A prospective study of implantation, maternal cardiovascular function and pregnancy outcome

Amita Mahendru MBBS MRCOG

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ABSTRACT

Events at embryonic implantation play a key role in the establishment of successful pregnancy. Not only is delayed implantation associated with an increased incidence of early pregnancy loss, but may also be associated with impaired trophoblastic invasion and uteroplacental insufficiency. Furthermore, uteroplacental vascular mal-adaptation may also be affected by pre-existing maternal cardiovascular function and associated with maternal cardiovascular maladaptation during pregnancy. There is limited understanding of events surrounding human implantation because of the difficulties in conducting prospective studies from prior to pregnancy and an inability to study events at the trophoblast-decidual interface *in vivo*.

The primary objective of this study was to test the feasibility of being able to conduct and complete a prospective study from prior to pregnancy to the postpartum period combining measures of ovulation, implantation, ultrasound measurements of fetal size and cardiovascular changes during pregnancy. The secondary objective was to investigate ovulation and implantation timing using digital home ovulation and pregnancy test kits along with cardiovascular changes in relation to various pregnancy complications and fetal growth to determine the power for a future prospective study.

This was a prospective cohort feasibility study of 143 women planning to conceive. Pre-pregnancy cardiovascular function was investigated in all women. We observed ovulation, implantation timing in 101 pregnancies and investigated the relationship between implantation timing, embryonic and fetal growth, birthweight and length of gestation in the 69 viable pregnancies. Longitudinal cardiovascular changes in viable pregnancies were examined in relation both to previous obstetric history and index pregnancy outcome.

Normal pregnancy was associated with profound cardiovascular changes, beginning from 6 weeks of gestation. Delayed implantation was associated with early pregnancy loss and a smaller first trimester fetal size. The incremental rise in cardiac output from before pregnancy to its peak in the second trimester was associated with birthweight.

It is feasible to conduct and complete a prospective study from prior to pregnancy to the postpartum period. Larger prospective studies of this nature will enable an understanding of the events surrounding implantation including the ‘cause and effect’ relationship of cardiovascular function with pregnancy complications such as preeclampsia and fetal growth restriction.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY AND ABBREVIATIONS

AC	Abdominal circumference
ADP	Adenosine diphosphate
AFI	Amniotic fluid index
AIx	Augmentation index
A-II	Angiotension II
ANOVA	Analysis of variance
AP	Augmentation pressure
APEC	Action on Preeclampsia
aPWV	Aortic pulse wave velocity
AUC	Area under the curve
BLISS	Baby Life Support Systems
BMI	Body mass index
BNF	British National Formulary
BP	Blood pressure
BPD	Biparietal diameter
CI	Confidence intervals
CI	Cardiac Index
CKD	Chronic kidney disease
CO	Cardiac output
CRL	Crown-rump length
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiography
EDTA	Ethylenediaminetetraacetic acid
EFW	Expected fetal weight
eGFR	Estimated glomerular filtration rate
FFA	Free fatty acids
FL	Femur length
FSH	Follicle stimulating hormone
GA	Gestational age
GA^{LMP}	Gestational age derived from last menstrual period
GA^{OV}	Gestational age adjusted by ovulation timing was derived by subtracting 14 days from the predicted ovulation date to the effective last menstrual period, as is convention for pregnancy

	dating
GA^{IMP}	Gestational age adjusted by implantation was derived by adding or subtracting the difference between the observed implantation day and the median implantation day (which was day 27 in this study) to the gestational age derived from last menstrual period
GH	Gestational hypertension
GP	General practitioner
HbA1c	Haemoglobin A1c
HC	Head circumference
hCG	Human chorionic gonadotrophin
HDL	High density lipoprotein
HR	Heart rate
IQR	Inter-quartile range
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
K^+	Potassium
LDL	Low density lipoprotein
LH	Luteinizing hormone
LIF	Leukemia inhibiting factor
LMP	Last menstrual period
LPL	Lipoprotein lipase
MAP	Mean arterial pressure
Na^+	Sodium
NCT	National Childbirth Trust
NKDEP	National Kidney Disease Education Program
N_2O	Nitrous Oxide
O-I	Ovulation to implantation
O_2	Oxygen
PAPP-A	Pregnancy-associated plasma protein-A
PE	Preeclampsia
PI	Pulsatility index
PP	Pulse pressure
PRF	Pulse repetition frequency
PVR	Peripheral vascular resistance
REC	Research Ethics Committee
RI	Resistance Index

RM	Recurrent Miscarriage
SBP	Systolic blood pressure
SD	Standard Deviation
SE	Standard Error of means
SF_6	Sulphur hexafluoride
SV	Stroke Volume
TC:HDL	Total cholesterol: High density lipoprotein
TG	Triglyceride
TOP	Termination of pregnancy
TVS	Transvaginal scan
UACR	Urine albumin creatinine ratio
UPCR	Urine protein creatinine ratio
VLDL	Very low density lipoprotein
WHO	World Health Organization

ACKNOWLEDGEMENTS

I would like to thank my supervisor Mr. Christoph Lees, of Cambridge University Hospitals NHS Foundation Trust, who gave me the opportunity to carry out this research project, to fund my research post, provide invaluable guidance, support and enthusiasm all throughout the research and in the writing up of this thesis. I am very grateful to Mr. Lees for protecting my dedicated research time and also for helping me secure the NIHR flexibility and sustainability fund in the second year of this research post to enable continuation of this research project.

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I must thank Dr. Carmel McEniery of the Clinical pharmacology Department of the University of Cambridge, who has been an invaluable mentor throughout the research project, a valuable source of advice and constructive criticism. I am very grateful for her patience in teaching me the principals behind the cardiovascular tests used in the study, constant motivation in striving for the highest quality data and writing good quality abstracts and papers.

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has become a good friend and in particular helped me to complete the last few research visits.

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During my research I worked closely with Dr. Thomas Everett, who became a very good friend and research colleague and provided valuable moral support during this project.

I am most grateful to all the women who participated in this study, dedicated their valuable time to come for their research appointments and provided us with valuable data.

CONTRIBUTION OF THE CANDIDATE

This study was conducted at Addenbrooke's Hospital, Cambridge in the Early Pregnancy Unit, Maternal Fetal Assessment Unit, Rosie Ultrasound, Fetal Medicine and Vascular Research Clinics in the Clinical Pharmacology department of the University of Cambridge.

The candidate prepared the research protocol, submitted the corresponding research ethics application to the ethical committee and designed all the patient information and data collection proformas for the study. The candidate liaised with the Swiss Precision Diagnostics, GmbH (SPD), Bedford for provision of the ovulation and pregnancy test kits for the research. The candidate liaised with the hospital communications team for advertisements in the hospital- by posters, website and newspaper advert; and with the GP liaison team for advertisement in the GP surgeries.

The candidate communicated with all prospective participants who approached her and gave them the necessary information and personally recruited those who volunteered to participate in the study. The candidate identified women with previous preeclampsia or intrauterine growth restriction from the hospital delivery database, fetal medicine diary, hypertension clinic lists, and women with unexplained recurrent miscarriage from the recurrent miscarriage lists and sent them invitation letters.

The candidate prepared packages of ovulation and pregnancy test kits with the necessary information and gave them to all the participants at their first visit. The candidate was also responsible to provide them with refills and instructions throughout the study as and when required, and made herself available to them any time for advice by phone or email.

The candidate obtained scan competence in early pregnancy transvaginal ultrasound scanning, uterine artery Doppler and growth scans to perform these for the study participants independently and also obtained competence in performing the cardiovascular assessments for the study. The candidate was responsible for arranging the subsequent visits, performing scans and cardiovascular tests, providing relevant advice and arranging referral in case problem was detected on a scan in all women who became pregnant during

the study. The candidate performed the scans in the Rosie ultrasound and early pregnancy unit and the cardiovascular assessments in the University of Cambridge and closely worked with the teams at all these sites to minimize any inconvenience to the participants.

The candidate kept the GP informed of all the participants in the study and especially if any abnormal scan or blood results were detected during the study. The candidate set up an access database after appropriate training for data recording on the Addenbrooke's Hospital server. The candidate was responsible for day to day management of the research appointments, data entry and collection of menstrual diaries and delivery outcomes.

The candidate performed all the statistical analysis of the study, prepared manuscripts for publications and abstracts for presentation under the supervision of and with the assistance of her supervisors and collaborators.

PUBLICATIONS

Publications in peer-reviewed journals arising directly from the research

- Mahendru AA, Everett TR, McEniery CM, Wilkinson IB, Lees CC. The feasibility of prospectively studying maternal cardiovascular changes from before conception. *Hypertension Research*. 11 April 2013;doi:101038/hr.2013.24
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Publications arising from investigations of maternal cardiovascular physiology

- Everett TR, Mahendru AA, McEniery CM, Wilkinson IB, Lees CC. Raised uterine artery impedance is associated with increased maternal arterial stiffness in the late second trimester. *Placenta*. 2012 Jul;33(7):572-7
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Published Abstracts

- A. Mahendru, A. Daemen, T. R. Everett, C. M. McEniery, I. B. Wilkinson, Y. Abdallah, D. Timmerman, T. Bourne and C. Lees. Abstract: OP32.02: Relationship between ovulation-implantation interval, first trimester crown-rump length, birth weight and gestation at delivery. *Ultrasound Obstet Gynecol*. 2012 | DOI: 10.1002/uog.11700
- Mahendru AA, Everett TR, McEniery CM, Wilkinson IB, Lees CC. Maternal cardiovascular function: longitudinal changes prior to and in pregnancy. *Arch Dis Child Fetal Neonatal Ed.* 2012 97: A30-A31
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- Mahendru AA, Lees CC, Everett TR, McEniery CM, Wilkinson IB. Abstracts: Oral Session 3.1 (Young investigators' presentations)) & Oral poster P5.01 (Clinical Science 1) Pre-pregnancy to early pregnancy changes in maternal cardiovascular physiology. *Artery Research*. 2011 Dec;5;4:119-206
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- Mahendru A, Everett TR, Hackett GA, McEniery CM, Wilkinson IB, Lees CC. Abstracts OC10.01: Early pregnancy changes in maternal haemodynamics using pulse wave analysis; OC10.05: Early pregnancy changes in maternal arterial stiffness; OP02.05: Ovulation-implantation interval in relation to early pregnancy outcome; OP36.05:Difference in pre-pregnancy arterial stiffness in women with previous severe pre-eclampsia/IUGR and women with prior normal pregnancies; P27.17 Ovulation implantation interval in relation to previous obstetric history. *Ultrasound Obstet Gynecol.* 2011;38;Supplement 1:S18-260

PRESENTATIONS

Oral presentations

27.04.2012 Ovulation implantation interval, impact on pregnancy dating and birth outcomes
EAOGS, Norwich (Second Prize)

16.03.2012 Maternal cardiovascular function: longitudinal changes prior to and in pregnancy
Herbert Reiss Prize Meeting, RSM, London

06.12.2011 Pre-pregnancy to early pregnancy changes in maternal cardiovascular physiology
Annual Blair Bell Research Society Meeting, RCOG, London

14.10.2011 Pre-pregnancy cardiovascular risk in women with previous preeclampsia (PET)/intrauterine growth restriction (IUGR) –
Young investigators' prize presentation category, Artery, Paris

06.10.2011 Pre-pregnancy to early pregnancy changes in maternal cardiovascular physiology
Young investigators' Award category, Euro ISSHP, Rome

20.09.2011 Early pregnancy changes in maternal haemodynamics using pulse wave analysis
21st World Congress ISUOG, Los Angeles, USA

20.09.2011 Early pregnancy changes in maternal arterial stiffness
21st World Congress ISUOG, Los Angeles, USA

01.07.2011 Ovulation, Implantation and Dating: is the CRL accurate?
Come to Cambridge Study day, Addenbrooke's Hospital

25.03.2011 Implantation Ovulation Study
Maternal haemodynamics workshop Churchill College, Cambridge

Oral Posters

13.09.2012 Impact of Ovulation-implantation interval on first trimester crown-rump length, fetal growth and birth weight

1st International Conference-Fetal growth 2012, Birmingham

(Prize- Best poster)

12.09.2012 Impact of Ovulation-implantation interval on first trimester crown-rump length, fetal growth and birth weight

22nd World Congress, ISUOG, Copenhagan

19.04.2012 Maternal cardiovascular function: longitudinal changes prior to and in pregnancy

BMFMS 2012, Glasgow

13.10.2011 Pre-pregnancy to early pregnancy changes in maternal cardiovascular physiology

Artery 2011, Paris

22.09.2011 Difference in pre-pregnancy arterial stiffness in women with previous severe pre-eclampsia/IUGR and women with prior normal pregnancies

21st World Congress, ISUOG, Los Angeles

19.09.2011 Ovulation-implantation interval in relation to early pregnancy outcome

21st World Congress, ISUOG, Los Angeles (Prize- Best short oral presentation in Early pregnancy category)

Posters

Dec 2012 A prospective study of changes in maternal cardiovascular function during pregnancy from prior to pregnancy

Blair Bell Research Society competition, RCOG

Oct 2011 "Pre-pregnancy cardiovascular risk in women with previous preeclampsia (PET)/intrauterine growth restriction (IUGR)",

Euro ISSHP, Rome

Sep 2011 "Ovulation implantation interval in relation to previous obstetric history"

21st World Congress, ISUOG, Los Angeles

DEDICATION

I dedicate this work to Ketan, Dipika, Alpa, my parents and my parents in law.

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Pregnancy complications range from those arising in early pregnancy such as miscarriage, to late pregnancy complications such as preeclampsia (PE) and intrauterine growth restriction (IUGR). Increasingly, the role of events around implantation is being highlighted in the etiology of recurrent early pregnancy loss[1,2] and in placental insufficiency in PE[3,4] and/or IUGR.

Normal implantation is the key step for the establishment of a successful pregnancy. The process of implantation is a result of a complex interaction between endometrium and the blastocyst, regulated by numerous endocrine, paracrine and autocrine signals.[5] Lack of endometrial preparedness, impaired communication between the embryo and the endometrium at the time of implantation and impaired trophoblastic invasion are likely to be pivotal in the disease process of recurrent miscarriage (RM).[2] This hypothesis is supported by an increased incidence of early pregnancy loss in cases with delayed implantation[6] and the association of RM with an increased risk of PE or IUGR in subsequent successful pregnancies.[7-9]

Although PE and IUGR are different pregnancy specific complications with varied maternal manifestations, both have underlying abnormalities of placental function.[10-12] Impaired trophoblastic invasion of the spiral arterioles, beginning around the time of implantation, and leading to impaired spiral artery remodelling and abnormal placentation is implicated in the aetiology of PE and IUGR.[3,13] Impaired trophoblastic invasion is seen even in women with spontaneous miscarriage.[14,15] This might explain the higher incidence of PE in women with unexplained sub fertility or recurrent miscarriage.[16,17] Therefore, it is plausible that impaired placentation and pregnancy complications such as PE and IUGR are also accompanied by delayed implantation.

Furthermore, pre-existing maternal cardiovascular function and maternal hemodynamic forces may also affect implantation events, trophoblastic invasion and uteroplacental vascular remodelling. This hypothesis is supported by the association of abnormal maternal cardiovascular parameters[18-20] and uterine artery Doppler (reflecting uteroplacental impedance) early in pregnancy in women who go on to develop PE or

IUGR.[21-23] Therefore, it is plausible that maternal systemic cardiovascular maladaptation may occur as early as at the time of implantation.

Profound maternal cardiovascular changes are seen as early as 5-6 weeks in normal pregnancy.[24-26] Pregnancies complicated by IUGR are associated with deficient plasma volume expansion very early in pregnancy.[19] Those complicated by preeclampsia are associated with higher cardiac output as early as 10-14 weeks, much before the fetal and maternal manifestations of the syndrome.[27,28] The relationship between implantation and cardiovascular changes is poorly understood in both normal and pregnancies complicated by uteroplacental insufficiency. Moreover, there are few prospective studies of maternal cardiovascular adaptation during normal pregnancies and pregnancy complications beginning from prior to conception.

Women with a history of both early and late pregnancy complications are also at increased risk of subsequent cardiovascular disease (CVD).[29-33] It has been suggested that this could be due to the deleterious effect of cardiovascular maladaptation in pregnancy complications but endothelial dysfunction, vascular disease or cardiovascular risk profile may already exist.[34] This may predispose these pregnancies to altered embryo-endometrial interaction, abnormal trophoblastic invasion and placental disease.

Our understanding of the events around natural human conception and implantation is very limited due to difficulties in conducting prospective studies of pregnancy where conception, implantation timing can be identified and the embryo-endometrial interaction can be studied in vivo. A prospective pre-pregnancy to postpartum study of comprehensive cardiovascular function combining markers of ovulation, implantation and ultrasound measures of fetal size and growth, would be an ideal study design to understand the biological process of pregnancy, identify the cause and effect relationship between implantation, cardiovascular function and pregnancy outcomes.

Improved understanding of the events around implantation, pre-pregnancy cardiovascular function and dynamics of the early pregnancy changes in maternal cardiovascular function may help to provide indirect insight into the pathophysiology of placentation. This may improve the ability to treat disorders related to implantation and placentation such as early pregnancy loss, PE or

IUGR, improve screening for PE and may lead to the development of new interventions in the pre-pregnancy period to prevent PE. An improved understanding of changes in the postpartum period may also enable to understand mechanisms of cardiovascular disease and prevention of CVD by controlling postpartum risks and early treatment opportunities.

The problems of conducting prospective studies from prior to pregnancy have been highlighted in the past.[35] Most pregnancies are uncomplicated and therefore, a large cohort of healthy women will need to be recruited to have sufficient power for pregnancy complications. A percentage of women may not conceive during the period of the study and amongst those who conceive, one third will miscarry.[36] Moreover, there may be a significant "drop out" rate, quoted to be about 3 out of 4 in a large pre-pregnancy study.[37]

The primary objective of this study was to test the feasibility of being able to recruit, conduct and complete a prospective pre-pregnancy to postpartum study, using digital home ovulation and pregnancy test kits to determine ovulation, implantation timing, serial ultrasound measures of embryonic and fetal growth and investigating changes in maternal cardiovascular function from prior to pregnancy using non-invasive tests. The secondary objective was to identify the trends in ovulation, implantation timing along with the pattern of cardiovascular changes in normal pregnancies, in women with previous history of RM, PE/IUGR and in those with PE/IUGR in their current pregnancy to provide information in order to enable us to determine power for a future prospective study.

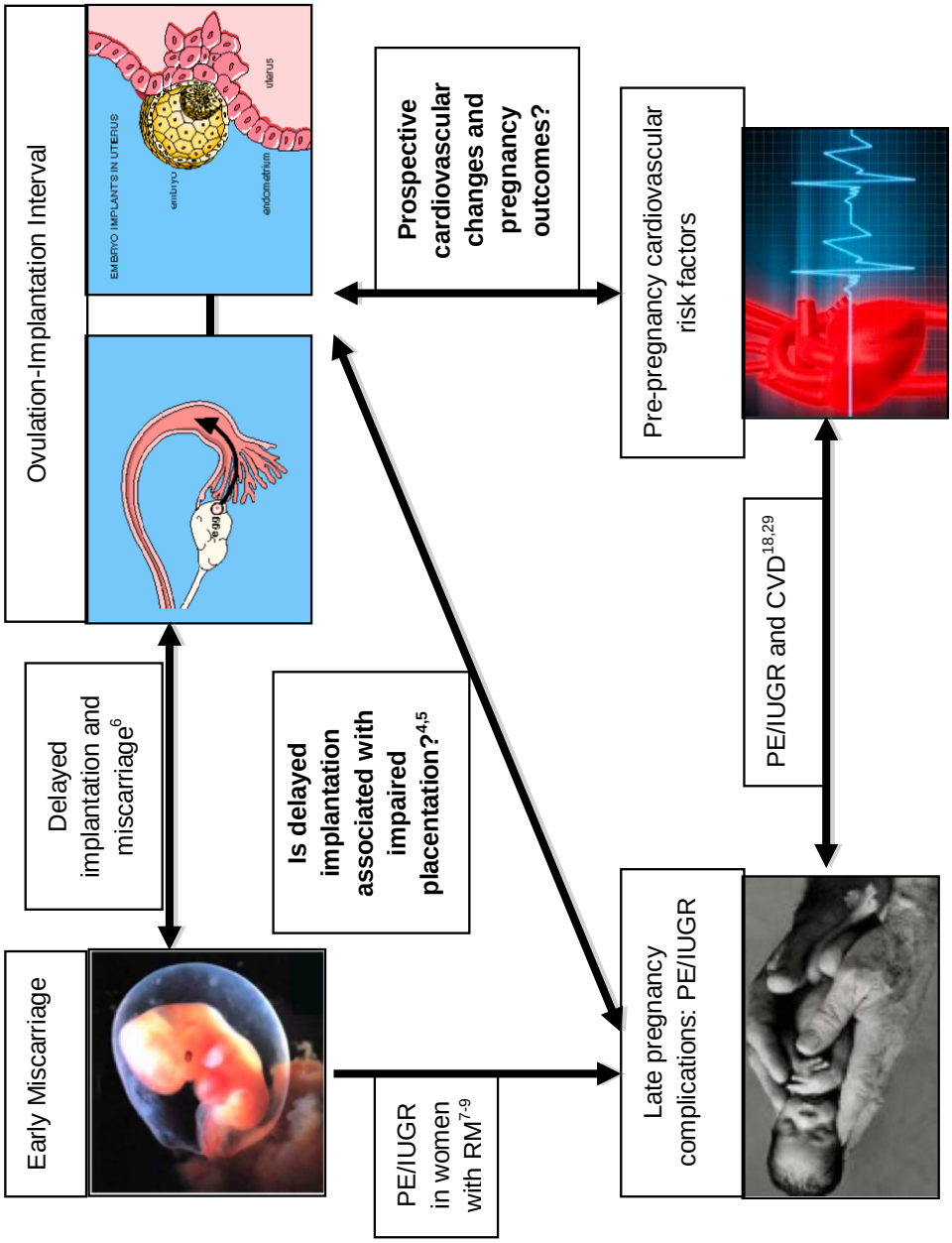


Figure 1.1 Background of the study hypotheses. The unexplored link between miscarriage, preeclampsia (PE), intrauterine growth restriction (IUGR), implantation timing and pre-pregnancy maternal cardiovascular function

1.2 Implantation

The process of implantation is as a result of interaction between the developing embryo and a receptive endometrium. There is a 25-30% probability of clinical pregnancy in a given menstrual cycle.[38] About a third of pregnancies are lost even before being recognized as clinical pregnancies.[36]

The events that lead to the formation of a conceptus involve ovulation followed by fertilization. The conceptus then develops into a morula and reaches the endometrial cavity and is implanted in a primed endometrium. The exact process of human conception, implantation, the timing of implantation and factors affecting the process of implantation in spontaneous conception are not well understood in humans. Much of the understanding of early human development comes from animal models, which may not be exactly representative of the steps in the process of human implantation.[39]

1.2.1 Ovulation

Ovulation is considered as a marker of ovarian function.[40] Determination of the ovulation day is important in longitudinal epidemiological studies of menstrual and reproductive function and to help in understanding the biological processes of pregnancy. The World Health Organization (WHO) Task Force on methods for the determination of the fertile period determined the temporal relationships between ovulation and changes in the concentration of plasma estradiol-17ß, LH, FSH and progesterone where ovulation was determined by morphological examination and biopsy of ovaries at laparotomy.[41] Ovulation could be predicted in 90% of cases within -3(±5) hours and 36(±5) hours of plasma LH surge.[41] Various other methods have been used to estimate ovulation such as, patient-performed charting of basal body temperature, cervical mucus scoring and daily mid-cycle sonographic monitoring of follicle development.[42-44] These methods may not be very reliable, are time-consuming and expensive. Estimation of ratio of urinary estrone-3-glucuronide and pregnanediol-3-glucuronide, is concordant with the day of peak urinary LH, with marked reduction of this ratio suggestive of the luteal phase transition, which can be reliably used in epidemiological studies.[40,45] Daily urinary LH testing is a reliable, simple and useful predictor of ovulation within 20 ± 3 hours [95% confidence intervals (CI);14-26] of LH surge with a

positive predictive value for ovulation within 0 to 24 hours being 73%(95% CI; 70.9%-74.7%) and 92%(95% CI; 89.8-93.6%) at 48 hours.[46]

1.2.2 Fertilization

The process of fertilization, involves interaction between sperm and oocyte. Neither do we know much about events around human conception nor do we have a reliable marker of detecting conception in vivo. Most of the spermatozoa may reach the mature oocyte within 1 hour after insemination.[47,48] A population of viable spermatozoa then becomes established in the isthmus of the fallopian tube and fertilizing potential is maintained by being stabilized by interaction between the isthmic epithelium and the spermatozoa.[49-51] The spermatozoa undergo the process of complex changes over a few hours, followed by the fusion of the acrosomal membrane of the sperm to the zona pellucida. This allows a series of events involving various receptors and mediators resulting in fusion of the male and female pronucleus to form a two-cell zygote.[47] The zygote undergoes a series of mitotic divisions and after three to four divisions the zygote is known as the morula, which then enters the uterus at 12 to 16 cell stage.[52]

Fertilization can occur only around the time of ovulation and is likely if intercourse occurs within 5 days before and on the day of ovulation itself (Figure 1.2).[53] In the North Carolina study,[53] the probability of conception progressively increased from about five days before ovulation to the day of ovulation, followed by a rapid drop after ovulation.[53] The drop in probability of conception after ovulation may be due to short survival time for ova believed to be about 12-24 hours after ovulation[54] or perhaps a change in the cervical mucus after ovulation that obstructs entry of other sperm. The sperm retains the ability to fertilize an ovum for about five days in the female reproductive tract.

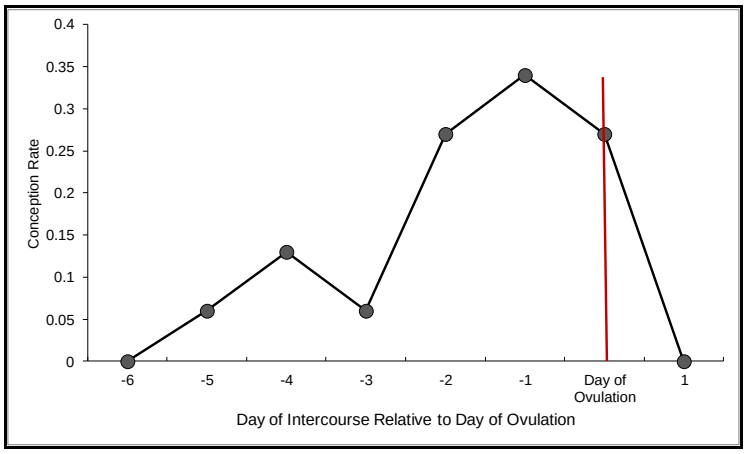


Figure 1.2 Probability of conception in relation to the ovulation day. The highest probability of conception is if the day of intercourse is 24 or 48 hours before the day of ovulation. The bold black line represents all the pregnancies and the red line represents the day of ovulation. (Adapted from Wilcox A.J. et al Post-ovulatory aging of the human oocyte and embryo failure. Human Reproduction. 1998;13:2:394-397)[54,55]

1.2.3 Pre-implantation conceptus

After fertilization the two-cell zygote develops into a 12 to 16 cell mass, known as the morula. The morula reaches the uterine cavity about 2 to 3 days after fertilization. Approximately five days after ovulation, the morula differentiates into a blastocyst with the appearance of a fluid-filled inner cavity and cellular differentiation of surface cells into trophectoderm, which contributes to development of the placenta, and the inner cell mass which gives rise to the embryonic structures. The embryo (blastocyst) undergoes a sequence of events to enhance its ability to implant in a receptive endometrium by about 6 days following fertilization.[5]

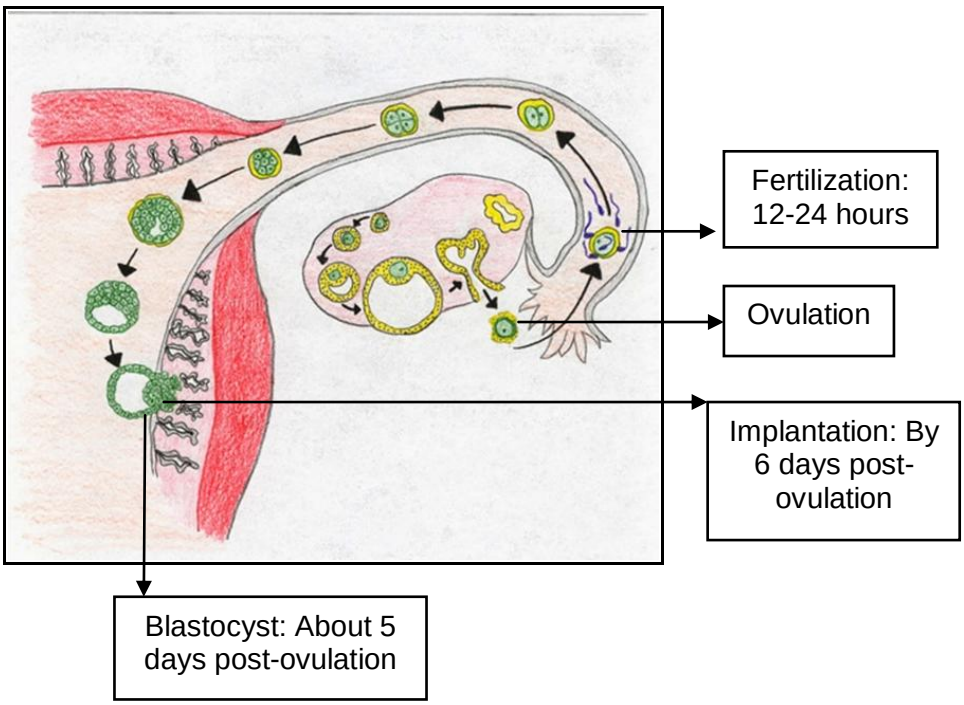


Figure 1.3: Timescale of events leading to implantation: Ovulation, fertilization and implantation (Adapted from Internet: google images by Alexis Dixis 2003)

1.2.4 Implantation

The human endometrium undergoes histological, biological and physiological changes during the menstrual cycle, which enable the endometrial tissue to be receptive for blastocyst adhesion.[56] The maximum endometrial receptivity is acquired during the mid-secretory phase about one week after ovulation and lasts for about 2 to 7 days in humans.[57,58] Further changes continue during the process of implantation, which include proliferation, differentiation of epithelial cells, structural changes, enhanced secretory activity of the endometrial glands, and increased vascularity. As a result of this the endometrial cells in the vicinity of the implantation site are transformed into decidual cells.

Regulation of events: The molecular mechanisms regulating the events around human implantation remain elusive. Hormones such as 17ß estradiol, progesterone and human chorionic gonadotrophin (hCG), maternally-derived growth factors secreted by uterine glands such as leukemia inhibiting factor (LIF), cytokines and growth factors secreted by the trophoblastic cells are examples of factors associated with implantation and maintenance of early pregnancy.[5]

Measures of implantation: The precise measures of implantation are not simple. The best in vivo measure of human implantation is hCG hormone. It is produced by the trophoblast surrounding the blastocyst and is secreted into the maternal circulation at the time of implantation.[59] It has a luteotrophic action and rescues the corpus luteum to produce increasing quantities of steroid hormone and thus maintain the pregnancy.

HCG subunits are already transcribed in six to eight-cell embryos,[60] and hCG becomes first detectable in the maternal circulation about 6 to 8 days postconception.[61] The first detection of hCG in the urine or maternal plasma may indicate successful implantation and the exponential rise in the maternal serum or urine hCG represents successful invasion of the maternal tissue by the conceptus although this may not be the earliest step in implantation.[61] The urinary hCG pattern during the first week following implantation may reflect the efficacy of placentation process.[62] Absolute hCG values in maternal serum and urine, the rates of rise in hCG hormone levels in the maternal serum and urine have been associated with the embryo's genetic quality, site of

implantation i.e. intrauterine pregnancy or ectopic pregnancy[63,64] and development of the pregnancy.

Ovulation to implantation (O-I) interval: The average timing between estimated day of ovulation to estimated day of implantation in spontaneous conception ranges from 6 to 12 days (mean 9.1± 1.12 days) using changes in urinary excretion of estrone 3-glucoronide and pregnanediol 3- glucuronide as indicator of ovulation and first detection of urinary hCG as indicator of implantation.[6]

Factors affecting O-I interval: It is plausible that the O-I interval is affected by the quality of the conceptus or endometrial receptivity. Later implantation is associated with increased risk of early pregnancy loss less than 6 weeks.[6] The time to implantation is also a significant predictor of the pattern of hCG rise in the first week after conception, when the process of placentation occurs.[65] Early implantation is associated with lower hCG levels on the day of implantation but higher rates of increase during the first week whereas late implantation is associated with a slower rise of hCG: an association that may suggest that time to implantation is related to embryo quality.[65] Since, the process of placentation begins in the first week as well, it is plausible that delayed implantation is also accompanied by less successful placentation and may be associated with conditions such as PE/IUGR.

Timing to implantation is also affected by a number of maternal factors. Current smoking and longer time between oocyte release and fertilization are associated with late implantation and increased incidence of early pregnancy loss.[66] A steeper rise in hCG is noted in the first week of pregnancy in women older than 29 years of age.[66]

1.3 Placentation

The process of placental development (placentation) begins following trophoblastic invasion of the endometrium at implantation. The human placenta is haemochorial because there is direct contact between maternal blood and chorionic (fetal) villi in the intervillous space. Therefore, establishment of optimal uteroplacental blood flow is a crucial step of human placentation.

As the blastocyst penetrates into the uterine epithelium, the trophoblastic cells differentiate into outer syncytiotrophoblast and inner cytotrophoblast. Lacunae in the syncytium form the intervillous spaces of the definitive placenta after establishment of the uteroplacental circulation. The trabeculae of syncytiotrophoblast develop into primary villi protruding in the intervillous spaces and later form secondary and tertiary villi.

Subsequently, both the trophoblastic cells differentiate further into villous and extravillous trophoblast. Those in contact with maternal blood are known as villous trophoblast and are involved in the transport of nutrients, oxygen to the fetus and secretion of hormones and proteins. The extravillous trophoblast forms placental anchoring villi, which anchor the fetal trophoblastic cells to the underlying decidua. These extravillous trophoblastic cells also invade the spiral arteries and make up the endovascular trophoblast. This extravillous trophoblastic invasion occurs as a two-wave phenomenon in the human:[3,67-69] the endovascular trophoblast appears in the decidual parts of the spiral arteries early in pregnancy upto the deciduo-myometrial junction, and 14-15 weeks onwards the myometrial spiral arteries are invaded by the endovascular trophoblast.

1.3.1 Histiotrophic embryonic nutrition

Although the process of endovascular invasion begins at about 7 to 8 weeks of gestation, maternal blood flow to the placenta is only fully established at about 10-12 weeks.[70,71] The low oxygen environment, facilitated by plugs of cytotrophoblastic cells may protect the developing embryo from free radical mediated damage.[72] Loss of these trophoblastic plugs has been shown to be associated with early miscarriage, or, dependent upon the timing, associated with preeclampsia.[72,73] The first trimester embryonic growth and development

is dependent upon nutrition derived from the endometrium and uterine glands, which is known as 'histiotrophic' nutrition.[74]

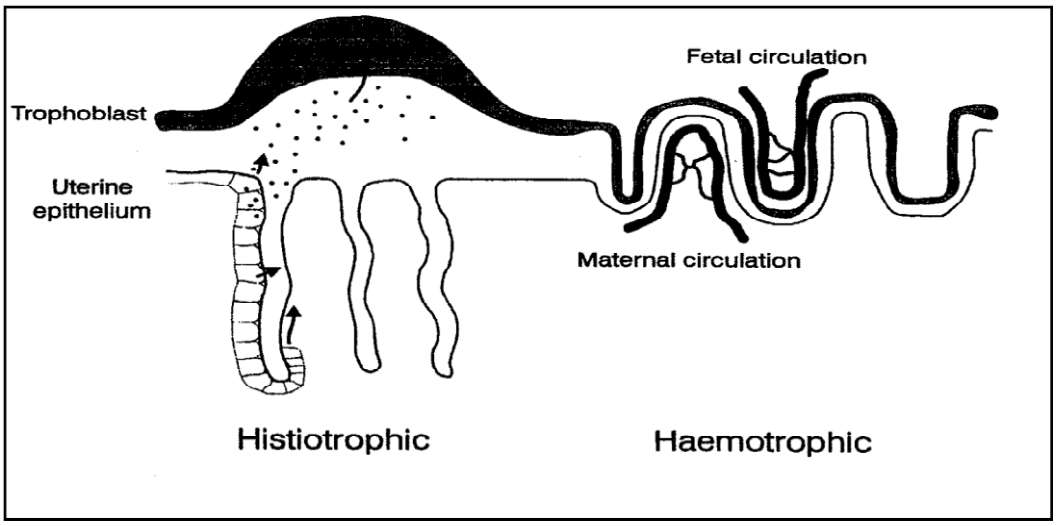
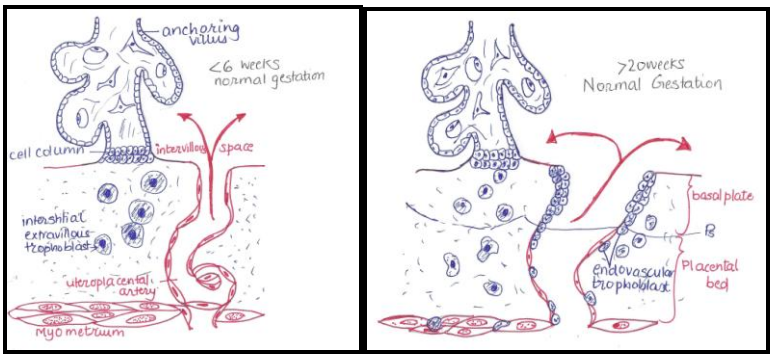


Figure 1.4 Schematic diagram of histiotrophic nutrition of the embryo till 8 to 10 weeks followed by haemotrophic nutrition after first trimester in human placenta. (Figure adapted from lecture notes on: Implantation, pregnancy loss and development of extra embryonic membranes from Human Reproduction module of Gonville and Caius College, Cambridge)

1.3.2 Haemotrophic nutrition

After the first trimester uteroplacental blood flow is an important determinant of fetal growth and development.[75] Maternal uterine spiral artery remodelling leads to a low resistance uteroplacental circulation and is the crucial step of human placentation.[11] The remodelling and dilatation of the uteroplacental arteries is believed to be a result of pregnancy-induced activation of the local decidual artery renin-angiotensin system leading to disorganized vascular smooth muscle and lumen dilatation followed by direct invasion of the smooth muscles of uteroplacental arteries by extravillous trophoblastic cells.[76] Figures 1.6 A and B describe spiral artery remodeling.[11]

A



B

Figures 1.5 A) Representation of endovascular trophoblastic invasion before 6 weeks of pregnancy and B) shows after 20 weeks of gestation in normal pregnancy. Blue: fetal tissues, Red: Maternal tissues. Ps: zone of placental separation, where the basal plate separates from the placental bed (Adapted from Kaufmann P et al. Biology of Reproduction. 2003;69: 1-7)[11]

Assessment of placentation: There are several approaches of assessing placental function in vivo. These include assessing biochemical markers: circulating trophoblast derived proteins such as pregnancy-associated plasma protein-A (PAPP-A) and free ßhCG and ultrasound markers: first and second trimester uterine artery blood flow indices[21] and placental volume.[77] All are indirect measures of placental function and are affected by various maternal and fetal parameters making them less reliable as surrogate markers of placental function. Uterine artery Doppler is often used to assess placental function along with maternal cardiovascular adaptation[78,79] and in screening high-risk women for preeclampsia clinically and for research purposes. We therefore used second trimester uterine artery Doppler to assess uteroplacental blood flow in this study.

1.4 Determination of gestational age

Gestational age (GA) assessment forms the basis for interpreting early pregnancy ultrasound scans and diagnosing fetal growth restriction. GA is routinely established by ultrasound measurement of fetal crown-rump length (CRL).[80-82] Fetal CRL charts were constructed from observed first trimester CRL measurements in relation to GA calculated from the LMP in women with regular menstrual cycles assuming that ovulation occurs mid-cycle.[80] Emphasis has been focused on a single first trimester CRL measurement in determining fetal growth.[77] However, there is a lack of prospective studies exploring the impact of biological variability due to ovulation and implantation timing on first trimester CRL. We have prospectively observed ovulation and implantation timing along with ultrasound measures of first trimester CRL in order to explore if this biological variability affects fetal CRL, fetal growth, birthweight or length of gestation.

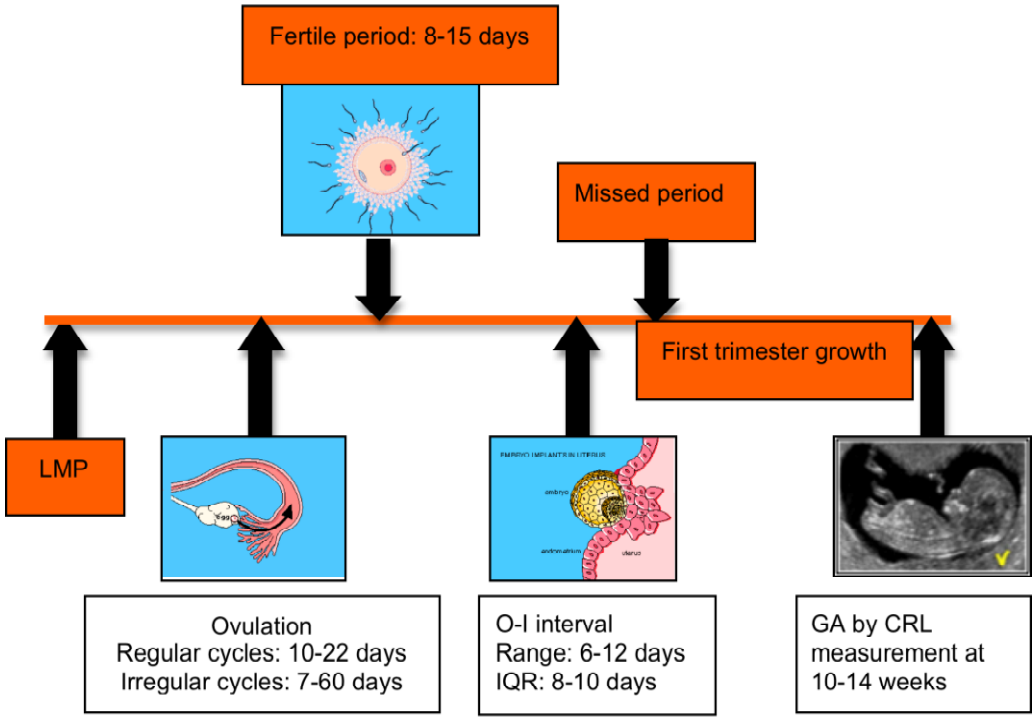


Figure 1.6 Schematic representation of timeline of events and variability that can contribute to the crown rump length measurement at 10-14 weeks from the first day of menstrual cycle (LMP): ovulation, conception, ovulation to implantation (O-I) interval (North Caroline Study)[6] and first trimester growth

1.5 Embryonic and fetal growth

Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to achieve its genetic growth potential in utero and implies placental dysfunction due to various causes originating in the mother, placenta or the fetus and occurs in about 4-7% of births.[83] The diagnosis of intrauterine growth restriction depends on the correct establishment of gestational age based on the fetal CRL at 10-14 weeks. In addition to the assumptions about ovulation and implantation timing, it is also assumed that growth is uniform in the first trimester.[84] However, first trimester growth is affected by age, smoking, ethnicity, maternal body mass index and fetal factors.[85] A relationship between a small first trimester embryonic measurement and low birth weight has been shown in IVF pregnancies[86] and in natural conceptions.[87,88] Fetal growth itself is a dynamic process and is controlled by maternal, fetal, placental and environmental factors. Fetal size at birth is a result of the interaction of all these factors. Fetal nutrition is provided by the placental and uterine vascular system and hence implantation, trophoblastic invasion, placentation are necessary for normal fetal growth. Maternal factors such as age[89], BMI, smoking, medical diseases and cardiovascular function can affect fetal growth and fetal size at birth.[90,91]

There are few prospective studies of serial ultrasound measurements in the first trimester exploring longitudinal embryonic and fetal growth along with biological variation in ovulation, implantation timing and late pregnancy growth and cardiovascular changes. In this study, we have explored the relationship between first, second, third trimester fetal growth, implantation timing, placental function using second trimester uterine artery Doppler, maternal characteristics and maternal cardiovascular changes.

1.6 Early pregnancy loss

Early pregnancy loss can have significant impact on woman's psychological wellbeing. Common causes of early pregnancy loss are: miscarriage or ectopic pregnancy. Most of the loss in early pregnancy is a result of impaired embryo-endometrial interaction and impaired trophoblastic invasion.[14] It is plausible that delayed implantation in early pregnancy loss less than 6 weeks is due to impaired trophoblastic invasion.[5]

1.6.1 Miscarriage and recurrent miscarriage

Miscarriage is the most common complication of pregnancy.[1] One third of embryos are lost prior to implantation[92] and a further third of early pregnancy loss occurs even before the pregnancy is recognized as a clinical pregnancy.[36] Recurrent miscarriage (RM) is defined as three consecutive miscarriages and affects about 1% of couples. Eighty five percent of cases of RM are unexplained[93] and heterogeneous in etiology.[2] RM is also associated with thrombophilia and antiphospholipid antibody syndrome.

1.6.2 Ectopic pregnancy

It occurs in about 1-2% of pregnancies. An ectopic pregnancy is characterised by the presence of an extra uterine gestational sac with or without a yolk sac or an adnexal mass or extra uterine sac like structure in the absence of an intrauterine pregnancy. It may compromise woman's health and future fertility.[94]

1.6.3 Pregnancy of unknown location

Women with a positive pregnancy test with no evidence of an intrauterine or extrauterine pregnancy on transvaginal ultrasound scan (TVS) are classified in the group to have pregnancy of unknown location. These women may either have an intrauterine pregnancy, miscarriage or ectopic pregnancy.[95]

We explored the relationship of ovulation, implantation timing and O-I interval in women who miscarried in the study population.

1.7 Impaired placentation and uteroplacental insufficiency: Preeclampsia and intrauterine growth restriction

Preeclampsia (PE) and intrauterine growth restriction (IUGR) are both common pregnancy complications and have different manifestations, but both are associated with underlying abnormalities of placental function. This association is supported by the presence of abnormal uterine artery Doppler and abnormal umbilical artery Doppler.[10,12]

1.7.1 Preeclampsia

It remains the second leading cause of maternal mortality in both developing and developed countries with the absolute numbers being higher in developing countries.[96] In the most recent report of confidential enquires into maternal deaths in the UK- PE and hypertensive disorders are responsible for 22/107 direct deaths.[97] PE affects about 3-5% of all pregnancies and is responsible for significant maternal and neonatal mortality and short and long-term morbidity.[98,99] Women with a history of PE are at increased risk of CVD later in life.[29,100] PE is associated with 15% of preterm births and a four-fold increase in the incidence of IUGR and is responsible for long-term health consequences such as obesity, cardiovascular disease, hypertension and diabetes later in life for the growth restricted newborns born to hypertensive mothers.[101-105]

Preeclampsia is commonly defined as a diastolic blood pressure (BP) of ≥ 110 mm of Hg on one or more occasions or ≥ 90mm of Hg on two or more occasions at least 4 hours apart, in combination with significant proteinuria (≥ 300mg/ 24hours or 2+ dipstick) developing after 20 weeks of gestation, in a previously normotensive woman.[106] PE is classed as severe if the systolic and diastolic BP rise above 160mmHg or 110mmHg respectively.[106]

1.7.2 Intrauterine growth restriction

IUGR even in the absence of PE is responsible for significant short term and long term neonatal morbidity and mortality.[103,104] It contributes to iatrogenic prematurity and short and long term complications associated with prematurity. Moreover, growth restricted neonates are likely to be at an

increased risk of hypertension, coronary heart disease and metabolic syndrome in adulthood.[104,107,108]

Although it is believed that fetal growth restriction begins in the first trimester, is associated with uteroplacental insufficiency and is associated with cardiovascular maladaptation,[91] there is lack of prospective studies of naturally conceived pregnancies, assessing the impact of biological variability in ovulation and implantation on measures of fetal size along with longitudinal ultrasound measures of fetal growth alongside maternal cardiovascular adaptation.[109] We have explored all these aspects in this feasibility study.

