The Clinical Importance of Retained Placenta Subtypes

'Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Achier Deng Akol'
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2019
Source Declaration

I fully declare that the studies and reviews presented in this thesis are the results of my independent research unless I otherwise acknowledge. The contents of my thesis have not and are not being currently submitted for any other degree.

Achier Deng Akol
Thesis Dedication

I dedicate this thesis to all mothers who bleed to death at birth and their babies that sadly remain as orphans, in the hope that it will help reduce such tragic loss and deep sorrow. In dedication to them, I say:

For the subsequent sorrow is so deep

When all children echo
As they start to grow
Words mamma and papa
They sound simple lingua
When they are so deep

When the wind stands still
And waves settle to nil
A sea seems shallow
To the unwary fellow
When it is so deep

When a mother bleeds to death
From a retained placenta at birth
The baby sheds endless tears later
Tears appear simply like water
When the sorrow is so deep

No mother should bleed to death
From a retained placenta enigma
For bringing new life to the earth
In this medically-advanced era
For the subsequent sorrow is so deep

Achier Deng Akol, Liverpool November 2019
Thesis Contribution

My thesis contributes to the area of the retained placenta in general and its subtypes in particular. It specifically addresses the clinical importance of these subtypes as well as their diagnosis and histological markers.

Both the thesis and publications are outcomes of positive collaboration with my supervisors Professor Andrew Weeks and Professor Zarko Alfirevic. I undertook the literature review, initially drafted the protocols and published papers included in the thesis. I have gone through the process of obtaining ethical approvals and steps of publishing journal articles, from submission of manuscripts and responses to reviewers' comments, up to the approval of final versions.

For ethical approval, I was assisted by Dot Lambert, the manager of Sanyu Research Unit and Professor Andrew Weeks, my primary supervisor, with whom I defended my ethical review application in the ethical review meeting.

My original idea of conducting research generally on maternal mortality was streamlined, with the expert guidance of Professor Andrew Weeks, to obstetric haemorrhage, as this is the leading cause of maternal death globally. Then this was fine-tuned down to the commonly neglected area of the retained placenta and its grey aspect of their subtypes.

I designed the recruitment process for all the studies, collected, analysed and summarised the data. But, for the retrospective cohort study, I was assisted by Miss Sandra Dramond (IT project midwife), Mr John McCormick (IT systems administrator) and Miss Caroline Cunningham (research midwife). They extracted the data from the Meditech and anonymised it in accordance with the Caldicott rule before submitting it to me.
The GE Voluson-i ultrasound machine I used to scan women with the retained placenta on the labour ward was kindly provided by Professor Zarko Alfirevic of the Fetal Medicine Unit.

For the laboratory work, I collected, sampled, stored and analysed the biopsies of retained placentas, supervised by Miss Dharani Hapangama (head of laboratories) and Jo Drury and Miss Lisa Heathcote (lab supervisors at the Centre for Women’s and Children’s Health Research).

Acknowledgements

It is difficult to do medical research, especially in emergency obstetrics, without the support of others. Invaluable inputs from many people made this thesis succeed.

First and foremost, I consider myself very lucky to have been primarily supervised by Andrew Weeks, Professor of International Women's Health, Sanyu Research Unit, Department of Women’s and Children’s Health, University of Liverpool. His unlimited availability, exceptional academic ability, superb publication skills and more were the cardinal keys for the success of this project. No words can fully express my extreme gratitude to you, Professor Weeks.

The input of Zarko Alfirevic, Professor of Fetal and Maternal Medicine, Department of Women's and Children's Health, University of Liverpool, was also indispensable. He provided the ultrasound machine that I used in the research to scan women with the retained placentas. He also equipped me with scanning skills via his team without which I would not have been able to conduct the prospective component of this study efficiently. I will remain grateful to him for these.

The support I received from Dr S. Lane of the Department of Biostatistics for statistical analyses of my data was indispensable, and I cannot find words enough to thank him abundantly.
English remains my second language despite using it since my childhood, and I am so grateful to Jo Weeks for helping me to proof-read and edit my thesis.

The retrospective part of my research could not also have succeeded without the help of Sandra Dramond and John McCormick of the IT department, who assisted me in extracting the data from the Meditech and anonymised it as a mandatory requirement. I am grateful to both of them.

The administrative backup that I constantly received from Dot Lambert, Caroline Cunningham and Miroslava Ebringer in the Sanyu Research Unit was key to the success of this thesis. I could not have wished for better friends who selflessly and endlessly supported me in the course of the five years.

My special thanks go to all clinicians and midwives of Liverpool Women's Hospital who kindly encouraged and facilitated me in recruiting women with retained placentas on the delivery suite. I am also very grateful to have received the support and supervision for my lab work from the staff of Liverpool Women Research Laboratory, particularly Professor Dharani Hapangama, Jo Drury and Lisa Heathcote as well as Professor Judith Bulmer of Newcastle University who taught me how to correctly score my IHC slides and Sofia Makrydima for counter-scoring them.

Above all, my thanks go to the women participants who helped me to gain more understanding on this subject.

I consider myself also lucky to have been sponsored by President Salva Kiir Mayardit of the Republic of South Sudan, whose interest in the welfare of women during pregnancy and childbirth is paramount. He made my life easier, personally and morally during the course of this research. Thank you very much, Mr President.

The motivation I received from my late father Deng Akol Ayay and late mother Adut Wol Dut to undertake and complete this thesis is second to none. I owe them full gratitude for bringing me to life and offering me the chance of education as well as the spirit of motivation. Where could I have got the energy and drive to be on call
24/7 for a complete year in my sixties recruiting women with the retained placentas, had it not been because of them? I dedicate this thesis to them.

My children Nyibol, Deng ‘Adhongweth’, Deng ‘Amatwuot’, Ayay ‘Wun Pachier’, Deng ‘Dhukbaai’, Akol ‘Nyuc Abun’, Awut, new-born Maa-Adut and grandchildren Adut, Kuol, Amiir, Ayak and Akon have been a huge source of my extra motivation. They tolerated my absence during the years of this project, and I hope this thesis will serve as a core stimulus and motivational limelight throughout their educational careers.

While I remain so grateful to my eldest sister Maria Pia Nyalou and blind brother Joseph Aguer for sticking by me till the end, I deeply lament the sudden departure of my best friend Justice Aleu Akechak Jok and Akuac Deng Madut Wek, during the period of my thesis. I wish they had waited to celebrate this thesis with me.

Finally, I am exceptionally grateful to my beloved wife Victoria Awit Bona But, Ambassador Sarah Victor Bol, and all my colleagues, relatives and friends, for continuing to encourage me and taking care of my children in my absence.
List of Published Papers

Papers Published


Manuscripts under Plan

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2. Akol AD, Mammarella S, Alfirevic Z, Weeks A, Ultrasound and Doppler can be used to reliably and accurately diagnose subtypes of the retained placenta.


Conference Presentations:

1. A poster on the role of ultrasound in the diagnosis of retained placenta subtypes, British intrapartum care society (BICS) conference, Leicester-UK, 2019

2. A poster on factors and outcomes associated with retained placenta subtypes, British intrapartum society (BICS) conference, Leicester-UK, 2019
Abstract

The Clinical Importance of Subtypes of Retained Placenta

By Achier Deng Akol

In the UK, a placenta is considered retained if it is not delivered within 30 minutes of active and 60 minutes of expectant management of the third stage of labour (NICE, 2014). If a retained placenta (RP) is not treated, it may lead to maternal death due to postpartum haemorrhage or sepsis. Currently, the standard treatment is manual removal of the placenta. This is associated with anaesthetic and surgical risks compared to medical alternatives. Unfortunately, these medical alternatives are not well investigated. This is partly because of current inability to rapidly and accurately diagnose the three known subtypes of the retained placenta prior to the theatre on the labour ward. These subtypes are defined as: placenta adherens, when there is failed contraction of the myometrium behind the placenta; partial accreta, when there is a small area of accreta preventing detachment; and trapped placenta, where a detached placenta is trapped behind a closed cervix. This study explored whether these subtypes can be diagnosed prior to surgery, whether the type is important and whether hydrogen sulphide expression is involved in the pathology of RP.

First, a retrospective epidemiological cohort study of Liverpool women examined the factors and outcomes associated with the various forms of the retained placenta from 2009 to 2014. Out of 48,546 women that gave birth, 31,120 had vaginal non-instrumental births. Of these, 464 of them had manual removal, giving a prevalence of 1.5% for MROP in this hospital. But, only 355 women out of 464 met the study criteria, with 264 having placenta adherens (74.4%), 72 having trapped placenta (20.3%), and 19 partial accreta (5.3%). All RP subtypes were associated with augmented labour, but placenta adherens was also associated with low BMI.
All the subtypes were associated with significant blood loss (p value = 0.0001), but partial accreta had the highest with median blood loss of 1300.00 mls and trapped placenta the lowest with median blood loss of 500.00 mls.

We also conducted a prospective cohort study to examine the diagnostic role of ultrasound amongst women at Liverpool with various forms of retained placenta. Thirty-four women with RP and 10 with non-retained placenta were recruited. Distinctive ultrasound images of the three retained placenta subtypes were obtained at this study. On statistical analysis, we found that the exact agreement between clinical assessment and the gold standard (manual removal of placenta) of these subtypes was low at 0.48 with a weak kappa of 0.02 compared to exact agreement of 0.83 for ultrasound assessment without Doppler with good kappa of 0.67, and exact agreement of 0.97 for ultrasound assessment with Doppler with a very good kappa of 0.93. We also assessed the role of the uterine artery resistance index (UARI) and found that the lower the uterine artery resistance index below 0.60, the more likely the diagnosis of partial accreta, followed by retained placenta adherens. Alternatively, resistance indices higher than 0.60 were associated with the diagnosis of the trapped placenta. Thus, ultrasound and Doppler significantly surpass clinical assessment in the diagnosis of retained placenta subtypes. They are as good as the findings at the manual removal of placenta (the gold standard). Therefore, they may be used to rapidly and reliably diagnose subtypes of the retained placenta before manual removal.

Nine ultrasound video clips were independently assessed by ten experts on ultrasonography to test intra-observer variability of ultrasound in the diagnosis of the subtypes of the RP. Out of the nine retained placenta videos, 6 were placenta adherens, two trapped placentas and one partial accreta, based on the diagnosis reached at manual removal of the placenta by the clinicians who were regarded as the gold standard. The intra-observer variability indicated significantly high reliability of ultrasound, with exact agreement of 0.70 for placenta adherens, 0.75 for the trapped placenta and 0.95 for partial accreta.
Women with placenta adherens were clearly seen on ultrasound to have uncontracted retro-placental myometrium, and it is hypothesised that this is caused by the placenta production of powerful, locally acting tocolytic. To explore whether this tocolytic was hydrogen sulphide, we assessed the expression of its three producing synthases (CSE, CBS and 3-MPS) in 20 Placenta adherens and ten normal controls using immunohistochemistry (IHC). The expression of the three hydrogen sulphide-producing enzymes is significantly reduced in retained placenta adherens compared to spontaneously delivered placentas. This suggests that hydrogen sulphide may contribute to the aetiology of retained placenta adherens.

**Acronyms and Abbreviations**

- BICS: British intrapartum care society
- BMI: Body mass index
- CBS: Cystathionine β-synthase
- CCT: Control cord traction
- CHUV: Vaud University Hospital Centre
- CI: Confidence intervals
- CP: Control placenta
- CS: Caesarean section
- CSE: Cystathionine γ-lyase
- CT: Computed tomography
- DAB: Diaminobenzidine
- ERPOC: Evacuation of retained products of conception
- FGR: Fetal growth restriction
- GOT-IT: Glyceryl trinitrate for retained placenta
- Gov: Government
- GTN: Glyceryl trinitrate
- H₂S: Hydrogen sulphide
- Hb: Haemoglobin
- hCG: Human chorionic gonadotropin
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<tr>
<td>HIER</td>
<td>Heat-induced epitope retrieval</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<td>hPL</td>
<td>Human placental lactogen</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>IT</td>
<td>Information technology</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
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<tr>
<td>LWH</td>
<td>Liverpool Women’s Hospital</td>
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<td>LWHTB</td>
<td>Liverpool Women’s Hospital tissue bank</td>
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<tr>
<td>MAB</td>
<td>Maternal abnormal</td>
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<tr>
<td>Mg</td>
<td>Milligram</td>
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<tr>
<td>MM</td>
<td>Millimetres</td>
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<tr>
<td>MM Hg</td>
<td>Millimetres of mercury</td>
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<tr>
<td>MN</td>
<td>Maternal normal</td>
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<tr>
<td>MPST</td>
<td>Mercaptopyruvate sulfurtransferase</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MROP</td>
<td>Manual removal of placenta</td>
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<tr>
<td>MTGCs</td>
<td>Multinucleated trophoblastic giant cells</td>
</tr>
<tr>
<td>NBF</td>
<td>Neutral buffered formalin</td>
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<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute of Clinical Excellence</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
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<tr>
<td>OH</td>
<td>Ohio</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PA</td>
<td>Placenta adhesive</td>
</tr>
<tr>
<td>PAD</td>
<td>Placenta adhesive disorder</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered solution</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PI</td>
<td>Pulsatility index</td>
</tr>
<tr>
<td>PI (AA)</td>
<td>Principal investigator (Achier Akol)</td>
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PLGF  Placental growth factor
PPE  Personal protective equipment
PPH  Postpartum haemorrhage
PPV  Positive predictive value
RCOG  Royal College of Obstetricians and Gynaecologists
RI  Resistance index
RNA  Ribonucleic acid
RP  Retained placenta
RPBF  Retro-placental blood flow
RPT  Retained placental tissue
SGA  Small for gestational age
SOP  Standard operation procedure
SQL  Structured query language
TBS  Tris Buffered Saline
UK  United Kingdom of Britain
uNK  Uterine natural killer cells
USB  Universal serial bus
USS  Ultrasound
UVI  Umbilical vein injection
VBAC  Vaginal birth after caesarean section
WHO  World Health Organisation
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Appendix 7 Patient Information Leaflet for LWHTB

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References
Chapter 1 Introduction
1.1 The placenta

1.1.1 Etymology

The term placenta originates from Latin words ‘plax plak’ in the late 17th century which translates as 'flat plate'. Mammals that develop placentas are called eutherians, placentalia or simply placentals. Unlike them, marsupials and monotremes do not develop placentas. Instead, they bear immature young that are developed in a pouch on the mother's abdomen while monotremes lay eggs.

The human placenta can be described as discoid (round), haemochorial and allantochorial: haemochorial, in that maternal blood is in direct contact with the chorion, and allantochorial, in that the allantois fuses with the chorion to form an extraembryonic compound membrane.

Some rodents have haemochorial and discoid placentas like humans. Other non-human mammals have different forms of placentas, such as:

- Epitheliochorial: where the non-eroded uterine epithelium is in contact with the chorion as in cows.
- Endotheliochorial: in which the endothelium of the maternal vessels is in contact with the chorion as in bitches.
- Syndesmochorial: where the eroded endometrial epithelium is attached to the chorion as in pigs

The word retained is also derived from the Latin term ‘retinere’ which means to hold. In animals, the placenta is considered retained if it has not been passed within 12 hours after the birth of the fetus. This interval is difficult to assess in carnivores as they rapidly eat the placentas. In humans, the period is reduced to 30 to 60 minutes, depending on the management of the third stage of labour, as discussed later.
1.1.2 Embryology

As described by Schoenwolf et al. in the Textbook of Larsen's Human Embryology (2014), the placenta is formed because of interactions between the invading blastocyst and the tissue of the uterine wall (Schoenwolf G; Bleyl S, 2014). Thus, the fully developed placenta is described as a feto-maternal organ. It is a combination of the chorion, a fetal portion, and decidua, a maternal uterine portion.

To establish the uteroplacental circulation, the embryonic trophoblast invades the myometrium of the uterine wall to form an association with the maternal bloodstream. Then, the cytotrophoblast projects into the syncytiotrophoblast to form the primary, secondary and tertiary villi respectively. Hence, in its final form, the placenta consists of stem villi that extend from the chorion to the decidua basalis.

1.1.3 Anatomy

Structurally, the placenta is a flattened discoid organ (Figure 1.4 and 1.5). The anatomical parts of a placenta include the umbilical cord, the amnion, the chorion, the villi, the basal plate and the cotyledon as depicted in Figure 1.1 (Punnett's., 2018).

In vivo, the placenta is attached to the fetus through the umbilical cord, and the uterus (figure 1.2).
Figure 1.1 The fundamental parts of the placenta (Punnett’s Square, 2018)
Figure 1.2 Utero-placental/fetal relationship (drawing from Ross Laboratories, Columbus)
Early on in pregnancy, the cytotrophoblasts invade the uterus and spiral arteries, thereby transforming them into large vessels of low resistance (Figure 1.3) (Jaffe, 2001) and (Wang, 2010). Failure of this invasion and transformation occurs in obstetric pathologies of pre-eclampsia and fetal growth restriction (FGR) (Lyall et al. 2001). This is thought to occur because of an inhibitor not yet identified.
Figure 1.3 Fetal and maternal blood vessels
The placental circulation has three components:

- **Intervillous space** - (choriodecidual space). This starts as a series of smaller spaces (lacunae) that fuse together to form the maternal blood-filled space.

- **Fetal vessels** – These consist of one umbilical vein, two umbilical arteries and fetal capillaries.

- **Maternal vessels** – These are spiral arteries of the uterine stroma that are modified to remain open into the intervillous space.

At birth, the placenta comprises a wrinkled rough maternal surface containing cotyledons and a smooth/shiny fetal side at which the umbilical cord is inserted (Figures 1.4, 1.5). The amniotic membranes consist of the amnion and the chorion. The amnion is the inner membrane that surrounds the embryo/placenta, while the chorion is the external membrane which surrounds the amnion.
Figure 1.4 Anatomy of placenta-maternal surface (image from the histology and embryology department of Masaryk University)
Figure 1.5 Anatomy of placenta-fetal surface (image from the histology and embryology department of Masaryk University).
There are interlocking uterine muscle bundles, consisting of tiny myofibrils, around the branches of the uterine arteries. They run through the wall of the uterus to the placental area. The placental site itself is usually located on the anterior, posterior or fundal wall of the uterus.

1.1.4 Oxidative stress

During pregnancy, active oxygen metabolism occurs in the placenta. Reactive oxygen and nitrogen species are overproduced, which can destroy normal placental function. Disturbances in the normal state of cells can cause the production of peroxidases and free radicals which are both toxic. Thus, reactive oxygen and nitrogen species are overproduced, which can destroy normal placental function. This continuously generates oxidative stress that is defined as an imbalance of free radicals and antioxidants in the body, which can lead to cell and tissue damage. Whereas oxidation is a normal vital process in the body, oxidative stress may be useful or harmful to the body. To counteract this oxidative stress, the feto-placental unit generates abundant antioxidants. Excess of free radicals more than antioxidants, can damage fatty tissue, DNA, and proteins in the body. This can lead to a vast number of diseases over time. Thus oxidative stress and related immune disturbances are associated with adverse pregnancy outcomes such as spontaneous abortion, preeclampsia and intrauterine growth restriction. (Wu et al. 2016) and (Endler, Saltvedt & Papadogiannakis 2016). They may also be associated with the retained placenta as discussed in chapter 6.

1.1.5 Physiology

a) Breathing function
In utero, the fetal lungs do not participate in gas exchange. Therefore the placenta plays the role of "fetal lungs"(Mushambi M 2016).
Oxygen crosses the placenta readily by passive diffusion. This is facilitated by the following:
• The partial pressure gradient between maternal blood in the intervillous space and fetal blood in the umbilical arteries is (~4 kPa).

• The double Bohr effect: maternal blood takes up carbon dioxide and becomes more acidotic. This favours oxygen release to the fetus by causing a rightward shift of the maternal oxyhaemoglobin dissociation curve. The fetal blood simultaneously releases carbon dioxide and becomes more alkalotic. This also enhances fetal uptake of oxygen by leading to a leftward shift of the fetal curve.

• Fetal haemoglobin binds to oxygen more strongly than adult haemoglobin, thereby facilitating the transfer of oxygen from the mother to the fetus.

• Carbon dioxide also crosses the placenta readily by passive diffusion. Its transfer from the fetus to the mother is mainly helped by the partial pressure gradient between fetal blood in the umbilical arteries and maternal blood in the intervillous space which is (~1.8 kPa). This transfer is also enhanced by the ‘double Haldane effect’ which is a combination of the following two effects:
  • The maternal blood releases oxygen and produces deoxyhaemoglobin, which is able to carry more carbon dioxide as bicarbonate and carbaminohaemoglobin.
  • The fetal blood simultaneously takes up oxygen to form oxyhaemoglobin, which has reduced affinity for carbon dioxide and therefore releases carbon dioxide to the mother.

b) Nutritive and excretory functions
A supply of nutrients from the mother reach the fetus via the placenta, which are essential for its growth and its energy use. These nutrients include water, electrolytes, glucose, proteins, amino acids, lipids, triglycerides and water-soluble vitamins. The placenta is also involved in the removal of waste products from the fetal metabolism. They diffuse into the maternal blood to be excreted by the mother. They include urea, creatinine and uric acid.

c) Placenta and the immunological barrier
The fetus is not rejected even though its set of chromosomes differs from that of its mother and represents an allogenic transplantation to the maternal organism (two individuals of the same kind, but genetically only half identical). However, after
birth, the mother would reject any tissue from the newborn, even though the same tissue was accepted, protected and nourished for nine months. During pregnancy, the mother develops a tolerance to her child. This phenomenon is based on the specific antigen property of the embryo and the placenta as well as on the transitory changes of the maternal immune system during pregnancy.

An embryo exhibits HLA proteins on its surface that differ from those of the mother as it has received half of its genes from the paternal pronucleus. To the maternal immune system, the embryo consists of foreign material and would be eliminated if there was no fetal protective mechanism. Thus, the fetal tissue that is in direct contact with the maternal system produces no tissue antigens.

e) Protein transfer
Maternal proteins do not traverse the placental barrier, except for immunoglobulin G (Ig), which passes over from the mother to the fetus (Chatuphonprasert, 2018). Through pinocytosis of syncitiothrophoblast cells, the mother transfers to the fetus the variety of IgG that she has synthesised during her life. This transfer occurs mainly towards the end of pregnancy. Thereby the fetus obtains a passive immunity that protects it against various infectious diseases in the first six months of its life. The other immunoglobulins, mainly IgM proteins, do not pass through the placental barrier. Transferrin is another important maternal protein that, as the name indicates, transports iron. On the surface of the placenta specific receptors exist for this protein, which, by means of active transport, enters fetal tissue. It can also be transferred from the fetus to the mother.

f) Protection function
The placenta forms a "protective barrier" against many infectious and toxic agents. Nevertheless, there are other things the placenta does not protect against, for example some microbes e.g. syphilis, the cytomegalovirus, toxoplasmosis, teratogenic drugs like tetracycline and some harmful substances such as alcohol and nicotine.
Few viruses can grow in the placenta and cross it to infect the baby. These must meet the following criteria (Pereira 2018):

- They should be blood borne, either as free-floating particles or inside blood cells. Many viruses are carried in blood, but not all.
- They have to remain circulating in the maternal blood for some time and make the right proteins that enable them to bind to placental receptors.
- They should also be in the right place, in huge amounts, at this critical time when the placental cells are making the right receptors.
- Furthermore, they should not be killed by maternal macrophages or degraded by Hofbauer cells.
- Others like Zika and CMV viruses infect the maternal monocytes rendering them unable to defend the fetus and crossing the placenta via these cells.

g) Endocrine function

The placenta, especially the syncytiotrophoblast is a large endocrine gland that is only present during pregnancy. It produces hormones, including human chorionic gonadotropin (hCG), progesterone, oestrogen, and human placental lactogen (hPL).

Human chorionic gonadotropin (hCG) is produced very early in the pregnancy by the fertilized egg after it implants in the uterus. This is the hormone that is tested for when performing a pregnancy test. If the egg is fertilised, it helps to maintain the corpus luteum during the early stages of pregnancy. This is essential as the corpus luteum produces progesterone, which ensures that the lining of the uterus stays intact and provides a nourishing environment for the egg to implant and develop. Without the progesterone from the corpus luteum, the lining of the uterus would slough off, thereby ending the pregnancy. If the egg is unfertilized, the corpus luteum degenerates and the woman undergoes her normal menstrual cycle.

The placenta also begins to produce oestrogen which, during pregnancy, helps to maintain the pregnancy and prepare the breasts for milk production.
The other hormone produced by the placenta is the human placental lactogen (hPL). This promotes the mammary gland growth in preparation for lactation.

Before implantation, the above hormones come from the ovary and pituitary gland. At the beginning of the pregnancy, oestrogen and progesterone are produced by the corpus luteum, which is in turn maintained by the human chorion-gonadotropin (hCG), from the trophoblast. The role of the corpus luteum decreases progressively from the beginning of the 8th week and is entirely replaced by the placenta at the end of the 1st trimester. During the pregnancy, the level of hormone concentration in the maternal blood is sustained and regulated by the cooperation of the placental, hypophysial and fetal suprarenal hormones as well as hormones from the gonads.

1.1.6 Characteristics

Yetter (1998) describes the clinical characteristics of the normal placenta as follows: The usual term placenta is about 22 cm in diameter and 2.0 to 2.5 cm thick (Yetter 1998). It generally weighs approximately 470 g. However, these measurements can vary considerably.

The maternal surface of the placenta is dark maroon in colour and divided into lobules or cotyledons. The structure normally appears complete, with no missing cotyledons. The fetal surface of the placenta is shiny, grey and translucent enough that the colour of the underlying maroon villous tissue may be seen.

At term, the typical umbilical cord is 55 to 60 cm in length, with a diameter of 2.0 to 2.5 cm. The structure has abundant Wharton's jelly, and no true knot or thrombosis is present. The normal cord contains two arteries and one vein.

Fetal membranes are usually grey, wrinkled, shiny and translucent. The membranes and the placenta have a distinctive metallic odour that is difficult to describe but is easily recognized with experience. Normally, the placenta and the fetal membranes are not malodorous.
1.2 The third stage of labour

1.2.1 Risks of placental delivery

Recently, Walker et al compared the risk of dying for different activities with the risk of dying on the day of childbirth using data from the Office of National Statistics and other sources (Walker et al. 2014). They found that the risk of the mother dying on the day of giving birth (0.43 per 1000, or 430 micromorts) exceeds that of any other average day of life until the 92nd year. It is comparable with other apparently more dangerous activities, such as undergoing major surgery.

The third stage of labour is the duration from birth of the baby to the expulsion of the placenta and membranes. It is the shortest of the three stages of labour, lasting normally for 5-10 minutes and rarely exceeding 30 minutes. It carries the greatest potential risk for the mother. This is because of the life-threatening complications of retained placenta and postpartum haemorrhage. Its physiological mechanisms that lead to the expulsion of the placenta have been clarified by ultrasound studies. WHO and UNICEF estimate that 35% of maternal deaths worldwide are due to PPH most of which occur in the first 24 hours of birth (WHO 2012).

1.2.2 Mechanisms of placental delivery

1.2.2 a) Description of methods

The placenta detaches as a result of uterine contraction and retraction, when the uterus becomes smaller. This process also prevents blood loss by the compression of the uterine blood vessels.

Historically, the separation of the placenta is described either by Schultze or Mathew Duncan.
Schultze method: In the majority of cases, separation starts in the centre of the placenta and this part descends first. The fetal surface therefore appears at the vulva with the membranes trailing behind. The retroplacental clot is contained within the inverted sac, so there is minimal blood loss.

Matthew Duncan method: less commonly, separation starts at the lower edge of the placenta. The placenta slips down sideways and the maternal surface appears first at the vulva. This method of separation is usually accompanied by some bleeding through the vagina, since blood from the placental site escapes immediately, and a retroplacental clot does not form. This method results in slower separation of the placenta and more bleeding.

Browne and O'Sullivan allow fundal pressure on the uterus as a method of assisting the delivery of the separated placenta (1955).

Fundal pressure (the Crede manoeuvre) involves placing one hand on the top of the uterus (uterine fundus) and squeezing it between the thumb and other fingers to help placental separation and delivery. Controlled cord traction (the Brandt-Andrews method) involves traction on the umbilical cord while maintaining counter-pressure upwards by placing a hand on the lower abdomen (Kimbell, 1958). Controlled cord traction should only follow signs of placental separation. Both these interventions, if not performed correctly, may have adverse outcomes including pain, haemorrhage and inversion of the uterus. Two other methods of placenta delivery are not advised because they may be dangerous: these are uterine manipulation and cord traction. A review by Pena-Marti and Comunian-Carrasco found no randomised controlled trials to assess the use of fundal pressure as part of the active management of the third stage of labour (Pena-Marti & Comunian-Carrasco 2007). Therefore, controlled cord traction should continue as the method of placental delivery in the active management of third stage of labour.

The Brandt-Andrews technique is described by De Lee and Greenhill (1947) and advocated because the Crede manoeuvre has potential and actual dangers. Norman
Kimbell in 1958 modified the technique as described by these authors (Kimbell 1958). Instead of using a hand to grasp the umbilical cord, he used forceps. The modified technique is as follows. A pair of forceps is placed on the umbilical cord as close to the vulva as possible. One hand grasps the forceps and the other hand is placed on the abdomen so that the palmar surface of the fingers is over the anterior surface of the uterus approximately at the junction of the corpus uteri with the lower segment. The uterus is now gently pressed backwards and upwards towards the patient's umbilicus, and at the same time steady but not too strong traction is made on the umbilical cord. If too great traction is made, the cord may break. This technique will usually lead to the expulsion of the placenta and membranes. Figure 1.6
Figure 1.6 Brandt-Andrews Manoeuvre: by Christy Krames: Firm traction is applied to the umbilical cord with one hand while the other applies suprapubic counter pressure.
1.2.2 b) Description of phases.

The role of ultrasound in the labour ward (intrapartum ultrasound) has now advanced. It is predominantly used to determine fetal presentation, position and station of the fetal head (Tutschek et al. 2013) as well as assessing liquor volume and fetal heart (Usman & Lees 2015). But its role in the diagnosis and management of the retained placenta is not apparent.

Previously, Herman et al (1993), had used the ultrasound in the third stage of labour to describe the phases of placental separation as (1) latent phase, characterized by thick, placenta-free wall and thin, placenta-site wall; (2) contraction phase, with thickening of placenta-site wall (from < 1 cm to > 2 cm); (3) detachment phase, in which the placenta completes its separation and detaches; and (4) expulsion phase, with a sliding movement of the placenta (Herman et al. 1993). This mechanism is completed only when the placenta-site wall attains full thickness.

The uterus is compressed by contractions and reduced in size and volume as the uterine muscle shortens in retraction. Subsequently, the placenta is tightly compressed in the uterus and the surface area of its site is reduced. Ultimately, the placenta shears off.

Herman described five cases with retained placenta. In all five the placenta-site wall was initially thin. In four of them it became thick, and the placenta was removed by traction of the cord, whereas in the fifth case the placenta-site wall remained thin and the placenta had to be removed manually, suggesting that contraction of the retro-placental myometrium is the critical factor in causing placental delivery (ibid.).

Herman went on to describe the ultrasound features of the third stage of labour in more detail in a further paper (Herman et al. 2002). He describes how the shearing mechanism can be either monophasic or multiphasic. If monophasic, the placenta is detached as a whole simultaneously. In a multiphasic mechanism, the separation occurs from different sites. Continuous real-time ultrasound was performed during
the third stage of labour in 101 normal deliveries to characterize the patterns of placental separation during the third stage of labour. Multiphasic separation was the more common, occurring in 97 out of 101 cases. Monophasic separation in which all parts of the placenta appeared to separate simultaneously was rare, occurring in two cases only. The placenta was anterior or posterior in the uterine wall in most cases 92/101 and fundal in few cases. Where the placenta was anterior or posterior, the process started at the lower pole (down–up separation) in 83/92 cases (90.2%) and began from the upper pole (up–down separation) in only 6/92 cases (6.5%). For the fundal placenta, the separation was also multiphasic but began sequentially from either the anterior or posterior pole, or simultaneously from both, so that the fundal part was separated last (bipolar separation).

In another earlier study, trans-abdominal Doppler velocity waveform measurements of the uterine arteries were performed during the third stage of labour on 25 patients with uncomplicated deliveries and 5 with prolonged 3rd stage. The aim was to further assess the third-stage mechanisms. Unlike the initial Herman study, only three phases of placental separation were observed. The latent and expulsion phases were noticed separately but the contraction and detachment ones occurred simultaneously. The Doppler changes are described in section 1.2.4.

