] In a large meta-analysis it was shown that an interval to a subsequent pregnancy of less than 12 months or more than 60 months after a previous birth is an independent risk factor for preterm delivery.[11] However, an interpregnancy interval after TOP of shorter than 12 month was, in contrast to an interval period longer than 12 month, not associated with an increased risk of preterm delivery.[28] The precise putative mechanism explaining the association between TOP and preterm delivery remains unknown and needs further investigation.

Threatened miscarriage and intrauterine haematoma

Ongoing pregnancies, which were complicated by first trimester blood loss, are at increased risk of preeclampsia, placental abruption, (very) preterm delivery, (very) low birthweight and congenital malformation. These risks increase with the severity of the blood loss and the presence of an intrauterine haematoma. Our findings are partially in concert with a recent published meta-analysis, Sarawat et al. observed increased odds for ante partum heamorrhage (OR 2.5, 95% CI 1.5-4.0), placental abruption (OR 1.4, 95% CI 1.0-2.1), PTD (OR 2.1, 95% CI 1.8-2.4), LBW (OR 1.8, 95% CI 1.5-2.3) and no increased odds for congenital abnormalities in women with a threatened miscarriage.[29] However, as opposed to our findings, they observed increased odds for placenta previa (OR 1.4, 95% CI 1.2-2.2), PPROM (OR 1.8, 95% CI 1.3-2.5), perinatal mortality (OR 2.2, 95% CI 1.4-2.3) and no increased odds for PIH and PE. These differences can be explained by the study design and the fact that the tests for heterogeneity for some associations were not significant. Our findings regarding pregnancy outcome after an intrauterine hematoma are partially in concert with a recent published meta-analysis, Tuuli et al. observed increased odds for placental abruption (OR 5.7, 95% CI 3.9-8.3), PPROM (OR 1.6, 95% CI 1.2-2.2) and PTD (OR 1.4, 95% CI 1.2-1.7).[30] However, they did not observe an association with PIH or PE (OR 1.5, 95% CI 0.4-5.9) and SGA (OR 1.7, 95% CI 0.9-3.2).

Intrauterine bleeding during early pregnancy most often originates from the utero-placental circulation between the forming chorionic membrane and the uterine wall.[31,32] The bleeding could be the result of an impaired placentation of a pregnancy already at risk for adverse obstetric outcome or a fetus with congenital malformation. Dislocation and disruption of the materno-placenta interface can result in full miscarriage if the damage expands rapidly to the definitive villous tissue. In the long term bleeding could cause disruption of the chorionic-amniotic plane which may make the membranes more susceptible to rupture. Persistent or recurrent placental hemorrhage could result in a chronic inflammatory reaction and stimulate premature myometrial activity that result in cervical change.[32-35] The prolonged presence of blood may act as a nidus for intrauterine infection, which, in turn, could stimulate myometrial.[34-36] Furthermore, it is thought that fetal growth restriction may be due to some degree of placental insufficiency secondary to scarring at the site of placental bleeding, possibly leading to impairment of oxygen transfer and fetal.[37,38] Obviously, the first priority in case of first trimester blood loss is detection of fetal viability. But after a positive heart beat, clinicians have to be aware that these women are at increased risk of adverse obstetric outcome in late pregnancy.

Some clinicians will treat a patient with a threatened miscarriage oestrogen and/ or progesterone to reassure or to improve the outcome in current pregnancy. As recently

published, there is no evidence that oestrogen and progesterone both influences the outcome.[39] Scars data with small number of patients and research of poor quality is available for progresterone treatment alone. The data suggest that the use of progesterone is an effective treatment of threatened miscarriage without any effect on obstetric outcome.[40] However more large randomized control studies are warranted to evaluate this effect. More emphasis needs to be given to long term outcome, especially neonatal congenital malformation as none of the studies included this endpoint.

Crown-rump length discrepancy

A smaller than expected CRL is associated with very preterm delivery, fetal growth restriction, SGA and LBW. It is often assumed that fetal growth is uniform in the first trimester.[41] However, variation in fetal growth may occur. Maternal factors such as age, smoking, ethnicity and body mass index, and fetal factors such as chromosomal abnormality and fetal sex have been reported to be associated with a discrepancy between the expected and observed CRL measurement.[42-44] A smaller than expected fetus could be the first sign for fetal growth restriction.[43,45] Hence, reassigning gestational age by first trimester ultrasound should be done with caution as this could mask early fetal growth restriction. Consecutive measurements in the first trimester, individual growth charts which take account of the maternal factors may contribute to a more accurate dating and identify pregnancies at risk of adverse obstetric outcome at an earlier stage.[42,46]

Vanishing twin phenomenon

The incidence of vanishing twin is slightly higher than that of clinical miscarriage (30 versus 20%) in first trimester singleton pregnancies but a vanishing twin is likely to have the same pathophysiology and similar aetiology, that is mainly the association with chromosomal abnormality.[47] Survivors of a vanishing twin phenomenon are at increased risk of (very) preterm delivery, SGA, (very) low birth weight and possibly perinatal death. The increased risk of low birth weight can be explained by the higher prevalence of preterm delivery. The increased risk of perinatal death was only described in one study and was associated with a late vanishing twin rather than an early vanishing twin.[48] The etiology of the increased risks of SGA and preterm delivery are not certain.

One explanation is that of early implantation crowding in multiple pregnancies, resulting in an unfavorable implantation site and/ or limiting placental expansion and growth.[49] These authors demonstrated that the risk of SGA increases with increasing initial number of fetuses in multifetal reduction pregnancies.[49] Another explanation is the presence of fetal growth determining factors or the decomposition of products segregated after the vanishing twin.[50] This theory is supported by the fact that the risk increases with increasing GA at the time of the occurrence of the vanishing twin phenomenon.[48] A third explanation is that a vanishing twin phenomenon could result in blood loss, which is an independent risk factor of SGA and preterm delivery.

Although the vanishing twin phenomenon occurs in both spontaneously conceived and artificially conceived multiple pregnancies, the effect of a vanishing twin on the obstetric outcome of the survivor twin has only been described in ART-population.[51] Although it is

likely that the found increased risks will also apply for survivor twins after a vanishing twin in a spontaneously conceived multiple pregnancies, confounders like type of ART treatment, time of (sub)infertility and diagnosis of (sub)infertility have to be taken into account. Most likely, the reason that the effect of a vanishing twin is only described in the ART-population is three-fold: (1) in spontaneously conceived pregnancies the first ultrasound examination is usually performed at the end of the first trimester, as opposed to in ART pregnancies; (2) in case of ART-pregnancies (and especially after double embryo transfer) more emphasis is given to multiple pregnancies or a vanishing twin phenomenon; and (3) it is better documented in ART-pregnancies. As the risk of adverse obstetric outcome is increased after a vanishing twin phenomenon, this indicates the necessity for early ultrasound in all pregnancies.

Hyperemesis gravidarum

Our review showed that women with hyperemesis gravidarum (HG) and a normal pregnancy weight are not at increased risks of adverse obstetric outcome. Only those with a pregnancy weight gain <7 kg have an increased risk of preterm delivery, LBW and SGA as compared with normal controls. However, comparable risks were observed when compared to women with also a pregnancy weight gain <7 kg and no HG.[52] This suggests that the adverse obstetric outcomes are the consequence of poor maternal weight gain throughout gestation rather than HG early in pregnancy. Our findings are in concert with a recent published meta-analysis, Veenendaal et al. observed increased odds for PTD (OR 1.32, 95% CI 1.04-1.68), LBW (OR 1.42, 95% CI 1.27-1.58), SGA (OR 1.28, 95% CI 1.02-1.60) and similar odds for perinatal deaths, low AS, and congenital anomalies, in women with hyperemesis gravidarum.[53]

Approximately 73% women experience nausea and vomiting during the first trimester.[54] Nausea and vomiting can lead to a mild, or in case of HG, to severe nutritional deprivation in early pregnancy. Data of the Dutch Hunger Winter shows that, as opposed to undernutrition throughout gestation, undernutrition in the first trimester leads to infants with birth weights within the normal range.[55] Furthermore, Lumey et al. found evidence of a compensatory placental growth after famine exposure limited to the first trimester.[55] This phenomenon is in concert with another study, which found higher placental weights after low calorie/ carbohydrate intake and lower placental weight after high calorie/ carbohydrate intake during first trimester.[56] Insulin and the Insulin-like Growth Factors, IGF-I and IGF-II, play an important role in placental and fetal growth. Both insulin and IGF-I levels are reduced by nutrient restriction, but the IGF-II level is less affected by nutrient restriction.[57] In late pregnancy IGF-I is the dominant factor for fetal growth, whereas in early pregnancy placental and fetal growth are predominantly regulated by IGF-II.[58] Hence, nutrient deprivation in late pregnancy, but not in early pregnancy, will result in SGA and LBW. This explains the finding of Dodds et al., that only women with HG and poor pregnancy weight gain are at increased risk of adverse obstetric outcome.[52] Nausea and vomiting and HG limited to the first trimester are associated with a favorable obstetric outcome because maternal nutrient restriction in first trimester favors placental development.[59,60]

Table II. Early pregnancy events and complications as risk factors for adverse obstetric outcome in the ongoing pregnancy.

Obstetric outcome	(Mild) blood loss	N	Heavy blood loss	N
Haemorrhage	**1.8 (1.7-1.9)**	2	no data	0
PIH	**1.4 (1.1-1.8)**	1	*1.1 (0.5-2.4)*	1
PE	*1.2 (0.9-1.6)*	2	*1.1 (0.5-2.4)*	1
Abruption	**1.8 (1.1-2.9)**	3	**3.6 (1.6-7.9)**	1
Previa	*1.5 (0.8-2.9)*	3	*2.5 (0.9-6.9)*	1
PPROM	*1.3 (1.0-1.7)*	3	***3.2 (1.8-5.7)***	1
Preterm <37 weeks	**1.6 (1.4-1.8)**	8	**2.4 (1.0-5.8)**	3
Preterm <34 weeks	**2.5 (1.6-3.9)**	4	no data	0
Perinatal outcome				
IUGR	no data	0	no data	0
SGA	*1.4 (1.0-1.9)*	2	**2.6 (1.2-5.6)**	1
LBW <2500g	**1.6 (1.1-2.2)**	5	*1.7 (0.9-3.3)*	1
LBW <1500g	**2.7 (1.4-5.2)**	3	no data	0
Cong. malformation	**1.5 (1.1-2.0)**	2	no data	0
5-Min Apgar score < 7	*1.1 (1.0-1.3)*	1	no data	0
Fetal death	*1.1 (0.8-1.4)*	4	no data	0
Perinatal death	*2.1 (1.0-4.4)*	4	no data	0

Data are reported as Odds Ratio (OR) with 95% Confidence Interval (CI); no data, no available study

Pregnancies with an intrauterine device in situ

Only a few studies addressed the association between IUD and adverse obstetric outcome, and they demonstrated an increased risk of late miscarriage and preterm delivery.[61,62] These studies were small, dated, and lacked control for confounders. Though, these findings were confirmed by a recently published well-controlled retrospective cohort study consisting of 196 pregnancies with a Copper T 380A IUD in situ.[63] After adjusting for important confounders - such as age, parity, obstetric history, gestational age at delivery, smoking and prepregnancy weight - they found an increased risk of late miscarriage (OR 16.8, 95% CI 10.6-26.7), antepartum hemorrhage (OR 3.1, 95% CI 2.1-4.7), placental abruption (OR 3.4, 95% CI 2.0-5.9), preterm delivery (OR 2.5, 95% CI 1.7-3.8), PPROM (OR 9.4, 95% CI 6.8-13.0) and clinical chorioamnionitis (OR 4.1, 95% CI 2.3-7.2). But, these women were not at increased risk for preeclampsia, SGA, fetal death and neonatal death. Placental histology showed a higher prevalence and a more severe histologic chorioamnionitis and funisitis (OR 3.4, 95% CI 2.2-5.3). Cultures isolated from the amnionitic fluid demonstrated for both pregnancies with and without an IUD a highest prevalence of Ureaplasma urealyticum (49%). Though, in pregnancies with an IUD a higher prevalence of candida species was isolated (31% vs. 6%).[63] This indicates the necessity of culturing in IUD pregnancies to determine the most optimal treatment.

Intrauterine haematoma	N	CRL discrepancy	N	Vanishing twin	N	Hyperemesis gravidarum	N
no data	0	no data	0	*0.9 (0.5-1.5)*	1	no data	0
2.1 (1.5-2.9)	1	*1.0 (0.8-1.2)*	1	*1.2 (0.6-2.1)*	1	*1.0 (0.9-1.3)*	1
4.0 (2.3-7.0)	1	no data	0	*1.8 (0.7-4.9)*	2	no data	0
6.4 (3.4-12.2)	2	no data	0	*1.9 (1.0-3.6)*	1	no data	0
no data	0	no data	0	*1.1 (0.5-2.4)*	1	no data	0
0.7 (0.1-3.2)	1	no data	0	no data	0	no data	0
2.4 (1.7-3.3)	4	*1.0 (0.7-1.5)*	1	**1.4 (1.1-1.7)**	6	*1.1 (1.0-1.4)*	4
no data	0	**2.0 (1.1-4.0)**	1	**2.3 (1.5-3.6)**	5	no data	0
no data	0	**2.8 (1.9-4.3)**	1	no data	0	no data	0
2.1 (1.4-3.3)	3	**1.1 (1.0-1.2)**	1	**1.7 (1.0-2.9)**	5	*1.3 (1.0-1.7)*	4
no data	0	**1.8 (1.2-2.3)**	1	**1.7 (1.3-2.2)**	3	**1.5 (1.3-1.7)**	2
no data	0	no data	0	**2.0 (1.3-3.2)**	3	**1.4 (1.0-2.0)**	1
1.6 (0.5-5.1)	1	no data	0	*1.0 (0.8-1.3)*	1	*1.1 (0.6-2.0)*	3
5.7 (2.5-12.7)	1	no data	0	no data	0	*1.2 (0.8-1.7)*	3
2.8 (0.9-8.4)	2	no data	0	no data	0	**1.6 (1.0-2.5)**	2
2.1 (0.8-5.4)	2	*0.8 (0.2-3.3)*	1	**3.3 (1.3-8.4)**	1	*1.2 (0.8-1.7)*	4

The increased adverse obstetric outcome in pregnancies with an IUD in situ can be explained by three mechanisms: (1) by direct pressure and disruption of the placental bed (2) by blood loss, causing a chronic inflammatory reaction[32] and (3) by microorganisms attached to the IUD.[63-65]

Clinical implications

Following this review, clinicians have to be aware that women after a first trimester complication are more at risk for adverse obstetric outcome. The antenatal identification of these parameters during the first half of pregnancy should enable better management protocols and new therapeutic guidelines aimed at improving the perinatal outcome in these groups of women at higher risks of abnormal pregnancy outcome. Although it remains questionable whether this knowledge could prevent obstetric complications to occur, but possibly by intensification of care the anticipated detrimental effects can be avoided or reduced.

Future research

Some associations are extensively investigated and distinct relations are demonstrated. Nevertheless, there is a paucity of evidence to demonstrate a relation for many associations.

This is especially the case for the risk of adverse obstetric outcome after recurrent miscarriage, intrauterine hematoma, vanishing twin and CRL-discrepancy.

For future research, it is essential that besides the obvious confounders such as maternal age, smoking, BMI, interpregnancy interval, socioeconomically status, ethnicity, stress, parity and diet patrons, that other first trimester complications have to be taken into account as well. Hereby, indicating the necessity of well-documenting all these risk factors in National Birth Registers.

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3

PLACENTAL VASCULOGENESIS AND ANGIOGENESIS IN EARLY PREGNANCY

3.1

VASCULOGENESIS AND ANGIOGENESIS IN THE FIRST TRIMESTER HUMAN PLACENTA; AN INNOVATIVE THREE-DIMENSIONAL STUDY USING AN IMMERSIVE VIRTUAL REALITY SYSTEM

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Placenta 2009; 30:220-2

ABSTRACT

Background

First trimester human villous vascularization is mainly studied by conventional two-dimensional (2D) microscopy. With this (2D) technique it is not possible to observe the spatial arrangement of the haemangioblastic cords and vessels, transition of cords into vessels and the transition of vasculogenesis to angiogenesis. The Confocal Laser Scanning Microscopy (CLSM) allows for a three-dimensional (3D) reconstruction of images of early pregnancy villous vascularization. Though, these 3D reconstructions are still analyzed on a 2D medium, lacking depth perception.

Methods

We performed a descriptive morphologic study, using an immersive Virtual Reality system to utilize the full third dimension completely. This innovative 3D technique visualizes 3D datasets as enlarged 3D holograms and provided detailed insight in the spatial arrangement of first trimester villous vascularization, the beginning of lumen formation within various junctions of haemangioblastic cords between 5 and 7 weeks gestational age and in the gradual transition of vasculogenesis to angiogenesis. This innovative immersive Virtual Reality system enables new perspectives for vascular research and will be implemented for future investigation.

INTRODUCTION

The fetal vascular system of the human placenta is formed by means of vasculogenesis and angiogenesis, resulting in a complex network of peripheral villous capillaries essential for nutrition and gas exchange during second and third trimester of human pregnancy.[1-3] Histopathology of the placenta often reveals an underdeveloped vascularization of the chorionic villi as a result of early deficient villous vasculogenesis and/or angiogenesis in miscarriage, early fetal growth restriction and preeclampsia.[4-6]

Normal and abnormal first trimester villous vascularization have primarily been investigated by the use of conventional 2D (electron) microscopy.[4,7-9] These studies provided a good insight of the maturation of lumenized vessels from primitive haemangiogenic cords and margination into peripherally located vessels. The spatial arrangement between lumenized vessels and cords, however, could not be elucidated using a 2D technique. Hence, in the last few years Confocal Laser Scanning Microscopy (CLSM) has been used, which allowed for a 3D reconstruction of the capillary bed in human first trimester and term placenta.[3,10,11] This technique enables to obtain perfectly registered stacks of thin serial optical sections from which computer generated 3D reconstructions can be made without problems to align images of successive sections. Although the CLSM proved to be a useful technique for 3D visualization of placental tissue and thus of the visualization of the spatial arrangement of vessels and cords, the analysis of these 3D images, however, was still limited by visualization on a 2D computer screen.

An innovative immersive Virtual Reality system – the I-Space – installed at the Erasmus MC allows for visualization of 3D datasets as enlarged 3D holograms floating in front of the viewers. The aim of our study is to investigate whether this 3D visualization allows for an improved analysis of the spatial arrangement of vessels and cords in first trimester chorionic villus vascularization

MATERIAL AND METHODS

Case selection

Fifteen placentae (four between 5 and 6 weeks, five between 7 and 8 weeks, three between 9 and 10 weeks and three between 11 and 12 weeks gestation) of uncomplicated singleton pregnancies were collected and studied after legal termination of pregnancy, terminated by dilatation and curettage. Ultrasound and clinical data were used to calculate the exact gestational age (GA). The Institutional Medical Ethics Committee approved the study design. All samples were included after informed consent of the patients.

A representative placental tissue of the free (terminal) villi of the chorion frondosum closest to the decidua basalis was drawn and fixed directly after collection in 4% (w/v) paraformaldehyde dissolved in PBS (10 mM H2NaPO4/HNa2PO4, 150 mM NaCl, pH 7.6). After fixation for 4 hr, the samples were stored in 70% ethanol at 4°C until further histological processing.

Immunofluorescent staining of placentae, CLSM

Placentae were stained whole mount as previously described by Van den Hoff and colleagues.[12] In short, a sample of the placenta was taken and hydrated in a graded series of ethanol-PBS (75%, 50% and 25%) and permeabilized by incubation in PBST (0.25% Triton_100 in PBS) for 30 min. To reduce background staining, the villi were incubated in PBS-A (1% BSA in PBS) with 5% Goat serum. The endothelial cells were stained using the CD31 monoclonal antibody (DAKO, Glostrup, Denmark) and Goat-anti-Mouse Alexa-568 (Molecular Probes), as secondary antibody. Following extensive washing in PBS-A, the villi were mounted in PBS with 50% (v/v) glycerol. Fluorescence was visualized using CLSM (Bio-Rad MRC 1024). Auto-fluorescence of the tissue was used to visualize the contours of the villi.

Morphometric analysis, Virtual Reality system – the I-Space

Digitized images (512 x 512 pixels) of serial optical sections (15-20 mm apart) of individual mesenchymal or immature intermediate villi were captured by the CLSM, Bio-Rad MRC 1024 using the planapochromat objective with a magnification of 10.

The BARCO I-Space (Barco, Kortrijk, Belgium) installed at the Erasmus MC is a so-called four-walled CAVE™-like Virtual Reality system.[13] In the I-Space researchers are surrounded by computer generated stereo images, which are projected by eight high quality DLP-projectors on three walls and the floor of the projection room. The CAVORE application[14] volume rendered the individual placental tissue sample datasets, obtained by CLSM, in the I-Space. This resulted in a visualization of an animated enlarged hologram of the dataset, floating in space in front of the viewers. The viewers wear a pair of lightweight glasses with polarizing lenses that allow perception of depth. Wireless tracking of the viewer's head allows the computer to provide a correct perspective and motion parallax that, in addition to the stereoscopic images, helps in discerning fine details and understanding of three-dimensional structures in the volumes. A wireless 6 degrees-of-freedom (DOF) controller that emits a virtual pointer is used for manipulation of and interaction with this hologram. The volume can be rotated and moved in all three dimensions to obtain an optimal view of the data. A cutting plane attached to the pointer allows assessment of the interior of the placental tissue. Measurements of structures can be taken by placing markers in the three-dimensional space of the volume with the virtual pointer. A study using ultrasound data demonstrated that measurements in the I-Space are at least as accurate as measurements taken on a 2D workstation.[15] A transfer function widget operated by the pointer controls the contrast and transparency of the rendered image. Manifestations of vasculogenesis and angiogenesis will be described using this innovative technique. The diameter of 15 randomly chosen peripherally located vessels will be measured using the virtual pointer for each placental tissue.

RESULTS

A total of 15 pregnancies between $5^{5/7}$ and $12^{3/7}$ weeks GA were studied. An intensive network of peripheral located cords, with sporadic capillaries with lumen, predominantly characterized the chorionic villi at $5^{5/7}$ until 7 weeks GA. In this period the networks of cords

were heavily connected with each other without any interruption (Fig 1A). As of 7 weeks GA onwards capillaries with lumen were more predominantly present and as of 9 weeks onwards the villi were predominantly characterized by an intensive network of lumenized capillaries with only sporadic cords (Fig 2). From $5^{5/7}$ until 7 weeks GA large luminal spaces, most pronounced within junctions of these haemangioblastic cords, were spotted (Fig 1B). The latter were discovered by zooming and using the cutting plane in real-time to assess the interior of the cords and vessels. At 8 weeks GA most cords had already made the transition to a capillary with lumen. Cords were still present, but were mostly seen between capillaries. At junctions of the capillaries and cords often an enlargement of the luminal space, at one or simultaneously at both sides, within the cords was observed (Fig 2).

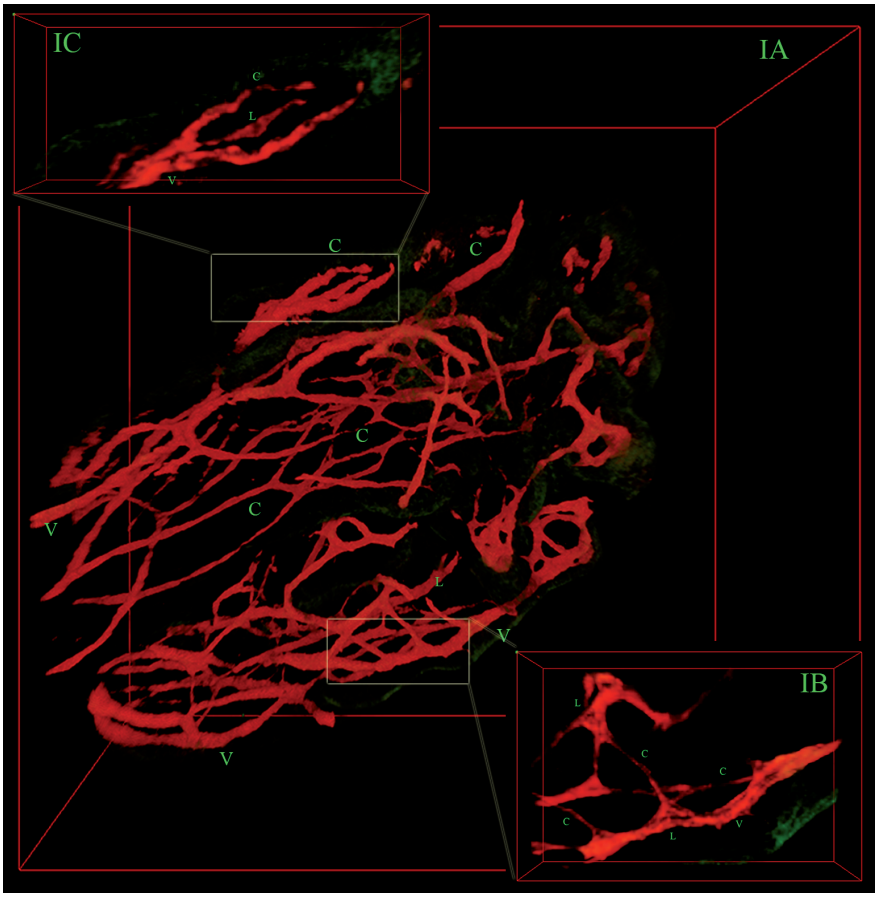

Figure 1. (1A) Direct volume rendering of a stack of optical sections of mesenchymal villi at $6^{4/7}$ weeks GA with a vascular network of mainly cords (C) and few capillaries (V); (1B) a close-up of the vascular network of capillaries (V) and cords (C). Large luminal spaces (L) are present at the junction of the vessels and cords; (1C) a close-up of a young sprout. The sprout consists of 3 cords in which lumenization has begun. A large luminal space (L) is seen in the second cord.

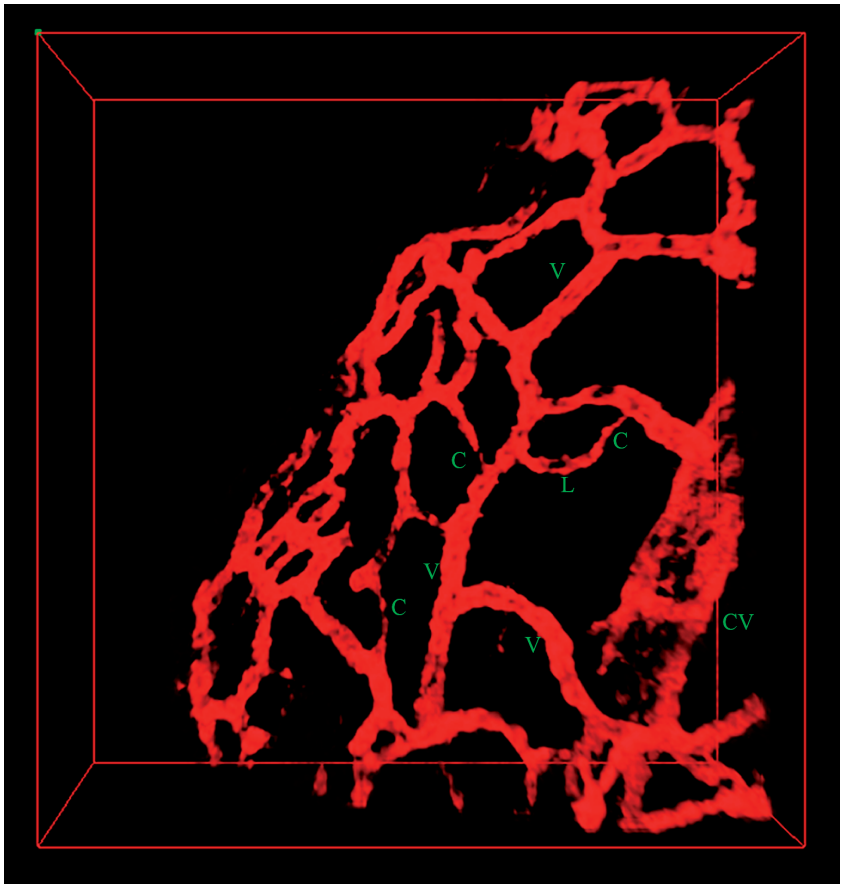

Figure 2. A close-up of direct volume rendering of a stack of optical sections of immature mesenchymal villi at $11^{4/7}$ weeks GA with a vascular network of mostly capillaries (V), a central vessel (CV) and sporadic cords in between vessels (C). Within some cords beginning of lumenization is observed (L).

