Assessing the relationship between maternal acidosis (MA) and postpartum haemorrhage (PPH); the MAPPH Study

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy, by Ms Helen Naomi Maver

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Glossary of Abbreviations

ACOG	American College of Obstetricians and Gynaecologists
ADP	Adenosine Diphosphate
AFL	Amniotic Fluid Level
aOR/ OR	Adjusted Odds Ratio/ Odds Ratio
ATP	Adenosine Trisphosphate
BK_{ca}	Calcium-activated Potassium Channels
BMC	Bimanual Compression
Ca^{2+}	Calcium
CaM	Calmodulin
CHUV	Centre Hospitalier Universitaire Vaudois
CI	Confidence Interval
CO_2	Carbon Dioxide
CS	Caesarean section
CSF	Cerebrospinal Fluid
DAG	Diacylglycerol
EBL	Estimated Blood Loss
ECC	Excitation-Contraction Coupling
EM	Emergency
fUApH	Fetal umbilical artery pH
fUVpH	Fetal umbilical vein pH
GPCR	G-Protein Coupled Receptor
H^+	Hydrogen Ion
H_2CO_3	Carbonic Acid
H_2O	Water
HCO_3-	Bicarbonate Ion
HRA	Health Research Authority
IP_3	inositol-tris-phosphate
K^+	Potassium Ion
LGA	Large for Gestational Age

MA	Maternal Acidosis
mApH	Maternal arterial pH
MAPPH	Maternal Acidosis and Postpartum Haemorrhage
mIVpH	Maternal intervillous pH
MLC20	Myosin Light Chain-20
MLCK	Myosin Light Chain Kinase
MLCP	Myosin Light Chain Phosphatase
mVpH:	Maternal venous pH
NAD+	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NMR	nuclear magnetic resonance
NPV	Negative Predictive Value
OT	Oxytocin
OTR	Oxytocin Receptor
PIP_2	phosphoinositide-bis-phosphate
PLC-β	Phospholipase-C β
PPH	Postpartum haemorrhage
PPHB	postpartum haemorrhage Butterfly
PPV	Positive Predictive Value
RBC	Red Blood Cell
RCT	Randomised Control Trial
REC	Research Ethics Committee
SPPH	Severe Postpartum Haemorrhage
SR	Sarcoplasmic Reticulum
TA	Tranexamic Acid
VOCC	Voltage Operated Calcium Channels
WHO	World Health Organisation

Acknowledgements

I don't think I can thank my supervisors enough for all the help they have given me in completing this thesis. They have gone above and beyond what has been expected of them in their role. I have met with them almost every single week since 2019, and sometimes even several times a week. Andrew's enthusiasm passion for his subject area is infectious, and he has been a great source of motivation for me in completing this project. Helen has been so understanding patient with me, always demonstrating such empathy and support. Both Andrew and Helen could not have done more to help me through this MPhil. They haven't just been my supervisors, they have also acted as emotional support, and even a lifeline for me during the tougher months of the lockdown.

I also would like to say a special thank you to Dr Steven Lane, who has also gone far above and far beyond his role in providing statistical advice on thesis. He has met with me countless times over Zoom to teach me the principals behind statistical tests and how to conduct such analyses. Since our first meeting, he has replied to hundreds of my emails and answered hundreds of my questions, no matter how seemingly obvious they may have been. Not to mention how many hours of his time he has given to me in helping me complete the analysis. Steven has even marked 'non-statistical' sections of my work, and has offered me so much helpful, constructive feedback, which I am so grateful for.

I cannot emphasize enough how the completion of this project has been a joint effort. I could not have possibly written this thesis without these people, and it was because of these people that you are reading these words today.

A big thank you must also go out to Professor Susan Wray for providing so much useful help and guidance in the early days of my project. Although Professor Wray retired from work at the end of 2020, her input and advice was invaluable, and her passion for improving women's health served as a huge source of inspiration for this study on postpartum haemorrhage.

Lastly, I would like to thank Professor David Baud, based over in Switzerland, for allowing me to use his data for my research. We wrote to Professor Baud to ask if he would be interested in working together to explore this possible relationship, and myself and my supervisory team were very grateful that he accepted our request. It was agreed that the thesis would be written as a collaboration between myself, my supervisory team and Professor Baud, where

we would all be included in the authorship of the thesis. Professor Baud not only allowed us to use his dataset, but he was also very helpful in replicating the data cleaning .do files and the univariate analysis on the full 33,000 database, despite having an extremely busy schedule. He has been extremely helpful throughout, and his generosity has made this MPhil possible.