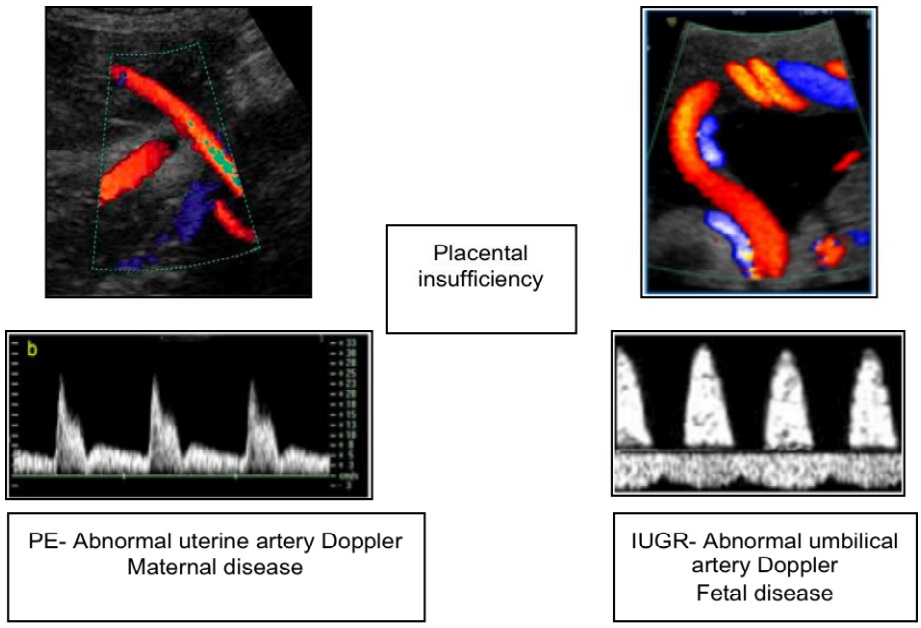


Figure 1.7 Evidence of abnormal placentation in preeclampsia (PE) and intrauterine growth restriction (IUGR) by uteroplacental Doppler (Doppler images have been obtained from Fetal Medicine Foundation website – online Doppler module)

1.8 Cardiovascular adaptation in pregnancy

1.8.1 Cardiovascular changes in normal pregnancy

Normal pregnancy is associated with profound cardiovascular adaptation in order to optimize uteroplacental perfusion without compromising maternal function and to ensure optimal conditions for fetal growth and development.[110] Lindhard first reported maternal cardiovascular function in pregnancy in 1915 using invasive investigations by dye-dilution techniques.[111] With development of newer, non-invasive and more accurate techniques it is now possible to determine patterns in maternal cardiovascular changes such as the time of onset of changes, the time of maximum changes, magnitude of change and behavior during and after pregnancy.[112] Understanding the nature and extent of cardiovascular adaptation in normal pregnancy enables assessment of cardiovascular changes associated with maternal complications such as PE or IUGR[113] and possible prediction of these pregnancy complications.

The haemodynamic changes in normal pregnancy start even before a fully functional placenta is formed.[26] Most of the current studies of maternal cardiovascular adaptation in pregnancy are cross-sectional and they may not account for individual variation during pregnancy. Existing longitudinal studies are small in number and most of them begin from late first trimester and use either first trimester or postpartum data as baseline data. However, it is known that significant cardiovascular changes begin as early as 5 to 6 weeks in pregnancy (Summary of longitudinal pre-pregnancy to postpartum studies in Table 1.1).[24-26] Moreover, certain cardiovascular parameters e.g. cardiac changes, may take longer to return to normal and may not return to baseline.[114]

Table 1.1 Summary of longitudinal studies investigating maternal cardiovascular changes in pregnancy

	Parameters studied	Study population (number and parity)	Timings of Research Visit
Atkins AFJ. 1981	BP, HR and SV using a Minnesota Impedance Cardiograph, PVR and CO	8 (Mixed parity)	Preconception, then monthly until delivery and on 3rd postpartum day, 6weeks and 4 to 16 months postpartum
Capeless E. 1989	M- mode echocardiograph left ventricular measurements, CO, ejection fraction, SV, MAP, systemic vascular resistance	8 (5 Nulliparous/ 3 multiparous)	Preconception within 3 months before conception, 6-8, 14-16, 22-24 weeks.
Robson SC. 1989	HR, BP, CO and SV using cross-sectional echocardiography and Doppler methods	13 (6 Nulliparous, 7 multiparous)	Preconception, early pregnancy at mean 35 days after the LMP and then monthly from 8 to 36 weeks and finally at 38 weeks
Chapman AB. 1998	MAP, CO, renal functions	10 7 normal/ 3 (one with PE, one with severe asthma and one with twins)	Preconception, 6, 8, 10, 12, 24 and 36 weeks
Ogueh O. 2009	Echocardiography for CO, SV, left ventricular measurements and BP	28 (Four groups): (Spontaneous and IVF pregnancies) 13 normal singleton, 5 singleton IVF, 4 singleton ovum donation, 6 multiple pregnancies	Preconception, at 6, 10, 16, 26, 36 weeks and 6 weeks postpartum.

Changes in Brachial blood pressure: Blood pressure (BP) is the most commonly measured clinical variable. It is well accepted that brachial blood pressure (BP) falls by about 10% in the midtrimester.[115,116] At least half of this fall in BP occurs in the first trimester, beginning very early in pregnancy at about 6weeks in pregnancy (≈13 ± 2.6 mmHg/ 20%), as observed in longitudinal studies from the pre-conception period[26], followed by a further drop in the midtrimester (Figure 1.9).[25,26,116,117] This is followed by an increase in BP towards term. These initial studies recorded BP using a sphygmomanometer. A cross-sectional study of automated BP in a large

cohort of pregnant women at various gestations has derived the 5th and 95th centiles of mean arterial pressure (MAP) values as 90/50mmHg and 130/80 mmHg respectively between 5 to 42 weeks of gestation (Figure 1.9).[118] The data regarding postpartum return of BP to normal are inconclusive because of lack of pre-pregnancy to postpartum follow up in most of the studies. It is suggested in a study from prior to conception that there is no difference at BP at 6 and 12 weeks postpartum and the MAP at 12 weeks returns to baseline.[114] Studies beginning during pregnancy have shown that SBP and DBP values are higher at up to 12 weeks postpartum compared to 36 weeks.[119] There is no difference in postpartum BP due to breastfeeding.[119] BP in Caucasians during pregnancy is affected significantly by parity, age and body mass index (BMI). BP is slightly lower in Asian and black women.[118] This could be because of lower BMI in Asian women and multiparity in black women. In addition, BP shows circadian rhythm and variability over a 24-hr time interval during pregnancy of 21-25 mmHg. The pattern of mean BP changes is maintained even after accounting for variability in normotensive pregnancies (Figure 1.8).[120,121]

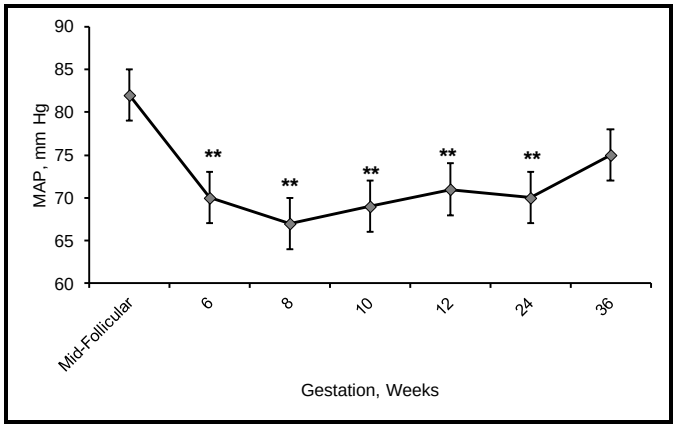


Figure 1.8 Changes in mean arterial pressure (MAP) during pregnancy. ** *P*<0.05 at that gestation in relation to Mid-Follicular MAP. (Adapted from Chapman AB et al. Kidney International. 1998;54:2056-63)[26]

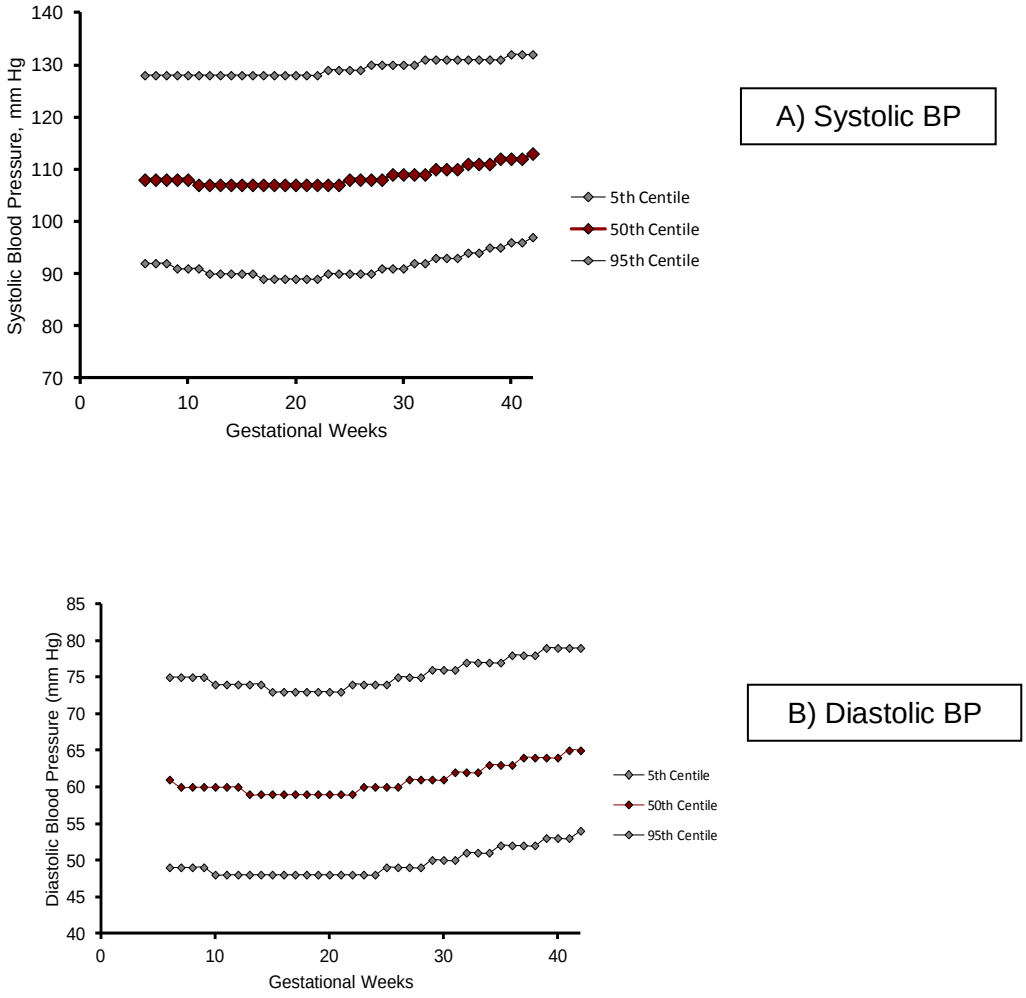


Figure 1.9 Reference ranges for A) Systolic BP and B) Diastolic BP during pregnancy (5-42 weeks) using automated BP. (Adapted from Ochsenbein-Kolble N et al. BJOG. 2004;111:319-25)[118]

Changes in Central (aortic) BP: Brachial artery BP is routinely used in day-to-day clinical practice and in research. However, brachial BP does not reflect BP changes in the central (aortic) BP. Central BP differs from brachial BP due to differences in the arterial stiffness and pulse wave reflections in the arterial tree and can differ by up to 20mmHg in young women.[122] Central BP is considered to be more relevant in understanding the pathophysiology of cardiovascular diseases than peripheral pressures due the proximity of aorta to the kidney, brain and heart[123] and also has superior prognostic value in prediction of cardiovascular disease later in life compared to brachial BP.[124] Due to proximity of the aorta to the uteroplacental bed central BP may represent the effects of pregnancy associated cardiovascular changes better than brachial BP. Central BP values during pregnancy in cross-sectional studies indicate that central systolic BP (SBP) is lower in pregnant than non-pregnant women (Figure 1.10) and lower in the second trimester during pregnancy.[125,126] Moreover, longitudinal data using late first trimester measurements as 'baseline' show that central SBP decreases in mid pregnancy[127,128] and to a greater extent than brachial SBP.[128] There is a lack of studies describing longitudinal changes in central SBP from prior to pregnancy to postpartum. It is plausible that the extent of changes during pregnancy is underestimated by using late first trimester measurements as 'baseline' measurements.

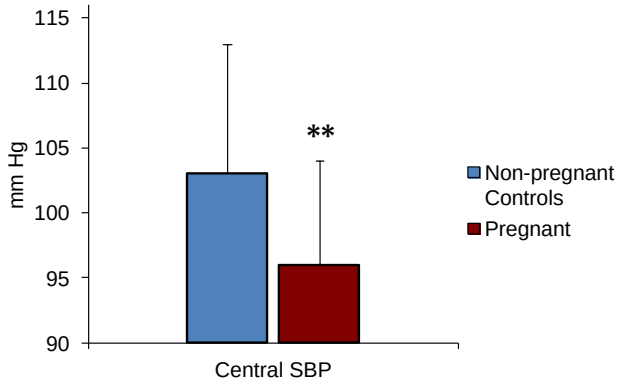


Figure 1.10 Difference between central BP in pregnant and non-pregnant women (Adapted from Macedo ML et al. Hypertension. 2008:51;4)[125]

Changes in cardiac function (cardiac output, stroke volume), morphology of the heart and heart rate: In normal pregnancy, there is an increase in cardiac output (CO), cardiac index (CI), heart rate (HR) and intravascular volume (Figure 1.11).[24,112,113]

The first cardiovascular change during pregnancy seems to be a rise in heart rate (HR).[129] Previous small prospective studies from prior to pregnancy have demonstrated that this rise starts between two and five weeks and continues well into the third trimester and plateaus after 32-34 weeks.[24-26,113]

In the last three decades, changes in CO, stroke volume (SV) and structural changes in the heart during pregnancy have been determined using the non-invasive, validated technique of M mode echocardiography.[112,130] Although there are a number of studies describing changes in heart during pregnancy, there is still controversy regarding left ventricular performance and global cardiac function.[131]

CO increases in pregnancy, with most of the increase being described in the first trimester.[132,133] This increase in CO is first seen by five weeks after the last menstrual period and a maximum increase of about 43-48% occurs (~ 2.5 L) by about 24 weeks.[24-26,129] The initial increase in CO is due to the increase in HR, followed by an increase in SV starting a little later than 8 weeks, with a maximum rise at about 20weeks.[24,113] The later increase of SV from 8 weeks onwards is believed to be a result of left ventricular remodelling, an increase in the left ventricular (LV) mass index and adaptation of the heart in response to physiological changes in the preload and afterload secondary to systemic vasodilatation.[129,134] The size, timing and mechanism of changes in CO and SV in the third trimester remain variable between different studies. Studies using dye dilution methods and impedance echocardiography have described a fall in CO due to a reduction in SV by 35 weeks, with heart rate remaining constant.[28,135] However, studies using echocardiography have reported that CO, SV and HR after 32 weeks plateau with a slight fall after 38 weeks and a further reduction back to pre-pregnancy levels by 2 weeks postpartum.[24,113,136] The increase in CO and SV in the third trimester are associated with structural changes in the heart such as progressive increase of LV end-diastolic and end-systolic diameters and volumes, increased LV muscle mass and septal[131,137,138] thickness and eccentric LV hypertrophy. These changes are

similar to those seen in athletes as a result of chronic adaptation of heart to an increased preload.

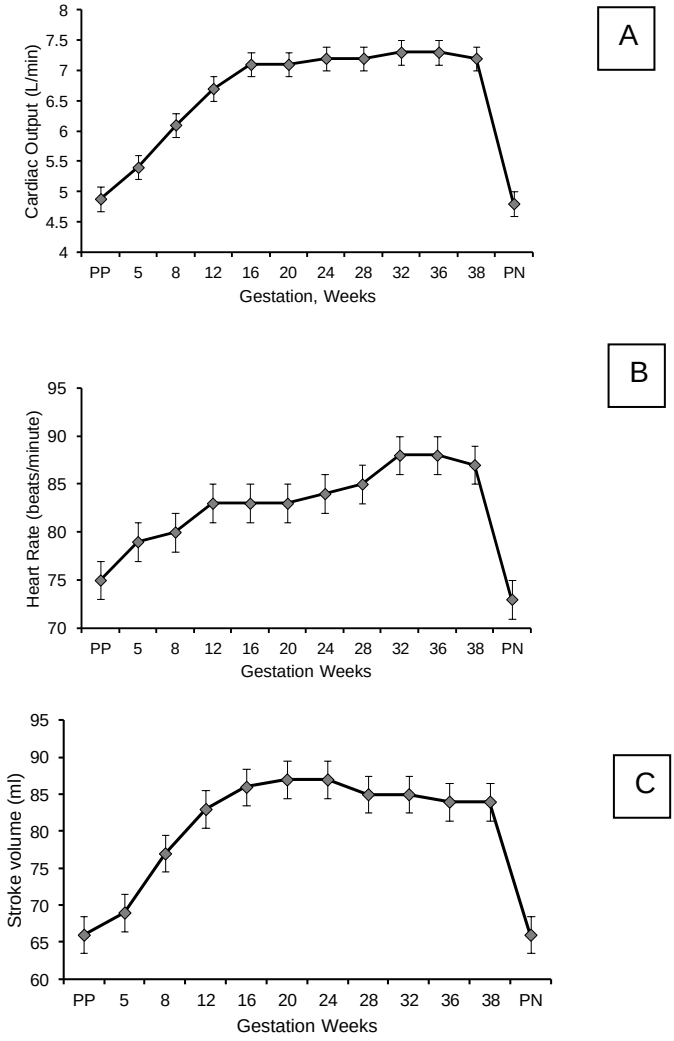


Figure 1.11 Changes in A) cardiac output, alongside changes in B) heart rate and C) stroke volume during normal pregnancy. (Adapted from Robson SC et al. Br Heart J. 1992;68:540-3)[113]

Changes in arterial and venous compliance, aortic stiffness, pulse wave reflection:

There is a growing awareness of the importance of large artery function in the understanding of the pathophysiology of CVD. Large artery stiffness, measured as the aortic pulse wave velocity (aPWV), and wave reflections, quantified by augmentation index (AIx) independently predict cardiovascular outcome later in life.[124] There is an increasing interest in the understanding the pregnancy related changes in the structural properties of large arteries such as the aorta, which can be measured using aortic compliance. Aortic compliance is affected by aortic vessel wall stiffness, its structural properties and wave reflections in the aorta.[139] Vessel wall stiffness and pulse wave reflections also have significant impact on central BP.[123]

Central BP, AIx and aPWV are higher in the preclinical[140] and clinical phases of pregnancies complicated by PE and IUGR.[126,141,142] However, in order to understand the extent and timing of changes in pregnancies complicated by PE/IUGR, it is important to understand changes in normal pregnancy from a pre-pregnancy baseline.

AIx is lower in pregnant than non-pregnant women and lower in mid pregnancy compared to the first trimester in cross-sectional studies[125] however there are few longitudinal studies of changes in AIx beginning from late first trimester.[126-128] The extent and relationship of maternal vascular function changes as reflected by arterial stiffness and arterial wave forms along with central haemodynamics in very early pregnancy from a pre-pregnancy baseline, their relationship to the process of implantation and pregnancy outcomes at the time of profound cardiovascular adaptation has not been reported before.

It is believed that aortic compliance increases during normal pregnancy with maximum increase in the third trimester, associated with a decrease in aPWV.[143,144] This change in aortic compliance and vessel compliance helps to regulate the cardiac output, accommodate the volume overload and the circulatory changes during pregnancy and maintain blood pressures.[139,143] However, it is difficult to interpret the existing evidence because of the limited numbers in the longitudinal studies performed, different parameters and

methods used to measure aortic compliance (or aortic distensibility) and varied times of performing the measurements (Summary of the existing longitudinal studies of aortic compliance is described in Table 1.2).[126,143-145]

Table 1.2 Summary of longitudinal studies assessing changes in maternal aortic compliance during pregnancy

	Parameter Technique used	Population Timing of tests	Changes during pregnancy
Edouard D.A., 1998	Aortic PWV Carotid-femoral, pulse transducers	9 normal pregnancies, First, third trimester and postpartum control	Decrease from first to third trimester
Mersich B., 2005	Aortic PWV, SphygmoCor® system (SCOR; PWV Medical, Sydney, Australia)	12 normal pregnancies, First, second, third trimester and postpartum control	Decrease from first to second to third trimester
Oyama-Kato M., 2006	Brachial-ankle PWV BP-203PRE, Colin Inc, Komaki, Japan	167 normal and 16 with hypertensive pregnancies First, second, third trimester and postpartum	Decrease from first to second with non-significant further decrease and increase in third trimester. No decrease in hypertensive pregnancies
Robb A., 2009	Carotid-femoral PWV SphygmoCor system (AtCor Medical)	22 normal pregnancies 16, 24, 32 and 37 weeks of gestation. 15 preeclamptic women at diagnosis and 7 weeks postpartum	An increase from 24 weeks to 7 weeks postpartum. Increase more in those with PE

Changes in renal haemodynamics: Pregnancy requires volume expansion for meeting the demands of maternal pregnancy adaptation and to meet the extra fluid demands of the growing conceptus.[146] This is met by changes in the osmoregulatory system and renal haemodynamics. Renal plasma flow and glomerular filtration rate is increased and renal vascular resistance decreases significantly by 6weeks. Maximum renal plasma flow, maximum glomerular filtration rate (about 45% above the follicular phase of the menstrual cycle) and minimum renal vascular resistance are seen at the eighth week of gestation.[129,146,147] The increase in the glomerular filtration rate remains until 36weeks with the peak glomerular filtration rate in the second trimester. Although still higher than the pre-pregnancy levels, there is a decline in later

pregnancy, which may be attributed to an increase in measurement errors in late pregnancy.[148] Renal function tests were performed in this study in the late first trimester in order to have an indirect estimate of the contribution of renal haemodynamics to pregnancy induced changes in early pregnancy and to assess if uteroplacental insufficiency was associated with reduced volume expansion modulated by renal haemodynamics.

Mechanisms: The initial cardiovascular changes in pregnancy are those of peripheral vasodilatation with a specific renal vasodilating effect and these lead to secondary changes in HR, CO followed by SV.[26,129] Normal human pregnancy is characterized by increased plasma renin concentration and activity[149], increased angiotensin II (A-II) and aldosterone however, an increased resistance to the pressor effects of infused A-II. Although the mechanisms for renal changes in normal human pregnancy are unclear, it is proposed that peripheral vasodilatation in very early pregnancy could be due to reduced sensitivity to A-II, and norepinephrine secondary to modulation of the renin-angiotensin-aldosterone system[150], increased nitric oxide production[151], a primary volume expansion[129] or relaxin mediated changes in arterial compliance.[152,153] It is unclear if the changes in large arteries later in pregnancy with minimal changes in blood pressure are due to passive changes in vessel wall properties due to exposure to enhanced preload following volume expansion, reduced distending pressure, vascular remodeling[154] or reduced smooth muscle tone mediated through estrogen receptors.[139]

Although we believe we know more about the sequence of cardiovascular adaptation we are nonetheless no wiser in determination of the mechanisms behind these changes. For decades it was believed that one or more feto-placental hormones such as sex steroids initiated a decrease in vascular reactivity, tone, systemic haemodilution, renal function and thus changes in effective circulating volume to trigger other hemodynamic changes.[155] The temporal relationship between cardiovascular, renal and hormonal changes from pre-pregnancy to very early in pregnancy also suggests that significant systemic and renal vasodilatation along with vasopressor activation occurs very early in pregnancy prior to full placentation.[26,129] Therefore, it is plausible that conceptus-derived factors may play a key role rather than maternal

hormones.[156] Unfortunately, due to the inability to perform *in vivo* studies at the trophoblast-decidual interface, complete understanding of these mechanisms may remain impossible.[110,112]

1.8.2 Cardiovascular changes in preeclampsia/intrauterine growth restriction

Recognition of changes in normal pregnancies has highlighted that pregnancies complicated by gestational hypertension, PE and/or IUGR are characterized by failure of normal cardiovascular adaptation.

Reduced volume expansion and insufficient CO changes very early in pregnancy are believed to precede later fetal growth restriction.[19]

The exact nature and timing of haemodynamic mal-adaptation in PE is unclear because of heterogeneity of various studies. The discrepancy between the results may be because of the heterogeneity in the study population, small cohorts of mixed parity and varying pre-existing risk factors or medical problems. Not only is the severity of PE varied in the various studies, but women may be already treated with intravenous fluids or vasoactive medications. Moreover, most studies are cross-sectional and have described maternal central haemodynamics during the clinical phase of PE. Most of these studies do not differentiate between early and late onset PE and it is now believed that early onset (<34weeks) PE (preterm PE) and late onset PE (≥34weeks) are two different disease entities[157] and therefore there is a difference in cardiovascular changes in both these conditions.

Changes in BP in PE and gestational hypertension (GH): Whilst it is known that BP reduces in pregnancy until 18-20 weeks of gestation, followed by a rise till delivery,[26,115,158,159] it is known to be higher in hypertensive disorders of pregnancy and BP of ≥ 140/90mmHg has been used as a cut off for the diagnosis of hypertension in pregnancy.[106] Higher BP prior to pregnancy,[18,158] and in late first trimester (11-14 weeks)[160,161] are associated with increased risk of developing hypertensive disorders of pregnancy. Women who develop PE/GH not only have a higher BP very early in pregnancy but also have a reduced initial first and second trimester drop in SBP and a faster elevation in BP from 18weeks onwards, with women likely to develop PE having a further

marked rise in BP from 30 weeks.[162] There is overall reduced variability within 24-hour ambulatory blood pressure readings in hypertensive pregnancies compared to good variability seen in normotensive pregnancies, although the overall pattern of change (such as the overall increase from 18 weeks onwards) in brachial SBP and DBP is maintained.[120,121]

Although the symptoms of PE resolve a few weeks after delivery, it is known that these women have higher BP at 2 yrs postpartum and also several years following the pregnancy.[163,164]

Changes in central haemodynamics- arterial, venous system and CO, SV and HR in preclinical phase, clinical phase of preeclampsia and postpartum: It remains debatable if PE is characterized by a high-output, low resistance haemodynamic state or a low output and high resistance hemodynamic state.[28,165-167] Easterling and Benedetti suggested that PE is characterized by an elevated CO,[28] which occurs early in pregnancy, before the development of hypertension and persists during the clinical phase of PE followed by elevated vascular resistance. Whereas a large, study of invasive haemodynamics in untreated preeclamptic women supports the concept of PE being associated with low CO in the presence of an increased left ventricular afterload.[167] Bosio PM in a longitudinal cohort study of 400 primigravidae proposed an initial hyperdynamic disease model characterized by elevated CO earlier in pregnancy but a subsequent hemodynamic crossover to low CO and high vascular resistance circulation coinciding with the onset of the clinical syndrome in PE and persistence of high CO and vascular resistance in gestational hypertension.[27]

Early PE (<34weeks) is associated with higher total vascular resistance (difference of ≈ 866 ± 248 dyn.s.cm-^{5}); lower CO (difference of ≈ 4.47 ± 1.09 L) and systemic under perfusion compared to late onset PE.[168] Both preclinical and clinical phases of PE are characterized by low plasma volume and low venous reserve capacity.[146,169]

Although not much is known about the temporal relationship between vascular resistance and cardiac function in PE, it is proposed that increased systemic vascular resistance and decreased vascular compliance leads to an increased systemic afterload in PE. This leads to left ventricular concentric remodeling in

order to minimize myocardial oxygen demand and thus preserve left ventricular performance.[137] This may lead to mild-moderate left ventricular global diastolic dysfunction, seen in both term and preterm PE. Preterm PE is associated with significant impairment of myocardial contractility and left ventricular systolic dysfunction.[170] Left atrial remodeling is only seen in cases of severe, preterm PE, due to increased left-sided chamber filling pressures following left ventricular global diastolic dysfunction from prolonged exposure to higher afterload.[134] Right ventricular hypertrophy and systolic dysfunction are only evident in severe preterm PE with advanced left ventricular impairment as a result of increased right ventricular afterload.[170] It is believed that 56% of women with preterm PE will have asymptomatic left ventricular dysfunction at about 2 years postpartum.[134,170]

Thus, PE and IUGR are associated with cardiovascular dysfunction, which is seen even in the pre-clinical phases. However, because of lack of pre-pregnancy data in all these studies, the baseline cardiovascular impairment in women with PE/IUGR remains unknown.

Aortic compliance, pulse wave reflection and aortic stiffness in the preclinical and clinical phase of pregnancy complications and postpartum: PE and IUGR are associated with impaired vascular function characterized by increased arterial stiffness during the clinical disease and even prior to the clinical disease, in the second trimester.[140,142,171] Both, AIx and aPWV are higher in women with PE as shown in cross-sectional and longitudinal studies.[126,172] It has recently been shown that the reduction in pulse pressure and vascular compliance from pre-pregnancy to very early in pregnancy is different in pregnancies complicated with hypertension and PE.[20] Most of the reports on AIx and aPWV in PE are from cross-sectional studies however, the longitudinal studies begin from late first or second trimester. There is lack of any information about arterial stiffness in women with PE or hypertension either prior to pregnancy or in very early pregnancy.

Mechanisms of cardiovascular changes in PE: The pathogenesis of cardiovascular maladaptation in PE is still unclear. It was first shown by Gant et al in 1973 that the pathophysiologic process of preeclampsia begins months before the onset of overt disease, may be present as early as 18-22nd weeks in pregnancy and is characterized by increased sensitivity to A-II.[173]

It was also hypothesized that imbalance between prostacyclin and thromboxane created a positive feedback loop of endothelial damage, platelet activation and increased endothelial damage in PE however their role in pathogenesis was not established because this imbalance is only seen in the clinical phase of PE. Women who develop uteroplacental insufficiency demonstrate hyperdynamic circulation prior to the development of PE to compensate for a lower degree of volume expansion and relatively under filled circulation.[174] Hypertension is the primary and ultimate manifestation of PE however this is in fact the end result of the inability of the various autoregulatory mechanisms to adapt to the pregnancy induced maternal cardiovascular changes.

It is believed that elevated CO precedes the development of hypertension[175]. An elevated CO during the latent phase before development of hypertension could result in compensatory vasodilatation to maintain near-normal blood pressure. Profound vasodilatation combined with significant BP changes may lead to damage of the endothelium of capillary beds and exhaust the vasodilatory rescue functions of the endothelium resulting in a damaging, fixed vasoconstricted state of severe PE and the absence of endothelial damage leading to persistently high CO and vascular resistance in gestational hypertension.[27] It is clear that the final cross over from high CO, normal BP to low CO, high BP and high peripheral vascular resistance (PVR) occurs before the clinical phase however the exact mechanism and timing of the initial CO increase remains poorly understood.

The elevated CO could be due to either primary chronic sympathetic overactivity[176] or failure of the vascular system to fill and increase in preload (primary failure of volume expansion in early pregnancy).[174] This could lead to compensatory higher CO which leads to compensatory vasodilatation, increased sensitivity to Angiotensin II and further reducing the vasodilatory reserves and ultimate decompensation leading to a low CO state in development of PE.

1.8.3 Metabolic changes in normal pregnancy

Pregnancy is characterized by transition to a catabolic state using lipids as energy sources and sparing glucose and amino acids for the fetus. The fetus requires maternal lipids, such as cholesterol for fetal use in building cell membranes, for cell proliferation, development of the growing body, and as a precursor of bile acids and steroid hormones. Thus, lipid metabolism is important for fetal growth but maternal hypertriglyceridaemia poses a stress to the maternal metabolic system, and appears to increase the risk of preeclampsia and preterm birth in predisposed women.[177] Not only do these changes predispose a woman to pregnancy complications, but also contribute towards increased maternal cardiovascular risk later in life.[178,179]

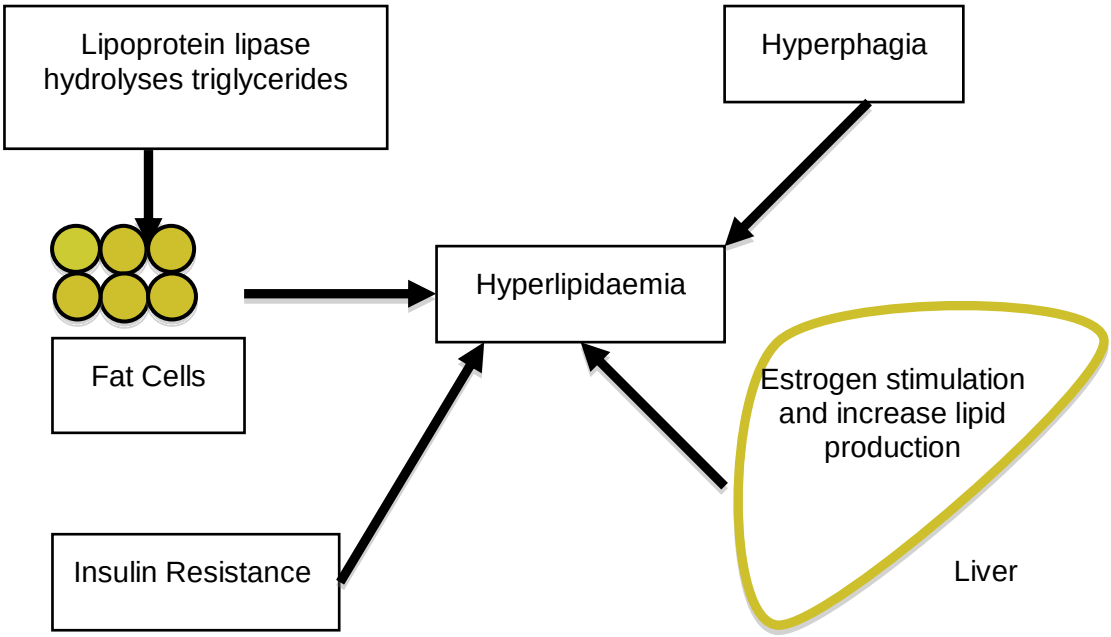


Figure 1.12 Schematic representation of mechanisms of hyperlipidaemia in pregnancy

Longitudinal and cross sectional studies of lipids in pregnancy from first trimester onwards and postpartum have shown that following initial first trimester decrease in HDL cholesterol and increase in LDL cholesterol, both LDL, HDL cholesterol and TG increase in the second trimester and maximum increase in total cholesterol (43%), LDL (36%), TC:HDL ratio and TG (about 60%) occurs at the end of third trimester.[180,181] Following an initial reduction,

there is increase in plasma lipids after 8 weeks of pregnancy due to increased intake, estrogen stimulation and insulin resistance leading to increased maternal fat accumulation. Lipoprotein lipase (LPL) in the extrahepatic tissues hydrolyses triglycerides (TG). Various maternal tissues such as the adipose tissues take up the fatty acids and glycerol produced as a result of this hydrolysis. This favors lipid metabolism thus sparing glucose and amnio acids for the fetus.[182,183] This is necessary for fetal metabolism, fetal growth especially brain and retinal development in the first trimester and then in the synthesis of steroids and bile acids via the placenta in later pregnancy.

In later pregnancy the increased lipolysis in the adipose tissues results in substantial increase in free fatty acids (FFA) and TG in the circulation, both of which cannot freely cross the placenta. As a result of which they are converted in the maternal liver to release very low -density lipoprotein (VLDL). These, along with increased insulin resistance lead to increased gluconeogenesis and ketogenesis. This results in increased maternal TG, phospholipids and cholesterol levels in the third trimester representing a pool of easily available energy source for the mother in fasting situations in order to spare glucose for the fetus.[182] Following delivery there is increased LPL expression and activity in the mammary glands to enable increased milk synthesis for subsequent lactation.[182]

It is known that the elevated TG and hypercholesterolemia in the third trimester predispose the mother to develop gestational diabetes and have atherogenic potential however the crucial levels for TG or cholesterol in order to predict this risk are not clear yet. Pregnancies complicated by preeclampsia are associated with significantly higher plasma lipids during the clinical phase of PE and it is believed that this hyperlipidaemia is responsible for endothelial injury and the systemic manifestations of PE.

1.8.4 Changes in platelet function in normal pregnancy

Pregnancy is believed to be a hypercoagulable state with an increase in clotting factors secondary to an increased estradiol. Platelet activation is increased in pregnancy and increases with increase in gestation; however this is not believed to be a feature of established PE.[184] There is limited evidence about the sequence of events that lead to platelet activation during normal

pregnancy due to a lack of prospective studies and variation in the methods for the assessment of platelet function in pregnancy.

Circulating platelets play a role in contributing towards the thrombotic tendency. The process of platelet activation involves stimulation of platelets following endothelial injury due to blood flow changes. Activated platelets change shape and express dendritic extensions. These changes mediate release of ADP from secretory granules within the platelets promoting platelet aggregation and coagulation, and mediate secretion of von Willebrand factor and platelet factor 4 from α granules. Platelet membrane glycoprotein receptors mediate initial platelet adhesion to the sub endothelial tissue followed by subsequent aggregation to form a haemostatic plug.[185]

It is plausible that the haemodynamic changes during pregnancy lead to platelet activation, which may lead to increased platelet aggregation as pregnancy advances. Platelet aggregation in very early pregnancy at the time of maximum cardiovascular changes has not been explored before.

8.5 Metabolic and hematological changes in preeclampsia/intrauterine growth restriction

Pregnancies complicated by IUGR are associated with the placental pathology similar to PE however without the systemic manifestations. It is proposed that gestational hyperlipidaemia should compensate for the placental insufficiency to provide nutrition to the fetus.[186] However, cross sectional studies in the third trimester have shown lower cholesterol, LDL cholesterol and VLDL mass in pregnancies complicated by IUGR compared to normal pregnancies.[186] If implantation and first trimester growth are related to both: placentation and birth weight then the changes in serum lipids by the end of the first trimester may reflect this. In this study we explore lipid changes in the end of first trimester and its relationship with first trimester fetal growth and pregnancy complications.

1.9 Recurrent miscarriage, placental insufficiency and cardiovascular disease later in life

PE and/or IUGR are associated with an increased maternal risk of cardiovascular disease (CVD) later in life.[29,31,100] Although the symptoms of PE resolve a few weeks after delivery, there is evidence of persistence of postpartum maternal endothelial dysfunction,[187] cardiac dysfunction in the form of asymptomatic left ventricular hypertrophy[170] and hypertension[188] for several months to years following the pregnancy. Physiological changes in the maternal cardiovascular system together with metabolic and hematological changes during a normal pregnancy predispose women to an increasingly pro-atherogenic metabolic state.[189] The cardiovascular dysfunction in women with PE/IUGR may therefore predispose them to long term CVD.

RM has also been inconclusively associated with hypertensive disorders in subsequent pregnancies. It is therefore possible though not proven that RM is also associated with cardiovascular dysfunction similar to PE. A previous study has suggested endothelial dysfunction and altered cardiovascular risk profile in women with RM, however this study included women with antiphospholipid antibody syndrome.[190] However, there is paucity of information on detailed cardiovascular function in women with unexplained RM.

It is known that RM, PE, IUGR and CVD share common risk factors. It is therefore, plausible that preexisting cardiovascular risk factors in women with RM or PE/IUGR may predispose them to impaired implantation, abnormal trophoblastic invasion and failure of the cardiovascular adaptation in pregnancy. Whether cardiovascular risk factors are the cause or effect of impaired cardiovascular adaptation in pregnancy is not yet determined. Only a prospective study from prior to pregnancy to the postpartum period could provide an insight into the cause and effect relationship of cardiovascular risk and pregnancy complications such as RM, PE or IUGR.

1.10 Feasibility study

In order to conduct a prospective pre-pregnancy to postpartum study powered to answer the questions related to ovulation, implantation timing, embryonic and fetal growth and cardiovascular adaptation in pregnancy complications, a large cohort of healthy women will need to be recruited. This would involve huge resources. A percentage of women may not conceive during the study period, a further third may miscarry and a significant number may drop out[37] and most women would have normal pregnancies.

A feasibility study is defined as a trial study carried out before a definitive study to assess the feasibility of the processes involved in the study and its management, resources needed, all of which are important for the success and add to the scientific value of the main study.[191] For a pre-pregnancy study the key processes are recruitment, retention and refusal rates, success rates of conception, rates of pregnancy loss, non-compliance rates, assessment of eligibility, time taken for the study and resources involved and pregnancy follow up rates. The scientific information regarding demographics, accuracy of the tests and extent of pregnancy changes using a pre-pregnancy baseline, usefulness and value of the non-invasive tests, blood tests, variability in the measurements from a feasibility study would enable establishing power for a future prospective study. This is the rationale behind this prospective feasibility study of ovulation implantation interval and maternal haemodynamics.

1.11 Hypotheses

1. Recurrent miscarriage is associated with delayed implantation and an adverse pre-conception cardiovascular risk factor profile

2. Women with a history of late complications in a previous pregnancy have delayed implantation in a subsequent pregnancy and an adverse pre-conception cardiovascular risk profile.

3. The timing of implantation is related to the maternal haemodynamic adaptation to pregnancy

Objectives

Primary: To assess the feasibility of both recruiting to and completing a future study of sufficient power to be able to test the hypotheses proposed above prospectively, using a cohort design.

Secondary: To be able to investigate the following relationships to help establish power calculations.

1. To establish whether a relationship exists between ovulation-implantation interval and subsequent pregnancy outcome.

2. To compare time from ovulation to implantation between women who have recurrent miscarriages, those with a previous late complication of pregnancy, and control healthy women.

3. To compare the pre-conception cardiovascular risk profile and cardiovascular changes in pregnancy between women who have recurrent miscarriage, those with a previous placental insufficiency, and control healthy women.

4. To characterize the changes in cardiovascular and metabolic function during pregnancy in relation to pregnancy outcome, using pulse wave reflection, aortic stiffness, central haemodynamics.

5. To compare the postpartum persistence of haemodynamic changes and changes in cardiovascular risk profile in relation to pregnancy outcome

CHAPTER 2: METHODS

This chapter is based on:

Mahendru AA, Everett TR, McEniery CM, Wilkinson IB, Lees CC. *The feasibility of prospectively studying maternal cardiovascular changes from before conception.* **Hypertension Research**. 11 April 2013;doi:10.1038/hr.2013.24

2.1 Design

This was a prospective cohort feasibility study combining measures of ovulation, implantation timing, fetal growth and maternal cardiovascular function from preconception to postpartum period.

2.1.1 Subjects

One hundred and forty three women planning to conceive were recruited in three groups: nulliparous women (70) or those with previous normal pregnancies who were healthy (35) (normal controls), history of unexplained recurrent miscarriages (RM) (26) and those with previous preeclampsia (PE)/intrauterine growth restriction (IUGR) (12). Additionally, three women were recruited in the PE/IUGR group for pre-pregnancy assessment but as they were not trying to conceive, they were not included within the cohort of 143 women for longitudinal study.

Unexplained recurrent miscarriage was defined as three or more consecutive miscarriages at less than 12 weeks, where investigations such as thrombophilia screen, cytogenetic examination of products of conception, pelvic ultrasound and parental peripheral blood karyotyping were negative and no specific problem was detected.[192]

PE was defined as diastolic blood pressure (BP) ≥ 110 mm Hg on ≥ one occasion, or ≥ 90mm Hg on ≥ two occasions at least 4 hours apart, in combination with significant proteinuria (≥ 300mg/ 24hours or 2+ dipstick; International Society for the Study of Hypertension in Pregnancy).[106]

IUGR was defined as birth weight below 3rd percentile,[193] in the absence of congenital infection or aneuploidy with abnormal umbilical artery Doppler, defined as umbilical artery pulsatility index (PI) of greater than 95th centile[194] and/or either absent or reversed end diastolic flow.

Participants were between 18-50 years of age and were not known to have thrombophilia or diabetes. One out of 15 women with previous PE/IUGR and one multiparous woman in the control group were known to be previously hypertensive and their hypertension was well controlled on antihypertensive medication.

Women with PE/IUGR in previous pregnancies have a higher recurrence rate of PE and IUGR in a subsequent pregnancy[195-197] and women with RM are also believed to have an increased risk of uteroplacental insufficiency in subsequent pregnancy.[7-9] We included both these subgroups of women in order to identify differences in their pre-pregnancy cardiovascular function and cardiovascular adaptation during pregnancy.

2.1.2 Recruitment of the participants (Figure 2.1)

Recruitment was achieved by advertisements over a period of 11 months from July 2010 to June 2011. Seventy nulliparous and 35 multiparous women with previous normal pregnancies (controls) were recruited prospectively in the study when they started trying to conceive. Study posters were put up in various wards and corridors in the Addenbrooke's hospital, in 95 GP surgeries in Cambridgeshire, on the Rosie hospital website, in the local newspapers, BLISS and APEC website (See Appendix 2). Furthermore, the study was advertised in preschool groups, NCT newsletter and in local GP newsletter. Information about the study was conveyed to GPs via the hospital GP liaison team and to the staff on the early pregnancy unit. The advertisements had the direct contact details of the lead research fellow and women who were interested to find more information would contact the research fellow by phone or email. Participants were given information about the visit by email or over the phone prior to each visit in advance.

One hundred and twenty five women with unexplained recurrent miscarriage were identified from the clinic lists and 250 women with previous PE and IUGR delivered in the past 4 years were identified from the delivery database and hypertension clinic & were invited to participate in the study. These women were sent invitation letters with reply slip to be returned (See Appendix 3) or to contact the lead research fellow by email or phone.

All 184 women who approached the research fellow were given the participant information sheet (See appendix 4) and detailed explanation about the study either by email, personally or over the phone. 146/184 were suitable and consented to participate in the study. Three women who were not actively trying in the PE/IUGR group were only included for their pre-pregnancy data but were not included in the prospective study. Women who were not pregnant

at the end of 6 cycles of the study were re-recruited if they wished to continue with the study. The total number of women including the women who were re-recruited following their first 6 unsuccessful cycles (n=27) or following an early pregnancy loss in their first pregnancy (n=11) in the study was 184.

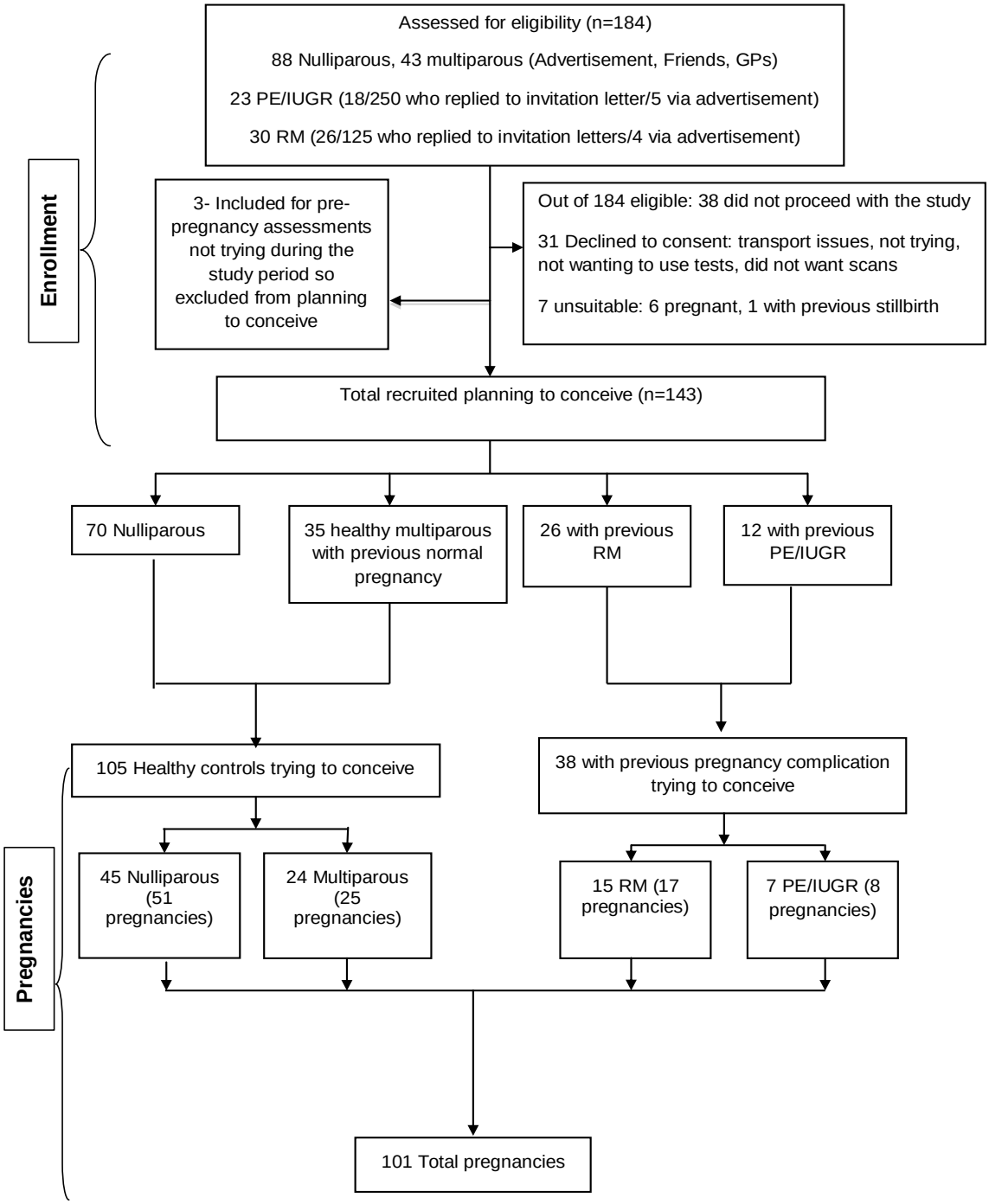


Figure 2.1 Flow chart of recruitment to the study

2.2 Study Protocol (Figure 2.2)

Women were recruited prior to conception and written consent was obtained at the time of recruitment or at the first research visit prior to any procedures/assessments. Baseline cardiovascular assessment and blood tests were performed as described below. They then performed digital home urine ovulation and pregnancy tests as described below. Women who became pregnant were followed up longitudinally with serial ultrasound scans and repeat cardiovascular assessments in very early pregnancy, second and third trimester and 3 to 4 months postpartum. Women who were re-recruited following a miscarriage or an ectopic pregnancy did not undergo repeat baseline cardiovascular assessments.