Blind-ending cords were observed in young villous sprouts until 7 weeks GA (Fig 1C). Hereafter, from 8 weeks GA onwards, blind-ending capillaries with lumen were observed in young villous sprouts. From 7 weeks GA central vessels, mainly characterized by two or more larger vessels connected by an intensive network of peripheral vessels and cords, were observed. By precisely placing calipers while using the cutting plane to assess the outer layer of the vessels in real-time it was possible to measure the diameter of the vessels. The mean diameters of the vessels (n=15) are presented in Table I.

DISCUSSION

The techniques to investigate normal villous vasculogenesis and angiogenesis, from the application of corrosion casts observed in a scanning electron microscope, injection of contrast

Table I. Luminal diameter of chorionic villous vessels in relation to gestational age

GA (weeks)	5-6	7-8	9-10	11-12	P-value[a]
Number of specimens	4	5	3	3	
Vessel diameter (μm)	12.9 (0.5)	14.9 (0.5)	15.5 (0.6)	16.5 (0.6)	<0.001

Data presented as means (SEM)
[a] One-way ANOVA

medium, by using a classical microscope to manual 3D reconstructions of paraffin sections and by CLSM, has improved over the years.[3,7-9,16-18] We present the results of a – to our knowledge – first 3D reconstruction of vasculogenesis and angiogenesis of human early pregnancy chorionic villi using an innovative and immersive 3D virtual reality system, the I-Space.

Although the disadvantages of the CLSM technique, such as the limited penetration depth of 1mm and the marked difference between the lateral and axial resolution, limits the analyses in the I-Space, the 3D enlarged hologram provided a more detailed insight of the vasculogenesis and angiogenesis. The real-time manipulation of the hologram by means of rotating, clipping, cutting, measuring and zooming into a region of interest provides a new dimension in 3D placental vascularization research.

In our study as of $5^{5/7}$ weeks GA onwards a complex network, primarily dominated by cords with redundant connections, was observed in the I-Space, as was also observed by Lisman and colleagues in their CLSM study.[3] Although the vascular network consisted mostly of cords, still capillaries with lumen were observed at this point as well. By zooming and using the cutting plane we observed large luminal spaces at junctions of haemangioblastic cords suggesting a beginning of lumen formation at these junctions, which then could progress into the cords (Fig 1B-C). This was observed at various junctions from $5^{5/7}$ weeks until 7 weeks GA suggesting that lumen formation does not occur synchronously in all cords but sequentially as was suggested by Kamei and colleagues.[19] Hereafter, lumen formation of these haemangioblastic cords, mostly located in between capillaries, was observed from one or simultaneously from both sides.

Benirschke and colleagues[1] observed vasculogenesis only until $6^{4/7}$ weeks GA, while others[3,9] observed vasculogenesis until at least 9-10 weeks GA. At 7 weeks GA we still observed blind-ending cords (Fig 1C), arising from the underlying vascular network, in sprouting villi suggesting vasculogenesis. From 8 weeks GA onwards, blind-ending vessels were noticed, arising from the underlying vascular network, suggesting branching angiogenesis. Though, until 11 weeks GA, haemangioblastic cords were still sporadically present between vessels (Fig 2). On basis of these observations we hypothesize a gradual transition from villous vasculogenesis to angiogenesis thorough the first trimester, in line with the suggestion of Demir and colleagues.[20]

In conclusion, the immersive Virtual Reality system allows for real-time depth perception and manipulation of 3D datasets in an intuitive manner. This innovative technique allowed for discovering complex details of first trimester villous vascular development and provides new perspectives for future investigation.

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3.2

THE EFFECT OF SMOKING ON EARLY CHORIONIC VILLOUS VASCULARIZATION

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Placenta 2012; 33:645-651

ABSTRACT

Background

The aim of the study was to investigate whether first trimester chorionic villous vascularisation is different in women who smoked cigarettes before and during pregnancy in comparison with women who did not smoke.

Methods

Placentas of smoking (>10 cigarettes/day, n=13) and nonsmoking women (n=13), scheduled for a legal termination of a viable first trimester pregnancy for social indications, were retrieved. Placental tissues of 3-5 mm^3 were whole mount CD31 immunofluorescence stained. Images of the CD31 immunofluorescence and contour of the villi were captured using an Optical Projection Tomography scanner. An immersive BARCO virtual reality system was used to create an enlarged interactive 3-dimensional hologram of the reconstructed images. Automatic volume measurements were performed using a flexible and robust segmentation algorithm that is based on a region-growing approach in combination with a neighbourhood variation threshold. The villous volume, vascular volume and vascular density were measured for the total chorionic villous tree as well as for its central and peripheral parts.

Results

No differences in maternal age and gestational age were found between nonsmoking and smoking women. No differences were found in the total, central and peripheral villous tree volume and vascular volume. The central (13.4% vs. 9.5%, p 0.03) and peripheral (8.4% vs. 6.4%, p 0.02) villous tree vascular densities were increased in the smoking women as compared with the nonsmoking women.

Conclusions

Chorionic villous vascularisation is already altered in first trimester of pregnancy in women who smoked cigarettes before and during pregnancy.

INTRODUCTION

Prenatal maternal smoking is one of the most important modifiable risk factors for pregnancy complications. The prevalence of prenatal smoking is 15 to 25%.[1-3] About 30 to 40% cease smoking in the first trimester of pregnancy, therefore 10-15% will continue smoking throughout pregnancy.[2,3] Smoking is associated with increased risks of adverse obstetric outcome such as intrauterine fetal growth restriction, preterm delivery and fetal death.[4,5] Although the association with adverse obstetric outcome is clear, the precise pathological mechanism is not yet completely understood. Studies on the effects of cigarette smoking on early first trimester placental development are sparse[6] and various histomorphometric studies on term placentas of smoking and nonsmoking mothers show contrasting effects of smoking on the villous vascularisation.[7-13] The differences in methology between the studies might explain the controversy in results found.

Villous vascularisation was primarily investigated by the use of conventional 2D microscopy.[14] Recently, vascularisation of chorionic villi was visualized in 3D by immunofluorescent staining of endothelial cells (CD31) and Confocal Laser Scanning Microscopy (CLSM).[15-18] The data were analyzed in 2D[17] and 3D[18]. Although the latter technique improved the possibility to investigate the spatial arrangement of the vessels, it was hampered by the limited penetration depth of 1mm and the marked difference between the lateral and axial resolution. Optical Projection Tomography (OPT scanner 3001, Bioptonics) does not have these limitations. Transferring these OPT dataset to the I-Space Virtual Reality (VR) system offers an excellent opportunity to study vasculature of the villi in 3D.

The aim of the present study is to investigate whether first trimester chorionic villous vascularisation is different in women who continued smoking cigarettes during pregnancy, in comparison with women who did not smoke before and during pregnancy, using an immersive VR system.

MATERIAL AND METHODS

Case selection

Between January and October 2009, at the Centre of Sexual Health (Amsterdam) smoking and nonsmoking women, who were already scheduled for a legal termination of pregnancy for social indications, were asked to participate in this study. The study design was approved by the Institutional Medical Ethics Committee.

Gestational age was determined by first trimester crown-rump length (CRL) measurements or in very early pregnancy by gestational sac measurements. Smoking was defined as women who self reported to smoke at least 10 cigarettes daily from the preconception period until the dilatation and curettage (D&C) and non-smoking women were defined as women who self reported to not have smoked any cigarettes at all. Ultrasonographic investigation was performed before each D&C to confirm fetal viability. Excluded were cases with a nonviable pregnancy, cases with no fetal growth with regard to prior ultrasound and cases with a negative discrepancy between ultrasound dating and last menstrual period dating of more then one week without any prior ultrasound to determine whether fetal growth has occurred.

Also, women who smoked less then 10 cigarettes daily, women who ceased smoking during pregnancy and women using drugs were excluded.

For each week of gestation, between $5^{4/7}$ and $11^{3/7}$ weeks, 3 cases were included. The inclusion period was initially set to six months, but was extended to nine months because of lack of inclusions. A total of 30 cases were collected, divided into non-smoking women (n=16) and smoking women (n=14). After examination, three cases of nonsmokers ($9^{2/7}$, $10^{4/7}$ and $11^{1/7}$ weeks GA) and one smoker ($9^{4/7}$ weeks GA) were excluded. For these cases it was not possible to perform independent measurements of the villous vascularisation and villous volume because of interference of the signals of both the vascularisation and the outer layer of the villus. Finally, 26 placental tissues, evenly divided between nonsmokers (n=13) and smokers (n=13) remained for further study. The observer was blinded for smoking status and gestational age.

Immunofluorescense staining

After confirmed consent and D&C the placental tissue was retrieved and fixed directly after collection in 4% (w/v) paraformaldehyde dissolved in PBS (10 mM H_2NaPO_4/HNa_2PO_4, 150 mM NaCl, pH 7.6). After fixation for 4 hours, the samples were stored in 70% ethanol at 4°C until further histological processing.

Placental tissues of approximately 3-5 mm^3 were whole mount CD31 immunofluorescence stained as previously described by van den Hoff *et al*..[19] In short, the placenta sample was hydrated in a graded series of ethanol-PBS (75%, 50% and 25%) and permeabilized by incubation in PBST (0.25% Triton x 100 in PBS) for 30 min. To reduce background staining, the villi were incubated in PBS-A (1% BSA in PBS) with 5% Goat serum. The endothelial cells were immunofluorescently stained using the CD31 monoclonal antibody (DAKO, Glostrup, Denmark) and Goat-anti-Mouse Alexa-568 (Molecular Probes), as secondary antibody. Following extensive washing in PBS-A, the villi were stored in PBS with 50% (v/v) glycerol.

Optical Projection Tomography

To scan with the OPT scanner 3001, the specimens were embedded in agarose and glued to a mount. Before embedding the tissue in agarose, the villi were washed in PBS. A 1% solution of low melting point agarose (gelling point 24-28˚C) in deionised water was made. The agarose was cooled to 60˚C, filtered through Whatman filter paper 113V and then placed in a 32˚C water bath. When cooled to 32˚C the agarose was put into a petri dish placed on a cold plate (-4˚C). The specimen was placed in the centre of the dish. After the agarose was completely set, the blocks were trimmed, leaving a small amount of agarose ~3mm at the edge. The block was dehydrated in methanol until no water was remaining. The methanol was replaced by BABB (66% Benzyl Benzoate and 33% Benzyl alcohol) and stored at 4˚C in dark until scanning.

Images were captured using a CCD-Camera with a resolution of 1024x1024 square pixels every 0.9° through 360°. Volumes of 1024^3 voxels were reconstructed from 400 1024^2 transmission/emission images. The resolution of the isotropic voxels was approximately 0.01 mm. The Alexa 568 was visualized using a GFR exciter 425 nm/ 40 nm, emitter LP475 nm fluorescence filter and stored in the red channel of the RGB dataset (Figure 1A). Immediately hereafter, auto-fluorescence of the tissue was used to visualize the contours of the villi, using

a Cy3 exciter 545 nm/ 30 nm, emitter 610/75 fluorescence filter and stored in the green channel of the RGB dataset (Figure 1B). The resulting datasets were stored, in preparation for analysis with the I-Space Virtual Reality (VR) system.

I-Space three-dimensional morphological measurements

The BARCO I-Space (Barco, Kortrijk, Belgium) installed at the Erasmus MC (Erasmus MC, Rotterdam, The Netherlands) is a so-called four-walled CAVE™-like VR system.[20] In the I-Space researchers are surrounded by computer generated stereo images, which are projected by eight high quality DLP-projectors on three walls and the floor of the projection room. The V-Scope[21] application is used to create an interactive hologram of the OPT image that can be manipulated by means of a virtual pointer, controlled by a wireless joystick. To perform volume measurements, this application includes a flexible and robust segmentation

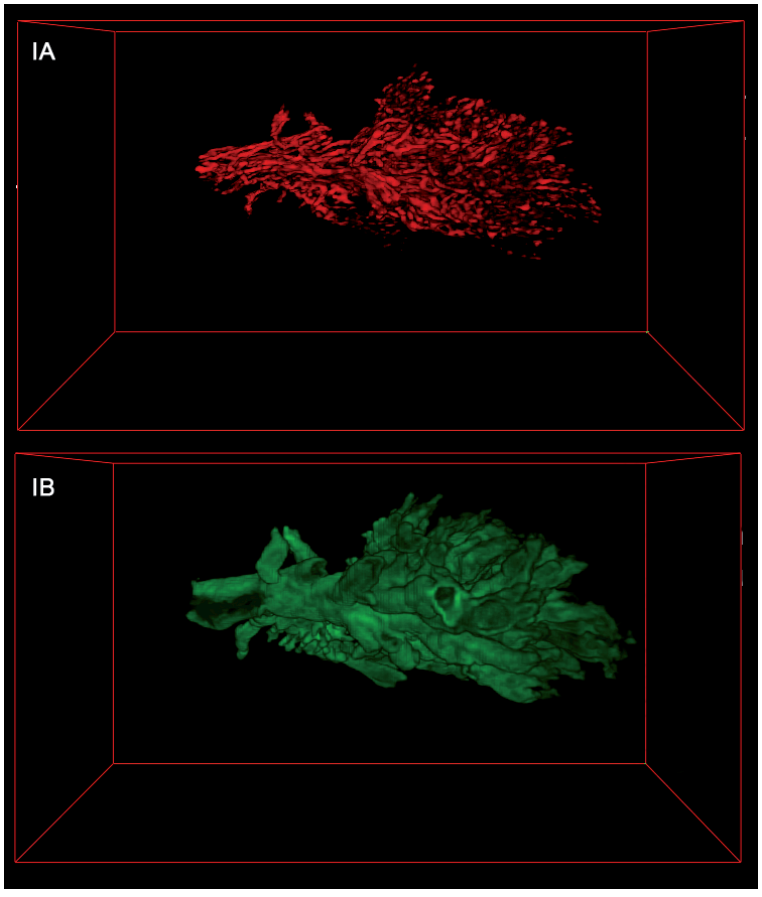

Figure 1. A screenshot of a direct volume rendering of a stack of optical sections of mesenchymal villus at $7^{4/7}$ weeks gestational age. (**IA**) the vascular network within the trophoblast is visualized in red. (**IB**) the outer layer of the villus is visualized in green.

algorithm that is based on a region-growing approach in combination with a neighbourhood variation threshold, as originally proposed for magnetic resonance imaging data by Myers and Brinkley.[22] The user selects an upper and lower red or green level threshold and an upper threshold for the SD of the voxels' neighbourhood. A seed point is placed and the algorithm will segment (grow) the region starting from the seed point. The SD threshold will stop the region growing when it reaches an interface.

As the autofluorescent signal was limited to the outermost part of the villus (Figure 1B), we could not obtain its volume by simple thresholding or region-growing. The quickest way to obtain the villus' volume therefore was to segment the empty space outside the villus by region growing, and subtract the empty volume from the total scanned volume (Figure 2A). If

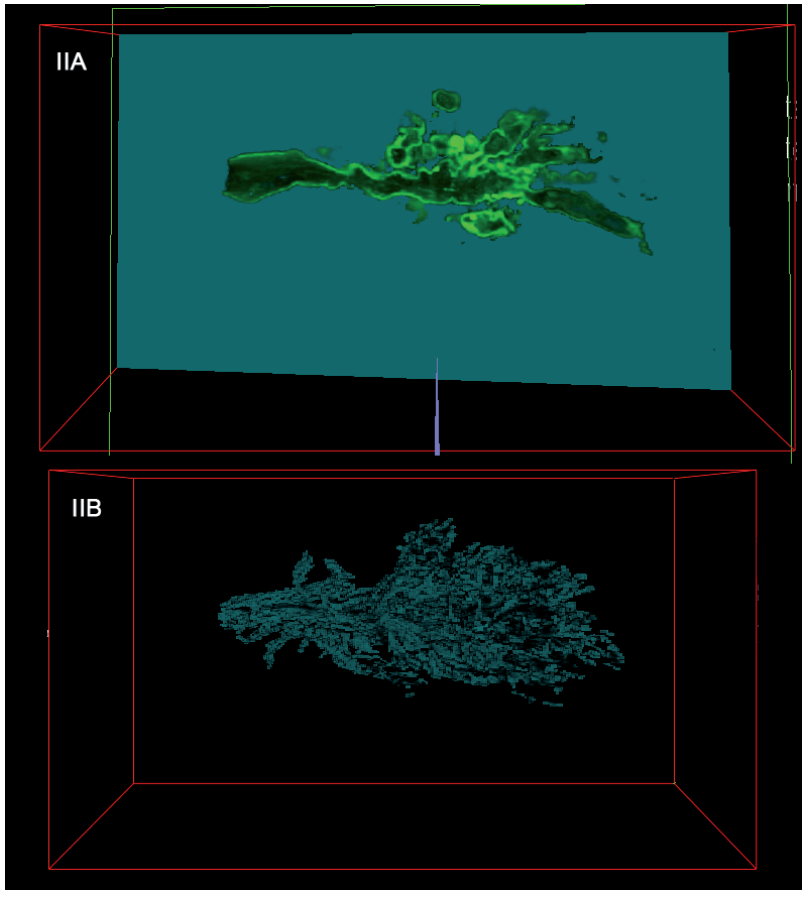

Figure 2. A screenshot of a direct volume rendering of a stack of optical sections of mesenchymal villus at $7^{4/7}$ weeks gestational age. (**IIA**) Example of the indirect villous volume measurement. The outer layer of the villus is visualized in green. The empty space is segmented and visualized in cyan. (**IIB**) Example of the direct chorionic vascular volume measurement. After placing a seed point in a vessel, the algorithm is segmented. The vascular volume is visualized in cyan.

the volume was incomplete, for example in case of a trapped air bubble, a spherical free hand 'paint brush' was used to remove these artefacts from the segmented structure as necessary.

The total villous vasculature volume was measured three times, as this was dependent on the threshold used and the selected seedpoint (Figure 2B). The measurements were performed by the same observer, who was blinded for the cases and thresholds used at prior measurements, with an interval of at least several days to avoid recall bias. The mean of these three assessments was used in the analysis. The Intraclass Correlation Coefficient (ICC) was calculated to quantify the intraobserver reliability of the volume measurements. For a good agreement, the ICC has to be 0.90 or higher. The ICC was 0.991 (95% CI 0.983-0.996). Hereafter, the central and peripheral vascularisation was only measured once. For the central villous tree measurements a region of interest (ROI) box was placed over the stem villi, defined as the trunk of the villus without branching villi. The vascularisation and the villous volume inside the ROI box were measured. For the peripheral villous tree measurements a ROI box was placed over three mesenchymal villous areas, defined as the branching villi (Figure 3). If more than three areas could be selected, then a ROI box was placed at the top of the tree and at opposing sides of the villous tree to get the best representation of the peripheral villous vascularisation. The mean of these three assessments was used for analysis. If the vascular volume measurement was incomplete, if, for example, the vessels were interrupted, the user could place another seed point. Because the vascular volume depends on the size of the specimen, the vascular density (vascular volume divided by villous volume in percentage) was calculated.

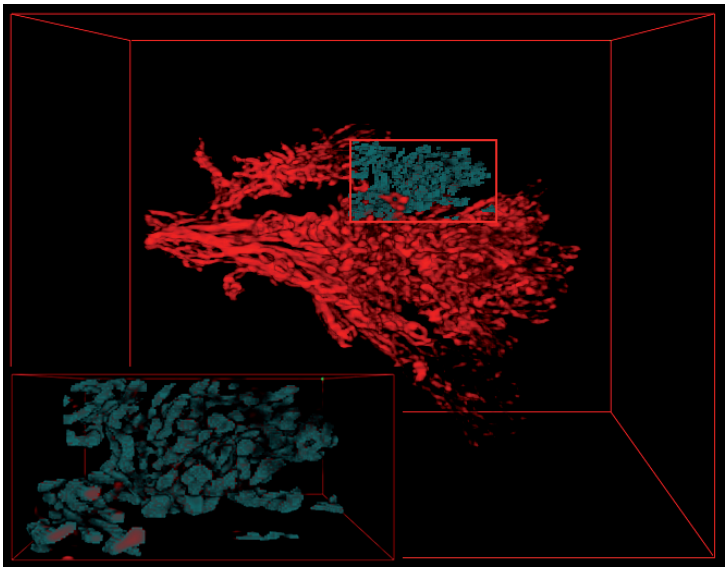

Figure 3. A screenshot of a direct volume rendering of a stack of optical sections of mesenchymal villus at $7^{4/7}$ weeks gestational age. An example of a peripheral villous tree vascular volume measurement. A region of interest box is placed and the vascular volume within is measured. The vascular network is visualized in red and the measured volume is visualized in cyan.

Statistical analysis

Patient characteristics were presented as means with standard error of the mean. Skewness was tested using the Shapiro Wilk test. A comparison between groups was made using a t-test. Correlation was tested using the Pearson correlation test. Differences in morphometrical measurements were analyzed using a general linear model adjusting for possible confounders. A probability value <0.05 was considered statistically significant in this study. The statistical analysis was performed using SPSS for Windows, version 15.0.0 (SPSS Inc., Chicago, Il).

RESULTS

Patient characteristics are presented in Table I. No differences in maternal age and gestational age were found between nonsmoking and smoking women. Neither difference was found in the total villous tree volume, nor in the total villous tree vascular volume (Table II). Also, no difference was found between the two groups with regard to the central and peripheral

Table I. Patient characteristics

	Non-Smokers (n=13)	Smokers (n=13)	p-value
Age (years)	25.0 (6.7)	23.3 (6.1)	0.50
Gestational age (days)	56.5 (11.5)	60.2 (11.5)	0.41

Date presented as means (SD) [a] Independent t-test

Table II. Results of morphological measurements

	Non-smokers (n=13)	Smokers (n=13)	p-value[a]	Adjusted p-value[b]
Total villous tree				
Villous volume (mm^3) *	15,0 (6,17)	20,1 (3,23)	0,34	0,40
Vascular volume (mm^3) *	0,83 (0,30)	1,57 (0,36)	0,050	0,057
Vascular density	6,18% (0,78)	8,54% (1,03)	0,08	0,053
Central villous tree				
Villous volume (mm^3) *	1,86 (0,73)	2,25 (0,63)	0,59	0,62
Vascular volume (mm^3) *	0,167 (0,063)	0,291 (0,081)	0,13	0,16
Vascular density	9,54% (1,25)	13,41% (1,03)	0,025	0,033
Peripheral villous tree				
Villous volume (mm^3) *	1,23 (0,23)	1,41 (0,26)	0,67	0,81
Vascular volume (mm^3)	0,090 (0,13)	0,129 (0,18)	0,09	0,12
Vascular density	6,38% (0,60)	8,40% (0,74)	0,046	0,018

Data presented as means (± SEM) or as geometric mean * (± SEM)
[a] Independent t-test, [b] Regression analysis, adjusted for gestational age

villous tree volume and vascular volume. A tendency for an increased total tree villous vascular density was observed in smoking women. The central and peripheral tree vascular densities were increased in the smoking women as compared with the nonsmoking women (Figure 4). After adjusting for gestational age the differences remained significant.

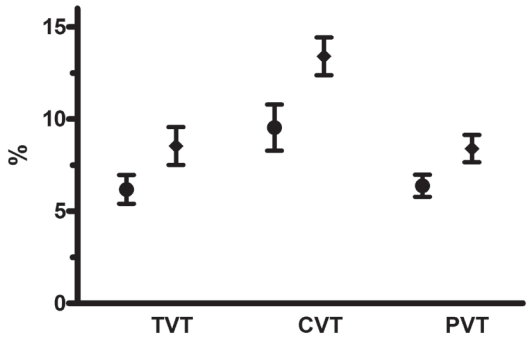

Figure 4. Shows the mean vascular densities ± the standard error of the mean (%) of placentas from smoking (diamond) and non-smoking women (circle). TVT = total vascular tree; CVT = central vascular tree; PVT = peripheral vascular tree.

DISCUSSION

This is the first study about three-dimensional chorionic villous vascularisation of first trimester placentas, using an immersive VR system. The advantage of this technique is the fact that it was possible to visualize an enhanced (1 m^3) three dimensional structure of the villus outer layer and vasculature of a whole mount villus (3-5 mm^3) in detail. The measurements were performed semi-automatically using segmentation. It was possible to perform measurements on the whole villus and to confirm, by scanning through the villus, whether the measurements were complete. The present study shows that the central and peripheral chorionic villous tree vascularisation density is higher in first trimester pregnancies of women who smoked cigarettes before and during pregnancy as compared with nonsmoking women. A trend to an increased total tree villous vascular density was observed as well, but probably due to the limited study size this just did not reach statistical significance (p 0.053). Another explanation could be that for the measurement of the central and peripheral vascularisation different lower thresholds needed to be used. Therefore the measurements of the total villous tree could be less accurate, hence explaining the relative high standard error of the mean (Table II).

Cigarettes contain a complex mixture of 4000 chemicals, and besides nicotine, several other toxic elements are present, such as cadmium, acrolein, acetaldehyde and free radicals.[23] Several studies have reported that nicotine acts as a pro-angiogenic factor.[23-24] Nicotine promotes migration, proliferation and in vitro vasculogenesis of endothelial progenitor cells.[24] This effect is dose-dependent and maximal at physiological nicotine serum

concentrations of smoking women. Genbacev et al.[25] demonstrated that the expression of VEGF-A, which stimulates vasculogenesis and branching angiogenesis, is increased by maternal smoking. The increased expression of VEGF could be an adaptive response to a decreased oxygen tension (relative hypoxia) caused by smoking.[25] This process could explain the increased central and peripheral vascular densities observed in the present study. A similar effect was previously demonstrated in term placentas of smoking women. With electron microscopy, an increased chorionic villous vascular branching as an adaptive response to increase the surface area for improving gas exchange was observed.[26] This is also in line with the proposed molecular cascade described by Zdravkovic et al.,[27] linking the pathophysiological changes of cigarette smoking to adverse obstetric outcome.