Abstract

Introduction

Worldwide, postpartum haemorrhage (PPH) remains the leading cause of death relating to pregnancy, with 25% of obstetric deaths caused by this medical complication each year. The most common cause of PPH is uterine atony, where myometrial contractions are too weak to occlude exposed blood vessels of the uterus after birth. It is estimated that atony is responsible for 70% to 80% of PPH cases. Uterine acidosis is defined as an increase in the acidity of the myometrial tissue and blood, causing myometrial blood pH to become abnormally low. Over the past few decades, various *in vivo* and *in vitro* studies have demonstrated a clear association between uterine acidosis and a decrease in myometrial contractility and uterine tone. It is thus hypothesised that postpartum blood loss may be higher in women with acidosis, and therefore acidosis may be a risk factor for uterine PPH by increasing the incidence of uterine atony. In order to investigate this hypothesis, data on both blood loss and myometrial pH needs to analysed to assess for any association between PPH rates and maternal acidosis.

The primary study objective was to determine whether patients with maternal acidosis have higher rates of PPH, compared to patients without acidosis. It was postulated that fetal umbilical vein pH (fUVpH) could be used as a surrogate measure for maternal arterial pH (mApH). Fetal umbilical vein pH (fUVpH) data is routinely collected as part of standard fetal monitoring, and therefore more widely available for analysis than mApH. In addition to this, findings from an extensive literature search suggests that fUVpH is often within 0.1 pH units of the mApH, and that they closely change together so that maternal acidosis can induce fetal acidosis.

The secondary study objective was to determine whether fUVpH is an independent risk factor for PPH. There are many other predictors for PPH (e.g. type of delivery, prolonged labour, fetal birth weight, parity, multiple pregnancy), and therefore their impact on blood loss needed to be investigated alongside fUVpH may have on PPH.

Methods

Fetal umbilical vein pH (fUVpH) was used as a surrogate measure for maternal arterial pH (mApH).

A secondary analysis was conducted on data gathered from a pre-existing Swiss database. Obstetrical data on 2000 live, cephalic- presenting, singleton, term pregnancies was collected between January 1997 and December 2006. Within that dataset, data on both maternal postpartum blood loss and on umbilical vein blood pH was gathered. Multiple logistic regression was carried out, where PPH was defined as blood loss >500ml for vaginal birth and >1000ml for caesarean birth. A sensitivity analysis was also conducted, where PPH was defined as blood loss >1000ml regardless of mode of birth.

To validate the of predictive properties of the model, receiver operating curves (ROC) curves were created to determine an optimum cut-off value to classify women as being either at risk of a PPH or not at risk, as well as negative and positive predictive values being calculated to assess the accuracy of the model.

Results

Results from the univariate model showed an association between umbilical venous pH and PPH (p=0.04). However, the multivariate model showed that umbilical venous pH and PPH were not independently associated (p=0.46), meaning that low umbilical venous pH did not have any significant effect on PPH incidence when controlling for other variables, regardless of definition used for PPH. However, the data did reveal that when PPH is defined as >500ml blood loss for a vaginal birth and >1000ml blood loss for a caesarean birth, the predictor variables that significantly increased PPH risk were; an operative vaginal birth, an emergency CS and a retained placenta. Results differed during the sensitivity analysis, where retained placenta remained a significant predictive factor for PPH but the other predictive factor was found to be induced labour. Emergency CS stopped becoming a significant predictor variable in the sensitivity analysis.

ROC curves proved that the model (including the sensitivity analysis) could discriminate between a woman who is likely to have a PPH and a woman that not likely to have a PPH.

Conclusion

It is believed that this study is the first to specifically look at the relationship between blood pH and PPH rates, and certainly the only one of its kind to use umbilical vein pH as a surrogate measure for myometrial capillary pH. When other predictors were included in the multivariate analysis, umbilical vein acidosis association with PPH became statistically

insignificant. Results also showed how one needs to be careful with how PPH is defined, because changes in definition resulted in changes in the explanatory variables that are associated with PPH. Despite this, retained placenta remained highly significant in the multivariate analysis for both PPH definitions. One might postulate that retained placenta may be the associated factor that was so highly significant to push umbilical vein pH out of the multivariate analysis.