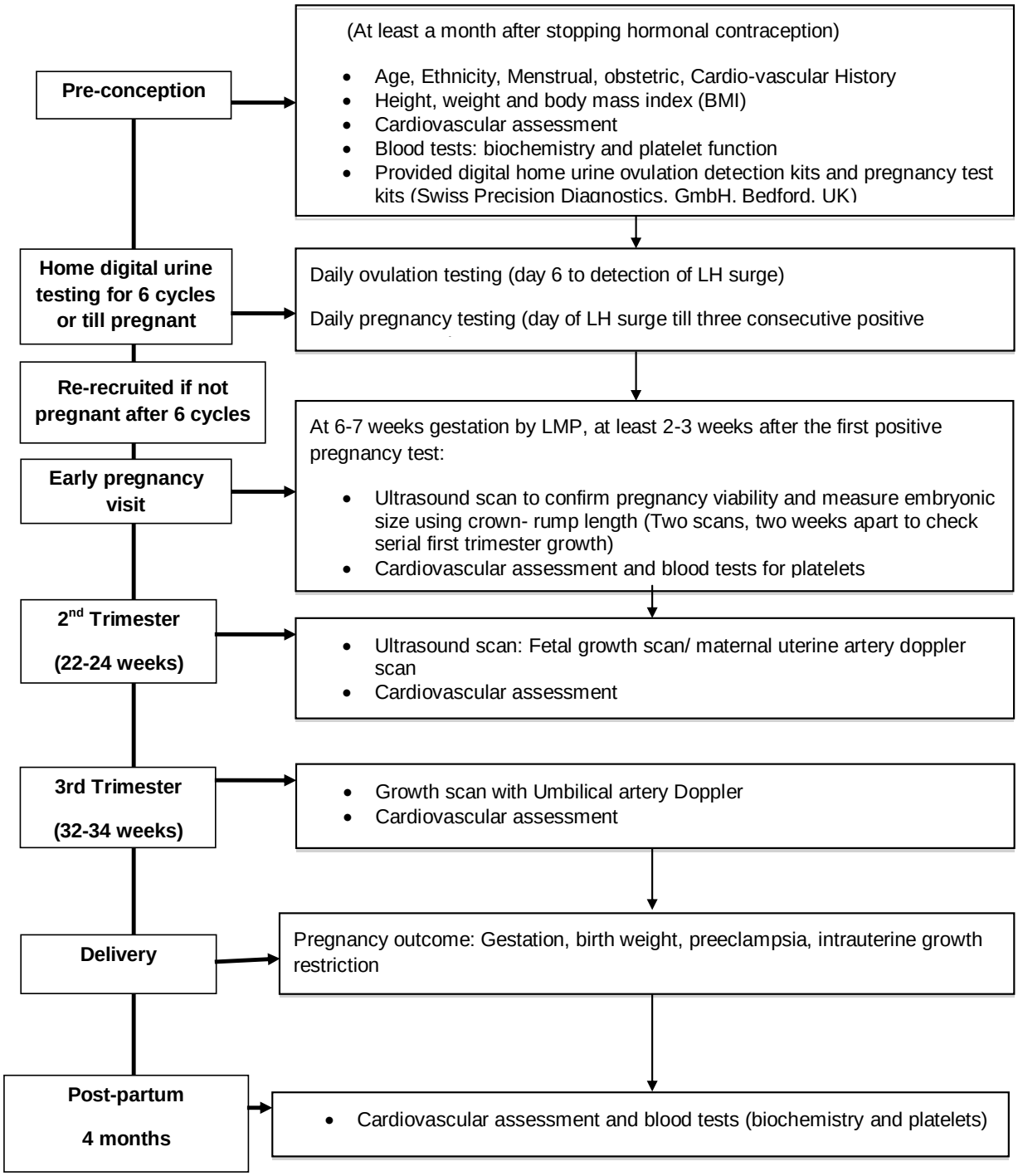


Figure 2.2 Summary of the study protocol

2.2.1 Setting

The study was undertaken at Addenbrooke's Hospital. The cardiovascular assessments and blood tests were performed in the vascular research clinic in the University of Cambridge and the ultrasound scans during the pregnancy were performed in the Rosie Ultrasound Department, Maternal Fetal Assessment Unit or on Daphne ward (Early Pregnancy Unit) in the Rosie Hospital. Participants were provided with car park tickets and travel expenses when necessary.

2.2.2 Ethics and data governance

The study received ethical approval from the Cambridgeshire 1 Research Ethics Committee (REC Ref. no. 10/H0304/28) (Appendix 1). The data collected from the study was anonymized to study numbers, the key to the study numbers from patient hospital numbers was kept in a paper diary marked as 'IMPOST' and locked in a safe non-clinical filing cabinet within Fetal Medicine Department. All data was entered on an access 2007 database, which was accessible on a password protected secure hospital server.

2.2.3 Pre-pregnancy visit

The first pre pregnancy visit was conducted at least a month after stopping contraception and in the luteal or follicular phase of the cycle. After taking written informed consent from these women personal details such as maternal age, ethnic group, smoking, detailed menstrual, obstetric and cardiovascular history, height, weight, body mass index (BMI) were recorded using a participant's questionnaire (Appendix 6). A copy of the consent form and the questionnaire were stored securely in the hospital in the research files.

2.2.4 Cardiovascular tests

Cardiovascular measurements were performed after 10 minutes rest in the supine left lateral position with arms relaxed and by their side with their head supported and legs uncrossed. All measurements were performed in a quiet, temperature-controlled room (21°C to 23°C). Participants were requested to abstain from caffeinated drinks for 4 hours before the visit and be fasted for at

least 4 hours before the first visit to enable performing blood tests for lipids and cholesterol. Cardiovascular function was assessed non-invasively by measuring brachial blood pressures (BP), central blood pressures (BP), pulse wave reflection quantified by augmentation index (AIx) (described later), aortic stiffness measured by carotid femoral (aortic) pulse wave velocity (aPWV), cardiac output (CO), cardiac index (CI) and stroke volume (SV).

Peripheral Blood pressures: Brachial BP and heart rate (HR) were measured in the non-dominant arm using an automated BP measuring device (Omron-M7) that has been validated for use in pregnancy using standard cuff size according to the arm circumference of the woman.[198]

Pulse wave reflection and central haemodynamics using pulse wave analysis: Central BP and arterial stiffness were investigated using pulse wave analysis with the SphygmoCor device (AtCor Medical, Sydney, Australia) (Figure 2.3). This technique of pulse wave analysis developed by Michael O'Rourke,[199] utilizes applanation tonometry to record high fidelity waveforms from peripheral arteries. The principal behind this is that when the curved surface of a cylinder is flattened, circumferential pressures are equalized and intraluminal pressures can be recorded accurately (Figure 2.4).

This device consists of a hand-held tonometer with a high-fidelity micromanometer located on its tip (SPC-301; Millar Instruments, USA); Radial artery waveforms were recorded by placing the tonometer on the wrist of the non-dominant arm (Figure 2.4)

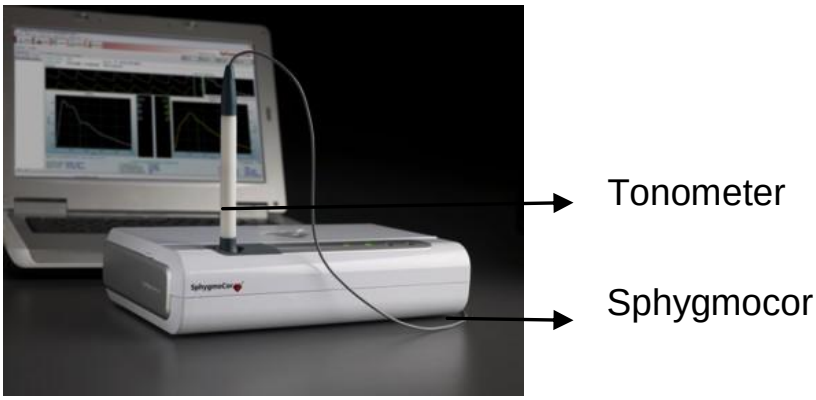


Figure 2.3: SphygmoCor device (AtCor Medical, Sydney, Australia) (From AtCor Medical website/ SphygmoCor technology /www.atcormedical.com/sphygmocor.html)

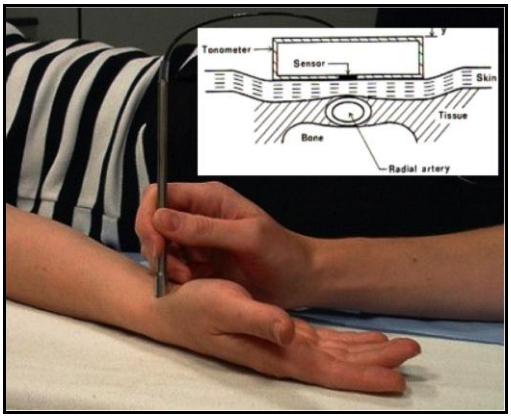


Figure 2.4 Recording of the radial artery waveform by placing the hand-held tonometer on the radial artery and using the principal of applanation tonometry (shown in the box on the right corner)

The recorded waveforms were calibrated by using the brachial systolic and diastolic BP, measured as above. A corresponding central waveform is generated with a validated transfer function (SphygmoCor; AtCor Medical, Sydney, Australia) (Figure 2.5).[199,200] Central systolic and diastolic BPs, mean arterial pressure (MAP), aortic augmentation index (AIx) were determined using the integrated software.

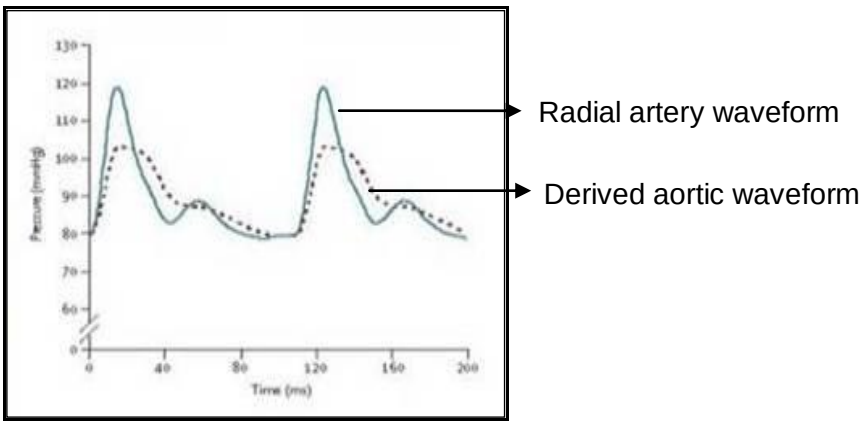


Figure 2.5: Pulse wave analysis using radial artery waveform. The green graph is the radial artery waveform and the dotted black graph is the aortic waveform derived by the computerized software using the generalized transfer function

Central BP can be measured non-invasively using applanation tonometry. A transcutaneous pressure transducer at the end of a probe is held against an artery to flatten the artery in order to equalize circumferential pressures and obtain pressure waveforms, which are identical to those obtained by intra-arterial measurements.[123,201] From these waveforms, central BP is estimated non-invasively either by using the carotid waveform as a surrogate for that of the aorta or by using a generalized transfer function where using a mathematical formula, an aortic waveform is derived from a radial waveform.[200] The second method is the most commonly used method because it is easy to use and has been shown to have repeatability.

Augmentation index (AIx), is a method of quantifying pulse wave reflection and it is the difference between the second and first systolic peaks (also known as augmentation pressure AP), expressed as a percentage of aortic pulse pressure.

AIx =100 x AP/PP (Figure 2.6).

The first systolic peak is the maximum pressure created by the forward going pressure wave and the second is a composite of forward and backward-going waveforms. Since the amplitude and velocity of wave reflection depends on the large artery stiffness, AIx provides an indirect measure of large artery stiffness.[202] Figure 2.6 is the schematic representation of derivation of AIx.

All measurements were performed at least twice and a third reading taken if the difference between two consecutive readings was more than 5 units and for waveforms derived using the SphygmoCor device operator index of at least more than 80 (quality control contained within the software) was considered acceptable. The average of the two or three readings was used for subsequent analysis.

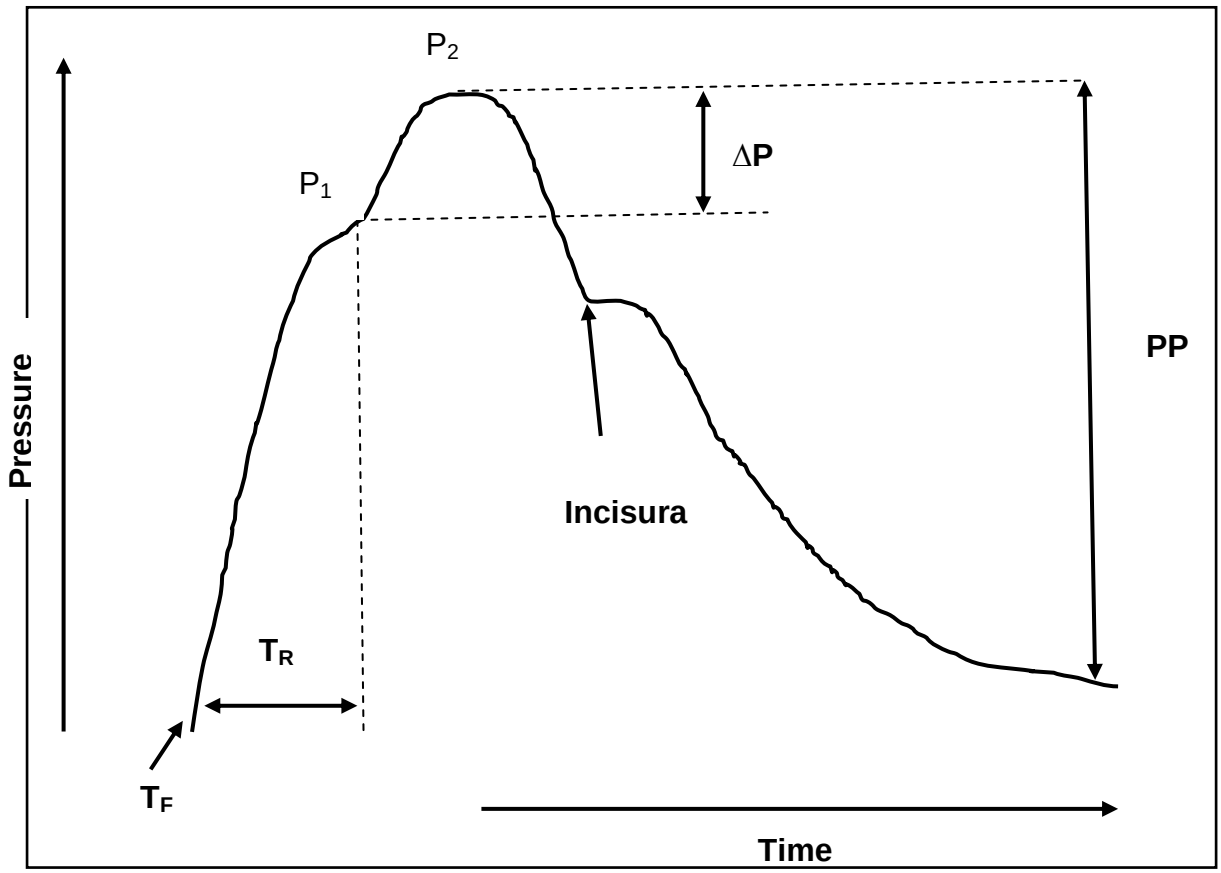


Figure 2.6 AIx representation and derivation of AIx: A typical central aortic pressure waveform- AIx= PP/ΔP. Augmentation index (AIx) is calculated as the difference between systolic peaks: P1 and P2 (ΔP), expressed as a percentage of the pulse pressure (PP). Ejection duration is calculated as the time between the foot of the wave (T_F) and the incisura, and T_R defined as the time between T_F and the inflection point. (Schematic figure adapted from Wilkinson I.B. Thesis title: Arterial stiffness, endothelial function and cardiovascular disease. Green College, Oxford. 2003)[203]

Aortic stiffness assessed by Carotid-femoral pulse wave velocity: Carotid-femoral PWV is the 'gold standard' measurement for arterial stiffness and has the largest amount of epidemiological evidence for its predictive value for cardiovascular events.[204] Carotid-femoral PWV was recorded non-invasively using SphygmoCor device (AtCor Medical, Sydney). For each subject the carotid-femoral distance was measured using calipers. ECG electrodes were attached to the participant to measure HR. After palpating the maximum palpable carotid artery pulse and femoral artery pulse the carotid-femoral path length was calculated using calipers. The carotid femoral path length = Distal

(distance in mm between the supra-sternal notch and the femoral artery site) – proximal distance (distance in mm between the supra-sternal notch and the carotid artery site). Two good quality and steady waveforms were obtained giving duplicate readings using the tonometer at the carotid and femoral site for at least 15 seconds. If readings differed by >0.5m/s then a third reading was taken & the two closest readings were averaged. The recording was obtained as shown in Figure 2.7.

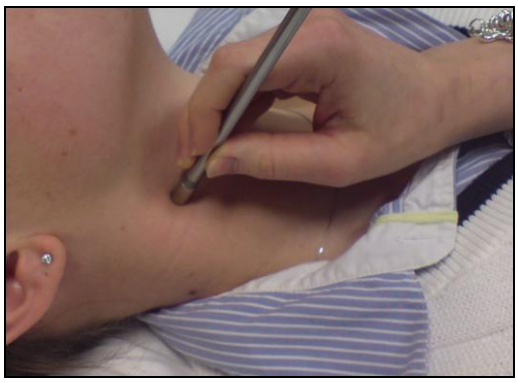


Figure 2.7 Measuring aPWV by placing the tonometer on the carotid followed by the femoral pulse

The carotid-femoral PWV was derived by the SphygmoCor from the carotid-femoral distance and the time delay between the foot of the carotid waveform and that of the femoral waveform obtained from the pressure waveforms at the carotid and the femoral site as shown in Figure 2.8.

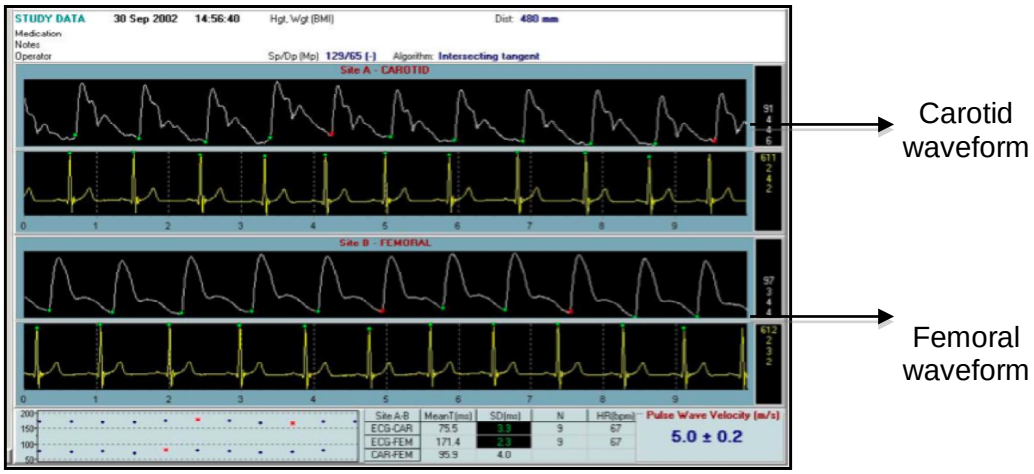


Figure 2.8 Recording of aortic pulse wave velocity: screen showing the recording of carotid waveform at the top and femoral waveform at the bottom part of the screen alongside an ECG analysis of heart rate

Cardiac output measurement: Cardiac output (CO) was assessed using a non-invasive, inert gas re-breathing technique known as Innocor, Innovision A/S, Denmark. (Figure 2.9) CO is the volume of blood pumped by the heart per unit of time (litres/minute). Innocor utilises pulmonary gas exchange as a means of measuring the pulmonary blood flow using a mixture of soluble and insoluble gases- some of which may be absorbed across the lungs.

Briefly, while resting, the subject continuously re-breathes a gas mixture: 1% sulphur hexafluoride (SF_6), 5% nitrous oxide (N_20) and 94% oxygen (O_2) over 20 seconds, with a breathing rate of 15/min (Figure 2.9). Expired gases were sampled continuously and analyzed by an infrared photoacoustic gas analyzer (InnoCor, Innovision A/S, Denmark), for the determination of CO and SV. The rate of disappearance of nitrous oxide (N_2O) is proportional to the pulmonary blood flow. Hence, the higher the cardiac output, the higher the disappearance rate of this gas. The inert/insoluble gas in the bag is sulphur hexafluoride and as it does not cross the lungs; it is used to quantify the lung volumes from which the N_2O is removed. A pulse oximeter simultaneously determines the HR from which SV (i.e. the volume of blood ejected per heartbeat) is computed by the machine. The machine also deduced cardiac index (CI). It is non-invasive, simple and easy to use- using the touch screen. This method has been validated against thermodilution in patients with cardiac failure.[205]

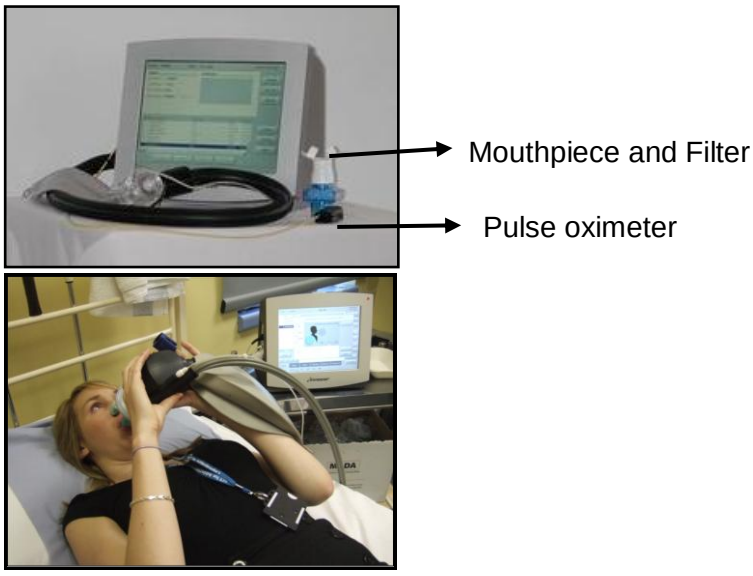


Figure 2.9 Innocor Gas re-breathing device (The top figure shows the device and the bottom one demonstrates the usage of the device)

Peripheral vascular resistance: Peripheral vascular resistance (PVR) was calculated from the formula:

$$PVR\ (dynes.s\text{-}^{1}.cm^{-5}) = MAP\ (mmHg) \times 80 / CO\ (L/min)$$

Variability of cardiovascular measurements: All measurements were performed in duplicate by a single, trained investigator (AAM), and the mean values were used in subsequent analyses. The within observer measurement reproducibility values for the AIx and aPWV described below were in agreement with previously published data.[206] The arterial wave reflections are believed also to have moderate reproducibility during the different phases of menstrual cycle (within subject absolute differences of AIx upto 10% between the different phases of menstrual cycle).[207] It has been shown that AIx is lower in the luteal compared to the ovulatory phase however overall the wave reflections and all other indices including central BP do not appear to vary significantly during the menstrual cycle.[126,207] Therefore, pre-pregnancy cardiovascular assessments were performed at any time in the menstrual cycle to make recruitment more flexible.

Intra-observer variability for AIx measurements: In 15 women (combination of pregnant and non-pregnant), the overall mean AIx was 13% ± 9% (95%CI: 8%, 18%). The mean difference between the readings was -0.5 ± 1.7% with no significant difference between the two readings (*P*=0.3) as has been shown before.[206]

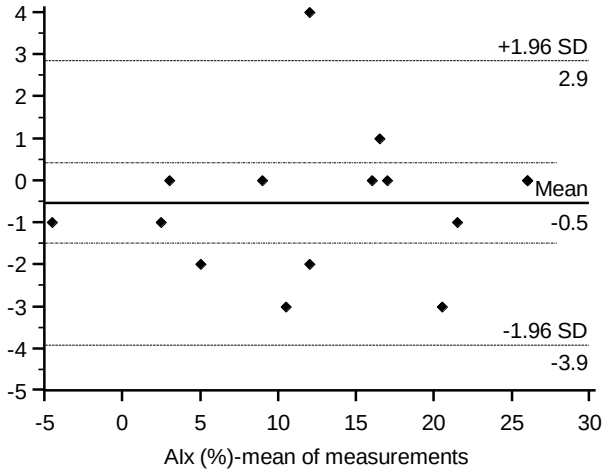


Figure 2.10 Within observer variability for AIx measurements using a Bland-Altman plot

Intra-observer variability for aPWV measurements: In 15 women (combination of pregnant and non-pregnant), the overall mean aPWV was 5.07 ± 0.44 m/s (95%CI: 4.8, 5.3). The mean difference between the readings was -0.01 ± 0.27m/s with no significant difference between the two readings (*P*=0.9) as has been shown before.[206]

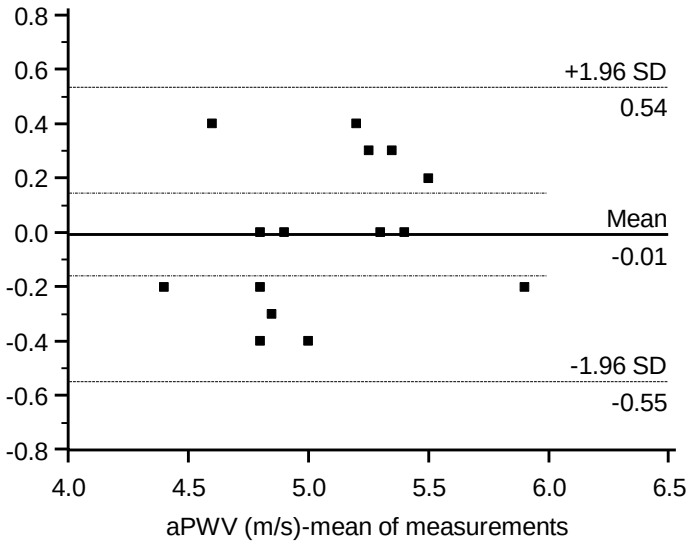


Figure 2.11 Within observer variability for aPWV measurements using a Bland-Altman plot

CO measurements: In 43 women (13 non-pregnant i.e. prior to pregnancy or in the postpartum period, 20 in second trimester and 10 in third trimester). The overall mean CO was 6.2 ± 0.4 L/min (range: 4.5-8.7 L/min), mean CI was 3.4 ±0.2 L/min per m^2 (range: 2.5-5 L/min per m^2) and mean SV was 83 ± 5 ml (range: 56-110 ml). There was no trend of the values to vary with any of the analyses. The within-observer differences (mean ± 2SD) were 0.10 ± 0.5 L/min for CO (95% CI of mean differences: -0.9,1.1) (Figure 2.12a), 0.07 ± 0.3 L/min per m^2 for CI (95% CI of mean differences:-0.5, 0.6)(Figure 2.12b) and for SV were 0.9 ± 7ml (95%CI: -12.9, 14.7) (Figure 2.12c). There was no difference in the values for reproducibility between the two readings of CO (P=0.2), CI (P=0.1) and SV (P =0.4) and the coefficient of variation between the two readings of CO, CI and SV was <6%.

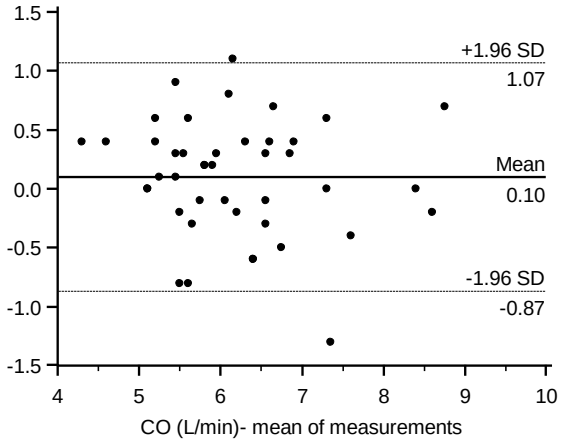


Figure 2.12a Within observer reproducibility of CO measurements using a Bland-Altman plot

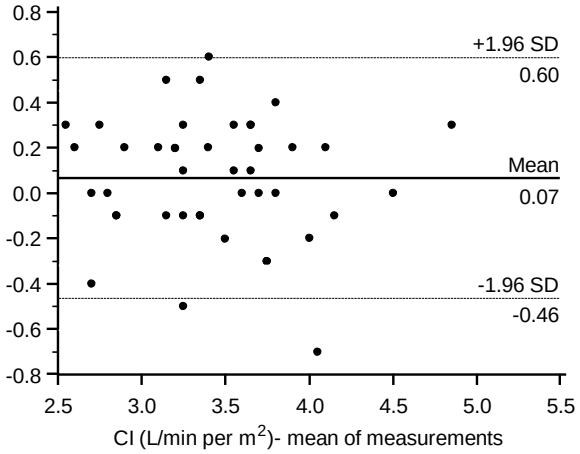


Figure 2.12b Within observer reproducibility of CI measurements using a Bland-Altman plot

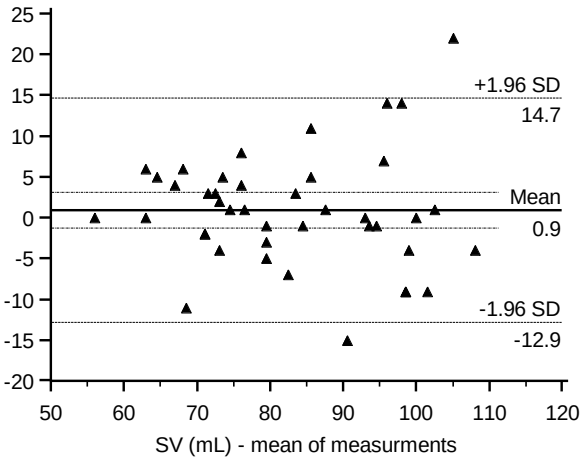


Figure 2.12c Within observer reproducibility of SV measurements using a Bland-Altman plot

2.2.5 Metabolic and kidney function assessment- blood and urine tests

In order to investigate the presence of unfavourable cardiovascular and metabolic risk profiles prior to pregnancy which may predispose to both preeclampsia and cardiovascular disease- baseline lipid profile, renal function, HbA1c were performed and the urine was tested to assess urine albumin creatinine ratio (UACR) (Urine Albumin-to-Creatinine Ratio (UACR).[208-210]

Blood samples were collected in accordance to the Addenbrooke's Hospital venepuncture guidelines: 5 millilitres (ml) in plain bottle, 2.5ml in yellow top fluoride bottle, 2.5ml in pink top EDTA bottle and 5ml in hirudin tube. The clotted blood was tested for total cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol and total triglycerides (TG); for renal function tests such as serum creatinine, estimated glomerular filtration rate (eGFR), urea and electrolytes. Blood sample in EDTA was tested for HbA1c and the sample in the fluoride bottle was analysed for serum glucose. These biochemistry tests were performed and results interpreted as per standard hospital protocols.(Table 2.1) The blood sample in hirudin tube was analysed for platelet aggregometry (described below).

These blood tests were performed at the first pre-pregnancy visit and repeated at 3 to 4 months postpartum. The platelet aggregation was repeated separately at 6 to 7 weeks in early pregnancy to investigate changes following implantation. The lipid profile and renal function tests were repeated separately at 10-14 weeks along with the routine blood tests to investigate changes in lipids and renal function in the first trimester.

Table 2.1 Reference ranges for blood biochemistry results (Addenbrooke's Hospital biochemistry ranges and BNF for lipid/cholesterol profile)

Test	**Reference range** (N adult female >18yrs)
Full Lipid Profile: Serum cholesterol HDL LDL TG	 <6mmol/L- normal Se. cholesterol ≥ 6mmol/L then refer to non-smoker/ under 50yrs chart of TC: HDL ratio (BNF-Cardiovascular risk prediction charts- see below figure 1) and inform GP
Serum creatinine Serum Urea eGFR Na^+ & K^+	35-12µmol/L <7.5mmol/ L ≥ 60ml/min/1.73m^2 Na^+: 135-145mmol/L & K^+: 3-4 to 5mmol/L
HbA1c Random Glucose	4.9-6.3% (non-diabetic) 3.5-5.5 mmol/L (Fasting) 3.5-9.0 mmol/L (Non-Fasting) >7mmol/L – Inform GP

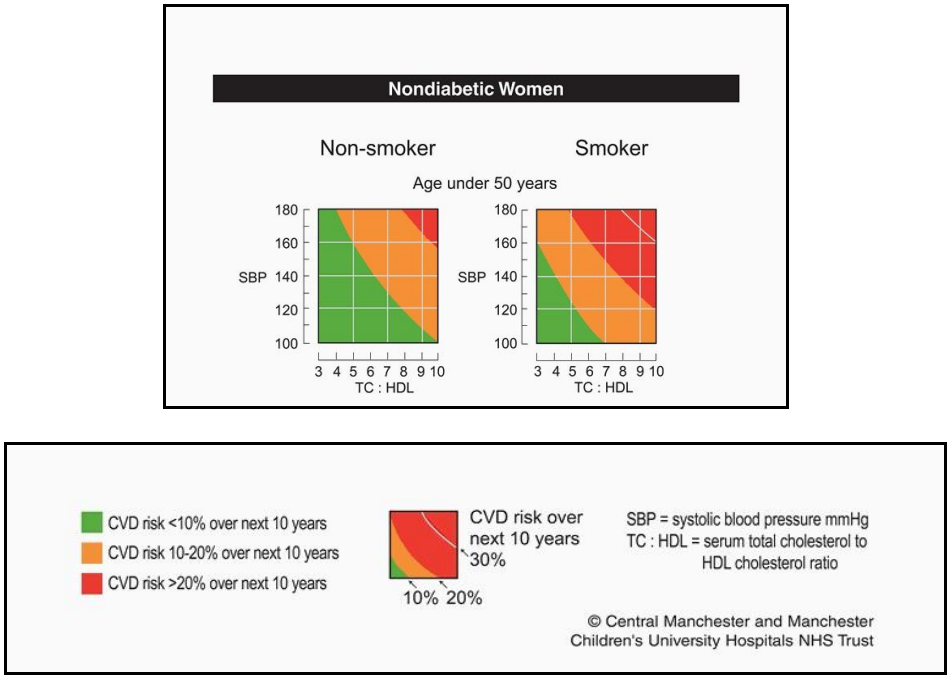


Figure 2.13 Cardiovascular risk prediction charts based on Total cholesterol:HDL cholesterol ratios. (Adapted from Wood D.A. Heart 2005; 91(supplement V: V1-V52)[211])

Urine test: Urine was tested for albumin/ creatinine ratio using urine dipstick (Siemens microalbustix). Spot protein creatinine ratio (UPCR) has good correlation with 24-hour urine protein excretion in kidney disease however urine albumin creatinine ratio (UACR) is useful to diagnose and monitor microalbuminuria. It is also used as a cardiovascular risk predictor.[208,209] The NKDEP (National Kidney Disease Education Program) suggests that albuminuria is present when UACR is greater than 30mg/g and is a marker of CKD (Chronic kidney disease) (Urine Albumin-to-Creatinine Ratio (UACR). (NKDEP factsheet; March 2010)

Table 2.2a Urine Microalbustix interpretation of albumin and creatinine depending on the colour on the strip (Adapted from Siemens Microalbustix Label. Product ref no. 04960872 (2087)

Creatinine (60 sec) mg/dl mmol/L g/dL	10 0,9 0.01	50 4,4 0.05	100 8,8 0.1	200 17,7 0.2	300 26,5 0.3
Albumin (50 sec) mg/L mg/dL	10 1		30 3	80 8	150 15

Table 2.2b Urine Microalbustix interpretation of results (Adapted from Siemens Microalbustix Label. Product ref no. 04960872 (2087)

Albumin mg/L	**Creatinine**				
	10	**50**	**100**	**200**	**300**
10	Recollect specimen	Normal	Normal	Normal	Normal
30	Slight abnormal	Abnormal	Abnormal	Normal	Normal
80	Medium abnormal	Abnormal	Abnormal	Abnormal	Normal
150	High abnormal	High abnormal	Abnormal	Abnormal	Abnormal

Table 2.3 Calculated value of Urine Albumin-to-Creatinine Ratio (UACR) in mg/g= Albumin excretion in mg/day

Albumin mg/dL	**Creatinine (g/dL)**				
	0.01	0.05	0.1	0.2	0.3
1	100	20	10	5	3.33
3	300	60	30	15	10
8	800	160	80	40	26.7
15	1500	300	150	75	50

2.2.6 Platelet function assessment[212,213]

Platelet function was assessed by whole blood aggregation using Multiple Electrode Aggregometry (MEA) on a new generation impedance aggregometer (Multiplate Analyzer, Dynabyte Medical, Munich) (Figure 2.14).

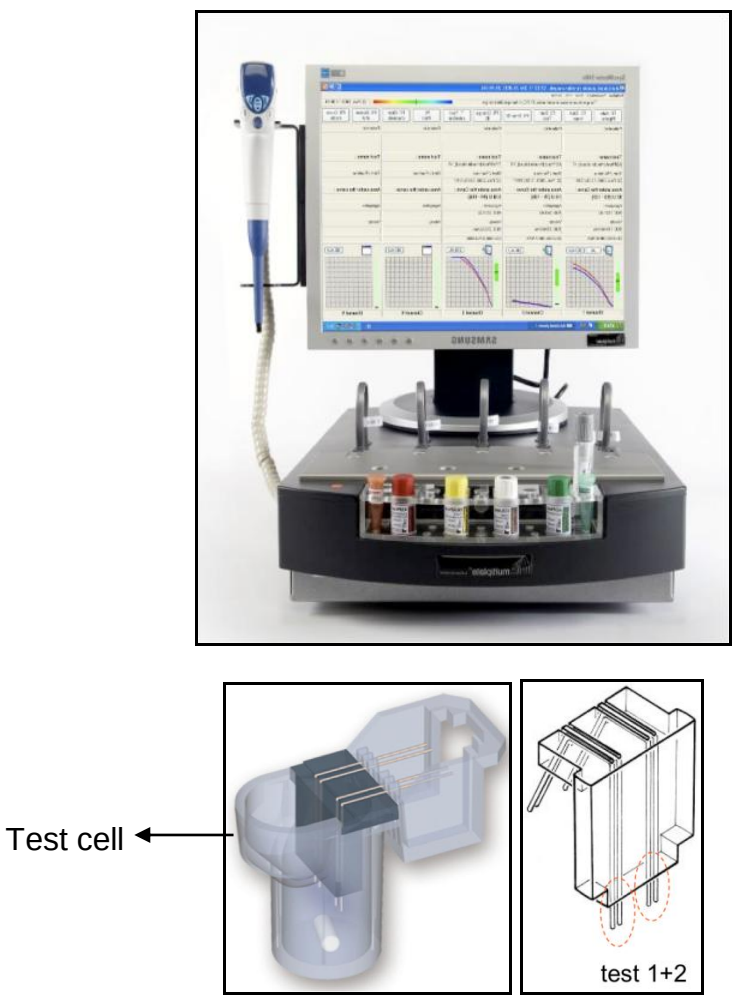


Figure 2.14 Multiplate® Analyzer, Dynabyte Medical, Munich (Adapted from multiplate® international marketing, Multiplate Services, GmbH, Munich, Germany-company information leaflet)

The blood platelets are non-thrombogenic in their resting state, but have exposed receptors on their surface which when activated, allow them to attach on the vascular injury and artificial surfaces. This system detects the electrical impedance change due to the adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette or test cells (Figure 2.15).

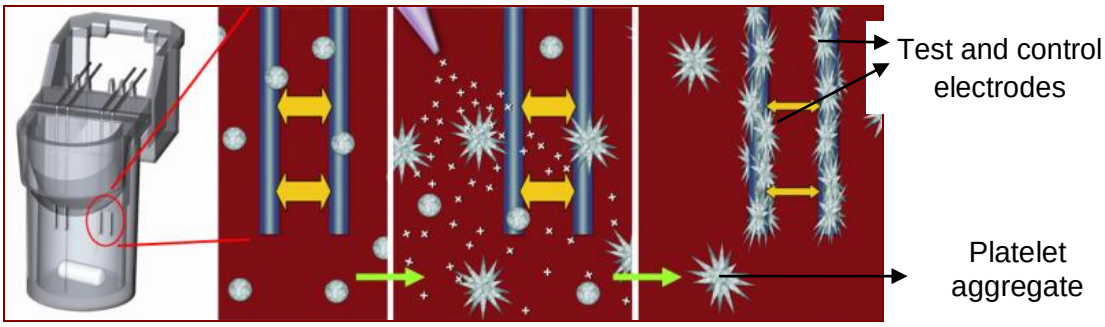


Figure 2.15 Aggregation of platelets on the electrodes in the test cells (Adapted from multiplate® international marketing, Multiplate Services, GmbH, Munich, Germany-company information leaflet)

The blood sample was collected in the hirudin tube, containing recombinant hirudin; which anticoagulates blood by direct inhibition of thrombin and allows platelet function analysis under physiological calcium concentration. Adenosine diphosphate (ADP) was used as an agonist for platelet activation. ADP was prepared by addition of 1ml purified water to ADP test vial and stored at 2-8°c for 7 days or at -80°c for 4 weeks.

A 1:2 dilution of whole blood anticoagulated with hirudin and 0.9% NaCl was stirred and incubated at 37°c for 3 minutes in the test cells. After addition of ADP agonist in serial dilution (10µl, 5µl, 2µl, 1µl and diluted ADP 1µl) to the incubated blood in the test cells, the increase in electrical impedance was recorded continuously for 6 minutes. The increase in impedance upon addition of ADP and platelet aggregation is transformed into arbitrary aggregation units (AU) and plotted against time. The most important parameter is the area under the aggregation curve (AUC). It is affected by the total height of aggregation curve as well as by its slope and is best suited to express the overall platelet activity (Figure 2.16). The other parameters reported are the aggregation as represented by the height of the curve and the velocity, which is the maximum slope of the curve. The internal quality control in the software gives the mean values of the curves from the two independent sensors in the Multiplate®- test cell.

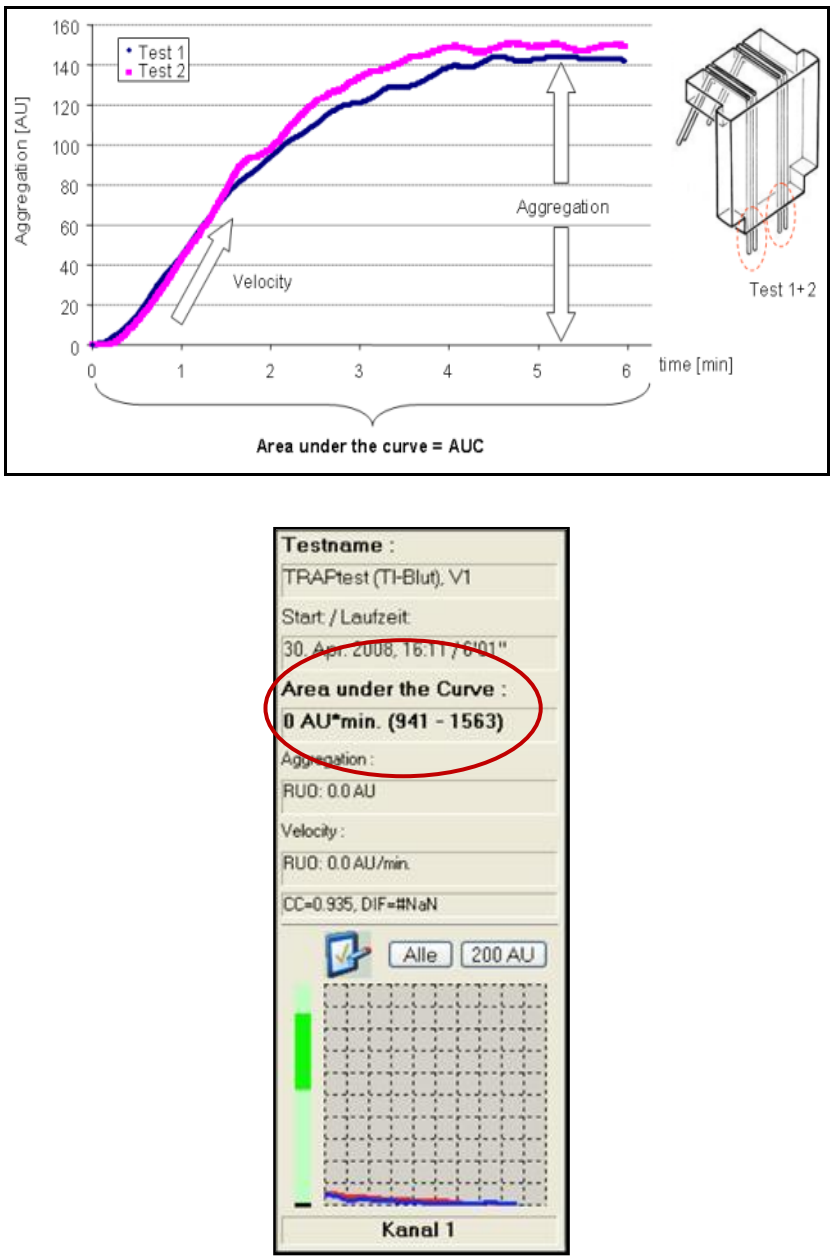


Figure 2.16: Description of derivation of the area under curve for platelet aggregation (AUC) and Aggregation Vs Time (Adapted from multiplate® international marketing, Multiplate Services, GmbH, Munich, Germany-company information leaflet)

2.3 Use of home ovulation and pregnancy test kits

Participants were given digital home ovulation and pregnancy test kits to identify the ovulation timing, implantation timing and ovulation to implantation interval (O-I interval). Tests were provided by Swiss Precision Diagnostics, GmbH (SPD), Bedford (Figure 2.17). They were instructed to use daily ovulation tests from the 6th day of their cycle until luteinizing hormone (LH) surge was detected in the urine identified by the 'smiley face' on the digital reader and perform daily pregnancy tests from 8 days after the LH surge (Figure 2.18) until they recorded three consecutive positive pregnancy tests or until their next period. These kits were provided with the menstrual diary and written instructions for initially three cycles (See appendix 5) and if the participants needed more for another three cycles then additional kits were sent either by post or personal collection. Participants were advised to contact the research fellow when they had three consecutive positive pregnancy tests to organize the early pregnancy visits and/or if they needed any more tests or any advice regarding the testing. Once pregnant the participants were followed up as described below however if they were not pregnant at the end of 6 cycles they were offered re-recruitment for another 3 to 6 cycles depending on the feasibility of the study time limits.

Figure 2.17 Ovulation and Pregnancy test kit pack with menstrual diary and information sheets provided to the participants

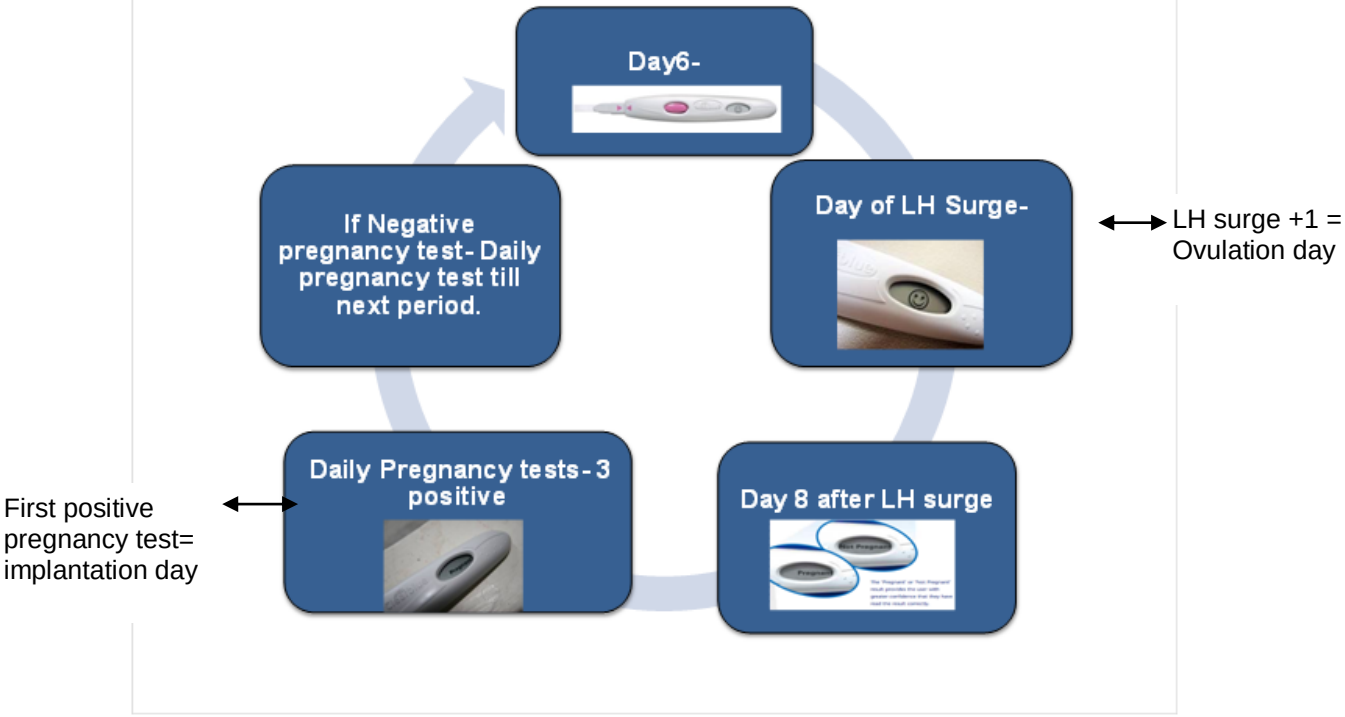


Figure 2.18 Flow chart: Instructions regarding use of ovulation and pregnancy tests in each menstrual cycle (LMP= Day 1 of the cycle). (The test results as shown in the figure have been adapted from the Clearblue- company information sheet)

A rise in urinary LH predicts ovulation at a mean of 20 hours from the initial LH rise. We therefore calculated the 'LH surge + one day' to define the day of ovulation as has been previously described.[46,214] The day of the first positive pregnancy test using sensitive digital urine pregnancy test kits was reported as the 'implantation day' in a similar way to previously described.[6] The ovulation-implantation (O-I) interval was calculated as the time interval between the presumed day of ovulation as indicated by the urinary LH test kits and the first positive pregnancy test.