In a conventional 2D study first trimester chorionic villous vascularisation in smoking and nonsmoking women was investigated using placentas of termination of pregnancy between 9-14 weeks gestation.[6] They showed an increase thickness of the villous membrane, trophoblast, and an increase of syncytial necrosis in women who smoked. In that study, not only there were no differences found between smoking and nonsmoking women in the trophoblast and stromal volume fraction, but, as in contrast to the present study, in the capillary lumen volume fraction as well. The authors stated that the latter could be explained by the fixation technique used.[6]

In contrast to most studies on term placentas, in which decreased capillary volume fraction in smoking compared to non-smoking women is reported,[7,10,11] we observed in the present study an increased first trimester villous vascular density in smoking women. A possible explanation could be that the detrimental effects of maternal smoking on the villous vasculature take place after onset of the uteroplacental circulation or after prolonged tobacco exposure. This could explain the fact that adverse obstetric outcomes, such as preterm delivery, fetal growth restriction, fetal birth weight and also preeclampsia, were similar between non-smoking women and women who ceased smoking before the second trimester of pregnancy.[28-30]

The effects of smoking on villous cytotrophoblasts and trophoblastic invasion have been demonstrated by histological studies of first trimester placentas and cultures of human trophoblastic cells. These studies demonstrated that cigarette smoking is associated with a thinner syncytiotrophoblast, reduced number of villous cytotrophoblasts, decreased cytotrophoblast proliferation and differentiation, decreased anchoring villi and trophoblastic migration.[31-33]

Much controversy exists on the effect of smoking on the villous vascularisation in term placentas (Table III). Most likely due to the variance of sampling procedures and stereologic methods used in the different studies. Some found no differences in the capillary diameter, length, surface area and volume whilst others found significant decrease in these parameters.[10-12] Increased villous vascular branching in smokers was reported in one study using scanning electron microscopy on the casts of the capillaries,[30] but two other conventional stereological studies reported similar branching degree between smokers and nonsmokers.[11-13] The incidence of placental infarction reported is controversial as well, in various studies it is reported to be decreased, similar or increased.[10,34,35] However, most

Table III. Effects of smoking on villous vascular changes and obstetric outcome

Villous vascular changes	
First trimester	Second / Third Trimester
Increased	Increased
Capillary density [Present study]	Branching degree[26]
Thickness of syncitiotrophoblast membrane[6]	Infarction[35]
	Thickness of vasculosyncytial membrane[7,8,13]
Decreased	Decreased
	Capillary diameter[11]
	Capillary density[7,13]
	Capillary length[7,10]
	Capillary surface area[10]
	Capillary volume[10,11]
	Infarction[34]
Similar	Similar
Capillary density[6]	Branching degree[11,13]
Number of capillaries[6]	Capillary diameter[7,10,11]
	Capillary length[11]
	Capillary surface area[11,12]
	Capillary volume[12]
	Infarction[10]
Adverse obstetric outcome	
Miscarriage	Placental abruption
Ectopic pregnancy	Placenta praevia
	Preterm delivery
	Fetal growth restriction
	Less preeclampsia
	Placenta accreta
	Stillbirth
	Premature rupture of membranes

studies have found that the intermediate and terminal villous vascular density is decreased in placentas of smokers as compared with nonsmokers.[10,11,13] Also, it is reported that the thickness of the villous and the vasculosyncitial membrane is increased, and that this effect can already been observed from the end of the first trimester of pregnancy.[6-8,13] The increased thickness of the vasculosyncitial membrane and the decreased terminal vascular

density in smokers could result in a reduced materno-fetal interface, compromising gas and nutrient transfer to the fetus. Hence, this could explain the subsequent adverse obstetric outcome in smokers.

The present study has some limitations. First, smoking of cigarettes was based on self-report rather than measurement of serum cotinine levels. Smoking is thought to be more likely to be underreported during pregnancy, mostly because of a feeling of guilt. However, McDonald et al.[36] and Yeager and Krosnick[37] demonstrated that the correlation between self reported smoking status and cotinine serum levels were high. Secondly, in a previous study placental vascularisation was visualized using a CLSM, which is limited by a penetration depth of 1mm and a marked difference between the lateral and axial resolution.[18] Although the OPT made it possible to investigate larger placental tissues in great resolution from all directions, it was not possible to visualize the lumen of smaller vessels: Therefore, in the present study a differentiation between vessels and haemangioblastic cords could not be made. Finally, it was not possible to obtain the volume of the whole placenta as, opposed to term pregnancies, after D&C the placenta was fragmented and often incomplete. Therefore, we could not determine the absolute vascular volume and density of the whole placenta. For both groups, the samples were at random chosen and therefore determined as a representative sample from every placenta.

In conclusion, we demonstrated that the first trimester peripheral and central chorionic villous tree vascular density is increased in placentas of smoking women as compared to non-smoking women. Thus, as early as the first trimester of pregnancy a chorionic villous vascular adaptive response is observed. As an early effect of smoking on the placenta is observed in the present and other studies, it would be advised to counsel for cessation of smoking even before conception. Especially because much controversy exist on the effect of smoking on placental vascularisation, more research is needed to gain better understanding in the underlying mechanisms of the effects of cigarette smoking on chorionic villous vascularisation.

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4

PLACENTAL VASCULOGENESIS AND ANGIOGENESIS IN MID AND LATE PREGNANCY

4.1

COMPROMISED CHORIONIC VILLOUS VASCULARIZATION IN IDIOPATHIC SECOND TRIMESTER FETAL LOSS

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Early Human Development 2010;86:469-472

ABSTRACT

Background

For normal fetal growth and development a well-developed chorionic villous vascularization is essential. The aim of this study is to investigate whether idiopathic second trimester fetal loss is associated with an underdeveloped chorionic villous vascularization.

Methods

38 placentas after late miscarriage, classified as idiopathic fetal loss (IFL, n = 16) or as fetal loss due to intrauterine infection (IUI, n = 22) were collected. After CD34 immunohistochemical staining the villous stromal area, number of villous vessels, vascular area and vascular area density (central, peripheral and total) were measured in randomly selected immature intermediate villi.

Results

The mean gestational age was $19^{4/7}$ weeks for the IFL group and $20^{6/7}$ weeks for the IUI group. After controlling for gestational age, we found no differences in fetal weight, placental weight, villous stromal area, number of vessels and central vascular features. The mean peripheral vascular area and peripheral vascular area density were, after adjusting for gestational age, reduced in the IFL group.

Conclusion

Idiopathic second trimester fetal loss is associated with a reduced peripheral chorionic villous vascularization. We hypothesize that in these cases, placentation is already disturbed in first trimester of pregnancy, leading to a reduced materno-fetal interface in second trimester, thus to early postplacental fetal hypoxia and fetal death.

INTRODUCTION

The incidence of a late miscarriage, defined as a spontaneous pregnancy loss between 12 and 24 weeks gestation, is approximately 1-2%.[1,2] The following causes for late miscarriage are described: intrauterine infection, uterine abnormalities, cervical incompetence, antiphospholipid syndrome and congenital malformations.[2-6] These mechanisms only explain a proportion of the late miscarriages. It is suggested that, in idiopathic late miscarriage, an inadequate placental development could play a role.[7,8]

In the first trimester of pregnancy, chorionic villous vascularization is formed by means of vasculogenesis and angiogenesis resulting in an intensive network of peripherally located mesenchymal capillaries at the end of the first trimester.[9-12] With increasing gestational age the materno-fetal diffusion distance will decrease due to margination.[9,13] This is a process in which the intravillous capillary position becomes closer to the villous surface due to decrease of villous stromal area and increase of capillary diameter. This results in a thin vasculosyncitial membrane, important for an optimal materno-fetal exchange of oxygen and nutrition.[9,14]

As compared with controls, in second trimester terminated aneuploid pregnancies a reduced villous vascularization with fewer peripheral capillaries has been observed.[15,16] This could lead to an underdeveloped vasculosyncitial membrane, and thus to a reduced materno-fetal exchange. Based on these observations we hypothesized that the reduced villous vascularization could be the underlying cause of intrauterine fetal growth restriction and intrauterine fetal death in aneuploid, but also in euploid pregnancies. The aim of our study is to investigate whether second trimester idiopathic fetal loss is associated with reduced chorionic villous vascularization.

METHODS

Patient Selection

We performed a retrospective study at the Liverpool Women's Hospital National Health Service Trust. The study was approved by the Local Research Ethics Committee. Placental tissue of spontaneous or, in case of intrauterine fetal death, induced miscarriages between 16 and 24 weeks gestation from January 2002 to December 2005 were retrieved and examined. We excluded twin pregnancies, late fetal loss due to congenital malformations, pregnancies with an intrauterine device in situ, women with antiphospholipid syndrome or uterine anomalies, pregnancies with an unknown gestational age and fetal loss with a retention time (time between the intrauterine fetal death and the delivery) of two or more weeks. The latter was based on the fact that chorionic villous vascularization is not influenced by prolonged retention time.[17]

The cases were divided into two groups: (I) cases with idiopathic fetal loss not caused by an intrauterine infection (IFL, n=16) and (II) cases with a live fetus at onset of labour with histopathological findings of intrauterine infection (IUI, n=22).

The diagnosis of intrauterine infection was based on histopathological findings of a perinatal pathologist (McP). A staging and grading system for maternal and fetal inflammatory response was used; stage S0 no inflammation, stage S1 early inflammation

(acute subchorionitis or early acute chorionitis), stage S2 intermediate inflammation (acute chorioamnionitis), stage S3 advanced inflammation (necrotizing chorioamnionitis), grade G1 mild and grade G2 severe.[18] Intrauterine infection was defined as at least S1G2 maternal inflammatory response or with fetal inflammatory response (S1G1) involvement. Cases of fetal death prior to labor and stillbirths, not due to intrauterine infection (S0G0 and S1G1), in whom the viability of the fetus at the time of miscarriage was not known, were assigned to the IFL group. The following patient characteristics were noted: Gestational age, gravidity, parity, fetal and placental weight. Gestational age was determined by first trimester crown-rump length (CRL) measurement in all cases.

Immunohistochemistry

In all cases paraffin blocks were retrieved and stained with hematoxylin and anti-CD34 antibody. Full depth cores of placental tissue sections of 5 mm thick were cut and mounted on polysine coated glass slides (VWR international, Leuven, Belgium). The paraffin embedded sections were de-waxed and re-hydrated through xylene and graded alcohols. Antigen retrieval was performed in 0.01M citrate buffer (pH6.0) for 1 minute using the pressure cooker method. Monoclonal mouse anti- CD34 antibody (DakoCytomatron clone QBEnd-10, diluted 1/100 in tris buffered saline and 0.5% bovine serum albumin) was applied for 30 minutes at room temperature. Blocking of endogenous peroxidase activity and detection of the primary antibody was performed according to the protocol described in the mouse Envision + HRP kit (Dako UK Ltd, Cambridgeshire, UK). Samples were counterstained for 30 seconds in filtered Harris Haematoxylin and permanently mounted in DPX solution (Themo Electron Corporation, Cheshire, UK).

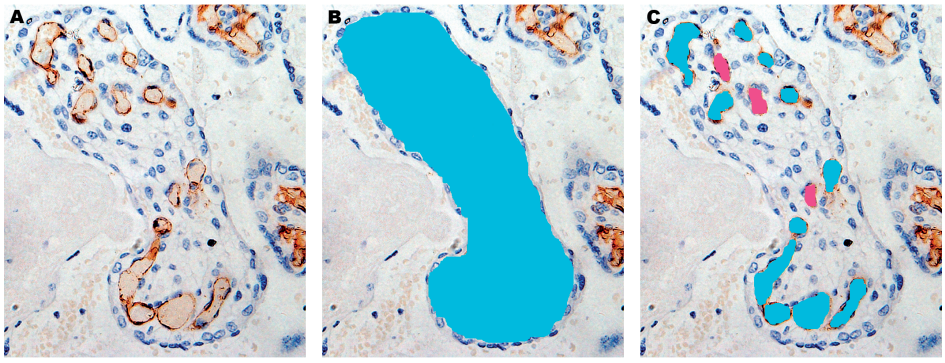
Analysis of extent of vascularization

The slides were examined at x400 magnification by one trained observer (RO), blinded to group and duration of pregnancy. From each placenta, fifteen mesenchymal or immature intermediate villi were randomly chosen. The diameter had to be greater than 80 mm to rule out terminal villi, and stromal connective tissue fibres and perivascular smooth muscle had to be absent to rule out stem villi.[19] In a pilot study, performed by two trained observers, an interobserver error of less then 10% and an intraobserver error of less then 10% were found when 15 villi per observer were randomly selected; this was consistent with Lisman and colleagues.[16]

The vessels in the 15 randomly selected villi were counted. The process of margination was illustrated by describing whether these vessels were located peripherally or centrally. A peripherally located vessel was defined as a vessel situated in direct contact with the syncitiotrophoblastic layer of the villus, as of such contributing to a vasculosyncytial membrane. A centrally located vessel was defined as a vessel without connection to the syncitiotrophoblastic layer.[13]

The microscopic field was transported onto a screen using a colour camera mounted on a standard light microscope. Contours of the stroma of the villi and all included vessels were traced manually on the computer monitor with a mouse-controlled cursor using an on-screen magnification of x400 (Figure 1A-C). Morphometrical measurements were performed using Eclipse Net (Version 1.20.0, laboratory-imaging ltd., Czech)

Figure 1 Analysis of CD34 stained chorionic villi



A, An immature intermediate villi of 20+1 weeks gestational age; **B**, The villous stromal area without the trophoblastic layer (cyan) was traced on screen; **C**, The centrally (cyan) and peripherally (magenta) located vessels were identified and traced on screen.

The following features were measured or calculated: the villous stromal area without the trophoblastic layer, the number of vessels per villus, vascular area (area of all vessels per villus) and vascular area density (percentage of villous stromal area occupied by vascular area). The vascular features were subdivided in centrally and peripherally located vessels.

Statistical Analysis

Patient characteristics were presented as means with standard error of the mean. Skewness was tested using Shapiro Wilk test. Variables with skewed distributions (total, central and peripheral vascular area) were logarithmically transformed and reported as back transformed geometric means. Comparison between groups was made using t-test or Mann-Whitney U test when appropriate. Correlation was tested using Pearson correlation test. Differences in morphometrical measurements were analyzed using a general linear model adjusting for possible confounders. A probability value <0.05 was considered statistically significant in this pilot study. The statistical analysis was performed using SPSS for Windows, version 15.0.0 (SPSS Inc., Chicago, Il).

RESULTS

In total, 38 placental tissues, divided in two groups, were examined. The IFL group (n=16) consisted of nine women in whom, after confirmation of fetal death, labor was induced within a few days and of seven women with a spontaneous miscarriage in whom the viability of the fetus at the time of miscarriage was not known. In nine cases no maternal inflammatory response (S0G0) and in seven a mild inflammatory response (S1G1) without fetal inflammatory involvement was observed. These subgroups showed similar patient characteristics and morphometrical measurements.

The IUI group consisted of 22 women in whom the placental examination revealed a severe maternal and/or a fetal inflammatory response. In 15 cases there was advanced maternal inflammation (S3G2) and in 17 cases a fetal inflammatory response was observed.

Patient characteristics are summarized in Table I. No significant differences in age, gravidity, parity and gestational age between the two groups were found. The results are presented in Table II. After adjusting for gestational age, there were no differences between the two groups for both fetal weight and placental weight. The stromal surface area of the examined immature intermediate villi, the number of vessels, the vascular area and the vascular area density were, after adjusting for gestational age, similar between the two groups.

After subdivision of the vascular features in centrally and peripherally located vessels we did not observe a difference in the number of vessels, the central vascular area and the central vascular area density between the two groups (Table III). The peripheral vascular area and peripheral vascular area density in the IFL group, were, after controlling for gestational age, significantly reduced in comparison with the IUI group.

Table I. Patient characteristics

	Idiopathic fetal loss IFL (n=16)	Intrauterine infection IUI (n=22)	p-value
Gestational age (days)	137 (14)	146 (13)	0.051[a]
Age (years)	29.9 (5.9)	29.7 (7.6)	0.94[a]
Gravidity	3 (2-4)	2.5 (2-4)	0.42[b]
Parity	1.5 (0.25-2.75)	1.0 (0.75-2.0)	0.36[b]

Date presented as means (SD) or as median (IQR) when appropriate
[a] Independent t-test
[b] Mann-Whitney U test

Table II. Outcome and morphometrical measurements

	Idiopathic fetal loss IFL (n=16)	Intrauterine infection IUI (n=22)	p-value[a]	Adjusted p-value[b]
Fetal weight	257 (232-292)	360 (328-392)	0.045	0.16
Placental weight	102 (92-114)	141 (131-151)	0.013	0.079
Villous stromal area	12.945 (12.663-13.227)	12.830 (12.412-13.248)	0.84	0.94
Number of vessels per villus (n)	5.3 (4.9-5.7)	6.1 (5.6-6.6)	0.20	0.59
Vascular area per villus	353 (304-410)	582 (510-663)	0.017	0.071
Vascular area density	2.7% (2.4-3.2)	4.6% (4.0-5.2)	0.014	0.064

Data presented as means (± SEM) or as back-transformed geometric mean (± SEM), weight in gram, area in μm^2
[a] Independent t-test
[b] p-value adjusted for gestational age

Table III. Morphometrical measurements

	Idiopathic fetal loss IFL (n=16)	Intrauterine infection IUI (n=22)	p-value[a]	Adjusted p-value[b]
Central Vessels				
Number per villus (n)	1.6 (1.4-1.8)	1.4 (1.3-1.5)	0.45	0.073
Vascular area per villus	112 (97-130)	137 (118-158)	0.35	0.77
Vascular area density	0.9% (0.8-1.0)	1.1% (0.9-1.2)	0.31	0.77
Peripheral vessels				
Number per villus (n)	3.5 (3.2-3.8)	4.5 (4.1-4.9)	0.053	0.15
Vascular area per villus	225 (187-270)	429 (371-495)	0.008	0.030
Vascular area density	1.7% (1.4-2.1)	3.4% (2.9-3.9)	0.006	0.025

Data presented as means (± SEM) or as back-transformed geometric mean (± SEM), area in μm^2, density in percentage

[a] Independent t-test

[b] p-value adjusted for gestational age

DISCUSSION

In this study we found that in cases of idiopathic second trimester fetal loss the peripheral chorionic villous vascularization was reduced in comparison with second trimester miscarriage due to intrauterine infection. The peripheral villous vascular area and villous vascular area density of the IFL group were, after adjusting for possible confounders, reduced, thus the villi were less vascularized as compared with the IUI group. These differences were in accordance with the work of Lisman and colleagues[13], who found a reduction in villous vascularization in first trimester fetal loss miscarriages as compared with cases of termination of pregnancy. In a few cases of the IFL group a normal developed chorionic villous vascularization was observed, indicating that other aetiologies for idiopathic fetal loss may play a role as well.

In this study, we could not provide evidence of a difference in placental weight between the two groups, though, statistical significance was nearly reached (p=0.08). This could be explained due to the lack of power of this study and due to other – not placental related – aetiologies of fetal loss in the IFL group.

Reduced placental vascularization was demonstrated at 16 weeks gestation. We hypothesize that in those cases placentation was already disturbed in first trimester of pregnancy. This resulted in a smaller placenta and reduced peripheral chorionic villous vascularization. In these cases normally oxygenated maternal blood enters the intervillous space, either at a normal or reduced rate, but there is a major reduced fetoplacental perfusion leading to fetal hypoxia.[9,20] In the models of postplacental hypoxia of Kingdom and Kaufmann the increased intraplacental oxygen concentration stimulates a predominantly non-branching angiogenesis which is associated with early fetal growth restriction with absent end-diastolic flow in the umbilical arteries.[20] Our results did not show evidence of an adequate adaptive vascular response, hence, explaining the fetal losses.

There are some limitations in this study. This study has a small sample size. A larger sample size would have strengthened the study. Power analysis was not possible because of a lack of similar studies. However, even with small numbers a clear difference was observed. Cases of both the IFL and IUI group are complicated pregnancies. Comparison with normal uncomplicated pregnancies, termination of pregnancy (TOP), would be most optimal. However, because of ethical issues, no TOP is performed at our clinic and therefore placental tissues of TOP were not accessible. Though on one hand, it is debatable whether the villous vascularization would be normal in all cases of TOP, as some pregnancies are terminated before complications eventually would occur.[9] On the other hand, we hypothesized that pregnancies complicated by intrauterine infection would have a similar distribution of deficient and normal placentation. This was because the assumed pathogenesis was ascending infection causing extremely premature labour. Hence, we maintain that these infected placentas are very reasonable to use as a comparative group in a situation when access to normal tissue is not possible.

Another limitation is the prolonged retention time, the time between fetal death and the delivery, as present in the IFL group. It could be argued that vascular elements change post-mortem and therefore altering the differences between the groups. Meegdes and colleagues[17] have observed similar number of villous vessels even after a prolonged intrauterine retention of more than 14 days and others have not found differences in the villous stroma fibrosis and villous vessel lumen obliteration within the first two days after fetal death.[21,22] Though, villous stroma fibrosis and villous vessel luminal changes are reported after a retention time of more than two days.[21,22] Consequently, an effect of the retention time after fetal death on the morphometrical measurements cannot be completely ruled out and thus the conclusions have to be taken with care. Cytogenetic analysis of the cases was not available. Therefore, it cannot be ruled out that aneuploid cases without any visible congenital malformation were included in our study.

In this study a large number of cases fell into the group where intrauterine infection was the apparent cause of the second trimester miscarriage. In 58% of the cases signs of an intrauterine infection were found; this prevalence is similar as that in other histological studies of intrauterine infection in second trimester miscarriages.[23,24] Only Srinivas and colleagues[25] observed a higher rate of infection. They found in 70% of second trimester miscarriages a stage 2-3 maternal inflammatory response, but they found a similar prevalence of fetal inflammatory response (54%) in comparison with our study (47%).

This study demonstrated that a proportion of idiopathic fetal losses could be well explained by a low placenta weight combined with a reduced chorionic villous vascularization, resulting in postplacental fetal hypoxia and subsequently fetal death. The reduced vascularization was demonstrated early in the second trimester, which implicates that the processes of vasculogenesis and angiogenesis are already disturbed in very early pregnancy. Furthermore, the fact that in most cases of second trimester miscarriage, signs of reduced chorionic villous vascularization and/ or intrauterine infection were found, indicates the necessity of histopathological examination of the placenta. This knowledge could help couples coping with late pregnancy loss. Further research is needed for understanding these aetiologies and for possible preventive treatment options to prevent (late) miscarriages in subsequent pregnancies.

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4.2

PLACENTAL VASCULARIZATION IN EARLY ONSET SMALL FOR GESTATIONAL AGE AND PREECLAMPSIA

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Reproductive Sciences 2011;18:586-593

ABSTRACT

Background

The objective was to determine whether chorionic villous vascularization is diminished in cases of early onset (<34 weeks) small for gestational age (SGA) and/or preeclampsia (PE).

Methods

Placental morphometrical measurements were performed in four gestational-age matched groups complicated by SGA, SGA with PE, PE, and spontaneous preterm delivery without SGA or PE as the reference group. Using a video image analysis system, in randomly selected intermediate and terminal villi, the stromal area and the following villous vascular parameters were manually traced and analyzed: number of total, centrally and peripherally localized vessels, vascular area and vascular area density.

Results

No differences were observed in intermediate and terminal villous vascular area. PE was associated with smaller terminal villous stromal area (Reference 2299 m^2, SGA 2412 m^2, SGA+PE 2073 m^2, PE 2164 m^2, p=0.011), whereas SGA was associated with an increased terminal villous vascular area density (Reference 26.1%, SGA 35.7%, SGA+PE 33.4% and PE 32.0%, p=0.029). Compared with preserved flow, lower terminal villous vascular area density was found in cases with absent or reversed end-diastolic (ARED) umbilical artery flow (39.3% vs. 30.3%, p=0.013).

Conclusion

These data demonstrate that villous vascularization was not influenced by PE, whereas in terminal villi an increased vascular area density was associated with SGA. Lower terminal villous vascular area density was associated with ARED flow in SGA pregnancies, indicating an increased risk of fetal compromise.

INTRODUCTION

Normal chorionic villous vascularization, resulting in an adequate gas and nutrient exchange between maternal and fetal blood, is essential for a normal development of pregnancy. In early placentation, the first capillaries are formed by vasculogenesis. From week 7 to 25 of gestation, vascular endothelial growth factor (VEGF) promotes branching angiogenesis, resulting in grape-like vascularized villi. From week 25 onwards, both branching and non-branching angiogenesis are involved[1]. The latter, by a decrease of VEGF and increase of placental growth factor (PlGF), results in elongated villi containing one or two poorly branched capillary loops[1]. With increasing gestational age, the number of vessels will increase due to maturation of haemangiogenetic cords to vessels and the materno-fetal diffusion distance will decrease due to margination.[1-3] In the latter process, the intravillous position of the fetal capillary becomes closer to the villous surface due to a decrease of villous stromal area and an increase in the capillary diameter. A minimal diffusion distance between maternal and fetal blood is important in order to obtain an optimal exchange of oxygen and nutrition across the vasculosyncytial membrane.[1,4]

In pathological pregnancies like anembryonic pregnancies, empty sac and gestational trophoblastic disease, placental vascularization is poorly developed.[3,5] In other cases of first trimester fetal loss, reduced maturation and margination of chorionic villous vessels have also been observed.[3] In the second trimester of pregnancy, reduced chorionic villous vascularization is observed in cases of fetal aneuploidy (trisomy 13,18 and 21), which could possibly play a role in the fetal growth restriction in these cases.[6] Finally, in second trimester, reduced margination is observed in cases of late miscarriage due to fetal loss.[7]

Consequently, poorly developed chorionic villous vascularization may lead to first and second trimester fetal loss and second trimester fetal growth restriction. The question arises whether diminished placental villous vascularization could also play a role in the manifestation of severe pregnancy complications and especially in early onset (before 34 weeks) small for gestational age (SGA) and preeclampsia (PE).[8] Both conditions may be partially attributable to placental villous and vascular abnormalities.[1,9]

The aim of the present study was to investigate whether chorionic villous vascularization and the process of margination are different in cases with early onset SGA and/or PE in comparison with a gestational age-matched reference group of spontaneous preterm deliveries without SGA and/or PE.

MATERIAL AND METHODS

Patient selection

A case-control study was performed at the Erasmus MC, University Medical Center Rotterdam. From the birth register of the Department of Obstetrics and Gynaecology, all deliveries in 2007 between 24 and 34 weeks of gestation (n= 315) were identified. Multiple pregnancies (n= 52), pregnancies complicated by chromosomal or congenital anomalies (n= 43), intrauterine fetal death (n= 12), women with alcohol and/or drugs abuse (n= 2) and sickle cell disease (n= 1) were excluded.

The remaining pregnancies (n= 205) were divided into four groups: early onset SGA (SGA, n= 17), early onset SGA and PE (SGA+PE, n= 20), early onset PE without SGA (PE, n= 43) and a reference group with spontaneous preterm deliveries which were not complicated by SGA, PE or other conditions such as diabetes and renal diseases (Reference, n= 125). In 33 cases (SGA n= 1; SGA+PE n= 1; PE n= 6; reference group n= 25) placental histology was not available. Cases in the PE, SGA and SGA+PE group and the reference group were matched for gestational age. For each week of gestation two cases per group, in total 72 cases, were included. If more cases were eligible for the same week, two cases were randomly selected for histological examination using a free random number generator program (www.random.org). Gestational age was calculated using the first day of the last menstrual period (LMP) and, in cases of unknown LMP or discrepancy of more than one week, gestational age was established by first trimester crown-rump length (CRL) measurements. SGA was defined as a birth weight below the fifth percentile adjusted for gender, parity and gestational age.[10] A recent large population-based study of over 19 million singleton births demonstrated that, in preterm (<37 weeks) gestations, the definition of small for gestational age may well be justified as a proxy for intrauterine fetal growth restriction.[11] PE was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP).[12]

The following patient characteristics were noted: age, obstetric history, body mass index prior to pregnancy, smoking habits (yes or no), pre-existing hypertension, highest systolic and diastolic blood pressure, proteinuria (mg/24 hour), urea, gestational age at delivery, SGA, PE, Haemolysis Elevated Liver enzymes and Low Platelets syndrome (HELLP, according to ISSHP criteria[12]), elevated pulsatility index (PI) of the umbilical artery within a week before delivery (>95th percentile[13]; yes or no) and whether the end-diastolic umbilical blood flow was preserved (PED), absent or reversed (ARED), birth weight, trimmed placental weight, fetal gender, 5-minutes Apgar score <7 and neonatal death (death within 30 days after delivery). Doppler measurements of the umbilical artery were not performed in the reference group and not systematically in the pathological groups due to fetal distress at admission, necessitating rapid delivery. According to the Dutch law, ethical approval and informed patient consent was not necessary for this study.