The significant predictive factors found in this study are also well recognised within the current literature to be associated with PPH. However, the literature is less clear on how valid fUVpH is as a surrogate measure for maternal myometrial acidosis. Therefore, it is advised that future research on this subject should scientifically test the accuracy of the fUVpH as a proxy for myometrial acidosis.

There is still no unanimous answer to whether acidosis can increase PPH risk. Indeed, the results from this current research show no association between low venous pH and volume of blood lost. However, research on the potential maternal acidosis and PPH association is well documented in the current literature. The methodology of this project is ultimately quite weak, mainly due to the small sample size and the fact that this work rests on an unproven hypothesis (that fUVpH is a proxy for mApH which in turn equates to myometrial capillary pH), which could even possibly explain the non-significant findings. It is hoped that future work would involve conducting a prospective clinical cohort study, as a more scientifically robust analysis on myometrial blood pH and post-partum blood loss volume could still show a positive association.

Covid Disruptions

The initial project

In October of 2019, work began on what was originally expected to be a PhD project. The aim was to conduct a prospective clinical cohort study to relate maternal and myometrial pH to atonic postpartum haemorrhage (PPH).

It was originally hypothesised that prolonged labour results in maternal systemic and myometrial acidosis, which in turn may cause myometrial atony and PPH. If this were to be the case, then it would have been envisaged that, in future, women having emergency caesarean section (CS) might be able to have their acid-base status assessed through a capillary lactate sample before a CS, so as to correct any acidosis and prevent a PPH.

The PhD project was thus assessing the relationship between uterine atony and maternal acidosis (both systemic and myometrial) in women having emergency caesarean sections. The specific research questions of this project were:

1. In women undergoing emergency caesarean sections, is there a difference in myometrial pH between those who require additional uterotonic therapy for atony (clinician decided), and those who don't?
2. How does myometrial pH correlate with myometrial lactate?
3. How does myometrial pH correlate with cutaneous pH (skin stab capillary pH)?
4. How does myometrial pH affect vaginal blood loss during CS?

This cohort observational study was planned to be carried out at Liverpool Women's Hospital. Patients undergoing emergency caesarean section for failure to progress (dysfunctional active labour), fetal distress, bradycardia, breech presentation and failed induction of labour (but never in active labour) would have been likely candidates for this study. Suitable patients would have been supplied with an information leaflet and a signed consent form, specific to this study, would first be obtained before recruitment. Tissue bank myometrial samples collected from women who underwent elective caesarean sections would have acted as a control, as these women would have never laboured.

The type of data that would have been collected for this study would have included data from contractility assays on myometrial biopsies, pH readings from capillary samples of myometrial and cutaneous blood and the weighing of vaginal blood loss during caesarean section. It was important that contractility data was collected so that any decrease in force, duration and amplitude of myometrial contraction, potentially caused by acidosis, could be closely documented. Data on whether any additional uterotonic therapy was used during the caesarean section, along with a clinician's assessment of uterine tone, would have also been recorded as part of this study.

Once written consent was obtained from suitable study candidates, tissue biopsy specimens would have been taken from the upper lip of the uterine incision during the caesarean section. Contractility assays would have been performed on the myometrium from these biopsies in the laboratories at the Liverpool Centre for Women's Health Research.

In order to conduct a contractility assay, 1mm by 5mm strips of myometrium are cut from the biopsy and superfused in a physiological saline solution at 37^{o}C. Using surgical suture, strips are then attached to a force transducer where readings on any myometrial contractions are recorded using Lab-Trax software. These ex-vivo contractility measurements collect data of force, amplitude, duration and AUC (area under curve) on each of the myometrial strips.

As well as gathering data on myometrial contractility, it was intended that maternal venous blood, or myometrial capillary pH, would have also been obtained at caesarean section in order to determine maternal acidosis levels. Samples would have then been immediately analysed in the blood gas analyser (Bayer) on delivery suite.

The amount of intrapartum vaginal blood loss would have also been measured using the gravimetric method (Inco pads and vaginal swabs to be weighed after the delivery of the placenta and the dry weight of each subtracted).

Lastly, in order assess levels of myometrial contractility, uterine tone would have been determined by clinician as a rating from 1-10 at 10 minutes following birth. Observations on the requirement for additional therapeutic uterotonics would have additionally been noted as an indication of uterine tone.