Establishment of Ovulation to implantation interval in pregnancies: Ovulation to "implantation" interval was estimated in these women using the day of LH surge and the first positive pregnancy test from their menstrual diary data. (See Appendix 6). This was recorded in the data collection form at the first early pregnancy visit or if the subject miscarried, they were offered the option of returning the data by post.

2.4 Ultrasound scans and pregnancy follow up

All ultrasound scans were reported using the Astraia® database and all participants were informed of the results and given the written report of the scan. Any pregnancy complication diagnosed on the scan or any abnormal scan result was managed according to the Rosie Hospital protocol.

2.4.1 Early pregnancy scans

The first early pregnancy visit was arranged at 6 to 7 weeks gestation by LMP similar to previous pre-pregnancy studies,[24,25] or from the ovulation day in case of irregular cycles. In order to demonstrate that we performed the cardiovascular measurements very early in pregnancy, at about 2 to 3 weeks after the first positive pregnancy test, representative of the 'implantation day' we have also reported the timing of the tests in days from the first positive pregnancy test. The purpose of the first scan was to assess gestational age, number, viability of the embryo and this gestational age was subsequently confirmed by the fetal crown rump length (CRL) measurement at 10-14 weeks.

Ultrasound scans were performed at 6-7, 8-9 weeks, and 10-14 weeks of gestation from either the LMP or gestational age estimated by ovulation. At each pregnancy visit gestational age (GA) was reported from LMP (GA^{LMP}), GA adjusted by ovulation timing and implantation timing (GA^{OV} and GA^{IMP}) respectively. GA adjusted for ovulation timing (GA^{OV}) was derived by subtracting 14 days from the predicted ovulation date (LH +1) to the effective LMP, as is convention for pregnancy dating. The median implantation day was 27 days and the GA^{IMP} was derived by adjusting to day 27 implantation. In other words if implantation occurred later than day 27 then the GA adjusted for implantation timing (GA^{IMP}) was obtained by subtracting the additional number of days from GA^{LMP}. Women with ongoing pregnancies at 10-14 weeks were followed up subsequently.

Early pregnancy transvaginal scans: The transvaginal probe utilizes frequencies of 7.0-8.0 MHz and is the preferred method of pregnancy assessment in the first 9 weeks of gestation. Transvaginal ultrasound scans were performed using Toshiba Xario/SSA-660A, Siemens ACUSON Antares, Toshiba (Powervision-6000)/SSA -370A, Hitachi Aloka (ProSound Alpha 7) depending on the availability of the machines. A gestational sac was located

by examining the entire uterus in a true longitudinal view of the uterus, with optimal longitudinal view of the uterine cavity, endometrium and cavity line. The site, size, number of gestational sac along with the decidual reaction and contents of the gestational sac such as the yolk sac, embryonic pole and fetal heart beat were identified (Figure 2.19)

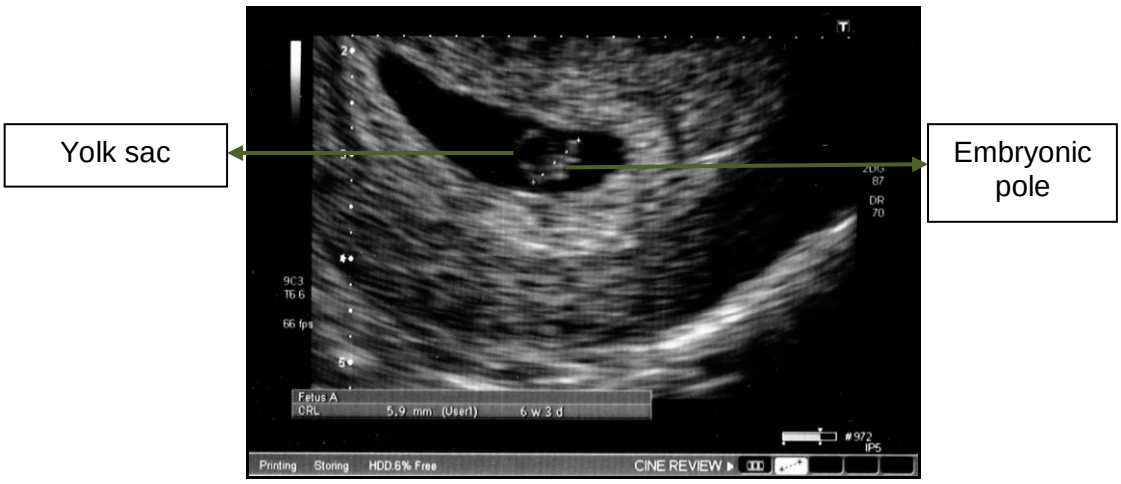


Figure 2.19 Transvaginal scan at 6 weeks showing singleton pregnancy, yolk sac and embryo

The mean gestational sac and crown rump length measurements were taken. As measurements of yolk sac do not correlate to the outcome of pregnancy only its presence or absence was reported. Mean gestational sac diameter was measured in three planes: the maximum longitudinal diameter (L) together with the maximum antero-posterior diameter (AP) was measured in the true longitudinal section and in a cross-sectional view maximum transverse diameter was obtained (Figure 2.20). The MSD (Mean Sac diameter = [L (cm) + AP (cm) + T (cm)]/ 3 was calculated from Astraia® once the measurements were inserted into the system. The values were recorded for the study as continuous variables and the mean gestational age derived from the charts by Grisolia et al (1993) [215]

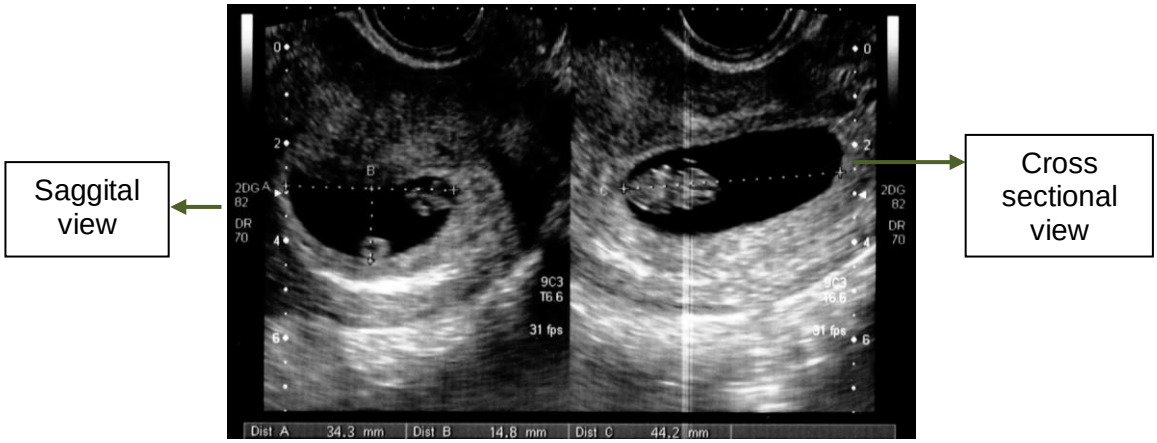


Figure 2.20 Gestational sac measurements in saggital (longitudinal and antero-posterior dimensions), and cross sectional (Transverse) view

Crown Rump length measurement: Three CRL measurements of a true, unflexed, longitudinal section of the embryo or fetus were taken by obtaining a longitudinal section of the uterus and gestation sac. The viability of the pregnancy was ascertained by the presence or absence of embryonic heart pulsations at this stage. The CRL was measured transvaginally at 6-7 and 8-9 weeks by placing the callipers at the outer side of the crown and rump of the embryo or fetus in a longitudinal, midsagittal section.[216,217] Of three CRL measurements taken, the one that most closely conformed to the standard described above was used for the analysis.

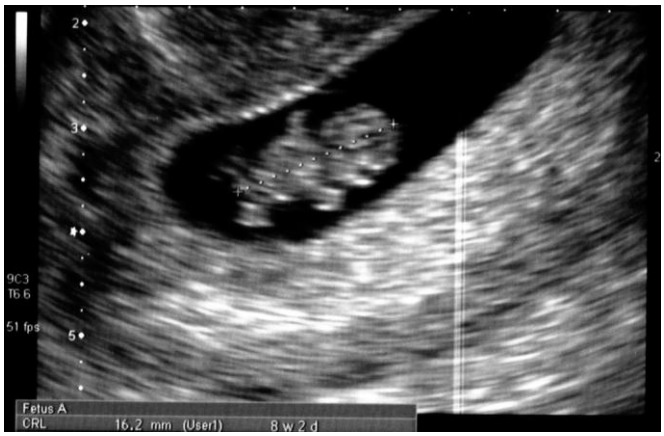


Figure 2.21 CRL measurements at 6 to 7 and 8 to 9 weeks on transvaginal scan

Early pregnancy complications: A pregnancy loss of less than 6 weeks was defined as either miscarriage or no demonstrable viable pregnancy at the 6 week scan followed by subsequent confirmation of missed miscarriage. Any early pregnancy problem that was suspected or detected had second observer

confirmation, and participants were referred to the early pregnancy unit (EPU) and managed according to the departmental protocols.

Any small bleeding around the gestational sac was reported and measurements were taken in three dimensions - longitudinal, transverse and antero-posterior. Incidental findings such as a simple ovarian cyst <5 cm in size and free fluid in the pouch of douglas were also reported.

Transabdominal dating scan (11-13+6 weeks routine scan)*:* The participants had routine departmental dating scan, where the fetal CRL at 10-14 weeks was measured transabdominally in a midsagittal plane with the fetal spine longitudinally in view as per the standards of dating (Figure 2.22).[218] The CRL measurements, results of the Down's syndrome screening, nuchal translucency (NT) and biochemical markers were recorded in the research records.

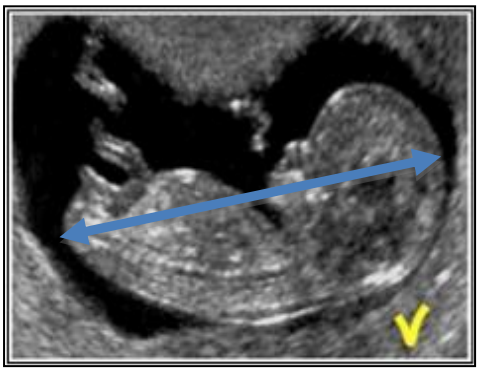


Figure 2.22 Measurement of fetal CRL in saggital plane and placement of calipers for gestational age assessment (Adapted from Fetal Medicine Foundation website)

2.4.2 Second trimester scans (18-21 weeks anomaly scan and 20-24 weeks uterine artery Doppler)

The participants had a routine departmental anomaly scan at 18-21 weeks in accordance with the National standards (FASP standards) and the findings of this scan including measurements of biometry and expected fetal weight (EFW) obtained using the computerized software (Astraia®) were recorded for longitudinal growth.

Uterine Artery Doppler: An extra transabdominal scan was performed at 20-24 weeks to assess uterine artery Doppler and fetal biometry using GE Voluson

E8 and GE Voluson 730 PRO ultrasound machines. Women with preexisting risk factors had this scan as a part of their routine antenatal care.

Pregnancies complicated by PE or IUGR have been associated with impaired trophoblastic invasion and impaired physiological changes in the spiral arteries.[219,220] Uterine artery Doppler has allowed non-invasive assessment of uteroplacental circulation by assessing the uterine artery waveform.[221] There is an agreement that there is increased impedance to uterine blood flow on uterine artery Doppler waveforms in women preceding development of PE/IUGR and during the clinical phase of PE/IUGR and uterine artery Doppler is thus, being used in screening for PE/IUGR. However, the sensitivity and specificity varies according to the study population, technique and site of measurement, gestational age at assessment, indices of the waveform that have been used and most importantly the definition of outcomes of PE or IUGR.

Technique of measurement of uterine artery Doppler: Transabdominally, the probe was placed longitudinally in the lower lateral quadrant of the abdomen angled medially.[222] The uterine artery was identified, at the site where it appears to cross the external iliac artery by identifying the external iliac artery on B-mode ultrasound. Colour flow mapping was used to visualize the uterine artery waveform. Once the artery was visualized, the sample volume was placed 1 cm downstream to the crossover point or just before the bifurcation of the uterine artery. Pulse repetition frequency (PRF) or in other words the scale of colour Doppler was adjusted in such a way that there was no aliasing of blood flow. The PRF was increased if there was aliasing and decreased if blood vessels were not clearly seen. Once the uterine artery was visualized at the cross over with the iliac blood vessels then by making further probe adjustments the uterine artery was positioned on the screen at an oblique angle so that the sample volume was obtained by placing the sample gate in the middle of the blood vessel. By Poiseuille's law the best estimation of the velocity of the blood flow would be obtained in the middle of the blood vessel. Once good quality, appropriate size waveforms with clear edges were captured using pulse wave Doppler, then the Doppler waveform indices are calculated either by manually placing the callipers on the waveform or by using the auto option.

Uterine artery Doppler indices:[223,224] Different indices have different predictive values, sensitivity and specificity in the prediction of various abnormal pregnancy outcomes associated with placental insufficiency. Table 2.4 describes the various Doppler indices, their advantages and disadvantages.

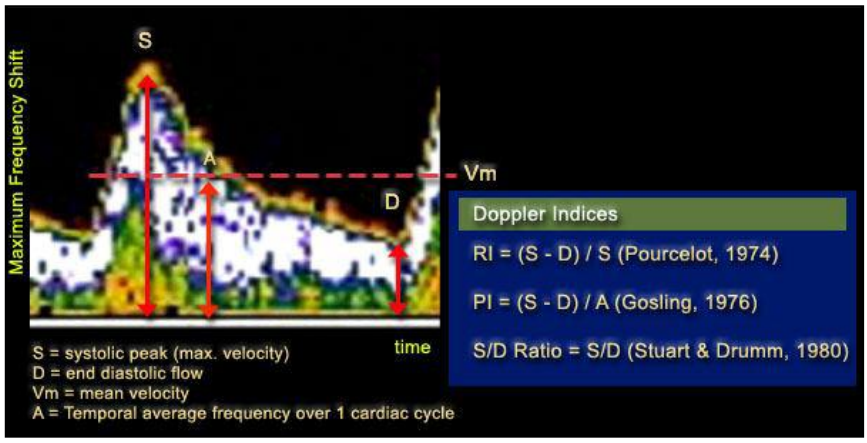


Figure 2.23 Indices of Doppler waveform (Taken from Fetal Medicine Foundation website)

Table 2.4 Explanation of the Doppler indices and their description

Index	Definition	Pros/cons
S/D ratio or A/B ratio	$Systolic^{max}/Diastolic^{max}$	Does not account for the entire wave form
RI (Resistance index)	$Systolic^{max}$ -$Diastolic^{max}$/ $Systolic^{max}$	• Only accounts for the systolic and diastolic velocities not the entire waveform. • If notching or if absent EDF then does not account for the entire waveform
PI (Pulsatility index)	$Systolic^{max}$ -$Diastolic^{max}$/ Mean velocity over one cardiac cycle	• Better as accounts for the entire waveform, mean velocity as well as the presence/absence of notch or EDF. • Determined by cardiac contraction force, heart rate, vessel compliance, blood viscosity and downstream impedance

We used two consecutive readings of uterine artery PI values on both sides and took the best of the two as the final result and reported the presence of absence of unilateral or bilateral notching. The cut off values to interpret the

results and management of the abnormal results was in accordance with the departmental guideline (Uterine artery Doppler screening guidance- The Rosie Hospital 2010).[225,226]

Table 2.5 Interpretation of Doppler results according to the Rosie Hospital protocol

Uterine artery Doppler result	Risk stratification	Management
Mean PI ≤1.3 (Figure 2.24)	Low risk-Normal	Routine antenatal care as planned
Mean PI > 1.3	Medium risk	Serial growth scans at 28 and 34 weeks and management as per the results
Mean PI >95th centile (1.45) or bilateral notches (Figure 2.25)	High risk	Three weekly growth scans/ 2 weekly BP and urine monitoring

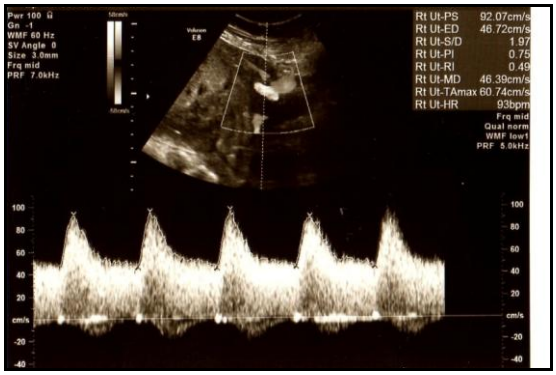


Figure 2.24 Normal uterine artery waveform

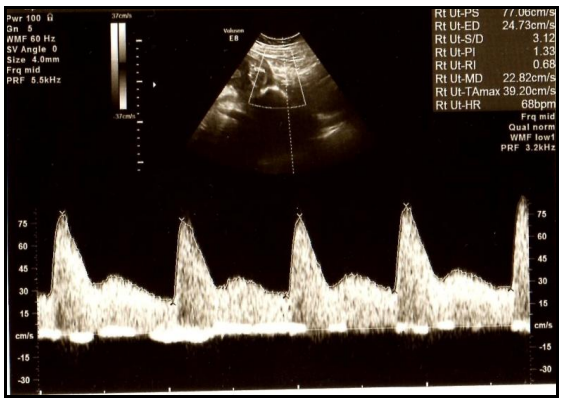


Figure 2.25 Abnormal uterine artery waveform

Fetal biometry and wellbeing[227,228] The measurements of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were performed to assess fetal size and growth. For each of the

parameters two measurements were obtained and the best of the two measurements was accepted.

Biparietal diameter (BPD) and head circumference (HC): The BPD is the maximum diameter of a transverse section of the fetal skull at the level of the parietal eminences.

Anatomical landmarks for obtaining BPD and HC on the thalamic view (Figure 2.26):

- A cross-sectional view of the fetal head was obtained at the level of the thalami.
- "Rugby football" shape of the head
- Centrally placed midline, the anterior midline presence of the cavum septum pellucidum (CSP) followed by the thalami interrupting the continuous midline echo of falx cerebri.
- Visualization of the basal cisterns.

Caliper placement (Figure 2.26):

- For BPD, the calipers were placed from outer to inner edge of the skull at the widest part of the skull using an angle perpendicular to the midline falx.
- For HC, the ellipse was placed around the skull bone echoes at the same thalamic view.
- The measurements of BPD and HC were plotted on the Chitty charts[229] on Astraia to determine serial growth velocity.

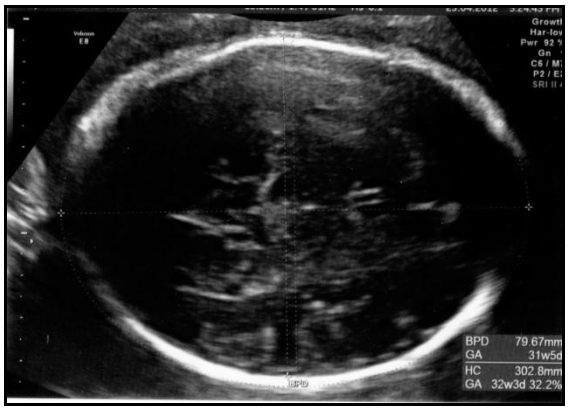


Figure 2.26 Measurement of BPD and HC by a thalamic view

Abdominal circumference (AC):

Anatomical landmarks (Figure 2.27):

- A circular section of the fetal abdomen with an unbroken and short rib echo of equal size on each side were taken.
- A cross-section of one vertebra was visualized.
- A short length of the umbilical vein was visible at the level of the portal sinus.
- The stomach was visualized as an hypoechoic area in the left side of the abdomen.

Caliper placement: The AC was measured by placing the calipers of the ellipse at the outer surface of the skin line (Figure 2.27) and then plotted on the Chitty charts.[230]

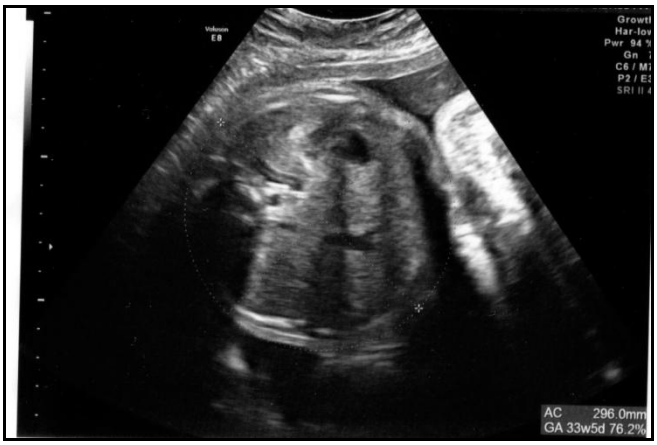


Figure 2.27 Measurement of fetal AC

Femur length (FL):

Anatomical landmarks (Figure 2.28): The femur was obtained in as horizontal view as possible with both ends of the ossified metaphysis clearly visible.

Caliper placement (Figure 2.28): Calipers were placed on either ends of the ossified diaphysis at the center of the 'U' shape of the bone without including the distal femoral epiphysis if it was visible and then measurements were plotted on the Chitty charts.[231]

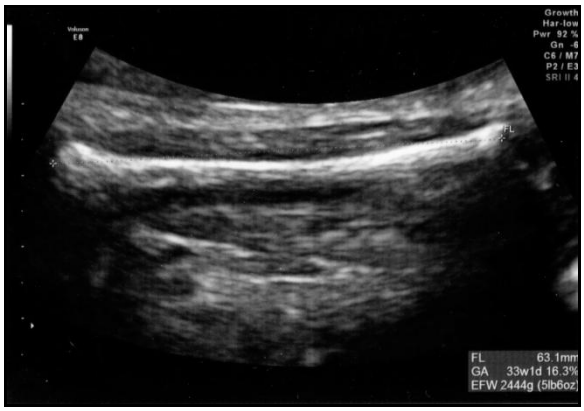


Figure 2.28 Measurement of fetal FL

Expected fetal weight (EFW): This was calculated using the Hadlock formula and reference charts used by Astraia® software.

2.4.3 Third trimester scans (31- 34 weeks)

Fetal biometry was performed along with amniotic fluid assessment using the ultrasound machines mentioned before. The biometry measurements were performed as described in the second trimester and EFW was calculated. Two measurements of each parameter were performed and the best of the two readings was accepted.

Amniotic fluid volume was evaluated by measuring the amniotic fluid index. Using the maternal umbilicus as a reference point, the abdomen is divided into four quarters. The largest vertical pool depth in each quadrant was recorded by placing the ultrasound probe perpendicular to the floor on the maternal abdomen. The sum of the measurements in all four quadrants represented the amniotic fluid index (AFI). Amniotic fluid of >25 cm was classed as polyhydramnios and <5 cm as oligohydramnios.

2.5 Postnatal Follow up

Women were followed up with the cardiovascular tests, blood biochemistry and the platelet function tests at three to four months postpartum. At this visit it was recorded if they were breast-feeding and if they were on any contraceptives.

2.6 Definition of pregnancy outcomes in the study

Table 2.6 Definitions of pregnancy outcomes in the study

Outcome	Definition
Miscarriage <6weeks	Early pregnancy loss of less than 6 weeks following a positive pregnancy test or no demonstrable viable pregnancy at the 6 weeks scan followed by a 'missed' miscarriage.
Miscarriage >6weeks	Early pregnancy loss between 6 to 12 weeks of pregnancy after a viable pregnancy was confirmed at the 6 weeks scan.
Ectopic pregnancy	Pregnancy located outside the uterus confirmed on ultrasound scan or by histological examination after surgery.
Preeclampsia	Diastolic blood pressure (BP) ≥ 110 mm Hg on ≥ one occasion, or ≥ 90mm Hg on ≥ two occasions at least 4 hours apart, in combination with significant proteinuria (≥ 300mg/ 24hours or 2+ dipstick; International Society for the Study of Hypertension in Pregnancy)[106]
IUGR	Birth weight below 3rd percentile for a given gestational age[193], in the absence of congenital infection or aneuploidy.

**Fetal anomaly not leading to termination of pregnancy was included in the study. Only singleton pregnancies were included for pregnancy follow up. We have combined the PE and IUGR cases within the category of uteroplacental insufficiency, as the study is too small for them to be considered separately.*

2.7 Communication with Primary Care

General practitioners were informed about the participation of their patient in the study with a standard letter approved by ethics committee (See Appendix 7). If any abnormal blood results were identified then the GP was informed of the results with a copy of the GP letter and copy of the results.

2.8 Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Version 18.0.0, 2009, SPSS Inc., Chicago, USA) and Medcalc software for windows (Medcalc Belgium, Version 11.6). The data on recruitment, conception, protocol adherence and outcome are presented in numbers and percentages. The data was assessed for normality of distribution by using frequency histograms and Kolmogorov-Smirnov test. Data are expressed as means ± standard deviation (SD) if normally distributed, and median ± inter-quartile range (IQR) or range if appropriate.

One-way analysis of variance (ANOVA) test was used to determine statistically significant difference between the means of three or more independent groups. This test was used for normally distributed continuous variables with equality of variances between the independent groups. When the overall difference between all the means was significant a *post-hoc Bonferroni* test was used to identify which two means were significantly different.

Kruskal-Wallis one-way analysis of variance was performed to determine statistically significant difference between non-normally distributed means of three or more independent groups. This test was also used when the groups were of unequal size.

Univariate general linear model (GLM) was performed to determine statistically significant difference between the means of two independent groups when the continuous dependent variable was affected by two or more factors. The mean AIx and mean aPWV between the groups were compared accounting for the fixed factors and covariates affecting them.

A repeated measures analysis of variance was performed to determine statistically significant difference between three or more observations of a dependent continuous variable in the same participant over time. When the overall difference between all the means was significant a *post-hoc Bonferroni* test was used to identify which two means were significantly different.

Paired or independent sample t-test was performed to determine statistical significance between the means of two observations if the data was normally

distributed and Mann-Whitney U test was performed if the data was not normally distributed or if the sample size was small.

The association between normally distributed variables was determined by Pearson's correlation coefficient (r) and that between non-normally distributed or small sample size was determined by Spearman's correlation coefficient rho (ρ). The value of the correlation coefficient varies between -1 to +1, with 1 being the strongest and 0 being no correlation between the variables. The sign "-" is suggestive of inverse and "+" is suggestive of positive correlation.

For all statistical tests $P < 0.05$ was determined as statistically significant. All P values were two-tailed.

Z-Score is the probability of a score of data occurring within the normal distribution and was determined using the observed and expected values from the means and SD of the population as described for the CRL measurement at 10-14 weeks and birthweight.

Bland Altman Plot[232] is a statistical method of plotting and comparing measurements obtained by two different techniques. The scatter plot represents the correlation between the two different methods. The mean of the two measurements is plotted on the X-axis and the difference between the measurements is plotted on the Y-axis. The limits of agreement are the 95% confidence interval of the difference between the two methods (average difference ± 1.96 SD of the difference). A narrower the 95% confidence interval implies better agreement between the two techniques.

2.8.1 Cardiovascular data

Pre-pregnancy comparisons: The pre-pregnancy measurements of MAP, central SBP, HR, CO and PVR in the four groups were compared using one-way analysis of variance (ANOVA) test and a *post-hoc Bonferroni* test was used to determine statistically significant differences between the groups. The AIx and carotid-femoral (aortic) aPWV between the groups were compared using ANOVA – generalized linear model univariate analysis using age, height and HR as covariates for AIx and MAP as a covariate for aPWV. The pre-pregnancy measurements of cardiovascular tests were also compared between the two groups: those with normal pregnancy outcome and those

with PE and/or IUGR in their current pregnancy using independent student t-test or Mann-Whitney U-test.

Longitudinal analysis: For the purpose of longitudinal analysis nulliparous and multiparous women with normal current pregnancies were considered as normal control group and the longitudinal changes were analyzed in this group separately as opposed to the other two groups- normal pregnancies in women with previous history of RM and normal pregnancies in women with previous IUGR/PE.

Longitudinal data on cardiovascular assessment, blood biochemistry and platelet aggregation data were analyzed using repeated measures ANOVA with the *post-hoc Bonferroni* test for pair wise comparisons. For the cases where the cardiovascular data was missing the data was imputed by calculating the estimated value using the mean difference of changes at that time in the pregnancy.[233]

2.8.2 Ovulation and implantation timing data

The ovulation, implantation timing and O-I interval in the ongoing pregnancies at 10 weeks, pregnancies that miscarried at less than 6 weeks and pregnancies that miscarried after 6 weeks were compared using Kruskal-Wallis Test.

Bland-Altman plots were constructed to compare the GA predicted by CRL (GA^{CRL}), GA estimated by adjusting for ovulation timing (GA^{OV}) and GA estimated by adjusting for implantation timing (GA^{IMP}).[232] The 95% confidence intervals (CI) were calculated of the differences between the gestational age estimated by single CRL measurement and that estimated based on adjustments for ovulation timing and implantation timing.

2.8.3 Ultrasound scan and birth weight data

Growth rate (mm/day) was calculated by dividing change in CRL by change in GA in the first trimester, changes in AC by change in GA in second and third trimesters respectively. The first trimester CRL growth rate was calculated as an average of the growth rate between scan 1 and 2 and the growth rate from scan 2 to 3. The second and third trimester growth rate was calculated as an

average of growth rate of AC between scan 1 and 2 in the second trimester and scan 2 to scan 3 in the third trimester.

CRL z-score at the 10-14 week scan was calculated as (measured CRL – expected CRL)/standard deviation, with respect to the Robinson & Fleming curve.[80] The birthweight z-score was calculated from the unstandardized residuals derived from the correlation between observed birthweight and observed GA at delivery. The relationship between implantation day, O-I interval, first-trimester growth, birthweight z-score, length of gestation and cardiovascular changes was investigated using spearman's correlation coefficient for non-normally distributed data and Pearson's correlation coefficient for normally distributed data.

CHAPTER 3: FEASIBILITY OF A PROSPECTIVE COHORT STUDY OF MATERNAL CARDIOVASCULAR CHANGES IN PREGNANCY FROM PRIOR TO CONCEPTION

Summary points:

- It is feasible to conduct a prospective study combining pre-pregnancy to postpartum study changes in cardiovascular function in relation to implantation events, fetal growth and pregnancy outcomes.
- This information would allow the design of a study, powered for pregnancy complications such as preeclampsia, to enable investigation of the 'cause and effect' relationship between abnormal cardiovascular function and pregnancy complications.

This chapter is based on:

Mahendru AA, Everett TR, McEniery CM, Wilkinson IB, Lees CC. *The feasibility of prospectively studying maternal cardiovascular changes from before conception.* **Hypertension Research**. 11 April 2013;doi:10.1038/hr.2013.24

3.1 Introduction

There is compelling evidence that factors prior to pregnancy and around implantation may have a bearing on maternal cardiovascular adaptation to pregnancy, pregnancy outcome and cardiovascular function later in life. The questions related to the cause and effect relationship between pre-pregnancy factors and pregnancy complications such as PE can only be answered by prospective cohort studies from prior to pregnancy. However, studies from prior to pregnancy are associated with difficulties in recruitment, low conception rates (25-30% probability of conception in a given menstrual cycle),[38] low retention of participants during pregnancy[37] along with further reduction in total numbers as a result of early pregnancy loss in about a third of pregnancies.[36] The incidence of pregnancy complications such as PE being 3-5% means a large number of pregnancies will be required to investigate the cause and effect relationship between cardiovascular dysfunction and PE.

Assessment of timing and events around human conception along with cardiovascular changes may also enable to provide insight into indirect evidence regarding the events around implantation. The objective of this study was to establish the feasibility of recruitment to and conducting and completing a prospective cohort study of ovulation, implantation timing and cardiovascular function from prior to pregnancy to the postpartum period.

This chapter describes the feasibility of recruitment prior to pregnancy in healthy women and in those with previous pregnancy complications, the pregnancy, miscarriage and livebirth rates, follow up, study completion and retention rates and the incidence of pregnancy complications such as PE and IUGR.

3.2 Methods

This was a prospective feasibility study in a single center. The study design, recruitment methods and the study protocol have been described in detail in chapter 2. There was no pre-existing data to help decide the sample size. The sample size for this study was a pragmatic choice rather than being based on a formal power calculation. We expected to recruit 80 participants in order for at least 20 pregnancies in each group to be followed up throughout the pregnancy. In this chapter the recruitment, conception, protocol adherence and outcome data are presented in numbers and percentages.

3.3 Results

3.3.1 Feasibility of recruitment and study participation (Flow chart in Chapter 2, Figure 2.1)

Out of the 184 women who responded to the advertisements/invitations, 143(80%) women planning to conceive were recruited in the pre-pregnancy period. Two hundred and fifty women with severe PE/IUGR were identified from the delivery unit database and clinic lists of 2006 to 2010. One hundred and twenty-five women with unexplained RM were identified from the clinic lists of 2009 to 2010. These women were invited to participate in the study if they were trying to conceive. Only 18 women (7%) with previous PE/IUGR and 26 women (21%) with unexplained RM replied to the invitation. Additionally, 5 women with severe PE/IUGR and 4 with RM in their previous pregnancy responded to the advertisements, had heard about the study from friends or from GPs and contacted by either email or over the phone for study information. Healthy volunteers contributed to 72% (105/143) of the study cohort. Nulliparous women formed 49% of the cohort, followed by 24% of healthy multiparous women; women with RM contributed to 18% of the cohort, and those with PE/IUGR contributed to 9% of the cohort.

3.3.2 Usage of home digital ovulation and pregnancy test kits as per protocol

Amongst women, who conceived and had a viable pregnancy, ovulation and/or implantation data was unavailable in about 15 pregnancies (21% cases). One of them had amenorrhea so did not start using the LH tests and 3 women did not detect LH surge in spite of using daily LH tests. Eleven women did not use LH and/or pregnancy tests as per protocol.

3.3.3 Conception rates (Figure 3.1)

One hundred and forty three women were trying to conceive at the time of recruitment. Three additional women recruited to the previous PE/IUGR group were not trying to conceive so were not included in the calculation of pregnancy rates. Ninety-one of one hundred and forty three women, (64%), became pregnant in 18 months. There were a total of 101 pregnancies in 143 women in 18 months including those who conceived twice during the study period. In those women who became pregnant, the median time to conceive (including the months they had been trying to conceive prior to participating in the research) was 5 (IQR 2 to 7) months and this constituted a median of 2 cycles after the study entry (IQR 1 to 5).

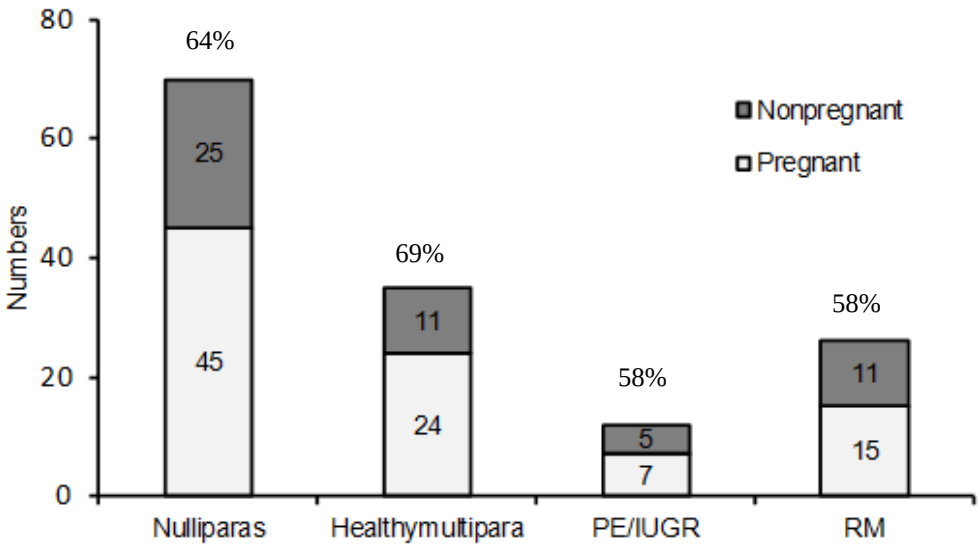


Figure 3.1: Number of participants who became pregnant in each group

3.3.4 Protocol adherence for cardiovascular tests (Table 3.1)

The cardiovascular assessments were performed at a median of 13 days in the menstrual cycle during the pre-pregnancy period, at 6 weeks in early pregnancy, 23 weeks in the second trimester, 33 weeks in the third trimester and at 16 weeks postpartum. 93% of pregnant participants completed all the cardiovascular tests as per protocol. 90% of participants completed the protocol for blood biochemistry tests and 86% completed the protocol for the platelet aggregometry.

Table 3.1 Protocol adherences for cardiovascular assessments, blood biochemistry and platelet aggregometry in 71 viable pregnancies

	Timing of assessment *	Cardiovascular assessments#	Blood biochemistry#	Platelet aggregometry#
Pre-pregnancy, days of menstrual cycle	13 (6-21)	71 (100%)	69 (97%)	69 (97%)
Early pregnancy, weeks of gestation	6 (6-7)	69 (97%)	-	66 (93%)
Late first trimester, weeks of gestation	12 (10-13)	-	63 (89%)	-
Second trimester, weeks of gestation	23 (23-24)	68 (96%)	-	-
Third trimester, weeks of gestation	33 (32-34)	67 (94%)	-	-
Postpartum, weeks from delivery	16 (14-17)	66 (93%)	64 (90%)	61 (86%)

**Data are median (IQR). #Data are number (percentage of total 71 viable pregnancies where data was complete)*

3.3.5 Pregnancy outcomes (Figure 3.2)

One third of pregnancies did not continue beyond the first trimester giving a pregnancy loss rate of 29/101 (29%) pregnancies. Early pregnancy loss included miscarriage<6 weeks (13%), miscarriage>6 weeks (12%) and ectopic pregnancy (4%). The miscarriage rate was higher in women with history of RM (53%) compared to all the other groups. Two participants were lost to follow up- one just after the pregnancy and another one in the middle of second trimester as she moved to another area. Therefore, the "dropout rate" following recruitment was 2% and was 1/71 (1.5%) amongst the participants with a viable pregnancy. Overall there was a very good follow up rate and a small dropout rate. Two out of seventy one women (3%) had a fetal abnormality (one had a fetus with triple X chromosomes and had a termination of pregnancy at 14 weeks and another one had a baby born with hypertrophic cardiomyopathy, who subsequently died in the neonatal period).

3.3.6 Pregnancy outcomes in live births (Figure 3.3)

Out of 57 (88%) controls. 50 had a normal pregnancy, 3 (5%) women had uteroplacental insufficiency (1 early onset IUGR <34 weeks, 1 late onset PE>37 weeks, 1 late onset PE with IUGR). All three were nulliparous women. 3 (5%) women had gestational diabetes. One fetus had hypertrophic cardiomyopathy and although the pregnancy was normal the baby died in the neonatal period. All women with RM had normal pregnancies (7). Amongst women with previous PE/IUGR, 2/6 (33%) had uteroplacental insufficiency (one had early onset PE and IUGR <34 weeks and the second one had late onset IUGR >37 weeks). Nulliparous women and those with previous healthy pregnancies were grouped together as controls and the cardiovascular changes in pregnancy have been described separately in normal pregnancies in this group. The pre-pregnancy cardiovascular function is different in women with previous PE/IUGR and they have been described separately.

3.3.7 Complete data for implantation timing and ultrasound scans

Amongst the 71 viable pregnancies, complete information on O-I interval was available in 56 (79%) pregnancies. Amongst these 56 pregnancies, first trimester growth rate was available in 45 (63%) pregnancies, second trimester

growth rate in 51 (72%) pregnancies, third trimester growth rate in 53 (75%) pregnancies and 54 (76%) cases had birthweight information.

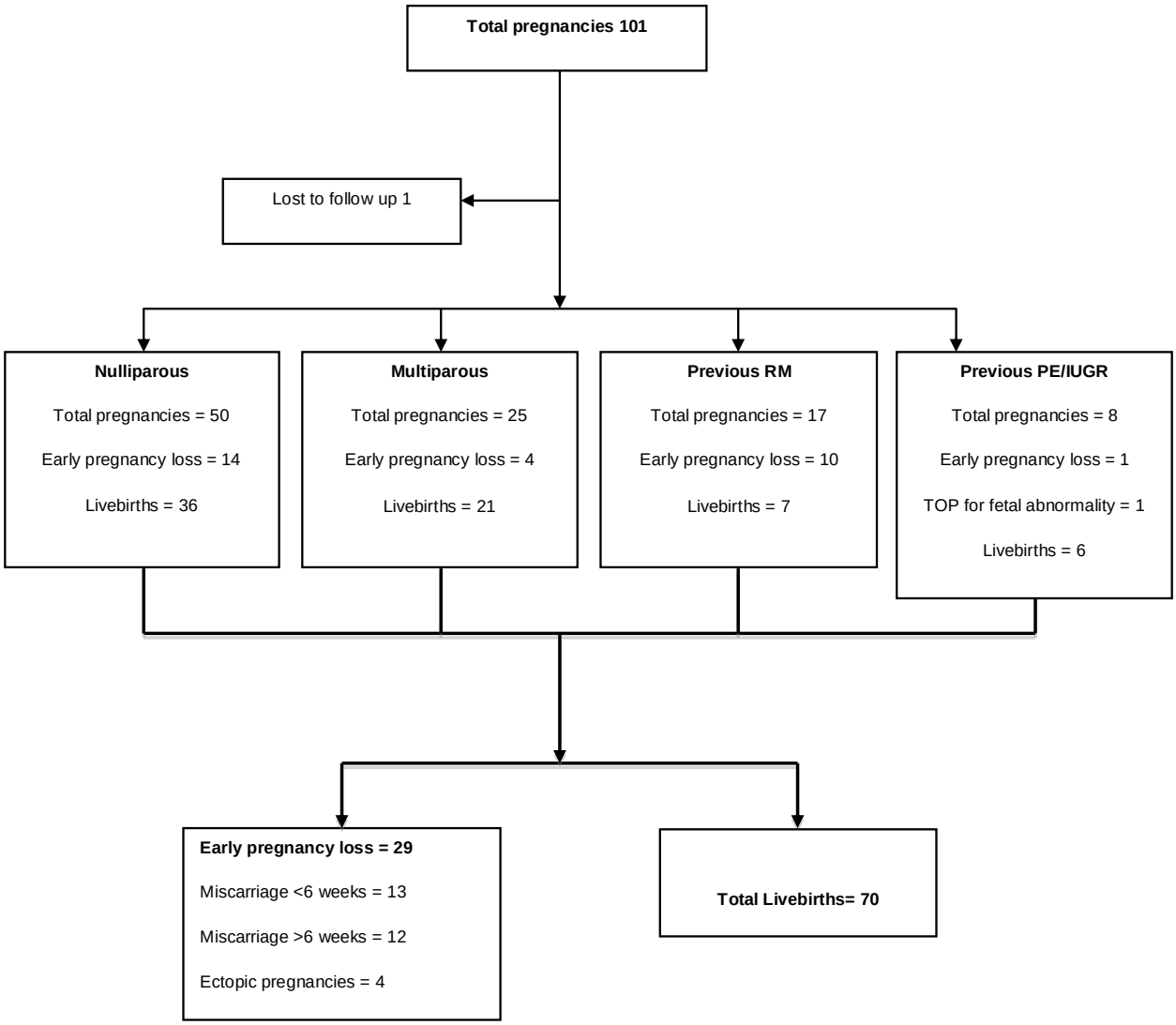


Figure 3.2 Flow chart of number of pregnancies, miscarriage and viable pregnancy in each group (Out of 100 pregnancies there were 29 early pregnancy losses, 1 TOP after 14 weeks and 70 livebirths)

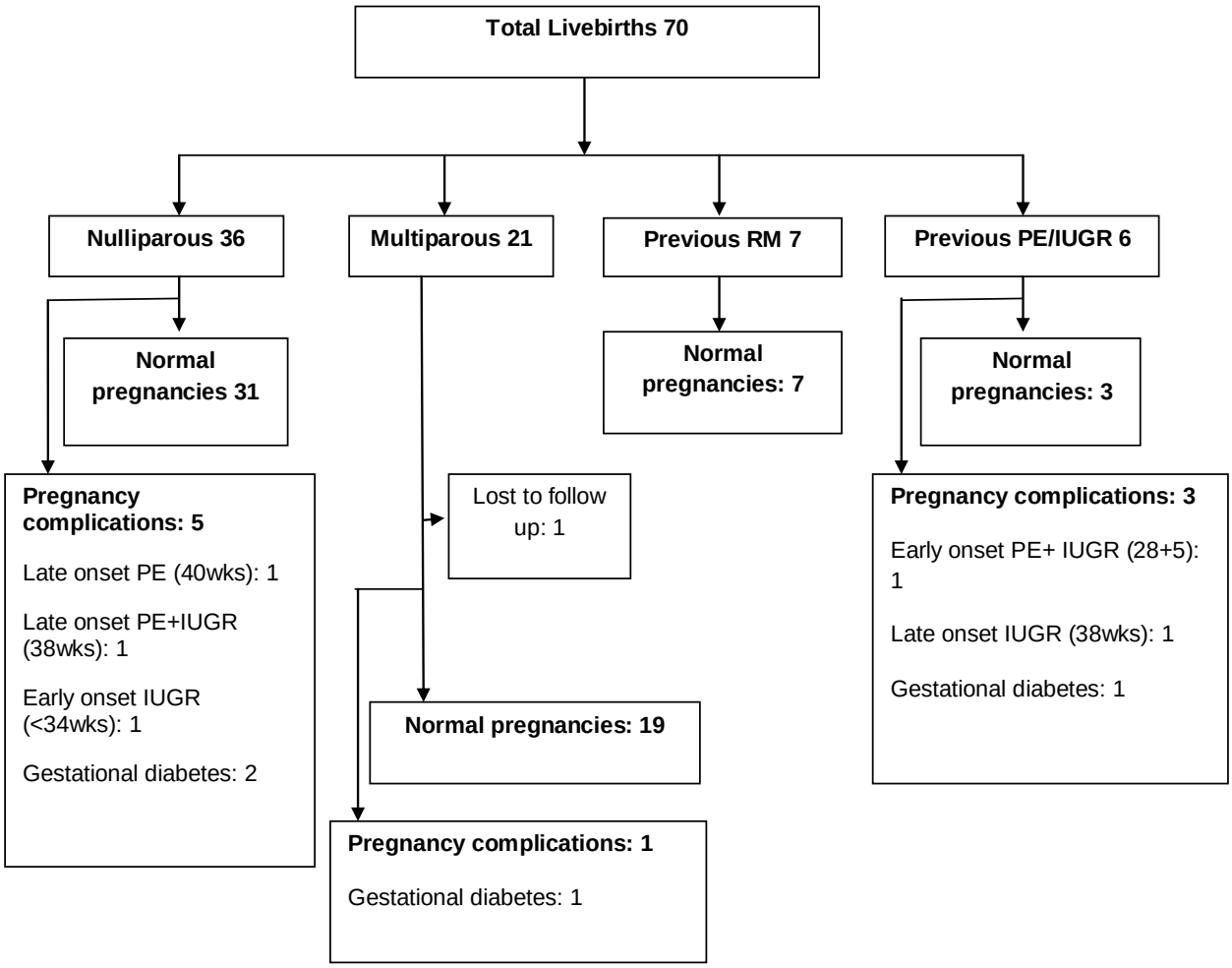


Figure 3.3 Flow chart of late pregnancy outcomes in all pregnancies with livebirth

3.4 Discussion

We have demonstrated that it is feasible to recruit, conduct and complete a study of implantation timing and cardiovascular function from prior to pregnancy. The feasibility of pre-pregnancy recruitment and prospect of a pregnancy follow up study has also recently been reported from a large prospective Chinese cohort study.[234]

The conception rate was almost similar between healthy nulliparous and multiparous women (64% and 69%). The information on ovulation and implantation timing was missing in about 21% of cases. The incidence of PE and/or IUGR is higher in women with previous PE/IUGR (33%), followed by nulliparous women (4%), consistent with what has been shown before.[3] The reported recurrence of PE in various studies varies between 6.8%[196,197] to 40%.[195] The high incidence in this study could be due to a selected population and small numbers. Although the incidence of uteroplacental insufficiency is higher in women with previous PE/IUGR, this group was difficult to recruit due to difficulties in identification of the cases prior to pregnancy. Moreover, having had PE/IUGR would have already affected the cardiovascular function in these women. Therefore, these women may not be ideal to study the 'cause and effect' relationship between pre-pregnancy cardiovascular function and uteroplacental insufficiency.