1.2.3 Management of the third stage

There are two main ways of managing the third stage of labour: expectant (or physiological) management, and active management.

1.2.3 a) Expectant or physiological management

This entails waiting for normal physiological processes to separate the placenta and establish haemostasis. Hence, no oxytocic is given to contract the uterus, the umbilical cord is allowed to stop pulsating before it is clamped and cut, and the placenta is delivered by maternal effort alone. Some have recommended immediate suckling of the baby after birth to reduce blood loss and postpartum haemorrhage,
pending the extraction of the placenta. There is no substantial evidence that this works.

With expectant management, the placenta usually delivers within 10-20 minutes in contrast to 5-10 minutes for active management. Women and their attendants who prefer limited intervention in the management of labour favour this approach. Begley et al (2019) conducted a systematic review to compare the effects of active versus expectant management of the third stage of labour on severe primary postpartum haemorrhage (PPH) and other maternal and infant outcomes. They showed that for “women at mixed levels of risk of bleeding, it is uncertain whether active management reduces the average risk of maternal severe primary PPH (more than 1000 mL) at time of birth (average risk ratio (RR) 0.34, 95% confidence interval (CI) 0.14 to 0.87. For maternal haemoglobin (Hb) less than 9 g/dL following birth, active management of the third stage may reduce the number of women with anaemia after birth (average RR 0.50, 95% CI 0.30 to 0.83.).” They also identified harms such as postnatal hypertension, pain and return to hospital due to bleeding.

Begley et al stated that in women at low risk of excessive bleeding, it was uncertain whether there was a difference between active and expectant management for severe PPH or maternal Hb less than 9 g/dL (at 24 to 72 hours) (Begley et al. 2019). Therefore, they suggested that women could be given information on the benefits and harms of both methods to support informed choice. They also added that, given the concerns about early cord clamping and the potential adverse effects of some uterotonics, it is critical to look at the individual components of third-stage management; and finally, that data is also required from low-income countries (ibid.).

An earlier study showed that active management of the third stage reduced the risk of PPH, irrespective of the woman’s posture (Rogers et al. 1998).
1.2.3 b) Active management

To reduce the risk of postpartum haemorrhage due to retained placenta, many units have a policy of active management of the third stage unless the woman requests physiological management. They base their choice on randomised control trials that have consistently shown that blood loss, postpartum haemorrhage and the need for blood transfusion are significantly reduced by 40-60% with active compared to physiological management. An example is the Hinchingbrooke trial (Rogers et al. 1998) that randomised 1512 women and showed a strong statistically significant reduction in blood loss with a p-value of <0.0001. The World Health Organisation, the International Federation of Gynaecology and Obstetrics, the Royal College of Obstetricians and Gynaecologists in the UK and the National Institute for Health and Clinical Excellence in UK all endorse active vis-à-vis expectant management (WHO 2012) (NICE 2014) (RCOG 2009).

Originally active management consisted of three components: oxytocic administration, cord clamping, and controlled cord traction. This has recently been modified by NICE and referred to as modified active management, as discussed later.

1.2.3.b i) Oxytocic administration

In a network meta-analysis, to identify the most effective uterotonic agent(s) to prevent PPH with the least side effects, all uterotonic agents were generally effective for preventing PPH when compared with placebo or no treatment. The analysis also suggested that ergometrine plus oxytocin combination, carbetocin, and misoprostol plus oxytocin combination might have some additional desirable effects compared with the current standard oxytocin. The two combination regimens, however, were associated with significant side effects. Carbetocin might be more effective than oxytocin for some outcomes without an increase in side effects (Gallos et al. 2018).
Oxytocin (10 IU, intravenously or intramuscularly) remains the recommended uterotonic of choice for all births according to WHO (Vogel et al. 2019). But, in settings where oxytocin is unavailable (or its quality cannot be guaranteed), the use of other injectable uterotonics (carbetocin, or if appropriate ergometrine/methylergometrine or oxytocin and ergometrine fixed-dose combination) or oral misoprostol is recommended for the prevention of PPH. Where skilled health personnel are not present to administer injectable uterotonics, the administration of misoprostol (400 µg or 600 µg orally) by community healthcare workers and lay health workers is recommended for the prevention of PPH.

But oxytocin requires cold storage which is not available in many countries. Therefore, in a large multinational WHO trial (CHAMPION study) intramuscular injections of heat-stable carbetocin (at a dose of 100 µg) and oxytocin (at a dose of 10 IU) were compared. This trial involved 29,645 women across 23 sites in 10 countries in a randomized, double-blind, non-inferiority trial. Heat-stable carbetocin was found to be non-inferior to oxytocin for the ‘prevention of blood loss of at least 500 ml’ and ‘use of additional uterotonic agents’. Non-inferiority was not shown for the outcome of blood loss of at least 1000 ml.

Liabsuetrakul et al. (2018) conducted a systematic review to assess the effectiveness and safety of prophylactic use of ergot alkaloids in the third stage of labour administered by any route (Liabsuetrakul et al. 2018). The study groups reviewed included those given IM or IV prophylactic ergot alkaloids, those given other uterotonics and those given placebos. They found that prophylactic IM or IV injections of ergot alkaloids may be effective in reducing blood loss and increasing maternal haemoglobin; that they may also decrease the use of therapeutic uterotonics; but that adverse effects may include hypertension and pain after birth. However, there were no differences between the groups in terms of other side effects (vomiting, nausea, headache or eclamptic convulsions).

Several earlier studies had implicated ergometrine as a causative factor in retained placenta. A randomised double-blind prospective study was conducted to compare
the effect of intramuscular ergometrine and oxytocin in the management of the third stage of labour among one thousand consecutive patients. The need for manual removal of the placenta was higher when ergometrine was used. A randomised, controlled trial of 1429 women was carried out to compare 'active' management of the third stage of labour, using i.v. ergometrine 0.5 mg, with a method of 'physiological' management, in women at 'low risk' to haemorrhage. In the "active" management group a higher incidence of manual removal of placenta (p < 0.0005) was found in association with ergometrine. This finding was also verified by the more recent WHO CCT Trial (WHO Reproductive Health Library 2012). It is thought that this occurs because ergometrine promptly induces powerful contractions that cause cervical constriction before the placenta is expelled.

Another systematic review and meta-analysis of randomized controlled trials compared the efficacy and safety profile of carbetocin (a synthetic analogue of oxytocin) with other uterotonic agents in preventing postpartum haemorrhage. It was found to be associated with a similar low incidence of adverse effects to oxytocin and at least as effective as ergometrine. It may become an alternative uterotonic agent for the prevention of postpartum haemorrhage. However, this systematic review recommended that further studies should be conducted to determine the safety and efficacy profile of carbetocin in women with cardiac disorders and to analyse its cost-effectiveness and its minimum effective dose. A large WHO trial is underway to assess the efficacy of this.

1.2.3. b ii) Cord clamping

A recent meta-analysis of 15 randomised controlled trials involving 3911 women and infant pairs determined the effects of early cord clamping compared with late cord clamping after term birth on maternal and neonatal outcomes. It concluded that a more liberal approach to delayed clamping in healthy term infants appears to be warranted, particularly in light of the growing evidence that delayed cord clamping increases early haemoglobin concentrations and iron stores in infants. It also added that delayed cord clamping is likely to be beneficial as long as access to treatment
for jaundice requiring phototherapy is available. No maternal benefits were reported to support early cord clamping. The American College of Obstetricians and Gynaecologists also recommend delayed cord clamping following their metanalysis (2017) (Committee on Obstetric Practice, 2017).

Recent NICE guidelines recommend that the cord should not be clamped earlier than 1 minute from the birth of the baby unless there is concern about the integrity of the cord or the baby has a heartbeat below 60 beats/minute that is not getting faster. They add that the cord should be clamped before 5 minutes to perform controlled cord traction as part of active management, and if the woman requests that the cord is clamped and cut later than 5 minutes, she should be supported in her choice.

1.2.3b iii) Control Cord Traction

A Cochrane Database systematic review was conducted to evaluate the effects of controlled cord traction during the third stage of labour, either with or without conventional active management. They concluded that CCT has the advantage of reducing the risk of manual removal of the placenta in some circumstances, can be routinely offered during the third stage of labour, by skilled birth attendants and should remain a core competence of the skilled birth attendants.

1.2.3b iv) Uterine massage

Hofmeyr et al conducted a recent meta-analysis of two controlled trials to determine the effectiveness of uterine massage, after birth and before or after expulsion of the placenta, in reducing postpartum blood loss and associated morbidity and mortality. The first trial included 200 women who were randomised to receive uterine massage or no massage following expulsion of the placenta, after active management of the third stage of labour, including use of oxytocin (Hofmeyr, Abdel-Aleem & Abdel-Aleem 2013). The other trial recruited 1964 women in Egypt and South Africa (Abdel-Aleem et al. 2010). Women were assigned to receive oxytocin alone or oxytocin with uterine massage after birth of the baby but before delivery of the
placenta. Hofmeyr et al. concluded that the result of the meta-analysis was inconclusive because of the limitations of the number of the included trials and the fact that all women had received an oxytocic; suggested that once an oxytocic has been given, there may be no added advantage of uterine massage in reducing blood loss; and stated that there are no controlled trials identified assessing the effectiveness of uterine massage in the absence of oxytocics.

1.2.4 Placental blood flow

Before birth there is blood flow to the placental bed at a rate of 500 ml per minute. If the uterus does not contract as the placenta detaches, this flow will continue, and the mother can exsanguinate within minutes. Great care is therefore taken to ensure that the placenta delivers completely and that the uterus contracts immediately thereafter.

Physiologically, the uterus continues to contract rhythmically after the birth of the baby. These contractions followed by retractions shorten the muscle fibres of the uterus that were overstretched to accommodate the fetus. The spiral arteries and veins pass through the uterine muscle fibres that are arranged in a crisscross fashion where they are compressed or 'physiologically ligated'. As the placenta is dissected through the decidua spongiosa layer, it leaves a very vascular placental bed with torn blood vessels. However, the rhythmical uterine contractions, retractions and 'physiological sutures’ or 'living ligatures’ establish haemostasis naturally after the separation of the placenta. It is only if the uterus relaxes again after complete or partial placental detachment that bleeding restarts.

The third stage of labour was examined in 62 patients by Krapp et al. using grey scale and colour Doppler sonography (Krapp et al. 2007). He indicated that cessation of blood flow between the basal placenta and myometrium following birth of the baby is the sonographic hallmark of normal placental separation while persistent blood flow demonstrated by colour Doppler sonography is suggestive of placenta accreta. However, the ultrasound features suggestive of retained placenta adherens and trapped placenta are not yet defined.
Doppler ultrasound has also been used to assess retained placental tissue after completion of the third stage. Van Den Bosch et al conducted a cross-sectional observational study involving 385 consecutive women presenting at their first visit after pregnancy, to evaluate the colour Doppler and grey-scale sonographic appearance of the uterus after pregnancy (Van den Bosch et al. 2002). They concurred with Krapp that areas of enhanced vascularity of the uterus, ranging from a focal vascular pedicle to a larger area of the myometrium are predominantly seen in the presence of placental remnants in the early postpartum period and after instrumental or manual removal of the placenta. They recommend that the knowledge of the ultrasound and colour Doppler features of the uterus after pregnancy may prove of practical value for the management of abnormal uterine bleeding in the postpartum period.

Another Doppler ultrasound study looked at resistance indices and hyper-vascularity areas among 20 women that were due to undergo evacuation of retained placental tissue (RPT) based on clinical and ultrasound diagnosis. It found that absence of a hyper-vascular area in the myometrium does not exclude RPT, but its presence is common finding associated with RPT and should not be misinterpreted as an arterio-vascular malformation. In our opinion, this presumably depends on whether the retained tissue is attached to the myometrium (i.e. was partial accreta) or is detached.

1.3 The retained placenta

1.3.1 Definition

There is no international consensus on the definition of retained placenta. A prospective observational study assessed 6,588 women delivering vaginally in a tertiary obstetric hospital for postpartum haemorrhage during a 24-month period, concluding that a third stage of labour longer than 18 minutes is associated with a significant risk of postpartum haemorrhage; and that after 30 minutes the odds of having postpartum haemorrhage are 6 times higher than before 30 minutes (Magann
et al. 2005). Combs et al. indicate the placenta to be considered retained if the third stage lasts longer than 30 minutes (Combs & Laros 1991). Hence the intrapartum guidelines of the UK National Institute for Health and Clinical Excellence (NICE) suggest that the placenta is considered retained if it exceeds 30 minutes during the active management of the third stage of labour (NICE 2014). However, the incidence depends on gestational age, being higher in pre-terms (Dombrowski et al. 1995).

The WHO guide, “Managing complications in pregnancy and childbirth”, states that if a placenta is not expelled within 30 minutes after the birth of a baby, the woman should be diagnosed as having a retained placenta (WHO 2007). But, it adds that since there is no evidence for or against this definition, the delay used before this condition is diagnosed is left to the judgment of the clinician.

1.3.2 Incidence

This depends on type of management used in the third stage, the time chosen, and the gestation. The placenta is delivered within 5-10 minutes with active and 10-20 minutes with expectant management. Generally, 90% of placentas deliver within 15 minutes, 96% within 30 minutes and 98% within one hour. The incidence and importance of retained placenta (RP) varies greatly around the world: in less developed countries, it affects about 0.1% of deliveries but has up to a 10% case fatality rate, while in more developed countries, it is more common (about 3% of vaginal deliveries) but very rarely associated with mortality (Weeks, 2008). This higher rate in well-resourced setting has increased over time from 0.66% in the 1920s to 2.34% in the 1980s (Cheung et al. 2011).

1.3.3 Classification

Weeks (2002) classifies retained placenta into three main subtypes (Figure 1.7):

- **Placenta adherens.** when there is failed contraction of the myometrium behind the placenta;
- **Partial accreta**, when there is a small area of accreta preventing detachment and;
- **Trapped placenta**, a detached placenta trapped behind a closed cervix.

Urner et al classifies the subtypes into placenta adherens, accreta and incarcerated placenta (Urner, Zimmermann & Krafft 2014). Others categorise them simply as trapped or adherent, omitting partial accreta.
Figure 1.7 Images of placenta adherens, partial accreta and trapped placenta. (Figures drawn by Mills Media Group in Wirral UK as commissioned by the author).
The morbidly adherent placenta known as placenta accreta is graded differently. This occurs when all or part of the placenta attaches abnormally to or invades the myometrium. Its three grades are defined: accreta, increta and percreta (Jauniaux et al. 2019).

In a placenta accreta, chorionic villi are attached to the myometrium and not restricted to the decidua basalis. The anchoring villi of placenta adherens, on the other hand, are merely attached to the decidua basalis.

With placenta increta, the chorionic villi invade into the myometrium whilst with placenta percreta the chorionic villi invade through the myometrium. Because of a morbid attachment to or through the myometrium, they are associated with an increased risk of heavy bleeding at the time of attempted placental expulsion. The need for transfusion of blood products is frequent, and hysterectomy is commonly indicated to control life-threatening haemorrhage.

A comprehensive classification of retained placentas by mode of birth that includes both normally and morbidly adherent forms is illustrated in Figure 1.8. Neither caesarean section nor retained products of conception are being studied in this thesis.
Figure 1.8 Classification of retained placenta by mode of placental birth
The number of women with full accreta after vaginal birth is very limited. Weeks estimates that it is only 1 in 400 women with retained placentas. In the previous Release trial on the retained placenta, only 1 out of 577 participants had a full accreta and she died following a failed normal removal and attempted hysterectomy to remove the placenta (Weeks et al, 2010).

1.3.4 Pathophysiology of retained placenta

A nested cohort study was conducted among 10,334 nulliparous singleton pregnancies that delivered vaginally in Hillerød Hospital (affiliated to the University of Copenhagen) in Denmark hospital during 2000–2009. Its objective was to estimate the prevalence and validate the diagnosis of retained placenta in nulliparous women and the risk of recurrence at subsequent vaginal birth. It found that 25.3% of women with a previous retained placenta and 5.3% without previously retained placenta experienced retained placenta in a subsequent birth. This shows that the risk of recurrence of retained placenta among women with a history of retained placenta after vaginal birth is increased five-fold compared with women without a history of retained placenta.

A large population-based cohort study recently reported the association between retained placenta and pre-eclampsia. The study included 386,607 primiparous women in Sweden with singleton vaginal deliveries between 1997 and 2009 at 32-41 weeks of gestation without placental abruption or infants with congenital malformations. The objective was to evaluate whether defective placentation disorders (pre-eclampsia, stillbirth, small for gestational age (SGA), and spontaneous preterm birth) are associated with risk of retained placenta. It concluded that these defective placentation disorders are significantly associated with an increased risk of retained placenta, virtually all adherens.

Another observational study included 45,852 women with singleton deliveries > or = 20 weeks' gestation from 1984 to 1992 (Dombrowski et al. 1995). The purpose was
to record gestational age-specific data for third-stage duration of labour, frequencies of retained placentas (undelivered at 30 minutes), manual removal of the placenta, and haemorrhage. It showed that the frequency of retained placentas (2.0% overall) was markedly increased among gestations < or = 26 weeks (odds ratio 20.8, 95% confidence interval 17.1 to 25.4) and < 37 weeks (odds ratio 3.0, 95% confidence interval 2.6 to 3.5) compared with term. Around 25% of all deliveries at 25 weeks have a retained placenta compared to only 3% at term.

Weeks (2003) assessed regional variations in intrapartum myometrial contractility during the first stage of labour using ultrasound. In 10 women with normal labours, the myometrial thickness increased in all areas during contractions, whilst in 10 women with slowly progressing labour, the retro-placental myometrium thinned during contractions (Weeks 2003). He suggested an association between retained placenta and dysfunctional labour and indicated that a locally acting placental tocolytic may be a cause of both dysfunctional labour and retained placenta.

The incidence of retained placenta is reported to be higher in developed countries compared to developing nations (Cheung et al. 2011). It has also been suggested that interventions common in the most developed countries such as abortions, uterine intervention, labour induction, and use of oxytocin could be contributing to the increased retained placenta (Weeks, 2008). Indeed, a more recent case-control study involving a total of 33,925 women who delivered vaginally was conducted to determine the incidence and risk factors for retained placenta between the years 2007 and 2012 (Ashwal et al. 2014). Women who delivered vaginally and who were diagnosed with retained placenta were compared to a control group of women with spontaneous vaginal birth with spontaneous non-complicated placental separation. Eligibility was limited to singleton fetuses with a vertex presentation with no history of more than one caesarean section, stillbirth or major fetal anomaly. Retained placenta was defined as the need of manual removal of the placenta or parts of it immediately following vaginal birth. It found that hypertensive disorders and oligohydramnios, as well as labour and birth interventions such as induction of labour (OR 1.84, 95% CI 1.30-2.59), neuro-axial analgesia (OR 1.60, 95% CI 1.27-
2.00) and vacuum extraction (OR 1.89, 95% CI 1.48-2.41) were independently associated with manual removal of the placenta. The authors suggest that the association with vacuum delivery may be due to doctors feeling the need to do manual removal of placenta within 30 minutes of delivering the baby instrumentally.

An earlier case-control study was carried out in the King Khalid University Hospital, Saudi Arabia, and involved 114 women who had retained placenta and 116 women with normal deliveries. It found that multiparity, induced labour, small placenta, and large amount of blood loss to be significantly associated with retained placenta (Adelusi et al. 1997).

As discussed under active management of labour, the prophylactic use of ergometrine is implicated in causing trapped placenta(Yuen et al. 1995). This association was confirmed in further trials by Gulmezoglu (Gulmezoglu et al. 2012).

A prospective observational cohort of 30,132 women who had caesarean section without labour was conducted in 19 academic centres in the United States over 4 years (1999-2002) to estimate the magnitude of increased maternal morbidity associated with increasing number of caesarean deliveries. It indicated that placenta acrreta was present in 15 (0.24%), 49 (0.31%), 36 (0.57%), 31 (2.13%), 6 (2.33%), and 6 (6.74%) women undergoing their first, second, third, fourth, fifth, and sixth or more caesarean deliveries, respectively.

A recent case-control study comparing 408 cases of retained placenta and an equivalent number of control individuals was conducted in Sweden to identify factors related to retained placenta in the context of contemporary obstetric practice. Epidemiological and birth-related variables were registered in computerized prenatal and in-hospital medical records. Univariate and multivariate logistic regressions were used to estimate risk ratios and statistical significance. In order of odd ratios, independent risk factors for retained placenta were found to be: previous retained placenta (odds ratio [OR] 12.61); oxytocin use more than 415 minutes (OR 6.55, 95% CI 3.42–12.54); preterm birth (OR 3.28, 95% CI 1.60–6.70); oxytocin use for
195–415 minutes (OR 2.00, 95% CI 1.20–3.34); preeclampsia (OR 2.85, 95% CI 1.20–6.78); two or more previous miscarriages (OR 2.62, 95% CI 1.31–5.20); and one or more previous abortion (OR 1.58, 95% CI 1.09–2.28). It concluded that identifying risk factors for retained placenta is important in the assessment of women after birth and that the increased risk associated with duration of oxytocin use is of interest, considering its widespread use (Endler, Grunewald & Saltvedt 2012). In a review of PPH, Weeks explains why women with postpartum bleeds might have little response to oxytocics such as dysfunctional labour and depletion of oxytocin receptors with intrapartum oxytocin use.

Weeks provides an ultrasound image of a retained placenta in a ruptured uterus, when the placenta extrudes into the peritoneal cavity through the rupture (Figure 1.9).
Figure 1.9 Retained placenta in a ruptured uterus in a 24-year old multiparous woman. Diagram (with permission from Prof Andrew Weeks). The ultrasound image (low abdomen transverse) shows the uterus with thin endometrial cavity, left sided lateral rupture and placenta outside of the uterus in the peritoneal cavity.
1.3.5 Aetiological Factors

Weeks (2008) explains how each type of retained placenta may have a different set of aetiological factors depending on underlying pathophysiology as summarised in table 1.1. For placenta adherens, the underlying pathogenesis is hypothesised to be due to retro-placental contractile failure by a localised placental inhibition (Herman et al. 1993).

<table>
<thead>
<tr>
<th>Type of retained placenta</th>
<th>Partial Accreta</th>
<th>Placenta Adherens</th>
<th>Trapped Placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathophysiology</td>
<td>Disruption of placenta—myometrial interface</td>
<td>Persistent placental inhibition of myometrial contraction</td>
<td>Loss of gravitational forces or cervical closure</td>
</tr>
<tr>
<td>Aetiological Factor</td>
<td>Pre-eclampsia, Small placenta, Previous abortion, Previous uterine injury, Uterine abnormalities</td>
<td>Prematurity, Augmented or dysfunctional labour, Induced labour</td>
<td>Birth in a labour bed, Use of prophylactic iv ergometrine</td>
</tr>
<tr>
<td>Optimal Treatment</td>
<td>Manual removal</td>
<td>Contraction of the retroplacental myometrium using oxytocics</td>
<td>Persistent controlled cord traction or uterine relaxation using tocolytics</td>
</tr>
</tbody>
</table>

Table 1.1 Pathophysiology, aetiological factors and optimal treatment of forms of RP (Weeks, 2008).
1.3.6 Diagnosis

1.3.6.1 Clinical

Generally, a placenta is diagnosed to be retained if it is not delivered within 30 minutes of the birth of the baby with active management or within 60 minutes with physiological management of the third stage of labour (NICE 2014).

Prior to manual removal of the placenta, Weeks indicates that the differentiation between a placenta that is ‘trapped’ and adherent (placenta adherens) is not easy unless ultrasound is used, because diagnosis by clinical examination is inadequate. Clues to a trapped placenta will be if the fundus feels high, but small and contracted, or if the edge of the placenta is palpable through a tight cervical os. By contrast, the fundus is usually soft and wide with placenta adherens (Weeks, 2008).

Placenta accreta is rare in women having a vaginal birth, and will usually be discovered only at the time of attempted manual removal.

1.3.6.2 Imaging

i) Grey-scale ultrasound

Weeks provides ultrasound images of two types of retained placenta (Weeks, 2008). With a trapped placenta, the myometrium is seen to be thickened all around the uterus and the uterus is empty. By contrast, with an adherent placenta, the myometrium is thickened in all areas except where the placenta is attached where it will be very thin or even invisible (Figure 1.10).

The potential for retained placenta has not been studied antenatally, except in the case of suspected placenta accreta. The ultrasound features of placenta accreta are shown in table 1.2. Women with a history of a previous caesarean birth, presenting
with a placenta praevia, have become the largest group with the highest risk of having placenta praevia accreta. The sensitivity and specificity of ultrasound imaging in this accreta presenting with anterior low placenta or placenta praevia, are >95% in prospective series when performed by skilled operators.
Figure 1.10 Ultrasound images (sagittal view) of placenta adherens and trapped placenta (sagittal view) of placenta adherens and trapped placenta. Image from (Weeks 2008).
<table>
<thead>
<tr>
<th>No.</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss of echolucent area between the uterus and placenta</td>
</tr>
<tr>
<td>2.</td>
<td>Multiple placenta lacunae (Swiss-cheese appearance)</td>
</tr>
<tr>
<td>3.</td>
<td>Loss of echolucent line between uterus and bladder</td>
</tr>
<tr>
<td>4.</td>
<td>Focal exophytic masses extending into the bladder</td>
</tr>
</tbody>
</table>

Table 1. 2 Ultrasound features of placenta accreta (Jauniaux et al, 2018 (Jauniaux & Bhide 2017); (D'Antonio, Iacovella & Bhide 2013).
ii) Doppler ultrasound

a) Antenatal diagnosis of placenta accretas

Ayati et al conducted a cross-sectional study, of 82 pregnant women who were high risk for placenta adhesive disorder (placenta accreta). They underwent colour Doppler ultrasound and MRI after 18 weeks of gestation. The sonographic and MRI findings were compared with the final pathologic or clinical findings (Ayati et al. 2017).

The diagnosis of placenta accreta spectrum (PAS) was found in 17 cases (21%). Doppler sonography sensitivity was 87% and MRI sensitivity was 76%. Doppler sonography specificity was 63% and MRI specificity was 83%. They concluded that women with high-risk factors for PAS should undergo Doppler ultrasonography first. When results on Doppler sonography are equivocal for PAS, MRI can be performed due to its high specificity.

b) Use in the third stage of labour

With regards to uterine artery Doppler parameters, normal ranges used antenatally in the literature were resistance index (RI) < 0.55 where there is no notch and < 0.65 with a notch. The pulsatility index (PI) was considered normal if the level was < 1.45 (Gomez et al. 2008) and (Bell 2018).

Krapp et al. had introduced the use of colour Doppler sonography in the third stage of labour (Krapp et al. 2000). They found cessation of blood flow between myometrium and placenta immediately after birth in cases with normal placental separation. In contrast, persistence of blood flow in the placenta vessels invading the myometrium was suggestive of placenta accreta. However, the authors did not explore the potential of using Doppler ultrasound to diagnose subtypes of the retained placenta.
iii) Computed tomography (CT) and magnetic resonance imaging (MRI)

a) The retained placenta

There are no studies of the need for CT/MRI postnatally to diagnose the retained placenta subtypes. None of the studies identified examined the role of CT/MRI in the third stage of labour for the diagnosis of the retained placenta.

b) Antenatal placenta praevia

A large although retrospective study evaluated 453 women with a diagnosis of placenta praevia, low-lying placenta with prior caesarean section, or myomectomy of whom 39 had abnormal placentation. This study revealed 77% sensitivity, 96% specificity, 65% positive predictive value (PPV), and 98% negative predictive value (NPV) for ultrasound and 88% sensitivity, 100% specificity, 100% PPV, and 82% NPV for MRI prediction of abnormal placentation.

Another study compared colour Doppler ultrasound with MRI in 50 women with placenta praevia during the third trimester and found 100% sensitivity (12/12) and 100% specificity for MRI and 91% sensitivity and 100% specificity for ultrasound diagnosis of abnormal placentation. Although these differences between MRI and ultrasound were not found to be statistically different for the diagnosis of abnormal placentation, MRI was considered significantly better at gauging the depth of placental invasion and topography.

c) Postnatal-Retained Placental Tissue

A small-sized study retrospectively assessed the role of MRI in diagnosis and predicting clinical outcome in women with retained placental tissue (RPT). It comprised eleven patients with pathologically proven RPT. All underwent MRI. On T2-weighted images, 10 cases showed high intensity and 9 cases were hypervascular. The myometrium was thinner at the attachment side than at the
opposite side. It therefore concluded that MRI is useful for diagnosis and follow-up of RPT.

1.3.7 Treatment of retained placenta

As previously defined, a placenta is retained if still undelivered after 30 minutes of active management or one hour of physiological management. Its standard treatment is surgical by manual removal of the placenta (Bewley 2009) but medical options have also been assessed, as discussed below.

1.3.7 a) History

A recent review of management of postpartum haemorrhage and retained placenta over 100 years from 1917 through the Ten Teachers Textbook revealed the following historical details in relation to the retained placenta (Kerr & Weeks 2015):

- **From 1917 to 1955,** an era before any access to antibiotics and blood transfusion, the uterus was massaged until it was firmly contracted and then squeezed repeatedly over five minutes to expel the placenta. If this failed, the last resort was to manually remove the placenta under general anaesthesia. However, the teachers warned of the high risk of complications, including death from sepsis.
- **From 1917 to 1931,** trapped placenta due to a constriction ring of the cervix or lower uterus was treated by dilating the ring with steady pressure with the fingertips. In 1935, however, the concept of uterine relaxation with amyl nitrite was introduced. This remained the teaching until 1990.
- **Between 1935 and 1942,** the use of an injection of saline into the intraumbilical vein was also recommended before any attempt at manual removal of placenta.
• **Between 1961 and 1980,** the recommended treatment for placenta trapped in the lower segment of the uterus was to push down on the fundus of the uterus so that the uterus acted as a piston against the placenta and expelled it.

• **From 1961 to 1995** cord traction was also described as an option to deliver the separated placenta (replaced by the Brandt-Andrews method from 1972).

The authors demonstrate that management practices in the past ebbed and flowed in the absence of scientific evidence, with more invasive procedures introduced as safe anaesthetics and antibiotics became available. Recent innovations like intravenous and sublingual glyceryl trinitrate are simply rediscoveries from the past when amyl nitrate for retained placenta had been introduced and later dropped.

The first confidential enquiries into maternal deaths, in the 1952-54 triennium, identified delayed manual removal of placenta as a common cause of maternal death, and recommended that this life-saving procedure be extended to the community via flying squads. This led to a dramatic reduction of retained placenta deaths in the UK from 53 deaths in 1952-54 to just 1 death in 1967-69. This trend of improvement has continued in the UK to the extent that there has been only one other death reported in 1991-2002 and nil thereafter.

1.3.7 b) Umbilical vein injection (UVI) of oxytocin

Various options have been tested for umbilical injections as a treatment for retained placenta as projected in Figure 1.11.

Nardin, Weeks and Carroli conducted a Cochrane database of systematic review of randomised trials comparing umbilical vein injection (UVI) of saline or other fluids, with or without oxytocics, either with expectant management or with an alternative solution or other uterotonic agent, in the management of retained placenta (Nardin, Weeks & Carroli 2011). They included 15 trials (1704 women) that were of variable quality. UVI of oxytocin solution is an inexpensive and simple intervention that
could be performed while placental expulsion is awaited. However, the overview of high-quality randomized trials show that the use of oxytocin has little or no effect. One of the trials was the Release study, a double blind placebo randomized controlled trial, conducted to assess the effect of high-dose oxytocin as a treatment for retained placenta (Weeks et al. 2010). It studied 577 women with retained placenta that were haemodynamically stable in the UK, Uganda and Pakistan. This high-quality research showed that the use of oxytocin has little or no effect on the need for manual removal of placenta for women with retained placenta.
Figure 1.11 Tested options for umbilical vein injection
To put the Release study results into context, its researchers undertook a meta-analysis of all randomised controlled trials comparing umbilical vein injection of oxytocin solution with placebo for the treatment of retained placenta. This update of the Cochrane review included all published studies up to January 2009, and included an assessment of methodological quality. They included 12 randomised trials (including the release study) with a total of 1288 women. This meta-analysis, which had previously suggested statistically and clinically significant benefit to umbilical oxytocin injection (odds ratio 0·79, 95% CI 0·69–0·91), now shows no significant difference in the primary outcome, having added the present study.

Two other studies suggested that UVI oxytocin may be associated with a clinically significant increase in postpartum haemorrhages, when compared with expectant management (van Beekhuizen et al. 2013; van Stralen et al. 2013). Therefore, the current clinical position is not to recommend UVI oxytocin.