Microscopy and Morphometrical measurements

Sections of formalin-fixed paraffin-embedded placental tissue were cut at 5 µm thickness and mounted on 3-aminoproppyl-triethoxy-silane coated slides. Slides were stained with haematoxylin and eosin (H&E). Because, the chorionic villous vessels are clearly visible by microscope, even without an immunohistological endothelial cell staining with CD31, no extra staining was performed. Examination of the slides was performed with a magnification of x100 by two trained observers (R.H.F.O. and N.E.B.) blinded for the groups and duration of pregnancy. Of each placenta, 15 randomly selected mesenchymal or immature/mature intermediate villi with a diameter >80 µm and 15 randomly selected terminal villi with a diameter <80 µm were examined. Villi with stromal connective tissue were excluded to rule out stem villi[3,14]. In a previous pilot study, an inter- and intra-observer variability of less than 15% was achieved, with 15 randomly selected villi.[5]

Digitalized images of the field were captured by a color camera connected to a standard light microscope. Using an on-screen magnification of x400, the contours of the stroma of the villi and the vessels were manually traced on the computer monitor with a mouse-controlled cursor. Morphometrical measurements were performed using Zeiss KS 400 image analysis system (version 3.0, Zeiss inc., New York).

The following features were measured: area of the villous stroma without the trophoblastic layer, vascular area and, secondarily calculated, the vascular area density (percentage of villous stroma occupied by vascular area). In the intermediate villi, the process of margination was investigated by counting the number of vessels and calculating the vascular area density for both centrally and peripherally located vessels. Peripherally located vessels were defined as vessels in contact with the trophoblastic layer whereas centrally located vessels did not contact this layer (Figure 1A and B).

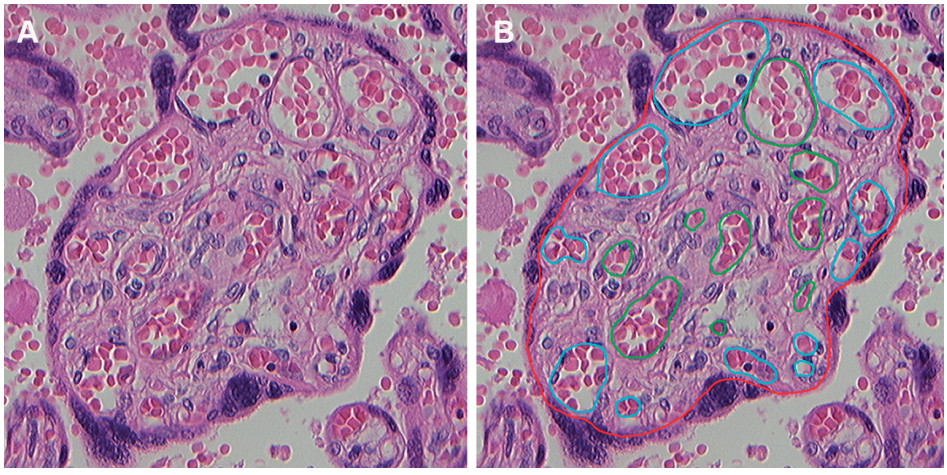

Figure 1. Morphometric measurement of chorionic villous vascularisation. (**A**) Intermediate villus after haematoxylin and eosin staining (original magnification x400); (**B**) The area of the villous stroma, without the trophoblastic layer, is marked in red; central vessels in green; and peripheral vessels, making contact with the trophoblastic layer, in blue.

Statistical analyses

Differences between groups in patient characteristics were tested for significance, using one-way analysis of variance (ANOVA), Kruskal-Wallis test, or Fisher's exact test when appropriate. Pregnancy outcome and morphometrical measurements were compared between the groups using two-way ANOVA (two factors analysed were SGA and PE, and SGA x PE as the interaction term). Variables with skewed distribution, analyzed using Shapiro-Wilk test, were normalized using logarithm transformation. Probability value <0.05 was considered statistically significant. Data were analyzed using Statistical Package for the Social Science (SPSS) 15.0.0 for windows (SPSS Inc., Chicago, Illinois).

RESULTS

Patient characteristics

Patient characteristics are presented in Table I. No statistically significant differences in maternal age, gestational age, gravidity, parity, preconceptional BMI and smoking habits were found. In the PE group the prevalence of pre-existing hypertension was higher.

Pregnancy characteristics and outcome

Pregnancy outcome is presented in Table II There were no significant differences in neonatal death and the low 5-minutes Apgar score. More females were born in the PE groups as compared with the SGA and the reference group. Proteinuria and urea were similar in both PE groups. No differences between SGA and PE could be found in the prevalence of elevated umbilical artery PI, whereas ARED umbilical blood flow was more frequent in SGA than in PE. Birth weight was lower in both the SGA and SGA+PE groups, but not in PE only. In the presence of SGA and SGA+PE the placental weight was significantly lower as compared with the PE and reference group.

Table I. Patient characteristics

	Reference (n=19)	SGA (n=16)	SGA+PE (n=19)	PE (n=18)	p-value
Age (years)	31.4 ± 5.2	29.8 ± 4.8	28.7 ± 5.6	30.7 ± 4.7	0.40[1]
Gestational age (weeks)	29.5 ± 3.0	29.3 ± 2.2	29.7 ± 2.4	30.5 ± 2.2	0.50[1]
Gravidity	2 (1-9)	2 (1-4)	2 (1-6)	1.0 (1-5)	0.69[2]
Parity	0 (0-4)	0 (0-1)	0 (0-2)	0 (0-2)	0.96[2]
Preconceptional BMI kg/m^2	21.9 (17.0-31.7)	22.4 (18.0-32.8)	23.4 (18.9-34.5)	25.7 (19.6-40.6)	0.06[2]
Smoking, *n* (%)	1 (5%)	2 (13%)	4 (21%)	3 (17%)	0.55[3]
Pre-existing hypertension, *n* (%)	0 (0%)	1 (6%)	1 (5%)	5 (28%)	**0.028**[3]

Abbreviations: SGA, small for gestational age; PE, preeclampsia
Data presented as mean ± SD, or as median (range), when appropriate
[1] One-way anova
[2] Kruskal Wallis test
[3] Fisher's exact test

Morphometrical measurements

There were no differences in the intermediate villous stromal area between groups (Table III). Also, no differences between SGA, PE and the reference group were shown with regard to the intermediate villous central, peripheral and total vascular area and vascular area density. Two-way ANOVA showed an interaction effect between SGA and PE for peripheral and total number of intermediate villous vessels. The number of peripheral intermediate villous vessels was increased in the SGA group as compared with the reference and SGA+PE group, whereas the total number of intermediate villous vessels was increased in the SGA

Table II. Pregnancy characteristics and outcome

	Reference (n=19)	SGA (n=16)	SGA+PE (n=19)	PE (n=18)	p-value SGA[1]	p-value PE[2]
Highest systolic blood pressure (mmHg)	124 ± 3	132 ± 6	**164 ± 5**	**172 ± 6**	0.98	**<0.001**
Highest diastolic blood pressure (mmHg)	76 ± 2	83 ± 2	**106 ± 2**	**106 ± 3**	0.14	**<0.001**
HELLP, *n* (%)	0 (0%)	0 (0%)	**7 (36%)**	**8 (44%)**	0.52	**<0.001**
Proteinuria, mg/24 hr	n.a.	n.a.	475 ± 151	288 ± 103		0.32*
Urea, mmol/L	n.a.	n.a.	7.7 ± 0.9	6.7 ± 0.6		0.35*
Pulsatility index of umbilical artery, *elevated / normal* (%)	n.a.	7/ 3 (70%)	13/ 5 (72%)	7/ 7 (50%)	0.21	0.91
Umbilical artery blood flow, *ARED / PED* (%)	n.a.	**5/ 5 (50%)**	**9/ 9 (50%)**	1/ 13 (7%)	**0.011**	1.00
Birth weight (g)	1447 ± 129	**826 ± 46**	**848 ± 58**	1269 ± 85	**<0.001**	0.37
Fetal gender, female *n* (%)	6 (32%)	7 (44%)	**10 (53%)**	**14 (78%)**	0.54	**0.018**
5 minutes Apgar score <7 (%)	3 (16%)	2 (13%)	0 (0%)	2 (11%)	0.28	0.22
Neonatal death, *n* (%)	3 (16%)	1 (6%)	2 (11%)	0 (0%)	0.90	0.36
Placental weight (g)	304 (133-495)	**185 (122-268)**	**182 (104-355)**	250 (182-386)	**<0.001**	0.12

Abbreviations: SGA, small for gestational age; PE, preeclampsia; ARED, absent or reversed end-diastolic blood flow; PED, preserved end-diastolic blood flow; n.a., not applicable
Data presented as mean ± SEM or as geometric mean (range), when appropriate
Two-way anova showed no interaction between PE and SGA for all features
[1] Significance of difference between SGA and PE and reference according to two-way anova
[2] Significance of difference between PE and SGA and reference according to two-way anova
* SGA+PE vs. PE, according to independent t-test

only in comparison with the SGA+PE group. With regard to the terminal villi, no differences in the number of vessels and the vascular area were demonstrated between SGA, PE and the reference group. In the presence of PE, the terminal villous stromal area was smaller as compared with SGA and the reference group. However, the vascular area density of the terminal villi was increased in the presence of SGA, whereas PE had no effect on this feature.

Additionally, we evaluated the influence of other clinical parameters on the morphometric results. The morphometric features were not affected by the presence of HELLP (Figure 2A), pre-existing hypertension or elevated PI of the umbilical artery. However, the vascular area density of the terminal villi was lower (30,3% vs. 39,3%, p=0,013) in the subgroup of SGA with ARED flow (n= 14) as compared with PED flow (n= 14, Figure 2B). This effect was not influenced by PE. Cases with PED and ARED did not differ in patient characteristics, birth weight and placental weight.

DISCUSSION

In this gestational-age-matched case-control study, we found an increased terminal villous vascular area density in placentas of pregnancies complicated by early onset SGA and

Table III. Morphometrical chorionic villous measurements

		Reference (n=19)	SGA (n=16)	SGA+PE (n=19)	PE (n=18)	p-value SGA[1]	p-value PE[2]
(Im)mature intermediate villi							
Villous stromal area (mm^2)		8324 ± 192	8696 ± 225	8195 ± 155	8179 ± 226	0.35	0.12
Number of vessels (n)	central	2.4 ± 0.2	2.4 ± 0.2	2.1 ± 0.2	2.3 ± 0.2	0.56	0.37
	peripheral	6.5 ± 0.4	**7.5 ± 0.2**	6.8 ± 0.2	7.0 ± 0.2	*	*
	total	8.9 ± 0.4	**9.9 ± 0.3**	8.8 ± 0.3	9.3 ± 0.4	#	#
Vascular area (mm^2)	central	357 ± 26	496 ± 49	454 ± 34	444 ± 38	0.06	0.52
	peripheral	1624 ± 191	2192 ± 211	2030 ± 205	2101 ± 192	0.24	0.41
	total	1981 ± 193	2688 ± 208	2484 ± 197	2544 ± 195	0.13	0.35
Vascular area density (%)	central	4.3% ± 0.3%	5.7% ± 0.6	5.6% ± 0.4	5.4% ± 0.4	0.08	0.26
	peripheral	19.7% ± 2.5%	24.9% ± 2.1%	24.8% ± 2.4%	25.7% ± 2.2	0.40	0.19
	total	24.0% ± 2.5%	30.6% ± 1.9%	30.4% ± 2.3%	31.1% ± 2.2%	0.24	0.13
Terminal villi							
Villous stromal area (mm^2)	total	2299 ± 80	2412 ± 79	**2073 ± 95**	**2164 ± 97**	0.93	**0.011**
Number of vessels (n)	total	3.5 ± 0.1	3.7 ± 0.1	3.5 ± 0.2	3.6 ± 0.1	0.70	0.84
Vascular area (mm^2)	total	609 ± 68	864 ± 75	708 ± 67	699 ± 57	0.06	0.67
Vascular area density (%)	total	26.1% ± 2.6%	**35.7% ± 2.8%**	**33.4% ± 2.2%**	32.0% ± 1.9%	**0.029**	0.43

Abbreviations: SGA, small for gestational age; PE, preeclampsia, data presented as mean ± SEM
[1] Significance of difference between SGA and PE and reference according to two-way anova
[2] Significance of difference between PE and SGA and reference according to two-way anova
*,# Two-way anova showed interaction effect between PE and SGA for number of peripheral (p=**0.027**) and total (p=**0.033**) intermediate villous vessels
* SGA vs. reference p=**0.024**; SGA vs. SGA+PE p=**0.021**; SGA vs. PE p=0.11; PE vs. reference p=0.26; PE vs. SGA+PE p=0.53
# SGA vs. reference p=0.05; SGA vs. SGA+PE p=**0.011**; SGA vs. PE p=0.21; PE vs. reference p=0.43; PE vs. SGA+PE p=0.30

a smaller terminal villous stromal area in pregnancies complicated by early onset PE. Furthermore, a lower terminal villous vascular area density was observed in SGA pregnancies with ARED as compared with PED umbilical artery blood flow.

The placental weight was significantly reduced in case of SGA and SGA+PE in comparison with the PE and reference group. These findings are consistent with other studies.[15-17] As in a previous study[18], we observed that intermediate villous stromal area, intermediate villous vascular area and vascular area density were similar in the different groups. We observed an increased number of peripheral intermediate villous vessels in the SGA group in

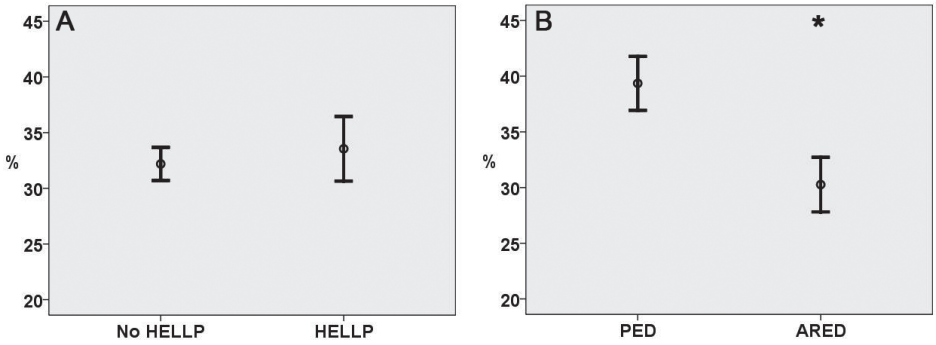

Figure 2. Morphometrical chorionic villous measurements; data presented as mean ± SEM. (**A**) Terminal villous vascular area density (%) in cases of PE with or without HELLP; (**B**) Terminal villous vascular area density (%) in cases of SGA with ARED or PED umbilical artery blood flow, * denotes significant effect of ARED.

comparison with the SGA+PE and reference group. However, in all four groups, we observed more peripherally than centrally located intermediate villous vessels, a larger peripheral than central vascular area and vascular area density, indicating that margination, which is necessary to develop a minimal diffusion distance between maternal and fetal blood, was similar in all groups.

The smaller terminal villous stromal area in pregnancies complicated by PE was not affected by the presence of the HELLP syndrome and pre-existing hypertension. One other study also demonstrated that the terminal villi volume and total terminal villi surface area were reduced in early onset PE but not in early onset SGA pregnancies.[18] In PE, the spiral artery remodelling is reduced, resulting in a limited uteroplacental blood flow.[19] The resulting reduced intraplacental oxygen concentration stimulates the expression of VEGF, favoring a predominantly branching angiogenesis.[1,9] Therefore, it was expected to find smaller terminal villi in the presence of PE.

Almost all terminal villous vessels are in direct contact with the trophoblastic layer, therefore, it was not appropriate to differentiate into peripherally and centrally located vessels. PE and SGA had no effect on the number of terminal villous vessels and the terminal villous vascular area. SGA, however, was associated with an increased terminal villous vascular area density as compared with PE and the reference group. The results of other studies are conflicting. Chen et al.[20] reported for the reference group a similar terminal villous vascular area density but a reduced, rather than increased, density in fetal growth restricted (FGR) pregnancies (n=9). Two studies did not report a difference in terminal villous vascular density,[15,16] and one study demonstrated a similar increased density as in the present study.[18] These studies were able, due to their prospective design, to calculate the total volume of the placental villi and vessels. One study found reduced total terminal villi and capillary volumes in placentas of pregnancies complicated by SGA and/or PE, but in contrast to our study, they did not find a difference in vascular area density.[21] Possibly,

this could be explained by the limited number of cases (n=5) included in this study and the 4 weeks difference in gestational age between the groups. Egbor et al. demonstrated a reduced total volume of terminal villi and vessels in placentas in cases of late onset SGA and/or PE,[15] but were unable to extrapolate these findings to cases with early onset SGA and/or PE, probably due to the small sample size of less than 10 cases per group.[18]

Although an increased terminal villous vascular density was found in the presence of SGA, this did not imply that the total materno-fetal interface was larger, as placental weight and thus the total terminal villous volume were smaller.[18,22] As a consequence, total terminal villous vascular surface area will be decreased[1]. Normally oxygenated maternal blood enters the intervillous space possibly even at a normal or slightly reduced rate,[9,19] but due to the severe compromised fetoplacental perfusion the resulting rising intraplacental oxygen concentration suppresses the expression of VEGF and leads to a relative dominance of PlGF.[1,9,23,24] This phenomenon, called postplacental fetal hypoxia,[1,9] results in long, filiform villi with poorly branched capillary loops instead of well branched, grape-like vascularized villi.[1,14,25-28] Already in the second trimester, this may lead to FGR and fetal death.[7] A possible compensating mechanism for the increase in blood flow impedance in these long capillary loops is a dilatation of the capillaries, resulting in an increased villous vascular area density.[1,9] This adaptation may provide better chances for fetal survival and postpone fetal compromise. This mechanism could explain the difference observed in terminal villous vascular area density in SGA pregnancies with ARED as compared with PED umbilical artery blood flow. If the placenta is less adaptive, this may result in a less increased terminal vascular area density, increased chorionic villous blood flow impedance and consequently ARED blood flow of the umbilical artery at an earlier stage. In a pilot study of preterm intrauterine fetal death pregnancies (n=8) we observed even lower terminal vascular area densities, suggesting that SGA pregnancies with lower terminal vascular area densities are at increased risk of fetal compromise.

This study has the following limitations. Due to the retrospective nature of this study, we were not able to reproduce the total volume of the villi and vessels as has been investigated by others.[1,15,18,21] However, we believe that the vascular area densities provide an unbiased estimate of real three dimensional quantities. Another probable limitation is the inclusion of SGA (birth weight <5th percentile) instead of FGR (flattening of growth curve or ARED umbilical artery blood flow). However, a very large population-based study[11] demonstrated increased risks of stillbirth and neonatal death following preterm (<37 weeks) SGA births (defined as <10th percentile) as compared with appropriate for gestational age birth (defined as 25-74th percentile). Based on these increased risks, Ananth and Vintzileos[11] concluded that preterm SGA neonates should be considered pathologically rather than constitutionally small.

The evidence, that the origin of both early onset FGR and PE can be found in the very early abnormal placental development, is growing.[29] Differences in first trimester serum markers, such as lower serum placental protein 13 and PlGF in pregnancies prone to develop FGR and/or PE as compared with controls,[30] underline this statement. A recent study demonstrated early villous vascular changes in first trimester chorionic villous

sampling in pregnancies later complicated by SGA or hypertensive disorders.[31] A recently published World Health Organization (WHO) report which, based on the lack of shared risk factors, suggest that SGA and PE are different entities.[32] Unfortunately, in this study no distinction was made between early and late onset SGA and PE. Egbor et al. demonstrated similar villous vascular abnormalities in both early onset SGA and PE, suggesting that both conditions might share a common pathophysiologic mechanism.[18] In a recent review, Burton and colleagues speculated that, although PE and FGR represent different placental pathologies, these conditions are secondary to deficient spiral artery conversion.[33] Also in the present study, a difference in placental pathology was identified between early onset SGA and PE.

In conclusion, early onset PE was associated with smaller terminal villous stromal area but had no effect on chorionic villous vascularization. In early onset SGA without PE, the number of peripheral intermediate villous vessels was increased, but with regard to the peripheral intermediate vascular area, vascular area density, and thus the process of margination, no differences were demonstrated between PE, SGA, and the reference group. Early onset SGA, regardless of PE, was associated with an increased terminal villous vascular area density. In pregnancies complicated by SGA, a less pronounced increase of the terminal villous vascular area density was associated with ARED umbilical artery blood flow, indicating that these less adaptive placentas may represent an increased risk of fetal compromise.

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5

GENERAL DISCUSSION

DISCUSSION

In this thesis, we investigated whether early pregnancy events will further affect placental development and function and may subsequently have an influence on both the foetus and its mother. After proposing an unambiguous early pregnancy Dutch nomenclature, essential for clinical and research purposes, we first investigated the influences of first trimester events on obstetric and perinatal outcome in current or subsequent pregnancy. Secondly, we investigated chorionic villous vascularization in normal and abnormal first, second and third trimester pregnancies.

In Chapter 2 we found that any event occurring in first trimester of pregnancy is associated with adverse obstetric and perinatal outcome. Misdating the date of the last menstrual period, previous miscarriages or terminations of pregnancy, threatened miscarriage with or without a hematoma, vanishing twin and hyperemesis gravidarum, are associated with an adverse effect on the obstetric and perinatal outcome in current or subsequent pregnancy.

In Chapter 3 and 4 we found that in early and late complicated pregnancies the chorionic villous vascularization is compromised. Effects of preconception and first trimester cigarette smoking are already present in early first trimester chorionic vascularization. Pregnancies complicated by foetal death in second trimester of pregnancies are associated with a less developed chorionic vascularization. Pregnancies complicated by preeclampsia have a normal terminal villous vascularization density but the terminal villi are smaller as compared with controls. Pregnancies complicated by small for gestational age (SGA) have similar size terminal villus but with an increased terminal villous vascularization density as compared with controls. However, in pregnancies with SGA complicated by absent or reversed end diastolic (ARED) umbilical artery blood flow and thus at risk for foetal demise, a lower terminal villous vascularization density as compared to those with a normal umbilical artery blood flow was observed.

Placentation

As we do not know the sequence of events, the question remains; what is the underlying cause and what are the consequences? Is first trimester placental maldevelopment causing first trimester events or do first trimester events cause subsequent placental maldevelopment. For example, in case of threatened miscarriage, it is not possible to perform *in vivo* investigation, thus the real pathogeneses remains unelucidated and can be different for each case. Logically, the effect on outcome will differ from case to case. To understand the pathogenesis of the possible chorionic vascular alterations in complicated pregnancies it is important to understand normal placentation.

During pregnancy, placental development is modulated by the intrauterine environment.[1] In the first 8 to 12 weeks' gestation placentation occurs in a low oxygen environment, supported by histiotrophic nutrition secreted from the endometrial glands.[2] Maternal circulation is prevented from entering the intervillus space by the presence of endovascular plugs of extravillous trophoblasts that occlude the tips of the spiral arteries.[3] The low oxygen environment during early placental development is essential for normal placental vasculogenesis and angiogenesis. Angiogenesis is promoted by hypoxia-

induced regulation of angiogenic factors, such as vascular endothelial growth factor-A (VEGFA), placental growth factor (PlGF), Angiopoietin-1 (ANG1) and Angiopoietin-2 (ANG2).[4] VEGFA favors branching angiogenesis whereas PlGF favors non-branching angiogenesis. ANG1 and ANG2 regulate angiogenesis. ANG1 helps stabilize newly formed capillaries whereas ANG2 destabilize vessels and as a result make them more susceptible to angiogenic stimulus. During pregnancy the expression of VEGFA and PlGF and the expression of ANG1 and ANG2 are regulated by the intraplacental oxygen concentration. Intraplacental low oxygen concentration, normal in early pregnancy, favors VEGFA expression and suppresses PlGF expression. Also, the balance between ANG1 and ANG2 shift towards ANG2, which favors vessel instability, angiogenesis and vessel remodelling. During pregnancy the intraplacental oxygen concentration increases after maternal circulation enters the intervillus space and leads to a slight favor of PlGF expression whereas VEGFA expression is downregulated.

In the last few weeks of the first trimester, the spiral artery plugs dislocate and maternal circulation perfuse the intervillous spaces. As a consequence, the nutrition switches from deciduochorial or histiotrophic to haemochorial or haemotrophic nutrition. The onset is first observed in the peripheral regions of the placenta rather than in the centre, probably the result of less extensive trophoblast invasion and thus incomplete plugging of the spiral arteries in the peripheral part of the placenta.[5,6] The presence of maternal circulation in the placenta will lead to a threefold rise of oxygen concentration.[7] Early onset intervillous circulation could lead to placental pathology by oxidative stress, nutritive stress and mechanical injury from turbulent, intermittent blood flow.[8]

Early pregnancy events could alter the interaction between endometrium and the invading trophoblasts in the ongoing pregnancy, while in women with previous miscarriages or terminations of pregnancy the intrauterine environment could be different resulting in an altered placentation in the subsequent pregnancy.[9] Cigarette smoking has an effect on endometrial receptivity and angiogenesis, and could therefore affect the intrauterine environment and thus the trophoblastic invasion negatively.[10] Threatened miscarriage cases could be the result of maternal circulation entering the intervillous space prematurely at the peripheral edge of the placenta due to incomplete plugging of the spiral arteries. The degree of placental involvement will determine the outcome. If only a very small region of the outer placenta is involved than most likely it would not influence the outcome significantly. However if a considerable region is involved, it could lead to oxidative stress and villous regression. And if the onset of the intervillous circulation occurs prematurely in a large part of the placenta, than it could result in miscarriage. If excessive premature flow occurs it could, by villous regression, lead to abnormal placental shapes and to smaller placenta volume.[1] After a steady state is reached, normally oxygenated maternal blood enters the intervillous space and due to the severe compromised fetoplacental perfusion the resulting rise of intraplacental oxygen concentration suppresses the expression of VEGF and leads to a relative dominance of PlGF.[11,12] Angiogenesis will shift from predominantly branching towards non-branching angiogenesis. This results in long, filiform villi with poorly branched capillary loops

instead of well-branched, grape-like vascularized villi as observed in FGR pregnancies.[12,13] A possible compensating mechanism for the increase in blood flow impedance in these long capillary loops is a dilatation of the capillaries, resulting in increased terminal villous vascular area density (**Chapter 4.2**). If this adaptation is less devolved than this may result in an increased chorionic villous blood flow impedance and consequently ARED blood flow of the umbilical artery at an earlier stage. Already in the second trimester, this may lead to fetal death (**Chapter 4.1**) or severe FGR (**Chapter 4.2**).In pregnancies complicated by PE, the spiral artery remodelling is impaired due to deficient trophoblast invasion. This will result in an impaired uteroplacental circulation with a relative low intraplacental oxygen concentration favouring VEGFA and ANG2 expression. During pregnancy branching angiogenesis will be predominantly promoted leading to richly branching capillary network with small terminal villi (**Chapter 4.2**).

Implication for clinical practice

Clinicians have to be aware that preconception, periconception and postconception patient characteristics can be associated with pregnancy outcome. Antenatal identification of these parameters such as maternal age, medication, and stimulants like alcohol, smoking and soft- and hard drugs, ethnicity, length, weight, dietary patterns, folic acid use, time of subfertility, ART, medical history, use of medication, obstetric history, interpregnancy interval, stress, marital status, socio-economic status, educational level, employment and – resulting from this thesis - the mode of determining the due date and early pregnancy events in current or previous pregnancy can contribute to individual risk stratification for adverse obstetric outcome. The use of unambiguous terminology to describe early pregnancy events is important in order to avoid confusion between researchers, doctors and patients. This enables better management protocols and new therapeutic guidelines aimed at improving the perinatal outcome in these groups of women at higher risks of abnormal pregnancy outcome. Although it remains questionable whether this knowledge could prevent obstetric complications to occur, the intensification of care may contribute to the anticipation of detrimental effects enabling secondary and tertiary prevention.