Complete data on O-I interval, embryonic and fetal growth and cardiovascular tests was available in 45(63%) cases. About 7% participants did not complete the entire study protocol for cardiovascular tests, 14% did not complete the entire study protocol for blood tests, 24% did not have entire O-I interval and/or cardiovascular test for a combination of reasons including moving away, inconvenience to attend postpartum visits, problems with equipment and transport of blood samples. A prospective study would have to account for the missing data as well as the incidence of uteroplacental insufficiency i.e. the outcome of interest in order to calculate the sample size.

3.5 Conclusion

It is possible to recruit women planning to conceive prospectively and conducting cohort studies using combination of measures of ovulation, implantation, fetal growth and cardiovascular function and provides estimates for a sample size for a definitive prospective cohort study from prior to pregnancy.

CHAPTER 4: DEMOGRAPHIC DATA OF THE STUDY POPULATION AND DIFFERENCES IN PRE-PREGNANCY CARDIOVASCULAR FUNCTION, BIOCHEMICAL PROFILE AND PLATELET AGGREGATION

Summary points:

- Pre-pregnancy cardiovascular BP and PVR were higher in women with previous PE/IUGR compared to normal multiparous or nulliparous women.
- Women who developed either PE and/or IUGR in their current pregnancy had a lower pre-pregnancy HR with higher PVR.
- Women with unexplained RM had no difference in their pre-pregnancy cardiovascular function.

This chapter is based on:

Mahendru AA, Everett TR, McEniery CM, Wilkinson IB, Lees CC. *Cardiovascular function in women with recurrent miscarriage, preeclampsia and/or intrauterine growth restriction*. **J Matern Fetal Neonatal Med**. 2013;26(4):351-356

4.1 Introduction

RM, PE and/or IUGR share the same risk factors as those for CVD such as obesity, hyperlipidaemia, insulin resistance and hypertension.[29,32,100,235] PE/IUGR are also associated with maternal endothelial dysfunction,[187,190] cardiac dysfunction, remodeling[236] and hypertension,[163] which persist postpartum and may contribute to CVD in the long term. Presence of underlying thrombophilia may predispose women with RM to an increased long term risk of CVD, however there is limited data on cardiovascular risk and cardiovascular function in women with unexplained RM to explain their risk of CVD.[33,190]

The knowledge of pre-pregnancy cardiovascular function and improved understanding of cardiovascular changes in normal pregnancies and pregnancy complications may enable us to understand the pathophysiology of long term CVD in these women. Early identification of cardiovascular risk factors may also enable primary prevention by controlling major CVD risk factors and early treatment opportunities to reduce morbidity and mortality.

This chapter describes the demographic data of the entire study population and the differences in pre-pregnancy cardiovascular function using brachial and central BP, AIx and aPWV along with metabolic function and platelet aggregation between healthy nulliparous, multiparous controls and women with previous PE/IUGR and RM in the first part. The second part of results describes differences in the pre-pregnancy cardiovascular function between women who had normal pregnancy outcome and those who had uteroplacental insufficiency during their current pregnancy.

4.2 Methods

Statistical analyses were performed using the Statistical Package for social sciences (Version 18.0.0, 2009, SPSS Inc., Chicago, USA). The normally distributed data was expressed as means ± SD and the non-normally distributed data as median [IQR or range]. The mean differences between the cardiovascular parameters were described as means ± standard error of means (SE). The brachial and central pulse pressures were calculated by subtracting the diastolic BP from the systolic BP separately for brachial and

central BP. The pulse pressure amplification (PP amplification) was then calculated by the following formula:

PP amplification = Brachial PP / Central PP

The demographics, measurements of the cardiovascular tests, blood tests for lipid profile, renal function, electrolytes and platelet aggregation were compared in all the four groups using one-way ANOVA and post-hoc test if normally distributed; and Kruskal-Wallis test if not normally distributed. The estimated glomerular filtration rate (eGFR) was calculated based on the creatinine clearance using the following formulae:

$$eGFR\ (ml/min/1.73m^2) = 186 \times (Creat/88.4)^{-1.154} \times Age^{0.203} \times (0.742 \text{ if female}) \text{ and } 1.210 \text{ if black.}$$

Previous studies have reported differences of 8 to 15 mm mmHg in SBP in women with previous PE and healthy controls.[163,190] The sample size required to detect a difference of 10mm Hg in SBP between controls and PE/IUGR group with the ratio of 1.4:1 assuming an SD of 10 at 80% power and with type 1 error of 0.05, was 20 controls to 15 PE/IUGR and for a ratio of 1.8:1 for RM it was 24 controls versus 13 RM.

The pre-pregnancy cardiovascular function in relation to the current pregnancy outcome was compared between those women who had a normal pregnancy (n=64) and those who had uteroplacental insufficiency (n=5) using Mann-Whitney U test. AIx and aPWV were compared after adjusting AIx for age, height and heart rate and aPWV for age and MAP using univariate logistic regression. A *P* value of <0.05 was considered significant. The estimated sample size to detect significant difference in SBP between normal pregnancy and PE/IUGR group assuming a standard deviation of 9 at 80% power and with type I error of 0.05 was at least 38 in each group.

4.3 Results

4.3.1 Pre-pregnancy differences in relation to previous obstetric history

Demographics of the study population (Table 4.1): Nulliparous women were significantly younger with a median age of 30 compared to multiparous women (healthy multiparous controls with a median age of 35 years and those with previous pregnancy complications with median age of 36 and 37 years respectively, *P*<0.001). The majority of women (125, 87%) were Caucasian in all groups, reflecting our local demographics.[237] Although women with RM were taller, the average BMI across all groups was normal (24 kg/m^2 in nulliparous women and 25 kg/m^2 in the rest of the groups). All women were non-smokers, except 2 nulliparous women, 2 RM women and 1 parous woman. The median time interval of the pre-pregnancy visit from the index pregnancy was 8 months in women with RM compared to 28 months in controls (*P*<0.001) and 40 months in women with PE/IUGR. The number of total pregnancies was significantly higher in the RM group than controls (*P*<0.001). The birthweight and GA at delivery were significantly lower in the PE/IUGR group compared to the controls (*P*<0.001). Seventy-nine percent of women with history of PE/IUGR had a family history of CVD compared to 24% of controls (*P*<0.05). Forty-eight percent of those with RM had family history of CVD, which was not significantly different from the controls. Information regarding the income and education was not collected and therefore, it was not possible to comment on the differences in socio-economic status within the study population.

Table 4.1 Demographics and obstetric history in all the groups

Characteristics	Nulliparous (n=70)	Healthy Multiparous (n=35)	PE/IUGR (n=15)	RM (n=26)
Age, years	30(28-32) #	35(33-38)	37(32-38)	36(33-41)
Caucasian	86%	91.4%	80%	88%
Height, m	1.64(1.61-1.69)	1.63(1.59-1.71)	1.61(1.57-1.65)	1.67(1.62-1.72)#
Weight, kg	65(59-73)	68(59-76)	63(57-71)	71(61-86)
BMI, kg/m^2	24(22-28)	25(22-29)	25(22-29)	25(23-32)
Previous pregnancies, n	0(0-2) (3 had miscarriage in past)	2(1-3)	2(1-3)	5(4-6)*
Primiparous/ Multiparous, n	-	27/8	11/4	19/2
Interval from last (index) pregnancy, months	-	28(15-38)	40(20-46)	8(5-15)*
Previous first trimester miscarriages, n	0(0-2)	1 (0-1)	1(0-2)	4(3-5)*
GA at miscarriage, wks	8(6-10)	9(6-10)	7(5-19)	8(6-10)
GA at delivery, wks	-	39(38-41)	36(32-38)*	40(38-41)
Birth weight, g	-	3560(3175-3719)	2259(1360-2640)*	3175(2721-3629)

*Data are median (IQR), BMI= Body mass index, GA= gestational age, *P<0.05 Mann-Whitney Test, #P<0.05 Kruskal-Wallis Test*

Differences in pre-pregnancy cardiovascular function in relation to previous obstetric history (Table 4.2, Figure 4.1)**:** Differences in the cardiovascular parameters between the groups are described in Table 4.2. Women with previous PE and/or IUGR had significantly higher brachial diastolic pressure (DBP) by 7 ± 2 mm Hg (*P* = 0.03), central systolic pressure (CSBP) by 8 ± 3 mm Hg (*P* = 0.01); and mean arterial pressure (MAP) by 8 ± 3mm of Hg (*P* = 0.02) compared to the multiparous women with previous healthy pregnancies. There was no significant difference between brachial and central BP in women

with RM compared to multiparous controls (Figure 4.1, Table 4.2). compared to multiparous controls The pulse pressure amplification was higher in the nulliparous controls and RM group compared to the multiparous controls and women with previous PE/IUGR (1.4 ± 0.2 versus 1.3 ± 0.4, *P* = 0.004).

Women with previous PE and/or IUGR had significantly higher PVR by 221 ± 75 dynes.s^{-1}.cm^{-5} than the multiparous control group of women (*P* = 0.02). The unadjusted AIx was higher in the women with PE/IUGR compared to nulliparous controls (*P* = 0.001), but after adjustment for age, height and heart rate (HR), there was no significant difference between the AIx in different groups. There were no significant differences between the CO, CI, HR and aPWV between the groups. The SV was higher by 15 ± 5 mL in the RM group compared to the multiparous controls (*P* =0.02).

Table 4.2: Differences in clinical characteristics of normal nulliparous and multiparous controls, with previous PE and/or IUGR and recurrent miscarriage (RM)

Cardiovascular parameter	Nulliparous (70)	Multiparous (35)	Previous PE/IUGR (15)	RM (26)	*P*
Brachial SBP, mmHg	109 ± 9	108 ± 9	115 ± 10	109 ± 12	0.1
Brachial DBP, mmHg	72 ± 7	71 ± 8*	78 ± 9*	72 ± 10	0.03*
Brachial PP, mmHg	36 ± 5	37 ± 6	37 ± 4	37 ± 7	0.9
Central SBP, mmHg	99 ± 9*	100 ± 9*	108 ± 10*	100 ± 11	0.01*
Central PP, mmHg	26 ± 5	28 ± 5	29 ± 4	27 ± 4	0.04*
PP amplification	1.4 ± 0.2*	1.3 ± 0.1*	1.3 ± 0.1*	1.4 ± 0.1	0.001*
MAP, mmHg	85 ± 8*	84 ± 9*	93 ± 9*	86 ± 10	0.02*
Heart rate, beats/min	69 ± 10	68 ± 9	68 ± 12	68 ± 12	0.9
CO, L/min	5.7 ± 1.0	5.5 ± 0.9	5.2 ± 1.1	5.9 ± 1.2	0.2
CI, L/min/m^2	3.3 ± 0.6	3.2 ± 0.5	3.1 ± 0.6	3.3 ± 0.7	0.6
SV, ml	80 ± 15	78 ± 12	68 ± 24	82 ± 14	0.02*
PVR, dynes.s^{-1}.cm^{-5}	1223 ± 240*	1248 ± 216*	1468 ± 311*	1206 ± 232*	0.004*
Unadjusted AIx, %	18 ± 10*	23 ± 7	28 ± 5*	22 ± 9	0.001
AIx_a, %	19 ± 9	22 ± 8	25 ± 8	20 ± 9	0.2
Unadjusted aPWV, m/sec	5.3 ± 0.7	5.3 ± 0.7	5.7 ± 0.7	5.4 ± 0.7	0.1
$aPWV_b$, m/sec	5.3 ± 0.8	5.3 ± 0.6	5.4 ± 0.8	5.3 ± 0.5	0.9

*Data are means ± S.D. a=adjusted for age, height and heart rate. b=adjusted for age and MAP. (SBP= Systolic blood pressure, DBP= Diastolic blood pressure, PP= pulse pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance) *P <0.05 using one way ANOVA. *P <0.05 for control versus PE/IUGR or RM is by post hoc comparison using Bonferroni test*

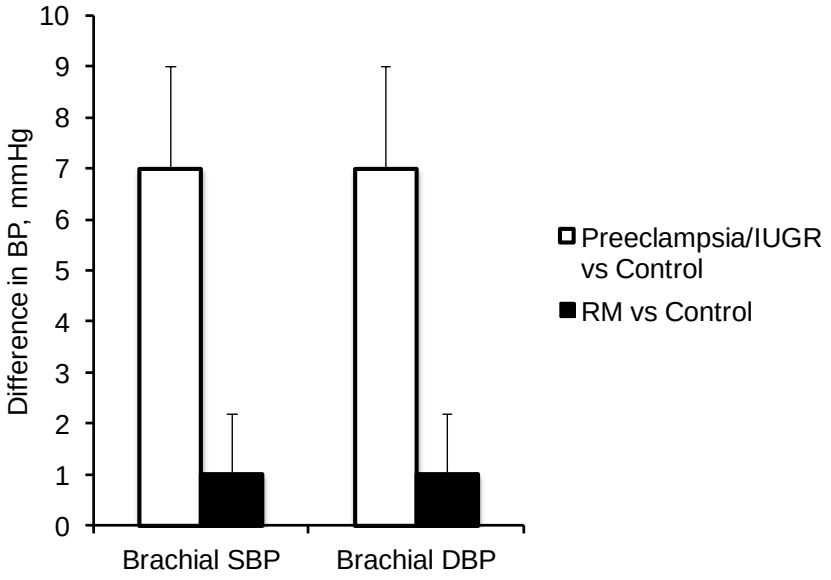


Figure 4.1 Differences in Brachial SBP and Brachial DBP between women with PE/IUGR versus controls and those with RM versus control

Differences in blood biochemistry and platelet aggregation between the groups (Table 4.3) Serum low-density lipoprotein concentration was higher in the RM group compared to the nulliparous group by 0.5 ± 0.2 mmol/l (*P* = 0.04) and the cholesterol:high-density lipoprotein (HDL) ratio was higher in the previous PE/IUGR group compared to the nulliparous controls by 0.5 ± 0.2 mmol/l (*P* = 0.06). However, there was no significant difference between the cholesterol, triglycerides, plasma glucose and platelet aggregation between the groups. Women with previous PE/IUGR had the lowest e-GFR, lower than the nulliparous women by 13 ± 5 ml/min/1.73m^2, (*P* = 0.04). e-GFR was lower in the healthy multiparous women in comparison to the nulliparous women by 9 ± 3 ml/min/1.73m^2, (*P*=0.04). There was no difference in renal function between RM and the multiparous controls and in the serum creatinine between all the four groups.

Table 4.3: Metabolic risk factors, renal function and platelet aggregometry

	Nulliparous (70)	Multiparous (35)	Previous PE/IUGR (15)	RM (25#)	*P**
Total cholesterol, mmol/L	4.5 ± 0.8	4.8 ± 0.9	4.8 ±0.9	5.0 ± 0.7	0.08
Triglyceride, mmol/L	0.8 ± 0.8	1.0 ± 0.8	1.1 ± 0.9	1.2 ± 1.2	0.4
HDL, mmol/L	1.6 ± 0.3	1.6 ± 0.3	1.5 ± 0.4	1.6 ± 0.3	0.5
LDL, mmol/L	2.7 ± 0.7*	2.9 ± 0.7	2.9 ± 0.7	3.2 ± 0.6*	0.04*
Cholesterol:HDL ratio	2.9 ± 0.6*	3.1 ± 0.6	3.4 ± 1.0*	3.3 ± 0.8	0.02*
Plasma Glucose, mmol/L	4.3 ± 0.4	4.4 ± 0.3	4.4 ± 0.3	4.3 ± 0.4	0.7
HbA1c, mmol/L	34 ± 3	35 ± 3	36 ± 4	36 ± 4	0.1
Creatinine, µmol/L	66 ± 13	68 ± 6	73 ± 20	66 ± 9	0.1
eGFR, ml/min/1.73m^2	101 ± 18*	92 ± 10*	88 ± 22*	96 ± 14	0.04*
AUC with 10µl ADP	64 ± 22	63 ± 21	74 ± 22	71 ± 21	0.2
AUC with 5µl ADP	58 ± 21	58 ± 22	61 ± 22	64 ± 23	0.7
AUC with 2µl ADP	43 ± 23	44 ± 20	44 ± 21	48 ± 26	0.8
AUC with 1µl ADP	31 ± 18	31 ± 20	31 ± 14	34 ± 21	0.9
AUC with1µl Low ADP	17 ± 12	14 ± 9	18 ±17	23 ± 16	0.07

*HDL: High-density lipoprotein, LDL: Low density lipoprotein, HbA1c: haemoglobin A1c, eGFR: estimated glomerular filteration rate, AUC: Area under the curve on aggregometry, ADP: Adenosine diphosphate. *P < 0.05 significant by one-way ANOVA between the three groups. *P <0.05 for control versus PE/IUGR or RM is by post hoc comparison using Bonferroni test with post hoc tests between groups. # n = 25: one missing data*

4.3.2 Pre-pregnancy differences in relation to the current pregnancy outcome

Seventy, out of the 143 women planning to conceive had livebirths. Of these one woman was lost to follow up, 5 pregnancies were complicated by PE and/or IUGR and 64 pregnancies were normal. There was no difference in the age (mean age of 32 years) or BMI between the women with normal pregnancy and those with PE/IUGR (26 ± 1 kg/m^2 in normal versus 29 ± 2 kg/m^2 in the PE/IUGR group respectively).

Differences in cardiovascular function (Table 4.4): On comparing the pre-pregnancy cardiovascular function between women who developed uteroplacental insufficiency in the form of either PE or IUGR and those with normal pregnancy there were no significant differences between the brachial SBP, DBP and MAP. The central SBP was 8 ± 4 mm Hg higher in the PE/IUGR group compared to those with normal pregnancy, (*P*=0.06, Figure 4.2).

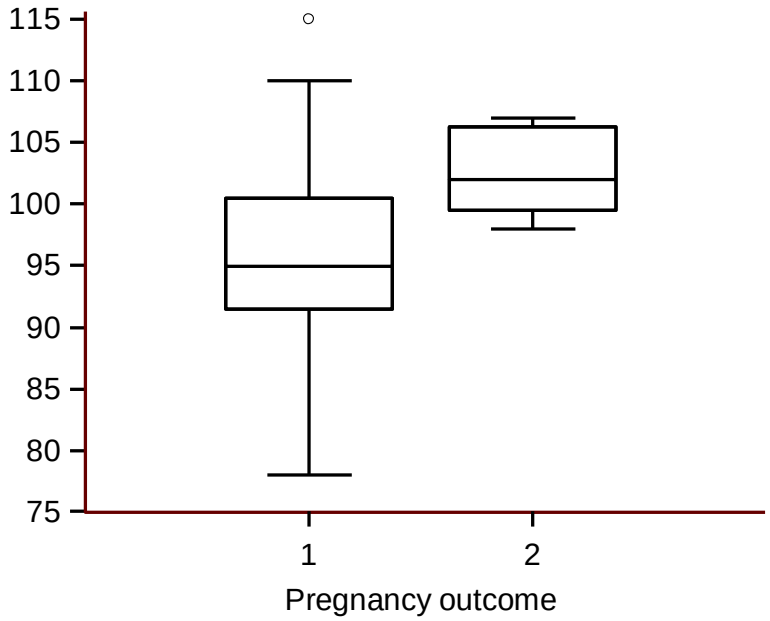


Figure 4.2 Pre-pregnancy central SBP is higher in women with PE/IUGR (1= Normal pregnancy, 2= women who developed PE/IUGR in their current pregnancy) P = 0.06 by Mann-Whitney U test.

The pre-pregnancy HR was 10 ± 4 beats/minute lower in women who developed PE/IUGR compared to those with normal pregnancy, (*P* = 0.02, Figure 4.3).

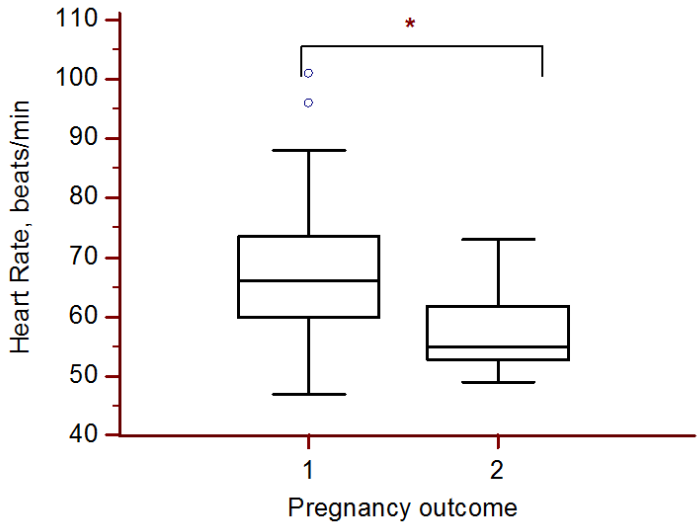


Figure 4.3 Pre-pregnancy HR is lower in women with PE/IUGR (1= Normal pregnancy, 2- women who developed PE/IUGR in their current pregnancy) **P* = 0.02 by Mann-Whitney U test.

The pre-pregnancy PVR was higher by 219 ± 82 dynes.s^{-1}.cm^{-5} in women who developed PE/IUGR compared to women with normal pregnancies, *(P* = 0.02, Figure 4.4).

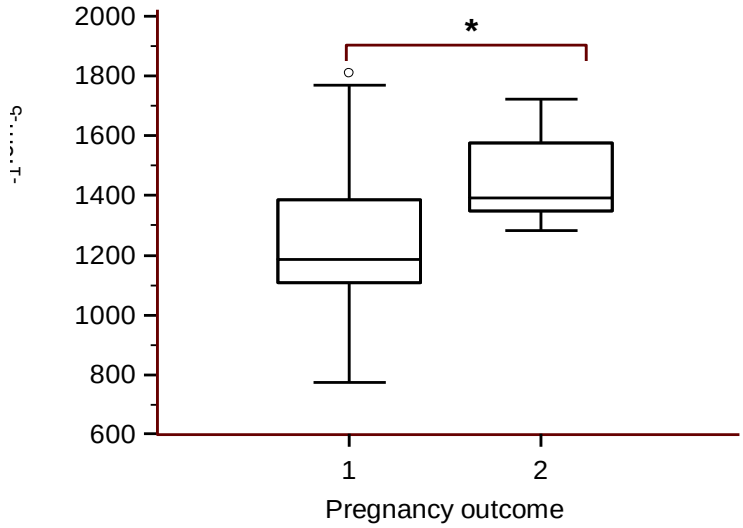


Figure 4.4 Higher pre-pregnancy PVR in women with PE/IUGR (1= normotensive women, 2 = women who develop PE/IUGR) (**P* = 0.02 by Mann-Whitney U test).

There was no significant difference between the CO, CI and SV between the two groups. There was no difference between AIx adjusted for age, height and HR prior to pregnancy between those who had normal pregnancy and had uteroplacental insufficiency. The unadjusted aPWV was higher is women who developed PE/IUGR compared to those with normal pregnancy (5.9 ± 0.6 versus 5.3 ± 0.6 m/sec, *P* = 0.03) however there was no difference between aPWV adjusted for age and MAP (5.6 ± 0.6 versus 5.3 ± 0.6 m/sec, *P* = 0.3)

Table 4.4 Differences in the pre-pregnancy cardiovascular parameters between women with normal pregnancy and those with utero-placental insufficiency

Cardiovascular parameter	Normal pregnancy (64)	PE/IUGR (5)	*P*
Brachial SBP, mmHg	108 ± 9	114 ± 9	0.2
Brachial DBP, mmHg	71 ± 7	77 ± 7	0.1
Brachial PP, mmHg	37 ± 6	37 ± 3	0.9
Central SBP, mmHg	99 ± 9	107 ± 9	0.06
Central PP, mmHg	27 ± 5	29 ± 6	0.4
PP amplification	1.4 ± 0.2	1.3 ± 0.2	0.1
MAP, mmHg	85 ± 8	91 ± 8	0.1
Heart rate, beats/min	68 ± 10	58 ± 9	0.02*
CO, L/min	5.6 ± 1.0	5.0 ± 0.6	0.3
CI, L/min/m^2	3.2 ± 0.5	2.7 ± 0.5	0.08
SV, ml	79 ± 12	83 ± 11	0.8
PVR, dynes.s^{-1}.cm^{-5}	1240 ± 224	1459 ± 172	0.02*
Unadjusted AIx, %	20 ± 9	27 ± 10	0.08
$AIx_{a,}$ %	20 ± 9	24 ± 4	0.3
Unadjusted $aPWV_{,}$ m/sec	5.3 ± 0.6	5.9 ± 0.6	0.03*
$aPWV_b$, m/sec	5.3 ± 0.6	5.6 ± 0.6	0.3

*Data are means ± S.D. a = adjusted for age, height and heart rate. b = adjusted for age and MAP. (SBP= Systolic blood pressure, DBP= Diastolic blood pressure, PP= pulse pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance). *P < 0.05 is significant by Mann-Whitney U test.*

Differences in pre-pregnancy blood biochemistry in relation to pregnancy outcome in the current pregnancy: We did not find any difference in the pre-pregnancy blood glucose, lipid profile or platelet aggregation between the normal pregnancies and those who had PE/IUGR. However, the number is too small to establish statistical significance.

Table 4.5 Differences in the pre-pregnancy blood biochemistry and platelet aggregometry between women with normal pregnancy and those with utero-placental insufficiency

	Normal pregnancy (64)	**PE/IUGR (5)**	***P****
Total cholesterol, mmol/L	4.7 ± 0.8	4.2 ± 0.4	0.09
Triglyceride, mmol/L	0.9 ± 0.8	0.7 ± 0.1	0.8
HDL, mmol/L	1.6 ± 0.3	1.4 ± 0.4	0.2
LDL, mmol/L	2.9 ± 0.7	2.5 ± 0.2	0.2
Cholesterol:HDL ratio	3.1 ± 0.7	3.1 ± 0.7	0.8
Plasma Glucose, mmol/L	4.4 ± 0.3	4.3 ± 0.5	0.6
HbA1c, mmol/L	34.8 ± 0.4	34.8 ± 1.1	0.9
Creatinine, µmol/L	67 ± 9	63 ± 9	0.5
eGFR, ml/min/1.73m^2	96 ± 2	103 ± 11	0.7
AUC with 10µl ADP	65 ± 21	75 ± 24	0.2
AUC with 5µl ADP	59 ± 20	68 ± 30	0.3
AUC with 2µl ADP	45 ± 23	40 ± 27	0.6
AUC with 1µl ADP	31 ± 19	38 ± 20	0.3
AUC with1µl Low ADP	16 ± 10	19 ± 11	0.3

HDL: High-density lipoprotein, LDL: Low density lipoprotein, HbA1c: Hemoglobin A1c, eGFR: estimated glomerular filteration rate, AUC: Area under the curve on aggregometry, ADP: Adenosine diphosphate. P <0.05 is significant by Mann- Whitney U test

4.4 Discussion

Women with previous PE/IUGR had higher DBP, central SBP, MAP, PVR and lower PP amplification compared to both controls and women with RM as has been shown before with no difference between cholesterol, triglycerides, glucose or platelet function contrary to what has been shown before.[163]

In contrast to women with previous PE/IUGR, women with previous unexplained RM had no difference in the cardiovascular function compared to normal controls. The women with RM had higher LDL cholesterol compared to nulliparous controls and those with PE/IUGR had higher total cholesterol:HDL ratio compared to multiparous controls. We found no difference in the adjusted AIx or aPWV between women with PE/IUGR and normal controls consistent with previous studies.[141,163] Women with previous PE/IUGR, in contrast to those with RM also had a strong family history of CVD compared to controls. This finding in PE/IUGR is consistent with previous studies,[238] although does not establish cause or effect.

On comparing the pre-pregnancy cardiovascular function in relation to development of utero-placental insufficiency in the current pregnancy, we found that the women who developed either PE or IUGR had higher PVR and lower HR with higher central SBP. There was no significant difference in brachial SBP, DBP, MAP, CO or CI between the groups. We did not find any difference in pre-pregnancy aortic stiffness between the women with normal pregnancy and those with PE/IUGR when the AIx was adjusted for age, height and HR, and the aPWV was adjusted for age and MAP.

4.5 Conclusion

Women with previous PE/IUGR had higher BP and PVR compared to normal multiparous or nulliparous women. Women with unexplained RM had no difference in their cardiovascular function. Pre-pregnancy HR was lower with higher PVR in women who subsequently developed either PE and/or IUGR in their current pregnancy. This highlights the importance of future prospective studies of maternal cardiovascular function from prior to conception in order to account for the pre-existing abnormalities in cardiovascular function and enable assessment of exact physiological maladaptation during pregnancy.

CHAPTER 5: CARDIOVASCULAR, METABOLIC AND PLATELET CHANGES IN NORMAL PREGNANCIES

Summary points:

Timing in pregnancy	Maximum change in cardiovascular function
Early pregnancy (6 weeks)	↑ HR
	↓ Brachial and central BP, MAP, PVR, AIx
	↑ Platelet aggregation
First trimester (10-12 weeks)	↑ eGFR, HDL cholesterol
Second trimester (23-24 weeks)	↑ HR
	↑ CO
	↓ Brachial and central BP, MAP, PVR, AIx and aPWV
Third trimester (33-34 weeks)	↑ HR
	↑ Brachial and central BP, MAP, PVR, AIx
	↓ CO
Postpartum	SBP lower and AIx higher compared to pre-pregnancy values

This chapter is based on:

Mahendru AA, Everett TR, Wilkinson IB, Lees CC, McEniery CM. *Maternal cardiovascular changes from pre-pregnancy to very early pregnancy.* ***J Hypertens.*** **2012 Nov; 30(11):2168-72**

5.1 Introduction

Normal pregnancy is associated with profound cardiovascular changes in BP, CO and HR, beginning as early as 5 to 6 weeks in pregnancy.[24-26] Recognition of changes in normal pregnancies has highlighted that pregnancies complicated by uteroplacental insufficiency may be associated with abnormal cardiovascular adaptation.[19]

The existing information on cardiovascular and metabolic changes in normal pregnancy is mostly from cross-sectional studies or longitudinal studies using either first trimester or postpartum measurements as baseline values. There is lack of longitudinal studies describing changes in pulse wave reflection, central BP and aortic stiffness from a pre-pregnancy baseline. The extent of cardiovascular changes may be underestimated by using late first trimester 'baseline' if these parameters have already altered by later first trimester and if the changes persist in the postpartum period.

The objective of this study was to investigate prospective changes in maternal cardiovascular haemodynamics during normal pregnancy in nulliparous women and those with previous normal pregnancy, from prior to pregnancy until the postpartum period along with changes in metabolic, renal function and platelet aggregation in first trimester.

5.2 Methods

Cardiovascular function was assessed by measurements of brachial and central BP, HR, CO, SV, peripheral vascular resistance (PVR), augmentation index (AIx), carotid-femoral pulse wave velocity (aPWV) prior to pregnancy, very early in pregnancy, second trimester, third trimester and 3 to 4 months postpartum in the 54 women in control group with normal pregnancies. The metabolic changes in lipids including total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and Cholesterol/HDL ratio were assessed from prior to pregnancy to late first trimester and then reassessed 3 to 4 months postpartum. The platelet function was assessed prior to pregnancy, very early in pregnancy and 3 to 4 months postpartum. Details of the cardiovascular tests, blood biochemistry and platelet function test are described in chapter 2. The timings of cardiovascular, metabolic and platelet tests are described in the figure below.

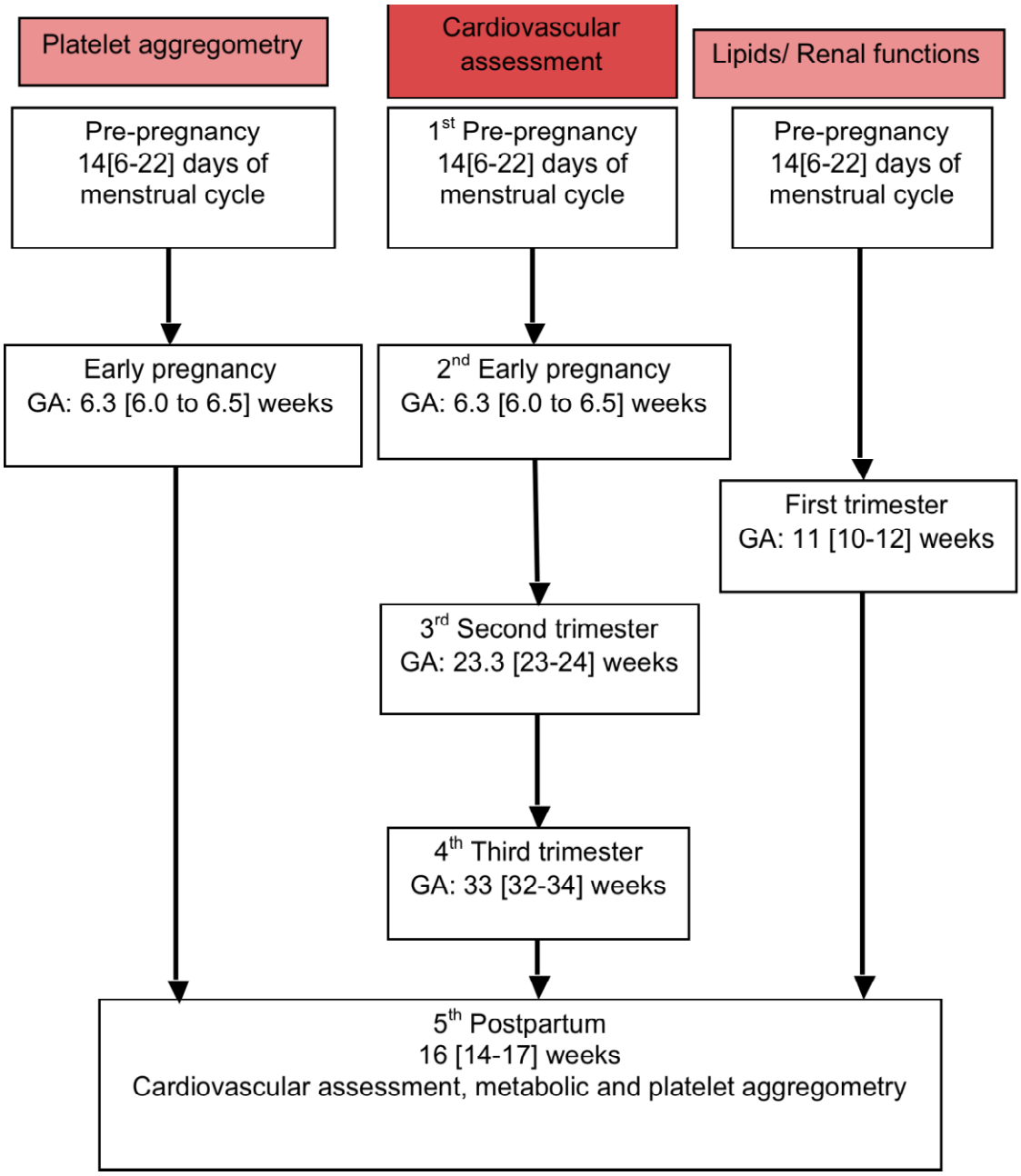


Figure 5.1 Timing of cardiovascular assessments (middle), blood biochemistry tests (3) and platelet tests (3) described as median [IQR]

Longitudinal data on cardiovascular assessment, blood biochemistry and platelet aggregation data were analyzed using repeated measures ANOVA with the Bonferroni post-hoc test for pair wise comparisons. Out of the total 54 cases, early pregnancy measurements were missing in one case, second and third trimester measurements were missing in two cases and postpartum measurements in other two cases. For the cases where the cardiovascular data was missing, this was entirely at random and by chance and therefore,

new data values was imputed at that time point by calculating the estimated value using the mean difference of changes at that time in the pregnancy.[233] The differences between the pre-pregnancy and postpartum values were assessed using paired student t- test for normally distributed data and Mann-Whitney U-test for non-normally distributed data. The AIx and carotid-femoral (aortic) aPWV at various times were compared using ANOVA – generalized linear model univariate analysis using age, height and heart rate as covariates for AIx and MAP as a covariate for aPWV. A value of $P<0.05$ was considered to be statistically significant. All *P* values were two-tailed.

5.3 Results

5.3.1 Demographics (Table 5.1)

The multiparous women were older than the nulliparous women (33years compared to 31 years, $P = 0.04$). Most of the women were Caucasian. There was no difference in the height, weight or body mass index (BMI) between the nulliparous and multiparous women.

Table 5.1: Demographic data of 54 women with normal pregnancies

	Nulliparous (33)	**Multiparous (21)**
Age, years	31 ± 3*	33 ± 5*
Ethnicity, Caucasian	29/33 (89%)	20/21 (95%)
Height, meters	1.64 ± 0.07	1.67 ± 0.08
Weight, Kg	67 ± 12	71 ± 13
BMI, Kg/meters^2	25 ± 5	26 ± 4

**Data are mean ± SD. *P<0.05 is significant by independent sample t-test*

5.3.2 Timing of pre-pregnancy tests

The pre-pregnancy visits were performed in both follicular and luteal phases of the cycle at a median of 14 days. There was no difference in pre-pregnancy to early pregnancy changes in maternal haemodynamics between the follicular or luteal phase of the menstrual cycle (Table 5.2). The timing of pre-pregnancy cardiovascular tests is reported as median days of the menstrual cycle.

Table 5.2 Pre to early pregnancy changes in maternal haemodynamics described from follicular phase to early pregnancy and luteal phase to early pregnancy

Cardiovascular parameter	Follicular phase to early pregnancy difference (n=30)	Luteal phase to early pregnancy difference (n=26)	*P*
HR, beats/min	3 ± 6	3 ± 8	0.7
Brachial SBP, mm Hg	6 ± 6	3 ± 8	0.2
Brachial DBP, mm Hg	7 ± 5	5 ± 7	0.4
MAP, mm Hg	8 ± 5	5 ± 8	0.2
Central SBP, mm Hg	8 ± 6	5 ± 8	0.1
CO, Litres/min	0.2 ± 0.7	0.2 ± 1.1	0.8
SV, Litres	0.1 ± 11.8	3.3 ± 15.1	0.8
PVR, dynes.s^{-1}.cm^{-5}	134 ± 144	61 ± 338	0.3
AIx, %	8 ± 6	5 ± 8	0.1
aPWV, m/sec	0.1 ± 0.6	0.1 ± 0.5	0.9

**Data are means ± SD*

5.3.3 Longitudinal cardiovascular changes in normal pregnancies (excluding three pregnancies with PE/IUGR) (Table 5.3)

Blood pressure: There was a significant reduction in brachial SBP (4 ± 7 mm Hg), DBP (6 ± 6 mm Hg), MAP (6 ± 7 mm Hg) and central SBP (7 ± 7 mm Hg) from pre-pregnancy to early pregnancy (Table 5.3, Figure 5.2a). Further reduction of brachial SBP, DBP, MAP and central SBP occurred from first to second trimester but to a lesser extent than that from pre pregnancy to early pregnancy. All BP increased in the third trimester but were still below the baseline pre-pregnancy values. Postpartum, the brachial and central SBP were still significantly lower than the pre-pregnancy level by 4 ± 8 mm of Hg (*P*< 0.001, Figure 5.2b) at 16 weeks postpartum. Follow up did not continue beyond 16 weeks.

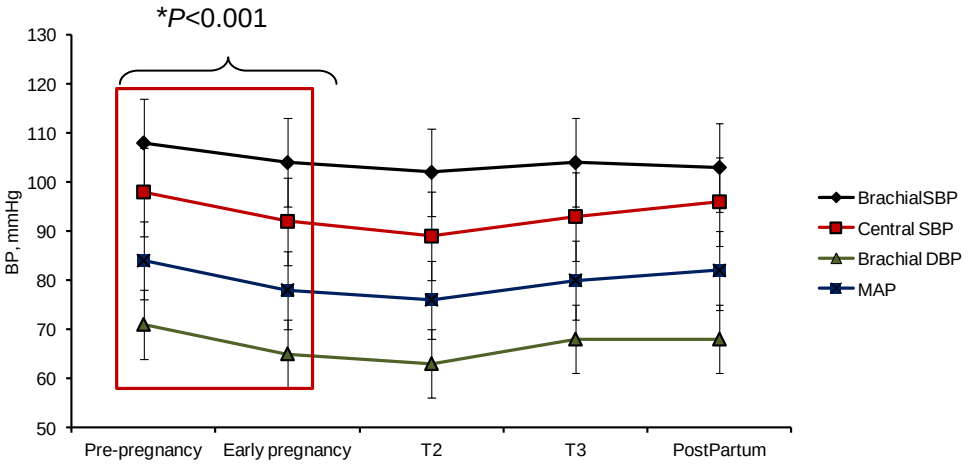


Figure 5.2a Changes in brachial, central SBP, DBP and MAP from pre-pregnancy to postpartum period. (T2 = second trimester and T3 = third trimester. The red box highlights the timing of significant changes in BP from pre pregnancy to early pregnancy)

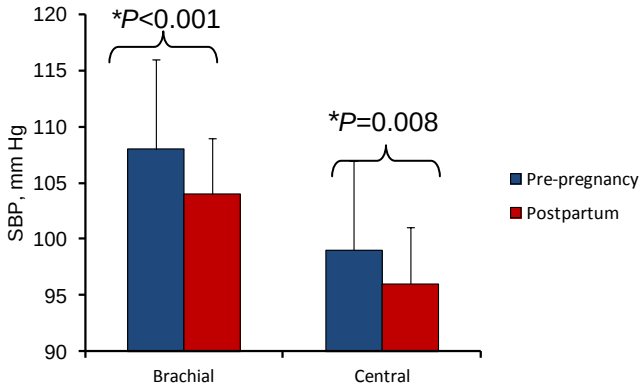


Figure 5.2b Persistence of reduced brachial SBP and central SBP in the postpartum period. * *P* < 0.05 is significant by paired student t- test.

CO and SV: The CO increased by 0.6 ± 1.0 L/min from the pre-pregnancy period to the second trimester with minimum change from pre-pregnancy period to very early in first trimester. It decreased slightly but non-significantly in the third trimester and fell to the pre-pregnancy baseline in the postpartum period. There was no significant change in SV during pregnancy, however SV was higher in the postpartum period compared to the third trimester (83ml versus 77ml, *P*=0.03). The increase in CO in second trimester was probably due to an increase in HR.

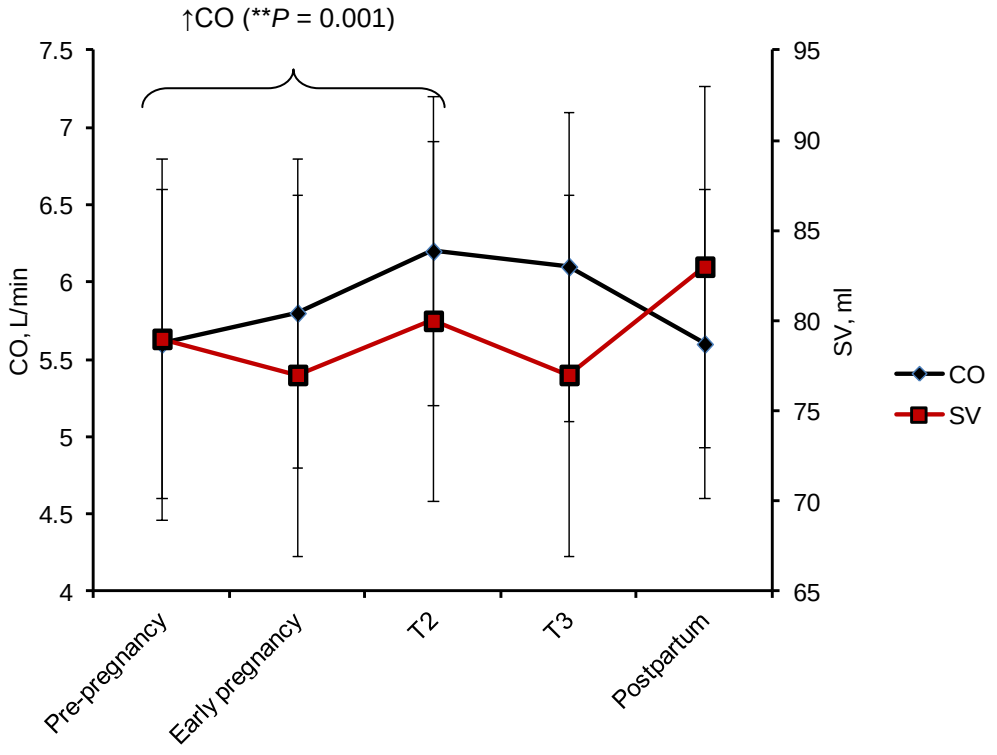


Figure 5.3 Changes in CO and SV during normal pregnancy. Significant increase was seen in CO in the second trimester. (T2 = second trimester and T3 = third trimester)

Heart Rate: There was a continuous increase in HR throughout pregnancy until the third trimester with a total increase of 13 ± 11bpm (overall 20% increase in the HR). The heart rate fell after delivery and with a decrease in HR from third trimester back to pre-pregnancy values in the postpartum period.

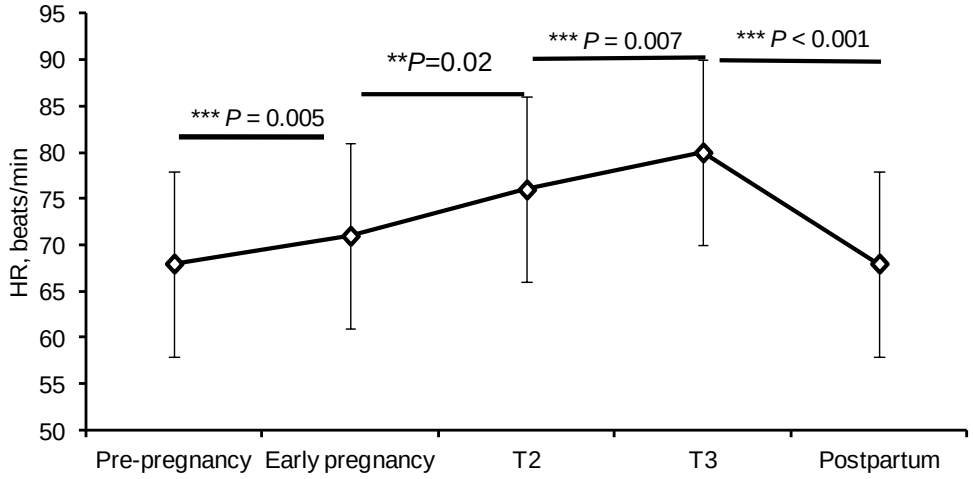


Figure 5.4 Changes in heart rate from prior to pregnancy to the postpartum period. HR increased throughout pregnancy. (T2 = second trimester and T3 = third trimester)

Pulse wave analysis (AIx): There was a significant reduction in both unadjusted AIx and AIx adjusted for heart rate from prior to pregnancy to early pregnancy (*P*=0.003), with further reduction in second trimester. However, most of the reduction in AIx (70% of the maximum reduction) occurred very early in pregnancy (Figure 5.5a). AIx increased in the third trimester and postpartum period with AIx in the postpartum period being significantly higher than the pre-pregnancy baseline (*P* = 0.01, Figure 5.5b).

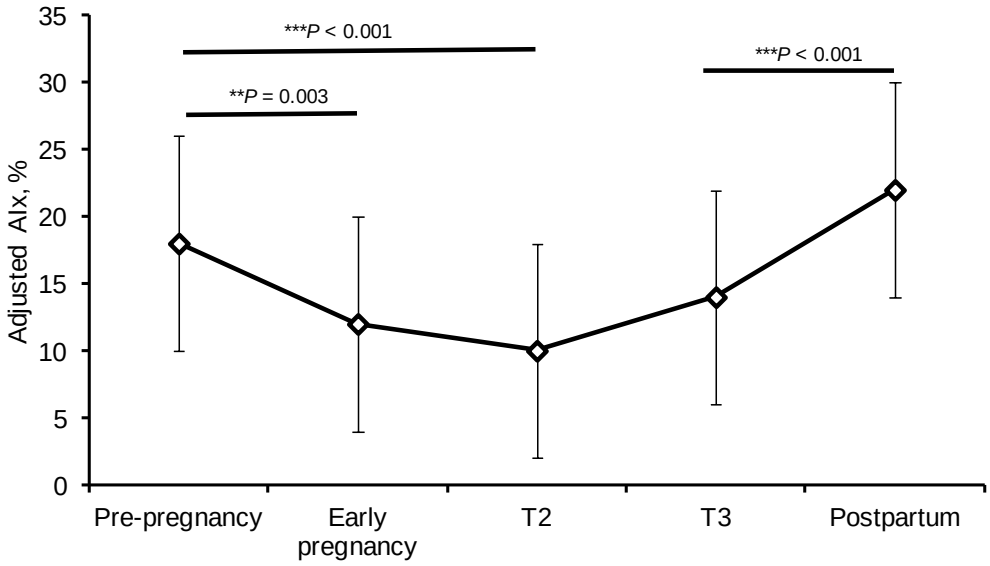


Figure 5.5a Changes in AIx from prior to pregnancy to the postpartum period (T2 = second trimester and T3 = third trimester)

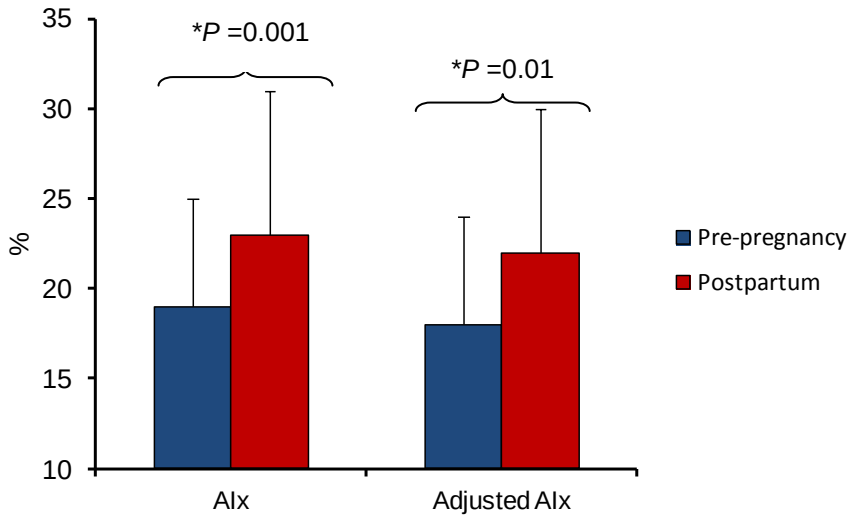


Figure 5.5b Higher AIx (Unadjusted and adjusted) in the postpartum period compared to the pre-pregnancy period. * *P* < 0.05 is significant by paired student t-test.