However, all studies to date have provided the treatment of all subtypes of RP, irrespective of their type. This is despite trapped placenta and placenta adherens being of contrasting pathophysiology. If they were restricted to certain subtypes of RP based on ultrasound diagnosis, they might have shown differentiated benefit.

The use of umbilical vein injections of oxytocics to overcome retroplacental contractile failure may yet allow retained placenta adherens to be treated medically. If an improvement in the delivery of the oxytocin to the placenta can be achieved, or alternative oxytocics succeed, then medical management of the retained placenta adherens could become the treatment of choice, even where theatre facilities are available.

1.3.7 c) Umbilical vein injection of prostaglandins
Apart from oxytocin and saline, other modes of treatments have been tried. Grillo-Ardila et al conducted a systematic review in which they identified three randomised controlled studies (involving 244 women) that compared the use of systemic prostaglandins with placebo (Grillo-Ardila, 2014). They found that the use of prostaglandins resulted in less need for manual removal of placenta, severe postpartum haemorrhage and need for blood transfusion, but none of the differences reached statistical significance. The prostaglandin was administered by intravenous infusion (E2 analogue sulprostone) in one study including 50 women and was orally or sublingually administered (E1 analogue misoprostol) in the other two studies including 194 women. All the trials were small and of poor methodological quality.

Meta-analyses demonstrated a significant reduction in the need for manual removal of the placenta following UVI of prostaglandin when compared to UVI oxytocin; and following IV sulprostone when compared to IV saline. However, the trials were few, sample sizes small and the effect was inconsistent or statistically insignificant. Hence a shift in practice towards the use of prostaglandins is not currently warranted in the UK.

There was no evidence of a difference in the need for a manual removal of the placenta following UVI oxytocin and UVI ergometrine. The study reporting this outcome was very small (n=53). No incidences of postpartum haemorrhage or side effects (n=53) were reported. The evidence was of very low to moderate quality.

1.3.7.d Glyceryl trinitrate (GTN)

<table>
<thead>
<tr>
<th>Study</th>
<th>Dose</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visalyaputra et all, 2011</td>
<td>200 mcg infusion</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Ekerhovd and Bullarbo, 2008</td>
<td>1 mg tablets</td>
<td>Sublingual</td>
</tr>
<tr>
<td>Bullarbo et al, 2012</td>
<td>1 mg tablets</td>
<td>Sublingual</td>
</tr>
<tr>
<td>Denison et al, 2017</td>
<td>800 mcg spray</td>
<td>Sublingual</td>
</tr>
</tbody>
</table>

Table 1. 3 Studies of glyceryl trinitrate (GTN) as a treatment for trapped placenta.
GTN acts by relaxing the myometrium. Whilst it is not expected to be of benefit in placenta adherens, it could benefit women with a trapped placenta where cervical relaxation could release the placenta. Various routes of administration have been tried (Table 1.3) besides intracervical (Rodgers, 2013); (Visalyaputra, 2011); (Ekerhovd, 2008); (Bullarbo, 2012) and (Denison, 2017).

In one trial, sublingual nitro-glycerine was shown to be associated with a significant reduction in manual removal of the placenta, when compared with a placebo. Although this trial involved only 24 women, it demonstrated a significant benefit. However, another small trial of intravenous nitro-glycerine comprising 40 women demonstrated no benefit.

Another small non-blinded trial was conducted by Ekerhovd and Bullarbo to examine the success rate and safety of sequential administration of intravenous oxytocin in combination with sublingual nitro-glycerine for the expulsion of retained placenta. 24 women with retained placenta despite a second dose of 10 IU of intravenously administered oxytocin, were given sublingual nitroglycerine (1 mg) to promote detachment of the placenta (Ekerhovd & Bullarbo 2008). Some 5 min after resorption of the tablets, controlled cord traction was carried out for a maximum of 5 min. In addition, changes in blood pressure following treatment with nitroglycerine and total blood loss during birth were registered. Twenty-one of the women delivered the placenta successfully. The procedure failed in 3 women and operative manual removal under regional or general anaesthesia was undertaken. No complications due to nitroglycerine were registered. Therefore, it concluded that sequential administration of oxytocin and nitroglycerine seems to be an effective and safe procedure in the management of retained placenta.

This small trial was followed up with a larger prospective double-blind randomized controlled multicentre study that was carried out at five Swedish hospitals between October 2008 and July 2010 (Bullarbo et al. 2012). The primary aim was to determine if sequential administration of oxytocin and nitroglycerine is effective for
management of retained placenta when performed by obstetricians with no experience of the method. Secondary aims were to examine possible adverse effects of nitroglycerine. One hundred and five women with retained placenta were randomly selected to receive either 1mg nitroglycerine or placebo tablets sublingually if intravenous oxytocin had failed to expel the placenta. It showed that an increased release of retained placenta following sequential administration of oxytocin and nitroglycerine compared to oxytocin and placebo. However, the difference in success rate did not reach statistical significance (p = 0.056). A tendency that clinical experience of the method could be of importance for removal of placenta was registered. Nitroglycerine did not cause any adverse effects of clinical importance.

The researches admit that one weakness of this study is that only obstetricians with no experience of the method were involved. On the other hand, they argued that their study describes a real-life clinical scenario when a new method is being introduced. It was simply not possible to carry out a multicentre study involving only obstetricians with great experience of the method.

Meanwhile the GOT-IT Trial: Glycerol Trinitrate for Retained Placenta (ClinicalTrials.gov Identifier: NCT02085213) aimed to determine the clinical and cost effectiveness of glyceryl trinitrate (GTN) in treating retained placenta. The investigators compared GTN against a placebo in a randomised controlled blinded trial (Denison et al. 2017). Women with RP of any type were treated with GTN spray or placebo. The need for manual removal of placenta was the same in each group, but those treated with GTN had increased rates of PPH (BMFMS 2018).

1.3.7e) Manual removal of placenta (MROP)

If a clear plane of cleavage cannot be defined during manual removal, placenta accreta is likely and various management options should be taken up with the involvement of an experienced multi-disciplinary team as per the RCOG Green-top Guideline No. 27 (RCOG 2011). As the placenta is partially separated it needs to be
delivered and any haemorrhage should be dealt with using standard protocols. Small adherent sections can be left but in such circumstances blood loss can be high and treatment for haemorrhage must be aggressive.

Ultimately, when any other treatment fails or if the patient with retained placenta is bleeding, manual removal of placenta is considered as depicted in Figure 1.13. This is a common low risk procedure. Since 2002 there has been no deaths in the UK despite an estimated 207,720 manual removals (2% of number of births). However, the earlier emphasis by Munro Kerr that this was an operation associated with an exceedingly high maternal mortality rate of 5-10% then, due to infection and shock, should serve as a constant reminder about the importance of aseptic technique, anaesthesia and need for safer medical therapies.

Figure 1.12 Diagram of manual removal of placenta: One hand supports and guards the uterine fundus abdominally whilst the other (with the fingers held tightly together) advances along the line of cleavage of the placenta, separating it in its entirety (Bewley, 2009).
Figure 1.12 Diagram of manual removal
1.3.7f) Ultrasound-guided surgical removal

A variation on the standard technique for surgical removal of retained placenta has been described. Rosenstein, Vargas and Drey demonstrate a technique of an ultrasound-guided surgical removal of a retained placenta in a 29-year-old gravida one woman who delivered vaginally without epidural anaesthesia (Rosenstein, Vargas & Drey 2014). Her placenta remained in situ after 30 minutes and she subsequently underwent ultrasound-guided instrumental removal of the intact placenta with forceps. She reported minimal pain with the procedure and required only 100 µg of fentanyl.

The researchers believe this technique causes less discomfort to the patient than a traditional manual extraction, because the instrument entering the uterus is much narrower than a hand. With the patient in dorsal lithotomy, they locate the cervix and stabilize it either with fingers or a ring forceps on the anterior lip. Then they introduce Bierer ovum forceps into the uterus under direct ultrasound guidance. The Bierer forceps are preferred because of their long length, large head, and serrated teeth that allow for a firm, secure grip on the placenta. They grasp the placental tissue with the forceps and apply slow, gentle traction in short strokes, re-grasping increasingly more distal areas of placenta as necessary to tease out the placenta. After 1-2 minutes, the placenta separates and can be pulled out of the uterus, usually intact.

They suggest that ultrasound-guided surgical removal of the placenta using Bierer forceps is an excellent option for management of the retained placenta, especially in a woman without regional anaesthesia or acute bleeding. They further recommend that further study is needed to quantify the amount of discomfort and anaesthesia that can be avoided with this technique, as well as whether this technique could decrease the frequency of infectious complications or the necessity of post-removal curettage. However, theirs is a single case study that requires further research for validation.
1.3.7g) Other methods

Another suggested option for the delivery of retained placenta is the windmill technique. This involves continuous 360° umbilical cord traction and rotation perpendicular to the direction of the birth canal at the level of the introitus. The 360° rotation is repeated slowly similar to the motion of the blades of a windmill. This is said to be suitable for both trapped placenta and placenta adherens. As assessed by Hinkson et al, this technique for the delivery of the retained placenta is simple, safe, effective and easy to teach (Hinkson et al. 2017). It reduces the use of the invasive operation of manual removal of the placenta, postpartum haemorrhage and delay in the delivery of the placenta. The authors assert that it can also be a lifesaving intervention especially in low resourced areas with limited or no access to operation theatre. The mechanism is not clear, but the motion may encourage a placenta adherens to separate with time and become a trapped placenta.

The repeated 360° rotation pulls of the windmill technique may then encourage dilatation and relaxation of the cervix leading to placental delivery. The concern would be that the technique could lead to premature detachment of the placenta from the uterus, causing bleeding. A randomised trial is required to formally assess the technique.

Later in the postpartum period, evacuation of retained products of conception (ERPOC) using suction and curettage is traditionally used to remove retained placental tissue. Alternatively, Legendre et al conducted a retrospective study on 12 consecutive patients to evaluate the feasibility and results of hysteroscopic removal of placental tissue after conservative management of retained placenta accreta (Legendre et al. 2014). A 24 F bipolar resectoscope was used and the mean retained placenta size on magnetic resonance imaging was 54 mm (range 13-110 mm). They concluded that hysteroscopic resection of retained placenta seems to be a safe and effective procedure to prevent major complications and to preserve fertility in cases of conservative management of placenta accreta.
1.4.7h Complications of retained placenta

Although it rarely causes death in developed countries, the case fatality rate of retained placenta is high up to 10% in rural areas of resource-poor countries (Harrison 1985). Onwudiegwu and Makinde analysed 91 cases of retained placentas at a university hospital in Nigeria and found 42% admitted in haemorrhagic shock, 56.5% anaemic, 17% with puerperal sepsis and 1% maternal death (Onwudiegwu & Makinde 1999). The 1% mortality stated may be an urban tip of a 10% rural iceberg referred to by Weeks. Memon also concurs with all that risks of massive blood transfusion, anaemia, shock and hysterectomy being examples of aspects of the morbidity (Memon, Talpur & Korejo 2011).

In cases of antenatally diagnosed placenta accreta spectrum, fertility may be preserved by leaving the placenta in situ. A prospective monitoring of two women with conservative treatment of placenta accreta describes the clinical course, placental regression, and recovery of the uterine anatomy using serial sonography, hysteroscopy and magnetic resonance imaging. There was no postpartum haemorrhage. Menstrual cyclicity resumed within 18 weeks. The human chorionic gonadotropin serum levels normalized within 10 weeks, whereas regression of placenta tissue was slow and continued up to nine months after birth. In both cases placental remnants persisted; in one woman they were removed and uterine anatomy restored, with a subsequent uneventful pregnancy afterwards. The presented systematic follow-up provides tools to monitor and treat other women in similar ways. However, Meller et al (2019) (Meller et al. 2019) have evaluated the feasibility and safety of the overall non-conservative management of PAS in the hybrid operating room (OR) to replace the classic two-step procedure (catheterization in the interventional radiology suite and transfer to conventional OR). They found that the overall non-conservative management of PAS in the hybrid OR has shown to be feasible and safe in our series, offering potential advantages to replace the classic two-step procedure.
1.3.8 Biochemical General

Studies have assessed hormonal levels in relation to retained placenta and placenta accreta. In 2 cases of placenta accreta where the entire placenta was left in situ, the menstrual cycle resumed remarkable quickly despite persistent placental tissue in both women. This was in accordance with the decline of the serum hCG levels even though placental tissue remained much longer and did not disappear completely. Menstrual cyclicity resumed within 18 weeks. The human chorionic gonadotropin serum levels normalized within 10 weeks, whereas regression of placenta tissue was slow and continued up to nine months after birth.

Progesterone and nitric oxide are major inhibitors produced by the placenta and it may be that the persistence of one or both of these might be responsible for the retroplacental contractile failure in some retained placentas. However, there are no studies examining hCG, progesterone, nitric oxide or other hormone levels for the 3 retained placenta subtypes in the third stage of labour.

1.3.9 Histological

Van Beekhuizen et al conducted a study on 23 retained placentas (RPs) clinically diagnosed as placenta adherens (placenta adherens) and 10 control placentas (CPs) to examine for differences in trophoblast fusion into multinucleated trophoblastic giant cells (MTGCs) (van Beekhuizen et al. 2009). They found the number of MTGCs in the basal decidua to be significantly smaller in RPs (0.23 MTGC/standard length) than in CPs (1.11 MTGC/standard length) (p<0.001). They speculated that defective fusion of trophoblastic cells into MTGCs plays a causative role in placenta adherens. However, they did not examine these levels among those clinically diagnosed as partial accretas and trapped placentas.

Another study was a descriptive analysis of immunohistochemical differences in 17 Placenta Adhesiva (PA) and 10 Control Placentas (CPs). It showed that in PA the amount of uterine natural killer (uNK) cells is significantly reduced (0.2 uNK cell/standardised area) as compared to CP (9.8 uNK cell/standardised area, p <
0.001), leading to the speculation that adequate numbers of uNK cells in the basal decidua are needed for normal expulsion of the placenta (van Beekhuizen et al. 2010).

There is evidence that pre-eclampsia has a common biological pathway with RP. Pre-eclampsia is found to be associated with an excess of proliferative immature intermediate trophoblast, lower (PLGF) and immunopathologic processes and coagulation (Chau, Hennessy & Makris 2017).

**1.4 A possible placental cause of retro-placental myometrial relaxation**

As reflected in Table 1.1 previously, the underlying pathogenesis of placenta adherens is hypothesised to be due to retro-placental contractile failure by a localised placental inhibition (Herman et al. 1993). However, the exact placental inhibitor which causes the retained placenta adherens is yet to be identified. Potentially, this may be progesterone (or its derivatives) being an important inhibitor of myometrial contractility during pregnancy (Mendelson, Montalbano & Gao 2017). It may also be nitric oxide which is a powerful smooth muscle relaxant (Sladek, Magness & Conrad 1997), or oxytocinase which inactivates oxytocin to relax the myometrium (Dicker & Whyley 1959). These potential inhibitors are produced in placenta and have been explored by previous researchers.

One of the potential inhibitors of myometrial contractility that has not been explored and also produced in placenta is hydrogen sulphide (H$_2$S) being a smooth muscle relaxant.

**1.4.1 Hydrogen sulphide**

This is a gas which is highly water-soluble, lipophilic, flammable and colourless with a characteristic rotten-egg odour (England 2009).
1.4.1 a) Production of H$_2$S

Endogenous H$_2$S is synthesised from cysteine, a product of methionine, by desulfuration using three enzymes: cystathionine $\beta$- synthase (CBS), cystathionine gamma-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MPST). (Zhao, Biggs & Xian 2014); figure 1.13).

H$_2$S was considered a toxic gas until recently as its increasing levels causes sore throat, eye irritation, dizziness, nausea, shortness of breath, chest tightness, central nervous system toxicity and depression. However, it is a gasotransmitter along with nitric oxide and carbon monoxide that are produced endogenously in low amounts and which acts as a signalling molecule (Papapetropoulos 2011).

1.4.1 b) Functions of H$_2$S

A major function of H$_2$S is relaxation of the smooth muscle. (Dunn et al. 2016). It inhibits murine myometrial contractility (Wakefield 2014). It is produced in the human uterus and placenta. It also relaxes the uterine smooth muscle, vasodilates the placental vasculature, and its CSE expression is reduced in placentas with high vascular resistance (Cindrova-Davies et al. 2013). Thus, it may be involved in inhibiting the retro-placental myometrium leading to retained placenta adherens.

Another function of endogenous H$_2$S is to maintain a healthy placental vasculature. Thus a decrease in production may contribute to the pathogenesis of preeclampsia (Ahmed 2013).
Figure 1.13 Endogenous production of hydrogen sulphide (courtesy of (Zhao, Biggs & Xian 2014))
H₂S also has potential therapeutic implications in reproductive disorders (Zhu, Gu & Ni 2011). It acts on the hypothalamic-pituitary-adrenal axis and may influence the gonadotrophins, releasing hormones with implications for fertility. Hydrogen sulphide could also inhibit the early onset of labour, being a vasodilator (Carson 2010). As it plays a role in angiogenesis, its topical administration promotes wound healing (Gersztenkorn et al. 2016) and (Papapetropoulos 2011).

1.4.1 c) Expression of H₂S enzymes

CSE is strongly expressed in the endothelial cells. Its H₂S has been implicated in retinal neovascularization (Gersztenkorn et al. 2016) and may lead to a novel therapy for proliferative retinopathy. CBS is mainly expressed in the brain, nervous system and liver. Hence, its deficiency may cause hyperhomocysteinaemia leading to preeclampsia and miscarriages (Wang et al. 2013). Both CSE and CBS expression are increased in term and labour placenta as well as the myometrium, (Vyas 2016).

The 3-MPST is expressed in the mitochondrial and cytosolic compartments of most tissues, and plays a role in vasodilation. (Kimura 2011). Therefore, under conditions of increased oxidative stress such as the retained placenta, H₂S production via the 3-MPST pathways is probably reduced.
Chapter 2 Factors and outcomes associated with retained placenta subtypes
2.1 Background

In the UK, a placenta is considered retained if it is not expelled within 30 minutes of active management and 60 minutes of expectant management of the third stage of labour. If a woman with a retained placenta (RP) is not treated, it may lead to maternal death due to postpartum haemorrhage or sepsis. Currently, the standard treatment for RP is the manual removal of placenta. Unfortunately, this is associated with anaesthetic and surgical risks compared to medical alternatives, and these medical alternatives have not been well investigated. This is partly because of our current inability to rapidly and accurately diagnose the subtype of the retained placenta while women are on the labour ward. A subsequent ultrasound study will seek to determine whether the subtype of RP could be diagnosed prior to surgery and whether this would make a difference in possible medical interventions (Chapter four). The importance of this is considerable; success would enable the investigation of safer medical alternatives to the risky processes of manual removal.

Initially, it is important to determine whether the three subtypes of the retained placenta are distinct clinical entities – do they differ in pathology only, or are the aetiological factors, outcomes, and underlying risk factors also different?

Researchers describe many factors and outcomes associated with the retained placenta, but they are not specified according to its various forms. Weeks hypothesises that aetiological factors are associated with retained placenta subtypes: placenta adherens is associated with prematurity, augmented or dysfunctional labour and induced labour; partial accreta is associated with pre-eclampsia, small placenta, previous abortion, previous uterine injury and uterine abnormalities; trapped placenta is associated with birth in a labour bed and use of prophylactic intravenous ergometrine (Weeks, 2008). This study assesses whether the three known subtypes actually differ in their association with aetiological factors and outcomes. The aetiological factors and outcomes examined have been taken from those studied by (Nikolajsen, Lokkegaard & Bergholt 2013); (Endler et al. 2014); (Dombrowski et al. 1995); (Yuen et al. 1995); and hypothesised in (Weeks, 2008).
A retained placenta is potentially fatal, especially in rural areas of less developed countries due to limited access to health services. In the UK, two women recently died following delayed manual removal of a retained placenta in the triennium of 2013-2015 (MBRRACE-UK 2017). This indicates that a retained placenta is potentially fatal even in developed countries, although it rarely happens there. On the contrary, the case fatality rate of retained placenta is high up to 10% in rural areas of less developed countries, whilst less than 1% in the UK (Weeks, 2008). Apart from mortality, a series of complications have been described by various researchers around the globe. Examples of these outcomes include haemorrhage, anaemia, puerperal sepsis and hysterectomy (Onwudiegwu & Makinde 1999) and (Memon, Talpur & Korejo 2011).

There are thought to be three pathological subtypes of RP that occur after vaginal birth: adherens, accreta and trapped. The mechanism of detachment is different for each and may affect the amount of associated blood loss.

After normal placental delivery, the rhythmical uterine contractions and retractions serve as ‘physiological sutures’ or ‘living ligatures’ to help establish haemostasis naturally after the separation and expulsion of the placenta. Therefore, care providers should ensure that the placenta delivers completely and the uterus contracts immediately to limit blood loss.

Maternal blood flows to the placental bed at a rate of 500 ml per minute, before birth. A mother can exsanguinate within minutes if the uterus does not contract as the placenta detaches because this flow will continue. This is more likely to happen in case of placenta adherens where the placenta is still partially attached to the uterus as the uterus cannot fully contract. However, it also happens in a trapped placenta although it is entirely detached from the decidua. This is because it interferes with effective uterine contraction and retraction at the uterine segment where it is trapped.
Where there is a small area of placenta accreta, it prevents complete detachment. Highest maternal mortality and morbidity is associated with full placenta accreta when managed as emergencies (Hull & Resnik 2010).

The pathological differences between the RP subtypes suggest that the level of maternal mortality and morbidity may differ according to retained placenta subtypes. We hypothesise that it would be higher in partial accreta, followed by placenta adherens, and least in the trapped placenta.

Since 2009 clinicians at LWH have been asked to define the type of retained placenta at the time of manual removal of placenta, on the electronic data collection form completed postoperatively. This study examined these subtypes to determine whether they differ in the rate and nature of complications. If so, it might allow different treatment; the trapped placenta with low morbidity could be treated expectantly whilst partial accreta with high morbidity should be treated expeditiously.

### 2.2 Methods

Data prospectively collected at the time of surgery was assessed as a retrospective cohort study of over 48,000 Liverpool women to examine the aetiological factors and outcomes associated with the various forms of the retained placenta from 2009 to 2014. The data for this was routinely collected at the Liverpool Women's Hospital from women that gave birth there during that period, both from those with the retained placenta (defined as the study group) and those without retained placenta (defined as the control group).

The options for clinicians to record when they complete the electronic RP form were:
• The placenta which was easily detached from the uterine wall and removed en bloc
• The placenta which was difficult to detach and removed piecemeal
• The placenta which was largely detached but had a small part still firmly attached

The clinicians were trained on how to use Meditech, but not on how to use the definitions of RP subtypes. However, there were brief descriptions beside each definition (Fig. 2.1) - they were expected to state the level of difficulty or ease in removing the placenta manually and to reflect this in their subgroups on Meditech. They were also expected to know whether the placenta was detached or still attached to the uterine wall and state the same in the records.

2.2.1 Statistical considerations

a) Setting

The data for this study was routinely collected at the Liverpool Women’s Hospital (LWH) from women that gave birth and had retained placenta (study group) and did not have retained placenta (control group). LWH is one of the largest women’s hospital in Western Europe with an annual birth rate of over 8500 women. It is a large tertiary referral unit that encompasses maternity, infertility and other specialised gynaecology and neonatal units. It also houses a university research centre and a specialised research laboratory/tissue bank.

b) Population size

This was the existing cohort of women from whom the data had been prospectively collected. Considering that retained placenta occurs in about 2% of women a year in the UK as described in Chapter 1, 1-2 women a week could have had that complication making an estimated total of 52-104 a year or 312-624 in 6 years.
Therefore, the overall size of the cohort was expected to range from 312-624. In this hospital, the retained placenta was defined as the failure to expel the placenta within 30 minutes of active management and 60 minutes of expectant management of the third stage of labour (NICE, 2014). This led to manual removal of placenta, typically carried out 30-60 minutes following diagnosis.

c) Inclusion criteria

The study group consisted of women delivering at gestation equal to or more than 24 weeks' gestation, who achieved normal non-instrumental vaginal birth and underwent manual removal of the placenta. The control group consisted of women that gave birth at gestation equal to or more than 24 weeks' gestation, who achieved normal non-instrumental vaginal births and expelled their placentas without undergoing manual removal.

d) Exclusion criteria

Women delivering at less than 24 weeks’ gestation and those that had a caesarean section or instrumental delivery were excluded. This is because, expectant management is prolonged or misoprostol used for gestations less than 24 weeks, whereas clinicians usually removed the placenta without waiting for it to be retained during caesarean sections or instrumental deliveries. Women with clashing and incompatible variables on Meditech were also excluded. For example, a maternal age recorded erroneously as 200 years was considered incompatible and removed rather than assuming that it was actually 20 years.

e) Data collection

Doctors that had performed the manual removal of placenta were requested to immediately complete a computerised operative summary (Figure 2.1) as part of the standard hospital data collection (MEDITECH, Massachusetts, USA). This included
details related to the delivery of the placenta such as the type of retained placenta
and level of difficulty of its removal. These data had already been entered into the
computer alongside all other maternal records, and no new data was collected for
this study. The stored data were extracted retrospectively from the Meditech
(Medical Information Technology, Westwood, Massachusetts, USA) by staff
working in the hospital’s information technology department. These data were
anonymised and then sent by secured internal e-mail to the PI (AA) via the primary
academic supervisor, in compliance with the Caldecott Rule, which restricts access
to patients’ data to their care-takers. As the PI was not a direct care-taker of the
participants at that time, he was not eligible to access their data directly.

Although this Meditech template depicts six subgroups. They can be summarised
into three subtypes as follows:

2+4 +6 = Partial accreta

1 = Placenta adherens

3 + 5 = Trapped placenta
Figure 2.1 Meditech template for retained placenta subtypes

f) Definitions

Each factor assessed was defined as reflected in table 2.1.
<table>
<thead>
<tr>
<th>Data</th>
<th>Definition</th>
<th>Meditech Source (Search Words and Free Text Areas)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>The period that has elapsed since birth in years as achieved at antenatal booking: the data was split into 3 groups: &lt;20 years, 20-39 and =/&gt;40.</td>
<td>From date of birth to booking date or age if stated</td>
</tr>
<tr>
<td>Gestation</td>
<td>Duration of pregnancy in weeks and days: &lt;37 weeks preterm, 37-40 as term and &gt;40 as postdates.</td>
<td>From current pregnancy or gestation if stated</td>
</tr>
<tr>
<td>Parity</td>
<td>The condition of having given birth to an infant or infants, alive or dead but over 24 weeks gestation, grouped as 0 for primiparous women, 1-4 for moderate parity and =/&gt;5 for grand multiparas*.</td>
<td>From current pregnancy or parity if stated</td>
</tr>
<tr>
<td>Gravidity</td>
<td>The number of pregnancies experienced by a woman: 1-4 as medium and =/&gt;5 as high*.</td>
<td>From current pregnancy or gravity if stated</td>
</tr>
<tr>
<td>BMI</td>
<td>The weight in kilograms divided by the square of the height in meters categorized as low when it was &lt;18, normal 18-29 and obese &gt;/&gt;30</td>
<td>From height and weight or BMI if stated</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Self-reported race dichotomised to White Caucasians or Others</td>
<td>Ethnic origin recorded at booking</td>
</tr>
<tr>
<td>Smoking</td>
<td>The inhalation of the smoke of burning tobacco encased in cigarettes, pipes, and cigars grouped under Non-smokers, Ex-smokers and Current smokers</td>
<td>Self-reported number of cigarettes smoked at booking</td>
</tr>
<tr>
<td>Induction of Labour</td>
<td>The artificial initiation of the processes of birth</td>
<td>Current Pregnancy: onset of labour; if induction method used.</td>
</tr>
<tr>
<td>Augmented/ Dysfunctional Labour</td>
<td>Abnormal progress of dilation and/or descent of the presenting part, augmented when oxytocin infusion is used to expedite.</td>
<td>Current pregnancy: was labour accelerated?</td>
</tr>
<tr>
<td>Home Birth</td>
<td>Where the birth was achieved at home</td>
<td>Current Pregnancy: where baby was born-home or hospital.</td>
</tr>
<tr>
<td>Hospital Midwifery Unit Birth</td>
<td>Where the birth was in hospital at a midwifery-led unit</td>
<td>Current Pregnancy: where baby was born- If hospital midwifery-led unit</td>
</tr>
<tr>
<td>Hospital Consultant Unit Birth</td>
<td>Where birth was in a hospital at a consultant-led unit</td>
<td>Current Pregnancy: where baby was born- If hospital: consultant-led unit</td>
</tr>
</tbody>
</table>
### Table 2.1 Definitions of factors and meditech sources

<table>
<thead>
<tr>
<th>Factor</th>
<th>Definition</th>
<th>Related Healthcare Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous Retained Placenta</td>
<td>The status of having failed to expel the placenta within 30 minutes of active management or 60 minutes of expectant management at prior to the index birth</td>
<td>Previous pregnancy: complications of labour</td>
</tr>
<tr>
<td>Previous Uterine Injury</td>
<td>Any previous operation or injury involving the uterus excluding caesarean sections</td>
<td>Previous gynaecological problems: any uterine surgery. For each previous pregnancy: labour &amp; birth complications Mode of birth e.g. trial of scar for previous caesarean section(s)</td>
</tr>
<tr>
<td>Previous Cervical Injury</td>
<td>Any previous injury, surgery or repair of the cervix</td>
<td>Previous gynaecological problems: any cervical surgery</td>
</tr>
<tr>
<td>Infertility Treatment</td>
<td>Any form of treatment for infertility</td>
<td>Current pregnancy: Infertility treatment</td>
</tr>
<tr>
<td>Expectant Management</td>
<td>No prophylactic oxytocics nor control cord traction in 3rd stage</td>
<td>Current Pregnancy: no oxytocic used prophylactically</td>
</tr>
<tr>
<td>Prophylactic Ergometrine</td>
<td>Syntometrine injection given at birth to prevent PPH</td>
<td>Current Pregnancy: Syntometrine given prophylactically</td>
</tr>
<tr>
<td>Prophylactic Oxytocin</td>
<td>Syntocinon injection given at birth to prevent PPH</td>
<td>Syntocinon given prophylactically</td>
</tr>
<tr>
<td>Current PET/Eclampsia</td>
<td>Hypertension and proteinuria with or without associated convulsions in the index pregnancy</td>
<td>Current Pregnancy: Antenatal or intrapartum complications</td>
</tr>
</tbody>
</table>

*For the purpose of this study, the index birth is ignored. Thus, a woman who has a retained placenta or who has just had a normal third stage with her first birth would be considered nulliparous and gravida 1.

Every outcome assessed was also defined as reflected in table 2.2.
<table>
<thead>
<tr>
<th>Data</th>
<th>Definition</th>
<th>Meditech source (search words and free text areas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss</td>
<td>Losing 500 ml of blood or more in 3rd stage of labour</td>
<td>Operation summary for manual removal of placenta</td>
</tr>
<tr>
<td>HB=/&gt;=11</td>
<td>Haemoglobin level of 11 g/dl or less within 24 hours of birth.</td>
<td>Discharge sheet</td>
</tr>
<tr>
<td>Uterine inversion</td>
<td>A uterus turned inside out in 3rd stage of labour</td>
<td>Current pregnancy: intrapartum complications</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>A surgical removal of the womb (within 48 hours of birth)</td>
<td>Current pregnancy: intrapartum or postpartum complications</td>
</tr>
<tr>
<td>Maternal Death</td>
<td>The death of a woman while pregnant or within 42 days of termination of pregnancy, irrespective of the duration and site of the pregnancy, from any cause related to or aggravated by the pregnancy or its management but not from accidental or incidental causes</td>
<td>Current pregnancy: intrapartum complications</td>
</tr>
</tbody>
</table>

Table 2.2 Definitions of outcomes associated with retained placenta subtypes.
g) Outcome measures

This part of the retained placenta study was intended to analyse data collected prospectively at the Liverpool Women’s Hospital to examine aetiological factors and outcomes that are associated with the various forms of retained placenta. The factors and outcomes examined were taken from those studied in (Nikolajsen, Lokkegaard & Bergholt 2013); (Endler et al. 2014); (Dombrowski et al. 1995); (Yuen et al. 1995); (Ashwal et al. 2014); (Silver et al. 2006) and hypothesised in (Weeks, 2008). They are reflected in table 1.1 in chapter 1.

h) Data analysis

After receiving the anonymised data, it was checked for errors and missing data. Every factor and outcome was then uploaded to SPSS and analysed separately against the control using chi-square tests to establish the level of significant association with the three known subtypes of the retained placenta. The statistical significance was set at < 0.05, but Bonferroni corrections were also deployed for outcomes. Emphasis was given to Fisher exact test considering the small data in our study. Where no value was obtained for Fisher exact, the values of Pearson Chi-square were considered. This analysis revealed whether the subtypes had different aetiological factors and morbidity rates, suggesting they were different clinical entities.