Pregnancies complicated by foetal death, intra-uterine growth restriction and preeclampsia are proceeded by diminished chorionic villous vascularization and/or changes in the intervillous circulation. Post partum placental histology is strongly recommend in complicated pregnancies to detect these changes. Some of these changes are already present at the end of the first trimester and can, for example, result in alterations to be detected by uterine artery Doppler measurements, three-dimensional placental vascularization indices and placental biomarkers.[15-17] Placental biomarkers such as PlGF, soluble endoglin (an anti-angiogenic factor found on placental syncitiotrophoblasts), Soluble Flt-1 (an anti-angiogenic factor binding VEGF and PlGF) and placental protein 13 (placenta specific protein) are promising in predicting adverse obstetric outcome.[18,19] But unfortunately, in despite of considerable research, still no superior first trimester screening tests are available to accurately predict those at risk for adverse pregnancy outcome and thus not recommended to use routinely in clinical practise.[15-17]

Implications for future research

Histological placental investigation is usually performed after the delivery and thus, in complicated pregnancy, after placental maldevelopment already has taken place. As many researchers have shown, placental vascularization is different in complicated pregnancies. As of such, to determine placental vascularisation at an earlier stage to assess which pregnancy is at risk for developing adverse obstetric outcome is essential. Currently, no superior tool to investigate *in vivo* first trimester placental vascularization to determine the outcome of the pregnancy exists. The use of surplus of trophoblast after chorionic villus surplus sampling at 11-14 weeks' gestation enables a possibility to examine placental tissue.[20] However, as only few villi are available, the vascularization cannot be accurately examined. With the rise of three-dimensional power Doppler it is possible to measure placental perfusion.[21] Several investigators have tried to link differences in placental flow indices, such as the vascularization index, flow index and vascularization flow index, in early pregnancy to adverse pregnancy outcome.[22-25] The first results are promising, lower first trimester indices are found in smaller CRL, SGA and PE.[22-25] However concerns are present, as the interobserver and intraobserver reproducibility are poor.[26,27] The results can be influenced by soft tissue motion, placenta localisation, obesity, the selected placental volumes and power Doppler settings.[26-28] Thus, currently these indices cannot be easily used in daily practise without gaining experience first. One other important downside of this technique, is the fact that it is impossible to distinguish between maternal and fetal flow within the placenta. From a theoretical point of view it would be vital to make such a distinction. With improvements of 3D ultrasound techniques or MRI techniques in the future it would be really interesting if vessels and blood flow indices could be followed and measured in detail from the umbilical cord or placental bed side and so determine both effects on the pregnancy outcome.

Using an immersive virtual reality system, as is available in the Erasmus MC, or other three-dimensional display technologies are beneficial in improving the visualization of the architecture of the chorionic villous vascularization and to perform semi-automated volume measurements.[28] This technique is unfortunately still limited to the resolution of the images captured by the scanning devices. Hopefully, in the future, the quality of the scanning devices will improve significantly to identify even the small vessels and blood flow. We are aware that the immersive VR system, such as the Barco I-Space in the Erasmus MC, is costly, however a less expensive desktop version is available and offers the same benefits.[29]

As current investigation, most insights on placental histology is based on a limited number of patients. Evidence is thus mostly based on small and limited studies. Larger studies comparing more numbers of placentas are needed to conform the findings, this would be more easily reached in a multicentre study. Comparing existing datasets is difficult because of heterogeneity in patient characteristics, placental sampling, placental storage and methodological factors. The Editors of the journal Placenta have published a technical note to include a table with suggested variables, so that confounding variables in subsequent placental studies could be identified and acknowledged.[30] Also, advises for optimising sample collection for placental research are published recently.[31] The authors state that a

standardized collection, processing and storage protocol (preferable an universal protocol) is essential for placental research to reflect the *in vivo state*, for experimental reliability and reproducibility. The authors discuss many factors such as patient characteristics, mode of delivery, medication use, oxygen use, collecting time, location of placenta sampling, which can influence data derived from studying the placenta.[31] Awareness of these potential confounders and standardized principles of stereological and morphometrical placental measurements could result in better insight in placental development and function in normal and abnormal pregnancies.

CONCLUSIONS

This thesis presents the effects of early pregnancy events on obstetric outcome and the changes in chorionic villous vascularization in normal and complicated pregnancies. Pregnancy events can induce or be the result of placental maldevelopment, subsequently, placental maldevelopment can cause adverse obstetric outcome to occur in early and late pregnancy. Early antenatal identification of these first trimester parameters should enable better management protocols and new therapeutic guidelines aimed at improving the perinatal outcome in these groups of women at higher risks of abnormal pregnancy outcome. For now, it remains questionable whether this knowledge could prevent obstetric complications to occur, but possibly by intensification of care the anticipated detrimental effects can be avoided or reduced.

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6

SUMMARY

SAMENVATTING

SUMMARY

The general introduction and the aim of this thesis are provided in **chapter 1.1**. Normal placental development in first trimester of pregnancy is of great importance for normal growth and development of the fetus in later pregnancy. Complications in early pregnancy could have its origin in abnormal placentation or the event itself could lead to changes in early placentation and , as a consequence, result in adverse outcome in the current or subsequent pregnancy. The aim of the thesis was to describe unambiguous Dutch terminology, to determine the effect of early pregnancy events on pregnancy outcome and to depict the chorionic villous vascularization in normal and complicated pregnancies.

Many terms used to describe early pregnancy events originated from before the ultrasound era. Some of these terms were confusing for both the clinician and the patient. Therefore, in accordance with the revision of the European English Revision and in collaboration with the members of the study group Early Pregnancy of the Dutch Society for Obstetrics and Gynaecology, a revision of the Dutch nomenclature used for early pregnancy events was made. The recommended Dutch terminology is outlined in **chapter 1.2**.

The discussion on how to date a pregnancy appears to end in favour of an early ultrasound measurement of the Crown-Rumps Length (CRL) instead of using the first day of the last menstrual period (LMP). One of the confounder of using the LMP is the preference for 'round' dates (1st, 5th, 10th, 15th, 20th, and 25th of the month) in women recalling their LMP. In **chapter 2.1** we describe whether this preference of certain dates was present in our population and which determinants were associated with this preference. In almost 25.000 records we observed more digit preference for especially the 1st and 15th of the month in women living in a deprived neighbourhood, in women with an uncertain LMP and women with an irregular cycle. In concordance with other studies, we observed more postterm pregnancies (≥ 42 weeks) in LMP dated pregnancies as compared with CRL dated pregnancies. Our data also indicate that this effect was larger in women with a digit preference.

The number of clinical miscarriages is an important determinant for the chance of a live birth in the subsequent pregnancy. In **Chapter 2.2** we investigated the incidence and the effect on the subsequent pregnancy of non-visualised pregnancy losses (biochemical pregnancy losses and failed pregnancies of unknown location). Biochemical pregnancy losses constitute 23% and failed PULs' 14% of all early pregnancy losses in women with recurrent miscarriage. Non-visualised pregnancy losses have a similar effect on live birth as compared to clinical miscarriages, both having a relative risk of 0.90 and 0.87 on live birth in the subsequent pregnancy. This effect counts for both biochemical pregnancy losses and failed PULs'. Even when using a strict definition of recurrent miscarriage of at least two or more clinical miscarriages this effect remains the same.

Complications and events are common in early pregnancy and these include: (recurrent) miscarriage, termination of pregnancy, vaginal bleeding, intrauterine haematoma, vanishing twin and hyperemesis gravidarum. Information of the long term effects of these early pregnancy events are sparse and scattered. Therefore, we performed a systematic review of the literature regarding each early pregnancy events and the association on adverse

obstetric and perinatal outcome in the ongoing or subsequent pregnancy. An extensive literature search in Medline and Cochrane for the period of 1980 – 2008 was performed. We found that almost all early pregnancy events are associated with certain adverse obstetric and perinatal outcome, these associations are outlined in **chapter 2.3.1**. The observed associations with adverse obstetric or perinatal outcome were related to the severity and recurrence of the early pregnancy event. In **chapter 2.3.2** the pathophysiology and aetiology of these early pregnancy complications and the effect on the utero-placental interface and thus on the subsequent pregnancy outcomes are depicted.

For normal fetal growth and development a well-developed chorionic villous vascularization is essential. The fetal vascular system of the human placenta is formed by means of vasculogenesis and angiogenesis, resulting in a complex network of peripheral villous capillaries essential for nutrition and gas exchange during second and third trimester of human pregnancy. By means of creating an enlarged three dimensional hologram of placental tissue using a virtual reality system, the Barco I-Space, we were able to investigate the transition of villous vasculogenesis to angiogenesis in early pregnancy. The study is described in **chapter 3.1**. Placental tissue of legally terminated intact intrauterine pregnancies between 5 and 12 weeks gestational age (GA) were stained with an endothelial marker CD31. Using a confocal laser scanning microscopy serial images of the villi were captured. A volume rendering application generated an animated enlarged hologram of the dataset. We observed as of $5^{5/7}$ weeks GA onwards a complex vascular network, primarily dominated by cords with redundant connections. Between 6 and 7 weeks GA large luminal spaces at junctions of haemangioblastic cords were observed suggesting a beginning of lumen formation at these junctions. This lumen formation does not occur synchronously in all cords but sequentially indicating a slow transition between vasculogenesis and angiogenesis. Hereafter, lumen formation of these haemangioblastic cords, mostly located in between capillaries, was observed from one or simultaneously from both sides.

In the subsequent study, covered in **chapter 3.2**, we investigated whether tobacco smoking had an influence on early chorionic villous vascularization. Placental tissue of terminated intact intrauterine pregnancies of 13 nonsmoking and 13 smoking women were compared. After staining with CD31, images of 3-5 mm^3 placental tissue were captured using an Optical Projection Tomography. A three dimensional hologram of the reconstructed images were created using the immersive BARCO virtual reality system. The villous volume, vascular volume and vascular density were determined. Already in the first trimester of pregnancy we observed different vascular densities in the group of smoking women as compared to the group of nonsmoking women, possibly due to an adaptive response, illustrating the necessity to counsel for cessation of smoking before conception.

In prior studies it was found that aneuploidy pregnancies (trisomy 13-18-21) which were terminated in second trimester have a less developed chorionic villous vascularization as compared with second trimester miscarriages. In **chapter 4.1** we investigate whether there was a difference in chorionic villous vascularization between second trimester miscarriage due to intrauterine fetal death and intrauterine infection. In 16 cases of second trimester idiopathic fetal loss and 22 cases of second trimester miscarriage, due to an intrauterine

infection, the placental tissue was stained for CD34, an endothelial marker. The central and peripheral vascular area and vascular density was measured by use of microscopy. Lower peripheral vascular area and density were observed in the fetal loss group as compared with the intrauterine infection group. We hypothesized that in these cases, placentation was already disturbed in first trimester of pregnancy, leading to a reduced materno-fetal interface in second trimester, thus to early postplacental fetal hypoxia and fetal death.

Placentas of pregnancies complicated by small for gestational age (SGA) and/ or preeclampsia (PE) before 34 weeks gestational age are smaller than those only complicated by a preterm birth. As previous studies have shown that poor developed chorinonic villous vascularization was associated with second trimester fetal growth restriction and fetal loss, we investigated in chapter 4.2 whether pregnancies complicated by PE and/or SGA have diminished villous vascularization as well. Placental morphometrical measurements were performed in four gestational-age matched groups between 24 and 34 weeks of GA complicated by SGA, SGA with PE, PE, and spontaneous preterm delivery without SGA or PE as the reference group. The data demonstrated smaller terminal villi with normal villous vascularization in pregnancies complicated by PE, whereas in SGA pregnancies normal size terminal villi with an increased vascular area density were observed. Lower terminal villous vascular area density was associated with absence or reversed end-diastolic umbilical artery flow in SGA pregnancies, indicating an increased risk of fetal compromise.

Chapter 5 provides a general discussion of the main findings, furthermore the implications for clinical practice and for future research are discussed.

SAMENVATTING

De algemene inleiding en de onderzoeksdoelen van dit proefschrift worden in **hoofdstuk 1.1.** Een normale ontwikkeling van de placenta in het eerste trimester van de zwangerschap is van groot belang voor de normale groei en ontwikkeling van de foetus in latere zwangerschap. Complicaties in de vroege zwangerschap kan zijn oorsprong hebben in abnormale placentatie of de gebeurtenis zelf kan leiden tot veranderingen in de vroege placentatie, dit kan vervolgens leiden tot ongunstige uitkomsten in de huidige of de volgende zwangerschap. Het doel van het onderzoek was om eenduidige Nederlandse terminologie te beschrijven, het effect van vroege zwangerschapscomplicaties op de zwangerschapsuitkomsten te bepalen en de chorionic villous vascularisatie in normale en gecompliceerde zwangerschappen vaststellen.

Veel termen die worden gebruikt om gebeurtenissen in de vroege zwangerschap te beschrijven ontspringen van voor het echo tijdperk. Sommige van deze termen werken verwarrend voor zowel de arts en de patiënt . Daarom werd, in overeenstemming met de herziening van het Europese Engels Herziening en in samenwerking met de leden van de studiegroep vroege zwangerschap van de Nederlandse Vereniging voor Obstetrie en Gynaecologie, een herziening van de Nederlandse nomenclatuur voor de vroege zwangerschap gebeurtenissen gemaakt . De aanbevolen Nederlandse terminologie wordt geschetst in **hoofdstuk 1.2**.

De discussie over de wijze hoe een zwangerschap gedateerd moet worden, lijkt te eindigen in het voordeel van een vroege echo meting van de Crown - Rump Lengte (CRL) in plaats van de eerste dag van de laatste menstruatie (LMP) . Een van de nadelen van het gebruik van de LMP is de voorkeur voor 'afgeronde ' data (1ste, 5de, 10de, 15de, 20ste, en 25ste van de maand). In **hoofdstuk 2.1** beschrijven we of deze voorkeur van bepaalde data aanwezig is in onze bevolking en welke determinanten geassocieerd werden met deze voorkeur. In een dataset van bijna 25.000 zwangerschappen constateerde we dat er vooral een voorkeur was voor de 1e en 15e van de maand. Deze voorkeur werd met name gezien bij vrouwen wonend in een achterstandswijk, bij vrouwen met een onzekere LMP en vrouwen met een onregelmatige cyclus. In overeenstemming met andere studies, zagen we meer serotiene zwangerschappen (≥ 42 weken) in LMP gedateerde zwangerschappen vergeleken met CRL gedateerde zwangerschappen. Onze gegevens geven ook aan dat dit effect groter was bij vrouwen met een voorkeur voor een afgeronde ELM datum.

Het is een bekend gegeven dat het aantal klinische miskramen een belangrijke determinant is voor de kans op een levend geborene in een volgende zwangerschap. In **Hoofdstuk 2.2** onderzochten wij wat de incidentie en wat het effect op een volgende zwangerschap is van niet geobjectiveerde miskramen (biochemische miskramen en zwangerschap met onbekende lokalisatie in regressie). Bij vrouwen met herhaalde miskraam zijn 23% van de miskramen een biochemische miskraam en 14% een ZOL in regressie. Niet geobjectiveerde miskramen hebben een vergelijkbaar effect op de kans van een levendgeborene al s een klinische miskraam, beiden hebben een Relatief Risico van 0.90 en 0.87 op een levendgeborene in een volgende zwangerschap. Dit effect geldt voor biochemische miskraam en voor ZOL in regressie. Ook bij het gebruik van een strictere definitie van herhaalde miskraam van twee of meer klinische miskramen wordt hetzelfde effect gevonden.

Complicaties en gebeurtenissen komen vaak voor in het begin van de zwangerschap en deze omvatten: (herhaalde) miskraam, zwangerschapsafbreking, vaginaal bloedverlies, intra-uteriene hematoom, verdwijnende tweeling en hyperemesis gravidarum. Informatie over de lange termijn effecten van deze vroege zwangerschapscomplicaties zijn schaars. Daarom hebben we een systematische review van de literatuur met betrekking tot elke vroege zwangerschap gebeurtenissen en de vereniging op ongunstige obstetrische en perinatale uitkomsten in het lopende of volgende zwangerschap . Een uitgebreid literatuuronderzoek in Medline en Cochrane voor de periode van 1980 - 2008 werd uitgevoerd . We vonden dat bijna alle vroege zwangerschap gebeurtenissen worden geassocieerd met bepaalde ongunstige obstetrische en perinatale uitkomsten. Deze associaties worden beschreven in **hoofdstuk 2.3.1**. De waargenomen associaties met ongunstige obstetrische of perinatale uitkomsten waren gerelateerd aan de ernst en de herhaling van de vroege zwangerschapscomplicatie. In **hoofdstuk 2.3.2** zijn de pathofysiologie en etiologie van deze vroege zwangerschapscomplicaties en het effect op de utero-placentaire interface en dus op de zwangerschapsuitkomsten beschreven.

Voor een normale foetale groei en ontwikkeling is een goed ontwikkelde chorionic villous (placentaire) vascularisatie essentieel. Het foetale vaatstelsel van de menselijke placenta wordt gevormd door middel van vasculogenese, de novo vaatvorming uit voorloper cellen, en angiogenese, vaatvorming door vertakking of verlenging van het al aanwezige vaatnetwerk. Hierbij ontstaat een complex netwerk van perifere capillairen aan de vasculo-syncitiale membraan essentieel voor adequaat uitwisseling van metabolieten en gassen in tweede en derde trimester van de zwangerschap. Door middel van het creëren van een sterk uitvergroot driedimensionaal hologram van placenta weefsel met behulp van een virtual reality systeem, de Barco I-Space, waren we in staat om de overgang van de placentaire vasculogenese naar angiogenese te onderzoeken. Het onderzoek wordt beschreven in **hoofdstuk 3.1**. Placentaweefsel van intacte intra-uteriene zwangerschappen tussen 5 en 12 weken amenorrhoeduur (AD), welke om sociale redenen werden beëindigd, werden gekleurd met een endotheel marker CD31. Met behulp van een Confocale Laser Scanning Microscopie (CLSM) werden serie beelden van de chorionvlokken gefotografeerd. Met behulp van een 'volume rendering' applicatie werden geanimeerde hologrammen van de dataset gegenereerd en uitvergroot in de I-Space driedimensionaal weergegeven. Vanaf $5^{5/7}$ week AD namen we een complex vasculaire netwerk waar, voornamelijk gedomineerd door vaatstrengen met redundante verbindingen. Tussen 6 en 7 weken AD werd met name lumen waargenomen op kruispunten van de vaatstrengen, suggererend dat lumen vorming begint op deze kruispunten. Dat lumen vorming niet synchroon voorkomt in alle vaatstrengen maar opeenvolgend wijst op een geleidelijke overgang van vasculogenese naar angiogenese. Hierna werd lumen vorming van deze vaatstrengen, meestal gelegen tussen capillairen, waargenomen vanaf een of beide zijden tegelijk.

In het daaropvolgende onderzoek, beschreven in **hoofdstuk 3.2**, hebben we onderzocht of het roken van tabak een invloed had op de vroege chorionic villous vascularisatie. Placentaweefsel van intacte intra-uteriene zwangerschappen, welke om sociale redenen werden beëindigd, van 13 niet-rokende en 13 rokende vrouwen werden vergeleken. Na kleuring met

CD31, werden beelden van 3-5 mm3 placentaweefsel vastgelegd met behulp van een Optische Projectie Tomografie (OPT). Een driedimensionaal hologram van de gereconstrueerde beelden werden gemaakt met het Barco VR systeem. Met behulp van dit systeem werden de volumes van de chorionvlokken en de aanwezige vasculair netwerk, en de vasculaire dichtheid bepaald. Reeds in het eerste trimester van de zwangerschap zagen we een verschil in de vasculaire dichtheid in de groep van rokende vrouwen in vergelijking met de groep van niet-rokende vrouwen, mogelijk als gevolg van een adaptieve respons. Dit onderzoek illustreert ook de noodzaak om te adviseren preconceptioneel te stoppen met roken.

In eerdere studies werd gevonden dat aneuploidie zwangerschappen (trisomie 13, 18 en 21), die in het tweede trimester werden beëindigd, een minder goed ontwikkelde placentaire vascularisatie hadden in vergelijking met tweede trimester vitale miskramen. In **hoofdstuk 4.1** onderzoeken we of er een verschil was in placentaire vascularisatie tussen tweede trimester miskramen als gevolg van intra-uteriene foetale dood (idiopathisch) en intra-uteriene infectie. In 16 gevallen van tweede trimester idiopathische miskramen en 22 gevallen van tweede trimester miskramen als gevolg van een intra-uteriene infectie, werden placentacoupes gekleurd op de endotheel marker CD34. De centrale en perifere vasculaire oppervlakken en de vasculaire dichtheid werden gemeten met behulp van klassieke microscopie. Lagere perifere vasculaire oppervlakken en dichtheid werden waargenomen in de idiopathisch groep in vergelijking met de infectie groep. Onze hypothese was dat in gevallen van idiopathische miskramen de placentatie al reeds verstoord was in eerste trimester van de zwangerschap, leidend tot een verminderde materna-foetale interface in het tweede trimester, en vervolgens leidend tot vroege postplacentale foetale hypoxie en foetale dood.

Placenta's van de zwangerschappen gecompliceerd door negatieve dyscongruentie (SGA) en/of pre-eclampsie (PE) vóór de 34 weken AD zijn kleiner dan die alleen gecompliceerd door een idiopathische vroeggeboorte. Aangezien eerdere studies aantoonden dat een vroege onderontwikkelde placentaire vascularisatie geassocieerd was met tweede trimester foetale groeivertraging en foetale dood, onderzochten we in **hoofdstuk 4.2** of zwangerschappen gecompliceerd door SGA en/of PE ook geassocieerd zijn met een onderontwikkelde placentaire vascularisatie. Placentaire morfometrische metingen werden uitgevoerd in vier zwangerschapsgroepen - gepaard op basis van amenorrhoeduur - tussen de 24 en 34 weken. De vier groepen bestonden uit zwangerschappen gecompliceerd door SGA, PE in combinatie met SGA, PE met normaal geboortegewicht, en spontane vroeggeboorte zonder PE en normaal geboortegewicht als de referentiegroep. In zwangerschappen gecompliceerd door vroege PE werden kleinere terminale chorionvlokken met een normale vasculaire dichtheid geobserveerd. Daarentegen werden in zwangerschappen gecompliceerd door vroege SGA normaal grootte terminale chorionvlokken met een verhoogde vasculaire dichtheid geobserveerd. In SGA gecompliceerde zwangerschappen was een lagere vasculaire dichtheid in de terminale chorionvlokken geassocieerd met een echoscopische afwezige of negatieve einddiastolische arteriële navelstreng doorstroming, wijzend op een verhoogd risico op foetale nood.

Hoofdstuk 5 geeft een algemene bespreking van de belangrijkste bevindingen en de implicaties voor de klinische praktijk en voor toekomstig onderzoek worden besproken.

7

ADDENDUM

7

SUPPLEMENTARY MATERIAL

Table III. Risk of adverse obstetric outcome in the subsequent pregnancy after a previous single miscarriage

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/ control			Low	High		
Pregnancy induced hypertension														
Eras[11]	2000	RC-C	3b	14	116	68	616	0/0	1,23		0,61	2,31	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Preeclampsia														
Bhattacharay[15]	2008	RC	2b	62	1404	811	21118	0/0	1,10		0,90	1,30	Age, BMI, interpregnancy interval, marital status, smoking and social class	
Dempsey[10]	2003	RC-C	4	10	29	89	227	0/0	0,80		0,33	1,95	Age, BMI, ethnicity, marital status and smoking	
Eras[11]	2000	RC-C	4	1	103	26	634	0/0	0,23		0,01	1,43	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Eskenazi[12]	1991	RC-C	3b	16	50	63	127	0/0	0,89		0,20	3,90	BMI, ethnicity, history of miscarriage and termination of pregnancy, hypertension, smoking and working in pregnancy	>=1 Miscarriage
Seidman[13]	1989	RC	3b	16	554	349	5502	0/0	0,46		0,27	0,76	Age, BMI, education level, ethnicity, marital status, religion, parity and smoking	Calculated OR
Stone[14]	1994	RC-C	4	14	3314	56	16010	x/x	1,21		0,67	2,17	None	Severe preeclampsia, calculated OR
Thom[8]	1992	RC	3b	116	2146	189	3099	0/0		1,00	0,80	1,30	Age and smoking	Definition preeclampsia not documented
Trogstad[142]	2008	RC	2b	133	2556	956	17687	0/0	0,94		0,78	1,13	Age, BMI, education, infertility treatment and smoking	

Placental abruption														
Bhattacharay[15]	2008	RC	2b	11	1404	150	21118	0/0	1,10		0,60	2,10	None	Crude OR
Thom[8]	1992	RC	2b	19	2146	25	3099	0/0		1,20	0,60	2,30	Age and gestational age	
Placenta preavia														
Bhattacharay[15]	2008	RC	2b	5	1404	44	21118	0/0	1,80		0,70	4,60	None	Crude OR
Thom[8]	1992	RC	2b	7	2146	6	3099	0/0		1,60	0,50	6,00	Age and gestational age	
Premature preterm rupture of membrane														
Buchmayer[17]	2004	RPB	2b	?	1293	?	31177	0/0	1,90		1,50	2,60	Age, ethnicity, marital status and smoking	Preterm delivery
El-Bastawissi[19]	2003	RC-C	3b	17	69	74	212	x/x	1,30		0,70	2,50	Age, ethnicity, medical payment status, parity and smoking	Preterm delivery
Hammoud[20]	2007	RPB	3b	192	5973	1354	52280	0/0	1,24		1,07	1,45	None	Calculated OR
Thom[8]	1992	RC	2b	52	2146	79	3099	0/0		1,00	0,70	1,40	Age and gestational age	
Preterm delivery <37 weeks														
Basso[21]	1998	RPB	2b	1333	21166	348	7410	0/0	1,36		1,20	1,54	Age and social status	
Bhattacharay[15]	2008	RC	2b	128	1404	1384	21118	0/0	1,40		1,30	1,60	Age, antepartum haemorrhage, BMI, induction of labour, interpregnancy interval, marital status, preeclampsia, smoking, social class and threatened miscarriage	
Buchmayer[17]	2004	RPB	2b	1293	21631	31177	578510	0/0	1,10		1,10	1,20	Age, ethnicity, marital status and smoking	>32 Weeks
El-Bastawissi[19]	2003	RC-C	3b	69	143	212	536	x/x	1,60		1,00	2,30	Age, ethnicity, medical payment status, parity and smoking	
Hammoud[20]	2007	RPB	2b	369	5973	2951	52280	0/0	1,13		1,01	1,26	Age, BMI, cerclage and smoking	>32 Weeks
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,30		1,00	1,70	Age, diethylstilbestrol exposure, incompetent cervix, prepregnant weight, parity, pregnancy weight gain, previous termination of pregnancy, stillbirth and preterm delivery, pyelonephritis and uterine anomaly	549 preterm/ 9.490 term

Table III. Risk of adverse obstetric outcome in the subsequent pregnancy after a previous single miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/ control			Low	High		
Lekea-Karanika[27]	1990	CS	2b	117	1291	342	4621	1/1	1,19		0,95	1,50	Logistic regression analysis	
Martius[28]	1998	RC-C	3b	1069	13461	5651	90274	x/x	1,30		1,22	1,39	Not significant after multivariate analysis, OR not documented	Calculated OR
Nguyen[29]	2004	PC	2b	16	164	177	1512	x/x	0,80		0,50	1,50	Age, economic status, height, prenatal care and weight gain	
Pickering and Deeks[23]	1991	RPB	3b	?	8589	?	104889	0/0		1,47	1,34	1,62	Age, marital height, marital status and social class	Hazards Ratio
Pickering and Forbes[24]	1985	RPB	3b	?	3927	?	45879	0/0	1,32		1,15	1,51	Age, marital height, marital status, sex of infant and social class	
Schoenbaum[16]	1980	RC	3b	17	189	118	1757	0/0	1,34		0,79	2,28	Ethnicity and social class	Calculated OR
Smith[25]	2006	RPB	2b	674	9215	4423	73384	0/0		1,31	1,18	1,45	AFP, age, BMI, hCG, height, marital status, smoking, socioeconomics and termination of pregnancy	>32 Weeks, Hazards Ratio
Thom[8]	1992	RC	2b	174	2146	220	3099	0/0		1,20	0,90	1,40	Age and smoking	
Very preterm delivery <34 weeks														
Basso[21]	1998	RPB	2b	466	21166	89	7410	0/0	1,73		1,38	2,18	Age and social status	<34 weeks
Bhattacharay[15]	2008	RC	2b	39	1404	456	21118	0/0	1,30		0,90	1,80	Age, antepartum haemorrhage, BMI, induction of labour, interpregnancy interval, marital status, preeclampsia, smoking, social class and threatened miscarriage	<34 weeks
Buchmayer[17]	2004	RPB	2b	203	21631	3379	578510	0/0	1,50		1,20	1,70	Age, ethnicity, marital status and smoking	28-31 weeks
El-Bastawissi[19]	2003	RC-C	3b	16	90	51	375	x/x	1,40		0,70	2,90	Age, ethnicity, medical payment status, parity and smoking	<34 weeks
Hammoud[20]	2007	RPB	2b	92	5973	436	52280	0/0	1,85		1,47	2,32	Age, BMI, cerclage and smoking	<32 weeks, calculated OR