Aortic Pulse wave velocity (aPWV): There was no change in aortic pulse wave velocity in the early pregnancy period. There was a trend towards reduction of aPWV from second to third trimester. This reduction in the aPWV was not statistically significant. It remained lower in third trimester and was followed by an increase after delivery to nearly the pre-pregnancy values.

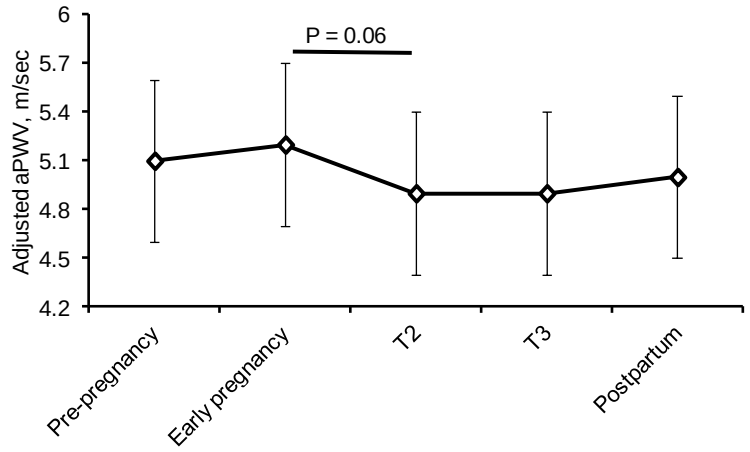


Figure 5.6 Changes in aortic pulse wave velocity from prior to pregnancy to postpartum period.

Peripheral vascular resistance: Peripheral vascular resistance reduced in very early pregnancy and further more in second trimester. The PVR reached its nadir in the second trimester and then continued to increase in the third trimester and postpartum period and reached to the value similar to the pre-pregnancy baseline in the postpartum period**.**

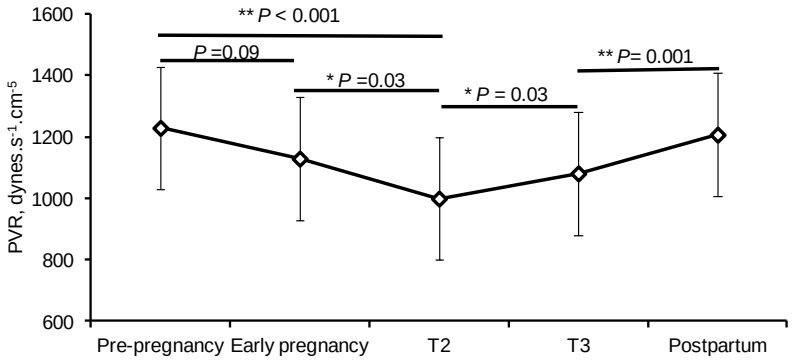


Figure 5.7 Changes in the PVR from a pre-pregnancy baseline to postpartum period

Table 5.3 Longitudinal changes in cardiovascular function in 54 normal pregnancies

Parameter	Pre-pregnancy	Early pregnancy	Second trimester (T2)	Third trimester (T3)	Postpartum
HR, beats/min	68 ± 10*	71 ± 10*	76 ± 10 *	80 ± 10*	68 ± 8*
Brachial SBP, mm Hg	108 ± 9*	104 ± 7*	103 ± 7	105 ± 8	104 ± 8*
Brachial DBP, mm Hg	71 ± 7*	65 ± 6*	63 ± 5*	68 ± 6*	69 ± 6
MAP, mm Hg	84 ± 8*	78 ± 6*	76 ± 5*	81 ± 6*	82 ± 7
Central SBP, mm Hg	99 ± 9*	92 ± 7*	90 ± 6	93 ± 7*	96 ± 7
CO, L/min	5.6 ± 1.0*	5.8 ± 1.2	6.2 ± 1.0*	6.1 ± 1.0	5.6 ± 1.0
CI, L/min/m^2	3.2 ± 0.6	3.3 ± 0.7	3.5 ± 0.5	3.3 ± 0.5	3.2 ± 0.5
SV, ml	79 ± 12	78 ± 18	80 ± 12	77 ± 13*	83 ± 18*
Unadjusted AIx, %	19 ± 10	12 ± 9*	9 ± 9*	12 ± 9	23 ± 6*
Adjusted AIx, %	18 ± 8*	12 ± 8*	10 ± 8*	14 ± 9	22 ± 8*
Unadjusted aPWV, m/sec	5.2 ± 0.6	5.1 ± 0.6	4.8 ± 0.5	4.9 ± 0.5	5.1 ± 0.6
Adjusted aPWV, m/sec	5.1 ± 0.7	5.2 ± 0.7	4.9 ± 0.7	4.9 ± 0.7	5.0 ± 0.7
PVR, dynes.s^{-1}.cm^{-5}	1229 ± 232*	1129 ± 291	999 ± 181*	1081 ± 201*	1207 ± 227*

*Data are means ± S.D. HR= heart rate, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance), *P<0.05 by repeat measures univariate analysis for overall significance and significance of difference between each visit in relation to the previous value calculated by post-hoc Bonferroni correction.*

5.3.4 Changes in renal function from pre-pregnancy to postpartum period (n=51) (Table 5.4)

There was significant decrease in serum creatinine (*P* <0.001) associated with an increase in eGFR (*P* <0.001) at the end of first trimester (Figure 5.8). The postpartum values were similar to the pre-pregnancy values.

Table 5.4 Changes in renal functions from pre-pregnancy to first trimester and postpartum

Renal function test	Pre-pregnancy	First trimester	Postpartum	*P
Creatinine, µmol/L	67 ± 9*	53 ± 8*	69 ± 11*	<0.001
eGFR, ml/min/1.73m^2	98 ± 17*	127 ± 25*	95 ± 20*	<0.001
Sodium, mmol/L	138 ± 2	137 ± 2*	140 ± 2*	<0.001*
Potassium, mmol/L	3.9 ± 0.3*	4.0 ± 0.4	4.1 ± 0.4*	0.002

**P<0.05 is significant by repeat measures analysis and significance of difference is between each visit in relation to the previous value calculated by post-hoc Bonferroni correction.*

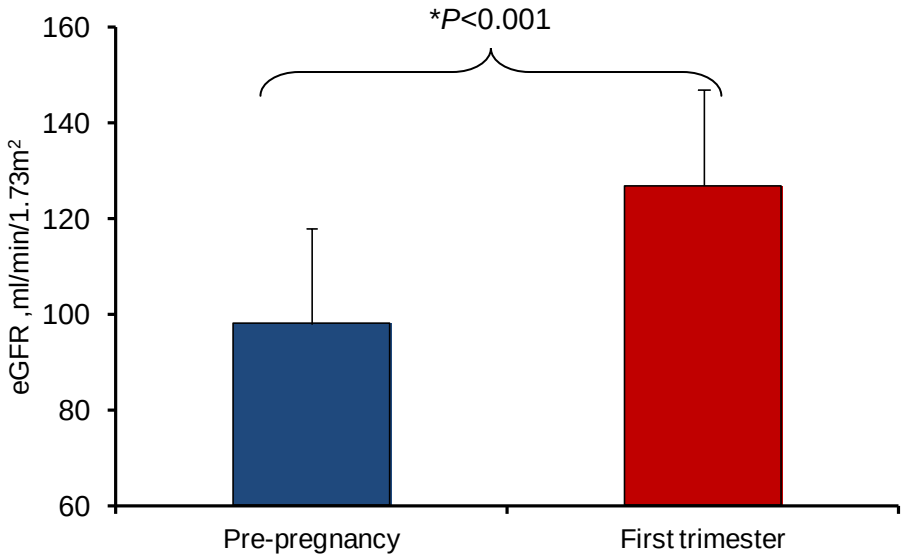


Figure 5.8 Estimated glomerular filtration rate (eGFR) increased significantly by late first trimester. (**P*<0.05 is significant by paired t-test between the pre-pregnancy and first trimester values)

5.3.5 Metabolic changes from pre-pregnancy to postpartum period (n=46)

Serum HDL increased, serum LDL and total cholesterol:HDL ratio decreased in the late first trimester in the 46 cases where tests were performed as per protocol (Figure 5.8). There was no difference between the pre-pregnancy and postpartum values of the lipid profile (Table 5.5). There was no change in plasma glucose in the first trimester.

Table 5.5 Changes in lipid profile from pre-pregnancy to first trimester and postpartum

	Pre-pregnancy	First trimester	Postpartum	*P*
Total cholesterol, mmol/L	4.7 ± 0.9	4.7 ± 0.8	4.6 ± 0.9	*NS*
Triglyceride, mmol/L	1.0 ± 0.9	1.0 ± 0.4	0.9 ± 0.7	*NS*
HDL, mmol/L	1.6 ± 0.4*	1.8 ± 0.4*	1.6 ± 0.4*	<0.001*
LDL, mmol/L	2.8 ± 0.7*	2.4 ± 0.7*	2.7 ± 0.7*	<0.001*
Total cholesterol:HDL ratio	3.0 ± 0.6	2.6 ± 0.5*	2.9 ± 0.7*	<0.001*
Plasma Glucose, mmol/L	4.3 ± 0.4	4.3 ± 0.6	4.3 ± 0.6	*NS*

**P<0.05 is significant by repeat measures analysis and significance of difference is between each visit in relation to the previous value calculated by post-hoc Bonferroni correction*

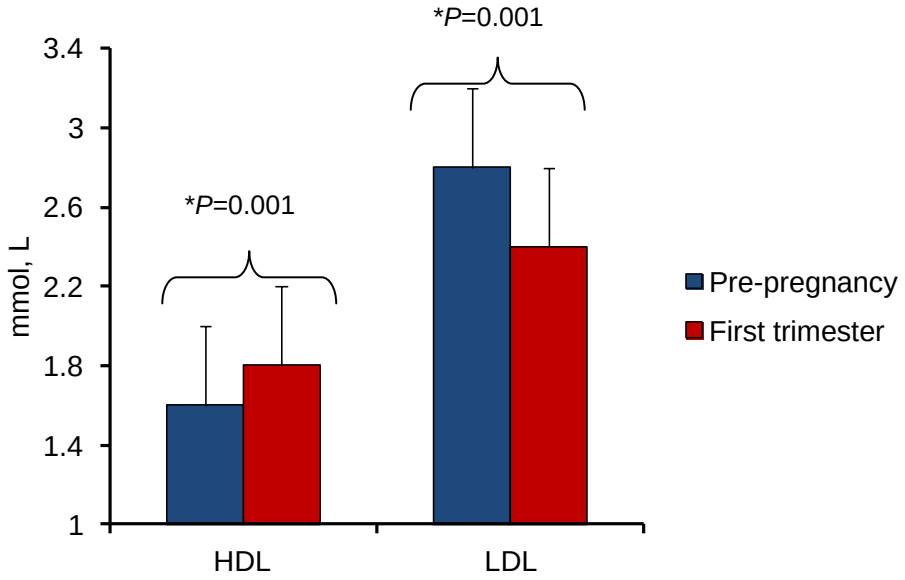


Figure 5.9 HDL increased in the first trimester and LDL decreased when compared to pre-pregnancy values. (**P*<0.05 is significant by paired t-test between the pre-pregnancy and first trimester values)

5.3.6 Changes in platelet aggregation during normal pregnancy (n=48)

There was increased platelet aggregation at higher doses of 10µl ADP and 5µl ADP in early pregnancy (*P*=0.002 and *P*=0.004 respectively, Figure 5.10) compared to at lower doses of 2µl ADP and below (Table 5.5). There was no difference between the pre-pregnancy and postpartum values.

Table 5.6 Changes in platelet aggregation at different concentrations of ADP

	Pre-pregnancy	Early pregnancy	Postpartum	*P*
Time from phlebotomy, minutes	15[13-21]	17[12-24]	22[17-25]	
AUC with 10µl ADP	64 ± 21*	73 ± 18*	70 ± 21	0.02*
AUC with 5µl ADP	59 ± 20*	66 ± 21*	65 ± 23	0.08
AUC with 2µl ADP	44 ± 22	49 ± 24	49 ± 26	0.2
AUC with 1µl ADP	32 ± 21	37 ± 22	38 ± 23	0.1
AUC with 1µlLow ADP	16 ± 11*	22 ± 13*	22 ± 15	0.01*

**P<0.05 is significant by repeat measures analysis and significance of difference is between each visit in relation to the previous value calculated by post-hoc Bonferroni correction*

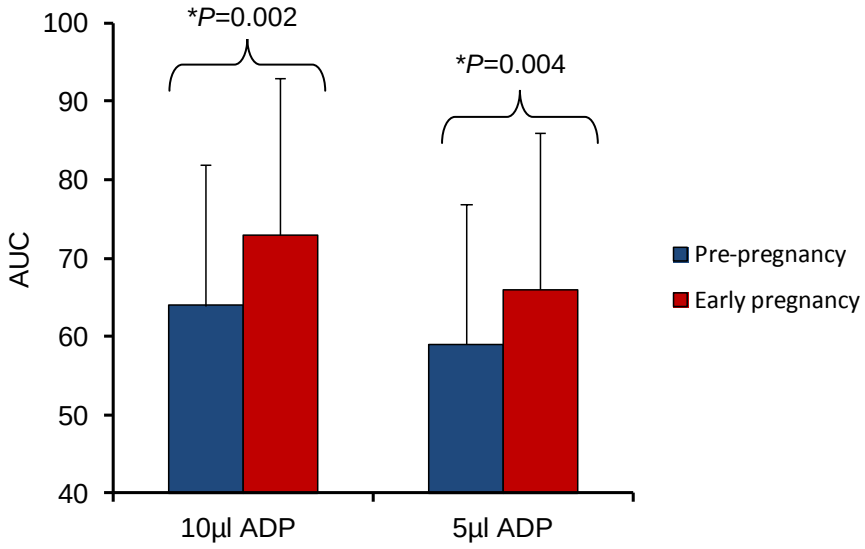


Figure 5.10 Significant platelet aggregation was seen in very early pregnancy compared to pre-pregnancy values. **P*<0.05 is significant by paired t-test

5.4 Discussion

The major novel finding was a significant reduction in central, brachial BP, AIx and PVR very early in pregnancy at about 6 weeks gestation in relation to the pre-pregnancy baseline values. This is similar to observations from previous smaller longitudinal studies beginning from prior to pregnancy.[24-26] This reduction was followed by a significant increase in the CO from the pre-pregnancy baseline by 23-24 weeks along with an increase in HR throughout pregnancy with non-significant changes in SV. The initial changes in BP, PVR and HR were associated with a significant increase in eGFR by the end of first trimester, consistent to previous studies,[146] suggesting that these changes were associated with volume expansion. The initial increase in CO could be explained by an increase in HR, however, persistent increase in preload due to volume expansion would explain the increase in SV and reduction in the aortic stiffness related to changes in structural properties of heart and aorta.

The brachial and central SBP were still lower than the pre-pregnancy value, whereas the AIx was higher at 3 to 4 months postpartum. Therefore, it is likely that longitudinal studies of cardiovascular changes in pregnancy underestimate the pregnancy changes using postpartum 'baseline' values. The higher AIx in the postpartum period could be due to higher SV in the postpartum period. Hence, it is instructive to report comprehensive haemodynamic changes rather than cardiovascular parameters in isolation in order to understand the pathophysiology of cardiovascular function in normal and complicated pregnancies.

We reported a significant increase in HDL cholesterol at the end of first trimester along with a significant reduction in LDL cholesterol, a reduction in the cholesterol:HDL ratio with all values returning to pre-pregnancy baseline in the post-partum period. Changes in total cholesterol and triglycerides are perhaps more significant in the latter part of pregnancy in association with fetal growth and may be influenced by hormonal changes at the feto-maternal interface later in pregnancy. Therefore, we did not find a significant change in total cholesterol or triglycerides in first trimester. The return to a pre-pregnancy baseline supports the hypotheses of pregnancy being a transient atherogenic event.

We reported increased platelet aggregation in very early pregnancy using platelet aggregometry consistent with previous studies suggesting platelet activation during pregnancy.[184] The changes in BP, systemic vascular resistance and blood flow very early in pregnancy may be responsible for the platelet aggregation.

5.5 Conclusion

This is the first prospective study to report longitudinal changes in maternal cardiovascular function during pregnancy including central BP, AIx and aPWV, along with brachial BP, CO and PVR, renal function, lipid profile and platelet activation from prior to pregnancy up to the postpartum period. We found significant reduction in central, brachial BP, AIx and PVR very early in pregnancy at about 6 weeks gestation and an associated increase in eGFR by the end of first trimester. This was followed by a maximum increase in the CO from the pre-pregnancy baseline by 23-24 weeks along with an increase in HR throughout pregnancy. The metabolic function and platelet activation being similar in the pre-pregnancy and post-partum period may suggest thereby that a normal pregnancy may not have residual atherogenic effect on a woman's cardiovascular system. Significant changes in the cardiovascular system in very early pregnancy together with the persistence of changes in the BP in the postpartum partum period suggest that cardiovascular changes from a late first trimester or postpartum 'baseline' may not give reliable estimates of pregnancy related changes. This highlights the value of conducting future longitudinal studies from prior to pregnancy in order to establish the exact extent and timing of changes during normal pregnancies and pregnancy complications.

CHAPTER 6: PREGNANCY CHANGES IN MATERNAL CARDIOVASCULAR, METABOLIC AND PLATELET FUNCTION IN WOMEN WITH PREGNANCY COMPLICATIONS

Summary points:

- Similar trends were observed in cardiovascular changes in HR, CO, PVR and arterial stiffness in all normal pregnancies.
- An increase in BP was noted in second trimester in women with previous PE/IUGR and all those women who had PE and/or IUGR in their current pregnancy.

6.1 Introduction

Nulliparous women are at higher risk of developing PE. Whereas normal pregnancy reduces the risk of development of PE in the subsequent pregnancy, women with previous PE/IUGR have a higher risk of recurrence of uteroplacental insufficiency in their subsequent pregnancy.[195] Women with previous PE have impaired vascular compliance and increased vascular resistance in subsequent pregnancy.[239] Recurrent PE/IUGR are associated with impaired cardiovascular adaptation in a subsequent pregnancy in the form of reduced plasma volume expansion, a lack of increase in the CO and an increase in the systemic vascular resistance in the second trimester.[240,241]

Pregnancies complicated by IUGR are associated with impaired volume expansion and impaired diastolic function very early in pregnancy,[19] whereas higher CO in late first trimester,[27] impaired cardiovascular function[236] and higher arterial stiffness in second trimester[140] are associated with PE.

In women with RM, there is thought to be higher resistance in the uterine blood flow prior to pregnancy and in very early pregnancy irrespective of the presence of anticardiolipin antibodies.[242,243] RM is believed to be associated with long-term cardiovascular dysfunction; however, the cardiovascular changes in subsequent pregnancies in women with RM have not been described before.

The objective of this chapter is to describe the longitudinal cardiovascular, metabolic and platelet changes in the pregnancies in women with previous PE/IUGR, those with RM and those women who had uteroplacental insufficiency in their current pregnancy. The study is not powered to compare the cardiovascular changes between the groups.

6.2 Methods

The cardiovascular parameters in all the normal pregnancies in women with previous RM or PE/IUGR are described as means ± standard deviation (SD). There were six normal pregnancies in women with previous RM and four normal pregnancies in women with previous PE/IUGR. Due to limited numbers repeated measures univariate analysis within the subgroups to determine the statistical significance of differences between the parameters in different

trimesters was not possible. The longitudinal trends of cardiovascular changes in these two groups have been described in graphs instead. The differences in the trends of cardiovascular changes between the groups were assessed using Kruskal Wallis test.

Amongst the 70 pregnancies, there were five cases with uteroplacental insufficiency in the form of PE/IUGR. The trends of cardiovascular changes from pre-pregnancy to early pregnancy, early pregnancy to second trimester, second to third trimester and third trimester to postpartum have been described using descriptive statistics in tables. The differences in the trends were compared between the 64 normal pregnancies and the five cases with uteroplacental insufficiency using Mann-Whitney U test. A *P* value of < 0.05 was considered as significant.

6.3 Results

6.3.1 Longitudinal changes in subsequent normal pregnancies in women with previous preeclampsia/intrauterine growth restriction

Amongst the women with previous PE/IUGR there were 6 viable pregnancies. Four out of the six pregnancies were normal. One woman had PE with IUGR and another one with IUGR. The cardiovascular changes in the four women with previous PE/IUGR who had normal pregnancies during the study are described in the Table 6.1.

Table 6.1 Longitudinal changes in cardiovascular function in 4 normal pregnancies in women with previous PE/IUGR

Parameter	Pre-pregnancy (1)	Early pregnancy (2)	Second trimester (3)	Third trimester (4)	Postpartum (5)
HR, beats/min	73 ± 17	81 ± 10	82 ± 12	84 ± 13	66 ± 11
Brachial SBP, mm Hg	114 ± 7	105 ± 5	107 ± 5	106 ± 8	100 ± 7
Brachial DBP, mm Hg	79 ± 9	68 ± 5	68 ± 4	70 ± 4	68 ± 8
MAP, mm Hg	93 ± 9	82 ± 5	82 ± 4	83 ± 5	81 ± 8
Central SBP, mm Hg	106 ± 7	94 ± 6	95 ± 4	95 ± 6	88 ± 9
CO, L/min	5.7 ± 0.5	6.2 ± 1.0	6.3 ± 0.8	5.8 ± 0.5	5.5 ± 1.2
CI, L/min/m^2	3.3 ± 0.2	3.6 ± 0.6	3.5 ± 0.6	3.1 ± 0.4	3.1 ± 0.6
SV, mL	76 ± 18	74 ± 19	76 ± 13	66 ± 10	81 ± 21
Unadjusted AIx, %	23 ± 3	14 ± 7	10 ± 9	14 ± 11	31 ± 5
Adjusted AIx, %	22 ± 3	15 ± 4	11 ± 3	16 ± 3	28 ± 4
Unadjusted aPWV, m/sec	5.5 ± 0.6	5.0 ± 0.5	4.9 ± 0.4	4.8 ± 0.3	4.9 ± 0.3
Adjusted aPWV, m/sec	5.5 ± 0.6	5.0 ± 0.5	4.9 ± 0.4	4.8 ± 0.3	4.9 ± 0.3
PVR, dynes.s^{-1}.cm^{-5}	1317 ± 243	1077 ± 200	1048 ± 103	1161 ± 148	1255 ± 477

Data are means ± S.D. HR= heart rate, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance

6.3.2 Longitudinal changes in subsequent normal pregnancies in women with previous recurrent miscarriage

All the six pregnancies in the RM group were normal. The descriptive statistics of the changes in the various cardiovascular parameters from pre-pregnancy to postpartum are described in Table 6.2.

Table 6.2 Cardiovascular changes in normal pregnancies in women with RM

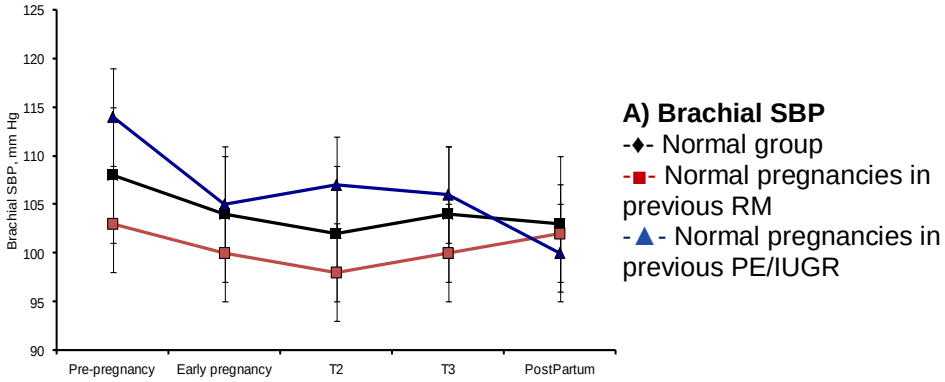
Parameter	Pre-pregnancy (1)	Early pregnancy (2)	Second trimester (3)	Third trimester (4)	Postpartum (5)
HR, beats/min	65 ± 3	75 ± 7	77 ± 1	86 ± 10	68 ± 12
Brachial SBP, mm Hg	103 ± 5	100 ± 6	98 ± 7	100 ± 5	102 ± 6
Brachial DBP, mm Hg	71 ± 3	66 ± 7	61 ± 4	67 ± 6	67 ± 5
MAP, mm Hg	83 ± 3	78 ± 8	74 ± 3	78 ± 6	81 ± 5
Central SBP, mm Hg	96 ± 4	90 ± 6	87 ± 6	89 ± 4	96 ± 7
CO, L/min	4.8 ± 0.3	6.2 ± 1.3	7.6 ± 1.2	5.6 ± 0.8	6.1 ± 0.8
CI,	2.6 ± 0.4	3.4 ± 0.6	4.0 ± 0.7	2.9 ± 0.4	3.3 ± 0.3
SV, Litres	71 ± 9	81 ± 14	95 ± 22	68 ± 11	93 ± 10
Unadjusted AIx, %	24 ± 12	16 ± 11	12 ± 9	10 ± 4	27 ± 11
Adjusted AIx, %	24 ± 12	13 ± 11	13 ± 9	7 ± 4	28 ± 11
Unadjusted aPWV, m/sec	5.1 ± 0.5	5.3 ± 0.6	4.8 ± 0.6	4.5 ± 0.5	4.9 ± 0.6
Adjusted aPWV, m/sec	5.2 ± 0.5	5.3 ± 0.6	4.9 ± 0.5	5.0 ± 0.5	4.9 ± 0.6
PVR, dynes.s^{-1}.cm^{-5}	1401 ± 115	1045 ± 282	789 ± 133	1126 ± 138	1071 ± 151

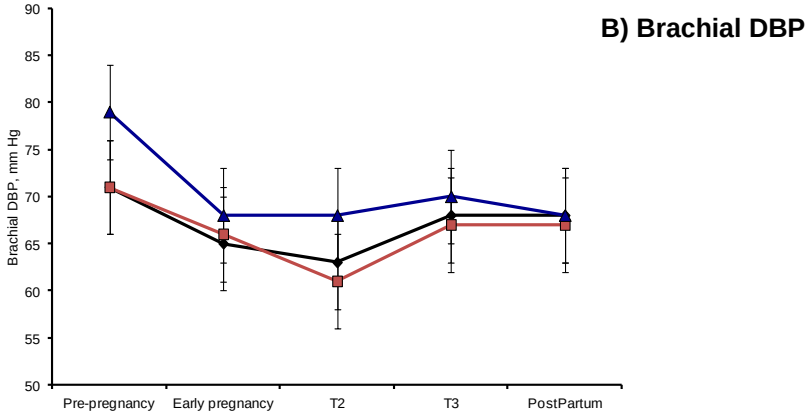
Data are means ± S.D. HR= heart rate, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance

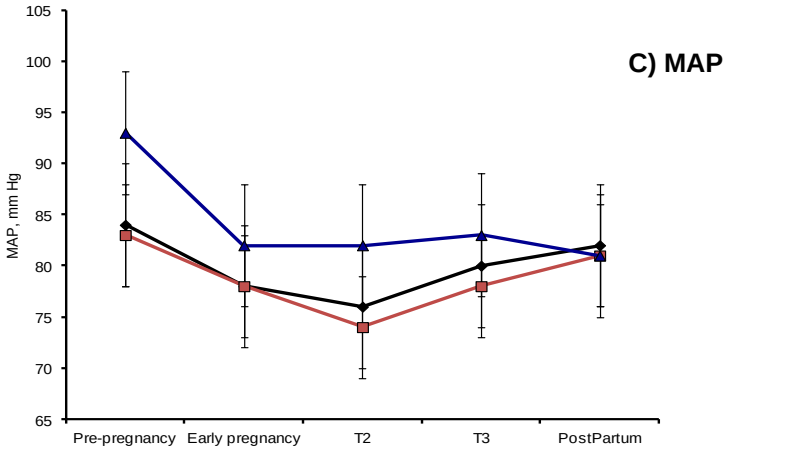
6.3.3 Comparison of trends in cardiovascular changes in all normal pregnancies (Total 64 pregnancies in nulliparous women, women with previous healthy pregnancies, previous preeclampsia/intrauterine growth restriction or recurrent miscarriage)

Changes in BP: In general there was a reduction in brachial and central SBP, brachial DBP and MAP in all normal pregnancies in very early pregnancy

(Trends of these changes in BP have been shown in Figures 6.1 (A, B, C and D). This was followed by a further non-significant fall of the BP in the second trimester in women in the control group and in those with previous RM. However, in contrast the BP either remained unchanged or increased in the second trimester in women with previous PE/IUGR. The differences in the changes in BP were not significantly different between the three groups with limited numbers of pregnancies within the groups.







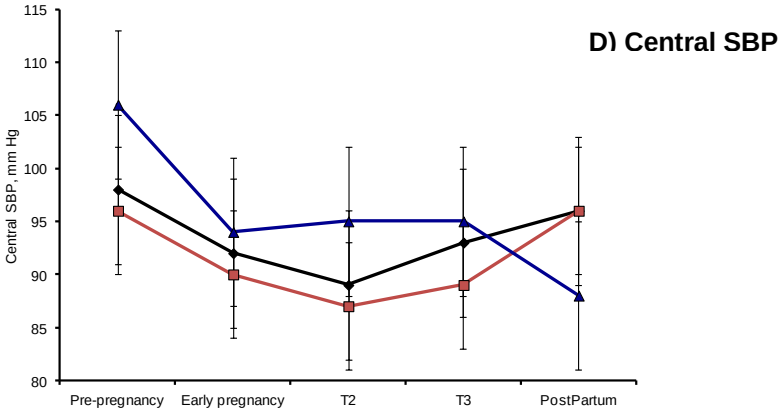


Figure 6.1 Longitudinal changes in A) brachial SBP, B) brachial DBP, C) MAP and D) central SBP in normal pregnancies. Normal pregnancies in the control group (black--♦--), normal pregnancies in women with previous RM (red--■--) and normal pregnancies in women with previous PE/IUGR group (blue--▲--)

Changes in CO, SV and HR: There was an increase in CO (Figure 6.2) associated with an increase in HR (Figure 6.3), beginning very early in pregnancy and significant by second trimester, also associated with an increase in the SV in the second trimester (Figure 6.4). The increase in CO and SV was more marked in women with RM compared to the control group (*P* = 0.02) as shown in the figures 6.5 and 6.7. There was marked increase in HR in all groups throughout pregnancy (Figure 6.6).

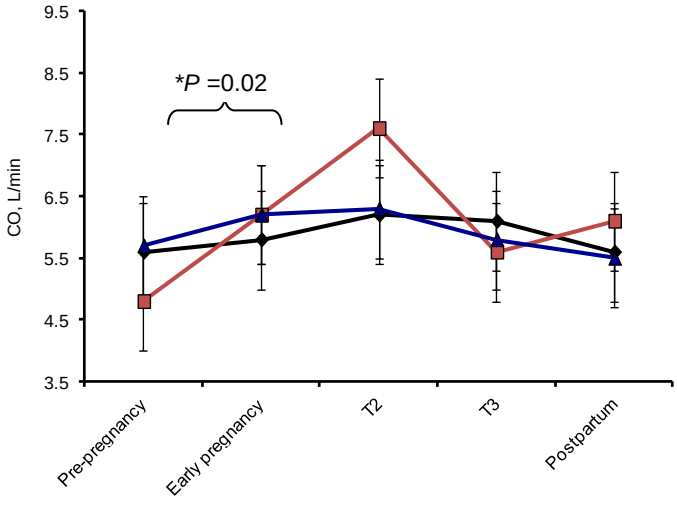


Figure 6.2 Longitudinal changes in CO in all normal pregnancies. Normal pregnancies in the control group (black--♦--), normal pregnancies in women with previous RM (red--■--) and normal pregnancies in women with previous PE/IUGR group (blue--▲--). ***P*** <0.05 is significant by Kruskal Wallis test between the groups.

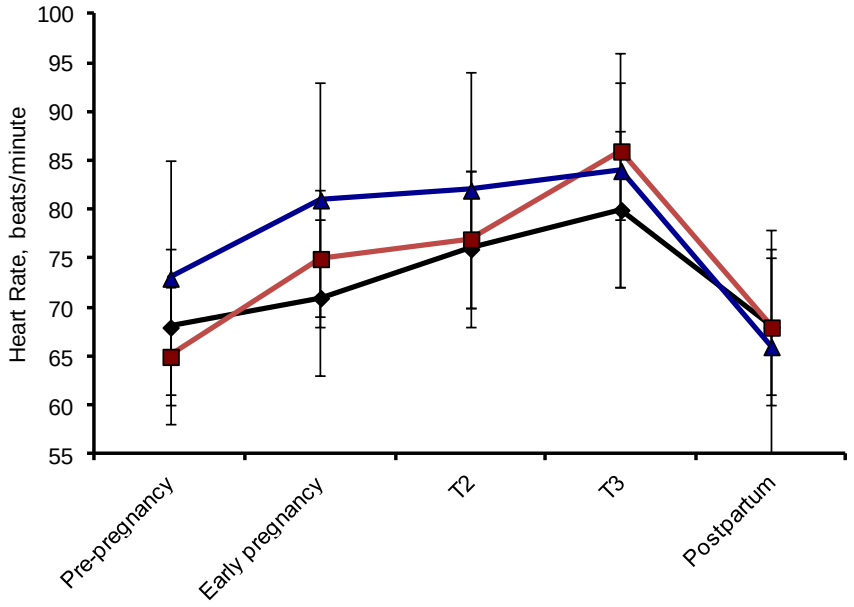


Figure 6.3 Longitudinal changes in heart rate in all normal pregnancies (Normal control group = black--♦--, women with previous RM = red--■-- and those with previous PE/IUGR = blue--▲--).

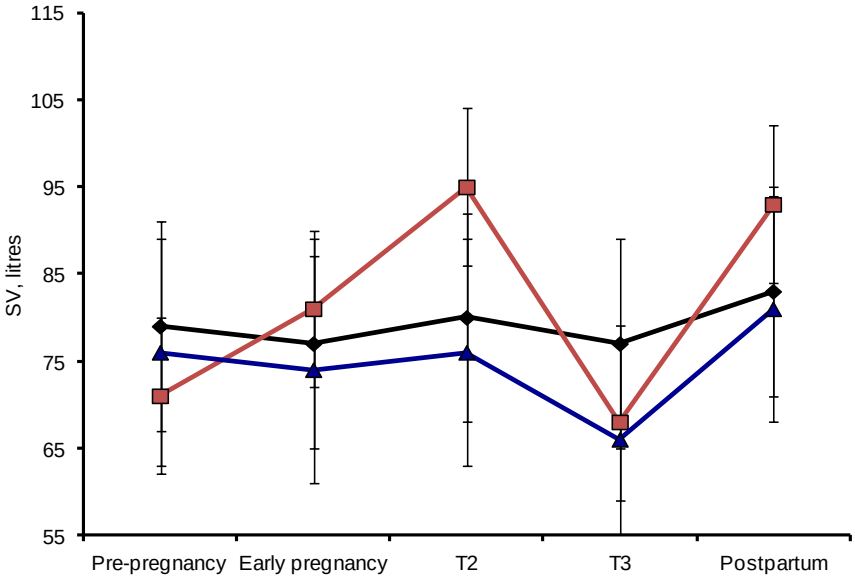


Figure 6.4 Longitudinal changes in stroke volume in normal pregnancies. (Normal control group = black--♦--, women with previous RM = red--■-- and those with previous PE/IUGR = blue--▲--).

Changes in PVR: There was a significant reduction in the PVR beginning from very early in pregnancy in all three groups. The early pregnancy drop in PVR was significant in the women with RM compared to the normal controls and PE/IUGR group (*P* =0.04). It continued to decrease to reach nadir in the second trimester in both control group and women with RM, but not in women with PE/IUGR, where it did not decrease significantly in the second trimester (Figure 6.5).

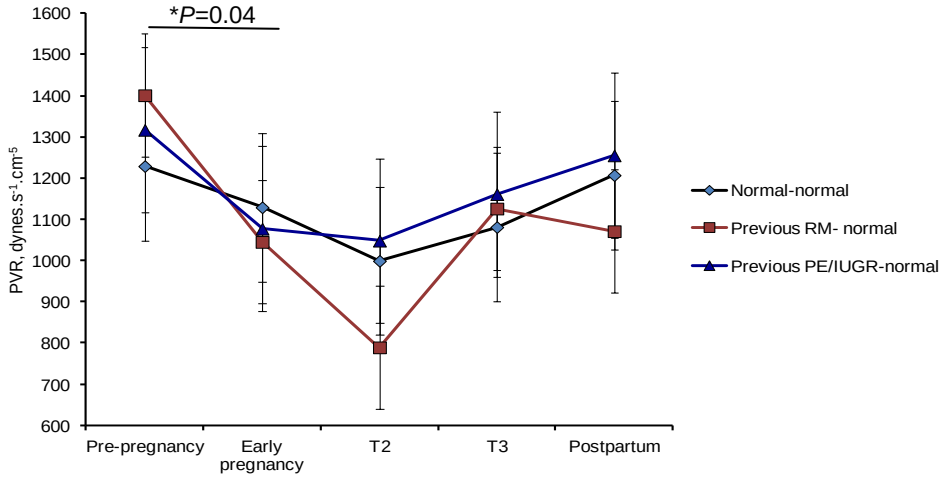


Figure 6.5 Longitudinal changes in the PVR in normal pregnancies in all groups. **P* <0.05 is significant by Kruskal Wallis test between the groups

AIx and aPWV: The adjusted AIx reduced significantly during pregnancy in all groups and remained higher than pre-pregnancy values in the postpartum period (Figure 6.6).

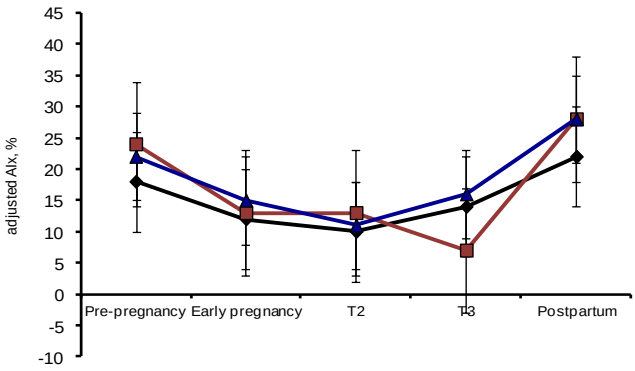


Figure 6.6 Longitudinal changes in adjusted AIx in normal pregnancies in the control group (black--♦--), women with previous RM (red--■--) and those with previous PE/IUGR (blue-▲--)

The aPWV adjusted for MAP reduced between second and third trimester in the control group however the reduction was not statistically significant. A similar trend was observed in normal pregnancies in the RM group, however, in the PE/IUGR group, aPWV reduced in very early pregnancy.

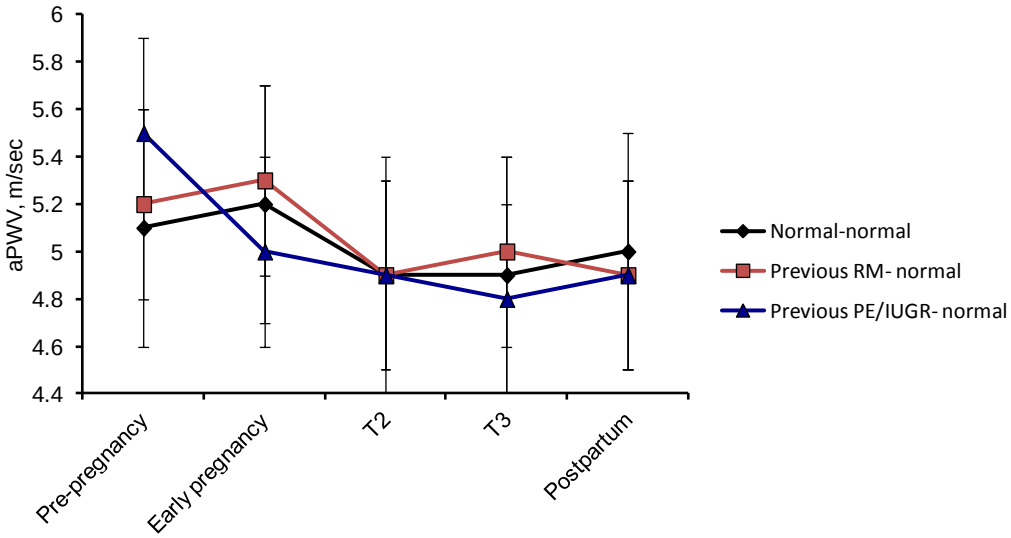


Figure 6.7 Longitudinal trends in aPWV in normal pregnancies in all groups (Control group (black-♦-), women with RM (red-■-) and those with PE/IUGR (blue-▲-)

Differences in pre-pregnancy to first trimester lipid changes: There was no difference in the trend of first trimester increase in HDL cholesterol between all the groups.

Differences in pre-pregnancy to first trimester changes in renal function: There was no difference in the increase in estimated GFR in late first trimester between all normal pregnancies.

Differences in pre-pregnancy to early pregnancy platelet aggregation: There was no difference between the pre-pregnancy to early pregnancy increase in platelet aggregation between the groups with normal pregnancies (mean difference in AUC with 10µl ADP of 7.9 ± 21 in controls, 9.4 ± 14 in women with PE/IUGR and 2.0 ± 15 in women with RM).

6.3.4 Longitudinal changes in current pregnancies complicated by preeclampsia and/or intrauterine growth restriction

The absolute values of the cardiovascular parameters in the 5 pregnancies complicated by PE/IUGR have been described below in Table 6.3.

Table 6.3 Absolute values of the cardiovascular parameters in pregnancies complicated by PE/IUGR (n=5)

Parameter	Pre-pregnancy	Early pregnancy	Second trimester	Third trimester	Postpartum
HR, beats/min	59 ± 10	72 ± 11	70 ± 6	72 ± 10	63 ± 8
Brachial SBP, mm Hg	115 ± 9	107 ± 5	111 ± 10	118 ± 4	111 ± 3
Brachial DBP, mm Hg	79 ± 7	66 ± 9	72 ± 4	75 ± 2	76 ± 3
MAP, mm Hg	93 ± 8	80 ± 6	85 ± 4	90 ± 2	89 ± 2
Central SBP, mm Hg	108 ± 10	94 ± 4	98 ± 5	105 ± 3	103 ± 4
CO, L/min	5.0 ± 0.7	5.6 ± 0.2	6.5 ± 1.3	6.3 ± 0.9	5.9 ± 0.2
CI, L/min/m^2	2.7 ± 0.5	3.0 ± 0.3	3.4 ± 0.5	3.2 ± 0.2	3.1 ± 0.3
SV, ml	78 ± 4	83 ± 21	96 ± 13	84 ± 5	91 ± 7
Unadjusted AIx, %	26 ± 11	13 ± 13	14 ± 7	14 ± 12	23 ± 9
Adjusted AIx, %	23 ± 10	16 ± 13	14 ± 6	18 ± 12	25 ± 11
Unadjusted aPWV, m/sec	5.7 ± 0.6	5.4 ± 0.5	5.3 ± 0.6	5.1 ± 0.1	5.5 ± 0.5
Adjusted aPWV, m/sec	5.7 ± 0.6	5.6 ± 0.5	5.2 ± 0.6	5.0 ± 0.1	5.3 ± 0.5
PVR, dynes.s^{-1}.cm^{-5}	1503 ± 162	1157 ± 91	1078 ± 171	1171 ± 201	1218 ± 64

Data are means ± S.D. HR= heart rate, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, MAP=Mean arterial pressure, CO=cardiac output, SV= stroke volume, PVR= peripheral vascular resistance.

Changes in BP in pregnancies complicated by PE/IUGR: Women who developed PE/IUGR during their index pregnancy in the study had a higher pre-pregnancy BP than women with normal pregnancies. These women had a significant reduction in the BP in very early pregnancy. However, unlike the further drop in BP from early pregnancy to second trimester seen in normal pregnancies, there was an increase in brachial DBP by 5 ± 7 mm Hg, in MAP

by 5 ± 5 mm Hg and in central SBP by 4 ± 5 mm Hg in the second trimester, followed by a further greater increase in the third trimester in women who developed PE/IUGR. (Figures 6.8 A, B and C)

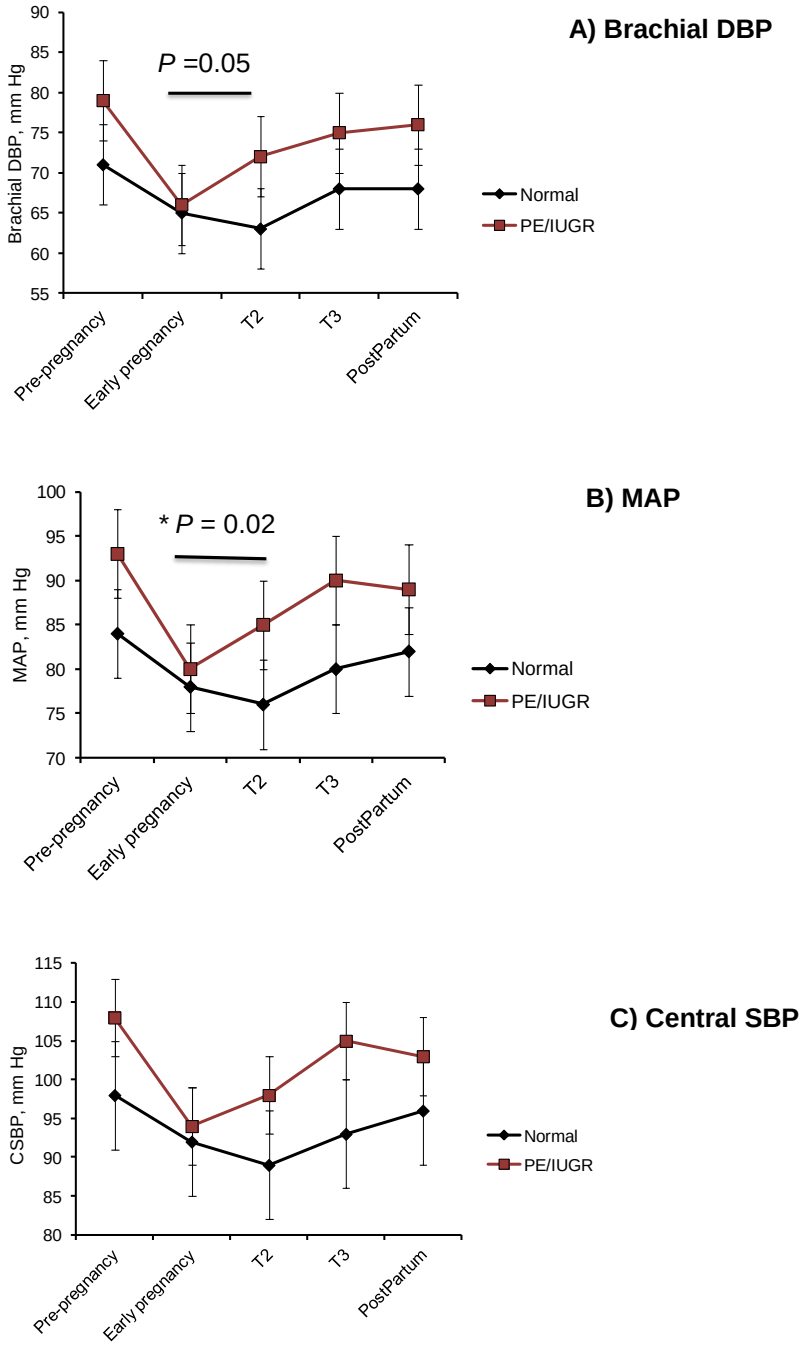


Figure 6.8 Changes in BP: A) Brachial DBP, B) MAP and C) Central SBP in normal pregnancies and pregnancies associated with PE/IUGR.T2= second trimester, T3= third trimester. *P<0.05 is significant by Mann-Whitney U test for differences between the mean change in BP at different time points in pregnancy between normal pregnancy and those with PE/IUGR.

Other cardiovascular changes: The trends of changes in CO, HR, SV, pulse wave analysis, aPWV and PVR were similar in the normal pregnancies and the pregnancies with PE/IUGR.