With the collection of all this data, the original study's primary objective was to examine whether patients with myometrial tissue acidosis have higher rates of intrapartum vaginal blood loss compared to patients without uterine acidosis. The project's secondary study objectives were to explore which method of measuring acidosis correlates best with myometrial capillary blood acidosis levels. As a final objective, we hoped to measure how clinicians' assessment of uterine contractility at 10 minutes postnatally are impacted by acidosis.

See Appendix 1 for the original PhD protocol draft

Lockdown and the decision to switch to an MPhil

In March of 2021 the nation was given the order to stay at home. This meant that all experiments in the laboratory ceased and myometrial sample recruitment came to a halt. Unfortunately, the lockdown came at a crucial time for the PhD. Final adjustments were being made to the protocol, the research passport, ethics and sponsorship approval were being processed and several preliminary experiments on elective CS biopsies had been conducted.

In the face of the rapidly spreading Coronavirus pandemic, observational studies on myometrial contractility were not at the top of the clinical agenda. It would be several months before biopsy recruitment could resume. The sensitive nature of gaining consent for emergency CS samples meant that they were difficult to obtain and rare to come by, even without a global pandemic. In addition to this, the hospital and university governance procedures drastically increased at this time, which made it even more unlikely that the original clinical project could continue.

After several months of trying to busy myself with stay-at-home 'research', it became apparent that the structure of the PhD project needed a rethink if any useful research was to come of it. As July approached, my supervisors and myself starting having frank discussions around the feasibility of continuing with the PhD. The PhD project was still in its relative infancy, and quite a bit more work was required to finalise the protocol and get around the difficult challenge of obtaining enough emergency CS samples. It became a concern that my relative in-experience and lack of independence (having just graduated from my undergraduate degree months earlier) would mean that it would be extremely difficult to make up for the time lost due to the lockdown. I was offered the opportunity to continue with

a PhD that was entirely desk based, but I did not feel that working independently from home, for the next few years, would have been beneficial for my mental health. At the same time, I was also having re-considerations as to whether a conducting a PhD was the right path for me. Having ADHD, for me at least, means that having external pressure and a structured routine imposed on me is beneficial, as I struggle to create that for myself. Additionally, I knew my personality type was better suited to working in a social environment, something which pursuing a PhD may not have been able to offer me. This re-appraisal of my future plans meant that completing a shorter MPhil project made a lot more sense. Therefore, my supervisors and I came to the mutual decision that it would be best to convert the PhD to an MPhil. The new plan offered a desk-based option that could still shed light on the original research question by using a pre-existing database to assess maternal acidosis and PPH. This decision has thus led to the MPhil project we have today.

The MPhil and analysis in Switzerland

As mentioned above, the new plan for the MPhil was to still investigate the effect lactate has on the myometrium with regards to PPH risk. However, rather than collecting my own data, I would search the internet for a database that contained blood loss values and pH values and re-analyse this data in a secondary analysis.

The first task was to identify a suitable database that could be used to conduct a secondary, multivariate analysis. After several weeks of compiling possible papers, the list of potential studies was reduced to 20 papers whose authors may or may not have access to a relevant obstetric database. This list of 20 was then reduced to 7 papers, and then to two papers. From the final two papers, the decision was made to reach out to Professor David Baud and request a collaboration.

	Paper	Date	Number of participants in the study	pH data	PPH data	Comments	Reference
1	Effects of delayed compared with early umbilical cord clamping on maternal postpartum hemorrhage and cord blood gas sampling: a randomized trial	2012	382	Blood gas analysis from umbilical vein	Blood loss estimated by midwife	This could be useful	Andersson, O., Hellström-Westas, L., Andersson, D., Clausen, J. and Domellöf, M., 2012. Effects of delayed compared with early umbilical cord clamping on maternal postpartum hemorrhage and cord blood gas sampling: a randomized trial. *Acta Obstetricia et Gynecologica Scandinavica*, 92(5), pp.567-574.

2	Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labor at term	2013	13971 (although this was a sample from a 33,000 patient dataset).	Risks of cesarean or instrumental delivery, postpartum hemorrhage >500 ml	arterial umbilical cord pH<7.1, no mention of umbilical vein	Retrospective cohort study . I hope they have unpublished data on umbilical vein.	Baud, D., Rouiller, S., Hohlfeld, P., Tolsa, J. and Vial, Y., 2013. Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labor at term. *The Journal of Maternal-Fetal & Neonatal Medicine*, 26(16), pp.1595-1601.