We had planned to conduct a univariate followed by a multivariate logistic regression. However, we dropped this option for the following reasons:

1. There were not enough significant factors and outcomes to make the analysis valid
2. There were no significant factors that predicted a trapped placenta. Therefore, the logistic regression model would have produced the same results as the simple analysis.
To assess for predictors of the trapped placenta, a predictor model was used to compare 72 women that had trapped placentas with both women that had placenta adherens and partial accreta totalling 283. A univariate analysis was to be followed with a multivariate one if there were significant predictive values for the trapped placenta.

i) Validation of data

Two staff from the information technology department of the LWH, Sandra Drummond and John McCormick) independently validated the integrity of the Meditech data used (that it was retrieved correctly from the overall database). They did so by comparing the data stored in the specific MEDITECH application with the data stored within the Data Repository SQL (Structured Query Language) tables. Both agreed 100%.

SQL stands for Structured Query Language. This is the standard language used to communicate with a database to perform tasks such as update data on a database, or retrieve data from a database (http://www.sqlcourse.com/intro.html, accessed 27/10/2018)

The master data set was compiled using SPSS software. This was further checked by the university statistician Dr Steven Lane and found to be consistent with the primary data.

j) Ethics approval

The ethics approval for this study was obtained from the East of Scotland Research Ethics Service (REC Reference 15/ES/0069), and it was sponsored by the University of Liverpool (Sponsor Reference UoL001133).
2.3 Results

2.3.1 Population size

The total number of women who gave birth at the Liverpool Women’s Hospital from 1 Jan 2009 to 31 Dec 2014 was 48 546. The total study population was calculated as 31 120 (as shown in figure 2.2) with a study group of 355 (adherens 264, trapped 72 and partial accreta 19) and a control of 30 765 based on the exclusion criteria. Out of the 31 120 women who achieved non-instrumental vaginal births, 464 had manual removal of placenta, which gives an incidence of 1.5% for manual removal in this hospital. The 464 who had manual removal comprised the study group of 355; there were 109 with no subtypes indicated, and two with missing data. See figure 2.2. The number of women omitted is based on the study exclusion criteria, as stated in subsection d above.
Figure 2.2 Calculation of the number of women in the study and control group
2.3.2 Predictor variables

The three subtypes of RP (placenta adherens, trapped placenta and partial accreta) were considered separately against the predictor variables.

Placenta adherens (Table 2.3)
Out of 20 factors considered in this study, we found a significant association between placenta adherens and maternal BMI (p-value 0.036), infertility treatment (p-value <0.001), augmented/dysfunctional Labour (p-value <0.001). However, there were only five women (1.9%) with placenta adherens that had infertility treatment. There was no significant association between placenta adherens and the other 19 factors.

Partial accreta (Table 2.3)
Partial accreta was significantly associated with 2 out of 20 factors, namely previous retained placenta (p-value 0.044) and augmented/dysfunctional labour (p-value <0.001).

Trapped placenta (Table 2.3)
The trapped placenta was only significantly associated with augmented or dysfunctional labour (p-value <0.001) out of the 20 factors studied.

The SPSS was used to automatically calculate a Fisher Exact Test or Pearson Chi-Square test p-values as appropriate. Due to very small numbers, Fisher Exact Test was used, and Pearson Chi-Square was only considered when the SPSS failed to provide a value for the Fisher Exact Test.

It was found that of all 20 possible factors, only augmented/dysfunctional labour was significantly associated with all three retained placenta subtypes. This was also the only factor significantly associated with the trapped placenta. In contrast, both placenta adherens and partial accreta were significantly associated with other aetiological factors (Table 2.3). If adjustment is made for multiple testing using the
Bonferroni correction, then the only augmentation of labour and infertility treatment are statistically significant.
<table>
<thead>
<tr>
<th>Factors</th>
<th>Normal Placenta (n=30,765)</th>
<th>Trapped Placenta (n=72)</th>
<th>P-Value</th>
<th>Placenta Adherens (n=264)</th>
<th>P-Value</th>
<th>Partial Accreta (n=19)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>&lt;20y</td>
<td>2,284 (7.4%)</td>
<td>3 (4.2%)</td>
<td>21 (8.0%)</td>
<td>0.937</td>
<td>1 (5.3%)</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>20-39y</td>
<td>27,133 (88.2%)</td>
<td>65 (90.3%)</td>
<td>231 (87.5%)</td>
<td></td>
<td>15 (78.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥40y</td>
<td>1,348 (4.4%)</td>
<td>4 (5.6%)</td>
<td>12 (4.5%)</td>
<td></td>
<td>3 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>Gestation</td>
<td>PT&lt;37 wks.</td>
<td>2,559 (8.3%)</td>
<td>8 (11.1%)</td>
<td>18 (6.8%)</td>
<td>0.445</td>
<td>3 (15.8%)</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td>T37-40 wks.</td>
<td>21,400 (69.6%)</td>
<td>47 (65.3%)</td>
<td>177 (67.0%)</td>
<td></td>
<td>13 (68.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PD&gt;40 wks.</td>
<td>6,688 (21.7%)</td>
<td>17 (23.6%)</td>
<td>69 (26.1%)</td>
<td></td>
<td>3 (15.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>118 (0.4%)</td>
<td>0 (0.0%)</td>
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It would be useful to predict a trapped placenta clinically. This is because it can be treated with medicines alone, controlled cord traction or Windmill technique as discussed earlier, thereby avoiding the need to take a woman to the theatre for anaesthesia and surgery. The trapped placenta is also associated with minimal blood loss. Therefore, it allows time to wait for the non-surgical treatment options to be conducted.

We attempted in our study to assess for aetiological factors that can be used to predict the trapped placenta. To do so, we had to compare the trapped placenta against the other two retained placenta subtypes (placenta adherens and partial accreta) (Table 2.4). We had expected to find aetiological factors, such as the use of ergometrine and history of previous cervical injury to be significantly predictive of the trapped placenta. However, we did not obtain any significant data that can be used to predict the trapped placenta.
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<td>72 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.0%)</td>
<td>283 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No P-value Calculated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophylactic Oxytocin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Yes</strong></td>
<td><strong>No</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (6.8%)</td>
<td>67 (93.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32 (11.3%)</td>
<td>251 (88.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.388&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current PET/Eclampsia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Yes</strong></td>
<td><strong>No</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.0%)</td>
<td>72 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.0%)</td>
<td>283 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No P-value Calculated</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4 Factors predictive of trapped placenta

**Key**

- Significant at p <0.05
- 3. Fisher Irvin Exact Test
- 4. Pearson Chi-square Test

### 2.3.3 Associated outcomes

Placenta adherens (table 2.5) was significantly associated with blood loss greater or equal to 500 mls and haemoglobin equal to or less than 11 gm/dl. It was not significantly associated with the other five factors studied.
Partial accreta (table 2.5) was significantly associated with uterine inversion at the third stage of labour, high blood loss and low haemoglobin. But, the number of women with partial accreta was only 19, and this significance could be a statistical anomaly due to very small numbers. However, partial accreta was not significantly associated with hypotension, hysterectomy and maternal death.

Trapped placenta (Table 2.5) was also significantly associated with postpartum haemorrhage (P-value <0.001). Table 3.2. However, this appears to be more of mild PPH with estimated blood loss of <500 mls, occurring in 35% of women compared to 13.9% for severe PPH. Thus, the trapped placenta is least associated with severe PPH compared to partial accreta and placenta adherens respectively.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Normal</th>
<th>Trapped</th>
<th>P-value</th>
<th>Adherens</th>
<th>P-value</th>
<th>Accreta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=30,765</td>
<td>N=72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N=19</td>
<td></td>
</tr>
<tr>
<td><strong>Specific Data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Loss in mls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>27505 (89.4%)</td>
<td>35 (48.6%)</td>
<td><strong>0.001</strong></td>
<td>90 (34.1%)</td>
<td><strong>0.001</strong></td>
<td>7 (36.84%)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>500-1000</td>
<td>2634 (8.6%)</td>
<td>27 (37.5%)</td>
<td>109 (41.3%)</td>
<td>5 (26.32%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1000</td>
<td>625 (2.0%)</td>
<td>10 (13.9%)</td>
<td>65 (24.6%)</td>
<td>7 ((36.84%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Free Text Search</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb&lt;=11 g/dl</td>
<td>Yes</td>
<td>5 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0.988</td>
<td>1 (0.4%)</td>
<td>0.058</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30760 (100.0%)</td>
<td>72 (100.0%)</td>
<td>263 (99.6%)</td>
<td>18 (94.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine Inversion</td>
<td>Yes</td>
<td>35 (0.1%)</td>
<td>0 (0.0%)</td>
<td>0.921</td>
<td>1 (0.4%)</td>
<td>0.271</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30730 (99.9%)</td>
<td>72 (100.0%)</td>
<td>263 (99.6%)</td>
<td>18 (94.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>Yes</td>
<td>6 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0.999²</td>
<td>0 (0.0%)</td>
<td>0.999²</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30759 (100.0%)</td>
<td>72 (100.0%)</td>
<td>264 (100.0%)</td>
<td>19 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Death</td>
<td>Yes</td>
<td>1(0.0%)</td>
<td>0 (0.0%)</td>
<td>0.999²</td>
<td>0 (0.0%)</td>
<td>0.999²</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30764 (100.0%)</td>
<td>72 (100.0%)</td>
<td>264 (100.0%)</td>
<td>19 (100.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.5 Outcomes associated with retained placenta subtypes

**Key**

*P-value < 0.05

1. Pearson Chi-Square Test
2. Fisher Exact Test
Although all the three subtypes of the retained placenta are significantly associated with estimated blood loss equal to or greater than 500 mls, they are differently associated with other outcomes studied. However, partial accreta is the only retained placenta subtype that is significantly associated with haemoglobin $\leq 11$ g/dl and uterine inversion in the third stage of labour. But, the number of women with partial accreta was very small, only 19, and both haemoglobin $\leq 11$ g/dl and uterine inversion occurred as only one event each out of 19 (Table 3.2). Hence, although the retained placenta accreta was found to be significantly associated with uterine inversion and haemoglobin less than 11 g/dl, this could be a statistical anomaly.

All four categories of retained placenta reflect the following p-values (Table 2.4) and median blood loss (Table 2.6).

Normal versus trapped p-value = <0.001; normal versus adherens p-value = <0.001; normal versus accreta p-value = <0.001; trapped versus adherens p-value <0.007; trapped versus accreta p-value = <0.001 and adherens versus accreta p-value = <0.005. These are statistically significant at both the standard 0.05 level, and at the 0.008 level (0.05/6) using the Bonferroni correction. The blood loss increases gradually from normal (non-retained) placenta, where it is lowest, to placenta accreta, where it is highest.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>Median Blood Loss</th>
<th>Interquartile</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30,765</td>
<td>250.00 mls</td>
<td>150</td>
<td>1-3300</td>
</tr>
<tr>
<td>Trapped</td>
<td>72</td>
<td>500.00 mls</td>
<td>450</td>
<td>100-2000</td>
</tr>
<tr>
<td>Adherens</td>
<td>264</td>
<td>700.00 mls</td>
<td>838</td>
<td>100-3500</td>
</tr>
<tr>
<td>Accreta</td>
<td>19</td>
<td>1300.00 mls</td>
<td>2000</td>
<td>300-4500</td>
</tr>
</tbody>
</table>

Table 2.6 Median blood loss associated with retained placenta subtypes. The interquartile range being between 25% to 75%.
Figure 3.1 below shows a box and whisker graph for the estimated blood loss in each group. The following p-values were obtained for this comparison using Mann-Whitney test (figure 2.3): The box and whisker plot represents the minimum quartile, first quartile, median, third quartile and maximum quartile ranges. A box plot alone displays the first quartile to the third quartile.
Figure 2.3 Box whisker plot of estimated blood loss with statistical hypothesis testing using Mann-Whitney test (significant p-value set at 0.008 following Bonferroni correction).
2.4 Discussion

2.4.1 Predictor variables

Our study explored whether trapped placenta can be identified using aetiological data by pitching trapped versus non-trapped placentas.

Weeks (2008) hypothesised aetiological factors predictive of trapped placenta to be birth in a labour bed and use of prophylactic intravenous ergometrine (Table 1.1). None of the 20 aetiological factors assessed was significantly associated with the trapped placenta. Hence, we did not identify any factors predictive of the trapped placenta (Table 2.2)

Prophylactic ergometrine and oxytocin were both free text data and so at high risk of reporting bias. The prophylactic use of ergometrine was implicated in causing trapped placenta (Yuen et al. 1995). However, our study did not find a significant association between any subtype of retained placenta with prophylactic use of ergometrine or oxytocin. Therefore, we could not verify the hypothesis that use of ergometrine is associated and predictive of the trapped placenta. Of the 42 women out of 31, 120 reported to have had prophylactic ergometrine in our study, none developed a retained placenta.

Over 90% of women were expected to have undergone active management of the third stage of labour using either oxytocin alone or combined with ergometrine in the form of syntometrine. This was not reflected in the data obtained since the number of women who used prophylactic oxytocin and ergometrine is very small. The drug use was picked up on a ‘medication used during labour’ free text box, and many midwives completing the form may have considered ‘routine’ medication not to be important. Therefore, it is more likely that these represent additional treatment above normal prophylactic doses.
Birth in a labour bed is another factor hypothesised to be predictive of the trapped placenta. However, our study did not examine this factor.

Potentially, a previous history of cervical injury could be predictive of the trapped placenta, considering the fact that a placenta may be trapped behind a stenosed or constricted cervix subsequent to scarring or post-traumatic adhesions. However, our study only detected two women out of 31,120 to have had a history of cervical injury and both had placenta adherens, not trapped placenta.

We did not assess birth in a labour bed as an aetiological factor. Hence, we could determine whether it is predictive of trapped placenta as hypothesised by Weeks (2008).

We also explored whether the three subtypes of the retained placenta are distinct clinical entities.

In this retrospective analysis of aetiological factors associated with retained placenta subtypes, the following was found. Placenta adherens was associated with maternal BMI, infertility treatment and augmented/dysfunctional labour. However, there were only five women (1.9%) with placenta adherens that had infertility treatment. Therefore, this significance can be a statistical anomaly due to very small numbers. Partial accreta was associated with previous retained placenta and augmented/dysfunctional labour. The trapped placenta was associated only with augmented/dysfunctional labour. Thus, all three subtypes of the retained placenta are significantly associated with augmented/dysfunctional labour, but differ in other aetiological factors. Clinically, significant aetiological factors may be used to predict the retained placenta subtypes. However, because of small numbers, some of these results may be misleading, e.g. for infertility treatment and partial accretas.

All the data in this and next chapter were from routinely collected computerised data. However, in the Meditech system, there are two different types of data: that in which there is a direct question/tick box (e.g. was the labour induced? Yes / No) and
the free text sections (e.g. record any complications ____ ). The former (specific data) are relatively accurate, whereas to assume that there was no pre-eclampsia because it was not mentioned in the free text box is a very inaccurate measure. In our study, we refer to the data we obtained from specific questions on the Meditech as specific data, and we consider it to be more accurate compared to the one obtained as a free text.

Our study is the first (to our knowledge) to examine the association of various aetiological factors by subtype of retained placenta. Most previous researchers have described the associations with the retained placenta irrespective of its subtypes, as discussed below.

### 2.4.1a Specific data

These data were recorded on the Meditech following specific questions such as age, parity, gravidity, BMI, ethnicity and place of birth. They are more reliable recorded on the Meditech compared to free text data as the specific questions prompted the clinicians to record them. Though more reliable, some may be affected by human error. For example, an age of 20 years may be recorded wrongly as 2000 years.

In our study, we noted that specific data were more reliably extracted and more likely to provide correct results as reflected in the findings below:

Augmented/dysfunctional labour was obtained as a specific data which gives more confidence about its accuracy. Weeks suggested an association between the retained placenta and dysfunctional labour, after finding that myometrial thickness of slowly-progressing labour to be thinner compared to that of normally-progressing labour (Weeks 2003). This is thought to occur because of the failure of contraction of retro-placenta myometrium.

Our study shows that the only factor significantly associated with all the three subtypes of the retained placenta is augmented/dysfunctional labour. Both
dysfunctional labour and placenta adherens occur because of inadequate contraction of the retro-placenta myometrium (Herman et al. 1993), 1993; (Weeks 2003). However, the data used by Herman was not strong at all, being a comparison of only 25 women with the normal placenta and five women with placenta adherens. Our finding is similar to that previously reported by Weeks (2003) and Coviello et al. (2015) who recently reported that longer first or second stages of labour among primiparous women are risk factors for the retained placenta (Coviello et al. 2015).

Considering that the underlying mechanism for placenta adherens is a failure of contraction of retro-placenta myometrium, it is understandable that dysfunctional labour is significantly associated with placenta adherens. However, it is difficult to understand why it is also significantly linked with partial accreta where the underlying mechanism is placental invasion (Greenbaum et al. 2017) unless the dysfunctional contractions are due to defective myometrium. But we could not identify evidence to this. It is also difficult to clearly explain its link with Trapped Placenta where the placenta is trapped probably following excessive contraction of the cervix and lower segment of the uterus (McDonald, Abbott & Higgins 2004; McDonald, Prendiville & Elbourne 2000).

Induction of labour was a specific data recorded on the Meditech following a direct question: was labour induced. Ashwal et al. (2014) found that hypertensive disorders, oligohydramnios, induction of labour, neuro-axial analgesia and vacuum extraction to be independently associated with manual removal of the placenta (Ashwal et al. 2014). Our study excluded women who had instrumental vaginal births. However, it found a similar significant association between induction of labour and trapped placenta.

Maternal BMI was a specific data with high confidence, in contrast to the history of pre-eclampsia/eclampsia and infertility treatment which were free texts with less confidence. Endler et al. (2014) found defective placentation disorders, i.e. pre-eclampsia, stillbirth, small for gestational age (SGA), and spontaneous preterm birth, to be associated with risk of retained placenta, virtually all adherens (Endler et al.
In contrast, we found a significant association between placenta adherens, maternal BMI, infertility treatment, augmented/dysfunctional labour. We assessed stillbirths in a different study.

Other specific data in our study such as age, gestation, parity ethnic group and place of birth were not significantly linked to any subtype of the retained placenta.

In principle, Meditech data should have been a considerable resource for research due to the very large study population it affords of over 48,546 (giving a sample size of 31,120 (30,765 control and 355 study)). This is a very large sample for statistical work of this sort. It compares well with previous studies: while not as large as the population used by Endler et al. (2014) (386,607), it is a similar size to the 45,852 studied by Dombrowski, (1995), 30,132 by Silver (2006), and 33,925 by Ashwal et al. (2012). (Endler et al. 2014); (Dombrowski et al. 1995); (Silver et al. 2006); (Ashwal et al. 2014)

Liverpool Women’s Hospital is one of the largest women’s hospitals in Western Europe; because of this our study could be a single-hospital study in contrast to those conducted in multiple hospitals or nation-wide (Endler et al. 2014); Release Trial, 2010 (Weeks et al. 2010) and GOT-IT Trial, 2018 (Denison et al. 2017). As with all the data used in this study, this confers a considerable advantage to the Meditech data, as a single hospital probably provides much more consistency of diagnoses in contrast to those from multiple hospitals.

The incidence of 1.5% we found for the manual removal of the placenta in Liverpool Women’s Hospital is half of the overall incidence of 3% for the retained placenta in developed nations, but more than 0.5-1% in developing countries (Weeks 2008) (Cheung et al. 2011) and (Ashwal et al. 2014). All these studies showed similar higher incidences in developed compared to developing countries. This may be explained by relatively increased rates of induction and augmentation of labour in developed countries. Indeed, our associations are age, BMI, infertility treatment and augmentation of labour, all of which are increased in developed countries.
A case-control study found that multiparity, induced labour and small placenta to be significantly associated with the retained placenta (Adelusi et al. 1997). Similarly, we found a history of repeated births to be significantly associated with placenta adherens. But we did not assess the size of the placenta.

A recent retrospective cohort study was similar to ours as it categorised epidemiological factors for the retained placenta by retained placenta subtypes (Greenbaum et al. 2017). But its focus was on underlying mechanisms of the retained placenta and not specific subtypes as in our study. We could not identify a similar study on the subtypes of the retained placenta.

2.4.1b Free text data

These were data recorded freely without specific questions. They depended on the memory of the clinician. If the clinician forgot to record them, they would not be available to extract later. Hence, most of them would be missing in the records. Therefore, they were less reliable and more likely to give wrong results than the specific closed questions. These included previous cervical/uterine injuries, prophylactic use of ergometrine/oxytocin, history of pre-eclampsia/eclampsia etc. as reflected below. Generally, the free-text data were so unreliable in our study to the extent that we rejected them.

Confidence in the study data.

Because the study used a retrospective cohort, the existing data was as recorded in clinical practice rather than research data. Some studies have demonstrated that in this respect, Meditech data is inferior and inaccurate compared to paper-based records. For instance, Corbell et al. found that using the paper-based clinical record as the gold standard, the reliability scores for variables on Meditech including
weight, haemoglobin, and CD4 counts ranged from 0.59 to 0.99 (Corbell et al. 2012).

The Meditech data was validated by the IT staff of LWH. Yet some of it was either missing or incompatible, e.g. for pre-eclampsia and previous caesarean sections. These were inserted on Meditech by clinicians as free text. If pre-eclampsia was not inserted, it could be assumed from relevant signs and symptoms if those were recorded. We could have traced the missing data and rectified incompatible ones using paper records, but the data set was too large to do so within the short time limit of a PhD thesis.

History of previous retained placenta was obtained as a free text in our study and therefore at high risk of reporting bias. Nikolajsen et al. found a recurrence of retained placenta to be increased five-fold among women with a history of retained placenta (Nikolajsen, Lokkegaard & Bergholt 2013). This is in line with our finding that a history of previous retained placenta is significantly associated with placenta accreta. But it was not significant for the trapped placenta and placenta adherens.

Prophylactic ergometrine and oxytocin were both free-text data and so at high risk of reporting bias. The prophylactic use of ergometrine was implicated in causing trapped placenta (WHO Reproductive Health Library 2012) and (Yuen et al. 1995). However, our study did not find a significant association between any subtype of the retained placenta with prophylactic use of ergometrine or oxytocin.

Overall, we only found five out of 20 factors to be significantly associated with one or the other subtype of the retained placenta. These were less than the ones showed by the previous researchers, as discussed below. However, unlike in this study, their data was not analysed according to the three subtypes of the retained placenta. Silver et al. (2006) found placenta accreta to be highly associated with multiple episodes of caesarean sections (Silver et al. 2006). We were unable to test this association as we excluded women who underwent caesarean sections. In the Meditech database, there
was no way of searching for a previous caesarean birth – this meant that we were unable to test that association.

We were surprised to find no significant association between placenta adherens and current pre-eclampsia/eclampsia, contrary to that of Endler et al. (Endler et al. 2014). This may be because our study was not set to pick up all women with the current history of pre-eclampsia and eclampsia. Our Meditech study could have missed most women with pre-eclampsia/eclampsia as these diagnoses were not specifically and comprehensively recorded on the Meditech, relying instead on the midwife mentioning the term postnatally in a list of complications. Surely a finding of only one woman out of 31120 to have had a pre-eclampsia/eclampsia must have been a gross underestimate, considering in the UK, the incidence of all pre-eclampsia is 3% (Hutcheon, Lisonkova & Joseph 2011) and severe pre-eclampsia is 5/1000 maternities, while the incidence of eclampsia is 4.9/10,000 maternities (Douglas & Redman 1994); (Tuffnell et al. 2005) and (Rudra 2011).

We were also surprised not to find a significant association between previous caesarean sections and partial accreta as commonly shown by other researchers ((Silver et al. 2006); (Jauniaux & Jurkovic 2012) and (Belachew et al. 2014). However, our study excluded women that had operative deliveries, some of whom could have had previous caesarean sections. Although we attempted to assess the history of previous caesarean section, we could only identify two women out of 31120 who achieved vaginal delivery following caesarean section. We believe they should have been more than that. But the history of previous caesarean section was not clearly recorded in the birth records on the Meditech.

The lack of significant link between ergometrine and trapped placenta in our study is unexpectedly different from the findings of others (Yuen et al. 1995), (Begley 1990) and (WHO Reproductive Health Library 2012), all of which showed an increase in the incidence of manual removal of placenta when ergometrine was used. This may be explained by a reduction in the usage of ergometrine prophylactically. Although it is more effective than oxytocin alone in the prevention of PPH (McDonald,
Prendiville & Elbourne 2000), its poor side effect profile, namely hypertension, nausea and vomiting, has led to its being used more for treatment rather than prophylaxis. Thus, Liverpool Women’s Hospital used oxytocin more than ergometrine for active management of the third stage of labour. However, our finding was like that of Harara et al., where the use of ergometrine was not associated with retained placenta, although their sample size of 53 was also small compared to ours (Harara et al. 2011).

Routinely collected data (RCD) are commonly utilised for biomedical research as in our study, where we used retrospective data routinely collected by clinicians. However, RCD have several strengths and many weaknesses (Hemkens et al., 2016). In terms of strengths, they minimise cost and effort. Hence, they are the only way of collecting the data in a short time period of the PhD as in this study. They also allow information to be captured and updated in large populations and clinical events. However, the routine data are generally collected in situations where the findings are difficult to generalise e.g. from tertiary hospitals which are not representative of primary health care centres. The use of routinely collected data is an inherent limitation as data collected in this way is more frequently inaccurate and missing than data collected by research staff, who may have time and a specific remit to collect accurate data. Furthermore, large sample sizes, collected routinely, without thorough analytical safeguards can lead to statistical type 1 error with false positive results.

Therefore, there is also a possibility that some of the junior doctors interpreted the Meditech questions incorrectly relating to the type of RP, when they originally compiled the data that we subsequently analysed.
Future improvements and research

Vital data, such as current pre-eclampsia and previous caesarean sections, should be clearly recorded on Meditech to be easily retrievable by future researchers or auditors. A repeat of this research, after proper records are established on the Meditech would provide a clearer picture. A better database would be required, maybe where the data is collected as part of a large research cohort study so that the data is validated and of proven accuracy.

Future research could also examine why augmented/dysfunctional labour is associated with partial accreta and trapped placenta, where the underlying mechanisms are inconsistent with failure of contraction of the retro-placenta myometrium. A possible hypothesis is that the myometrium fails to contract because it is invaded by the placenta in case of placenta accreta. Another hypothesis is that the myometrium fails to contract efficiently because it is compressed by a trapped placenta.

2.4.2 Associated outcomes

In this retrospective analysis of outcomes associated with retained placenta subtypes, all the three subtypes were significantly associated with blood loss of 500 mls or more. Only placenta accreta was also associated with a haemoglobin level of 11 g/dl or less as well as uterine inversion.

We found only three out of five outcomes assessed to be significantly associated with either or all the three subtypes of the retained placenta. This falls short of the findings of previous researchers (Nikolajsen, Lokkegaard & Bergholt 2013); (Endler et al. 2014); (Dombrowski et al. 1995); (Yuen et al. 1995); (Ashwal et al. 2014), (Silver et al. 2006) and hypothesised in (Weeks 2008). They also found significant associations with previous caesarean sections, use of ergometrine, stillbirths and sepsis amongst others. This may be due to a limited number of the retained placenta subtypes in our study, but also due to inaccuracies in the electronic data records.
Our study is the first to our knowledge to assess the association of various outcomes according to the three subtypes of the retained placenta. Most previous researchers have described the outcomes of combined retained placenta irrespective of its three subtypes.

Our large study population of over 48,546 and sample size of 31,120 (30,765 control and 355 study) is another aspect of its strength, which similar in population size to other studies (Dombrowski et al. 1995, Silver et al. 2006 and Ashwal et al. 2012).

2.4.2a Specific data

Estimated blood loss was obtained as specific data on the Meditech with high reliability. In this study, placenta adherens leads to significant postpartum haemorrhage with a p-value <0.001. However, severe PPH occurs only in 24.6% of women with placenta adherens compared to 36.8% of women with partial accreta. This suggests that severe PPH is more frequently associated with partial accretas than placenta adherens, which is significant at a chi-square value of 49.2289 and a P-value of <0.05.

The trapped placenta was also significantly associated with postpartum haemorrhage (P-value <0.001). However, this appeared to be more of mild PPH with estimated blood loss of <500 mls, occurring in 35% of women compared to 13.9% for severe PPH. Thus, the trapped placenta is least associated with severe PPH compared to partial accreta and placenta adherens respectively.

Retained placenta leads to postpartum haemorrhage as it interferes with uterine contractions. Partial accreta causes more severe postpartum haemorrhage compared to placenta adherens and trapped placenta. Our study verifies this with a significant P-value <0.001.
Our study shows that all subtypes of the retained placenta are significantly associated with postpartum haemorrhage (P= <0.001). This association is in line with the fact that retained placenta is a significant contributor to postpartum haemorrhage RCOG (2009) and concurs with data from Onwudieigo and Makinde (1999) and Memon et al. (2011). But these studies did not categorise postpartum haemorrhage according to retained placenta subtypes with which to compare our findings.

The significant association of estimated blood loss with retained placenta subtypes was verified using various statistical tests (Chi-square, Kruskal-Wallis and Mann-Whitney). It was also maintained following Bonferroni correction to indicate that it was not due to pure chance nor type one error. The median blood loss (Table 2.5) and Box whisker plot (Figure 2.3) clearly showed a step-wise increase in blood loss from trapped where it is lowest through adherens in the middle up to accreta with the highest blood loss. However, we acknowledge that some of the blood loss might have been estimated without weighing as this was the clinical practice before weighing became mandatory.

Knowledge of blood transfusion rate would have also helped to indicate the number of women that had severe blood loss. However, individual data on whether a woman had a blood transfusion or not is not collected on the same Meditech system and so we were not able to obtain data for this.

### 2.4.2b Free text data

Maternal mortality was obtained on the Meditech as a free text with lower reliability. However, our finding of a 0% maternal mortality rate amongst women with retained placenta over a period of 6 years verifies the 0.1% case fatality rate reported by Weeks for developing nations (Weeks 2008). This is in contrast to case fatality rates of up to 10% in low middle-income countries. The single maternal mortality reported during our study period occurred in the year 2011 and was not due to the retained
placenta. It was caused by pulmonary embolism and reported as the first case of maternal mortality in 15 years.

Haemoglobin less than or equal to 11 g/dl was also obtained as free text data. This was found to be significantly associated with placenta accreta, which is linked to severe blood loss in this subtype. In this study, only 2 women out of 355 with retained placenta were reported to have postnatal haemoglobin less than 11 g/dl. This is probably incorrect considering that many women with retained placenta suffer from postpartum haemorrhage. The data were extracted from free text which is unreliable and the results were not checked on the pathology system. Furthermore, the number of women that had no haemoglobin checked or who had it checked more than once postnatally was not reported. This demonstrates the problem of using retrospective data from a single data source which is not linked to other hospital systems.

Another free text data obtained was a uterine inversion. Partial accreta was also found to be significantly associated with it. This life-threatening complication was reported by others to be associated with the retained placenta (Bewley 2009). This may be due to poor technique of controlled cord traction, such as excessive traction on an unsuspected accreta (Pena-Marti & Comunian-Carrasco 2007). More cases of uterine inversion have been reported in association with placenta accreta (Tsivos et al. 2009).

Stillbirth was not assessed as an outcome in this study as done by other researchers (Endler et al. 2014). Disorders of defective or impaired deep placentation are known to be associated with enhanced oxidative stress and apoptosis in the placenta (Jauniaux, Poston & Burton 2006) and (Roje et al. 2011). Consequently, uteroplacental insufficiency leads to stillbirth.

Uterine sepsis is reported to occur in up to 17% of women with retained placenta. (Onwudiegwu & Makinde 1999). We did not examine this outcome at our study because the data related to it was not provided as requested.
Although our study was limited as a single hospital study, it was conducted in Liverpool Women’s Hospital, one of the largest women’s hospital in Western Europe, with a birth rate of over 8 500 per annum.

Another weakness of this study is that it was a retrospective cohort, which means we had to use the existing data as recorded in clinical practice rather than research data. Nevertheless, the data examined was collected prospectively.

The main weakness of our study though is that it is a Meditech based study and as stated in Chapter 2, our experience showed that some of the data in it were either not recorded or recorded incomplete or incompatible. This is in line with a study that validated the Meditech compared to paper records (Corbell et al. 2012).