Differences in pre-pregnancy to first trimester lipid changes: There was no difference between the first trimester increase in lipids (HDL) between normal pregnancies and those with PE/IUGR.

Differences in pre-pregnancy to first trimester changes in renal function: There was no difference in the increase in estimated GFR between all normal pregnancies.

Differences in pre-pregnancy to early pregnancy platelet aggregation: The platelet aggregation was higher in women with PE/IUGR (mean difference in AUC for aggregation between pre-pregnancy to early pregnancy with 10µl of ADP being 11 ± 22 and with 5µl of ADP being 16 ± 20) than in women with normal pregnancies (7 ± 20 with 10µl of ADP and 5 ± 16 with 5µl of ADP). However, this difference was not statistically significant.

6.4 Discussion

We found that the cardiovascular changes in early pregnancy were similar in all normal pregnancies. Women with previous PE/IUGR had a higher pre-pregnancy BP and had a significant reduction in the BP in early pregnancy, however, in the second trimester instead of a decrease in BP as noted in all women in the normal group and women with previous unexplained RM; the brachial DBP, central SBP and MAP were found to increase. This finding supports that the theory of maternal cardiovascular maladaptation may be related to pre-existing higher BP in women with previous PE/IUGR. There was no difference in the cardiovascular adaptation in normal pregnancies between women with previous unexplained RM and women with previous normal pregnancies. We did not find any significant differences in the trends in renal function, platelet aggregation or first trimester changes in lipids between women with previous PE/IUGR, RM or normal pregnancies. There are no previous studies of platelet aggregation or lipids changes in relation to previous history of PE/IUGR or unexplained RM to compare our findings.

In the women who developed PE/IUGR in their current pregnancy, there was a trend of an increase rather than decrease in BP, in particular of the MAP in the second trimester. Although, limited by numbers to make any conclusions and a heterogeneous outcome group i.e. combination of PE and/or IUGR, the findings are consistent with those of a previous large cohort study of BP changes.[162]

6.5 Conclusion

An increase rather than a decrease in BP was observed in the second trimester in women with previous PE/IUGR and those who developed uteroplacental insufficiency in their current pregnancy. Apart from the changes in BP, similar cardiovascular changes were observed in all women with normal pregnancies including nulliparous women, those with previous normal pregnancies, unexplained RM and previous PE/IUGR.

CHAPTER 7: OVULATION IMPLANTATION TIMING, FETAL GROWTH, CARDIOVASCULAR CHANGES AND PREGNANCY OUTCOME

Summary points:

- Implantation timing was a key determinant of fetal size at 10-14 weeks and influenced gestational age assessment but did not influence first, second or third trimester fetal growth, placentation, or birth weight z-score.
- Second to third trimester fetal growth, uterine artery Doppler PI and a maximum second trimester CO increase were significantly correlated with birth weight z-score.

This chapter is based on:

Mahendru AA, Daemen A, Everett TR, Wilkinson IB, McEniery CM, Abdallah Y, Timmerman D, Bourne T, Lees CC. *Impact of ovulation and implantation timing on first trimester crown-rump length and gestational age*. **Ultrasound Obstet Gynecol**. 2012 Dec; 40(6):630-5

7.1 Introduction

Normal implantation is crucial for the establishment of a successful pregnancy. Impaired embryo-endometrial interaction and abnormal implantation are likely to be pivotal in the disease process of recurrent miscarriage.[1] This hypothesis is supported by an increased incidence of early pregnancy loss in cases with delayed implantation.[6] Impaired trophoblastic invasion of the spiral arterioles is believed to be responsible for PE and IUGR. Since the process of spiral artery remodeling begins along with trophoblastic differentiation around the time of implantation, it is possible that abnormal implantation underlies the pathogenesis of PE and fetal growth restriction. However, the events surrounding implantation in natural human conception are unclear. We hypothesize that both early and late pregnancy complications could be due to the same underlying pathology: namely impaired embryo-endometrial interaction around the time of implantation and impaired endometrial preparation as evidenced by delayed implantation. We test this hypothesis by observing the relationship between ovulation to implantation timing (O-I interval) in relation to previous history of healthy pregnancy, recurrent miscarriage and PE/IUGR. We explore the relationship of O-I interval with early pregnancy loss, placental function using uterine artery Doppler, fetal growth in the first, second and third trimester and birth weight in a cohort of women who conceived naturally.

The timing of implantation may also have an impact on gestational age assessment (GA) and may influence embryonic and fetal growth. GA itself is a determinant of perinatal outcome.[244] GA is established based on measurement of fetal crown-rump length (CRL) at 10-14 weeks.[245] We hypothesize that variation in fetal CRL at 10-14 weeks could be attributed to variation in the ovulation to implantation (O-I) interval and that differences in O-I interval may correlate to birth weight and GA at delivery.

Pregnancies complicated by fetal growth restriction are associated with abnormal cardiovascular adaptation very early in pregnancy. We explore this hypothesis by investigating the relationship between fetal growth and birth weight of the neonate with the cardiovascular adaptation during pregnancy.

7.2 Methods

101 women became pregnant out of the 143 initially recruited in the study. The differences in the demographics and menstrual cycle characteristics such as ovulation day, implantation day and ovulation implantation timing, were investigated using Kruskal- Wallis test between groups of women with previous RM, PE/IUGR and those with previous healthy pregnancies. The differences in ovulation, implantation timing and menstrual cycle data were also investigated in all pregnancies in relation to the current pregnancy outcome.

Ultrasound scans were performed at 6-7, 8-9 weeks, and 10-14 weeks of gestation from LMP in all women as described in the chapter 2 to establish first trimester growth rate. CRL growth rate (mm/day) was calculated by dividing change in CRL by change in gestational age. In the case of 3 scans, the average was taken from the growth rate between scan 1 and 2 and the growth rate from scan 2 to 3.[246]

Fetal size at 10-14 weeks was determined by the CRL measurement at 10-14 weeks and CRL z-score at the 10-14 week scan was calculated as:

CRL z-score = Measured CRL – expected CRL/standard deviation

The expected CRL was based on the CRL values derived by Robinson and Fleming[80,216] for a GA derived from ovulation timing. The relationship between implantation day, O-I interval, first-trimester growth and fetal size i.e. CRL z-score at 10-14 weeks was investigated in the 56 women with viable pregnancies, where complete data was available regarding ovulation timing, implantation timing and O-I interval.

GA was estimated by LMP (GA^{LMP}), adjusted from ovulation timing (GA^{OV}) and implantation timing (GA^{IMP}). GA^{OV} was derived by subtracting 14 days from the predicted ovulation date (LH +1) to the effective LMP, as is convention for pregnancy dating. GA^{IMP} was derived by adding the difference between the observed implantation day and the median implantation day (which was day 27 in this study) if the observed implantation day was earlier than day 27. Similarly we subtracted the difference if the observed implantation day was later than day 27. Bland-Altman plots were constructed to compare the GA

predicted by CRL (GA^{CRL}), GA^{OV} and GA^{IMP}. The 95% confidence intervals (CI) were calculated of the differences between the GA estimated by single CRL measurement and that estimated based on adjustments for ovulation and implantation timing.

The second to third trimester growth rates were derived from differences between second and third trimester AC measurements divided by the differences in GA between the scans in days. AC measurements were used in growth rate estimation because they correlate better with birth weight. The relationship between second, third trimester growth assessed using serial growth scans, length of gestation at delivery, birthweight and O-I interval was investigated using linear correlation. The first, second and third trimester growth rates were then compared to cardiovascular changes in the pregnancy.

7.3 Results

7.3.1 Clinical characteristics and menstrual cycle data in all pregnancies

The age, ethnicity in all the women who became pregnant is described in table 7.1 in relation to their previous obstetric history. Women with previous PE/IUGR were significantly older compared to the nulliparous women. There was no significant difference between the time to conceive in the women with previous healthy pregnancies, those with RM or previous PE/IUGR (median of 4 months versus 6 months in RM and PE/IUGR group, *P* = 0.5). There was no significant difference between the ovulation or implantation days between the groups of women in relation to their previous obstetric history, however, the O-I interval was longer in the women with previous RM (13 days compared to 11 days in those with previous healthy pregnancies and previous PE/IUGR respectively, *P* =0.03).

Table 7.1 Characteristics of all pregnant women and menstrual cycle details in relation to their previous obstetric history

Characteristics	**Nulliparous 51**	**Previous healthy pregnancies 25**	**Recurrent Miscarriage 17**	**Previous PE/IUGR 8**	***P****
Age, years	31 (28-33)	34 (31-35)	35 (32-39)	37 (36-38)	0.001*
Time to conceive, months	5 (3-8)	4 (2-7)	6 (4-11)	6 (2-11)	0.6
Ovulation day, days	16 (14-19)	15 (14-20)	16 (15-18)	15 (14-18)	0.9
Implantation day, days	27 (25-31)	28 (26-33)	29 (26-32)	27 (26-29)	0.5
O-I interval, days	11 (10-12)	11 (10-12)	13 (11-15)	11 (9-12)	0.03*

*O-I interval = ovulation to implantation interval. *P <0.05 is significant by Kruskal Wallis test for comparison between the ovulation day, implantation day and O-I interval women with previous healthy pregnancies in comparison to those with previous RM and PE/IUGR*

7.3.2 Menstrual cycle data in relation to the current pregnancy outcome in all pregnancies

Miscarriages less than 6 weeks had a delayed implantation day (31 versus 27 and 25 days in viable pregnancies and those with miscarriages at > 6 weeks respectively, *P* =0.004) and a prolonged O-I interval (14 days versus 11 days in both: pregnancies viable at >10 weeks and in miscarriages greater than 6 weeks) (Table 7.2).

Table 7.2: Menstrual cycle data of all pregnancies described in relation to the current pregnancy outcome

Days	Viable pregnancies at > 10 weeks	Miscarriage <6wks (*n=13)	Miscarriage >6wks (*n=12)	$P^{\dagger}$
Ovulation day	16 (11 to39)	15 (14 to 23)	14 (12 to 20)	0.2
Menstrual cycle length, days	28 (21 to 60)	30 (27 to 47)	28 (25 to 35)	0.2
Implantation day	27 (23 to 44)	31 (26 to 37)	25 (23 to 32)	0.004*
O-I interval, days	11 (9 to 20)	14 (11 to 17)	11 (9 to 13)	<0.001*

*O-I interval = Ovulation to implantation interval, * P <0.05 is significant using Kruskal-Wallis Test. Complete information was available on ovulation day in 59/71 viable pregnancies, menstrual cycle length in 69/71 viable pregnancies, implantation day in 58/71 viable pregnancies and O-I interval in 56/71 viable pregnancies.*

7.3.3 Demographics and clinical characteristics of women with viable pregnancies

Seventy-one out of the 101 pregnancies were viable at the end of first trimester. The median age, ethnicity and parity of the 71 women with viable pregnancies have been described in the table below. The O-I interval data was available for only 56 of the 71 pregnancies. One of these 56 was lost to follow up during the pregnancy and one of them had a termination of pregnancy (TOP) for chromosomal abnormality after the first trimester. Therefore, first trimester fetal growth, first trimester fetal crown rump length, second trimester growth, third trimester growth, birth weight and gestational age at delivery have been described in 54 cases with complete data on the O-I interval (described in Figure 7.1)

Table 7.3 Demographics and clinical characteristics of the 71 women with viable pregnancies

Characteristics	**Participants (n=71)**
Age, years	32 (29-35)
Ethnicity	
White	65 (91.6)
Black	2 (2.8)
Asian	2 (2.8)
Others	2 (2.8)
Parity	
Nulliparous	37 (52)
Multiparous	34 (48)

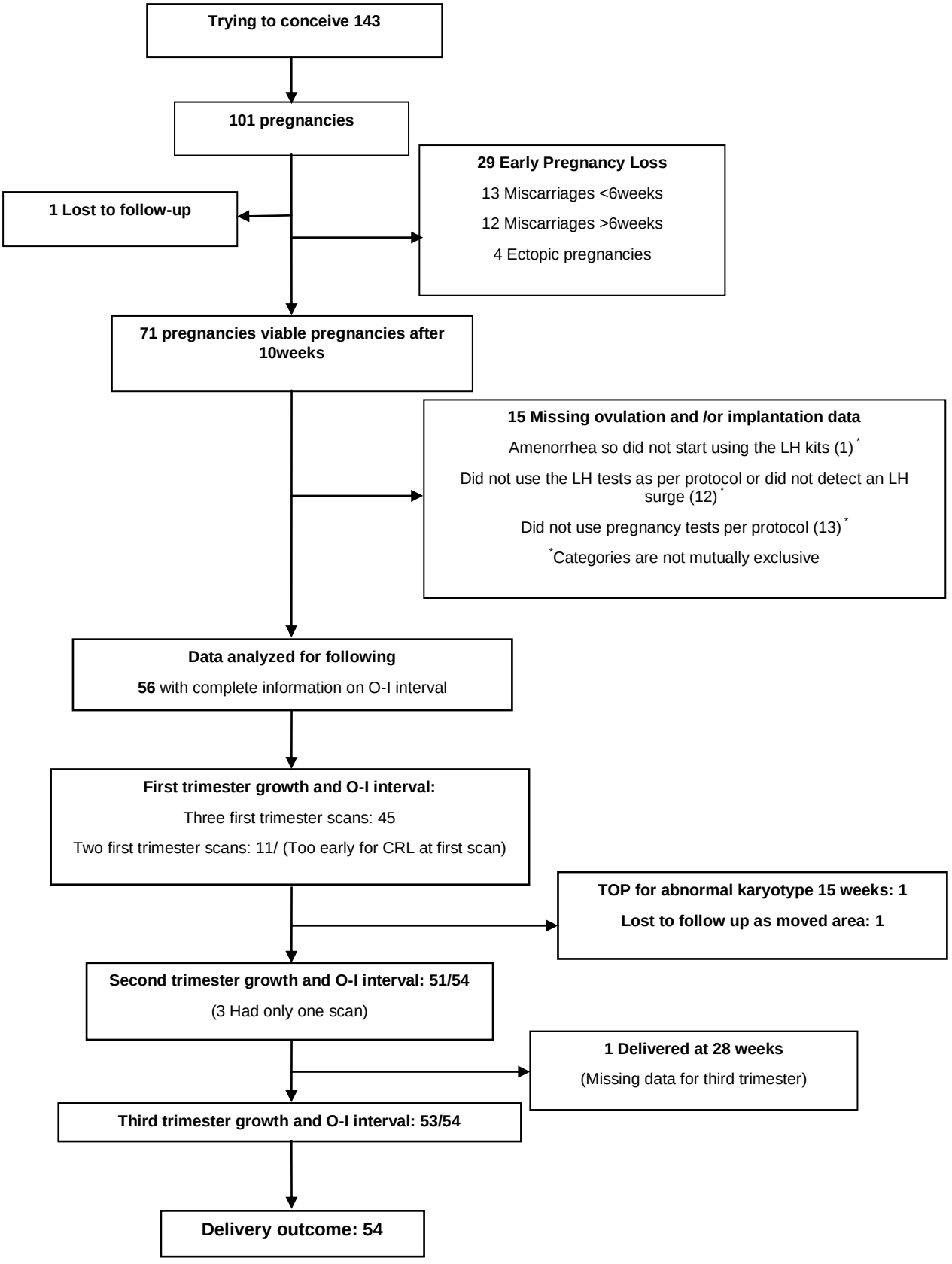


Figure 7.1 Flow chart of pregnancies with complete data on the O-I (Ovulation implantation) interval, first, second and third trimester growth

7.3.4 Ovulation and implantation in relation to fetal growth, size, placental function, GA and birth weight

O-I interval and first trimester growth: We did not find any significant relationship between first trimester CRL growth rate and O-I interval (Spearman's correlation coefficient rho 'ρ' = 0.1, P = 0.7). First trimester growth rate was not related to the difference between observed and expected CRL based on GA^{LMP} (ρ= 0.2, P = 0.1). However, there was a significant relationship between CRL growth rate and the difference between observed and expected CRL based on GA^{OV} (ρ = 0.4, P = 0.003, Figure 7.2) and that between observed and expected CRL based on GA^{IMP} (ρ = 0.5, P < 0.001, Figure 7.3).

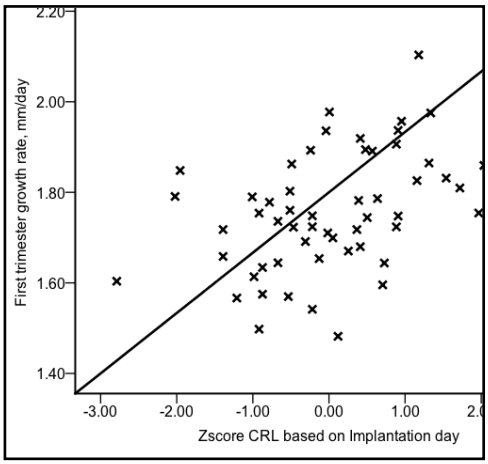


Figure 7.2 Correlation between first trimester growth rate and Z-score CRL based on GA^{OV} (ρ = 0.4, P = 0.003)

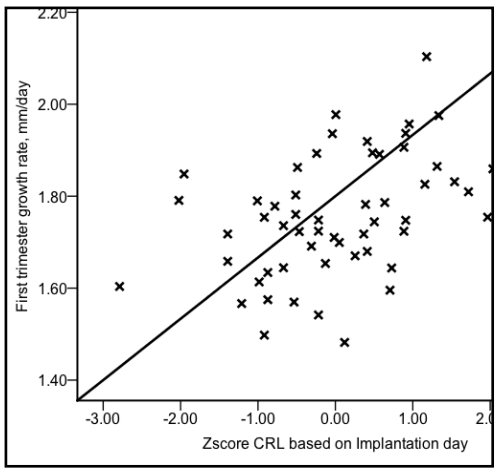


Figure 7.3 Correlation between first trimester growth rate and Z-score CRL based on GA^{IMP} (ρ = 0.5, P < 0.001)

O-I interval and Z-score CRL at 10-14 weeks: CRL Z-score at the 10-14 week scan shows a negative relationship with the O-I interval (ρ = -0.431, *P* = 0.0009, Figure7.4). In a multiple regression model, O-I interval correlated best with the Z-score CRL (based on GA by ovulation) at 10-14 weeks (R^2 = 8.9, *P* <0.001), followed by first trimester growth rate (R^2 = 2.2, *P*=0.04, Figure 7.4)

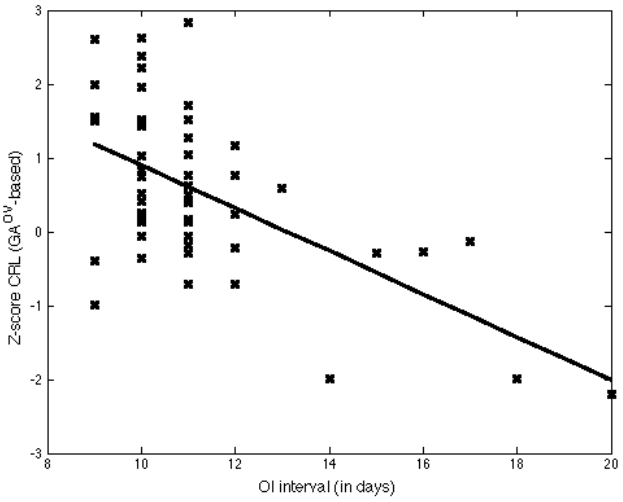


Figure 7.4 Scatter plot of crown-rump length (CRL) Z-score at 10-14 weeks showing the inverse relationship between CRL Z-score and the ovulation implantation (O-I) interval (ρ = -0.431, *P* = 0.0009)

O-I interval and GA estimation at 10-14 weeks: The difference between GA^{CRL} and GA^{OV} showed a similar inverse relationship with O-I interval (ρ = -0.430 P = 0.0009; Figure 7.5).

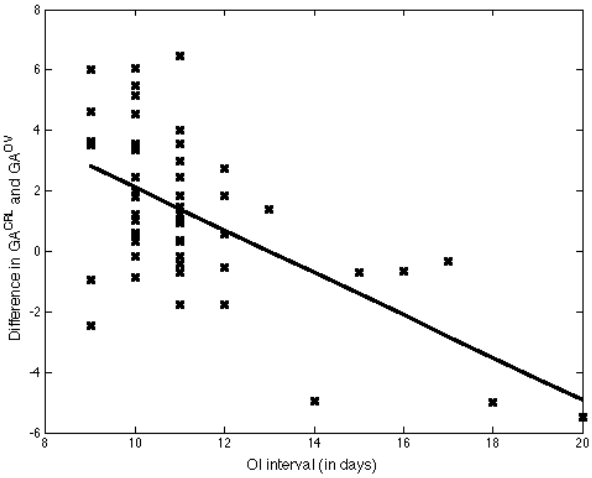


Figure 7.5 Scatterplot of difference between GA observed by CRL (GA^{CRL}) and GA derived from the ovulation day (GA^{OV}). The smaller the O-I interval, the greater the difference between GA^{CRL} and GA^{OV} (ρ = -0.430, *P* = 0.0009)

GA is routinely assigned from fetal CRL measurement at 10-14 weeks. Since this study showed that implantation timing is better related to the fetal size at 10-14 weeks and to the difference in GA derived by fetal size at 10-14 weeks, we explored the impact of ovulation timing and implantation timing on GA assessment. The differences between GA^{CRL} and GA^{LMP}, GA^{CRL} and GA^{OV} and that between GA^{CRL} and GA^{IMP} were determined in comparison with the GA derived from LMP using Bland-Altman plots. The mean difference between GA^{CRL} and GA^{LMP} was -0.8 days (95% LOA, -11.8 to 10.1 days) (Figure 7.6), that between GA^{CRL} and GA^{OV} was 1.3 days (95% LOA, -3.8 TO 6.4 days) (Figure 7.7) and that between GA^{CRL} and GA^{IMP} was 0.4 days (95% LOA, -4.0 to -4.9 days) (Figure 7.8).

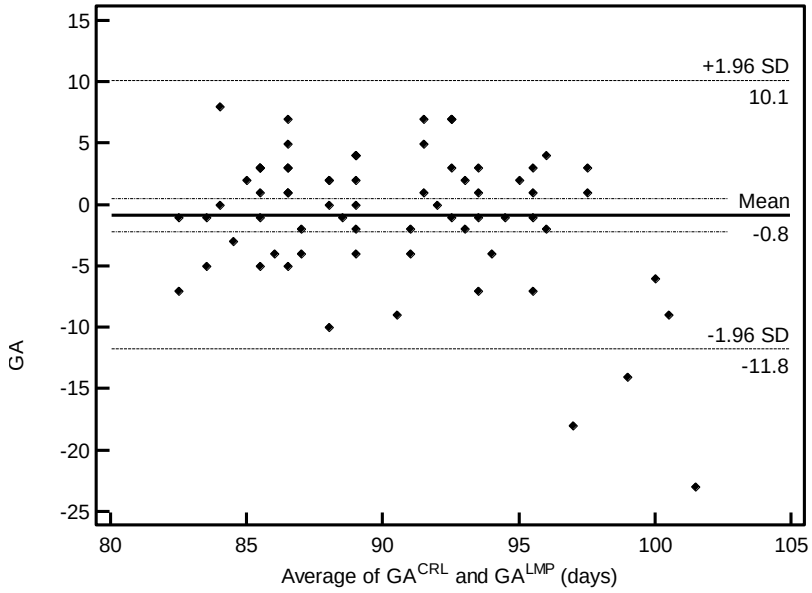


Figure 7.6 Bland-Altman plot, showing comparison of gestational age (GA) estimated from crown-rump length measurement at 10-14 weeks' gestation (GA^{CRL}) with GA based on the last menstrual period (GA^{LMP}). The bold line in the middle of the graph represents the mean difference (with 95% CI shown (_._._.) and dashed lines (_ _ _) at the either end represent 95% limits of agreement (± 1.96 SD)

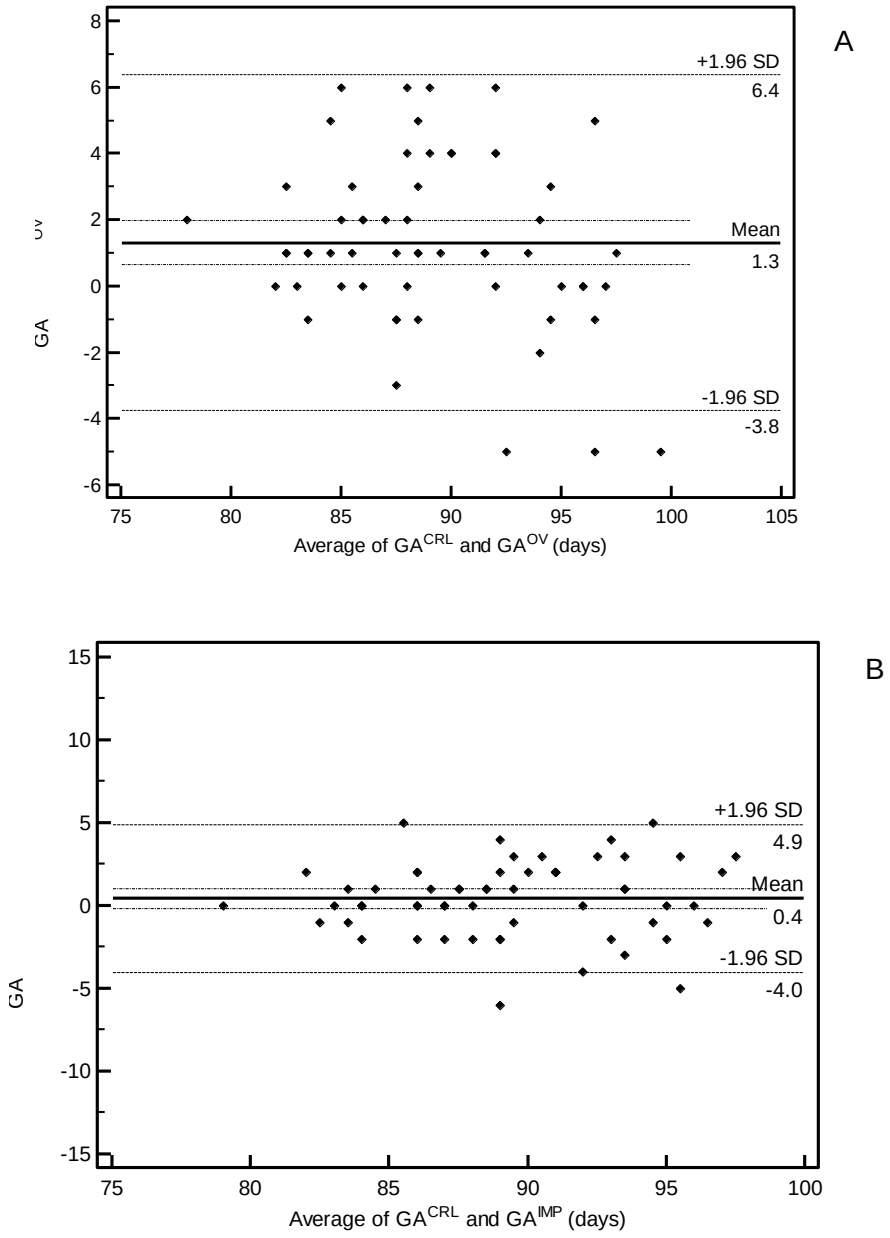


Figure 7.7 A) Bland-Altman plot showing comparison of gestational age (GA) estimated from crown-rump length measurement at 10-14 weeks' gestation (GA^{CRL}) with GA adjusted by ovulation day (GA^{OV}). B) Bland-Altman plot showing comparison of gestational age (GA) estimated from crown-rump length measurement at 10-14 weeks' gestation (GA^{CRL}) with GA adjusted by implantation day (GA^{IMP}). The bold line in the middle represents the mean difference (with 95% CI shown (_._._.) and dashed lines at the far ends (_ _ _) represent 95% limits of agreement (± 1.96 SD)

O-I interval and placental function (Uterine artery Doppler at 23-24 weeks): The O-I interval did not correlate to the median uterine artery pulsatility index (PI) (median PI: 0.82[0.75-0.97], ρ = 0.2, *P* = 0.2).

O-I interval and late pregnancy growth rate, birth weight and GA at delivery: The O-I interval did not correlate to the combined second and third trimester AC growth rate (ρ = 0.09, *P* = 0.5), but did correlate with borderline significance to the second to third trimester growth rate (ρ = 0.3, *P* = 0.05). The O-I interval did not correlate to the z-score birth weight at delivery (ρ = 0.2, *P* = 0.2; Figure 7.8).

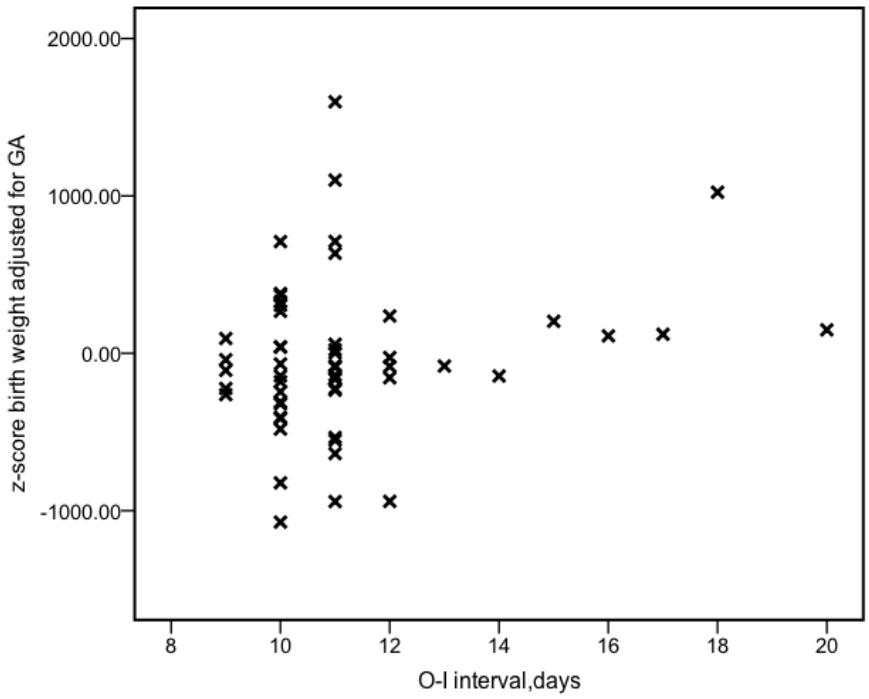


Figure 7.8 No relationship between z-score birth weight adjusted for GA and O-I interval (ρ = 0.2, *P* = 0.2)

However, O-I interval was inversely correlated to the difference in the length of gestation derived by LMP and derived from implantation day at the time of delivery i.e. longer the O-I interval, the greater the difference between the length of gestation based on LMP and implantation timing (ρ = 0.3, *P* = 0.04, Figure 7.9).

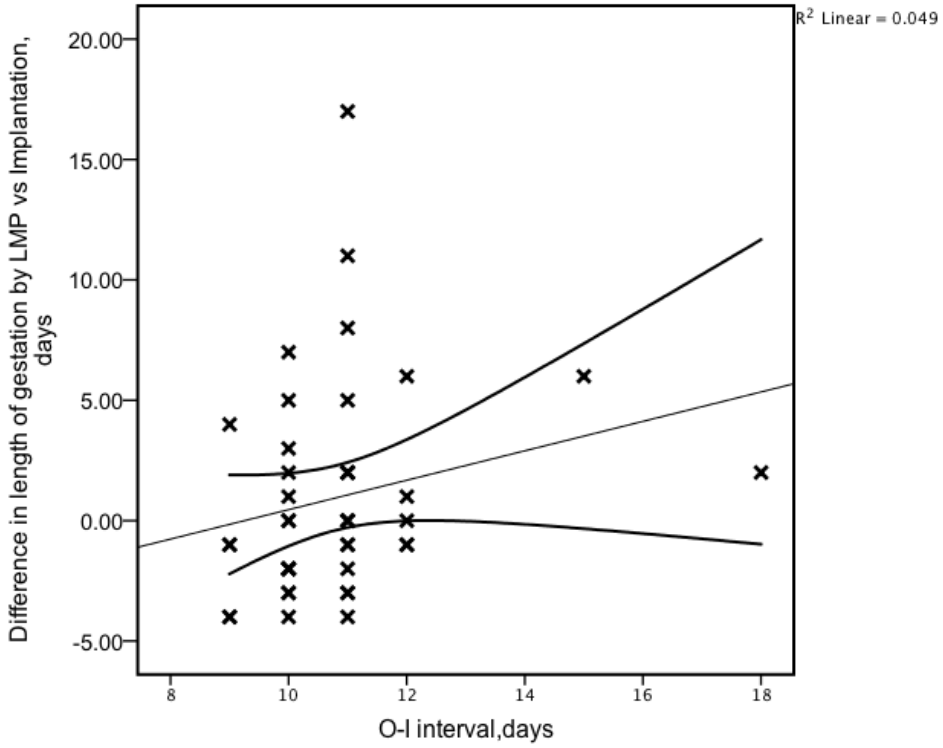


Figure 7.9 The difference in the length of gestation by LMP and implantation timing is correlated to the O-I interval (ρ = 0.3, *P* = 0.04). The bold lines represent the mean and 95% CI.

7.3.5 Factors affecting birth weight at delivery

Maternal demographics: We found no correlation between maternal age and parity with either fetal CRL at 10-14 weeks (ρ = 0.1, *P* = 0.6 and ρ = 0.1, *P* =0.3) or with z-score birth weight (ρ = 0.2, *P* = 0.1 and ρ = 0.2, *P* = 0.2). Maternal weight gain in pregnancy from pre-pregnancy to late third trimester: median of 11kg [8-14kg] was significantly correlated with z-score birth weight (ρ = 0.3, *P* = 0.02).

Birth weight and first trimester embryonic growth, fetal size at 10-14 weeks: There was no correlation between mean first trimester growth rate of 1.8 [1.7-1.9] mm/day and second to third trimester growth rate of fetal AC of 1.6 [1.4-1.7] mm/day (ρ = 0.130, *P* =0.3). The first trimester CRL z-score at 10-14 weeks did not correlate with second to third trimester fetal AC growth rate (ρ =-0.083, *P* =0.6). The first trimester growth rate did not correlate with the z-score birthweight (ρ = 2, *P* = 0.2) nor did the CRL z-score at 10-14 weeks (ρ = 0.1, *P*= 0.3).

Placental function and 23-24 weeks uterine artery Doppler: The mean uterine artery PI (0.88 ± 0.3) was inversely related to birth weight z-score (Pearson's correlation coefficient r = -0.3, *P*= 0.03), however was not related first trimester CRL growth rate (r = 0.06, *P*= 0.6), CRL z-score at 10-14 weeks (r =-0.1, *P* = 0.5) and growth rate of AC from second to third trimester (r = -0.045, *P* = 0.7).

Second and third trimester growth rates: The second to third trimester fetal AC growth rate and fetal EFW growth rates were linearly correlated to the z-score birth weight at delivery (ρ =0.598, *P* <0.001 and ρ =0.758, *P*<0.001 respectively, Figure 7.10) respectively.

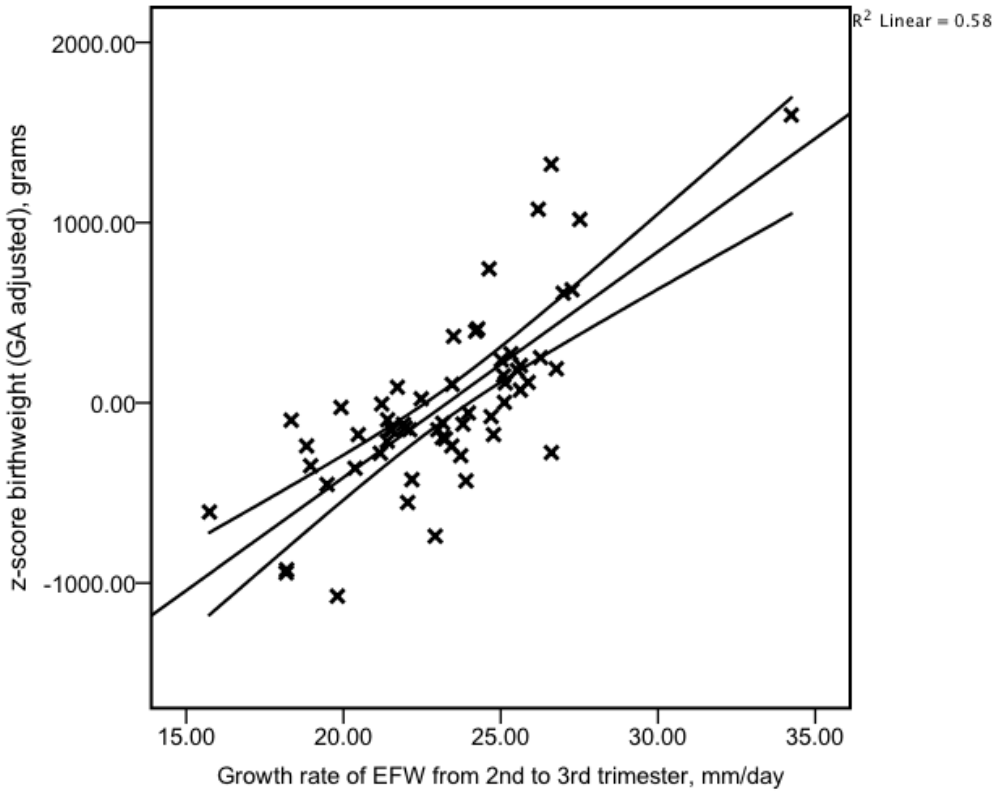


Figure 7.10 An increase in second to third trimester EFW growth rate is correlated with an increase in z-score birth weight (ρ =0.758, *P*<0.001). The bold lines represent the mean and 95% CI.

7.3.5 Cardiovascular changes in relation to fetal size and fetal growth

The details of cardiovascular changes in pregnancy have been described in the chapter 5. A maximum increase was seen in the HR from prior to pregnancy to third trimester of 13 ± 11 beats/min. There was a significant decrease of BP from pre-pregnancy to first trimester followed by a further reduction in the second trimester. The maximum increase in CO was in the second trimester. The maximum increase in CO from pre-pregnancy to second trimester was significantly correlated to the z-score birth weight (ρ = 0.3, *P* = 0.04, Figure 7.11), but not to the second to third trimester growth rate (ρ = 0.2, *P* = 0.2) or to the EFW in second trimester.

The maximum increase in HR during pregnancy did not correlate with second to third trimester growth rate of AC (r = 0.144, *P*= 0.3) or with the z-score birth weight (r= 0.120, *P* =0.3). The maximum decrease in BP from pre-pregnancy to second trimester did not correlate with second to third trimester growth rate of AC (Decrease in MAP: r=0.14, 0.3 and decrease in CSBP: r = -0.14, *P* =0.3). Neither the maximum decrease in the MAP or CSBP from pre-pregnancy to second trimester correlated to the z-score birth weight (ρ = -0.123, *P* = 0.3 & ρ = -0.079, *P* = 0.5), nor did a maximum decrease in the PVR from pre-pregnancy to early pregnancy (r =0.03, *P*= 0.8). We did not find any correlation between changes in AIx or aortic stiffness and birth weight or fetal growth rate.

7.4 Discussion

This is the first prospective study describing the ovulation and implantation timing in relation to the ultrasound measurements of embryonic growth in the first trimester, variations in GA assessments and length of gestation, fetal growth in late pregnancy and maternal cardiovascular changes in natural conception.

7.4.1 Ovulation-implantation interval, growth and fetal size

The major novel finding was that O-I interval was a major determinant of fetal size at 10-14 weeks. An early implanting embryo (short O-I interval) was larger than expected and a later implanting embryo (long O-I interval) was smaller than expected by almost exactly the number of days of variance about the median O-I interval. The O-I interval did not correlate with the first, second or third trimester growth, nor with the z-score birth weight. However, the O-I interval correlated with the difference in the length of LMP derived gestation versus implantation timing based length of gestation. The median ovulation timing in this study was 16 days as opposed to the assumption of day 14 ovulation made by Robinson and Fleming's CRL charts that are routinely used for dating.[80] It is therefore, plausible that it is likely that where ovulation occurred later on day 16, there will be a systematic over-estimation of post-implantation gestation. This could explain the observed 1.3 days overestimation from GA^{CRL} as shown in Figure 7.7(A).

We did not find a significant correlation between first trimester growth rate and z-score CRL based on LMP at 10-14 weeks. However, the CRL growth rate was related to the z-score CRL adjusted for ovulation and implantation timing, meaning thereby, that the first trimester CRL at 10-14 weeks depends on the implantation timing and subsequent embryonic growth. Contrary to what has been shown before,[86-88] we did not find any correlation between first trimester CRL measurement, first trimester growth and birth weight. These previous studies assumed a single first trimester CRL measurement as representative of first trimester growth. Although this study has limited numbers to make any conclusions, it is possible that the fetal birth weight is rather dependent on later pregnancy fetal growth.

7.4.2 Cardiovascular changes, growth and fetal size

The factors that affected birth weight significantly were total weight gain in pregnancy, uterine artery doppler PI, second to third trimester fetal growth and the maximum increase in CO in the second trimester. Uterine artery doppler PI is an indirect indicator of uteroplacental blood flow and therefore, lower resistance may suggested better perfusion of the uteroplacental bed and therefore, lead to better fetal growth.

Interestingly, of all the cardiovascular changes, maximum increase in CO from pre-pregnancy to second trimester was significantly associated with birth weight. It was however, not related to either the second trimester EFW or to second to third trimester fetal growth. It is plausible that it is the changes in CO that are dependent on the uteroplacental flow eventually affect the birth weight independent of fetal growth.

7. 5 Conclusion

Fetal size at 10-14 weeks was dependent on the implantation timing and therefore O-I interval influenced GA assessment. However, the implantation timing did not impact on first trimester embryonic growth, placentation and uterine artery Doppler, late pregnancy fetal growth or birth weight. Birth weight was influenced by maternal weight gain in pregnancy, placental function, second to third trimester growth and changes in maternal CO to improve the uteroplacental flow.

CHAPTER 8: DISCUSSION AND FUTURE PROSPECT

Summary of findings:

- It is feasible to conduct and complete a prospective cohort study from prior to conception, combining measures of ovulation and implantation with measures of fetal size, cardiovascular changes and pregnancy outcomes in naturally conceived pregnancies.
- Pre-pregnancy BP and PVR are higher in women with previous PE and/or IUGR.
- Normal pregnancies are characterized by an increase in HR during pregnancy, an early pregnancy and second trimester fall in BP, AIx and PVR along with second trimester increase in CO when compared to pre-pregnancy values.
- All normal pregnancies in nulliparous women, women with previous healthy pregnancies and those with unexplained RM were characterized by similar cardiovascular changes.
- A second trimester increase in BP was observed in pregnancies associated with PE/IUGR and in normal subsequent pregnancies in women with previous PE/IUGR.
- Women with previous unexplained RM had delayed implantation in subsequent pregnancies.
- Delayed implantation was associated with an early pregnancy loss less than 6weeks gestation.
- Implantation timing was the key determinant of fetal size at 10-14 weeks and influenced GA assessment but did not influence first, second or third trimester fetal growth, placentation or birth weight z-score.
- Second trimester increase in CO correlated with the birth weight z-score.

8.1 Background

Early risk prediction and diagnosis, increased surveillance and early delivery remain the management options for PE. Although it is known that women with PE have abnormal cardiovascular function[134] and increased risk of CVD in long term,[29,100] the exact mechanism of cardiovascular maladaptation in PE and long-term CVD is not known. It has been proposed that events around human implantation determine trophoblastic invasion and placentation and may therefore be important in predicting events preceding uteroplacental insufficiency in very early pregnancy. However, due to the inability to perform in vivo studies at the trophoblast-decidual interface, complete understanding of the events around implantation and their relationship with cardiovascular changes in pregnancy remain unclear.[110,112]

Only a prospective study from prior to pregnancy and in very early pregnancy may help in understanding the 'cause and effect' relationship, improve screening for PE and may lead to development of new interventions in the pre-pregnancy period to prevent PE.[34] An improved understanding of changes in the post partum period may also enable us to understand mechanisms of cardiovascular disease and prevention of CVD by controlling postpartum risks and early treatment opportunities. Most of the available data on cardiovascular changes in pregnancies: both normal and those with PE/IUGR consists of cross-sectional studies. Recruitment to prospective studies from prior to pregnancy can be challenging compounded with an increased incidence of pregnancy loss,[36] "drop out rate"[37] and requirement of large number of normal pregnancies in view of the rarity of occurrence of pregnancy complications such as PE/IUGR.

8.2 Feasibility of a prospective cohort study

This was a prospective cohort feasibility study combining pre-pregnancy to postpartum changes in cardiovascular function in relation to implantation events, fetal growth and pregnancy outcomes. This study was conducted in a single centre in Addenbrooke's Hospital. One hundred and forty three women planning to conceive were recruited over a period of 11months from July 2010 to June 2011. There were 101 pregnancies: 25 pregnancies miscarried; there were 4 ectopic pregnancies, 70 live births and one TOP after 12 weeks for

abnormal chromosomes. The incidence of PE and/or IUGR was 33% (2/6) in women with previous PE/IUGR and 4% (3/70) in nulliparous women.

This study shows that it is feasible to recruit women who are planning to conceive, conduct pre-pregnancy cardiovascular assessments and follow them up during pregnancy at various time points for cardiovascular assessments and fetal growth. Based on the current data, approximately half the women recruited will have healthy ongoing pregnancies. About 7 to 14% participants did not complete the entire study protocol for cardiovascular or blood tests and complete data for O-I interval, embryonic and fetal growth and cardiovascular changes was available in 63% cases. This information regarding conception rates, pregnancy loss rate, incidence of pregnancy complications and missing data, would allow the design of a prospective study from prior to conception, powered for pregnancy complications such as PE.

The availability of ovulation and pregnancy test kits, extra early and late pregnancy scans and extensive advertising enabled good recruitment to this study. The flexibility of research visits, a single person dedicated for study recruitment, performing the scans and cardiovascular tests with good rapport and communication with the participants enabled good follow up and completeness of data in the study. A recent large Chinese pre-conception cohort study has also shown the feasibility of pre-pregnancy recruitment and pregnancy follow up for BP measurements along with biochemical markers of placentation in large numbers.[234] The challenges were recruiting women who had previous PE/IUGR because it was not only difficult to identify them, but also to identify those who were trying to conceive again. Those women who experienced early pregnancy loss also required additional support and counseling. The completeness of data after delivery required good rapport and communication with the participants in order to get delivery details from other hospitals. Arranging postpartum follow up was challenging due to difficulties in contacting these participants and then arranging the visit with a 3 to 4 month old baby.

8.3 Pre-pregnancy cardiovascular function in relation to previous pregnancy outcome

In this study, women with PE/IUGR began a subsequent pregnancy with higher BP (DBP, central SBP, and MAP), lower pulse pressure (PP) amplification and PVR compared to women with previous healthy pregnancies, consistent with findings of the previous studies.[163] However, this does not prove the 'cause and effect' relationship because the higher BP and PVR may be a result of a previous pregnancy. The limitations of this study are the small numbers in these groups, selection bias in the women with PE/IUGR- as these women were self-selected and there may have been a selection bias towards more severe and earlier onset disease. Although this is a small cohort, the number is consistent with the recruitment protocols of previous studies.[163]

On the other hand, there was no difference in the cardiovascular function, lipid, cholesterol, renal function and platelet aggregation in women with unexplained RM and those with previous normal pregnancies. RM has been associated with an increased risk of CVD in epidemiological studies but most studies of CVD and RM include women with antiphospholipid syndrome,[32,190] which is a common cause of RM and, in itself can lead to endothelial dysfunction and hypertension. There is limited data regarding detailed cardiovascular function in women with RM and the link between cardiovascular dysfunction and long term CVD remains unexplored. No prospective data on cardiovascular function and arterial stiffness in women with RM is available to compare against our findings.

Although, PE/IUGR and RM may all have underlying abnormal implantation, their link to cardiovascular dysfunction remains uncertain. While it is plausible that underlying maternal vascular disease predisposes to development of PE and CVD later in life, RM in itself may not be associated with pre-existing vascular disease or have an immediate deleterious effect on maternal cardiovascular function. Oestrogen is believed to have a protective effect on vascular endothelium[247] and pregnancy duration influences the length of time a women is exposed to oestrogen with the highest exposure being at the end of a full-term pregnancy. It has been previously hypothesized that the shorter

duration of exposure to lower levels of oestrogen in women with RM may predispose them to CVD which manifests later in life.[248]

However, in the absence of pre-pregnancy and prospective data on these women the 'cause and effect' relationship cannot be explored. This emphasizes the importance of a larger prospective pre-pregnancy cohort study.

8.4 Pre-pregnancy cardiovascular function in relation to current pregnancy outcome

There were only five pregnancies with PE and/or IUGR in this study. We therefore, combined early and late onset disease in all women irrespective of their previous history into a group of uteroplacental insufficiency. Those women who developed either PE or IUGR had higher pre-pregnancy central SBP and PVR, implying the presence of vascular dysfunction prior to pregnancy. These women also had a lower HR, which may suggest a lower plasma volume reserve prior to pregnancy. There was no significant difference in BP, CO, AIx or aPWV or in metabolic function, renal function or platelet aggregation in comparison with those with normal pregnancies.

This study does not have the power to answer the questions on pre-pregnancy cardiovascular dysfunction in women who subsequently develop PE and especially early-onset disease, which is more likely to be associated with long-term cardiovascular dysfunction.