Martius[28]	1998	RC-C	3b	195	13461	829	90274	x/x	1,90		1,38	1,89	Not significant after multivariate analysis, OR not documented	<32 weeks, calculated OR
Smith[25]	2006	RPB	2b	96	9215	656	73384	0/0	1,23		0,90	1,68	AFP, age, BMI, hCG, height, marital status, smoking, socioeconomics and termination of pregnancy	29-32 weeks, hazard ratio
Thom[8]	1992	RC	2b	26	2146	47	3099	0/0		0,80	0,50	1,40	Age and smoking	30-33 weeks
Small for gestational age <10th percentile														
Basso[21]	1998	RPB	2b	2625	21166	926	7410	0/0	1,03		0,95	1,12	Age and social status	
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,10		0,90	1,30	Age, diethylstilbestrol exposure, ethnicity, height, prepregnant weight, parity, pregnancy weight gain, previous adverse pregnancy outcome, three or more previous termination of pregnancy, smoking and uterine anomaly	1.162 SGA/ 10.889 control
Parazzini[51]	2007	RC-C	3b	96	439	459	2082	x/x	1,00		0,80	1,40	Age, history of small for gestation, hypertension, parity and smoking	>=1 miscarriage
Pickering and Forbes[24]	1985	RPB	3b	?	3927	?	45879	0/0	1,02		0,92	1,14	Age, marital height, marital status, sex of fetus and social class	
Thom[8]	1992	RC	2b	94	2146	133	3099	0/0		0,90	0,70	1,20	Age and smoking	
Low birth weight (<2500g)														
Basso[21]	1998	RPB	2b	1291	21166	348	7410	0/0	1,08		0,92	1,28	Age and social status	
Bhattacharay[15]	2008	RC	2b	120	1404	1509	21118	0/0	1,20		0,90	1,40	Age, BMI, interpregnancy interval, marital status, preterm delivery, sex of fetus, smoking and social class	
Lekea-Karanika[27]	1994	CS	2b	81	1269	123	3482	1/1	1,18		0,90	1,55	Logistic regression analysis	
Schoenbaum[16]	1980	RC	4	23	189	144	1757	0/0	1,48		0,93	2,36	Ethnicity and social class	Calculated OR
Thom[8]	1992	RC	2b	124	2146	141	3099	0/0		1,20	0,90	1,60	Age and smoking	

Table III. Risk of adverse obstetric outcome in the subsequent pregnancy after a previous single miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/ control			Low	High		
Congenital malformation														
Paz[30]	1992	RC-C	3b	383	2334	2012	16455	x/x		1,40	1,20	1,60	Age, consanguinity, chronic and acute diseases, interpregnancy interval, previous infertility and socioeconomics	Cases with multiple congenital malformations
Schoenbaum[16]	1980	RC	3b	23	189	111	1757	0/0	1,93		1,20	3,10	Ethnicity and social class	Calculated OR
Thom[8]	1992	RC	3b	36	2146	53	3099	0/0		1,00	0,60	1,50	None	
5-minute Apgar score <7														
Bhattacharay[15]	2008	RC	2b	48	1404	646	21118	0/0	1,10		0,90	1,20	None	Crude OR
Thom[8]	1992	RC	2b	36	2146	64	3099	0/0		0,90	0,60	1,40	Age, gestational age and smoking	
Fetal death														
Bhattacharay[15]	2008	RC	2b	14	1404	133	21118	0/0	1,60		0,90	2,80	Age, BMI, interpregnancy interval, marital status, smoking and social class	
Schoenbaum[16]	1980	RC	3b	3	189	12	1757	0/0	2,32		0,65	8,31	Ethnicity and social class	Calculated OR
Neonatal death														
Bhattacharay[15]	2008	RC	2b	11	1404	77	21118	0/0	2,10		1,00	3,90	Age, BMI, interpregnancy interval, marital status, preterm delivery, smoking and social class	
Schoenbaum[16]	1980	RC	3b	2	189	5	1757	0/0	3,72		0,72	19,3	Ethnicity and social class	Calculated OR

1 Design: PC, prospective cohort; RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 Parity: 0/0, gravida 2 and nulliparous versus primigravidae; 1/1, primiparous versus primiparous; x/x, nulliparous and multiparous versus nulliparous and multiparous
4 OR, odds ratio; RR, relative risk; CI, confidence interval

Table IV. Risk of adverse obstetric outcome in the subsequent pregnancy after two or more previous miscarriages

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/ control			Low	High		
Pregnancy induced hypertension														
Eras[11]	2000	RC-C	4	4	20	68	616	0/0	2,24		0,53	7,19	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Preeclampsia														
Dempsey[10]	2003	RC-C	4	8	13	89	227	0/0	2,11		0,59	7,50	Age, BMI, ethnicity, marital status and smoking	
Sheiner[7]	2005	RPB	2b	120	7503	1615	146791	x/x	1,50		1,30	1,80	Multivariate analysis	Severe preeclampsia
Sheiner[7]	2005	RPB	2b	263	7503	4991	146791	x/x	1,00		0,90	1,20	Multivariate analysis	Mild preeclampsia
Trogstad[143]	2008	RC	2b	32	603	956	17687	0/0	0,91		0,63	1,32	Age, BMI, education, infertility treatment and smoking	
Placental abruption														
Sheiner[7]	2005	RPB	2b	83	7503	1028	146791	x/x	1,50		1,10	1,70	Multivariate analysis	
Placenta preavia														
Sheiner[7]	2005	RPB	2b	68	7503	587	146791	x/x	1,70		1,30	2,30	Multivariate analysis	
Premature preterm rupture of membrane														
Buchmayer[17]	2004	RPB	3b	?	146	?	31177	0/0	1,50		1,00	2,40	Age, ethnicity, marital status and smoking	
El-Bastawissi[19]	2003	RC-C	3b	8	31	74	222	x/x	1,80		0,70	4,40	Age, ethnicity, medical payment status, parity and smoking	
Hammoud[20]	2007	RPB	3b	46	908	1354	52280	0/0	1,96		1,45	2,64	None	Calculated OR
Sheiner[7]	2005	RPB	2b	488	7503	8220	146791	x/x	1,20		1,10	1,30	Multivariate analysis	
Preterm delivery <37 weeks														
Basso[21]	1998	RPB	2b	432	5268	380	9752	x/x	2,13		1,79	2,53	Age, interpregnancy interval and social status	

Table IV. Risk of adverse obstetric outcome in the subsequent pregnancy after two or more previous miscarriages (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/ control			Low	High		
Buchmayer[17]	2004	RPB	2b	146	1742	31177	578510	0/0	1,60		1,30	1,90	Age, ethnicity, marital status and smoking	>32 Weeks
El-Bastawissi[19]	2003	RC-C	3b	31	57	212	536	x/x	2,20		0,70	2,00	Age, ethnicity, medical payment status, parity and smoking	
Hammoud[20]	2007	RPB	2b	88	908	2951	52280	0/0	1,56		1,24	1,97	Age, BMI, cerclage and smoking	>32 Weeks
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,80		1,10	2,90	Age, diethylstilbestrol exposure, incompetent cervix, prepregnant weight, parity, pregnancy weight gain, previous termination of pregnancy, stillbirth and preterm delivery, pyelonephritis and uterine anomaly	549 preterm/ 9.490 term
Lekea-Karanika[27]	1990	CS	2b	73	439	342	4621	1/1	1,89		1,31	2,71	Logistic regression analysis	
Martius[28]	1998	RC-C	3b	309	2788	5651	84623	x/x	1,90		1,66	2,12	Not significant after multivariate analysis, OR not documented	
Nguyen[29]	2004	PC	2b	8	33	177	1512	x/x	2,60		1,10	6,20	Age, economic status, height, prenatal care and weight gain	
Pickering and Deeks[23]	1991	RPB	3b	?	1524	?	104889	0/0		2,75	2,35	3,23	Age, marital height, marital status and social class	Hazards Ratio
Pickering and Forbes[24]	1985	RPB	3b	?	689	?	45879	0/0	1,91		1,47	2,50	Age, marital height, marital status, sex of infant and social class	Hazards Ratio
Smith[25]	2006	RPB	2b	178	1792	4423	73384	0/0	1,50		1,22	1,84	AFP, age, BMI, hCG, height, marital status, smoking, socioeconomics and termination of pregnancy	Hazards Ratio
Very preterm delivery <34 weeks														
Basso[21]	1998	RPB	2b	158	5268	98	9752	x/x	3,40		2,53	4,58	Age, interpregnancy interval and social status	<34 weeks
Buchmayer[17]	2004	RPB	2b	26	1742	3379	578510	0/0	2,70		1,80	4,00	Age, ethnicity, marital status and smoking	28-31 Weeks
El-Bastawissi[19]	2003	RC-C	3b	6	32	51	375	x/x	1,40		0,40	4,20	Age, ethnicity, medical payment status, parity and smoking	<34 weeks

Hammoud[20]	2007	RPB	3b	6	908	269	52280	0/0	1,28	0,57	2,89	None	<32 weeks, calculated OR
Martius[28]	1998	RC-C	3b	71	2788	829	84623	x/x	2,90	2,28	3,72	Not significant after multivariate analysis, OR not documented	<32 weeks
Smith[25]	2006	RPB	2b	38	1792	656	73384	0/0	2,24	1,36	3,68	AFP, age, BMI, hCG, height, marital status, smoking, socioeconomics and termination of pregnancy	29-32 weeks, hazard ratio
Small for gestational age <10th percentile													
Basso[21]	1998	RPB	2b	537	5268	790	9752	x/x	1,41	1,23	1,63	Age, interpregnancy interval and social status	
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,00	0,70	1,60	Age, diethylstilbestrol exposure, ethnicity, height, prepregnant weight, parity, pregnancy weight gain, previous adverse pregnancy outcome, three or more previous termination of pregnancy, smoking and uterine anomaly	1.162 SGA/ 10.889 control
Pickering and Forbes[24]	1985	RPB	3b	?	689	?	45879	0/0	1,20	0,95	1,51	Age, marital height, marital status, sex of infant and social class	
Low birth weight (<2500g)													
Basso[21]	1998	RPB	2b	395	5268	312	9752	x/x	1,87	1,46	2,40	Age, interpregnancy interval and social status	
Lekea-Karanika[27]	1994	CS	3b	?	?	123	3482	1/1	1,87	1,24	2,84	Logistic regression analysis	
Sheiner[7]	2005	RPB	3b	713	7503	14239	146791	x/x	0,98	0,91	1,06	None	calculated OR
5-minute Apgar score <7													
Sheiner[7]	2005	RPB	2b	45	7503	881	146791	x/x	1,00	0,80	1,40	Multivariate analysis	
Neonatal death													
Sheiner[7]	2005	RPB	2b	128	7503	2055	146791	x/x	1,20	0,90	1,40	Multivariate analysis	

1 Design: PC, prospective cohort; CS, cross-sectional; RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 Parity: 0/0, gravida 2 and nulliparous versus primigravidae; 1/1, primiparous versus primiparous; x/x, nulliparous and multiparous versus nulliparous and multiparous
4 OR, odds ratio; RR, relative risk; CI, confidence interval

Table V. Risk of adverse obstetric outcome in the subsequent pregnancy after recurrent miscarriage

Study	Year	Design[1]	Level[2]	Idiopathic[3]	Cases		Controls		Parity[4]	OR[5]	RR[5]	95% CI[5]		Adjusted for	Comment
					N	Total	N	Total	Case/control			Low	High		
Preeclampsia															
Hughes[35]	1991	RC	4	No	2	88	333	12860	1/1	0,88		0,22	3,35	None	Definition preeclampsia not documented, calculated OR
Thom[8]	1992	RC	3b	No	26	638	189	3099	0/0		0,80	0,50	1,30	Age and smoking	Definition preeclampsia not documented
Trogstad[143]	2009	RC	2b	No	11	130	956	17687	0/0	1,51		0,80	2,83	Age, BMI, education, infertility treatment and smoking	
Placental abruption															
Thom[8]	1992	RC	3b	No	5	638	25	3099	0/0		1,20	0,40	3,10	Age and gestational age	
Placenta previa															
Thom[8]	1992	RC	3b	No	8	638	6	3099	0/0		6,00	1,60	22,20	Age and gestational age	
Premature preterm rupture of membranes															
Hammoud[20]	2007	RPB	3b	No	15	225	1354	52280	0/0	2,57		1,52	4,35	None	Calculated OR
Thom[8]	1992	RC	3b	No	33	638	79	3099	0/0		1,80	1,20	2,90	Age and gestational age	
Preterm delivery <37 weeks															
Hammoud[20]	2007	RPB	3b	No	36	225	2951	52280	0/0	2,46		1,68	3,60	Age, BMI, cerclage and smoking	>32 Weeks
Hughes[35]	1991	RC	4	No	11	88	1075	12860	1/1	1,50		0,80	2,81	None	Calculated OR
Jivraj[34]	2001	RC	4	Yes	7	61	959	24699	0/?	2,96		1,35	6,48	None	Calculated OR
Jivrajj[34]	2001	RC	4	No	22	162	959	24699	0/?	3,50		2,23	5,49	None	Calculated OR
Lang[22]	1996	RC-C	3b	No					x/x	2,70		1,40	5,30	Age, diethylstilbestrol exposure, incompetent cervix, prepregnant weight, parity, pregnancy weight gain, previous termination of	549 preterm/ 9.490 term

													pregnancy, stillbirth and preterm delivery, pyelonephritis and uterine anomaly	
Lekea-Karanika[27]	1990	CS	3b	No			342	4621	1/1	3,00	1,59	5,65	Logistic regression analysis	
Martius[28]	1998	RC-C	3b	No	151	639	5651	90274	x/x	3,10	2,62	3,75	Not significant after multivariate analysis	
Thom[8]	1992	RC	2b	No	63	638	220	3099	0/0	1,50	1,10	2,10	Age and smoking	
Very preterm delivery <34 weeks														
Hammoud[20]	2007	RPB	3b	No	5	225	436	52280	0/0	2,66	1,09	6,50	None	<32 weeks, calculated OR
Martius[28]	1998	RC-C	3b	No	52	639	829	90274	x/x	7,40	5,50	9,87	Not significant after multivariate analysis	<32 weeks
Thom[8]	1992	RC	3b	No	19	638	47	3099	0/0	2,40	1,40	4,30	Age and smoking	30-33 weeks
Small for gestational age <10th percentile														
Hughes[35]	1991	RC	4	No	3	88	180	12860	1/1	2,43	0,76	7,77	None	Calculated OR
Jivrajj[34]	2001	RC	4	Yes	5	61	523	24699	0/?	3,87	1,55	9,67	None	Calculated OR
Jivrajj[34]	2001	RC	4	No	21	162	523	24699	0/?	6,12	3,85	9,72	None	Calculated OR
Lang[22]	1996	RC-C	3b	No					x/x	1,40	0,80	2,60	Age, diethylstilbestrol exposure, ethnicity, height, prepregnant weight, parity, pregnancy weight gain, previous adverse pregnancy outcome, three or more previous termination of pregnancy, smoking and uterine anomaly	1.162 SGA/ 10.889 control
Thom[8]	1992	RC	2b	No	41	638	133	3099	0/0	1,20	0,80	1,70	Age and smoking	
Low birth weight (<2500g)														
Hughes[35]	1991	RC	4	No	11	88	867	12860	1/1	1,85	0,99	3,48	None	Calculated OR
Lekea-Karanika[27]	1994	CS	3b	No			123	3482	1/1	1,87	0,86	4,09	Logistic regression analysis	
Thom[8]	1992	RC	2b	No	60	638	141	3099	0/0	2,00	1,40	2,80	Age and smoking	

Table V. Risk of adverse obstetric outcome in the subsequent pregnancy after recurrent miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Idiopathic[3]	Cases		Controls		Parity[4]	OR[5]	RR[5]	95% CI[5]		Adjusted for	Comment
					N	Total	N	Total	Case/control			Low	High		
Congenital malformation															
Thom[8]	1992	RC	3b	No	21	638	53	3099	0/0		1,80	1,10	3,00	None	
5-minute Apgar score <7															
Thom[8]	1992	RC	2b	No	22	638	64	3099	0/0		0,60	0,30	1,60	Age, gestational age and smoking	
Fetal and neonatal death															
Hughes[35]	1991	RC	4	No	0	88	58	12860	1/1					None	Stillbirth and neonatal death
Jivrajj[34]	2001	RC	4	Yes	1	61	247	24699	0/?	1,64		0,23	11,87	None	Neonatal death
Jivrajj[34]	2001	RC	4	No	4	162	247	24699	0/?	2,47		0,91	6,71	None	Neonatal death

1 Design: PC, prospective cohort; CS, cross-sectional; RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 Cases with idiopathic recurrent miscarriage
4 Parity: 0/0, gravida 2 and nulliparous versus primigravidae; 1/1, primiparous versus primiparous; x/x, nulliparous and multiparous versus nulliparous and multiparous; ? Parity not documented
5 OR, odds ratio; RR, relative risk; CI, confidence interval

Table VI. Risk of adverse obstetric outcome in the subsequent pregnancy after a single previous termination of pregnancy

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control			Low	High		
Pregnancy induced hypertension														
Eras[11]	2000	RC-C	3b	19	188	68	616	0/0	1,01		0,55	1,75	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Preeclampsia														
Dempsey[10]	2003	RC-C	4	20	43	89	227	0/0	1,51		0,73	3,14	Age, BMI, ethnicity, marital status and smoking	
Eras[11]	2000	RC-C	4	3	172	26	634	0/0	0,42		0,01	1,38	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Eskanazi[12]	1991	RC-C	3b	44	79	35	97	x/x	2,16		1,18	3,96	None	
Linn[44]	1983	RC	3b	35	827	243	4331	0/0	0,75		0,53	1,08	None	Calculated OR
Raatikainen[40]	2006	RPB	3b	85	2364	752	24248	x/x	1,16		0,92	1,46	None	Calculated OR
Seidman[13]	1989	RC	3b	5	418	349	5402	0/0	0,19		0,08	0,45	Age, BMI, education level, ethnicity, marital status, religion, parity and smoking	Calculated OR
Stone[14]	1994	RC-C	3b	18	5935	52	13099	x/x	0,76		0,45	1,31	None	Severe preeclampsia, calculated OR
Trogstad[142]	2008	RC	2b	116	2469	997	17992	0/0	0,87		0,71	1,06	Age, BMI, education, infertility treatment and smoking	
Placental abruption														
Linn[44]	1983	RC	3b	16	827	48	4331	0/0	1,75		0,99	3,09	None	Calculated OR
Placenta preavia														
Johnsons[145]	2003	RC-C	3b	40	175	111	520	x/x	1,00		0,70	1,60	Age, parity and smoking	
Linn[44]	1983	RC	3b	2	827	13	4331	0/0	0,81		0,18	3,58	None	Calculated OR
Zhou[46]	2001	RC	3b	34	12553	122	40758	0/1	0,90		0,62	1,32	None	Calculated OR, Not adjusted for parity. Matched for gravidity

Table VI. Risk of adverse obstetric outcome in the subsequent pregnancy after a single previous termination of pregnancy (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control			Low	High		
Premature preterm rupture of membranes														
Ancel[45]	2004	RC-C	3b	?	964	?	7719	x/x	1,35		1,10	1,66	Age, country, marital status, parity, social class and smoking	EUROPOP study
El-Bastawissi[19]	2003	RC-C	3b	21	64	64	209	x/x	1,60		0,90	2,80	Age, ethnicity, medical payment status, parity and smoking	Preterm delivery
Linn[44]	1983	RC	3b	56	1342	333	8122	x/x	0,95		0,71	1,27	Age, ethnicity, economics, parity and smoking	
Moreau[47]	2005	RC-C	3b	?	238	?	1644	x/x	1,70		1,20	2,50	Age, education level, employment during pregnancy, marital status, parity, previous preterm birth, smoking and weight before pregnancy	EPIPAGE study <32 weeks
Preterm delivery <37 weeks														
Ancel[45]	2004	RC-C	3b	389	964	2938	7719	x/x	1,15		0,99	1,33	Age, country, marital status, parity, social class and smoking	EUROPOP study
El-Bastawissi[19]	2003	RC-C	3b	64	133	209	524	x/x	1,50		1,00	2,30	Age, ethnicity, medical payment status, parity and smoking	
Henriet and Kaminski[49]	1995	RPB	2b	83	1487	443	10563	x/x	1,30		1,00	1,70	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,10		0,80	1,50	Age, diethylstilbestrol exposure, incompetent cervix, prepregnant weight, parity, pregnancy weight gain, previous miscarriage, stillbirth and preterm delivery, pyelonephritis and uterine anomaly	549 preterm/ 9.490 term
Lao and Ho[146]	1998	RC	4	12	118	10	118	x/x	1,20		0,50	2,89	None, matched for age and parity	>=1 TOP, calculated OR
Linn[44]	1983	RC	3b	102	1342	536	8122	x/x	1,07		0,86	1,34	Age, ethnicity, economics, parity and smoking	

Martius[28]	1998	RC-C	3b	251	2596	7181	99164	x/x	1,30		1,21	1,45	Multivariate analysis	
Meirik and Bergstrom[147]	1983	RC	4	32	532	29	483	0/0		1,00	0,55	1,82	None	Matched pairs
Nguyen[29]	2004	PC	2b	26	281	161	1274	x/x	0,80		0,50	1,30	Age, economic status, height, prenatal care and weight gain	
Pickering and Deeks[23]	1991	RPB	3b	?	7449	?	104889	0/0	1,51		1,38	1,67	>=1 TOP	
Pickering and Forbes[24]	1985	RPB	3b	?	2795	?	45879	0/0	1,35		1,16	1,57	Age, marital height, marital status, sex of infant and social class	
Raatikainen[40]	2006	RPB	2b	173	2364	1503	24248	x/x	1,13		0,94	1,35	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Reime[48]	2008	RC	3b	17	211	275	7845	0/0	1,90		0,77	4,69	BMI, ethnicity, marital status, prenatal care and smoking	In adolescents
Schoenbaum[16]	1980	RC	4	10	205	118	1757	0/0	0,73		0,38	1,41	Ethnicity and social class	Calculated OR
Smith[25]	2006	RPB	2b	503	7340	4700	76267	0/0	1,19		1,06	1,34	AFP, age, BMI, hCG, height, marital status, miscarriage, smoking and socioeconomics	hazard ratio
Zhou[42]	1999	RC	3b	615	11394	1467	40758	0/1	1,82		1,63	2,04	Age, interpregnancy interval, number of previous preterm delivery and residence.	Not adjusted for parity. Matched for gravidity
Very preterm delivery <34 weeks														
Ancel[45]	2004	RC-C	3b		964		7719	x/x	1,34		1,08	1,68	Age, country, marital status, parity, social class and smoking	EUROPOP study, <32 weeks, number of cases not documented
El-Bastawissi[19]	2003	RC-C	3b	13	82	48	360	x/x	1,10		0,50	2,40	Age, ethnicity, medical payment status, parity and smoking	<34 weeks
Henriet and Kaminski[49]	1995	RPB	2b	18	1487	74	10563	x/x	1,40		1,10	1,80	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	<33 weeks, >=1 TOP

Table VI. Risk of adverse obstetric outcome in the subsequent pregnancy after a single previous termination of pregnancy (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control			Low	High		
Martius[28]	1998	RC-C	3b	64	2596	1146	99164	x/x	1,80		1,57	2,13	Multivariate analysis	<32 weeks
Moreau[47]	2005	RC-C	3b	238	294	1644	2199	x/x	1,50		1,10	2,00	Age, education level, employment during pregnancy, marital status, parity, previous preterm birth, smoking and weight before pregnancy	EPIPAGE study, <32 weeks
Smith[25]	2006	RPB	2b	88	7340	696	76267	0/0	1,31		1,05	1,64	Not significant after multivariate analysis, AFP, age, BMI, hCG, height, marital status, miscarriage, smoking and socioeconomics	<32 weeks, calculated OR
Small for gestational age <10th percentile														
Henriet and Kaminski[49]	1995	RPB	2b	143	1478	767	10563	x/x	1,20		1,00	1,50	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,10		0,90	1,30	Age, diethylstilbestrol exposure, ethnicity, height, prepregnant weight, parity, pregnancy weight gain, previous adverse pregnancy outcome, three or more previous termination of pregnancy, smoking and uterine anomaly	1.162 SGA/ 10.889 control
Lao and Ho[146]	1998	RC	4	10	118	13	118	x/x	0,77		0,33	1,82	None, matched for age and parity	>=1 TOP, calculated OR
Parazzini[51]	2007	RC-C	3b	37	163	508	2312	x/x	1,00		0,60	1,70	Age, history of small for gestation, hypertension, parity and smoking	>=1 TOP
Pickering and Forbes[24]	1985	RPB	3b	?	2795	?	45879	0/0	0,90		0,79	1,02	Age, marital height, marital status, sex of infant and social class	
Raatikainen[40]	2006	RPB	2b	236	2364	2304	24248	x/x	0,96		0,83	1,11	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	

Low birth weight (<2500g)													
Henriet and Kaminski[49]	1995	RPB	2b	81	1494	456	10563	x/x	1,10		0,90	1,40	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	
Mandelson[52]	1992	RC-C	4	111	1944	85	1941	?/?	1,20		0,90	1,50	Age, antenatal care, economics status, marital status and smoking	
Lao and Ho[146]	1998	RC	4	11	118	10	118	x/x	1,10		0,45	2,69	None, matched for age and parity	>=1 TOP, calculated OR
Lekea-Karanika[53]	1994	CS	2b	94	1581	123	3482	1/1	1,36		1,03	1,78	Logistic regression analysis	
Linn[44]	1983	RC	3b	97	1342	569	8122	x/x	0,93		0,74	1,17	Age, ethnicity, economics, parity and smoking	
Lumley[55]	1986	RC	3b	679	7759	6042	111453	x/x	1,61		1,49	1,75	None	>=1 TOP, calculated OR
Meirik and Bergstrom[147]	1983	RC	4	23	532	21	483	0/0		1,13	0,55	2,41	None	Matched pairs
Raatikainen[40]	2006	RPB	2b	125	2364	1140	24248	x/x	1,03		0,83	1,37	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Schoenbaum[16]	1980	RC	4	14	208	144	1757	0/0	0,82		0,47	1,45	Ethnicity and social class	Calculated OR
Seidman[142]	1988	RC	2b	145	1791	817	14857	x/x	1,47		1,23	1,77	Multiple regression analysis	>=1 TOP, calculated OR
Zhou[54]	2000	RC	3b	570	11394	1427	40758	0/1	1,90		1,60	2,30	Age, gender of fetus, interpregnancy interval, number of previous low birth weights and residence.	Not adjusted for parity. Matched for gravidity
Low birth weight (<1500g)														
Lumley[55]	1986	RC	3b	79	7759	560	111453	x/x	2,03		1,60	2,57	None	>=1 TOP, birth weight <1000g, calculated OR