Clinical implications and research

Despite the weaknesses in the studies, we have shown that the three subtypes of the retained placenta appear to differ in outcomes and could be separate clinical entities, especially in blood loss.

If the three subtypes of the retained placenta are separate clinical entities, they should be treated differently. The contemporary approach of treating the retained placenta as a single entity may need to be revised. This could lead to a smaller number of women undergoing the invasive procedure of manual removal of the placenta with surgical and anaesthetic complications. This is because some of the subtypes of the retained placenta can be treated with simple medicines only. Of the three subtypes, the trapped placenta is the safest, considering it causes less severe bleeding compared to adherens and accreta. If so, when a trapped placenta is diagnosed, it might be delivered spontaneously by waiting longer, but for less than 60 minutes in case it might be a partial placenta accreta, which can lead to heavy vaginal bleeding. However, once a retained placenta is diagnosed, and the subtype is unknown, obstetric review and transfer to the theatre should be expedited, and
careful recording of observations should be performed, as concealed bleeding can be marked, and deterioration is likely. (MBRRACE-UK 2017).

When records on the Meditech are improved, our findings can be validated through further research. Furthermore, considering the subtypes of the retained placenta as separate clinical entities, future research can examine the effectiveness of type-specific treatment compared to single entity therapy.

2.5 Conclusion

2.5.1 Predictor variables

This study was based on routinely collected data using the hospital’s Meditech electronic data collection system. We found evidence, however, that the Meditech data had high levels of reporting bias, especially for items that came from a free text search and were not routinely collected as a specific item. Nevertheless, it was possible to derive the following from the paper-based data that was used, with regards to aetiological factors associated with subtypes of the retained placenta:

- Although it would be clinically useful to predict a trapped placenta, considering it can be more easily treated and less associated with heavy PPH compared to the placenta adherens and partial accreta, our study failed to establish aetiological factors that can be used to predict it.

- The three subtypes of the retained placenta may have different aetiological factors. This suggests that they may be different clinical entities that should be treated differently.

- The only factor that is significantly shared by all the three retained placenta subtypes is augmented/dysfunctional labour, which indicates inadequate contraction of the retro-myometrium as the underlying cause of the retained
placenta. However, this does not fit with our understanding of underlying mechanisms for either placenta accreta or trapped placenta.

- Whereas it is understandable that augmented/dysfunctional labour is associated with placenta adherens, as the underlying aetiology is a failure of contraction of the retro-placenta myometrium, it is not clear why it is also linked to placenta accreta and trapped placenta, where the underlying mechanisms are thought to be different.

- This unexpected finding, among others in this study, may be because of the flawed nature of the Meditech data.

- Therefore, this study highlights some ways in which Meditech data could be considerably improved, so that in future, it could be more reliably used for research. In particular, for studies of pregnancy and labour, it is suggested that pre-eclampsia, previous retained placenta, previous cervical injury, previous uterine injury, infertility treatment, induction of labour, prophylactic ergometrine, prophylactic oxytocin and current pre-eclampsia/eclampsia should be mandatory questions (specific data), not free text data.

- With improved Meditech data, suggestions for future research include validating our findings on augmented/dysfunctional labour and exploring why it is also associated with partial accreta and trapped placenta.

2.5.2 Associated outcomes

As stated above, compared to paper-based data, the entered Meditech was too unreliable to be used in research and derive meaningful conclusions. Nevertheless, our study provided the following hints about complications associated with subtypes of the retained placenta:
• Although the three subtypes of the retained placenta share a significant association with postpartum haemorrhage, they differ in other outcomes. Even in PPH which they share, they vary in its severity. This suggests that they may be separate clinical entities, and there may be a benefit in treating them separately.

• If the three subtypes of the retained placenta are separate clinical entities, they should be treated differently. The contemporary approach of treating the retained placenta as a single entity may need to be revised. The lower PPH rate with trapped placenta means it can be managed expectantly. Placenta adherens, with a significantly lower risk of severe haemorrhage compared to placenta accreta, may be treated with medicines alone, if an effective medication is developed. However, partial placenta accreta, which is most likely to lead to severe postpartum haemorrhage, needs urgent surgical intervention once it is diagnosed.

After improving the data on Meditech and considering the subtypes of the retained placenta as separate clinical entities, future research can examine the effectiveness of type-specific treatment compared to a single entity approach.
Chapter 3 The role of ultrasound in diagnosis of retained placenta subtypes
3.1 Background

As described in Chapter 1, the three subtypes of RP are placenta adherens (when there is a failed contraction of the myometrium behind the placenta); trapped placenta (a detached placenta trapped behind a closed cervix); and partial accreta (when there is a small area of accreta preventing detachment).

The standard treatment of RP is manual removal of the placenta. However, this is associated with anaesthetic and surgical risks, which could be avoided with medical alternatives. Unfortunately, these therapeutic alternatives are not well investigated. This is partly because of the current inability to rapidly and accurately diagnose the various forms of the retained placenta before manual removal. Medical therapies are therefore studied in all RP and not in its subtypes, even though the oxytocic and uterotonic effects may benefit one and hinder other pathologies. For instance, uterotonics may help placenta adherens but delay further the delivery of a trapped placenta.

Conversely, GTN may help deliver trapped placentas but delay spontaneous expulsion of placenta adherens. An overall finding of 'no effect' may, therefore, hide a benefit in one pathology and a worsening of another. A method of accurate diagnosis of the pathological subtypes of RP is, therefore, crucial if progress is to be made.

This ultrasound study relates purely to the use of ultrasound as a diagnostic tool. It will seek to determine whether the subtypes of RP can be diagnosed before manual removal.
3.2 Methods

This study was a cohort one conducted prospectively at the Liverpool Women's Hospital in the UK. The specific objective was to determine the accuracy of ultrasound assessment in diagnosing the type of retained placenta, using a clinical evaluation at the time of manual removal shortly afterwards as the gold standard. Ultrasound images and Doppler parameters were thoroughly evaluated at the ultrasound diagnosis and compared to the findings at manual removal.

Before recruitment, meetings were held with teams of midwives and clinicians on delivery suite to brief them about the research and solicit their engagement with the study.

Patient information posters were also displayed in public areas, and information extracts were inserted into the patient held records. This publicity was to increase awareness of the study and their participation if they develop retained placenta.

The midwives and clinicians were provided with contact details for the PI (AA) so that they could contact him if a woman was diagnosed with RP. The PI (AA) planned to be available promptly when contacted to discuss the study with the woman, take informed consent if the woman wished to participate in the research, undertake the ultrasound scanning and finally complete a case report form.

The following criteria were used to include or exclude participants:

3.2.1 Inclusion criteria

- Retained placenta for at least 30 minutes (or 60 minutes with physiological management)
- Written informed consent
- Estimated gestation of at least 24 weeks
- Participants should be aged over 18 years, or over 16 and 'Gillick competent'.
3.2.2 Exclusion Criteria

- Women who expelled the placentas spontaneously
- PPH
- Still birth
- Estimated gestation less than 24 weeks
- Participants aged under should 18 years, or over 16 and not 'Gillick competent'.

3.2.3 Diagnostic tests

The following diagnostic tests were evaluated:

a. Clinical assessment without ultrasound

The clinician was asked to palpate the uterus, conduct a vaginal examination and indicate in the case record form whether they considered that the retained placenta was adherens, trapped or partial accreta. These decisions were recorded prior to manual removal so that they were not influenced by the operative findings.

b. Ultrasound and Doppler assessment

The GE Voluson-I SN BC02634 portable ultrasound machine was used. The following parameters were assessed and recorded in the proforma before manual removal:

An ultrasound assessment was conducted to study the relationship between the placenta and myometrium. This allowed a visual assessment as to whether the placenta was trapped, accreta or adherens.

The uterine artery Doppler parameters were assessed as follows. Trans-abdominally, the probe was placed longitudinally in the lower lateral quadrant of the abdomen and
angled medially. Colour flowmetry mapping was used to identify the uterine artery where it was seen crossing the external iliac artery. The sample volume was placed 1 cm downstream from this crossover point. If the uterine artery branched before the intersection of the external iliac artery, the sample volume was placed on it just before the uterine artery bifurcation. The same process was repeated on the contralateral site of the uterine artery. The levels of bilateral uterine artery Doppler resistance index (RI) was measured and recorded in the proforma.

Recording of video images: When time allowed, video clips were recorded to be used later to assess the intrarater and interrater variation in the use of ultrasound to diagnose retained placenta subtypes (see Chapter 5).

c. Quantitative assessment

The aim was to conduct the USS assessment just before transfer to the theatre so that the manual removal was performed within 30 minutes of the ultrasound assessment. (If greater than 30 minutes elapsed before manual removal, ultrasound findings may not remain accurate as the retained placenta subtype may have changed due to further spontaneous separation, for example, from placenta adherens to trapped placenta). A clinical assessment in the theatre was made based upon whether the placenta was still attached or separated from the decidua/myometrium, and the ease or difficulty of the separation. The clinician who performed the manual removal was asked to indicate the subtype of the placenta, whether adherens, trapped or partial accreta. These findings at manual removal were used as the gold standard against which the clinical and ultrasound/Doppler assessments before manual removal were judged.

3.2.4 Sample size calculation

A formal sample size calculation was not conducted as this was the first study of its kind and so there was no prior data. We, therefore, had time-limited recruitment, with the aim of recruiting as many as possible in one year. In the Release trial previously conducted in the same hospital with similar inclusion criteria (Weeks et al, 2010), we had recruited one participant per week or 52 in one year. However, in that study, there were multiple recruiters, whereas in this one, only one person was
recruiting. It was therefore expected we would recruit around 30 women in the 12 months available.

3.2.5 Statistical analysis

Computerised data analysis was conducted with SSPS statistical software (IBM Corp., 2013) with the support from Dr. S. Lane from the department of biostatistics, University of Liverpool. Diagnostic accuracy was described using estimates of kappa agreement and confidence intervals. The kappa values were interpreted according to Cohen (1960) as follows: (Cohen 1960)

- A poor agreement = or less than 0.20
- A fair agreement = or between 0.20 to 0.40
- A moderate agreement = or between 0.40 to 0.60
- A good agreement = or between 0.60 to 0.80
- A very good agreement = or between 0.80 to 1.00

The outcome measures of this study were the ultrasound diagnoses of the retained placenta subtypes, namely: placenta adherens, partial accreta and trapped placenta, as judged by the gold standard i.e. the findings at manual removal of the placenta.

The ethical approval for this study was granted by the Liverpool Central NRES Committee (REC Reference 15/NW/0484) and research and development of the Liverpool Women's Hospital NHS Trust. The study was sponsored by the University of Liverpool (Sponsor Reference UoL 001134). It was publicly publicised with posters (appendices 5 and 10), and informed consent was comprehensively obtained with consent forms and information sheets (appendices 8 and 9).
3.3 Results

3.3.1 Number of women recruited

There were 145 women with a third stage between 30 minutes (with active management) and 60 minutes (with expectant management). Of these, 34 were recruited. However, five women spontaneously delivered while awaiting theatre, which prevented a clinical diagnosis of the manual removal, and so 29 ultimately underwent manual removal of the placenta (figure 3.1).

Out of 34 women recruited, 33 had active 3rd stage (97.1%) and one did not (2.9%).

Ultrasound video clips were recorded from 9 of the women to be used for validation (see chapter 4). The distribution of diagnoses of retained placenta subtypes at various assessments is reflected in table 3.1.
Figure 3.1 Calculation of recruited women who underwent the MROP
The exact agreement between clinical assessment and the gold standard was low at 0.48 with a weak kappa of 0.02 compared to the agreement of 0.83 for ultrasound assessment without Doppler with good kappa of 0.67, and the agreement of 0.97 for ultrasound assessment with Doppler with a very good kappa of 0.93 (tables 3.2, 3.3 and 3.4).

<table>
<thead>
<tr>
<th></th>
<th>Diagnosis at Manual Removal (Gold Standard)</th>
<th>Clinical Diagnosis By Palpation</th>
<th>Grey-scale Ultrasound without Doppler</th>
<th>Grey-scale Ultrasound with Doppler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta Adherens</td>
<td>20</td>
<td>15</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Trapped Placenta</td>
<td>7</td>
<td>13</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Partial Accreta</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 3.1 Distribution of diagnoses of retained placenta subtypes at various assessments
Table 3.2 Agreement of clinical assessment by palpation with the gold standard

<table>
<thead>
<tr>
<th>Clinical Assessment By Palpation</th>
<th>Surgical Diagnosis (Gold Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherens</td>
</tr>
<tr>
<td>Adherens</td>
<td>10</td>
</tr>
<tr>
<td>Accreta</td>
<td>1</td>
</tr>
<tr>
<td>Trapped</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

- **Exact Agreement**: 0.48
- **Chance Agreement**: 0.47
- **Kappa**: 0.02
- **Standard error**: 0.11
- **95% Confidence interval**: -0.23 0.24

Table 3.3 Agreement of ultrasound assessment without Doppler with the gold standard

<table>
<thead>
<tr>
<th>Ultrasound Assessment Without Doppler</th>
<th>Surgical Diagnosis (Gold Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherens</td>
</tr>
<tr>
<td>Adherens</td>
<td>16</td>
</tr>
<tr>
<td>Accreta</td>
<td>0</td>
</tr>
<tr>
<td>Trapped</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

- **Exact Agreement**: 0.83
- **Chance Agreement**: 0.48
- **Kappa**: 0.67
- **Standard error**: 0.08
- **95% Confidence interval**: 0.51 0.83
Table 3.4 Agreement of ultrasound assessment with Doppler against the gold standard

<table>
<thead>
<tr>
<th>Ultrasound Assessment With Doppler</th>
<th>Surgical Diagnosis (Gold Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherens</td>
</tr>
<tr>
<td>Adherens</td>
<td>20</td>
</tr>
<tr>
<td>Accreta</td>
<td>0</td>
</tr>
<tr>
<td>Trapped</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td><strong>Exact Agreement</strong></td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Chance Agreement</strong></td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Kappa</strong></td>
<td><strong>0.93</strong></td>
</tr>
<tr>
<td><strong>Standard error</strong></td>
<td>0.04</td>
</tr>
<tr>
<td><strong>95% Confidence interval</strong></td>
<td>0.85</td>
</tr>
</tbody>
</table>

3.3.3 Ultrasound images

Some of the ultrasound images of the three retained placenta subtypes obtained in this study are reflected in Figure 3.2, along with diagram illustrations.
Figure 3.2 Ultrasound images of retained placenta subtypes

1 Partial accreta  2 Placenta adherens  3 Trapped placenta
Figure 3.3 Uterine artery Doppler in placenta adherens
3.3.4 Uterine artery resistance index (UARI)

Figure 3.4 Use of uterine artery resistance index to diagnose subtypes of the RP. A box and whisker plot represent the minimum quartile, first quartile, median, third quartile and maximum quartile ranges. A box plot alone displays the first quartile to the third quartile.
The lower the uterine artery resistance index below 0.60, the more likely the diagnosis of partial accreta, followed by retained placenta adherens. Alternatively, resistance indices higher than 0.60 were associated with the determination of the trapped placenta (Figure 3.3 and 3.4).

3.4 Discussion

To our knowledge, this is the first study to assess the role of ultrasound in diagnosing the subtypes of the retained placenta. We have shown that assessment using a combination of ultrasound and Doppler significantly surpasses clinical assessment in the diagnosis of the retained placenta subtypes. They are as good as the findings at the manual removal of placenta (the gold standard). Therefore, they may be used to rapidly and reliably diagnose subtypes of the retained placenta before manual removal.

We found gross placental imaging using ultrasound combined with uterine artery resistance indices to be the diagnostic parameters for the retained placenta subtypes in our study.

Strengths of this study include it being a prospective study where the PI (AA) and the clinicians were blinded to the gold standard outcomes. Both clinical and ultrasound assessments were completed and documented before manual removal.

There are also weaknesses for this study. The sample size in this study for the three subtypes (especially partial accreta) is low with only 2 for partial accreta. A lack of standard descriptions of subtypes and a lack of specialist training of the PI (AA) were additional weaknesses. However, it remains the largest study into subtypes.

Of the 29 women that had manual removal of the placenta, 20 (69%) had placenta adherens, 7 (24%) trapped and 2 (7%) partial accreta. This compares with findings of the Release trial (Weeks et al, 2010).
Although the PI (AA) was specifically trained for this research, he did not hold a higher qualification in ultrasonography. However, this mimics clinical practice where the vast majority of retained placentas are dealt with by trainees. We therefore believe that it was appropriate that a trained clinical fellow conducted this study. For, if a trained research fellow can satisfactorily use the ultrasound, then registrars and other junior clinicians will also be able and available to do so as part of their routine duties.

Our recruitment size of 34 women falls below the estimated 52/year. This is because of the rigorous criteria for safety which were followed to ensure that the lives of women that bled heavily were not compromised by delaying their transfer to theatre to allow for scanning. Compared to the Release trial in 2010, the incidence of retained placenta was also slightly lower during the period of this study at 1.5% compared to 2% (Weeks et al. 2010).

The level of agreement with the gold standard suggests that retained placenta subtypes are more accurately diagnosed by the use of combined ultrasound and Doppler than by clinical assessment alone. The very good kappa agreement of 0.93 (CI 0.85-1.00) for combined ultrasound and Doppler indicates they are as good as the gold standard (the manual removal of placenta). This contrasts with clinical assessment which has a poor kappa agreement of 0.02 (CI -0.23 to 0.24). The use of ultrasound alone without Doppler is also better than clinical assessment with a good kappa of 0.67 (CI 0.51-0.83) although less than ultrasound and Doppler combined. This is because there are no specific and reliable diagnostic features for accurate clinical diagnosis, whereas ultrasound has diagnostic features of myometrial thickness, Doppler parameters and retroplacental blood flow as described by Krapp, Weeks, Hull and Resnik (Hull & Resnik 2010; Krapp et al. 2007; Weeks 2008).

The cut off for the uterine artery resistance index (RI) which we used is 0.6, with lower readings indicating placenta adherens and accreta where there is persistent blood flow and higher readings pointing at trapped placenta with no blood flow.
Similarly, we considered a cut off of 1.45 for the uterine artery pulsatility index as done by other researchers (Gomez et al. 2008).

From 24 weeks gestation, the resistance index performs better than pulsatility index in the presence of bilateral notches (Albaiges, 2003). Therefore, we chose to use the resistance index in case any of our women had bilateral notches.

To our knowledge, there is no previous ultrasound study on retained placenta subtypes to compare our results with.

The lower the uterine artery resistance index, below 0.6, the more likely the diagnosis of partial accreta, followed by retained placenta adherens. Alternatively, the resistance indices higher than 0.6 were associated with a diagnosis of trapped placenta (see figures 4.3 and 4.4.). Thus, the use of the Doppler uterine artery resistance index raised the diagnostic accuracy of the ultrasound from a kappa of 0.67 up to a kappa of 0.93. This is line with findings of other researchers. (Chou, Ho & Lee 2000; Hull & Resnik 2010; Krapp et al. 2007; Weeks 2008). However, this is the first study to assess all the three subtypes simultaneously. Some of the retained placenta may have changed from placenta adherens to trapped or accreta between the USS and manual removal in theatre. Note in the results that there were five ‘wrong’ USS diagnoses – but all were thought to be adherens on USS and changed, none the other way. So, it may be that the USS was actually 100% accurate at the time. These parameters also require skill and time to measure them. This may compromise the safety of a woman who is bleeding heavily. Therefore, they have to be omitted when a woman is bleeding heavily to expedite the operation of manual removal in the interest of safety, as done in this study.

Despite the fact that the use of the Doppler uterine artery resistance index raises the diagnostic accuracy of retained placenta subtypes as in our study, we acknowledge that there is no true control group with regards to RI as we have no examples of these values in postnatal women without RP.
If ultrasound could be used to diagnose the subtypes of the retained placenta before manual removal, it would have the following implications:

- Two out of three subtypes, i.e. placenta adherens and trapped placenta, could be treatable by simple medicines alone, or by persistent control cord traction as hypothesized by (Weeks 2008). This would reduce the number of women who otherwise would ultimately undergo the more invasive and risky surgical operation of manual removal. It would also improve the outcome of medical management, since this would be offered as type-specific therapy.
- Women with partial accreta would be expeditiously taken to theatre for manual removal of placenta if they were at higher risk of developing severe postpartum haemorrhage because of delayed surgery. A senior surgeon would need to be involved as they are more difficult to remove.
- It would also validate the outcome of clinical trials on the retained placenta, as they could be conducted on a type-specific basis.
- It would also avoid the iatrogenic complications of giving medicines blindly irrespective of the retained placenta subtypes. For example, excessive bleeding because of the inadvertent administration of glycerine trinitrate to women with placenta adherens or partial accreta, or aggravating cervical constriction with an oxytocic in women with trapped placenta.

Although it would be advantageous clinically to use ultrasound on the labour ward to diagnose the subtypes, it might raise the cost of care considering the extra training of clinicians that would be required. However, this extra cost would be offset by the saving that would arise from reduced numbers of manual removal of placenta operations in theatre.

The use of ultrasound and Doppler can be added to clinical trials to undertake type-specific studies on retained placenta and explore whether the outcomes would be more valid. For example, the GOT IT trial could be repeated by targeting only women with trapped placenta (GOT IT, 2018). GOT IT was a nation-wide study in
the UK which examined whether glycerine trinitrate (GTN) could effectively treat retained placenta. The recently produced results were found to be ineffective. This might have been because GTN was offered to women with retained placenta irrespective of its subtype, whereas it is expected to treat only trapped placenta and not placenta adherens or partial accreta. If ultrasound had been used to identify and target trapped placetas in that study, the outcome might have been positive.

Other researchers, with better ultrasound skills and equipment, could conduct a similar but longer prospective cohort study, to increase the recruitment rate and strength of the study.

### 3.5 Conclusion

Ultrasound and Doppler significantly surpass clinical assessment in the diagnosis of the retained placenta subtypes. They are as good as the findings at the manual removal of the placenta (the gold standard).

Therefore, they could be used to rapidly and reliably diagnose the subtypes of the retained placenta before manual removal.

This rapid and reliable diagnosis of retained placenta subtypes would pave the way for safer, type-specific and effective medical therapy to be developed and offered in the future.

However, given the smaller sample size in this study, a more extensive prospective study is recommended.
Chapter 4 Intra-observer variation in USS assessment of retained placenta subtypes
4.1 Background

If retained placenta subtypes (RP) are rapidly and accurately diagnosed, they can be treated promptly to prevent potential maternal mortality from life-threatening complications of haemorrhage and sepsis. Any method used to determine this diagnosis should provide a consistent diagnosis when used by different experienced clinicians in obstetrics.

In a normal clinical setting, Weeks (2008) indicated that the differentiation between a placenta that has an area of accreta and those that are trapped or adherent is not easy unless ultrasound is used. Trapped placenta and adherens are easier to diagnose - it is the partial accreta that is difficult. Generally, the diagnosis by clinical examination is inadequate (Weeks 2008). There are no definitive diagnostic features on clinical examination. Without ultrasound, only clinical clues obtained by palpation of the uterine fundus suggest the type of placenta, as discussed in chapter 1.

However, as discussed in Chapter 4, the potential for deploying ultrasound and Doppler to rapidly and accurately diagnose the three retained placenta subtypes preoperatively in the delivery suite is high (Section 4.3.2). Krapp pioneered the use of greyscale and coloured Doppler ultrasonography to diagnose placenta accreta (Krapp et al. 2007). However, the authors did not explore the potential of using uterine artery Doppler ultrasound to diagnose the other retained placenta subtypes.

Ultrasound is subject to considerable inter-observer and intra-observer variability. Inter-observer variation is defined as the amount of variation between the results obtained by two or more observers that examined the same material. Intra-observer variation is defined as the amount of variation one observer experiences when examining the same material more than once (Shiel, 2020). This study examined both the intra-observer and inter-observer variation.
Sarris et al. assessed intra- and inter-observer variability in fetal ultrasound measurements and Verburg et al. conducted an intra- and inter-observer reproducibility study of early fetal growth parameters (Sarris & Altman 2012) and (Verburg et al. 2008). However, those studies were not on subtypes of the retained placenta. Furthermore, they were not done by junior doctors in an emergency situation, with a portable ultrasound scan. The potential for inaccuracy is higher in those situations. Hence, the primary goal of this study is to examine the ability of ultrasound to detect RP subtypes as well as explore both intra-observer and inter variability among the practitioners in the ultrasound assessment of these subtypes.

4.2 Methods

As detailed in Chapter 4, an ultrasound study was conducted among women who had retained placenta to determine whether the subtypes of RP could be diagnosed prior to manual removal of placenta. The PI (AA) assessed this diagnosis in real-time during emergency scanning in the delivery suite. Video clips of this ultrasound scan were recorded for approximately 15 seconds on the integral digital video recorder. These were downloaded and assessed by experts in obstetric ultrasound to obtain their respective diagnoses of the three known subtypes of RP, i.e. placenta adherens, trapped placenta and partial accreta. To assess intra-observer variation, the assessment of the videos was conducted by the ten experts twice, with an interval between assessments ranging from 6 hours to two weeks. The ten experts had received advanced training in ultrasound and were working at Liverpool Women's Hospital. The experts included senior specialist registrars and consultant obstetricians. Ultrasound videos included sagittal and transverse views of the uterus. These views were mixed and not systematic due to the speed required in taking ultrasound videos in an emergency situation without delaying the transfer of the women to theatre for manual removal operation. The video clips were anonymised before they were presented to the experts for assessment to maintain confidentiality.
The experts were required to examine the video clips and report their diagnosis of the subtype of retained placenta. The gold standard was the diagnosis of RP types at manual removal of the placenta.

4.2.1 Analysis of data

The data generated from the diagnoses made by the experts were analysed on SPSS software. The frequencies of the agreements and disagreements of the diagnoses with the gold standard were summarised initially. This was followed by calculation of the Cohen kappa coefficient (including 95% confidence intervals) to determine the level of agreement between the ultrasound diagnosis and gold standard, whilst adjusting for agreement by chance alone according to the following corresponding coefficients: 0, by chance alone, <0.21 poor, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial and >0.80 very good intra-observer agreement (Cohen, 1960). Pre-designed Excel sheets were created to calculate kappa statistics applicable for multiple raters.

For small data as in our study, exact agreements were used to compare the variability.

4.2.2 Ethics approval

Ethics approval was obtained from Liverpool Central Research Ethics Committee (REC Reference (15/NW/0484) and was sponsored by the University of Liverpool (Sponsor reference UoL001134). Consent was obtained from participants (appendix 8).
4.3 Results

A total of 9 ultrasound video clips were assessed by ten experts in obstetric ultrasound. The mean length of the video clips was 15.9 seconds (range 6.5-18.0), and the interval between the first and second diagnosis ranged from 6 hours to two weeks. Details are shown in Table 5.1.

Out of the nine retained placenta videos, 6 were placenta adherens, two trapped placentas and one partial accreta, based on the diagnosis reached at manual removal of the placenta by the clinicians who were regarded as the gold standard.

4.3.1 Intra-observer variation

Whereas 1 indicated a correct diagnosis, 0 reflected a wrong diagnosis (table 4.1), the agreements were reflected as 1 1 or 0 0 and disagreements 1 0 or 0 1 for first and second diagnosis of every expert and/or video clip. Expert one (E1), for example agreed with himself on 6 out of 9 (67%) on two separate diagnoses. Thus, the intra-observer variation, expressed as percentage agreement gained per every expert was high for consultants and senior registrars (67%-100%) but low for the junior research registrar (33%) (Table 4.1).
Initially, we were looking for consistency among the experts in the ultrasound diagnosis of the retained placenta subtypes irrespective of whether the diagnosis was correct or incorrect. Therefore, the high agreements and related percentages achieved did not necessarily reflect the correctness in the diagnosis achieved Table 4.1.

Table 4.1 Intra-observer variation in ultrasound diagnosis of retained placenta [E =Expert; 1=Right; 0=Wrong; C=Consultant; SR=Senior registrar; RR=Research registrar].
<table>
<thead>
<tr>
<th>Expert</th>
<th>Both right</th>
<th>Both wrong</th>
<th>Right first-Wrong second</th>
<th>Wrong first-Right second</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expert 1</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Expert 2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Expert 3</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Expert 4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Expert 5</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Expert 6</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Expert 7</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Expert 8</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Expert 9</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Expert 10</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>44 (48.9%)</td>
<td>22 (24.4%)</td>
<td>17 (18.9%)</td>
<td>7 (7.8%)</td>
<td>90 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Both right</th>
<th>Both wrong</th>
<th>Right first-Wrong second</th>
<th>Wrong first-Right second</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherens</td>
<td>12 (20.0%)</td>
<td>10 (16.7%)</td>
<td>6 (10.0%)</td>
<td>32 (53.3%)</td>
<td>60 (100%)</td>
</tr>
<tr>
<td>Trapped</td>
<td>4 (20%)</td>
<td>5 (25%)</td>
<td>1 (5%)</td>
<td>10 (50%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>P. accreta</td>
<td>0 (0%)</td>
<td>7 (70%)</td>
<td>1 (10%)</td>
<td>2 (20%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

Table 4.2 Overall distribution of correct and incorrect video diagnoses

4.3.2 Ability of ultrasound to detect RP subtypes

Next, we checked the ability of the experts to correctly diagnose the RP subtypes from the ultrasound video clips. The correct diagnoses on both occasions were most commonly seen in placenta adherens and trapped placenta, with just over half being correctly identified on both occasions (53%, 50% respectively). Only two experts (20%) diagnosed partial accreta correctly on both occasions. In other words, partial
accreta was misdiagnosed twice by 7 out of 10 experts (70%). As a note of caution, this is one video, and the misdiagnosis could be due to poor video quality (table 4.2).

The quality of the videos was poor. They were taken in haste before women were transferred to the theatre for the manual removal of the placenta. Others were interrupted by the onset of severe bleeding and had to be curtailed for safety. Thus, the duration of the videos was variable.

There were additional explanations for the poor quality of the videos: a lower-quality portable scanner used by a clinical research fellow without a higher qualification in ultrasonography. Hence, the gain was generally suboptimal, and the videos were not taken uniformly.

4.3.3 Inter-observer variation
4.3.3 a) Placenta adherens
The 6 cases of placenta adherens were diagnosed by ten experts on two separate occasions, giving diagnostic episodes of 20 per placenta adherens video and a total of 120 for all 6. We found the exact agreement for interobserver variation in the video diagnosis of the retained placenta adherens to be 0.70 (table 4.3).
### Placenta adherens

<table>
<thead>
<tr>
<th></th>
<th>Correct</th>
<th>Not correct</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First assessment</strong></td>
<td>32</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td><strong>Exact agreement</strong></td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chance agreement</strong></td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kappa</strong></td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Standard error</strong></td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>95% Confidence interval</strong></td>
<td>0.18</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3 Inter-observer variation in video diagnosis for placenta adherens.
4.3.3 b) Trapped placenta

The two cases of trapped placenta were diagnosed twice by each of the ten experts, giving diagnostic episodes of 20 per trapped placenta video and a total of 40 for both. The exact agreement for inter-observer variation in the study for the trapped placenta was 0.75 (table 4.4).

**Trapped placenta**

<table>
<thead>
<tr>
<th>Second assessment</th>
<th>Correct</th>
<th>Not correct</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>First assessment</td>
<td>10</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Exact agreement</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chance agreement</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>0.26</td>
<td>0.70</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4 Inter-observer variation in video diagnosis for trapped placenta.

4.3.3 c) Partial accreta

All ten experts attempted to diagnose the single case of partial accreta on two separate occasions, giving a total of 2 diagnostic episodes per expert and 20 overall for the ten experts. The exact agreement for interobserver variation was found to be 0.90 (table 4.5).
Partial accreta

<table>
<thead>
<tr>
<th>Second assessment</th>
<th>Correct</th>
<th>Not correct</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>First assessment</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Exact agreement</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chance agreement</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>0.31</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5 Interobserver variation in video diagnosis for placenta accreta

Thus, the interobserver variation in the diagnosis of all the three known subtypes of the retained placenta (adherens, trapped and partial accreta) was high at exact agreements of 0.70, 0.75 and 0.90 respectively (tables 4.3, 4.4 and 4.5).

4.4 Discussion

A total of 9 ultrasound video clips were assessed by ten experts in obstetric ultrasound to explore both intra-observer and inter-observer variation in USS assessment of RP subtypes as well as the ability to correctly diagnose these subtypes from the ultrasound video clips. Out of the nine video clips, 6 were placenta adherens, two trapped placentas and one partial accreta.