Table 1: Above is a table containing the final two papers whose authors may have had access to the relevant obstetric data. The decision was made to reach out to Professor David Baud (paper 2) and request a collaboration.

See Appendix 2 for the original table of 20 papers; sourcing data for analysis.

In Professor David Baud's 2013 paper ('*Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labour at term'*) (Baud *et al.*, 2013), it appeared that Baud et al had gathered data on more than 33,000 women, over a ten-year period, from the Lausanne University Hospital in Switzerland. Within that database, there was data on estimated postpartum blood loss and also on umbilical vein pH. After carrying out an extensive search on the literature, I found that maternal myometrial blood pH was rarely tested, whereas umbilical vein pH was. Because blood from the maternal side of the placenta is in close contact with blood that enters the umbilical vein, fetal umbilical vein pH should mirror that of maternal uterine vein and myometrial capillary pH. Another search of the literature did indeed confirm this to be the case. This data was of particular relevance to the proposed research, as it could be used to provide evidence for a correlation between maternal acidosis and PPH. We then wrote to Professor Baud to ask if he would be interested in working together to explore this possible relationship. Myself and my supervisory team were very grateful that David accepted our request. It was agreed that the thesis would be written as a collaboration between myself, my supervisory team and Professor David Baud, where we would all be included in the authorship of the thesis.

It was initially hoped that I would travel to Lausanne, Switzerland, and carry out the statistical analysis on the data myself. This would have avoided the issue of data governance; where the raw data could not be sent outside of Centre Hospitalier Universitaire Vaudois (CHUV)

hospital, but the results from the analysis could be used in my work. However, as the COVID-19 pandemic continued to affect travel into 2021, the feasibility of me travelling to Lausanne to run the data soon became a concern. As the data could not be sent to me, due to patient data protection laws, Professor Baud suggested the next best option; that I send over a detailed statistical analysis plan, containing all the STATA commands (.do files), so he could replicate analysis on the full 33,000 patient database. Professor Baud would then send me back the results of the analysis to be used in my work. For me to write the .do files, it was hoped that a dataset sample of 2000 patients records would be sufficient. Unfortunately, it soon became apparent that because only Professor Baud could carry out the analysis, conducting multiple regression would have been extremely difficult to do remotely. Writing .do files on STATA for multiple regression requires each variable to be added and subsequently removed if and when they become non-significant. This could only work if I were able to work on the 33,000-patient dataset in real-time. However, it was fortunate that the sample dataset of 2000, anonymised obstetric records proved to be large enough to be used for the purposes of this MPhil thesis. Indeed, it would have been preferable to have access to the full 33,000 patient database, but after the number of obstacles we had already faced in getting this study off the ground, we were grateful that statistical analysis could finally begin.

Negative Implications

Switching from the original PhD project to an MPhil does still allow, in essence, the original hypothesis to be investigated. The MPhil hypothesis is that prolonged labour results in maternal acidosis, as mirrored in the fetal umbilical vein pH (fUVpH). This acidosis will, in turn, cause myometrial atony and thus increase risk of the mother suffering from a PPH. This is defined by blood loss >500ml for a vaginal birth and >1000ml for an emergency caesarean section.

However, these project changes that the pandemic brought (along with changes to many of our lives) have had some negative implications on the rate of progress of this research. Many of us experienced a decrease in mental wellbeing during this difficult time, which often resulted in decreased levels of motivation. I, myself, was no exception to this. Feelings of having failed at the original PhD project, along with feelings of loneliness and isolation that

working from home brought, have been persistent since the first national lockdown. The impact of which is reflected in a thesis deadline extension until March 2022.

To add to this, it is worth mentioning that external postgraduate courses, conferences, events and opportunities were also put on hold. These would have been, under normal circumstances, part of the academic calendar in my area of research. Participating in online webinars did not have the same impact as attending in-person taught courses. For example, I required a fair amount of statistical training in order to comprehend the analysis that was required for my project.