Table VI. Risk of adverse obstetric outcome in the subsequent pregnancy after a single previous termination of pregnancy (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control			Low	High		
Reime[48]	2008	RC	3b	6	211	110	7845	0/0	2,74		1,06	7,09	BMI, ethnicity, marital status, prenatal care and smoking	In adolescents
Congenital malformation														
Linn[44]	1983	RC	3b	28	1342	219	8122	x/x	0,76		0,51	1,13	Age, ethnicity, economics, parity and smoking	Major malformations
Linn[44]	1983	RC	3b	73	827	346	4331	0/0	1,10		0,85	1,44	None	Any malformation, Calculated OR
Schoenbaum[16]	1980	RC	4	12	205	111	1757	0/0	0,93		0,50	1,71	Ethnicity and social class	Calculated OR
Seidman[142]	1988	RC	2b	19	1791	152	14857	x/x	1,04		0,64	1,68	Multiple regression analysis	Major malformations, >=1 TOP, calculated OR
Seidman[142]	1988	RC	2b	122	1791	969	14857	x/x	1,04		0,86	1,27	Multiple regression analysis	Major malformations, >=1 TOP, calculated OR
5-minute Apgar score <7														
Raatikainen[40]	2006	RPB	2b	47	2364	461	24248	x/x	1,12		0,81	1,55	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Fetal death														
Frank[148]	1991	RC	3b	11	1311	19	2131	x/x	0,94		0,45	1,98	None	Calculated OR
Linn[44]	1983	RC	3b	3	827	26	4331	0/0	0,61		0,18	2,00	None	Calculated OR
Raatikainen[40]	2006	RPB	3b	5	2364	121	24248	x/x	0,42		0,17	1,04	None	Calculated OR
Reime[48]	2008	RC	3b	3	211	36	7845	0/0	1,23		0,17	9,15	BMI, ethnicity, marital status, prenatal care and smoking	In adolescents
Schoenbaum[16]	1980	RC	4	2	205	12	1757	0/0	1,43		0,32	6,43	Ethnicity and social class	Calculated OR

Seidman[142]	1988	RC	3b	6	1791	126	14857	x/x	0,40	0,17	0,90	Multiple regression analysis	>=1 TOP, calculated OR
Neonatal death													
Linn[44]	1983	RC	3b	5	827	17	4331	0/0	1,54	0,57	4,19	None	Calculated OR
Raatikainen[40]	2006	RPB	2b	14	2364	145	24248	x/x	0,97	0,53	1,78	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Reime[48]	2008	RC	3b	1	211	16	7845	0/0	4,64	0,58	37,50	BMI, ethnicity, marital status, prenatal care and smoking	In adolescents
Schoenbaum[16]	1980	RC	4	0	205	5	1757	0/0				Ethnicity and social class	Calculated OR

1 Design: PC, prospective cohort; CS, cross-sectional; RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 Parity: 0/0, gravida 2 and nulliparous versus primigravidae; 0/1 gravida 2 and nulliparous versus gravida 2 and primiparous; 1/1, primiparous versus primiparous; x/x, nulliparous and multiparous versus nulliparous and multiparous; ?, parity not documented
4 OR, odds ratio; RR, relative risk; CI, confidence interval

Table VII. Risk of adverse obstetric outcome in the subsequent pregnancy after ≥ 2 previous terminations of pregnancy

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control		Low	High		
Pregnancy induced hypertension													
Eras[11]	2000	RC-C	4	1	80	68	616	0/0	0,11	0,00	0,67	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Preeclampsia													
Dempsey[10]	2003	RC-C	4	9	17	89	227	0/0	1,48	0,50	4,40	Age, BMI, ethnicity, marital status and smoking	
Eras[11]	2000	RC-C	4	1	80	26	634	0/0	0,30	0,01	1,86	Age, alcohol and coffee consumption, BMI, education level, ethnicity, marital status and smoking	
Linn[44]	1983	RC	3b	12	194	243	4331	0/0	1,10	0,61	2,00	None	Calculated OR
Raatikainen[40]	2006	RPB	3b	13	355	752	24248	x/x	1,18	0,68	2,06	None	Calculated OR
Trogstad[142]	2008	RC	2b	8	385	997	17687	0/0	0,39	0,19	0,80	Age, BMI, education, infertility treatment and smoking	
Placental abruption													
Linn[44]	1983	RC	3b	2	194	48	4331	0/0	0,93	0,22	3,89	None	Calculated OR
Placenta preavia													
Johnsons[145]	2003	RC-C	3b	20	69	111	520	x/x	1,40	0,80	2,50	Age, parity and smoking	
Linn[44]	1983	RC	3b	0	194	13	4331	0/0				None	
Zhou[46]	2001	RPB	3b	7	2381	86	21602	1/2	0,74	0,34	1,60	None, matched for gravidity	Calculated OR
Premature preterm rupture of membranes													
El-Bastawissi[19]	2003	RC-C	3b	14	39	64	209	x/x	1,50	0,70	3,50	Age, ethnicity, medical payment status, parity and smoking	
Linn[44]	1983	RC	3b	27	359	238	8122	x/x	1,90	1,26	2,87	Age, ethnicity, economics, parity and smoking	
Preterm delivery <37 weeks													
Ancel[45]	2004	RC-C	3b	215	218	2938	4781	x/x	1,63	1,31	2,03	Age, country, marital status, parity, social class and smoking	EUROPOP study
El-Bastawissi[19]	2003	RC-C	3b	39	82	209	521	x/x	1,20	0,70	2,00	Age, ethnicity, medical payment status, parity and smoking	
Henriet and Kaminski[49]	1995	RPB	2b	21	313	443	10536	x/x	1,90	1,20	2,80	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	

Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,90	1,20	3,00	Age, diethylstilbestrol exposure, incompetent cervix, prepregnant weight, parity, pregnancy weight gain, previous miscarriage, stillbirth and preterm delivery, pyelonephritis and uterine anomaly	549 preterm/ 9.490 term
Linn[44]	1983	RC	3b	34	359	536	8122	x/x	1,31	0,91	1,89	Age, ethnicity, economics, parity and smoking	
Lopes[50]	1991	RC	4	15	285	13	285	0/0	1,15	0,54	2,47	None	Calculated OR
Martius[28]	1998	RC-C	3b	50	347	7181	99164	x/x	2,30	1,73	3,15	Multivariate analysis	
Nguyen[29]	2004	PC	2b	14	154	161	1274	x/x	0,90	0,50	1,60	Age, economic status, height, prenatal care and weight gain	
Pickering and Forbes[24]	1985	RPB	3b	?	168	?	45879	0/0	1,27	0,69	2,33	Age, marital height, marital status, sex of infant and social class	
Raatikainen[40]	2006	RPB	2b	31	355	1503	24248	x/x	1,35	0,91	2,02	Age, alcohol, diabetes, education level, employment, intrauterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Smith[25]	2006	RPB	2b	72	784	4700	76267	0/0	1,90	1,44	2,49	AFP, age, BMI, hCG, height, marital status, miscarriage, smoking and socioeconomics	hazard ratio
Zhou[42]	1999	RPB	3b	127	1921	573	15486	1/2	2,45	1,90	3,17	Age, interpregnancy interval, number of previous preterm delivery and residence.	Not adjusted for parity. Matched for gravidity
Very preterm delivery <34 weeks													
Ancel[45]	2004	RC-C	3b	?	433	?	7719	x/x	1,82	1,34	2,49	Age, country, marital status, parity, social class and smoking	EUROPOP study, <32 weeks
El-Bastawissi[19]	2003	RC-C	3b	12	55	48	360	x/x	1,40	0,60	3,20	Age, ethnicity, medical payment status, parity and smoking	<34 weeks
Martius[28]	1998	RC-C	3b	19	347	1146	99164	x/x	5,60	3,52	8,96	Multivariate analysis	EPIPAGE study, <32 weeks
Moreau[47]	2005	RC-C	3b	61	68	1644	2199	x/x	2,60	1,10	5,90	Age, education level, employment during pregnancy, marital status, parity, previous preterm birth, smoking and weight before pregnancy	<32 weeks
Smith[25]	2006	RPB	3b	6	784	696	76267	0/0	0,84	0,37	1,88	Not significant after multivariate analysis, AFP, age, BMI, hCG, height, marital status, miscarriage, smoking and socioeconomics	<32 weeks, calculated OR

Table VII. Risk of adverse obstetric outcome in the subsequent pregnancy after ≥ 2 previous terminations of pregnancy (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		Parity[3]	OR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	Case/control		Low	High		
Small for gestational age <10th percentile													
Henriet and Kaminski[49]	1995	RPB	2b	29	310	767	10536	x/x	1,10	0,70	1,60	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	
Lang[22]	1996	RC-C	3b	?	?	?	?	x/x	1,00	0,70	1,60	Age, diethylstilbestrol exposure, ethnicity, height, prepregnant weight, parity, pregnancy weight gain, previous adverse pregnancy outcome, smoking and uterine anomaly	1.162 SGA/ 10.889 control
Lekea-Karanika[53]	1994	CS	3b	?	?	123	3482	1/1	1,19	0,80	1,77	Logistic regression analysis	
Lopes[50]	1991	RC	3b	24	285	26	285	0/0	0,92	0,52	1,65	None	Calculated OR
Pickering and Forbes[24]	1985	RPB	3b	?	168	?	45879	0/0	1,13	0,70	1,81	Age, marital height, marital status, sex of infant and social class	
Raatikainen[40]	2006	RPB	2b	42	355	2304	24248	x/x	0,99	0,70	1,40	Age, alcohol, diabetes, education level, employment, intra uterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Low birth weight (<2500g)													
Henriet and Kaminski[49]	1995	RPB	2b	25	315	456	10536	x/x	1,40	0,90	2,30	Age, antenatal care, education level, employment, ethnicity, marital status, parity, previous obstetric outcome, smoking and weight before pregnancy	
Linn[44]	1983	RC	3b	37	359	569	8122	x/x	1,25	0,88	1,79	Age, ethnicity, economics, parity and smoking	
Lopes[50]	1991	RC	3b	13	285	18	285	0/0	0,72	0,37	1,50	None	Calculated OR
Mandelson[52]	1992	RC-C	3b	138	1788	85	1941	?/?	1,50	1,10	2,00	Age, antenatal care, economics status, marital status and smoking	
Raatikainen[40]	2006	RPB	2b	35	355	1140	24248	x/x	1,26	0,79	2,00	Age, alcohol, diabetes, education level, employment, intrauterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	

Zhou[54]	2000	RC	3b	104	1921	557	15486	1/2	1,90	1,30	2,70	Age, gender of fetus, interpregnancy interval, number of previous low birth weights and residence.	Not adjusted for parity. Matched for gravidity
Low birth weight (<1000g)													
Lumley[55]	1986	RC	3b	22	1231	560	111453	x/x	3,56	2,31	5,47	None	Calculated OR
Congenital malformation													
Linn[44]	1983	RC	4	8	359	219	8122	x/x	1,28	0,71	2,32	Age, ethnicity, economics, parity and smoking	Major Malformation
Linn[44]	1983	RC	4	15	194	346	4331	0/0	0,97	0,57	1,66	None	Any malformation, calculated OR
5-minute Apgar score <7													
Raatikainen[40]	2006	RPB	3b	7	355	461	24248	x/x	0,81	0,33	1,99	Age, alcohol, diabetes, education level, employment, intrauterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	
Fetal death													
Linn[44]	1983	RC	4	0	194	26	4331	0/0				None	Calculated OR
Raatikainen[40]	2006	RPB	3b	2	355	121	24248	x/x	1,13	0,28	4,59	None	Calculated OR
Neonatal death													
Linn[44]	1983	RC	4	1	194	17	4331	0/0	1,31	0,17	9,92	None	Calculated OR
Lopes[50]	1991	RC	4	0	285	0	285	0/0				None	
Raatikainen[40]	2006	RPB	3b	2	355	145	24248	x/x	0,52	0,07	3,75	Age, alcohol, diabetes, education level, employment, intrauterine device, marital status, parity, preeclampsia, prolonged gravidity, smoking, surgical scarred uterus and weight	

1 Design: PC, prospective cohort; CS, cross-sectional; RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 Parity: 0/0, gravida 2 and nulliparous versus primigravidae; 1/2 gravida 3 and nulliparous versus gravida 3 and parity 2; 1/1, primiparous versus primiparous; x/x, nulliparous and multiparous versus nulliparous and multiparous; ?, parity not documented
4 OR, odds ratio; CI, confidence interval

Table VIII. Risk of adverse obstetric outcome in the index pregnancy after threatened miscarriage

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	RR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Ante Partum Haemorrhage													
De Sutter[68]	2006	RC	3b	31	253	35	1179	4,56		2,76	7,56	None	ART population, 2nd trimester
De Sutter[68]	2006	RC	3b	13	253	22	1179	2,85		1,42	5,73	None	ART population, 3rd trimester
Mulik[67]	2004	RC	2b	75	458	389	6445	2,30		1,10	5,10	Logistic regression analysis	
Wijesiriwardana[61]	2006	RC	2b	1109	7627	2651	31633	1,80		1,73	2,01	Marital status, smoking and social class	
Pregnancy induced hypertension													
Johns[69]	2003	RC-C	3b	3	129	4	143		0,83	0,19	3,64	None	
Weiss[60]	2004	PC	1b	128	2094	807	14160	1,40		1,10	1,80	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	20	252	807	14160	1,10		0,50	2,40	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Preeclampsia													
Johns and Jauniaux[59]	2006	PC	2b	?	214	?	214					Controls matched for age, p=0,47	
Weiss[60]	2004	PC	1b	73	2094	340	14160	1,40		1,10	1,80	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	7	252	340	14160	1,10		0,50	2,40	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Wijesiriwardana[61]	2006	RC	3b	432	7627	1716	31633	1,04		0,94	1,16	None	Calculated OR

Placental Abruption												
Johns[69]	2003	RC-C	3b	2	129	0	143				None	
Johns and Jauniaux[59]	2006	PC	2b	?	214	?	214				Controls matched for age, p=0,22	
Mulik[67]	2004	RC	3b	9	458	50	6445	2,80	2,00	3,70	Logistic regression analysis	
Weiss[60]	2004	PC	1b	25	2094	113	14160	1,60	1,10	2,60	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	7	252	113	14160	3,60	1,60	7,90	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Wijesiriwardana[61]	2006	RC	3b	49	7627	150	31633	1,35	0,98	1,87	None	Calculated OR
Placenta praevia												
Johns and Jauniaux[59]	2006	PC	2b	?	214	?	214				Controls matched for age, p=0,1	
Mulik[67]	2004	RC	3b	4	458	17	6445	3,30	1,00	11,00	None	
Weiss[60]	2004	PC	1b	13	2094	85	14160	0,90	0,50	1,80	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	4	252	85	14160	2,50	0,90	6,90	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Wijesiriwardana[61]	2006	RC	2b	24	7627	54	31633	1,77	1,09	2,87	Marital status, smoking and social class	
Premature preterm rupture of membranes												
De Sutter[68]	2006	RC	3b	19	253	38	1179	2,44	1,83	4,31	None	ART population
Hertz and Heisterberg[70]	1985	PC	2b	8	156	4	282	3,61	1,07	12,20	None	Calculated OR

Table VIII. Risk of adverse obstetric outcome in the index pregnancy after threatened miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	RR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Johns[69]	2003	RC-C	3b	3	129	1	143		3,55	0,37	33,60	None	
Johns and Jauniaux[59]	2006	PC	2b	13	214	4	214		3,70	1,20	11,20	Controls matched for age	
Weiss[60]	2004	PC	1b	48	2094	227	14160	1,30		0,90	1,90	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	13	252	227	14160	3,20		1,80	5,70	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Wijesiriwardana[61]	2006	RC	3b	57	7627	217	31633	1,09		0,81	1,46	None	Calculated OR
Yang[71]	2004	PC	2b	?	472	?	2119		1,90	1,10	3,30	Age, drugs, exercise, genital tract infection and prior adverse pregnancy outcomes	
Preterm delivery <37 weeks													
Das[72]	1996	PC	2b	3	55	2	55	1,50		0,24	9,33	None	Calculated OR
De Sutter[68]	2006	RC	3b	29	253	87	1179	1,64		1,05	2,55	None	ART population
Funderburk[73]	1980	RC	3b	33	259	1356	25118	2,36		1,64	3,41	None	Calculated OR
Hertz and Heisterberg[70]	1985	PC	2b	16	156	13	282	2,22		1,04	4,75	None	Calculated OR
Johns[69]	2003	RC-C	3b	11	129	4	143		3,05	0,99	9,34	None	
Johns and Jauniaux[59]	2006	PC	2b	22	214	11	214		2,30	1,40	4,60	Controls matched for age	
Mulik[67]	2004	RC	2b	66	458	387	6445	2,00		1,30	3,30	Logistic regression analysis	
Nguyen[29]	2004	PC	2b	31	141	165	1555	2,40		1,50	3,60	Logistic regression analysis, no confounders found	Light bleeding
Nguyen[29]	2004	PC	2b	5	13	165	1555	5,30		1,70	16,30	Logistic regression analysis, no confounders found	Heavy bleeding

Sipilä[65]	1992	PC	2b	40	601	279	7911	1,80		1,30	2,50	Age, contraception method, infertility treatment, parity, previous low birth weight, preterm birth and miscarriage and previous stillbirths and perinatal mortality	
Strobino and Pantel-Silverman[66]	1989	PC	1b	41	611	154	2602	1,30		0,90	1,90	Age, ethnicity, gynaecological conditions, place of birth, parity, prepregnancy weight, previous miscarriage and termination of pregnancy, sex of fetus, smoking and working during pregnancy	Light bleeding, <36 weeks
Strobino and Pantel-Silverman[66]	1989	PC	1b	9	133	171	2676	0,90		0,40	2,20	Age, ethnicity, gynaecological conditions, place of birth, parity, prepregnancy weight, previous miscarriage and termination of pregnancy, sex of fetus, smoking and working during pregnancy	Heavy bleeding, <36 weeks
Weiss[60]	2004	PC	1b	191	2094	850	14160	1,30		1,10	1,70	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	35	252	850	14160	3,00		1,90	4,50	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Wijesiriwardana[61]	2006	RC	2b	737	7627	1906	31633	1,56		1,43	1,71	Antepartum haemorrhage, marital status, smoking and social class	
Williams[64]	1991	CS	2b	124	1174	536	10055		2,00	1,60	2,50	Age, cervix incompetence, ethnicity, education level, economic status, intrauterine diethylstilbestrol exposure, parity, previous miscarriage and termination of pregnancy, sex of fetus, smoking, stillbirth,	
Yang[71]	2004	PC	2b	?	472	?	2119		1,20	1,00	1,60	Age, drugs, exercise, genital tract infection and prior adverse pregnancy outcomes	
Very preterm delivery <34 weeks													
De Sutter[68]	2006	RC	3b	6	253	9	1179	3,05		1,12	8,31	None	<32 wks, ART population
Hertz and Heisterberg[70]	1985	PC	2b	6	156	1	282	10,85		1,29	90,91	None	<34 wks, Calculated OR
Johns and Jauniaux[59]	2006	PC	2b	13	214	4	214		3,70	1,20	11,20	Controls matched for age	<35 wks

Table VIII. Risk of adverse obstetric outcome in the index pregnancy after threatened miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	RR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Mulik[67]	2004	RC	3b	22	458	54	6445	5,73		3,46	9,50	None	Calculated OR
Wijesiriwardana[61]	2006	RC	2b	274	7627	561	31633	1,89		1,62	2,19	Antepartum haemorrhage, marital status, smoking and social class	<34 wks
Williams[64]	1991	CS	2b	47	1174	171	10055		2,70	1,80	3,90	Age, cervix incompetence, ethnicity, education level, economic status, intrauterine diethylstilbestrol exposure, parity, previous miscarriage and termination of pregnancy, sex of fetus, smoking, stillbirth,	<33 wks
Yang[71]	2004	PC	2b	?	472	?	2119		1,60	1,10	2,40	Age, drugs, exercise, genital tract infection and prior adverse pregnancy outcomes	<35 wks
Small for gestational age <10th percentile													
Das[72]	1996	PC	3b	1	55	0	55					None	
De Sutter[68]	2006	RC	3b	8	253	65	1179	0,57		0,27	1,21	None	ART population
Johns[69]	2003	RC-C	3b	3	129	2	143		1,66	0,28	9,79	None	
Sipilä[65]	1992	PC	1b	14	601	147	7911	1,30		0,80	2,20	Age, contraception method, infertility treatment, parity, previous low birth weight, preterm birth and miscarriage and previous stillbirths and perinatal mortality	
Weiss[60]	2004	PC	1b	31	2094	142	14160	1,40		0,90	2,10	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Light bleeding
Weiss[60]	2004	PC	1b	7	252	142	14160	2,60		1,20	5,60	Age, ART, education, ethnicity, marital status, parity, previous miscarriages and terminations of pregnancy, previous term and preterm deliveries, previous pregnancy with genetic or neural tube abnormalities	Heavy bleeding
Low birth weight <2500g													
Das[72]	1996	PC	3b	8	55	6	55	1,33		0,43	4,10	None	Calculated OR
De Sutter[68]	2006	RC	3b	22	253	85	1179	1,24		0,76	2,02	None	ART population
Funderburk[73]	1980	RC	3b	44	259	1780	25118	2,40		1,74	3,31	None	Calculated OR

Hertz and Heisterberg[70]	1985	PC	2b	21	156	19	282	2,00		1,04	3,83	None	Calculated OR
Mulik[67]	2004	RC	2b	67	458	457	6445	1,30		0,80	1,90	Logistic regression analysis	
Sipilä[65]	1992	PC	1b	33	601	182	7911	2,10		1,50	3,10	Age, contraception method, infertility treatment, parity, previous low birth weight, preterm birth and miscarriage and previous stillbirths and perinatal mortality	
Strobino and Pantel-Silverman[66]	1989	PC	1b	43	619	171	2676	1,10		0,70	1,60	Age, ethnicity, gynaecological conditions, place of birth, parity, prepregnancy weight, previous miscarriage and termination of pregnancy, sex of fetus, smoking and working during pregnancy	Light bleeding
Strobino and Pantel-Silverman[66]	1989	PC	1b	18	141	171	2676	1,70		0,90	3,30	Age, ethnicity, gynaecological conditions, place of birth, parity, prepregnancy weight, previous miscarriage and termination of pregnancy, sex of fetus, smoking and working during pregnancy	Heavy bleeding
Wijesiriwardana[61]	2006	RC	2b	732	7627	2078	31633	1,22		1,09	1,37	Marital status, preterm delivery, smoking and social class	
Williams[64]	1991	CS	2b	136	1174	593	10055		2,30	1,90	2,70	Age, cervix incompetence, ethnicity, education level, economic status, intrauterine diethylstilbestrol exposure, parity, previous miscarriage and termination of pregnancy, sex of fetus, smoking, stillbirth,	
Very low birth weight <1500g													
De Sutter[68]	2006	RC	4	6	253	8	1179	3,56		1,28	9,90	None	ART population
Funderburk[73]	1980	RC	3b	23	259	334	25118	6,68		4,30	10,37	None	Calculated OR
Hertz and Heisterberg[70]	1985	PC	3b	1	156	1	282	1,81		0,11	29,10	None	Calculated OR
Lekea-Karanika	1994	RC	3b		?	123	3482	1,70		1,20	2,30	Logistic regression analysis	In multigravidae
Mulik[67]	2004	RC	3b	23	458	61	6445	5,30		3,25	8,65	None	Calculated OR
Williams[64]	1991	CS	2b	33	1174	131	10055		2,20	1,30	3,50	Age, cervix incompetence, ethnicity, education level, economic status, intrauterine diethylstilbestrol exposure, parity, previous miscarriage and termination of pregnancy, sex of fetus, smoking, stillbirth,	

Table VIII. Risk of adverse obstetric outcome in the index pregnancy after threatened miscarriage (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	RR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Congenital malformation													
Das[72]	1996	PC	3b	0	55	0	55					None	
Funderburk[73]	1980	RC	3b	7	259	409	25118	1,66		0,78	3,54	None	Calculated OR
Hertz and Heisterberg[70]	1985	PC	2b	12	156	15	282	1,45		0,66	3,17	None	Calculated OR
Sipilä[65]	1992	PC	1b	28	601	252	7911	1,40		1,00	2,10	Age, contraception method, infertility treatment, parity, previous low birth weight, preterm birth and miscarriage and previous stillbirths and perinatal mortality	
Strobino and Pantel-Silverman[66]	1989	PC	2b	19	597	62	2746	1,70		1,00	2,90	None	
5-minute Apgar score <7													
De Sutter[68]	2006	RC	3b	5	253	31	1179	0,80		0,32	2,03	None documented	ART population
Wijesiriwardana[61]	2006	RC	3b	208	7627	775	31633	1,11		0,95	1,30	None	Calculated OR
Fetal death													
Johns and Jauniaux[59]	2006	PC	2b	3	214	0	214					Controls matched for age	
Mulik[67]	2004	RC	3b	2	458	32	6445	0,90		0,40	2,20	None	Calculated OR
Strobino and Pantel-Silverman[66]	1989	PC	2b	3	597	21	2746	1,00		0,30	4,10	None	
Wijesiriwardana[61]	2006	RC	2b	56	7627	212	31633	1,10		0,82	1,47	None	Calculated OR
Williams[64]	1991	CS	2b	6	1174	50	10055		1,10	0,50	2,70	Age, ethnicity and economic status	
Neonatal death													
Das[72]	1996	PC	3b	0	55	0	55					None	
De Sutter[68]	2006	RC	3b	3	253	17	1179	0,87		0,25	3,02	None	ART population

Funderburk[73]	1980	RC	3b	20	259	502	25118	3,86		2,43	6,14	None	Calculated OR
Hertz and Heisterberg[70]	1985	PC	3b	2	156	0	282					None	
Mulik[67]	2004	RC	3b	8	458	18	6445	6,25		2,71	14,46	None	Calculated OR
Sipilä[65]	1992	PC	2b	6	601	53	7911	1,10		0,40	2,70	Age, contraception method, infertility treatment, parity, previous low birth weight, preterm birth and miscarriage and previous stillbirths and perinatal mortality	<7 days
Wijesiriwardana[61]	2006	RC	2b	57	7627	121	31633	1,27		0,91	1,78	Antepartum haemorrhage, low Apgar score, malpresentation, marital status, preterm delivery, smoking and social class	
Williams[64]	1991	CS	2b	7	1174	20	10055		2,50	1,10	5,50	Age, ethnicity and economic status	

1 Design: PC, prospective cohort; CS, cross-sectional; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 OR, odds ratio; RR, relative risk; CI, confidence interval

Table IX. Risk of adverse obstetric outcome in the index pregnancy after intrauterine haematoma