Nevertheless, although it is known that arterial stiffness is higher in the disease phase of PE, there are no data regarding pre-pregnancy arterial stiffness or cardiovascular function in women who develop PE.[172] It is plausible that arterial stiffness is higher as a result of the cardiovascular dysfunction during PE but may perhaps be normal prior to and after the pregnancy. On the other hand, there may be some cardiovascular dysfunction in the form of higher central SBP even before pregnancy. Again only a prospective study powered for the outcomes of PE may be able to answer this question and may enable assessment of the cardiovascular maladaptation during pregnancy.

8.5 Cardiovascular, metabolic and platelet changes in normal pregnancies

There were significant changes in BP, HR, CO, AIx, renal function and platelet aggregation during pregnancy, beginning from very early in pregnancy.

The significant reductions in brachial SBP, DBP and MAP very early in pregnancy were consistent with those reported in previous, albeit smaller, prospective studies.[24-26] The mid-pregnancy decrease in brachial BP had been questioned by a longitudinal study beginning from 14 to 16 weeks of pregnancy,[249] however, it is likely that the most significant reductions in BP were missed due to the relatively late 'baseline' used in this study. We observed a further decrease in the BP at 23-24 weeks consistent with what has been shown previously. We also observed a greater reduction of central versus brachial SBP in very early pregnancy, which confirms and extends previous cross-sectional studies demonstrating that the central SBP is lower in pregnant than non-pregnant women.[126,127] Interestingly, all BP measurements were lower in the postpartum period compared to the pre-pregnancy values however, only the SBPs were significantly lower at 3 to 4 months postpartum compared to the pre-pregnancy values. Therefore, using postpartum values as baseline may lead to underestimation of pregnancy related changes in BP.

We observed a significant increase in HR throughout pregnancy beginning from very early in pregnancy until the third trimester, with return of the HR to baseline at about 3 to 4 months postpartum, consistent with previous studies.[24] There was a significant increase in the CO at 23-24weeks rather than in very early pregnancy despite a significant increase in the HR in very early pregnancy, followed by a non-significant drop in the third trimester. This is in contrast to previous studies where CO increases significantly in early pregnancy and an increase in CO and SV is also seen in the third trimester in normal pregnancies.[24,112] The early pregnancy measurements were performed before 8 weeks when the SV increases and this may explain the lack of early pregnancy increase in the CO in this study. Alternatively, this could be because the Innocor technique uses a gas re-breathing method which may be affected by changes in the respiratory function during pregnancy. The later increase in CO could be due to structural changes in the heart secondary to

exposure to an increased preload in the first trimester. Since echocardiography was not performed in this study, we are unable to explain cardiac structural changes during normal pregnancies.

AIx decreased significantly very early in pregnancy followed by a further smaller decrease in the second trimester. This reduction has been underestimated in previous studies beginning from late first-trimester.[127,128] Interestingly, the AIx was found to be higher than pre-pregnancy values at 3 to 4 months postpartum. This may be because of higher SV in the postpartum period. Since cardiovascular parameters are interdependent on each other, only comprehensive understanding of all cardiovascular parameters can establish the pathophysiology of cardiovascular changes.

We observed a reduction in aPWV between the second and third trimester consistent with previous studies, demonstrating an increase in aortic compliance during pregnancy.[139,144] The later onset of changes in aortic stiffness may be because structural changes in the aorta are secondary to continued exposure to higher preload in the earlier part of pregnancy.

The eGFR increased significantly by the late first trimester suggesting the renal vasodilatation and volume expansion. The increase in HDL cholesterol and decrease in LDL cholesterol in late first trimester with no significant changes in total cholesterol or triglycerides was contrary to what has been shown previously.[177] It is possible that changes in cholesterol perhaps occur later in pregnancy as a result of the fetal effects on maternal metabolism. This has been shown previously in studies of lipids and cholesterol in the second and third trimester.[183] Platelet aggregation was elevated only at higher concentration of ADP in early pregnancy, suggesting that pregnancy induced changes probably start very early in pregnancy alongside the haemodynamic and blood flow changes, even before placental function begins.

An understanding of the cardiovascular changes in normal pregnancies is a key to understanding the pathophysiology of cardiovascular changes in pregnancies associated with uteroplacental insufficiency. So far, few prospective cohort studies with limited numbers from pre-pregnancy to the postpartum period including very early pregnancy measurements have assessed cardiovascular, metabolic or haematological changes in normal

pregnancy. The role of normal pregnancy in 'programming' and modifying the pre-existing cardiovascular, metabolic and platelet function has not been explored so far. Previous studies have used either late first trimester or postpartum values as 'baseline', whereas, we have demonstrated that significant changes would have already occurred before the first trimester and some of them would persist postpartum. Recently a Chinese pre-pregnancy cohort study has recruited a large number of women prior to pregnancy and is the largest prospective study from prior to pregnancy. It does not include measurements in very early pregnancy, nor does it include data on comprehensive cardiovascular function including central BP, AIx, aortic stiffness or cardiac output and their results are yet to be reported.[234]

This is the first prospective study from pre-pregnancy to postpartum period including early pregnancy measurements of comprehensive maternal cardiovascular, renal, metabolic and platelet changes in pregnancy including central BP, AIx and aortic stiffness. We have shown that it is feasible but also important to evaluate comprehensive changes from prior to pregnancy, in very early pregnancy and postpartum in all future pregnancy studies of maternal cardiovascular function.

8.6 Cardiovascular, metabolic and platelet changes in women with pregnancy complications

8.6.1 Previous history of preeclampsia/intrauterine growth restriction/ or recurrent miscarriage

Similar cardiovascular changes in HR, CO, PVR, arterial stiffness, renal function, lipid changes and platelet activation were noted in normal pregnancies in all groups of women irrespective of their previous obstetric history. Women with previous PE/IUGR already began with a higher pre-pregnancy 'baseline' BP and it appeared that the only difference in the pregnancy adaptation was an increase rather than a decrease in DBP and MAP in the second trimester. Although we cannot conclude based on the small numbers in this study, we hypothesize that in the women with previous PE/IUGR, there is cardiovascular maladaptation even in subsequent normal pregnancy.

8.6.2 Preeclampsia and/or intrauterine growth restriction in index pregnancy

We observed an increase rather than a decrease in the mid trimester BP in women who developed uteroplacental insufficiency in their current pregnancy, evident by either early or late onset PE/IUGR . There was no difference in other cardiovascular parameters, which may be due to limited numbers in the study.

We also observed higher platelet aggregation in early pregnancy in women with PE/IUGR compared to those with normal pregnancies using higher concentrations of ADP. However, this difference was not statistically significant. If this is true it may imply that platelet dysfunction in PE or uteroplacental insufficiency may occur very early in pregnancy lead to impaired placentation at the time of trophoblastic invasion in late first trimester. These findings emphasize the importance of a large prospective study incorporating all the cardiovascular parameters and involving larger number of pregnancies with PE/IUGR.

8.7 Ovulation, implantation timing, fetal growth, cardiovascular changes and pregnancy outcome

Implantation timing was the key determinant of fetal size at the 10-14 weeks scan and influenced GA assessment. This study reported an 11 day range of ovulation to implantation timing using digital home ovulation and pregancy test kits. Women with a previous history of RM had a median implantation day 13 compared to day 11 in all other groups implying the possibility of impaired implantation as a cause of their recurrent pregnancy failure. This is supported by recent data suggesting the role of impaired implantation in RM women.[1] Delayed implantation was associated with early pregnancy loss at less than 6 weeks consistent with previous studies.[6]

The novel finding of this study was the association of delayed implantation with a smaller CRL measurement at 10-14 weeks which would affect GA assessment. Not only this, the median ovulation day in this study was day 16 as opposed to day 14 assumed by the Robsinson CRL charts.[80] The 95% CI of differences between GA^{CRL} versus GA^{LMP} reduced from 21.9 days to 10.2

days by adjusting for ovulation and 8.9 days by adjusting for implantation day, thereby implying that the precision of GA estimation in a pregnancy by CRL, improves greatly by taking into account ovulation timing and furthermore, by accounting for the implantation date. Neither the implantation timing nor the CRL measurement at 10-14 weeks correlated with birthweight.

First trimester growth also appeared to be independent of implantation timing and did not affect second or third trimester growth or birthweight z-score. However, this study has insufficent numbers to firmly draw these conclusions.

None of the biological factors such as ovulation or implantation timing affected the later pregnancy fetal growth or placentation as demonstrated by uterine artery Doppler or birth weight z-score at delivery.

Factors affecting birth weight included maternal weight gain in pregnancy, second to third trimester fetal growth, uterine artery doppler PI and an increase in CO in the second trimester compared to the pre-pregnancy values. None of the other cardiovascular changes appeared to correlate to birthweight z-score at delivery.

This is the first study to investigate fetal growth and fetal size in relation to ovulation and implantation timing and highlights the importance of prospective studies from prior to conception in order to enable futhur discoveries about the processes around implantation which might in future help clinicians to monitor and support the progress of healthy pregnancies.[109]

8.8 Future studies

This study shows not only that it is feasible to recruit, conduct and complete a study of cardiovascular function, measures of ovulation, implantation along with measures of fetal size but at the same time pre-pregnancy and early pregnancy period is very important. Future studies from preconception period may provide valuable insight into mechanisms of maternal and fetal disease. This might lead to newer developments in the prediction and prevention of pregnancy complications such as PE.

Pregnancy is believed to be a unique condition where cardiovascular adaptation occurs in response to volume changes, changes in preload and afterload[131] along with metabolic adaptation in order to optimize uteroplacental

perfusion for a successful pregnancy outcome. Pregnancy complications such as preeclampsia, fetal growth restriction or gestational diabetes are examples of cardiovascular maladaptation and are all associated with an increased future cardiovascular risk.

Future studies might elucidate the changes that occur in fetal growth restriction, early and late onset preeclampsia separately as these conditions are often, possibly incorrectly considered together. Pre-pregnancy to postpartum changes in diabetic pregnancies are also not well described.

Although in this study cardiovascular adaptation in normal pregnancy is relatively well described, the changes in lipids and renal function still need to be better delineated.

Prospective studies of fetal growth will determine the relative contribution of implantation timing, first, second and third trimester growth velocity and cardiovascular risk factors in determination of birthweight. Even in assisted conception (where embryo transfer dates are known) very little is known about implantation timing.

The above studies can most easily be performed in either nulliparous women or those with previously healthy pregnancies, however, consideration should be given for targeted studies recruiting women with previous pregnancy complications. In particular, those at higher risk of abnormal cardiovascular adaptation in relation to preeclampsia and fetal growth restriction.

Further we have not addressed whether the maternal cardiovascular response to pregnancy is similar to cardiovascular adaptation to exercise. If the maternal response to exercise and pregnancy are similar, one might speculate that exercise or other sympathetic stimulation may be used in the pre-pregnancy period to simulate the cardiovascular challenges of pregnancy. This could unmask and predict the occurrence of maladaptation in response to pregnancy. Various physiological parameters can affect measurements of cardiovascular parameters and therefore, 24 hour readings of these measurements may be more applicable. With the availability of newer non-invasive techniques, it may be possible to use 24-hour ambulatory readings and measure various cardiovascular parameters more accurately in larger population studies.

Based on the observations of this study we propose sample size calculations to conduct a prospective pre-pregnancy study powered for PE. Assuming an incidence of PE of 5%, this means that for one woman developing PE, 40 women must be recruited pre-pregnancy, of whom 30 will become pregnant and 20 will have viable pregnancies. The ratio of 40 recruited: 1 case of PE cardiovascular changes in a large number of healthy pregnancies need to be investigated for every one case of PE. Therefore in order to be able to have at least 50 women with PE/IUGR, 2000 women will have to be recruited (Figure 8.1).

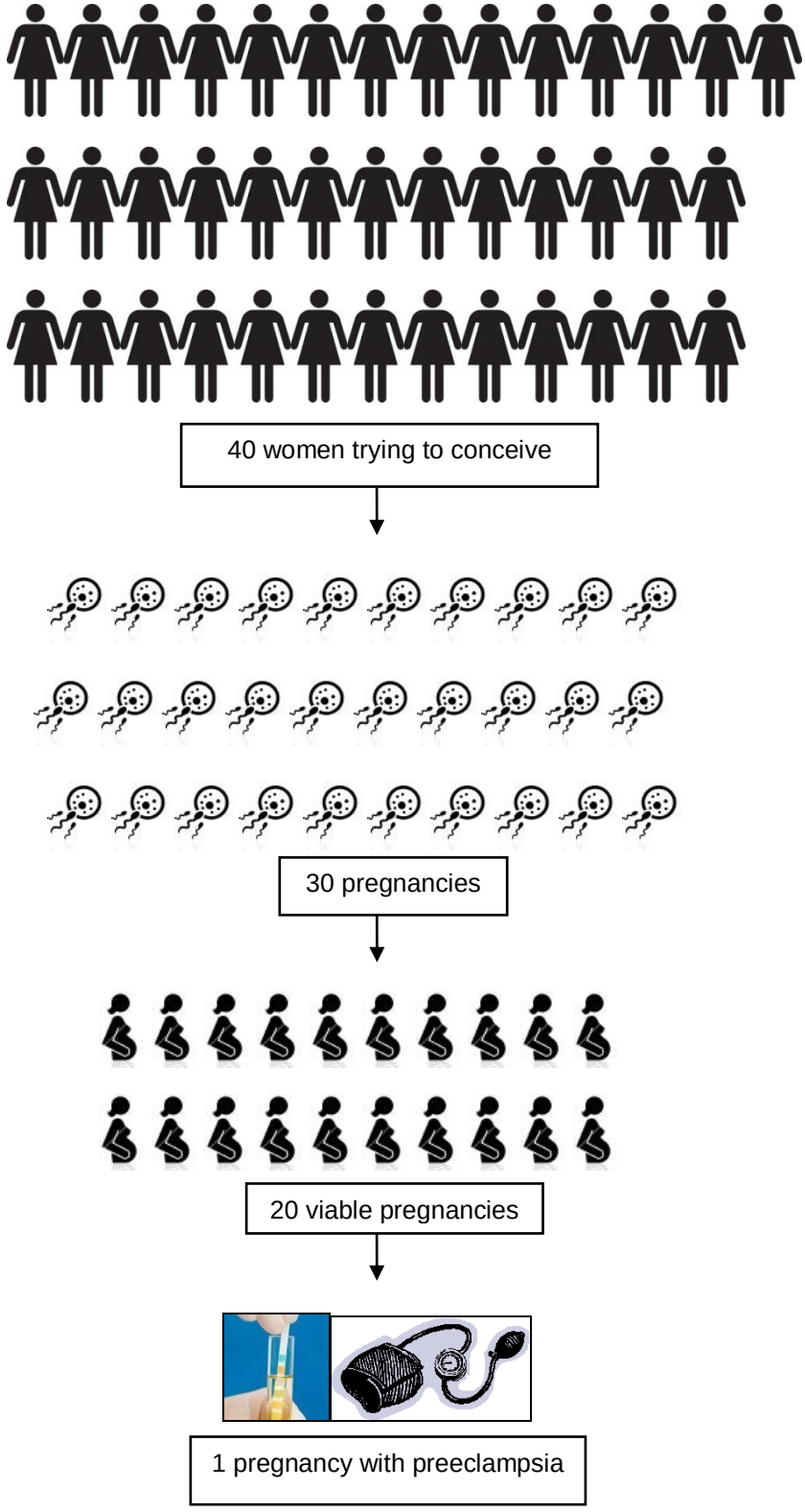


Figure 8.1 Pictorial representation of planning for recruitment of a prospective study

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APPENDICES

APPENDIX 1 ETHICS APPROVAL LETTERS

NHS

National Research Ethics Service

Cambridgeshire 1 Research Ethics Committee

Victoria House
Capital Park
Fulbourn
Cambridge
CB21 5XB

Telephone: 01223 597653
Facsimile: 01223 597645

20 MAY 2010

Copy → Aranta original in letters - I kept in 25/5/10

20 May 2010

Mr. Christoph Lees
Consultant in Obstetrics and Fetal-Maternal Medicine
Rosie Maternity
Addenbrookes Hospital Cambridge University Hospitals NHS Foundation Trust
Hills Road
Cambridge CB2 2QQ

Dear Mr. Lees

Study Title: **Implantation ovulation study- IMPOST**
REC reference number: **10/H0304/28**
Protocol number: **Version 6.2**

Thank you for your letter of 12 May 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

Other conditions specified by the REC:

- The description added on cardiovascular function under **Before becoming pregnant:** in the Participant Information Sheets is too technical and lacks lay person terminology. It is suggested amending the third sentence in the second paragraph to read "..... to breathe some air and chemically inactive gas in and out of a tube for about 20 seconds and measurement of activity of your blood vessels. Blood vessel activity is measured using a probe which is a device the size of a small pencil placed gently on the skin surface over the artery at the wrist, neck and upper leg"
- The two participant groups also require separate consent forms relative to that group, which should be coded accordingly.

On behalf of the committee, favourable opinion is granted subject to the above points being satisfactorily amended.

Authority is delegated to the Co-ordinator to ensure compliance with the above conditional points.

Final versions of documents should be provided to the committee for information.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter from Mr Christoph Lees		06 April 2010
REC application	Submission code: 45615/110865/1/239	06 April 2010
Investigator CV - Christoph C Lees		24 March 2010
Referees or other scientific critique report - Notification of Independent Review from Prof Dirk Timmerman MD, PhD		31 March 2010
Summary/Synopsis	Version 6.1	01 April 2010
CV - Dr Ian Boden Wilkinson		23 March 2010
CV - Dr Carmel Mary McEniery		22 March 2010
CV - Mr Gerald Anthony Hackett		31 March 2010
CV - Dr Amita Mahendru		26 March 2010
CV - Dr Thomas Richard Everett		23 March 2010
Covering Letter email attaching response to committee from Amita Mahendru		17 May 2010

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Protocol	Version 6.2	11 May 2010
Participant Information Sheet: Participant Information Sheet (1) - Healthy women / women with previous normal pregnancies	V6.2	11 May 2010
Participant Information Sheet: Participant Information Sheet (2) - Women with previous pregnancy problems	V6.2	11 May 2010
Participant Consent Form	V6.2	11 May 2010
Letter of invitation to participant	V6.1	07 May 2010
GP/Consultant Information Sheets	V3.1	07 May 2010
Sample Diary/Patient Card - Menstrual diary	Version 1	11 May 2010
Questionnaire	V6.2	11 May 2010
Advertisement 'Are you thinking of getting pregnant?'		
Flowchart	Version 6.2	11 May 2010
Response to Request for Further Information from Mr Christoph Lees, Principal Investigator		12 May 2010

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/H0304/28	**Please quote this number on all correspondence**

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Yours sincerely

pp S. Davies

Dr Daryl Rees
Chair

Email: susan.davies@eoe.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: Mr Stephen Kelleher
R & D Department
Cambridge University Hospitals NHS Foundation Trust
Box 277 - Addenbrooke's Hospital
Hills Road
Cambridge CB2 0QQ

Dr John Bradley
R & D Department
Cambridge University Hospitals NHS Foundation Trust
Box 277 - Addenbrooke's Hospital
Hills Road
Cambridge CB2 0QQ

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

NHS
Health Research Authority

NRES Committee East of England - Cambridge East

Victoria House
Capital Park
Fulbourn
Cambridge
CB21 5XB

Tel: 01223 597750
Fax: 01223 597645

14 March 2012

Mr. Christoph Lees
Consultant in Obstetrics and Fetal-Maternal Medicine
Rosie Maternity
Addenbrookes Hospital Cambridge
Hills Road
Cambridge CB2 2QQ

Dear Christoph Lees

Study title: **Implantation ovulation study- IMPOST**
REC reference: **10/H0304/28**
Amendment number: **10/H0304/28/AM02**
Amendment date: **07 March 2012**
Amendment Summary: **Minor Amendment extension to study end date from July 2012 to July 2013 due to follow up of pregnancies.**

Thank you for your letter of 07 March 2012, notifying the Committee of the above amendment.

The amendment has been considered by the Committee Coordinator on behalf of the Chair.

The Committee does not consider this to be a "substantial amendment" as defined in the Standard Operating Procedures for Research Ethics Committees. The amendment does not therefore require an ethical opinion from the Committee and may be implemented immediately, provided that it does not affect the approval for the research given by the R&D office for the relevant NHS care organisation.

Documents received

The documents received were as follows:

Document	Version	Date
Notification of a Minor Amendment		07 March 2012
Covering Letter		07 March 2012

Statement of compliance

A Research Ethics Committee established by the Health Research Authority

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

10/H0304/28:	Please quote this number on all correspondence

Yours sincerely

Mrs Melanie Johnson
Administration Assistant

E-mail: melanie.johnson@eoe.nhs.uk

Copy to:

Mr Stephen Kelleher
R & D Department
Cambridge University Hospitals NHS Foundation Trust
Box 277 - Addenbrooke's Hospital
Hills Road
Cambridge CB2 0QQ

Dr John Bradley
R & D Department
Cambridge University Hospitals NHS Foundation Trust
Box 277 - Addenbrooke's Hospital
Hills Road
Cambridge CB2 0QQ

APPENDIX 2 STUDY POSTER

Cambridge University Hospitals NHS
NHS Foundation Trust

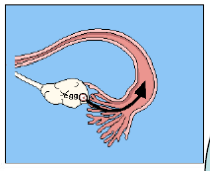
Are you thinking of getting pregnant?

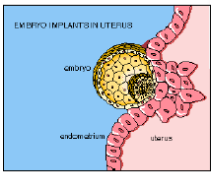
You are invited to participate in this research study.

We are interested in understanding why certain pregnancy problems occur.

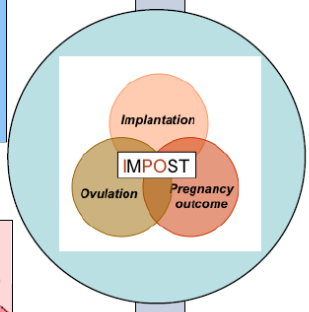
If you take part in this study, we will give you pregnancy tests and ovulation kits. We will also do some simple cardiovascular tests before and during pregnancy, and once after you have had the baby.

Egg release: Ovulation

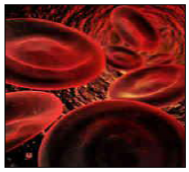




Embryo implantation



Cardiovascular status



Platelet function

If you would like further information, please contact:

Dr Amita Mahendru
via email amita.mahendru@addenbrookes.nhs.uk
or mobile telephone 07947814778

Investigators:
Mr Christoph Lees, Mr Gerald Hackett, Dr Ian Wilkinson, Dr Carmel Mc Eniery, Dr Thomas Everett
IMPOST study, Rosie Hospital, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ

Addenbrooke's Hospital | Rosie Hospital

APPENDIX 3 PARTICIPANT INVITATION LETTER

Invitation Letter to participant- IMPOST V6.1; 07/05/10

Cambridge University Hospitals NHS
NHS Foundation Trust

Addenbrooke's Hospital

Hills Road

Cambridge CB2 2QQ

Name:

Address:

Postcode:

Invitation to participate in the IMPOST study

Dear ,

If you are thinking of getting pregnant, we would like to invite you to participate in a research study in the Rosie Maternity at Addenbrooke's Hospital

We are interested in understanding why certain complications occur in pregnancy. If you take part in this study, we will give you free pregnancy tests and ovulation kits for a maximum of 6 cycles. We will also perform some simple, safe, non-invasive tests of your circulatory system before you become pregnant, at various times during pregnancy and few months after you deliver.

If you would like further information then please contact Dr Amita Mahendru via email amita.mahendru@addenbrookes.nhs.uk or mobile telephone 07947814778 or please reply using the reply slip and we will send you details by post.

We appreciate you taking time to read this letter.

Yours sincerely,

Mr. Christoph Lees

Principal Investigator-IMPOST study

Consultant in Obstetrics and Fetal-Maternal medicine

Rosie Hospital/ Addenbrooke's Hospital.

Invitation Letter to participant- IMPOST V6.1; 07/05/10

Reply slip:

IMPOST Study

Please return to: Dr. Amita Mahendru, Rosie Maternity, Addenbrooke's Hospital, Hills Road, Cambridge. CB2 2QQ

I am interested to find out more about the study and would like further information about the study.

My Details:

Name: _____

Address: _____

_____

Postcode: _____

Email (If information to be provided by email): _____

Telephone no (If information to be conveyed by phone):_____

I would like the information provided by:

Sent by post to the above address: _____

Sent by email: _____

Telephone call (any preferred times-please state): _____

APPENDIX 4 PARTICIPANT INFORMATION SHEETS AND CONSENT FORMS

Cambridge University Hospitals NHS
NHS Foundation Trust
Rosie Maternity
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 2QQ

Participant Information Sheet–V6.4; 18/09/10

Information Sheet for Research Participants (1)

Healthy women/ women with previous normal pregnancies

Study title: Implantation Ovulation Study- IMPOST

Principal Investigator: Mr. Christoph Lees MD MRCOG

You are being invited to take part in this research study, which aims to investigate pre-pregnancy predictors for development of pregnancy problems.

Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and members of hospital staff.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.
- Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part in the study.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. If you decide not to take part or to withdraw at any other time without explanation, your future care will not be affected by your decision. Thank you for reading this.

Part 1 of the information sheet

What is the purpose of the study? Most pregnancies have a good outcome. Some women may develop problems in pregnancy such as recurrent miscarriage, high blood pressure with protein in the urine (pre-eclampsia), reduced growth of the baby during pregnancy (known as intrauterine growth restriction). Early and late pregnancy problems may both be associated with delayed implantation, which could be used to predict these problems pre-pregnancy.
We want to establish whether it is possible to determine if delayed attachment of the embryo to the lining of womb (The process of attachment is known as implantation) or pre-pregnancy cardiovascular risk factors are linked to pregnancy problems.

By assessing the cardiovascular function (heart and blood vessel function) using non-invasive tests pre-pregnancy, at various times during pregnancy and 4 months post-delivery, any existing association between the cardiovascular risk factors and adverse early and late pregnancy outcomes can be investigated.

Why have I been invited? You are healthy and either never been pregnant before or never had any previous pregnancy problems and are trying to get pregnant.

Do I have to take part? It is up to you to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive in the future.

What do I have to do? During your visits to various clinics, you will be asked whether you would like to participate in the study. If you would like to participate you will be given the contact details of a member of the research group who will explain the purpose of this study, explain the tests involved in further detail and answer your questions. You will be given an information leaflet to read and decide if you want to participate.

What will happen to me if I take part? If you want to take part you will be enrolled in the study **before becoming** pregnant. You will be seen in a research clinic where you will be asked to sign a consent form.

Before becoming pregnant: You will be asked questions about your previous pregnancies and general wellbeing. The research team member will help you to complete a questionnaire, which would take about 15 minutes. Your urine will also be tested. We will ask you not to have food for 4 hours before the visit.

The study will involve a visit to the Vascular Research Clinic, where the researcher will measure your height and weight. She/he will then perform some simple, non-invasive tests to assess your heart and blood vessel function (cardiovascular function tests), which are completely safe in pregnancy. This will take about 30 minutes. These tests involve measuring your pulse, blood pressure, and assessing heart function by asking you to breathe some air and chemically inactive gas in and out of a tube for about 20 seconds and measurement of activity of your blood vessels. Blood vessel activity is measured using a probe, which is a device the size of a small pencil placed gently on the skin surface over the artery at the wrist, neck and upper leg.

We will take an extra 20ml (approximately 4 teaspoons) of blood from you. To give you some idea, normally approximately the same amount of blood would be taken anyway, as part of your routine care. This sample is used to measure various levels of naturally occurring substances in the blood for example- glucose, cholesterol and to check your platelet function (blood cells which help clotting). If the result is abnormal then we will inform you and your GP.

You will be given a free urine test kit to be able to assess ovulation (egg release) and to detect whether you are pregnant. The kits are easy to use, reliable and user

friendly. The research team member will show you how to use this kit. You will be requested to record when you are having your period and when you perform the urine test for ovulation and the test for detection of pregnancy.

You will be given contact details of the research team member. If you don't become pregnant following the cycle you will be given another kit for the next cycle. If you are not pregnant by the end of 6th cycle after recruitment you will be offered re-recruitment for another 6 cycles or you can opt out if you want.

Once you are pregnant: You will be asked to attend the research clinic for ultrasound scan at 6 and 8 weeks to check on the growth of the pregnancy. **These will not be your dating scans. You will have a normal departmental dating scan and anomaly scan as per routine antenatal care.**

Your cardiovascular function will be checked again at 6 weeks, 18-24 weeks when you come for a routine scan, at 34 weeks and then at about 4 months after delivery.

The blood tests performed prior to pregnancy will be repeated late in first trimester (at the time of your routine booking bloods) and then at 4 months after delivery. You will be requested not to have food 4 hours before this visit.

You will also have ultrasound scans at 18-24 weeks and at 34 weeks.

Taking part in this study will not affect the standard treatment that you receive. You can opt out of the study at any stage. Any complications in the pregnancy will be managed as per our routine protocols. We will be following you up during pregnancy and after you deliver to check on the outcome of the pregnancy and the wellbeing of the baby once the baby is born.

What are the possible disadvantages and risks of taking part? There are no disadvantages or major risks associated with taking part. The cardiovascular tests and ultrasound scan have no known adverse effects on the mother or the baby. Staff who are trained and experienced in the field will perform the ultrasound scans. The blood tests may cause some discomfort and slight skin bruising.

What are the possible benefits of taking part? You will get free ovulation and pregnancy detection kits for 6 cycles, which will detect pregnancy at an early stage. Having extra ultrasound examinations may help reduce the anxiety associated with pregnancy.

Will my taking part in this study be kept confidential? Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2

Thank you for taking the time to read this information and consider this study. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2 of the information sheet

What will happen if I don't want to carry on with the study? If you decide that you don't want to continue to take part in the study, there is no problem with this. All the information that had been collected about you will be destroyed and you can continue to have your pregnancy care as per the hospital guidelines.

Complaints If you have a concern or question about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions, or to: Mr C C Lees, Principal Investigator, Fetal Medicine Department, Rosie Hospital 01223 217972

If you are unhappy with any aspect of the study and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the PALS office at Addenbrooke's Hospital.

Harm in NHS based research If you are harmed due to someone's negligence, then you may have grounds for a legal action for compensation against Cambridge University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential? All information that is collected about you during the course of the research will be kept strictly confidential. Your name and address will be removed from the information when it is shown to other medical staff outside the study. Some parts of your medical records and the data collected for the study will be looked at by the research team only, and kept on a secure hospital server with password protection. The data will be kept for a minimum of five years after the end of the study. The data may also be looked at by representatives of regulatory authorities and by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty. All data obtained as part of a research study can only be used in the way that has been agreed by the ethics committee, and is under the overall auspices of the hospital's Caldicott Guardian, an independent person who has responsibility to ensure that all data is kept legally and correctly.

Involvement of the General Practitioner/Family Doctor (GP): With your agreement we will write to your GP informing them that you have taken part in this study.

What will happen to any samples I give? All samples will be analysed within 24 hours and then disposed of.

Will any genetic tests be done? No

What will happen to the results of the research study? The results will be published in peer-reviewed publications in scientific journals, or presented at scientific meetings. You will not be identified in any report or publication under any circumstances, as all data will be anonymised prior to publication.

Will I know the results? If yes, how? We will not be able to identify the participants in particular however; the common research results will be passed on to you. Only the research team will be able to access your personal details stored in the questionnaire, which will be kept in a secure research area in the hospital. You would receive results of all your other investigations as per normal routine.

Who is organising and funding the research? This study is being organised by a team of clinicians and researchers at the Addenbrooke's Hospital. The funding covers the salary of a research doctor. No further payments are made to the investigators, nor is any member of staff's salary dependent on whether you take part or not.

Who has reviewed the study? All research in the NHS is reviewed by independent group of professionals and lay people. This is called a Research Ethics Committee, and it is their job to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Cambridgeshire 1 Research Ethics Committee.

Further information and contact details If you would like any further information about the study, please tell a member of the research team who will give you copies of some of the scientific papers that have already been published on this topic. If you have a concern or question about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

You could contact the research fellow Dr. Amita Mahendru on mobile no. 07947814778 or email: amita.mahendru@addenbrookes.nhs.uk or at Rosie Hospital 01223 348137.

If the research team members are unable to answer your questions or if you wish you could ask: Mr C C Lees, Principal Investigator, Fetal Medicine Department, Rosie Hospital 01223 217972

If you are unhappy with any aspect of the study and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the PALS office at Addenbrooke's Hospital.

Cambridge University Hospitals NHS
NHS Foundation Trust

Participant Consent Form V6.4; 18/09/10
(1- Healthy women/ women with previous normal pregnancies)

Participant Code No.: _____

Study title: Implantation-Ovulation Study- IMPOST

Principal Investigator: Mr. Christoph Lees

Please initial the box.

1. I confirm that I have read and I understand the information sheet (1) dated 18/09/10 (Version 6.4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to have the tests as described in the participant information sheet (1) dated 18/09/10 (Version 6.4). ☐
4. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals of the research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
5. I agree to my GP being informed of my participation in the study. ☐
6. Do you wish to be informed of the final results of the study? Please initial in the box against the option you prefer.
   - Yes ☐
   - No ☐

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7. If you wish to be informed of the final results of the study please initial in the box against the method you wish to be informed by.
   - By Post ☐
   - By Email ☐
8. I agree to take part in the above study. ☐

Name of the participant	Date	Signature
Name of the Person taking consent	Date	Signature

1 copy for participant, 1 copy for Principal Investigator, 1 copy for hospital notes

Rosie Maternity, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ

Cambridge University Hospitals NHS
NHS Foundation Trust
Rosie Maternity
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 2QQ

Participant Information Sheet–V6.4; 18/09/10

Information Sheet for Research Participants (2)

Women with previous pregnancy problems

Study title: Implantation Ovulation Study- IMPOST

Principal Investigator: Mr. Christoph Lees MD MRCOG

You are being invited to take part in this research study, which aims to investigate pre-pregnancy predictors for development of pregnancy problems.

Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and members of hospital staff.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.
- Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part in the study.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. If you decide not to take part or to withdraw at any other time without explanation, your future care will not be affected by your decision. Thank you for reading this.

Part 1 of the information sheet

What is the purpose of the study? Most pregnancies have a good outcome. Some women may develop problems in pregnancy such as recurrent miscarriage, high blood pressure with protein in the urine (pre-eclampsia), reduced growth of the baby during pregnancy (known as intrauterine growth restriction). Early and late pregnancy problems may both be associated with delayed implantation, which could be used to predict these problems pre-pregnancy.
We want to establish whether it is possible to determine if delayed attachment of the embryo to the lining of womb (The process of attachment is known as implantation) or pre-pregnancy cardiovascular risk factors are linked to pregnancy problems.

By assessing the cardiovascular function (heart and blood vessel function) using non-invasive tests pre-pregnancy, at various times during pregnancy and 4 months post-delivery, any existing association between the cardiovascular risk factors and adverse early and late pregnancy outcomes can be investigated.

Why have I been invited? You are trying to get pregnant and had problems in your previous pregnancy such as three or more consecutive miscarriages; pre-eclampsia (high blood pressure in pregnancy with protein in the urine), reduced growth of the baby during pregnancy or death of the baby in the womb.

Do I have to take part? It is up to you to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive in the future.

What do I have to do? During your visits to various clinics, you will be asked whether you would like to participate in the study. If you would like to participate you will be given the contact details of a member of the research group who will explain the purpose of this study, explain the tests involved in further detail and answer your questions. You will be given an information leaflet to read and decide if you want to participate.

What will happen to me if I take part? If you want to take part you will be enrolled in the study **before becoming** pregnant. You will be seen in a research clinic where you will be asked to sign a consent form.

Before becoming pregnant: You will be asked questions about your previous pregnancies and general wellbeing. The research team member will help you to complete a questionnaire, which would take about 15 minutes. Your urine will also be tested. We will ask you not to have food for 4 hours before the visit.

The study will involve a visit to the Vascular Research Clinic, where the researcher will measure your height and weight. She/he will then perform some simple, non-invasive tests to assess your heart and blood vessel function (cardiovascular function tests), which are completely safe in pregnancy. This will take about 30 minutes. These tests involve measuring your pulse, blood pressure, and assessing heart function by asking you to breathe some air and chemically inactive gas in and out of a tube for about 20 seconds and measurement of activity of your blood vessels. Blood vessel activity is measured using a probe, which is a device the size of a small pencil placed gently on the skin surface over the artery at the wrist, neck and upper leg.

We will take an extra 20ml (approximately 4 teaspoons) of blood from you. To give you some idea, normally approximately the same amount of blood would be taken anyway, as part of your routine care. This sample is used to measure various levels of naturally occurring substances in the blood for example- glucose, cholesterol and to check your platelet function (blood cells which help clotting). If the result is abnormal then we will inform you and your GP.

You will be given a free urine test kit to be able to assess ovulation (egg release) and to detect whether you are pregnant. The kits are easy to use, reliable and user friendly. The research team member will show you how to use this kit. You will be requested to record when you are having your period and when you perform the urine test for ovulation and the test for detection of pregnancy.

You will be given contact details of the research team member. If you don't become pregnant following the cycle you will be given another kit for the next cycle. If you are not pregnant by the end of 6th cycle after recruitment you will be offered re-recruitment for another 6 cycles or you can opt out if you want.

Once you are pregnant: You will be asked to attend the research clinic for ultrasound scan at 6 and 8 weeks to check on the growth of the pregnancy. **These will not be your dating scans. You will have a normal departmental dating scan and anomaly scan as per routine antenatal care.**

Your cardiovascular function will be checked again at 6 weeks, 18-24 weeks when you come for a routine scan, at 34 weeks and then at about 4 months after delivery.

The blood tests performed prior to pregnancy will be repeated late in first trimester (at the time of your routine booking bloods) and then at 4 months after delivery. You will be requested not to have food 4 hours before this visit.

You will also have ultrasound scans at 18-24 weeks and at 34 weeks.

Taking part in this study will not affect the standard treatment that you receive. You can opt out of the study at any stage. Any complications in the pregnancy will be managed as per our routine protocols. We will be following you up during pregnancy and after you deliver to check on the outcome of the pregnancy and the wellbeing of the baby once the baby is born.

What are the possible disadvantages and risks of taking part? There are no disadvantages or major risks associated with taking part. The cardiovascular tests and ultrasound scan have no known adverse effects on the mother or the baby. Staff who are trained and experienced in the field will perform the ultrasound scans. The blood tests may cause some discomfort and slight skin bruising.

What are the possible benefits of taking part? You will get free ovulation and pregnancy detection kits for 6 cycles, which will detect pregnancy at an early stage. Having extra ultrasound examinations may help reduce the anxiety associated with pregnancy.

Will my taking part in this study be kept confidential? Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2

Thank you for taking the time to read this information and consider this study. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2 of the information sheet

What will happen if I don't want to carry on with the study? If you decide that you don't want to continue to take part in the study, there is no problem with this. All the information that had been collected about you will be destroyed and you can continue to have your pregnancy care as per the hospital guidelines.

Complaints If you have a concern or question about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions, or to: Mr C C Lees, Principal Investigator, Fetal Medicine Department, Rosie Hospital 01223 217972

If you are unhappy with any aspect of the study and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the PALS office at Addenbrooke's Hospital.

Harm in NHS based research If you are harmed due to someone's negligence, then you may have grounds for a legal action for compensation against Cambridge University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential? All information that is collected about you during the course of the research will be kept strictly confidential. Your name and address will be removed from the information when it is shown to other medical staff outside the study. Some parts of your medical records and the data collected for the study will be looked at by the research team only, and kept on a secure hospital server with password protection. The data will be kept for a minimum of five years after the end of the study. The data may also be looked at by representatives of regulatory authorities and by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty. All data obtained as part of a research study can only be used in the way that has been agreed by the ethics committee, and is under the overall auspices of the hospital's Caldicott Guardian, an independent person who has responsibility to ensure that all data is kept legally and correctly.

Involvement of the General Practitioner/Family Doctor (GP): With your agreement we will write to your GP informing them that you have taken part in this study.

What will happen to any samples I give? All samples will be analysed within 24 hours and then disposed of.

Will any genetic tests be done? No

What will happen to the results of the research study? The results will be published in peer-reviewed publications in scientific journals, or presented at scientific meetings. You will not be identified in any report or publication under any circumstances, as all data will be anonymised prior to publication.

Will I know the results? If yes, how? We will not be able to identify the participants in particular however; the common research results will be passed on to you. Only the research team will be able to access your personal details stored in the questionnaire, which will be kept in a secure research area in the hospital. You would receive results of all your other investigations as per normal routine.

Who is organising and funding the research? This study is being organised by a team of clinicians and researchers at the Addenbrooke's Hospital. The funding covers the salary of a research doctor. No further payments are made to the investigators, nor is any member of staff's salary dependent on whether you take part or not.

Who has reviewed the study? All research in the NHS is reviewed by independent group of professionals and lay people. This is called a Research Ethics Committee, and it is their job to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Cambridgeshire 1 Research Ethics Committee.

Further information and contact details If you would like any further information about the study, please tell a member of the research team who will give you copies of some of the scientific papers that have already been published on this topic. If you have a concern or question about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

You could contact the research fellow Dr. Amita Mahendru on mobile no. 07947814778 or email: amita.mahendru@addenbrookes.nhs.uk or at Rosie Hospital 01223 348137.

If the research team members are unable to answer your questions or if you wish you could ask: Mr C C Lees, Principal Investigator, Fetal Medicine Department, Rosie Hospital 01223 217972

If you are unhappy with any aspect of the study and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the PALS office at Addenbrooke's Hospital.

Cambridge University Hospitals NHS
NHS Foundation Trust

Participant Consent Form V6.4; 18/09/10
(2-Women with previous pregnancy problems)

Participant Code No.: _____

Study title: **Implantation-Ovulation Study- IMPOST**

Principal Investigator: Mr. Christoph Lees

Please initial the box.

1. I confirm that I have read and I understand the information sheet (2) dated 18/09/10 (Version 6.4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to have the tests as described in the participant information sheet (2) dated 18/09/10 (Version 6.4). ☐
4. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals of the research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
5. I agree to my GP being informed of my participation in the study. ☐
6. Do you wish to be informed of the final results of the study? Please initial in the box against the option you prefer.
   - Yes ☐
   - No ☐

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7. If you wish to be informed of the final results of the study please initial in the box against the method you wish to be informed by.
   - By Post ☐
   - By Email ☐
8. I agree to take part in the above study. ☐

Name of the participant	Date	Signature
Name of the Person taking consent	Date	Signature

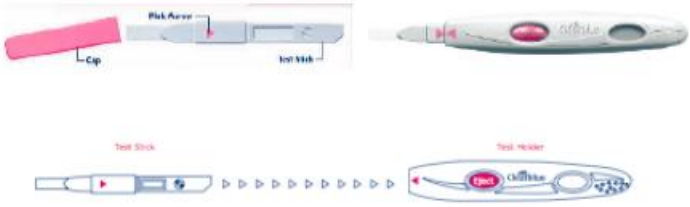
1 copy for participant, 1 copy for Principal Investigator, 1 copy for hospital notes

Rosie Maternity, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ

APPENDIX 5 STUDY INSTRUCTIONS FOR OVULATION AND PREGNANCY TESTS

Study instructions for the volunteers regarding use of the Ovulation and pregnancy test kit:
The first day of your first menstrual cycle is the day that you start to bleed and is marked as Cycle 1, Day 1.
You must begin your menstrual diary on that day, mark the date and tick the box that says Day 1 and then continue with the diary throughout the cycle. This will help in keeping a track of which day in your cycle you are and when to perform the tests.

A) Using the Clearblue® Easy ONE-MONTH Digital Ovulation Kit:
Read the information leaflet provided with the kit
For this study- irrespective of the your cycle length- you are required to begin testing your urine on the Day 6 of your menstrual cycle (Day 1 being the 1st day of start of Menstruation)



Remove the cap and assemble the test in such a way that the pink arrow on the test strip faces the pink arrow on the digital reader and clicks and the test ready symbol appears.

Do not use the test until the test ready symbol appears.
When the symbol appears- either test
1.By placing the absorbent sampler pointing downwards in the urine stream for 5-7 seconds
2.By dipping the absorbent sampler in the urine sample collected in a clean, dry container for about 15 seconds.

1. Under urine stream

2. Dipping in collected urine

Information Leaflet-Use of Ovulation and Pregnancy Test IMPOST V1; 140610

Wait:
Keep the absorbent sampler pointing downwards or lie the test flat.
The Test Ready Symbol will start flashing after 20-40 seconds to show that the test is working.
Do not eject the test Stick.

See your result:
Within 3 minutes the display will show you the result:
1. **If the result is "O" on the display screen then the test is negative.**
If the result is negative –repeat the test again tomorrow at the same time using a new Test Stick.

2. If the result is  then the test is positive which means that you are likely to ovulate about a day later.

3. Tick opposite the day of the cycle in the menstrual diary when  is seen and the next day is the day when you are likely to ovulate.

4. If the test is positive you donot need to perform further ovulation tests in this cycle and now could wait for 9th day after the  was seen on the test to start performing the pregnancy tests.

After you have obtained the result:

- Press the Eject Button and remove the test stick.
- The result will remain on the display for 8 minutes.
- If your test stick is still inserted and the display is blank, eject the test stick and your result will re-appear for 2 minutes.
- **Do not: Re-insert a used Test Stick.**
- The result of the test can only be read on the Test Holder Display. You cannot determine your result from any lines that you see on the Stick.
- If the display screen shows error symbols please follow the instructions on the bottom of the information leaflet.
- Please mark the day of the cycle on each test stick opposite the arrow with a marker pen provided.
- Please collect all the used test sticks in a bag provided.
- Please preserve the spare test sticks and they can be used in the next cycle or if spare test sticks are left and if you are pregnant then please return them to the researcher at any research visit.

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B) Performing a pregnancy test using the Clearblue® Easy Pregnancy Test:
The Clearblue Digital Pregnancy test is available in a pack with one holder and three cartridges. Please read the information leaflet provided with it carefully.
You will start performing this test from the 8th day after the smiley face is seen on the ovulation test kit. Perform one test /day. If it is positive perform 3 tests (one/day for 3 positive results and inform the researcher that the test is positive). If it is negative repeat it till you get your next period.

- Remove the cartridge from its foil wrapper and remove the cap.
- The cartridge must then be inserted into the holder by aligning the blue arrow on the cartridge with the blue arrow on the holder.
- When it is inserted correctly, the cartridge will automatically "click" into place, the Holder will automatically switch on, and the "Test Ready" symbol will appear on the Display.

- The user is then required to hold the Absorbent Sample in her urine stream for 5-7 seconds as shown before for the ovulation test. Alternatively she can collect a sample of urine in a container, and immerse just the Absorbent Sampler in the collected sample for 15 seconds.
- When the system detects that the sample has been applied, the "Test Ready" symbol on the display begins to flash to show that the Test is working.

- The holder also measures the flow of the urine along the cartridge – so if it insufficient urine has been applied an error symbol will appear on the Display- a static Test and "see leaflet". If the fluid moves through the Test too fast then this indicates over sampling and the same error symbol will be displayed.
- You may want to reapply the cap and leave the holder flat for about 3 minutes.
- In three minutes the display will either show a "Pregnant" or "Not Pregnant" result in words, which will be displayed for approximately 60 minutes.

- A further test is possible when the display has returned to a blank screen.
- If the cartridge is removed from the holder at any time before the result appears, the Display will show a flashing Test and "see leaflet" symbol. The user should re-insert the cartridge immediately. If it is not re-inserted quickly enough the error symbol will change to a static Test and "see leaflet".

APPENDIX 6 MENSTRUAL DIARY

Menstrual Diary- Implantation Ovulation Study Version 1; 11/05/2010

Participant's Menstrual Diary: Participant's Code: _____
When was the first day of your last period? _____This is the first day of your cycle. Please write this date in the first column which is marked as the day 1 of your cycle. Please perform the ovulation and pregnancy tests as instructed in the leaflet. 1st Cycle:

Date	Day of Cycle	Tick if you are having your period	Ovulation test		Pregnancy test	
			Tick if you performed the test	Result of the Ovulation Test (Tick if the result shows "smiley face"	Tick if you performed the test	Result "P" if Pregnant "NP" if non-pregnant
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	3					
	4					
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APPENDIX 7 GP LETTER

GP information letter V3.1:07/05/10

Cambridge University Hospitals NHS
NHS Foundation Trust

Addenbrooke's Hospital

Hills Road,

Cambridge CB2 2QQ

Name of GP

Address of Surgery:

Postcode:

Participation of your patient in the IMPOST research study

Dear Dr ,

I am writing to inform you that (Name of Participant) is participating in a research study by the name of IMPOST at Addenbrooke's Hospital.

This study is being conducted to be able to understand why certain pregnancy problems like recurrent miscarriage, pre-eclampsia, intra-uterine growth restriction occur. Women who take part in this study will be given free pregnancy tests and ovulation kits for 6 months, will have some basic non-invasive cardiovascular tests prior to pregnancy, during pregnancy and after delivery. They will also have extra ultrasound scans at 6 and 8 weeks and possibly an extra growth scan at 34 weeks. Their pregnancy will in every other way be managed routinely as per hospital protocols.

If you would like further information then please contact Dr Amita Mahendru via email amita.mahendru@addenbrookes.nhs.uk or mobile telephone 07947814778.

Yours sincerely,

Mr. Christoph Lees

Principal Investigator-IMPOST study

Consultant in Obstetrics and Fetal-Maternal medicine

Rosie Hospital/Addenbrooke's Hospital.