The intra-observer variation of the individual experts ranged from 67 to 100 percentage agreements among senior research registrars and consultants, while it was low at 33% for a junior research registrar. This probably reflects different levels of experience in the interpretation of ultrasound.
The study also showed that the interobserver consistency in the ultrasound assessment of the retained placenta subtypes were high being 0.70, 0.75 and 0.90 for placenta adherens, trapped placenta and partial accreta respectively. This suggests that experts in obstetric ultrasound have a good to excellent inter-observer correlation for differentiating among three subtypes of the retained placenta on recorded video clips (Cohen 1960).

The results further explain the high level of difficulty in the correct ultrasound diagnosis of partial accreta (20.0%) compared to placenta adherens (53.3%) and trapped placenta (50.0%) respectively, considering the percentages obtained for both right diagnoses. This is in line with the findings in Chapter 3, where there was only 50% exact agreement between USS and surgical diagnosis; this accuracy increased to 100% when Doppler results were added.

The strengths of this study are that it is prospective, it used one of the highest quality portable ultrasound scanners available, with images viewed by experts practising in a tertiary centre. It also appears to be the first to assess intra-observer and interobserver variation in the ultrasound assessment of the subtypes of the retained placenta. We could not identify a previous study that had similarly assessed these variations on the subtypes of RP. Although this may be a strength for this study, it deprives us of comparative data.

A weakness of our study was that the variable level of experience among the ten experts, including a research registrar, senior specialist registrars and consultants (Table 4.1). Thus, the accuracy in the diagnosis was higher for consultants and senior registrars compared to research registrar (% agreement of 0.7-0.9 versus 0.3). This may reflect the difference in the level of experience in ultrasound. However, the research registrar had a % agreement value far less than the 0.88 obtained by the PI (AA). This is probably because the PI conducted the US assessments in real-time, allowing to explore the moving image and direct the probe according to his own wishes rather than relying on someone else's short video clips.
The study had other limitations. The small sample size of only nine videos was clearly one of them. The time-lapse between the first and second diagnosis was also inconsistent among the experts. It varied from 6 hours to two weeks, depending on the availability of the raters for the second diagnosis. We recognise that the timings may have an effect on bias. The experts that reattempted the diagnosis following a shorter interval may have recalled some of their earlier diagnoses. Ideally, the maximum interval should be less than two weeks between comparisons for consistency (Menga et al. 2016).

The fact that ultrasound is an interactive procedure and does not translate to pre-recorded video images was another weakness. The original plan had been to obtain a standardised 3D image, but this did not prove possible due to time constraints in the emergency situation. Future research using 3D which the clinician can explore in real time could provide a more realistic picture.

The videos were not recorded uniformly, and their duration was different. The chances of achieving a correct diagnosis may be better with longer than shorter videos. The technical quality of the videos was also suboptimal at times because of the emergency setting, need for haste and the portable scanner. High-quality videos may have improved the prospects of obtaining the correct diagnosis. In future, it may be able to create a virtual 3D image of a woman's uterus that can subsequently be 'scanned' in real-time by an operator in a simulation of that woman's uterus. This would allow correct scanning of a 'real' uterus away from the bedside and at leisure, thus also allowing a more accurate assessment of intra and inter-observer error.

There is also a problem related to the kappa statistics in general. Kappa agreements do not differentiate between true and false diagnosis. Thus, an excellent correlation may simply reflect a consistently wrong diagnosis and not the accuracy of obtaining the correct diagnosis. It also does not take into account the accuracy of the gold standard. Yet, kappa is generally thought to be a more robust measure than simple per cent agreement, since it considers the possibility of the agreement occurring by
chance. (Landis and Koch, 1977). A discussion of diagnostic accuracy compared to the gold standard was covered in chapter 4.

Our study showed a good to excellent inter-observer variability in ultrasound assessment. This is similar to the inter-observer agreements obtained by Zosmer et al. (2017). This study focussed on morbidly adherent placentas at the antenatal period (Zosmer et al. 2017). This gave the researchers the luxury of time to carefully select the images for the sonographers to perform the scans without haste. Their sample size of 175 was larger than in this study; it was conducted on live scans; and data was analysed using Z-scores rather than Kappa, either of which can be used as indicators of variance, although the latter, which we used, is considered as the standard method.

The findings of this study may have implications for clinical practice. Our study demonstrated that experts in obstetric ultrasound have a good to excellent inter-observer correlation for differentiating among three subtypes of the retained placenta on recorded video clips, especially placenta adherens and trapped placenta. As these two subtypes have the potential to be treated with simple medicines alone, it could substantially reduce the number of women who would otherwise undergo the more invasive and risky surgical procedure of manual removal of the placenta.

Retained placentas are generally managed by registrars, many of whose ultrasound experience falls well below the level of senior registrars and consultants in this study. Therefore, they may not achieve the same level of diagnostic ability or agreement during the clinical practice as obtained in this study. However, the fact that the PI (AA), a registrar without formal USS training, was able to, suggests that they can with appropriate training.

Given the importance of the retained placenta, it might be possible in the future for NHS trusts to incorporate ultrasound diagnosis of retained placenta subtypes into the ongoing training of speciality registrars. This would provide them with relevant
skills to achieve higher agreements, as occurred with the PI (AA) in this study. Although that would come with its own cost implications, it would be cheaper than training consultants and senior ultrasonographers. However, there would be little benefit in this until effective medical management becomes available as currently, all women have manual removal.

A larger study is needed to validate our results, considering our small sample size. It would also be better for raters to use live ultrasound scans, which would omit the errors of recorded videos. However, it would be difficult for the same raters to independently scan one woman with a retained placenta and complete their processes before she is taken to theatre for manual removal. It may be simpler to increase the number of participants and use systematically recorded ultrasound video clips in a future study. As the computing power increases, it may become possible to conduct a rapid 3D scan which can later be assessed off-live.

Whereas this study assessed reliability and reproducibility, it could not determine sensitivities and specificities of the ultrasound in the diagnoses of the subtypes of the retained placenta. This would require a larger study.

4.5 Conclusion

The study demonstrates that experts in obstetric ultrasound have a good to excellent inter-observer correlation for differentiating among three subtypes of the retained placenta on recorded video clips. The intra-observer agreements were consistently high among the more experienced experts and low in the single less experienced participant.

This suggests ultrasound may be used in the future to consistently diagnose subtypes of the retained placenta and thereby allow some women to be offered type-specific medical therapies to treat retained placenta. However, the ability for ultrasound to correctly detect the retained placenta subtypes is higher in placenta adherens followed by trapped placenta but lower in partial accreta.
Chapter 5 An exploration of a possible placental cause of retro-placental myometrial relaxation
5.1 Background

Although cases of retained placenta (RP) are well known medically, the exact causes of this phenomenon have not yet been precisely identified. Of its three subtypes, placenta adherens is particularly interesting as it is usually caused by a failure of the retroplacental myometrium to contract (Weeks 2014). It has been suggested that this is because of the presence of a potent locally-acting tocolytic produced by the placenta (Weeks 2001) and (Weeks & Mirembe 2002). This is particularly likely as these cases frequently exhibit histological features of maternal under-perfusion and oxidative stress (Endler, Saltvedt & Papadogiannakis 2016).

It was hypothesised that if the exact tocolytic or inhibitor causing placenta adherens could be correctly identified, specific medical treatment might be successfully developed. This would end the current confusion over medical treatment and should be safer than manual removal of the placenta.

Multiple smooth muscle relaxants are present in the placenta, including progesterone, nitric oxide, oxytocinase and hydrogen sulphide and its derivatives, (Weeks & Mirembe 2002). Progesterone inhibits labour (Mendelson, Montalbano & Gao 2017) while the anti-progesterone mifepristone induces labour (Baev et al. 2017). Nitric oxide, which is produced in the placenta by nitric oxide synthetase, is a powerful muscle relaxant (Sladek, Magness & Conrad 1997), while exogenous administration of nitric oxide seems to relax both cervical and uterine contraction, although it induces labour when given locally to the cervix (Ledingham et al. 2000). Oxytocinase inactivates natural and synthetic oxytocin to delay the onset of parturition (Dicker & Whiley 1959). Although progesterone, progesterone derivatives and nitric oxide have all been explored by previous researchers as potential tocolytics, hydrogen sulphide (H$_2$S) has not. H$_2$S is a volatile soluble gas produced from cysteine by 3 enzymes: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercapropyrurate sulfurtransferase (3-MPST). CSE is normally expressed in smooth muscle (vascular and non-vascular) and pancreatic cells (Kaneko et al. 2006) and (Hosoki, Matsuki & Kimura 1997); CBS is mainly
found in the cytosol cells of the liver, brain, kidney and nervous system (Abe & Kimura 1996) and (Enokido et al. 2005); 3-MPST is mainly expressed in the mitochondria cells of the brain (Hu et al. 2011). In a recent study, H$_2$S was found to be significantly produced in human placenta via CBS and CSE, but not rat placenta (Patel et al. 2009). The reason for this variation was not clear.

Several studies have examined the expression of H$_2$S producing enzymes in the placenta, and have identified the presence of CSE and CBS within the placenta (Patel et al, 2009; Wang et al, 2013; Endler et al., 2014). Furthermore, Endler et al. found CSE to be reduced in retained placentas. However, none of these studies considered individual retained placenta subtypes, and none examined the three H$_2$S-producing enzymes simultaneously (Patel et al. 2009); (Wang et al. 2013) and (Endler et al. 2014).

Endogenous H$_2$S is required for healthy placental vasculature as described in section 1.4.1 and a decrease in CSE/ H$_2$S activity may contribute to the pathogenesis of pre-eclampsia (Wang et al. 2013). In this study, the plasma levels of H$_2$S were significantly decreased in women with pre-eclampsia (P<0.01) compared to women without pre-eclampsia. As pre-eclampsia is associated with placenta adherens (Endler et al. 2014), a decrease in H$_2$S activity may similarly contribute to the pathogenesis of the retained placenta. Specifically, as a proangiogenic vasodilator and a potential inhibitor of the myometrium, it is suggested that increased levels of endogenous H$_2$S may contribute to persistent placental inhibition of myometrial contraction leading to retained placenta adherens.

H$_2$S is a volatile gas that is difficult to measure directly in a lab. However, its potential/likely levels can be indirectly assessed via the expression or activity of the enzymes by which it is produced; CSE, 3MPS and CBS (Alexander et al. 2015). The aim of this study, therefore, was to investigate the expression of these three H$_2$S - producing enzymes in placenta adherens, using immunohistochemistry (IHC), to explore whether H$_2$S plays a role in the aetiology of placenta adherens.
For decades, IHC has been an invaluable tool for the detection, localization, and quantification of antigens in preserved tissue for research and diagnostic purposes (Brandtzaeg 1998). This study used immunohistochemistry antibodies recommended and previously validated by (Fitzgibbons et al. 2014).

5.2 Methods

5.2.1 Ethical approval:
This study was conducted at the Liverpool Women’s Hospital (LWH) in the UK. Approval was obtained from the LWH tissue bank research laboratory (LWHTB), the Liverpool Central NRES committee (REC Reference 15/NW/0484) and the NHS research and development unit of the LWH NHS trust. It was sponsored by the University of Liverpool (Sponsor Reference UofL001134). Written informed consent was gained from every woman prior to the collection of the placentas (appendices 8 and 9).

5.2.2 Recruitment

Identification of participants
Women who had retained or normal placentas at the main delivery suite of LWH were identified. These women were then approached and informed consent gained. Donated placentas were then collected with the full consent of the participants. In effect, the participants consisted of two groups with two separate consent/recruitment processes: one through the retained placenta project for retained placentas, the other via the LWHTB for normal (non-retained) placentas (see below).

Groups
Women with retained placenta adherens were the study group, whereas those with normal, non-retained placentas constituted the control group. The inclusion and exclusion criteria were as follows:
Women with retained placenta adherens: inclusion criteria

- Retained placenta for at least 30 minutes (or 60 minutes in physiological management)
- Written informed consent
- Estimated gestation of at least 24 weeks
- Over 18 years of age, or over 16 and ‘Gillick competent’.

5.2.3 Women with retained placenta adherens: exclusion criteria

- Significant vaginal bleeding or maternal haemodynamic instability (pulse > 100bpm or systolic blood pressure <100mmHg) necessitating immediate placental removal
- Stillborn baby
- Women requiring English translation
- Women without time for ultrasound assessment before going to theatre, or for whom ultrasound would delay normal management.

5.2.4 Women with non-retained placentas

The inclusion and exclusion criteria were the same as the above, with the following exceptions:

- Placenta delivered within 30 minutes of active management or 60 minutes of physiological management of the third stage of labour.
- No ultrasound assessment.
- Normal vaginal births in LWH.

The control samples thus collected were subjected to the same process of IHC as the RPs in LWHTB. All the biopsies were assessed for expression of hydrogen sulphide enzymes (Appendices 2, 3 and 4), using the standard operating procedures (SOPs) of the LWHTB.
5.2.5 Sample size

At the outset it was hoped to be able to compare the levels of H₂S producing enzymes in samples of 30 placentas from women with retained placentas (ten trapped, ten adherent and ten accreta) with placental samples from ten normal controls. It was not possible however because of recruitment difficulties (see chapter 4). By the time of the analysis there were only ten samples from adherent placentas and ten from controls.

5.2.6 Placenta sampling

A sampling kit was prepared (for both study and control samples) with all the materials necessary for tissue collection: sterile forceps, dissecting scissors, formaldehyde 37% and sterile phosphate buffered solution (PBS).

To prevent tissue degradation all the placentas (study and controls) were freshly collected and transferred to the laboratory as soon as possible after delivery (ideally within 30 minutes), for processing. The time between delivery and start of laboratory processing was recorded. This ranged from 60 minutes to 24 hours from birth to placenta sampling. Local risk assessment and safety procedures were strictly followed throughout, such as use of personal protective equipment (PPE), biosafety facilities and sharps policy.

Each placenta was initially oriented on a plastic tray with the maternal aspect of the placenta uppermost. Appropriate containment and spillage prevention processes were put in place to protect staff during placental dissection. After collecting the maternal side samples, the placenta was turned over to obtain fetal side samples. From each placenta (study and control sample), or normally delivered placenta (control sample), four biopsies 2-3 cm in size were taken from the placenta using tissue forceps and dissecting scissors, after consulting the LWHTB and Professor Judith Bulmer (Appendix 2). The following sites were sampled:
1. Maternal side (three samples): two were biopsied from any normal-looking sites of the placenta and one from any area with an abnormal appearance.

2. Fetal side (one sample): the biopsy was taken from midway between the cord insertion and the upper periphery of the placenta.

The decidual part of the maternal samples, and the chorionic part of the fetal sample, were retained to allow for correct orientation.

Each sample was immediately placed in separate universal bottles containing 4% methanol for fixation.

5.2.7 Tissue processing

Slides from ten controls and ten studies were mixed and processed with an enzyme simultaneously to minimise bias. As there were three biopsies from each placental sample, a total of 60 slides were processed for every enzyme, making a total of 180 for all the three enzymes (CSE, CBS and 3-MPST). These were grouped into nine batches of 20 slides each and run over a period of two days for each batch, after baking the slides overnight.

After the placental biopsy, samples for immunostaining were obtained. They were placed directly into 10 ml of neutral buffered formalin (NBF; Sigma-Aldrich, UK) contained within a universal tube. NBF consists of approximately 4% formaldehyde, which preserves the tissues as close as possible to their original biological state. Prior to processing, the samples were incubated for at least 24 hours in NBF. The Shandon Citadel 1000 machine was used to dehydrate and impregnate these tissue samples with paraffin.

Briefly, formalin fixed tissue was dehydrated, cleared, then impregnated with paraffin wax using the automated Shandon Citadel 1000 processing machine. The samples were processed overnight for a period of 18 hours 45 minutes. Samples were then embedded in paraffin moulds using the Shandon Histocentre in order to
allow thin sections to be prepared. 3 µm sections were prepared using a rotary microtome and floated onto 3-aminopropyltriethoxysilane (APES) coated glass slides. The slides were then labelled, allowed to air-dry, then filed in labelled plastic slide cases.

Preparation of slides prior to IHC:
Appropriate tissue samples were selected and labelled with the experiment criterion: date, sample code, H2S enzyme type and concentration of antibody used. Thereafter, the slides were transferred to a metal staining rack and baked in a heated chamber at 60°C for one hour or at 37°C overnight. This ensured the removal of residual moisture between the tissue section and slide that might impede adhesion. At this juncture, the utmost care was taken not to ‘overbake’ the slides which can cause degradation of proteins within the tissue and lead to divergent staining outcomes.
<table>
<thead>
<tr>
<th>Antibody</th>
<th>CBS</th>
<th>CSE</th>
<th>3-MPST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplier</td>
<td>Abcam</td>
<td>Abnova</td>
<td>Santa Cruz</td>
</tr>
<tr>
<td>Antigen retrieval solution</td>
<td>10 mM Tris- 1mM EDTA, pH 8</td>
<td>0.1 M Citrate, pH 6</td>
<td>0.1M Citrate, 0.2 pH 6</td>
</tr>
<tr>
<td>Time in pressure cooker</td>
<td>3 minutes</td>
<td>4 minutes</td>
<td>4 minutes</td>
</tr>
<tr>
<td>Company</td>
<td>Abcam</td>
<td>Abnova</td>
<td>Santa Cruz</td>
</tr>
<tr>
<td>Catalogue number</td>
<td>Ab-54883</td>
<td>H00001491-MOIA</td>
<td>Sc-376168</td>
</tr>
<tr>
<td>Concentration</td>
<td>1:300</td>
<td>1:3000</td>
<td>1:400</td>
</tr>
<tr>
<td>Positive control</td>
<td>Liver</td>
<td>Liver</td>
<td>Mouse gut</td>
</tr>
<tr>
<td>Negative control</td>
<td>Mouse IgG 1:10000</td>
<td>Mouse IgG 1:10000</td>
<td>Mouse IgG 1:10000</td>
</tr>
<tr>
<td>Incubation period</td>
<td>4°C overnight</td>
<td>4°C overnight</td>
<td>4°C overnight</td>
</tr>
<tr>
<td>Mono/poly clonal</td>
<td>Monoclonal</td>
<td>Monoclonal</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone</td>
<td>3E1 (ab 124276)</td>
<td>4E1-1B7</td>
<td>H-11</td>
</tr>
<tr>
<td>Suggested dilutions</td>
<td>5 µg/ml</td>
<td>0.5 mg/ml</td>
<td>200 µg/ml</td>
</tr>
</tbody>
</table>

Table 5.1. Conditions for the H$_2$S enzymes
6.2.8 Immunohistochemistry

Immunohistochemistry (IHC) is a process used to detect specific antigens within tissue samples. It utilizes primary antibodies that specifically bind to the antigens. Then a secondary antibody labelled with horseradish peroxidase, is added. This produces a brown coloured product, which allows the antibody to be visualized and localized using light microscopy. In 1966, Nakane described a method of antigen detection in tissue using an antibody conjugated to an enzyme (horseradish peroxidase) and utilized a colorimetric substrate that could be detected by light microscopy, which is the theoretical basis of most modern tissue-based immunohistochemical assays (Nakane and Pierce, 1966). This chapter will focus on detection of H$_2$S enzymes in formalin-fixed, paraffin-embedded placental tissues.

The conditions for the three antibodies (CBS, CSE and 3-MPST) were initially identified as reflected in table 5.1. These were optimised by the LWHTB.

**De-paraffinisation**

The tissue sections were de-paraffinised in xylene, then rehydrated in descending grades of alcohol to ensure successful adherence and tissue penetration prior to heat-based antigen retrieval. The de-paraffinisation regime followed is indicated in table 5.2. After the samples were dewaxed, they were placed in metal racks and emerged in a staining dish filled with tap water.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Minimum period in solution in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene 1</td>
<td>10</td>
</tr>
<tr>
<td>Xylene 2</td>
<td>10</td>
</tr>
<tr>
<td>Ethanol 100%-1</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol 100%-2</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol 90%</td>
<td>1</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5.2. Deparaffinisation regime

**Epitope (antigen) recovery**
During de-paraffinisation, antigens undergo chemical reactions which could result in masking of their sites, leading to false negative results. Therefore, the epitomes were unmasked with a heated solution of citric acid or Tris-EDTA. The citrate-based buffer (pH 6) was used for CSE+ 3-MPST, while the Tris-EDTA buffer (pH 9) was for CBS. Both buffer solutions were pre-prepared as 10x stock solutions and diluted with distilled water: citrate with 150mls buffer and 1350mls distilled water; Tris-EDTA with 300mls buffer and 1200mls distilled water. A pressure cooker was used to heat the samples at full pressure, a process referred to as a heat-induced epitome retrieval (HIER). The duration of heating varied from 3-4 minutes according to the enzyme antibody as reflected in table 3. This heating breaks protein cross-links, thereby unmasking antigens and epitopes in the tissue sections. When the alarm clock sounded at the end of 3-4 minutes, the slides were immersed into the cooker and its lid locked. At the sound of a continuous hiss, the pressure was instantly released and the cooker and slides rapidly cooled with tap water in an adjacent sink. Care was taken to direct the water from the tap away from the slides. The cooled slides were then returned to the staining dishes filled with water, and transferred into tris buffered saline (TBS) for 5 minutes.

**Blocking endogenous peroxidase activity**

Any remaining endogenous peroxidase activity was then quenched by incubating the specimen for 10 minutes with a peroxidase block known as hydrogen peroxide in a concentration of hydrogen peroxide (30%) and water (70%). This reduces background staining from non-specific binding which could mask the target antigen.

**Staining of primary and secondary antibodies**

The slides were washed and tissue samples encircled with DAKO hydrophobic marker pen (DAKO, Cambridge, UK). Primary antibodies (CSE, CBS or 3-MPST) were applied, 50µl per tissue site. They were then kept in a humidified chamber (to prevent samples from drying out) and incubated overnight at 4°C.

An HRP labelled polymer conjugated with secondary antibodies was then applied. The secondary antibodies bind with primary antibodies and make them stand out
more clearly. The polymer did not contain avidin or biotin, thereby preventing non-specific staining resulting from endogenous avidin-biotin activity. The primary staining process was completed by incubation with 3’3-Diaminobenzindine (DAB) + substrate – chromogen, which resulted in a brown-coloured precipitate. Further secondary staining was applied using haematoxylin. This was to provide contrast and make the architecture of the tissue stand out.

Dehydration and mounting of slides
The slides were then dehydrated using alcohol washes of increasing concentrations and cleared using a xylene detergent. Next, they were mounted, cover-slipped and left to dry in a hood before they were finally examined and scored under a microscope.

Controls
One positively staining sample (MN001) was cut and included in every run. These were biopsies from the first donated normal placenta, taken from the normal site of the maternal surface. They were used as positive internal controls to compare the intensity of staining in each run. If the intensity of staining was consistent throughout all runs, the samples were ultimately scored.

Every run also included at least one negative IgG control in one slide to ensure specificity of staining in each run. These were randomly selected slides into which mouse IgG at a concentration of 1: 10 000 was added to prevent staining and provide a negative control. External tissue positive control sections were also included in a run for each antibody (liver for CSE and CBS; mouse gut for 3-MPST). This allowed an accurate assessment of staining to be made in comparison with a tissue in which the staining pattern was well established.

Image capture

High resolution images of the stained placenta sections were captured utilising the Nikon eclipse microscope and camera head (Nikon, Tokyo, Japan). The focus in this
A semi-quantitative modified quick score

The immunohistochemistry (IHC) slides were scored using a modified “quick score” method (Schiessl et al. 2009) as applied by Professor Judith Bulmer, taking into account both intensity of staining (0 = negative, 1 = weak, 2 = moderate and 3 = strong), and the percentage or proportion of cells for each staining intensity (1 = 0-25%, 2 = 26-50%, 3 = 51-75 % and 4 >75%). For this quick score, the maximum score achievable by the use of this method is 12 (sum of % x intensity of score) and possible combinations range from 0-12 as reflected in table 5.3.

The samples were thoroughly examined on a lower power at x4 or x10 initially to examine the slides thoroughly to obtain an overall picture of staining. Next, the samples were visualised at a higher magnification x20 and images captured at x40.

<table>
<thead>
<tr>
<th></th>
<th>0 (Negative)</th>
<th>1 (Weak)</th>
<th>2 (Moderate)</th>
<th>3 (Strong)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25%</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>25-50%</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>51-75%</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 5.3 Quick score table, showing possible combinations.

A total of 180 slides were scored: 60 per enzyme (CSE, CBS and 3-MPST). The fourth biopsy sample for RNA-Later was omitted as it was not required for immunohistochemistry. A mean and level of significance was later calculated statistically for each enzyme (section 3.12).

Validation and counter-scoring
The scoring method for the slides was validated and the slides themselves were counter-scored and validated.

5.2.9 Statistical analysis

The two-tailed student t-test was used to directly compare the means of the two pairs of variables per every enzyme. P values < 0.05 were considered to be significant between retained placenta adherens and non-retained placentas.

The women participants in this study were identified by the midwives and consented by the author. The donated placentas were collected and sampled by the author. The tissue processing, including preparation of slides, described here were carried out by the laboratory technicians and laboratory scientist J Drury. The immunohistochemistry was conducted by the author using SOP 12 of LWHTB. The scoring method for the slides was validated by Professor Judith Bulmer (University of Newcastle). She taught the author the scoring method on 6 slides (2 for each enzyme). Then, she validated this scoring on a further 12 slides in which she had complete agreement with the researcher. The slides themselves were counter-scored by Dr Sofia Makrydima (trained with the author by a paediatric placental pathologist, then trained by the author on the semi-quantitative quick score). She counter-scored 120 slides (66%) and only disagreed with the author in 7 out of 120 slides (6%). High resolution images were captured by the author. The statistical analysis of the data was then conducted by the author under the close supervision of Dr Steven Lane (university statistician) and Professor Andrew Weeks (academic supervisor).

5.3 Results

As stated in table 3 in chapter 4 there were only two trapped placentas and one partial accreta alongside the ten normal placentas. Only placenta adherens therefore had sufficient numbers recruited for the study.
All the participants were women who had achieved normal, non-instrumental live vaginal deliveries at more than 24 weeks gestation, as stipulated by the inclusion criteria.

The immunohistochemistry for the expression of H$_2$S enzymes was limited to ten consecutive placenta adherens alongside ten normal non-retained placentas.

The means and standard deviations for the demographics of the two groups are reflected in table 5.4.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Retained placenta (placenta adherens)</th>
<th>Normal placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years and SD)</td>
<td>31.2 (2.2)</td>
<td>29.2 (2.5)</td>
</tr>
<tr>
<td>Parity (mean and SD)</td>
<td>2.1 (0.4)</td>
<td>0.8 (0.4)</td>
</tr>
<tr>
<td>Gestation (weeks and SD)</td>
<td>34.3 (1.6)</td>
<td>37.7 (0.7)</td>
</tr>
<tr>
<td>Newborn centile (mean and SD)</td>
<td>47.3 (2.1)</td>
<td>57.3 (6.1)</td>
</tr>
<tr>
<td>Placenta removal-to-sample fixing (minutes and SD)</td>
<td>62.8 (6.3)</td>
<td>63.5 (6.1)</td>
</tr>
</tbody>
</table>

Table 5.4 Demographics of participants (mean and standard deviations)
<table>
<thead>
<tr>
<th>Slide code</th>
<th>CSE Normal placenta</th>
<th>CSE Placenta adherens</th>
<th>CBS Normal placenta</th>
<th>CBS Placenta adherens</th>
<th>3-MPST Normal placenta</th>
<th>3-MPST Placenta adherens</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>4.7</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>02</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>03</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>04</td>
<td>5.3</td>
<td>1</td>
<td>6.0</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>05</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>06</td>
<td>4.7</td>
<td>1</td>
<td>6.0</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>07</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.7</td>
<td>1</td>
</tr>
<tr>
<td>08</td>
<td>4.7</td>
<td>1</td>
<td>6.0</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>09</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>5.3</td>
<td>1</td>
<td>5.3</td>
<td>0.7</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>5.1</td>
<td>1</td>
<td>5.5</td>
<td>0.7</td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>t-value</td>
<td>45.0</td>
<td></td>
<td>17.4</td>
<td></td>
<td>43.9</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.00001</td>
<td></td>
<td>&lt; 0.00001</td>
<td></td>
<td>&lt; 0.00001</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5 Expression of H$_2$S enzymes in placenta adherens

Values given in table 5.5 are mean quick scores with a possible range of 0-12 (see text for details) For each slide three biopsies were taken from a normal placenta and three from a retained placenta adherens. The mean of the three values is shown in the table.

The expression of all the three H$_2$S producing enzymes (CSE, CBS and 3-MPST) was significantly reduced (p-value < 0.0001) in retained placenta adherens compared to normal spontaneously delivered placenta (Table 5.5 and Figures 5.1, 5.2 and 5.3).
The mean scores were 1.0 for placenta adherens versus 5.1 for normal placentas for CSE; 0.7 versus 5.5 for CBS and 1.0 versus 4.1 for 3-MPST (Table 5.5).
Figure 5.1 Expression of CSE hydrogen sulphide in placenta adherens
Figure 5.2 Expression of CBS hydrogen sulphide in placenta adherens
Figure 5.3 Expression of 3-MPST hydrogen sulphide enzyme in placenta adherens
5.4 Discussion

This study is the first to assess all three H$_2$S producing enzymes in retained placentas. Other previous studies have studied only one or two of the three.

Wang et al also found a reduction of CSE in pre-eclamptic placentas compared to normal non-eclamptic placentas (Wang et al. 2013). Although their study is similar to this study, it was on pre-eclamptic not retained placentas.

Endler et al also found a reduction in CSE in pre-eclamptic retained placenta adherens compared to normal non-eclamptic placentas and suggested a link between eclampsia and retained placenta adherens (Endler et al. 2014). Our study of placenta adherens had only ten cases, while theirs was double (20). However, their study was country-wide whereas this study was a single hospital base.

Other studies have also detected similar expressions of CBS and CSE enzymes in placentas (Patel et al. 2009); (Holwerda et al. 2012) and (Cindrova-Davies et al. 2013). However, these studies neither assessed retained placentas nor examined all 3 enzymes simultaneously.

Vyas et al found that CSE and CBS expression was increased in term and labour placentas compared to pre-term and non-labouring placentas, similar to their findings for the myometrium (Vyas 2016). However, our study did not involve the myometrium, and their study was not of retained placentas.

No previous study (including this study) has thus so far shown high levels of a hydrogen-sulphide producing enzyme in a retained placenta adherens. Thus, speculation that levels of H$_2$S maybe high in retained placenta adherens, as a potential inhibitor of retro-placental myometrial contraction, is not reflected in this study or others.
This study benefits from being a prospective and lab-based study. Women with retained and normal placentas were recruited prospectively as they gave birth in LWH during the study period. This minimised the risk of bias and provided stronger and more reliable evidence than retrospective studies.

Immunohistochemistry is a well-established method, suitable to this study. It is relatively inexpensive and routinely available, compared to other methods such as polymerase chain reaction (PCR) and metabolomics.

Adequate training was also provided in laboratory techniques, thus ensuring the quality and validity of this study.

The quick score seems to be less prone to variation than the H-score because the observer evaluates the whole section, estimating an overall impression of intensity when scoring, and not just ten representatives but randomly selected high-power fields (Dere 1995). The modified semi-quantitative quick score used in this study was adopted from (Schiessl et al. 2009), as modified and applied by Bulmer in a series of placental studies (Bulmer J, University of Newcastle). That a leading expert on placenta studies validated the data is an important strength. This is over and above the close and thorough supervision of the study itself in the LWHTB, a university research laboratory in LWH with experience of IHC.

However, this study has limitations. We acknowledge that there could be bias in the placental sample counting of staining as the reporter was not blinded to the knowledge of RP or non-RP. The number of participants was low and not enough participants were found to make it possible to examine trapped placentas or partial accretas. Therefore, the study fell short of its intended plan to compare all three retained placenta subtypes with normal placentas. This would have also provided controls that were matched for time post-delivery, which could not be done with non-retained controls.
The recruitment period was limited to one year for this PhD study and the incidence of the retained placenta in the UK is high, more than 2% as in other developed nations. In this study, it was 1.5%, slightly less than that of the Release study in 2010 (Weeks et al. 2010). Whilst placenta adherens is common, trapped placentas and partial acertas are comparatively rare. Furthermore, the actual sampling of placentas was started late in this study due to the need for an ethical amendment. This led to the relatively small sample size. Immunohistochemistry itself, used in this study, has inherent shortfalls: difficulty in quantification, lack of standardisation and dependency on experimental technique are among them. Future studies with higher levels of funding could consider the use of PCR or metabolomics to automate the process and provide more valid results.