Study	Year	Design[1]	Level[2]	Cases		Controls			OR[4]	RR[4]	95% CI[4]		Adjusted for	Comment
				N	Total	N	Total	TM/N[3]			Low	High		
Pregnancy induced hypertension														
John[69]	2003	RC-C	4	1	51	2	78	TM	0,76	0,75	0,07	8,22	None	
Nagy[78]	2003	PC	2b	29	187	252	6488	N	3,99	2,10	1,50	2,90	None	
Preeclampsia														
Makikallio[82]	2001	PC	3b	0	22	1	16	N						
Nagy[78]	2003	PC	2b	15	187	130	6488	N		4,00	2,40	6,70	None	
Placental abruption														
Ball[77]	1996	RC	3b	8	238	2	648	N	11,20		2,70	46,40	Matched for age, gestational at scan and invasive procedures	
Ball[77]	1996	RC	3b	8	238	7	558	TM	2,70		1,00	7,40	Matched for age, gestational at scan and invasive procedures	
John[69]	2003	RC-C	4	1	51	2	78	TM	0,76		0,07	8,65	None	Calculated OR
Nagy[78]	2003	PC	2b	9	187	56	6488	N		5,60	2,80	11,10	None	
Premature preterm rupture of membranes														
Ball[77]	1996	RC-C	3b	2	238	8	648	N	0,68		0,14	3,23	Matched for age, gestational at scan and invasive procedures	Calculated OR
Ball[77]	1996	RC-C	3b	2	238	7	558	TM	0,67		0,14	3,25	Matched for age, gestational at scan and invasive procedures	
John[69]	2003	RC-C	4	1	51	2	78	TM		0,77	0,07	8,22	None	
Preterm delivery <37 weeks														
Ball[77]	1996	RC	3b	27	238	32	648	N	2,60		1,50	4,60	Matched for age, gestational at scan and invasive procedures	
Ball[77]	1996	RC	3b	27	238	26	558	TM	2,50		1,40	4,50	Matched for age, gestational at scan and invasive procedures	
Borlum[149]	1989	PC	2b	7	67	16	278	TM	1,82		0,72	4,59	None	Calculated OR
John[69]	2003	RC-C	4	5	51	6	78	TM		1,27	0,41	3,96	None	
Makikallio[82]	2001	PC	3b	2	22	1	16	N	1,45		0,12	17,46	None	Calculated OR
Nagy[78]	2003	PC	2b	30	187	459	6488	N		2,30	1,60	3,20	None	

Pedersen and Mantoni[76]	1990	PC	3b	7	62	31	280	TM	1,02		0,43	2,42	None	Calculated OR
Sauerbrei and Pham[150]	1986	RC	4	7	22	3	30	N	3,18		0,74	13,70	None	Risk related to size hematoma
Small for gestational age <10th percentile														
Ball[77]	1996	RC	3b	14	238	19	648	N	2,00		1,00	4,07	Matched for age, gestational at scan and invasive procedures	Calculated OR
Ball[77]	1996	RC	3b	14	238	18	558	TM	1,82		0,89	3,73	Matched for age, gestational at scan and invasive procedures	Calculated OR
John[69]	2003	RC-C	4	2	51	1	78	TM	3,06	1,21	0,27	34,62	None	
Makikallio[82]	2001	PC	3b	1	22	1	16	N	0,73		0,04	12,52	None	Calculated OR
Nagy[78]	2003	PC	2b	13	187	195	6488	N		2,40	1,40	4,10	None	
Congenital malformation														
Nagy[78]	2003	PC	2b	3	187	65	6488	N	1,60		0,50	5,00	None	
5-minute Apgar score <7														
Nagy[78]	2003	PC	2b	7	187	43	6488	N	5,65		2,51	12,72	None	
Fetal death														
Ball[77]	1996	RC	3b	8	238	5	648	N	4,50		1,50	13,20	Matched for age, gestational at scan and invasive procedures	
Ball[77]	1996	RC	3b	8	238	5	558	TM	3,90		1,30	11,40	Matched for age, gestational at scan and invasive procedures	
Nagy[78]	2003	PC	2b	2	187	48	6488	N		1,40	0,30	5,90	None	
Sauerbrei and Pham[150]	1986	RC	4	4	22	0	30	N					None	Risk related to size hematoma
Neonatal death														
Ball[77]	1996	RC	3b	2	238	1	648	N	5,44		0,49	60,33	Matched for age, gestational at scan and invasive procedures	Calculated OR
Ball[77]	1996	RC	3b	2	238	2	558	TM	2,34		0,33	16,74	Matched for age, gestational at scan and invasive procedures	Calculated OR
Nagy[78]	2003	PC	2b	4	187	78	6488	N		1,80	0,70	4,80	None	

1 Design: PC, prospective cohort; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 TM, threatened miscarriage population; N, uncomplicated pregnancies population
4 OR, odds ratio; RR, relative risk; CI, confidence interval

Table X. Risk of adverse obstetric outcome in the index pregnancy after a Crown-Rump Length discrepancy

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	RR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Pregnancy induced hypertension													
Smith[96]	1998	RC	2b	141	1289	235	2108	1,0		0,8	1,2	None	
Preterm delivery <37 weeks													
Smith[96]	1998	RC	2b	50	1289	78	2108		1,0	0,7	1,5	None	Between 33-36 weeks
Very preterm delivery <32 weeks													
Smith[96]	1998	RC	2b	22	1289	17	2108	2,0		1,1	4,0	Age, parity, pregnancy induced hypertension, previous termination of pregnancy and miscarriage, sex of the fetus and vaginal bleeding	
Small for gestational age <10th percentile													
Bukowski[95]	2007	PC	1b	8	55	16	168	1,1		1,0	1,2	Age, BMI, education level, ethnicity, height, placental abruption, placental previa, preeclampsia, previous term and preterm deliveries, miscarriages and termination of pregnancy, pprom, sex of fetus and weight	In ART population
Intrauterine growth restriction <5th percentile													
Smith[96]	1998	RC	2b	65	1289	36	2108	2,8		1,9	4,3	Age, parity, pregnancy induced hypertension, previous termination of pregnancy and miscarriage, sex of the fetus and vaginal bleeding	
Low birth weight <2500g													
Smith[96]	1998	RC	2b	83	1289	53	2108	1,7		1,2	2,3	Age, parity, pregnancy induced hypertension, previous termination of pregnancy and miscarriage, sex of the fetus and vaginal bleeding	
Neonatal death													
Smith[96]	1998	RC	2b	3	1289	6	2108		0,8	0,2	3,3	None	

1 Design: PC, prospective cohort and; RC, retrospective cohort
2 Level of evidence
3 OR, odds ratio; RR, relative risk; CI, confidence interval

Table XI. Risk of adverse obstetric outcome in the index pregnancy after a vanishing twin phenomenon in assisted reproductive techniques (ART) population

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total		Low	High		
Ante Partum Haemorrhage												
Pinborg[115]	2007	RC	3b	13	642	123	5237	0,86	0,48	1,54	None	Calculated OR
Pregnancy induced hypertension												
Pinborg[115]	2007	RC	3b	13	642	92	5237	1,15	0,64	2,07	None	Calculated OR
Preeclampsia												
Chasen[116]	2006	RC	3b	5	55	4	168	3,81	0,99	14,70	None	Calculated OR
Pinborg[115]	2007	RC	3b	33	642	210	5237	1,28	0,88	1,87	None	Calculated OR
Placental abruption												
Pinborg[115]	2007	RC	3b	11	642	48	5237	1,87	0,97	3,62	None	Calculated OR
Placenta praevia												
Pinborg[115]	2007	RC	3b	8	642	57	5237	1,14	0,54	2,41	None	Calculated OR
Preterm delivery <37 weeks												
Chasen[116]	2006	RC	4	7	55	15	168	1,43	0,55	3,68	None	Calculated OR
Dickey[109]	2002	PC	2b	16	140	389	4683	1,38	0,81	2,33	None	Calculated OR
Dickey[151]	2004	RC	4	5	41	19	261	1,68	0,59	4,73	None	Calculated OR
La Sala[117]	2004	RC	3b	12	62	73	437	1,16	0,60	2,26	None	Calculated OR
Pinborg[111]	2005	RC	2b	70	611	471	5237	1,30	1,00	1,70	Age, ICSI versus IVF, parity and spontaneous reduction	
Shebl[118]	2008	RC-C	4	9	46	8	92	2,25	0,82	6,22	None	Calculated OR
Very preterm delivery <32 weeks												
Chasen[116]	2006	RC	4	4	55	3	168	4,07	0,88	18,76	None	Calculated OR
Dickey[151]	2004	RC	4	1	41	3	261	2,12	0,22	20,89	None	Calculated OR
La Sala[117]	2004	RC	4	3	62	12	437	1,76	0,48	6,42	None	Calculated OR
Pinborg[111]	2005	RC	2b	18	611	68	5237	2,30	1,40	4,00	Age, ICSI versus IVF, parity and spontaneous reduction	
Shebl[118]	2008	RC-C	4	2	46	2	92	2,00	0,27	14,66	None	Calculated OR

Table XI. Risk of adverse obstetric outcome in the index pregnancy after a vanishing twin phenomenon in assisted reproductive techniques (ART) population (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		OR[3]	95% CI[3]		Adjusted for	Comment
				N	Total	N	Total		Low	High		
Small for gestational age <10th percentile												
Chasen[116]	2006	RC	4	8	55	16	168	1,53	0,62	3,76	None	Calculated OR
Dickey[109]	2002	PC	2b	22	140	211	4683	3,49	2,18	5,58	None	Calculated OR
La Sala[117]	2004	RC	4	6	62	68	437	0,62	0,26	1,49	None	Calculated OR
Pinborg[115]	2007	RC	3b	34	642	189	5237	1,56	1,10	2,30	None	Crude OR
Shebl[118]	2008	RC-C	4	15	46	15	92	2,00	0,90	4,44	None	Calculated OR
Low birth weight <2500g												
La Sala[117]	2004	RC	4	8	62	51	437	1,11	0,50	2,44	None	Calculated OR
Pinborg[111]	2005	RC	2b	59	611	330	5237	1,70	1,20	2,20	Age, ICSI versus IVF, parity and spontaneous reduction	
Shebl[118]	2008	RC-C	4	12	46	11	92	2,18	0,90	5,32	None	Calculated OR
Very low birth weight <1500g												
La Sala[117]	2004	RC	4	2	62	12	437	1,17	0,26	5,38	None	Calculated OR
Pinborg[111]	2005	RC	2b	18	611	79	5237	2,10	1,30	3,60	Age, ICSI versus IVF, parity and spontaneous reduction	
Shebl[118]	2008	RC-C	4	2	46	1	92	4,00	0,35	45,28	None	Calculated OR
Congenital malformation												
Pinborg[115]	2007	RC	3b	59	642	487	5237	0,99	0,75	1,31	None	Calculated OR
Neonatal death												
Pinborg[111]	2005	RC	3b	6	611	16	5237	3,30	1,30	8,40	None	Calculated OR
Shebl[118]	2008	RC-C	4	0	46	0	92				None	Calculated OR

1 Design: PC, prospective cohort; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 OR, odds ratio; RR, relative risk; CI, confidence interval

Table XII. Risk of adverse obstetric outcome in the index pregnancy after hyperemesis gravidarum

Study	Year	Design[1]	Level[2]	Cases		Controls		OR	RR	95% CI		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Pregnancy induced hypertension													
Dodds[122]	2006	RPB	2b	129	1270	14449	154821		1,00	0,90	1,30	Age and time period	
Dodds[122]	2006	RPB	2b	14	144	12241	127835		3,00	1,90	4,30	Age and time period	Pregnancy weight gain <7kg in cases
Dodds[122]	2006	RPB	2b	14	144	?	?		1,40	0,90	2,10	Age and time period	Pregnancy weight gain <7kg in cases and controls
Preterm delivery <37 weeks													
Dodds[122]	2006	RPB	2b	82	1270	8233	154821		1,20	1,00	1,50	Age and time period	
Dodds[122]	2006	RPB	2b	20	144	6134	127835		3,00	1,90	4,30	Age and time period	Pregnancy weight gain <7kg in cases
Dodds[122]	2006	RPB	2b	20	144	?	?		1,40	0,90	2,10	Age and time period	Pregnancy weight gain <7kg in cases and controls
Gross[126]	1989	RC-C	4	3	30	3	34	1,13		0,21	6,04	None	Hyperemesis & weight loss >5% vs. hyperemesis & weight loss <5%
Hallak[131]	1996	RC	4	11	98	1320	12335	1,05		0,56	1,96	None	Calculated OR
Tsang[134]	1996	RC	3b	44	193	2829	12857	1,04		0,75	1,44	None	Calculated OR
Small for gestation <10th percentile													
Bailit[125]	2005	RPB	3b	663	2270	101193	486505	1,40		1,29	1,53	None	Singleton pregnancies, Calculated OR
Bailit[125]	2005	RPB	3b	123	163	10104	13728	1,03		0,81	1,30	None	Multiple gestation pregnancies, Calculated OR
Dodds[122]	2006	RPB	2b	137	1270	15217	154821		1,10	0,90	1,30	Age, time period and smoking	
Dodds[122]	2006	RPB	2b	21	144	12541	127835		1,50	1,00	2,20	Age, time period and smoking	Pregnancy weight gain <7kg in cases
Dodds[122]	2006	RPB	2b	21	144	?	?		1,10	0,70	1,50	Age, time period and smoking	Pregnancy weight gain <7kg in cases and controls
Gross[126]	1989	RC-C	4	9	28	2	34	5,46		1,09	27,39	None	Hyperemesis & weight loss >5% vs. hyperemesis & weight loss <5%

Table XII. Risk of adverse obstetric outcome in the index pregnancy after hyperemesis gravidarum (*Continued*)

Study	Year	Design[1]	Level[2]	Cases		Controls		OR	RR	95% CI		Adjusted for	Comment
				N	Total	N	Total			Low	High		
Low birth weight <2500g													
Bailit[125]	2005	RPB	3b	177	2270	24811	486505	1,53		1,31	1,78	None	Singleton pregnancies, Calculated OR
Bailit[125]	2005	RPB	3b	106	163	7056	13728	1,27		0,99	1,62	None	Multiple gestation pregnancies, Calculated OR
Dodds[122]	2006	RPB	2b	72	1270	7143	154821		1,30	1,00	1,70	Age, time period and smoking	
Dodds[122]	2006	RPB	2b	18	144	5326	127835		2,80	1,70	4,30	Age, time period and smoking	Pregnancy weight gain <7kg in cases
Dodds[122]	2006	RPB	2b	18	144	?	?		1,30	0,80	2,00	Age, time period and smoking	Pregnancy weight gain <7kg in cases and controls
Very low birth weight <1500g													
Bailit[125]	2005	RPB	3b	35	2270	5352	486505	1,40		1,00	1,96	None	Singleton pregnancies, Calculated OR
Bailit[125]	2005	RPB	3b	22	163	1496	13728	1,24		0,79	1,94	None	Multiple gestation pregnancies, Calculated OR
5-minute Apgar score <7													
Dodds[122]	2006	RPB	2b	19	1270	1898	154821		1,20	0,80	1,90	Age and time period	
Dodds[122]	2006	RPB	2b	8	144	1471	127835		5,00	2,60	9,60	Age and time period	Pregnancy weight gain <7kg in cases
Dodds[122]	2006	RPB	2b	8	144	?	?		3,10	1,50	6,50	Age and time period	Pregnancy weight gain <7kg in cases and controls
Gross[126]	1989	RC-C	4	1	28	1	33	1,18		0,07	19,71	None	Hyperemesis & weight loss >5% vs. hyperemesis & weight loss <5%
Hallak[131]	1996	RC	4	1	98	259	12335	0,49		0,07	3,50	None	Calculated OR
Congenital Malformation													
Bashiri[124]	2005	RC	4	1	164	2	209	0,64		0,06	7,09	None	Calculated OR, Multiple pregnancy in respectively 8,6% and 4,3%
Hallak[131]	1996	RC	4	2	98	192	12335	1,31		0,32	5,36	None	Calculated OR
Tsang[134]	1996	RC	4	10	193	591	12857	1,13		0,59	2,14	None	Calculated OR

Fetal Death												
Bailit[125]	2005	RPB	3b	16	2270	2092	486505	1,64	1,00	2,69	None	Singleton pregnancies, Calculated OR
Bailit[125]	2005	RPB	3b	4	163	202	13728	1,67	0,61	4,54	None	Multiple gestation pregnancies, Calculated OR
Hallak[131]	1996	RC	4	0	98	70	12335				None	Calculated OR
Tsang[134]	1996	RC	4	4	193	180	12857	1,48	0,54	4,03	None	Calculated OR
Neonatal Death												
Bailit[125]	2005	RPB	3b	10	2270	1460	486505	1,47	0,79	2,74	None	Singleton pregnancies, Calculated OR
Bailit[125]	2005	RPB	3b	6	163	291	13728	1,74	0,76	3,96	None	Multiple gestation pregnancies, Calculated OR
Bashiri[124]	2005	RC	4	3	164	4	209	0,96	0,21	4,33	None	Calculated OR, Multiple pregnancy in respectively 8,6% and 4,3%
Dodds[122]	2006	RPB	2b	6	1270	929	154821	0,70	0,30	1,60	Age and time period	
Tsang[134]	1996	RC	4	6	193	309	12857	1,29	0,57	2,94	None	Calculated OR

1 Design: RPB, retrospective population-based; RC, retrospective cohort and; RC-C, retrospective case-control
2 Level of evidence
3 OR, odds ratio; RR, relative risk; CI, confidence interval

7

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ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
ANG1	Angiopoietin-1
ANG2	Angiopoietin-2
APH	Ante Partum Hemorrhage
ARED	Absent or Reversed End-Diastolic
ART	Assisted Reproductive Technology
ASRM	American Society for Reproductive Medicine
BMI	Body Mass Index
CI	Confidence Interval
CLSM	Confocal Laser Scanning Microscopy
CRL	Crown-Rump Length
D&C	Dilatation and Curettage
EP	Ectopic Pregnancy
ESHRE	European Society for Human Reproduction and Embryology
EUG	Extra-Uteriene Graviditeit
FGR	Fetal Growth Restriction
HG	Hyperemesis Gravidarum
ICSI	Intracytoplastmatic Sperm Injection
IFL	Ideopathic Fetal Loss
IUD	Intrauterine Device
IUI	Intrauterine Infection
IUGR	Intra Uterine Growth Restriction
IUH	Intrauterine Hematoma
IVF	In Vitro Fertilisation
IVIg	Intravenous Immunoglobulin
GA	Gestational Age
hCG	Human Chorionic Gonadotropin
HELLP	Haemolysis Elevated Liver Enzymes and Low Platelets syndrome
LBW	Low Birth Weight <2500 gram
LMP	Last Menstrual Period
OPT	Optical Projection Tomography
OR	Odds Ratio
PAPP-A	Pregnancy-Associated Plasma Protein - A
PE	Pre-eclampsia
PED	Positive End-Diastolic
PI	Pulsatility Index
PIH	Pregnancy Induced Hypertension
PlGF	Placental Growth Factor
PRM	Primary Recurrent Miscarriage
PPROM	Preterm Premature Rupture of Membranes
PTD	Preterm Delivery <37 weeks gestation
PUL	Pregnancy of Unknown Location
RCOG	Royal College of Obstetricians and Gynaecologists
RM	Recurrent Miscarriage
RR	Relative Risk
SGA	Small for Gestational Age
SRM	Secondary Recurrent Miscarriage
TLC	Tender Loving Care
TOP	Termination of Pregnancy
TVS	Transvaginal Ultrasound
VEGF	Vascular Endothelial Growth Factor
VLBW	Very Low Birth Weight <1500 gram
VPTD	Very Preterm Delivery <34 weeks gestation
VR	Virtual Reality
ZOL	Zwangerschap met Onbekende Lokalisatie

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Van Oppenraaij RHF, Goddijn M, Lok CAR, Exalto N. De jonge zwangerschap; revisie van de Nederlandse benamingen voor klinische en echoscopische bevindingen. (Early pregnancy; Revision of Dutch terminology for clinical and ultrasound findings) NTvG 2008; 152:20-4.

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Chapter 2.3.1

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Jauniaux E, van Oppenraaij RHF, Burton GJ. Obstetric outcome after early placental complications. Curr Opin Obstet Gynecol. 2010;22:452-7.

Chapter 3.1

Van Oppenraaij RHF, Koning AHJ, Lisman BA, van den Hoff MJB, Boer K, van der Spek PJ, Steegers EAP, Exalto N. Vasculogenesis and angiogenesis in the human placenta; an innovative 3 dimensional study using an immersive Virtual Reality system. Placenta 2009; 30:220-2.

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Van Oppenraaij RHF, Koning AH, van den Hoff MJ, van der Spek PJ, Steegers EAP, Exalto N. The effect of smoking on early chorionic villous vascularisation. Placenta 2012;33:645-51.

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Van Oppenraaij RHF, Nik H, Heathcote L, McPartland J, Turner MA, Quenby S, Steegers EAP, Exalto, N. Compromised chorionic villous vascularization in idiopathic second trimester fetal loss. Early Hum Dev 2010;86:469-72.

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PHD PORTFOLIO

PHD PORTFOLIO

Name PhD student: R.H.F. van Oppenraaij
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Research School: Erasmus MC
PhD period: 2007-2013
Promotor: Prof. Dr. E.A.P. Steegers
Co-Promotor: Dr. N. Exalto

1. PhD training

	Year	Workload (Hours/ECTS)
Research skills		
Biostatistics for clinicians	01-2009	1.0
Regression analysis for clinicians	02-2009	1.9
Oral Presentations		
Uterine rupture, vaginal birth after caesarean. Breakfast Meeting. Liverpool Women's Hospital Foundation NHS Trust, Liverpool, Engeland.	03-2007	1.0
uNK cells in unexplained second trimester miscarriage. The North of England Obstetrical and gynaecological society. Liverpool Meeting. Liverpool Women's Hospital Foundation NHS Trust, Liverpool, Engeland.	03-2007	1.0
De jonge zwangerschap; revisie van de Nederlandse benamingen voor klinische en echoscopische bevindingen. Vakgroep gynaecologie & obstetrie AMC, Amsterdam	09-2007	1.0
'*Eerste trimester complicaties als voorspeller voor late zwangerschapscomplicaties*' NVOG Symposium 'De jonge zwangerschap' Erasmus MC	06-2008	1.0
Vasculogenesis en angiogenesis in humane placenta: een innovatieve 3 dimensionale studie gebruikmakende van virtual reality. Researchbespreking verloskunde & prenatale geneeskunde Erasmus MC	09-2008	1.0
Predicting adverse obstetric outcome after early pregnancy complications. NEDWEP symposium, Utrecht	11-2008	1.0
Predicting adverse obstetric outcome after early pregnancy complications. Early pregnancy winter course. SIGEP of ESHRE, Milaan, Italy	12-2008	1.0
Vasculogenesis in human placenta; an innovative 3 dimensional study using an immersive Virtual Reality system. SGI 56th annual meeting, Glasgow, Scotland. Winner of SGI President's presenter award.	03-2009	1.0

1. PhD training (*Continued*)

	Year	Workload (Hours/ECTS)
Predicting adverse obstetric outcome after early pregnancy complications. ESHRE 25th annual meeting, Amsterdam	06-2009	1.0
Predicting adverse obstetric outcome after early pregnancy complications. Early pregnancy winter course. Invited speaker, SIGEP of ESHRE, Rotterdam	12-2009	1.0
Poster Presentations		
Uterine Natural Killer cells in second trimester miscarriage. R.H.F. van Oppenraaij, H. Nik, L. Heathcote, J. McPartland, R. Shukla, G. Kokai, M.A Turner, S. Quenby. IFPA Meeting, Kingston Canada, Placenta 2007;28(8-9):A27.	08-2007	1.0
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Acquired and inherited thrombophilia disorders in formerly preeclamptic women. R.H.F. van Oppenraaij, D. Berks, M. Hoedjes, W. Visser, E.A.P. Steegers, J.J. Duvekot. ISSHP 16th world congress, Washington DC, USA	09-2008	1.0
Vasculogenesis and angiogenesis in human placenta; an innovative 3 dimensional study using an immersive Virtual Reality system – the I-Space. Oppenraaij RHF van, Koning AHJ, Lisman BA, van den Hoff MJB, Boer K, van der Spek PJ, Steegers EAP, Exalto N. NVOG gynaecongres, Utrecht	11-2009	1.0
International conferences		
ESHRE: 'New trends in diagnosis and management of early pregnancy failure'. Poznan, Polen	12-2006	1.0
International symposium 'The management of postpartum heamorrhage'. Liverpool, Engeland	01-2007	1.0
IFPA 2007 Meeting. Placenta. Kingston, Canada	09-2007	1.0
Placental bed meeting, Leuven, Belgie	11-2007	1.0
ESHRE: 'Early pregnancy winter course, Brussel, Belgie	12-2007	1.0
ISSHP: 16th world congress, Washington DC, USA	09-2008	1.0
ESHRE: Early pregnancy winter course, Milaan, Italie	12-2008	1.0
SGI: 56th annual meeting, Glasgow, Scotland	03-2009	1.0
ESHRE: 25th annual meeting, Amsterdam	06-2009	1.0

1. PhD training (*Continued*)

	Year	Workload (Hours/ECTS)
ESHRE: Early pregnancy winter course, Rotterdam	12-2009	1.0
ESHRE: annual Meeting, Istanbul, Turkij	07-2012	1.0
ESHRE: Early Pregnancy Wintercourse, Amsterdam	12-2012	1.0
ESHRE: annual meeting, London, Engeland	07-2013	1.0
ESHRE: Early Pregnancy Wintercourse, Brussel, België	11-2013	1.0
ESHRE: annual meeting,Munchen, Duitsland	07-2014	1.0
Seminars and workshops		
NVOG: De jonge zwangerschap. Spaarne symposium. Hoofddorp	04-2007	0.2
NEDWEP symposium, AMC, Amsterdam	11-2007	0.2
NVOG: De jonge zwangerschap symposium. Erasmus MC, Rotterdam	06-2008	0.4
NEDWEP & DSMP symposium, Utrecht	11-2008	0.2
Didactic skills		
Other		
Junior Deputy Special interest Group Early Pregnancy of ESHRE	2012-2014	5.0

2. Teaching activities

	Year	Workload (Hours/ECTS)
Lecturing		
Prenatal ethics (3x), training medicine students	10-2008	1
Gynaecological investigation, training emergency ward nurses	2012	0.5
Gynaecological presentations, training medicine students	2012	0.5
Supervising medical students: Nienke Bergen	2010	0.5
		1.0
Awards		
Winner of SGI President's presenter award	09-2009	

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ABOUT THE AUTHOR

Robbert Henricus Franciscus van Oppenraaij was born on June 19th 1978 in Amsterdam, The Netherlands. He grew up in Woerden and went to secondary school at the Minkema College in Woerden. He attended medical school at the University of Amsterdam (UvA) from 1996-2003. During the internship he developed his interest for Obstetrics and Gynaecology and after his graduation he started working as an Obstetrics and Gynaecology resident at the Slotervaart Hospital in Amsterdam. From 2005-2007 he worked as a resident at the Department of Obstetrics and Gynaecology of the Spaarne Hospital in Hoofddorp. Here he met his co-promotores Dr. Niek Exalto, with whom he started his first research project. The collaboration resulted in a three month research project at the Department of Obstetrics and Gynaecology of the Liverpool Women's Hospital in Liverpool, United Kingdoms and proved later to be the beginning of the thesis. Hereafter he worked for a short period as a Obstetrics and Gynaecology resident at the Erasmus MC in Rotterdam. From August 2007 he started the research described in this thesis at the Department of Obstetrics and Gynaecology, Division Obstetrics and Prenatal Medicine of the Erasmus MC in Rotterdam under the supervision of Prof. Dr. E.A.P. Steegers and Dr. Niek Exalto. In March 2010 he started his training in Obstetrics and Gynaecology, from March 2010 till December 2011 and from September 2013 till August 2014 at the Reinier de Graaf Groep in Delft (Dr. W.A. Ter Harmsel / Dr. H.A. Bremer), and from January 2012 till August 2013 and from September 2014 onwards at the Erasmus Medical Center in Rotterdam (Prof. Dr. C.W. Burger / Dr. M. Ten Kate-Booij). He was junior Deputy of the Special Interest Group of Early Pregnancy (SIGEP) of the European Society of Human Reproduction and Embryology (ESHRE) from 2012 till 2014. After the defence of his thesis he hopes to finish his residency in the middle of 2015. In his spare time he likes to play golf, play strategic games, and travel to distant places to enjoy the nature. He has a relationship with Inge van Hooft and they are expecting a baby in March 2015.

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WORD OF THANKS

DANK

DANK

DANK

Aan Eric, Niek, de lees- en grote commissie, en de paranimfen. Aan alle co-auteurs (co-authors) en allen die mij ondersteund hebben in mijn onderzoek.

DANK

DANK

DANK

Aan al mijn collega's, mijn ouders Bernard en Mieke, mijn zus Martine, mijn familie, schoonfamilie en vrienden.
En dank aan mijn lieve Inge.