Although all three H$_2$S producing enzymes were reduced in the retained placentas, 3-MPST was very much less expressed compared to CBS and CSE. The expression of CBS and CSE was also noted to be different from 3-MPST among the various cells of the placentas. This may be because cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are pyridoxal phosphate (PLP)-dependent enzymes while 3-MPST is not. CBS and CSE are also predominantly cytosolic enzymes, while 3-MPST is both cytosolic and mitochondrial (Kashfi & Olson 2013). Yet, this does not fully explain why 3-MPST is less expressed in the placenta compared to both CSE and CBS.

It is not evident why the three hydrogen sulphide enzymes were so significantly reduced in the retained placenta adherens compared to normal non-retained placentas. The interval between birth and collection of the placenta/delivery to the lab did not differ significantly between the samples as they were taken to the lab promptly. However, we acknowledge that the delay in the duration of storage in the lab to biopsy (60 minutes to 24 hours) could have affected the quality of the samples. The same applies to the period from freezing the samples to processing in the lab which varied between the samples. If these enzymes degrade with time, it could explain why they were so low in the retained placentas. The other plausible explanation may be the fact that the mean gestation for the women with the retained
placentas was pre-term at 34.3 weeks but term at 37.7 weeks for those with normal placentas.

5.4.1 Meaning of the paper

The fact that H\textsubscript{2}S is significantly less expressed in retained placenta adherens compared to spontaneously delivered placentas suggests that it may contribute to the aetiology of the retained placenta. None of the ten participants with retained placenta adherens had pre-eclampsia. Therefore, this reduction was not due to pre-eclampsia. However, the time between expulsion and storage of the placentas was lower in the controls. Therefore, one could argue that this reduction is an artefact as levels of H\textsubscript{2}S (as a gas) reduces with time post-delivery. However, the enzyme and not the gas was studied, and enzyme expression is unlikely to change remarkably in the 30-60 minutes that is the difference between the RP and the controls. Furthermore, the high level of statistical significance suggests that this reduction is valid, especially with a two-tailed t-test.

An increase in the expression of the H\textsubscript{2}S producing enzymes, not a reduction, was expected. Being a vasodilator, an increase in endogenous H\textsubscript{2}S would inhibit the contraction of the retro-placental myometrium. On the contrary, the study found a significant reduction in the expression of its enzymes. This reduction and its link to retained placenta might:

- be the effect of poor placental function, with the reduction being a secondary outcome of other conditions such as pre-eclampsia.

- originate from poor placental vascularity, which in turn leads to retained placenta through another mechanism. As H\textsubscript{2}S is responsible for normal vascularity, it may be reduced in cases of abnormal vascularity e.g. failure of trophoblastic invasion of the uterus and spiral blood vessels, which is associated with intra-uterine growth restriction and pre-eclampsia.
be because H$_2$S is part of a group of bioactive agents that attenuate myometrial contractility. Other bioactive agents could be nitric oxide, oxygenase, progesterone or progesterone derivatives, and their inhibition could overpower the effect of H$_2$S.

be a compensatory mechanism to try to prevent RP. As RP is said to be due to the failure of the contraction of the retroplacental myometrium, H$_2$S may be used up and thereby reduced in the process of trying to counteract this aetiology.

If its reduction causes RP, a treatment for retained placentas can potentially be developed in relation to H$_2$S, which may be simpler and safer than the manual removal of the placenta that is associated with surgical and anaesthetic risks.

5.4.2 Future research

More specialised studies such as polymerase chain reaction and placenta metabolomics may be used to validate these findings. With the full consent of the participants in this study, RNA samples have been frozen in the LWHTB, which can be used for this purpose if funding becomes available.

There is also a need to study the expression of H$_2$S enzymes in the other two subtypes of the retained placenta, i.e. trapped placenta and partial accreta, which was not possible in this study because of the limited number of biopsy samples. As they have different underlying aetiological mechanisms, they may show different findings to that of placenta adherens.

This study was also restricted to biopsies of the placenta. It might be useful to extend it to the retro-placenta myometrium to determine how H$_2$S is expressed there. Being the site where the expulsion of the placenta originates, biopsies of the myometrium may provide a clearer picture. Logistically however, this would be complex to do as
access to this site in the immediate postpartum period for biopsy is limited, and obtaining biopsies of this area postnatally would be highly invasive.

5.5 Conclusion

The expression of the three hydrogen-sulphide producing enzymes (CSE, CBS and 3-MPST) is significantly reduced in retained placenta adherens compared to spontaneously delivered placentas.

This suggests that placental changes may be involved in the aetiology of the retained placenta adherens. If so, it potentially opens the door for a simpler and safer medical treatment to be developed for retained placenta adherens.
Chapter 6 Final discussion and conclusion
6.1 Summary of research findings

This chapter recaps the present state of research, the overall findings of the thesis; a critique of the methods used, and finally, implications for present practice are discussed, with suggestions for future research.

Weeks and Mirembe hypothesised that there are three known subtypes of the retained placenta: placenta adherens, which is still entirely attached to the decidua; partial accreta, which is largely detached from the decidua but with a small part still deeply attached to the myometrium, and trapped placenta that is entirely detached from the decidua but trapped inside the lower segment of the uterus (figure 1.7, chapter 1) (Weeks & Mirembe 2002).

For both contemporary management and current research, a retained placenta is treated as a single entity. Generally, every woman with a retained placenta is taken to the theatre in the UK for manual removal of placenta irrespective of its subtype. Most researchers globally describe a retained placenta as a single entity.

To date, very limited knowledge has been gained on these subtypes of the retained placenta. Their clinical importance largely remains unclear as limited studies have been conducted on them. This research has focused on the clinical importance of these three subtypes. The discussion of the research findings and implications from here on is based on the reliable findings that could be made, despite the difficulties with the data which were discussed in the thesis and will be summarised separately in this chapter.

First, the thesis examined whether these subtypes of the retained placenta were separate clinical entities by exploring the aetiological factors (chapter 2) and outcomes (chapter 3) associated with them. This was to explore whether the subtypes differed in pathology only or also in outcomes and aetiological factors, as postulated by Weeks (2008). This is a question of considerable importance, for as separate
clinical entities, different modes of treatment could be more effective than the present practice of treatment as a single entity.

Based on the exclusion criteria, we examined 31120 women out of a total of 48 546 that gave birth at the Liverpool Women’s Hospital from 01 Jan 2009 to 31 Dec 2014 in a retrospective Meditech-based cohort study. The aetiological factors studied were those examined by previous researchers between 1995 and 2014. We found that the three subtypes of the retained placenta might be different in both aetiological factors and outcomes. Furthermore, the outcomes suggest that they might present different risks to the woman. (section 2.4, chapter 2).

Associations were found with age, BMI, infertility treatment, repeated births and augmentation of labour, (Adelusi et al. 1997; Nikolajsen, Lokkegaard & Bergholt 2013) confirming the findings of Weeks (Weeks 2008). However, we did not confirm the associations with caesarean section (Silver et al. 2006), ergometrine (Yuen et al. 1995) and defective placentation disorders, i.e. pre-eclampsia, stillbirth, small for gestational age (SGA), and spontaneous preterm birth, associated with placenta adherens (Endler et al. 2014). As discussed, this may relate to methodological difficulties with the data collected from the computerised systems.

The retained placenta was found to be associated with increased rates of postpartum haemorrhage and uterine inversion in line with previous research (RCOG 2009), (Onwudieguwu & Makinde 1999), (Memon, Talpur & Korejo 2011) and (Bewley 2009). The number of complications assessed in our study were less than those described in Nikolajsen et al. (2013), Endler et al. (2014), Dombrowski et al. (1995), Yuen et al. (1995), Ashwal et al. (2014), Silver et al. (2006) and hypothesized in (Weeks 2008). We did not examine sepsis as examined in Onwudiegu and Makinde (1999). Our study on outcomes associated with retained placenta subtypes found partial accreta to be associated with the highest levels of PPH (chapter 3).
We attempted to explore factors which can be used to predict the trapped placenta and failed to find significant ones. This could also be due to the poor data on the Meditec.

Next, we examined the role of ultrasound in the diagnosis of the subtypes of the retained placenta (chapter 4). Ultrasound has been used on the retained placenta as an entity as well as retained placenta tissue. However, this is the first study to assess its diagnostic role for the subtypes of the retained placenta. We examined whether ultrasound can be used to accurately and reliably diagnose the three subtypes of the retained placenta before manual removal of the placenta. We found that ultrasound examination significantly surpasses clinical assessment for this diagnosis, and it is as good as the findings at the manual removal of placenta, which is the gold standard.

To assess the reliability of ultrasound diagnosis, we compared the findings of ten experts in obstetric ultrasound using nine ultrasound video clips recorded prospectively. Our study showed that in experienced hands, obstetric ultrasound has a good to excellent intra-observer correlation for differentiating the three subtypes of the retained placenta.

The exact inhibitor that causes the failure of the contraction of the retro-placenta myometrium in placenta adherens is not known. Hydrogen sulphide is present in the myometrium and is a potent smooth muscle inhibitor. We, therefore, examined the expression of three H$_2$S enzymes in ten retained placenta adherens and ten non-retained placentas collected prospectively. We found markedly different levels of H$_2$S in placenta adherens and normally delivered placentas, suggesting that H$_2$S might contribute to the aetiology of the retained placenta adherens. However, the expression of the three hydrogen-sulphide producing enzymes (CSE, CBS and 3-MPST) was significantly reduced not increased as expected, suggesting that the underlying mechanism is more complex. See chapter 6.

6.2 Comparison with previous research
A considerable body of knowledge has already been gained on the retained placenta (chapter 1, section 1.3). Our research has attempted to explore the clinical importance of RP subtypes.

6.2.1 Placenta adherens

Weeks hypothesizes that it is associated with prematurity, dysfunctional/augmented and induced labour (2008). It is also recently found to be associated with pre-eclampsia, intrauterine growth restriction and stillbirth (Endler et al. 2014). In contrast, we found it to be significantly linked to maternal BMI, infertility treatment and augmented/dysfunctional labour. (See chapter 2).

In terms of complications, we verify that placenta adherens is associated with moderately severe PPH, which is less than that caused by partial accreta and more than that of the trapped placenta (see chapter 3). This is in line with findings of other researchers and the RCOG reviews (RCOG, 2009; Onwudiego and Makinde, 1999; Memon et al., 2011)

Krapp et al. had pioneered the use of greyscale ultrasound to diagnose a retained placenta (Kapp et al. 2007). Using greyscale alone, we accurately diagnosed placenta adherens in 9 out of 10 (90%), compared to 5 out of 7 (71%) for the trapped placenta and 1 out of 2 (50%) for partial accreta (see Table 3 in Chapter 4). This indicates that placenta adherens is more easily diagnosed by ultrasound alone compared to the other two subtypes of the RP.

The exact agreement of 0.70 for intrarater variation of placenta adherens suggests that ultrasound can be used consistently to accurately and reliably diagnose placenta adherens (see Tables 4.4 and 4.5 in chapter 4).

It has been long postulated that the pathogenesis of placenta adherens is a failure of the contraction of retro-placental myometrium due to an unknown inhibitor (Weeks, 2001, 2014; Weeks and Mirende 2002). Our finding of a significant reduction in the
expression of the three hydrogen enzymes in placenta adherens compared to normal non-retained placentas, suggests that hydrogen sulphide may be implicated, although not through a direct effect.

6.2.2 Partial accreta

Weeks (2008) hypothesized that partial accreta is associated with pre-eclampsia, small placenta, previous abortion, previous uterine injury and uterine abnormalities. In this study, however, we found it to be significantly associated with only previous retained placenta and augmented/dysfunctional labour (chapter 2).

We excluded women who had operative births, including caesarean sections in the current pregnancy. Surprisingly, we identified only two women out of 31,120 to have had previous caesarean sections. This is clearly untrue as the rates of caesarean section at Liverpool Women's Hospital is 23%, and a significant proportion of them opt for vaginal births after caesarean sections (VBACs, LWH internal audit data 2018). We believe that this error occurred because VBACs are recorded as 'free text' on the Meditech system and might not have all been recorded by the clinicians. Therefore, the instability of the Meditech data meant that we could not validate its association with previous caesarean sections as established by other researchers (Silver et al., 2006; Jauniaux et al., 2012 and Belachew et al., 2014).

Its link with dysfunctional/augmented labour, as shown in our study, is difficult to understand, as its underlying mechanism is thought to be placental invasion (Greenbaum et al. 2017). One possible mechanism is that the invasion leads to myometrial dysfunction, but we have no evidence for this. Wray (2015) asserted that there is an increased risk of subsequent stillbirth and placenta accreta (retained placenta) in cases of dysfunctional labour. She explains that stillbirth may be due to the fact that dysfunctional labours increase the risk of fetal asphyxia as well as sepsis and haemorrhage (Wray 2015).
Considering that partial accreta has been associated with uterine inversion at the third stage of labour (Bewley 2009) and (Tsivos et al. 2009) and severe blood loss, it is the riskiest subtype of retained placenta. We found that it is also the most difficult one to diagnose accurately. In our study, it could only be identified correctly by only 40% of the ultrasound experts in contrast to placenta adherens and trapped placenta which were diagnosed by 80% and 70% of the same experts respectively.

If diagnosed, women should be expeditiously taken to theatre for manual removal of the placenta to avert the risk of life-threatening postpartum haemorrhage.

6.2.3 Trapped placenta

The trapped placenta is thought to be due to loss of gravitational forces or premature cervical closure as previous studies have found it to be associated with birth in a labour bed and prophylactic ergometrine (Weeks, 2008). In our study, it was only significantly associated with augmented/dysfunctional labour, which does not fit with either cervical closure or gravitational forces. Surprisingly, our study did not find a link between ergometrine and trapped placenta, contrary to the findings of others (Yuen et al. 1995), (Begley 1990) and (Gulmezoglu et al. 2012) a Gulmezoglu, WHO CCT Trial in 2012. But our finding was similar to that of Harara et al. (Harara et al. 2011).

The trapped placenta is thought to lead to mild PPH compared to accreta and placenta adherens. This is in line with the findings of our study (chapter 3), within the limits of the shortcoming of the Meditech records. It is not expected to cause severe blood loss after it is entirely detached from the myometrium, as the myometrial contraction is not inhibited. The lower PPH rate with trapped placenta means that it is safe for it to be managed expectantly or with persistent controlled cord traction. One medical option for its management has been the smooth muscle relaxant glyceryl trinitrate (GTN). The use of GTN to treat RP has had conflicting results over the years (Bullarbo, Tjugum & Ekerhovd 2005), (Visalyaputra et al. 2011).
2011) and (BMFMS 2018). Other alternative ways of managing it are the ‘windmill’ technique of cord traction and bearing down efforts involving valasava manoeuvre.

The trapped placenta is easier to diagnose by ultrasound than partial accreta. In our study, it was correctly diagnosed in 5 out of 7 (71%) in contrast to 1 out of 2 (50%) for accreta, with a kappa coefficient of >80, which is substantial to excellent according to Cohen (Cohen 1960). However, this finding might not be true because our sample size was so small.

6.3 Strengths and limitations

6.3.1 Strengths

To our knowledge, this is the first study to focus on the subtypes of the retained placenta by examining their association with factors and outcomes, the role of ultrasound in their diagnosis, the interrater and intrarater variation of this diagnosis. This was made possible by the prospective collection of routine data for placental subtypes at the time of manual removal.

Our control group sample size for the retrospective cohort was large (31,120 out of a total of 48,546) although the sample size for our study group was small at 355. Unlike other multi-hospital studies (Endler et al. 2014); Release Trial, 2010 (Weeks et al. 2010) and (BMFMS 2018), ours was data from a single hospital. This has provided consistency of diagnosis, in contrast to the multiple hospital studies.

6.3.2 Limitations

Our study has shortcomings. First and foremost, there were significant problems with the routine data collected through the Meditech computer system. Extracting full data from the Meditech records proved to be challenging. Some of the vital records were either not recorded at all or incorrectly recorded. There were two main problems:
1. One problem is inaccuracies in data entry (e.g. entering 1000 rather than 100 – some can be detected (e.g. if the age is put at 400 rather than 40), but others cannot (e.g. blood loss of 4000 rather than 400, or previous PPH ‘yes’ rather than ‘no’). Others may be errors of omission (e.g. a woman’s pre-eclampsia may not be mentioned in the free text).

2. The other problem is the use of free-text fields rather than asking specific closed questions.

Other database studies have had similar problems. Lliodromiti and Smith G et al. (2017) for example conducted a large population-based linkage study of 979,912 term singleton pregnancies to explore the link between birth weight and stillbirth and infant death and neonatal morbidity (Lliodromiti. S. 2017). Although this study was successful in verifying the link, it also faced similar problems of missing data. They were unable to fully customise centile charts because they lacked data on maternal weight and ethnicity. Hence, they recommended that replication of the analysis with fully customised centiles accounting for ethnicity was warranted.

Endler, M et al. (Endler et al. 2014) also conducted a large population-based cohort of primiparous women in Sweden with singleton vaginal deliveries between 1997 and 2009 at 32–41 weeks of gestation (n=386,607) to evaluate whether defective placentation disorders were associated with the retained placenta. Like our study, this nation-wide epidemiological study also lacked some data. The authors admitted that the birth register included data on only 98% of deliveries, not 100%. However, unlike our study, additional data, which was missing from their birth register, such as the mother’s country of birth, was obtained from other linked records, such as the register of population and population changes. Our study was only limited to records on the Meditech.

There is no doubt that inadequate/inaccurate data on Meditech was a major limitation for this study on aetiological factors and outcomes. However, the move for fully electronic patient records provides an opportunity to ensure that they have all the information needed for large scale research in the future, based on the knowledge
gained in our study about the problems with Meditech. New systems of data collection should ensure that these errors are avoided by utilising ‘specific data’ recording and avoiding ‘free text’. But how this can be resolved without a vast amount of work for the overworked maternity staff, is challenging.

A formal comparison of Meditech derived data and data collected in hand-held notes is needed to check reliability. Opportunities to do this exist where large clinical studies have taken place in the trust, and the recorded data can be compared.

6.4 Implications and future research

6.4.1 Theoretical implications

The contemporary treatment of retained placenta is to take all women to the theatre (irrespective of its subtypes) for the invasive procedure of manual removal of placenta, a procedure associated with surgical and anaesthetic risks. The finding that the retained placenta subtypes results can be reliably and accurately diagnosed with ultrasound opens the possibility of offering type-specific treatments instead. When diagnosed, 2 out of 3 subtypes (adherens and trapped) could be treated with simple techniques or medicines.

If the trapped placenta is caused by contraction of the cervix, a muscle relaxant such as GTN is expected to relax the cervix and lead to its expulsion. Unfortunately, this simple medicine is found to be ineffective for the treatment of retained placenta (Abdel-Aleem, Abdel-Aleem & Shaaban 2015; Denison et al. 2017). However, these researchers did not specifically target women with trapped placenta but offered GTN to all women with retained placenta whatever the subtype. Perhaps, the outcome could have been positive had they first diagnosed trapped placentas using ultrasound before administering the GTN. Presumably, if there was a decrease in MROP for the trapped placenta, then it would have been seen as an overall reduction in MROP, unless there was an increase in the need for MROP in those with placenta adherens (as would be suggested by our knowledge of the underlying pathology). If
this happened then, the overall results would show no difference, but would mean a reduction in MROP for those with the trapped placenta and an increase in those with placenta adherens.

Potential medical treatments for placenta adherens include umbilical oxytocin, umbilical prostaglandins and systemic oxytocics. None of them has yet been proved to work in a controlled trial or systemic review, and the search for effective medical treatment for the retained placenta adherens continues. The first approach is to identify the specific inhibitor causing the retained placenta adherens. Then, its antidote can be explored, developed and tried as its medical treatment.

If successful, type-specific treatments can be identified, then only around a third of women with the retained placenta (those with partial accreta) would need to be expeditiously taken to theatre for surgical management to avert the risk of severe haemorrhage. This new approach could cut down the number of women requiring surgery with probable cost-saving implications.

The use of ultrasound has already been established in the labour wards. It can now be extended to diagnose the subtypes of the retained placenta. This will entail further training of clinicians on how to diagnose them using ultrasound. This training should be incorporated into the existing RCOG courses and curriculum on ultrasound, such as the RCOG basic ultrasound course.

6.4.2 Practical implications of the research
An algorithm that may be used to diagnose the subtypes of the retained placenta is reflected in Figure 6.1
Figure 6.1 A flow chart for ultrasound diagnosis of retained placenta subtypes.
If the uterus is empty, then this cannot be placenta accreta. If it is empty, then the placenta is trapped. If it is unclear, however, then the clinician should use uterine artery Doppler blood flow; if blood flow is seen, it is placenta accreta. If there is no flow, it is trapped (Figure 6.1).

6.4.3 Future research

6.4.3a Direct extensions of the study

Our study offers possibilities for further exploration of the subtypes of the retained placenta. Apart from validating our study, it offers other opportunities. For, example, the frozen samples we have stored in the lab is an opportunity for another researcher to extend our study to more specialized studies such as PCR and metabolomics before they are discarded. A direct extension would also be to examine the expression of hydrogen sulphide in the other two subtypes (partial accreta and trapped placenta) to compare with our findings for placenta adherens. More women will have to be recruited to get adequate sample sizes for all the subtypes.

Considering the shortcomings of Meditech and small sample sizes, a larger study, after proper records are established on the Meditech or supplemented with paper-records, might provide a better accurate assessment. This could be designed as a prospective cohort study.

A trial of subtype-specific treatment following ultrasound diagnosis is needed to compare with the current approach of manual removal for all subtypes. This would show which approach is better in terms of safety, cost and effectiveness. If required, our findings on the role of ultrasound in the diagnosis of the subtypes of the retained placenta should first be validated by other researchers before embarking on this comparative study. However, umbilical oxytocin is unlikely to be effective against adherens. The Release study (Weeks et al., 2010) collected data on the retained placenta subtypes at manual removal; there was no difference in the % of each in the placebo or umbilical oxytocin arms.
6.4.3b Broader issues to be covered in future work

Many previous studies on medical treatment of the retained placenta have fallen through. Example of drugs that have been explored includes various forms of glycerine trinitrate, umbilical oxytocin injection, normal saline and others. A possible explanation for the failure of these trials is the fact that the medicines were administered blindly, irrespective of the diagnosis of the subtypes of the retained placenta. It was only in the Release study that subtypes were considered by the clinician conducting the MROP, and they found no difference in proportions between the placebo and intervention arm. Now that our study has shown the diagnostic role of ultrasound, these studies could be repeated, with the addition of ultrasound, so as to select the appropriate intervention for the subtype. In this way, the treatments could be offered to the relevant subtypes of the retained placenta with a higher probability of success. For instance, if glycerine trinitrate had been offered specifically to women with trapped placentas, following ultrasound diagnosis, the outcome of the recent UK-wide GOT-IT trial might have shown that it was effective. Likewise, the previous Release trial of oxytocin injection (Weeks et al. 2010) might have been positive if women with placenta adherens were correctly identified and specifically targeted.

6.5 Conclusion

We have explored the clinical importance of the three subtypes of the retained placenta. The data suggests that they may be separate clinical entities that should be treated differently. We also know that the subgroups can be distinguished prior to treatment using ultrasound. Furthermore, we have shown that the expression of three hydrogen sulphide -producing enzymes is reduced in placenta adherens compared to the non-retained placenta. We hope this work will encourage clinicians and future researchers to use the three subtypes in future research to explore ways to reduce the morbidity and mortality of this common pregnancy complication.
Appendices
Appendix 1 Liverpool Women’s Tissue Bank: Collection and storage

Liverpool Women’s Research Tissue Bank
Collection and storage of tissue

Protocol

The aim of the Liverpool Women’s Research Tissue Bank (LWRTB) is to collect and store tissue, surplus to diagnostic requirement, from patients undergoing a caesarean section, normal delivery or gynaecological procedures. This will enable the LWRTB to build an extensive and valuable collection for use by research groups investigating pregnancy, labour and its complications as well as other common (e.g. endometriosis) and life threatening (endometrial cancer) complications affecting the female reproductive system. The informed consent process employed is to obtain samples for broad research purposes, it is not project specific.

IDENTIFICATION AND RECRUITMENT OF PATIENTS

Tissue is donated to the LWRTB using an informed consent process. Potential donors would be identified by the clinical teams responsible for their care. Most patients would be approached by a member of the clinical team during a pre-operative clinic, whilst some will be approached at an outpatient clinic, or at the time between admission and surgery. Potential donors will be given the LWRTB Patient Information Leaflet and Consent Form and a verbal description of what consenting to donate will entail. The patient will have the opportunity to ask any questions relating to the use of their donated tissue and read the Patient Information Leaflet, which they may keep. Patients will be made aware that they are also consenting to allow LWRTB staff access to medical records and other data relating to their clinical information. This is to allow meaningful clinical research so samples can be linked to relevant clinical and pathological data. The patients would then be asked if they would be willing to donate tissue. If they agree, they are asked to sign and date the Consent Form which will be countersigned by the consenting individual. The
consent form is in three parts; one part is kept in the Tissue Bank as a record of informed consent, one is kept in the patient medical records and one copy is given to the patient to keep. It will be made clear that if patients are unwilling to donate, the standard of clinical care they receive will not be affected in any way. Consent will only be sought by individuals trained to take consent for research purposes. Only approved versions of the Consent Form and Patient Information Leaflet may be used, which will have been approved by the Research Ethics Committee. Changes to this consent form can only be made through direct consultation with the Research Ethics Committee.

COLLECTION OF SAMPLES

Surgical/theatre samples: On the day of surgery/delivery, theatre staff will be made aware of patients who have consented to the LWRTB. Resected specimens would be placed into 10% formalin or a physiological solution by theatre staff. LWRTB staff would either be notified by phone that a specimen is ready for collection, or will have checked the theatre schedule and be aware of the approximate time when a sample would be ready for collection. The specimen should be collected immediately from specimen reception point in theatres. Specimens would only be collected if all paperwork is present, complete and corresponds to the specimen. Specimens would then be brought to the Centre for Women’s Health Research laboratory room 0618 and booked in.

Non-surgical samples: One the day of the clinic, clinical staff would notify the LWRTB staff of potential tissue donors, following which the LWRTB staff will prepare a ‘sample collection box’ containing the appropriate containers and solutions along with crushed ice for transportation. LWRTB staff would be notified by telephone that a specimen is ready for collection together with the clinic location. The specimen would be collected immediately, having checked that all paperwork is present and correct, and transported to the Centre for Women’s Health Research laboratory, Room 0618 and booked in.
All samples entering the laboratory are logged in the sample log binder located next to the incubator in Room 0618.

All samples would require the completion of the LWRTB Sample Reception Form. The top portion is removed and attached to the consent form. The clinical information and LWRTB Sample Reception Form are forwarded to the LWRTB staff for data entry onto the Laboratory Samples Database which is stored securely on an NHS server with limited access and password protection. Following data entry, all paperwork is stored securely in a locked filing cabinet in Room 0631 which has a keycode entry system.

PROCESSING OF SAMPLES

The processing and storage of samples for the LWRTB is detailed in SOP 33. Laboratory specific SOPs for individual steps/procedures are also in place (SOP3, SOP4, SOP5 & SOP22). In summary, blood samples for plasma/serum are centrifuged and aliquoted in labelled tubes according to SOP 22. Whole blood is aliquoted and stored frozen for DNA extraction and hormone analysis. Urine samples are aliquoted and stored frozen. RNA samples are stored overnight at 4°C prior to the removal of the solution and being frozen. Tissue biopsies are processed for freezing or paraffin embedding according to SOP 03. Following at least 24 hours fixation in NBF, tissues are processed and embedded in paraffin wax prior to storage according to SOP 04. Haematoxylin and eosin (H&E) staining is performed on all biopsies in order to assess the cycle stage of normal endometrium, grade and type of endometrial cancer and appropriate orientation of the tissues according to SOP 13. Pathology reports are accessed where available and checked against the findings from the research biopsy. The above information is also recorded on the LWRTB database.

STORAGE OF SAMPLES
All frozen samples are stored in freezer 3 at -80°C in Room 0630. The location and time of freezing should be recorded manually on the Freezer log sheet, LWRTB Sample Reception Form and on the LWRTB database. The freezer is constantly monitored by the Tscan system, with alerts automatically notifying of any deviation from the preset temperature range. FFPE samples (formalin fixed paraffin embedded) are stored in the block storage cabinets in room 0625.

The number of samples obtained and storage location should be logged onto the LWRTB Sample Reception Form and the LWRTB database.

The primary data which will include minimal patient identifiable data as well as relevant clinical information, will be kept on a password protected database. This data is stored on an NHS server available only to LWRTB staff in the laboratory. Samples from each donor are assigned a unique identifier number which is used to identify samples. The LWRTB team will update the database periodically with relevant information from the patient medical records.

All data released to researchers along with samples is released in an anonymised form; no patient identifiable data is released. Only the LWRTB will be able to link individual samples to individual donors.

RELEASE OF SAMPLES

Samples can be released to researchers working in the academic and NHS sectors. All applications will be considered by the LWRTB Management Group to view the scientific merit of the proposed study. Informal applications are encouraged to determine whether the LWRTB has the available samples and whether the proposed project falls within the LWRTB’s remit. The aims and purpose of the study, along with a plan of investigation and study, background and rationale for using LWRTB samples must be stated. The source of funding for the project, to cover any costs for the LWRTB material or processing must also be stated. It is important to assess whether the project falls under the LWRTB’s informed consent process.
In addition, researchers must have ethical approval for their proposed study, which has to be obtained from a NHS Research Ethics Committee. Only when the Management Group have approved a project which has received ethical approval can samples and data be released. A Materials Transfers Agreement may need to be signed between the LWRTB and the researcher. The terms of the Material Transfer Agreement state the conditions concerning the release, use, distribution and return of samples.

All material released is logged onto the main LWRTB database as being released, as well as on the Requests Database which logs all material sent out to researchers.

REFERENCES

SOP3  Sample reception processing tissue biopsies, Version 2.0, May 2014
SOP4  Tissue Processing, Version 1.3, May 2014
SOP5: Embedding Samples on the Shandon Histocentre 3, Version 11.3, May 2014
SOP22 Sample Reception: Processing Blood Samples, Version 3.0 May 2014
SOP33 Liverpool Women’s Research Tissue Bank: Patient identification, sample acquisition, sample reception and processing Sample Reception and storage, Version 2.0, May 2014
Appendix 2 Placental Sampling for Hydrogen Sulphide Enzymes

Placenta Sampling for Hydrogen Sulphide Enzymes

Version 4a Dated 25 January 2016

1.0 BACKGROUND

Recently, endogenous hydrogen sulphide (H\textsubscript{2}S) is found to be required for healthy placental vasculature; a decrease in cystathionine γ-lyase activity may contribute to the pathogenesis of preeclampsia (Wang et al. 2013) and retained placenta (RP) is found to be associated with pre-eclampsia (Endler et al. 2014). The level of this activity has not been assessed in relation to the retained placenta (RP). The expression of all three hydrogen sulphide-producing synthases (Cystathionine γ-lyase, 3-mercapropionate sulfurtransferase, Cystathionine β-synthase) may be quantified in the RP using PCR with the view to assessing whether these can be used as markers for the subtypes of the RP. Up to 40 placentas will be sampled from labouring women as reflected in Figure 1 to compare the levels of H\textsubscript{2}S enzymes.
Classification of Placentas that will be sampled Figure 2.1
2.0 PURPOSE
To describe the process for the collection of placenta samples

3.0 ASSOCIATED DOCUMENTS
Retained Placenta Study (RPS) Part II Protocol

4.0 RESPONSIBILITY
It is the responsibility of the Principal Investigator for the RPS Part II to obtain biopsies from the retained placentas and submit them for processing in the Research Laboratory in the Liverpool Women’s Hospital.

5.0 SAMPLING KIT
A sampling kit will be provided with all the materials necessary for tissue collection.

Each kit will contain
Forceps x1
Dissecting scissors x1

The following materials will need to be provided locally
Formaldehyde 37% aq. Solution
Sterile Phosphate Buffered Solution (PBS)

6.0 TISSUE COLLECTION

To prevent tissue degradation the placenta should be collected and transferred to the laboratory as soon as possible after delivery, ideally within 30 minutes, for processing (the time between delivery and start of laboratory processing will be recorded).

Local risk assessment and safety procedures should be followed throughout, such as use of Personal protective equipment (PPE), biosafety facilities and sharps policy.

The placenta should be oriented on a plastic tray with the maternal aspect of the placenta uppermost (Fig. 2). The process of placental dissection can be
messy and appropriate containment and spillage prevention processes should be in place to protect staff.

Correct Orientation with Maternal Side Up (Figure 2).

Placental Sampling

4 biopsies will be taken from a retained placenta (study sample), or normally delivered placenta (control sample), from the following sites of the placenta:

1. Maternal Side-three samples: 1 normal looking site, 1 abnormal looking site (fibrotic etc.) and 1 any other site (Figure 3). The first 2 are for immunohistochemistry and the last one is to be divided into RNAlater® one and Frozen one for PCR. The frozen one will be taken straight into the freezer. It will not be snap-frozen using nitrogen sulphide to minimise the risk of a student doing that alone, after working hours.
2. Foetal side-one sample - Midway between cord insertion and upper periphery
3. The decidual part of the maternal samples and chorionic part of the foetal sample should not be sliced off. They should be left to allow for correct orientation, i.e. to differentiate between maternal and foetal samples.
3 biopsy sites from the maternal side (Figure 3).

Maternal side sample incision (Fig. 3).
Cut square block first as shown

Sample thickness and size (Figure 3)

Each sample should be of full thickness and 2-3cm³ size as shown in Fig. 4
Washing sample in PBS (Fig. 4).
Wash the sample three times in sterile phosphate buffered saline (PBS) to remove maternal blood contamination (Fig. 5). After washing the sample in ice-cold PBS x3, leave it in PBS whilst other samples are removed from the placenta.
Washing samples in PBS (Figure 4)

Fetal side sample removal (Fig. 5). (Use an image showing fetal side)
This is the same procedure independent of position. Avoid areas of frank fibrosis.

Removal of foetal side sample (Figure 5))

7.0 TISSUE PREPARATION

Dissection of sample into 5mm blocks (Fig. 6).

The third maternal sample is divided into two smaller parts for preservation: one for RNALater® and the other for direct freezing.
Cutting Third Maternal Sample into Two (Figure 6)

Samples are prepared for freezing (Fig. 7).

A single sample of tissue is placed into each Eppendorf.

Collect tissue as above and transfer 3 x 5-10mm³ pieces to a labelled cryovial as either “Maternal Normal”, “Maternal Abnormal” or “Maternal Other” and “Fetal” with the study ID number (x3; Fig. 10). Each cryovial should contain only one fragment of tissue.

Cryovials should be kept on ice during the procedure and for transport to storage – do not allow the sample to warm to room temperature.
Freeze samples in cryovials by speedily taking them into the freezer and store at -80°.

RNALater® tissue preparation (Fig. 8).

Collect tissue as above and transfer 3 x 5-10mm3 pieces from each sample. Wash samples rapidly again (x3) in ice-cold PBS to remove blood. Transfer sample to the provided universal container containing RNA later® and ensure that the sample is thoroughly immersed by gentle shaking. Label as before (Fig.8).

Tissue Preparation for RNALater® (Figure 8).

Store samples for 24 hours at 4°C to allow RNALater® to permeate the tissue. The following day, remove the tissue sample from the solution with sterile forceps and place into a sterile cryovial. The cryovial should also be labelled to indicate that tissue has been processed with RNAlater®. Samples should then be transferred to a -80°C freezer.

Store all samples at -80°C and record the time of freezing for all samples in the Placenta Sample log Appendix 1.

RPS Placental Sample Log (Appendix 1.).
<table>
<thead>
<tr>
<th>Date of Placenta collection (DD/MM/YYYY)</th>
<th>Subject ID</th>
<th>Collection Time Point</th>
<th>Specimen Type</th>
<th>Freeze time</th>
<th>Long term Storage -80°C</th>
<th>Cryo number &amp; sample location</th>
<th>Freezer Location Reference Rack shelf freezer</th>
<th>PI Initials</th>
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</table>

8.0 TISSUE ANALYSIS

The placental samples are analysed by the PI using immunohistochemistry and polymerase chain reaction to detect and record the levels of the 3 enzymes of hydrogen sulphide.
Appendix 3 Cutting Paraffin Sections

STANDARD OPERATING PROCEDURE

<table>
<thead>
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<th>Number</th>
<th>06</th>
<th>Version &amp; Issue</th>
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<tr>
<td>Title</td>
<td>CUTTING PARAFFIN SECTIONS</td>
<td>Author</td>
<td>Jo Drury &amp; Lisa Heathcote</td>
</tr>
<tr>
<td>Date</td>
<td>21/5/2007</td>
<td>Approved</td>
<td>Dr Siobhan Quenby</td>
</tr>
<tr>
<td>Date</td>
<td>23/5/2007</td>
<td>Last Reviewed</td>
<td>November 2012</td>
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BACKGROUND

Preparation of 3-5 micron thick sections from wax-embedded tissue is a commonly used histological technique. This SOP will describe how to prepare these using the Microm HM335 rotary microtome and attaching sections onto aminopropyl triethoxy silane (APES) or polysine coated slides.

HEALTH AND SAFETY REQUIREMENTS

Wear a laboratory coat and nitrile gloves. Care needs to be taken when performing this procedure because there is a risk of sharps injury from the microtome blades. Refer to the physical hazards safety circular, risk assessments and COSHH forms before starting procedure. Also ensure waste wax sections are cleaned from the floor and the general working area at the end of the procedure to prevent risk of slip/trip injuries. Place all waste tissue sections in the clinical waste bags and the used microtome blades and slides into the yellow clinical waste sharps bins. DO NOT COMMENCE WORK WITH THE MICROTOME UNTIL SUFFICIENT
TRAINING HAS BEEN PROVIDED AND AUTHORISATION HAS BEEN RECEIVED FROM LISA HEATHCOTE OR JO DRURY.

EQUIPMENT INFORMATION:

Microm HM335 rotary microtome (Microm)
Microscope slides (twin frost size 26 x 76mm-Printed “IVD CE” 90° ground edges, catalogue number MAE-1000-03P Pack of 1000) are purchased from Liverpool Women’s Hospital (NHS) purchasing department.
polysine coated slides (VWR)
Coverslips 22 x 22mm, 22 x 40mm and 22 x 50 are purchased from Liverpool Women’s Hospital (NHS) purchasing department.
Microtome blades (MB Dynasharp Catalogue Reference 3050836) (Thermo Scientific)
Forceps, paint brush, section dryer and water bath (Thermo Scientific)

SUPPLIER INFORMATION
MICROM. Microm UK Ltd., 8 Thame Park Business Centre, Leinman Road, Thame, OX9 3XA. www.microm-online.com.
Thermo Scientific. (Fisher Scientific UK Ltd) Bishop Meadow Road, Loughborough, LE11 5RG. Tel: 01509 555 500. Web: www.fisher.co.uk
VWR International Ltd. Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, LE17 4XN, England. Tel: 0800 22 33 44. uksales@uk.vwr.com

METHOD:

1. Carefully fill the water bath with distilled water ensuring the electrical connection points remain dry. Set the water bath to ~ 40°C by turning the dial to the maximum setting and set the power switch from O to I. Turn the dial down to mark 4 when the correct temperature is reached (check using a thermometer).

2. Scrape away any surplus wax from the edges of the cassette to ensure a secure fit in the holder then place the tissue block in the refrigerator. Cool the block
for at least 30 min prior to cutting (alternatively place the block in the freezer for quicker and more effective cooling).

3. Ensure that the hand wheel is locked in the upper range of the vertical movement by turning the lever downwards. Insert the specimen against the ‘fixed jaw’ of the universal cassette clamp and secure by pulling the lever to the front. Ensure that each specimen cassette is always inserted in the same orientation (horizontal placement in the universal cassette clamp with the specimen number on the left).

4. Press the reverse course feed button to move the specimen away from the knife carrier.

5. Turn the clamping lever on the blade holder to the front and swing the protective bracket forwards. Insert the feather S35 blade into the slot behind the clamping plate ensuring the blade is level on the rail (use forceps to manipulate the blade if necessary). Take care with this step!

6. Return the clamping lever to the original position to lock the blade in place. The protective bracket should also be returned to the original position when the microtome is not in use to minimise the risk of sharps injury.

7. The blade angle is pre-set to 10-12° and should not require adjustment. These settings should remain the same to ensure minimal amounts of tissue are wasted when different laboratory workers prepare sections from the same block.

8. Unlock the hand wheel by turning the lever upwards. Rotate hand wheel in a clockwise direction until the centre of the tissue block is level with the blade holder. Then lock the hand wheel by turning the lever downwards. Press the forward course feed button to move the specimen near to the blade carrier.

9. The required section and trimming thickness are set by means of a circular knob on the left of the instrument. Press the circular control knob to switch between section (FEED) and trimming (TRIM) thickness. The corresponding LED will be displayed on the operating control panel:
   
   - Green LED lights up when the FEED function is used
   - Yellow LED lights up when the TRIM function is selected.
When a new tissue block is used, select TRIM and turn the control knob until the corresponding LED is set at 10-20µm.

10. Unlock the handwheel and trim (by rotating the handwheel in a clockwise direction) until a representative amount of tissue is exposed in the tissue block.

11. Select FEED and turn the control knob until the corresponding LED is set at the required section thickness (usually 3-5µm). Rotate the handwheel until the first section is generated. Gently hold the end of the section with forceps and continue to cut until a ribbon (consisting of approximately 6 sections) is produced.

12. Carefully float the sections onto the pre-warmed water bath. Separate the sections by applying gentle pressure using the rounded edge of forceps. Select the best sections and float onto APES-coated slides. It is considered to be good laboratory practice to attach each section in the same orientation.

13. Label the frosted part of the slide with the specimen ID and thickness (using a pencil). Place slides in a rack to dry at room temperature for several hours (preferably overnight).

14. If the sections start to wrinkle during the cutting procedure, then the tissue block has become too warm and needs to be refrigerated for at least 10 minutes, or the blade needs changing (see step 16). To remove the tissue block, press the reverse course feed button until the specimen is safely away from the blade holder. Return the safety bracket to the upright position. Remove the tissue block by pressing the lever on the universal cassette clamp.

15. Remove any unused tissue sections from the water bath surface using paper towel.

16. When the microtome blade needs to be replaced, return the tissue block to the furthest position using the course feed button and lock the handwheel. Unlock the lever on the blade carrier and place the microtome blade into the yellow clinical waste sharp bin. NEVER try to remove the microtome blade when the tissue block is close to the blade edge because there is a risk of sharps injury and also the risk of damaging the tissue block.

17. When the sections have dried, place the slides into a staining rack and bake for 60 minutes in a slide drier (pre-warmed to 60°C), or 37°C overnight. Place
slides in a suitable ‘dust free’ container until required for the immunohistochemical staining procedure.

Other useful SOPs associated with 06 Procedure
Embedding samples using the Shandon Histocentre 3 (SOP 05).
Preparation of APES coated slides (SOP 07).

SOP History
Version 1.1 Updated to include changes in both Liverpool Women’s Hospital and University of Liverpool new logos. In addition, supplier changes for some of the consumables utilised have been updated.
Version 1.2 Updated prepared to include additional equipment and supplier.
Version 1.3 Prepared to update procedures following relocation of laboratories.

Appendices - Associated Documents

<table>
<thead>
<tr>
<th>Document</th>
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<tbody>
<tr>
<td>1 Risk assessment for preparation of sections</td>
<td>Risk assessment folder in the deputy departmental safety advisors office</td>
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<tr>
<td>2 Physical Hazards Code of Practice</td>
<td>Electronic copies are available on the University of Liverpool health and safety intranet: <a href="https://www.liv.ac.uk/intranet/safety/codes_of_practice/physical_hazards.pdf">https://www.liv.ac.uk/intranet/safety/codes_of_practice/physical_hazards.pdf</a></td>
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Review Date: November 2013
Appendix 4 Antigen Retrieval

STANDARD OPERATING PROCEDURE

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<tr>
<td>Jo Drury</td>
<td>November 2010</td>
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<tr>
<td>Date</td>
<td>November 2013</td>
<td>November 2012</td>
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BACKGROUND

The mechanisms of formalin-fixation are thought to be due to the formation of cross-linking bonds between tissue proteins, stabilising them to withstand subsequent processing (Mason and O’Leary 1991). Whilst preserving tissue morphology, the formation of cross-linking bonds may, however, modify the antigen’s epitopes and/or its electrostatic charges, thus producing weak or false negative staining during immunohistochemical detection of certain proteins. Restoring the epitopes enables the antigen to react with the paratope of the antibody. Methods of proteolytic pre-treatment and heat retrieval of tissue antigenicity aim to restore the avidity of the immune reaction.

Proteinase K is a type of proteolytic agent which is isolated from the saprophytic fungus Tritirachium album. It possesses a high specific activity which remains stable over a wide range of temperatures and pH values with substantially increased activity at higher temperature. It particularly unmasks antigens of proteins found in the basement membranes including laminin and collagen IV.
There are 2 commonly used heat retrieval buffers, which vary in their pH. Heat causes cross-linked protein epitopes to ‘unfold’ (in manner similar to DNA denaturation), while buffer solutions aid in maintaining the conformation of the unfolded protein. The citrate based solution is designed to break the protein cross-links, therefore unmask the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing staining intensity of antibodies. Tris-EDTA is very useful for low affinity antibodies or when tissue antigens are not intense. This buffer works well for many antibodies, but it often gives high background staining (maybe due to endogenous biotin revealed after this pre-treatment), so primary antibody can often be highly diluted.

HEALTH AND SAFETY REQUIREMENTS

Care needs to be taken when performing heat based retrieval procedures because there is a risk of burns from the hotplate and pressure cooker. There is also a risk of chemical burns from sodium hydroxide and there is a mild irritant effect from citric acid. Suitable personal protection equipment (Nitrile gloves, safety glasses and a lab coat etc.) should be worn. Refer to the physical hazards safety circular, risk assessments and COSHH forms before starting procedure. DO NOT COMMENCE WORK WITH THE PRESSURE COOKER UNTIL SUFFICIENT TRAINING HAS BEEN PROVIDED AND RECEIVED AUTHORISATION FROM LISA HEATHCOTE OR JO DRURY. THE SEAL ON THE PRESSURE COOKER SHOULD BE INSPECTED PRIOR TO EVERY USE AND REPLACED ANNUALLY.

EQUIPMENT INFORMATION:

- Proteinase K supplied by QIAGEN. QIAGEN HOUSE, Fleming Way, Crawley
  West Sussex, RH10 9NQ
• Diluent
• Humidity Chamber
• Sodium Hydroxide pellets and citric acid are supplied by VWR International Ltd, Poole, BH15 1TD England (Future orders will be obtained from Sigma or Thermo Fisher).
• Trizma base (T1503, Sigma-Aldrich Chemical Company Ltd, The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT. Tel: 0800 717181. Fax: 0800 378785. Web: www.sigma-aldrich.com)
• EDTA (disodium, dehydrate) is supplied by VWR International Ltd, Pole, BH15 1TD. Future orders will be obtained from Sigma (E4884).
• Tefal Clipso Easy 6L pressure cooker and Russell Hobbs hotplate are obtained commercially from John Lewis department store. Annual Insurance check TBC. The seal/gasket needs to be replaced annually. Gasket (part number SA793145) is obtained directly from: http://www.homeandcook.co.uk refer to section accessories/pressure cookers. 2010 price £8.50 +£1.50 delivery
• Slide racks are supplied by Raymond A Lamb Ltd. Manufacturer’s address: Units 4 & 5, Parkview industrial estate, Eastbourne, East Sussex, BN23 6QE England.

• Tris-EDTA Buffer (10mM Tris Base, 1mM EDTA Solution, pH 9.0):
  Tris Base 12.1 g  
  EDTA 3.7 g  
  Distilled water 2 l to make 5x stock solution  
  Mix to dissolve, pH is usually at 9.0. Store this solution at room temperature for 3 months or at 4°C for longer storage.

METHOD A (Proteinase K):

• Prepare a volume of proteinase K with a 1:25 concentration of the working stock that is sufficient for 50μl per section. Allow larger volume for larger sections.
• Once all slides have been de-waxed place into distilled water
• Prepare humidified chamber
• Remove slides from the staining dish, wipe the backs of the slides and place onto the humidified chamber.
• Distribute 50μl of proteinase K onto each section and spread over whole of tissue using a piece of parafilm. Take care whilst spreading to ensure sections do not get dried or damaged in any way.
• All sections should be incubated with proteinase K for 5 minutes at room temperature.
• After 5 minutes tap the solution off the slide and place slides in a glass staining dish filled with TBS.
• See SOP 12 for immunohistochemistry protocol.

METHOD B (Heat based antigen retrieval):

1. Either:
   (a) Prepare a 10mM solution of citrate buffer, pH=6.0 by adding 3.15g citric acid to 1.5 litre of distilled water and adjust pH to 6.0 with 2M NaOH.

Or:
   (b) Dilute stock 5x Tris-EDTA buffer, pH=9.0 by adding 300 ml to 1.2 l of distilled water.

2. Place buffer in the pressure cooker and place on the hotplate.
3. Turn on the hotplate to the maximum heat setting.
4. Loosely place the lid back on pressure cooker. Bring the buffer to a rolling boil.
5. Immerse the slide rack in the buffer using long forceps.
6. Engage the lid, turn the valve to pressure symbol and press the lid down until it clicks.
7. Set timer for 1 minute - when steam starts to vent, start timer. After 1 minute, turn off the hotplate and release the pressure by turning the vent to the “steam” symbol, and cool as quickly as possible by transferring the pressure cooker to the sink and running under cold water.
8. Transfer the slides back to the glass staining jar containing distilled water.
9. Transfer to TBS ensuring that the tissue does not dry out.
10. See SOP 12 for immunohistochemistry protocol.

Other useful SOPs associated with 06 Procedure
Embedding samples using the Shandon Histocentre 3 machine (SOP 05).
Preparation of RM biopsy samples (SOP 03).
Preparation of APES coated slides (SOP 07).
Tissue processing using Shandon processing machine (04).
Immunohistochemistry (SOP12).

SOP History
Updated version was prepared in July 2009 to include changes in both Liverpool Women’s Hospital and University of Liverpool logos.
Version 2.1 was prepared by K Palial to include Proteinase K antigen retrieval
Version 2.2 was prepared by J Drury to include Tris-EDTA antigen retrieval and generally update information.
Version 2.3 was prepared to update procedures following relocation of laboratories.

Appendices - Associated Documents

<table>
<thead>
<tr>
<th>Document</th>
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<tr>
<td>1</td>
<td>Risk assessment for antigen retrieval</td>
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<td>2</td>
<td>Physical Hazards Code of Practice</td>
</tr>
<tr>
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</table>
Appendix 5 Participant Information Poster

Will you help us learn about Retained Placenta?

When a placenta or afterbirth remains inside the womb after the baby is born, it is called a retained placenta. This puts a woman at risk of bleeding and infection. The standard treatment is to take the placenta out with a gloved hand in an operating theatre. This is called a ‘manual removal of placenta’.

There are three known types of retained placenta and we may be able to treat some of them using medicines alone. This would avoid going to theatre and having an anaesthetic. However, at the moment, these types cannot be accurately diagnosed before theatre. Therefore, everyone has a ‘manual removal’ whatever their type of retained placenta.

But ultrasound of a retained placenta may help!

If your placenta is retained, would you be willing to have a scan?

It won’t delay your treatment - but may help us know more about this condition.

You do not need to contact us now, but if you have a retained placenta you could be approached to take part.

Research Team
Prof Andrew Weeks
Dr Achier Deng Akol

For more information:
email achier@liv.ac.uk
phone 0151 7959800
Appendix 6 Patient Information Sheet for RPS Part II

Patient information sheet: (version 4; 03/09/2015)

A prospective study of the diagnostic role of ultrasound amongst women with retained placentas

We would like to invite you to take part in a research study. Please read this information carefully and discuss it with others if you like.

Ask us if there is anything that is not clear and take time to decide whether or not you wish to take part.

Why are we doing this research?

For one woman in every 50 births, the placenta or afterbirth remains inside the womb after the baby is born. This is called a retained placenta and puts the woman at risk of vaginal bleeding and infection. Usually we wait for a while to see if it will come out on its own (so long as you are not bleeding). If it does not deliver after an hour, then we would give you an anaesthetic in the operating theatre (either an epidural – or similar – or put you to sleep with a general anaesthetic) and then take the placenta out using a gloved hand. This is called a ‘manual removal of placenta’.

There are three known retained placenta subtypes and some may be possible to treat using non-surgical methods. This avoids the additional risks of surgery and anaesthesia. However, at the moment, the 3 retained placenta subtypes cannot be accurately diagnosed before operation. This means that everyone has to undergo a manual removal of placenta, whatever their type of retained placenta. We are trying to find ways to work out what type of retained placenta women have so that they can be treated with medicines instead of having to go to theatre. We hope that an ultrasound scan may help us to diagnose the type of placenta retained in you.

Do I have to take part in this study?
No, it is your choice and if you choose not to take part it will not affect your treatment.

What will happen if I take part?
You will have an ultrasound scan before you go to theatre to have your placenta removed. We may also take a biopsy from your placenta. Then you will continue to receive the usual care from your medical team. If you choose to take part it will not
delay your treatment. Neither you nor your care team will be told the results of our scan.

**What happens if I don’t want to be in the study?**
Then you will have a manual removal of placenta as usual, without a scan beforehand.

**What if something goes wrong?**
No medication will be given to you for the study apart from the ultrasound scan which is known to be completely safe. So we do not expect anything serious to go wrong. However, in the unlikely event of anything going wrong with the scanning, or if you wish to complain, or have any concerns about the way you have been approached or treated during the course of this study, then the normal NHS complaints system will be available to you on telephone number 0151 702 4416. You can also contact PALS (Patient Advice and Liaison Service) if you wish to speak to someone independent of the research team (telephone number 0151 702 4353). If you choose to take part it will not delay your treatment. If you are harmed due to someone’s negligence, then you may have grounds for a legal action. There are, however, no special compensation arrangements in place in case of problems with the research.

**Will my information be kept confidential?**
All information that is collected about you during the course of the research will be kept confidential. If you agree to take part in the research, the research team may look at any part of your medical records to collect further data. Your results may also be read by people from regulatory authorities to check that the study is being carried out correctly.

**Who is organising the research?**
The research is being organised by the Department of Women’s and Children’s Health at the University of Liverpool.

**Who has approved the study?**
This study has been approved by one of the National Research Ethics Service committees in the UK.

**Contact for Further Information**
For further information about the study please contact:
Dr. Achier Deng Akol at the Department of Women’s and Children’s Health, Liverpool Women’s Hospital, Crown Street, Liverpool L8 7SS (Tel: 0151 795 9800; e-mail achier@liv.ac.uk).

Thank you for taking part in this study!
Patient Information Leaflet

Collection and storage of tissue, blood and other biological samples for research from women

Consent to storage and use of tissue for research

Version 1.0: 09.07.2014

Contact Information for LWRTB
Professor James Neilson
University of Liverpool Department
Liverpool Women’s NHS Foundation Trust
Liverpool
L8 7SS
We would like to invite you to take part in clinical research by donating tissue to be stored in the Liverpool Women’s Research Tissue Bank held at Liverpool Women’s NHS Foundation Trust. The tissue will then be used for future research. Before you decide whether to donate tissue, you need to understand why we are requesting tissue and what collection of tissue will involve. Please take time to read the following information carefully. Talk to others about the research if you wish.

**What is the Research Tissue Bank?**
The Liverpool Women’s Research Tissue Bank (LWRTB) collects and stores tissue and other samples taken at surgery or in clinic for use in medical and scientific research. By doing this the LWRTB is able to build up a valuable collection that will be used by research groups undertaking women’s health research. There are many common conditions such as endometriosis, heavy periods and prolapse, malignant conditions such as endometrial cancer and pregnancy complications that our research teams are trying to find new treatments for. Therefore, it is hoped that the resulting knowledge from this research will help other women in the future.

**Why have I been chosen?**
Either, you are about to undergo a surgical operation or biopsy procedure, which will involve the removal of tissue as a part of your treatment. Or, you will be asked to give a biological sample as part of your routine appointment. We are asking for your consent for a small amount of the sample to be stored and used later in research.

**What will happen to me if I take part?**
The tissue removed at your surgical operation is in most cases sent to the Department of Pathology to be tested by doctors and scientists to help in the diagnosis and treatment of your medical condition. In other cases, the tissue would simply be discarded. Often, it is not necessary to test the whole sample, which means that there will be tissue left over.
If you agree to take part in this study, we are asking for your informed consent to store some of this tissue in the LWRTB for use in research projects. The tissue sample the LWRTB will store will be very small, usually no bigger than the size of a one pound coin.

If as part of your routine treatment, a doctor would normally take a blood sample, we may ask for your informed consent to take a small additional amount at the same time as this routine sample. This will usually be taken at the time of your surgery or in clinic and will be approximately 20ml, around 4 teaspoons worth.

In certain cases, if it is relevant to your illness, we would also like your informed consent to take an additional biological sample (e.g. urine, saliva, amniotic fluid, endometrial biopsy). We would ask you to provide a small sample of one or more of these, according to what is appropriate and we would store this together with the tissue and blood sample.

We would also like your consent to access your health records. These will be reviewed by LWRTB staff on an annual basis in order to update information on the Tissue Bank database. All information will be treated with the strictest confidence and held securely within the LWRTB. In addition, the LWRTB may seek to access information held by other sources such as NHS Trusts, Disease Registries (such as the North West Cancer Intelligence Service, NWCIS) and the UK Statistics Authority.

This information is essential to help researchers understand what your illness was like and relate what is found in the laboratory to what happens to patients. Your name, address and any other personal data will be removed before any information is given to research groups, so you will not be identifiable to the researchers.

**What are the advantages and disadvantages of taking part?**
The samples taken for storage and research are only small and are taken during your surgery/clinic appointment or alongside routine clinical samples once the necessary
diagnostic tests have been performed. The results of research carried out using your
tissue, blood and/or biological samples, and those of others, may help in the future
discovery of new drugs and treatments for women with a variety of conditions.
There will be no direct benefit to yourself as you will not be identifiable to the
research team. There will be no additional risks if you choose to participate. The
risks associated with surgery will be explained separately by the medical team as
part of your treatment. If a blood sample is taken, occasionally, this may require an
additional entry site to the routine blood sample. There is a small chance that you
may experience some bruising at the site.

Do I have to take part?
No. The choice to allow us to collect samples of your tissue for the LWRTB is up to
you. If you do decide to consent to donate tissue then you can keep this information
document and you will be asked to sign a consent form. Even if you do decide to
donate tissue, you are free to change your mind at any time and without saying why.
Whatever your decision it will not affect the standard of care that you receive.

What will happen if I change my mind?
If you do decide to take part you are still free to change your mind at any time. You
have the right to withdraw your consent to store your tissue, blood and/or biological
samples without giving a reason. If you do withdraw, then it will not affect in any
way the treatment that you are receiving. You can withdraw your consent by
contacting the tissue bank on:

Telephone number: 0151 702 4346 / 4241 or write to:

Professor James Neilson,
University Department, First Floor, Centre for Women’s Health Research
Liverpool Women’s NHS Foundation Trust
Crown Street
Liverpool, L8 7SS
The tissue, blood and/or biological samples stored in the LWRTB, along with any information held about you, will be destroyed and a letter of confirmation will be sent to you. If you change your mind a long time after the samples were donated then some research may have already taken place on your samples. The LWRTB would not be able to recall samples and information once they have been used, but we would ensure that no further research work will be undertaken on your tissue, blood and/or biological samples.

**What will happen to my tissue or blood?**

Academic research groups will use your gift of tissue, blood and/or biological samples to understand the causes of conditions affecting women and the best treatments and care for women in the future. It may also be used to test or develop new assays in the laboratory. In all cases research studies will be ethically approved and monitored. Your tissue will not be used for transplantation or reproductive cloning. Nor will the tissue be used for non-medical or non-scientific purposes.

The research may be carried out in Academic Institutions and the NHS throughout the UK. In all cases, you will be anonymous to the researcher.

When we store samples, we will use some of them to obtain genetic material (DNA and RNA) and protein. We are asking you to allow us to obtain DNA, RNA and protein so that this can also be made available to research groups. We will not use DNA, RNA or protein samples for any purpose other than research and the research team will not be able to identify you in any way.

**What if Researchers find new information about my health?**

Usually the information discovered during research is not relevant to your care and treatment. If information is discovered that will affect your health then you will be informed via the medical team responsible for your care.

**Will anybody make a profit from my tissue, blood and/or biological samples?**
You are asked to donate your tissue for research and will not receive a financial reward either now or in the future. Similarly, the LWRTB will not sell your tissue for profit to other researchers. A charge will be made only to cover processing and staff costs. However, your tissue may be used in a research project that may lead to the development of new drugs or treatments. It will not be possible for you to make a claim for money for tissue you donate. Any drug, treatment or test developed may help women in the future.

**What will happen to the results of the research study?**
Research studies using tissue, blood and/or biological samples may take several years to complete. Results will be published when appropriate in scientific papers and magazines and at scientific meetings. The LWRTB will request updates on the progress of research projects.

You will not be able to be identified if research using your tissue, blood and/or biological samples is published in any scientific papers.

**Will my taking part in this study be kept confidential?**
All information that is collected related to your medical condition will be kept strictly confidential. Your name, address and other personal information will be removed before any information is released to researchers using your tissue, blood and/or biological samples. You will not be able to be identified by the researcher.

**Who has reviewed the study?**
The collection and storage of tissue, blood and/or biological samples by the LWRTB has been ethically approved by the NHS Research Ethics Service; an independent body external to the LWRTB. The LWRTB is licensed by the Human Tissue Authority as required by law.
Appendix 8 Consent Form for RPS Part II

CONSENT FORM

A Prospective Study of the diagnostic role of ultrasound amongst women with retained placentas

Name of Researcher: Dr. Achier Deng Akol, Clinical Research Fellow, University of Liverpool

Please initial box

1. I confirm that I have read and understand this information sheet (version 2, dated March 2015) for the above study

2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected.

3. I agree that my medical notes may be looked at by the research team.

4. I understand that my medical notes may be looked at by the research team, supervisors or inspectors as part of this research. I give permission for these individuals to have access to my records.

5. I agree to take part in the above study.

6. I agree for my placenta to be used for the above study and to be transferred to the University of Liverpool for analysis and storage

Name of Patient ___________________________ Date _____________

Signature _____________________________

Researcher _____________________________ Date _____________

Signature _____________________________

1 copy for patient, 1 copy for research, 1 copy to be kept with hospital notes
Appendix 9 Consent form for LWHTB

Liverpool Women’s Research Tissue Bank Consent Form
Collection and Storage of Tissue, blood and other biological samples for Research

Liverpool Women’s Research Tissue Bank, University Department, Liverpool
Women’s NHS Foundation Trust, Liverpool L8 7SS. Tel: 0151 702 4346
If you agree to take part, please initial each box and sign and date this form

1. I have read and understood the information leaflet (Version…….) on
   the above research project and have been given a copy to keep. I have
   had the opportunity to ask questions about the project and understand
   the benefits and risks of donating.

2. I agree to give samples of my tissue from any hospital appointment I
   attend in relation to my condition.

3. I agree to give samples of my blood from any hospital appointment I
   attend in relation to my condition.

4. I agree to give another biological sample, if appropriate, from any
   hospital appointment I attend in relation to my condition (Please
   state)…………………………………………………

5. I understand how the samples will be collected, that giving a sample is
   voluntary and that I am free to withdraw my approval for use of the
   samples at any time without giving a reason and without my care or
   legal rights being affected.

6. I agree that the University of Liverpool will become custodian of this
   tissue, blood and/or biological sample for use in regulated research
   projects.

7. I agree that tissue bank staff can collect and store information on an
   ongoing basis for updating the tissue bank database from my health
   records for research that uses my samples and that this information
   may be viewed by regulatory authorities. I understand that some
   information may be held at different sources such as Disease
Registries. I understand that information about me will be treated confidentially and stored securely.

8. I understand that any information given to research groups will be anonymised and my identity will be protected. I agree to samples being sent to research groups based in the UK.

9. I agree that it may be appropriate for genetic assessment of the samples to be carried out to determine whether genetic makeup has any influence on my condition.

10. I understand that I will not benefit financially if research using my samples leads to new treatments or medical tests.

Patient Statement I agree to give tissue and blood for use in medical and scientific research.

Signed……………………………….Print
Name……………………………….Date…………………

Clinical/Research Practitioner Statement I have explained the request for samples for research purposes and have answered such questions as the patient has asked.

Signed……………………………….Print
Name……………………………….Date…………………

Please complete the following patient details:

Hospital Unit Number………………………………
NHS Number……………………………………
Appendix 10 Poster on the role of ultrasound in diagnosis of retained placenta subtypes: Best poster in BICS Conference 2019
Appendix 11 Poster on factors and outcomes associated with retained placenta subtypes: 2nd poster for BICS Conference 2019
References


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