Nonetheless, this project was able to see its eventual completion, not least for the huge amount of help and support I received from my supervisory team. Fortnightly meetings (and most often weekly meetings) between myself, my supervisors; Professor Andrew Weeks and Dr. Helen Wallace, and statistician; Dr. Steven Lane, were conducted remotely. Furthermore, Professor David Baud has been more than helpful with sharing his obstetric data and running the statistical analysis on his end. The many hours of advice and support I have received has been paramount in helping me to complete an MPhil project during this uniquely challenging time.

Chapter 1. Background

Physiology of myometrial contraction

The uterus is a muscular reproductive organ that sits in the pelvic cavity of the woman. The uterus is made up three main segments; the fundus (top portion of the uterus and connects to the fallopian tubes), the body (main portion of the uterus and the typical site of embryo implantation) and the cervix, which links the uterus to the vagina. Both the fundus and body of the uterus are made up of three distinct tissue layers. The inner most layer is known as the endometrium, which lines the inner uterine surface and is shed at the end of each menstrual cycle. Above the endometrium lies the myometrium. This is a thick layer of smooth muscle that is responsible for the contractions that expel the fetus during labour and to help shed the lining of the endometrium during menstruation. The myometrium itself consists of three muscle layers; an inner circular layer, a vascularized middle layer, and an outer longitudinal muscle layer. Muscle fibres of the circular layer are arranged in a concentric fashion, parallel to the longitudinal muscle fibres of the outer myometrial layer. The middle layer, which contains many blood vessels, has muscle fibres which are interlacing and irregular in arrangement; lying in transverse, oblique and longitudinal directions. The middle myometrial layer can be described as "mesh-like" in appearance (Escalante and Pino, 2017). During childbirth, this arrangement of muscle fibres allows the uterus to contract down on itself, helping to expel the fetus and then to occlude sheared blood vessels after the delivery of the placenta. Covering the outermost surface of the uterus is the peritoneum, which is made up of two membranous layers and is continuous with the abdominal peritoneum.

The uterus is an active organ, rhythmically and spontaneously contracting throughout a woman's life. Even in the post-menopausal state, studies have shown the uterus to still be active. Karl de Vries et al have reported that rhythmic myometrial contractions were seen in ultrasound examinations of post-menopausal women in 35 studies (de Vries et al., 1990). In the non-pregnant state, the myometrium exhibits different types of contractions depending on the different phases of the menstrual cycle; from rhythmic contractions (peristalsis) to sustained contractions, which help the movement of sperm and the removal of old endometrium (Aguilar and Mitchell, 2010). Under the influence of elevated oestrogen and progesterone levels, the labouring, pregnant state sees the myometrium contracting far more forcefully to deliver the fetus. The frequency, amplitude and duration of uterine contractions

are predominantly regulated by the intracellular calcium concentration of the myocytes (muscle cells). Contraction involves the interplay of many specialised proteins, two of which are known as thin actin filaments and thick myosin filaments.

Oxytocin in Uterine Contraction

Oxytocin (OT) is an important nonapeptide hormone in stimulating uterine contraction and in lactation, where it is made in the hypothalamus and is stored and then released from the posterior pituitary gland. Oxytocin can also be synthesized in the female reproductive anatomy; in the lining of the uterus, placenta, amnion and corpus luteum. (Arrowsmith and Wray, 2014). However, OT can also be administered intravenously by clinicians as a uterotonic agent to augment labour and to treat PPH. In the body, release of OT from posterior pituitary gland is said to create a positive feedback loop, were the hormone's initial release is responsible for stimulating further oxytocin release. The stimulation of uterine contractions by OT is mediated through a g-protein coupled receptor (GPCR), known as the oxytocin receptor (OTR) (Willets *et al.*, 2009). Oxytocin binding to its receptor allows intracellular calcium (Ca^{2+}) to be released from the sarcoplasmic reticulum (SR), causing intracellular levels to rise through a series of signalling events:

Oxytocin binds to the OTR and activates a g-protein called $G\alpha_{q/11}$. Activation of $G\alpha_{q/11}$ results in the activation of the enzyme phospholipase C-β (PLC-β). This enzyme hydrolyses phosphoinositide-bis-phosphate (PIP_2) into two molecules: diacylglycerol (DAG) and inositol-tris-phosphate (IP_3). IP_3 is responsible for the release of Ca^{2+} from the sarcoplasmic reticulum. Activation of $G\alpha_{q/11}$ is also thought to open voltage gated L-type Ca^{2+} channels (VOCC) present in the cell membrane (Arrowsmith and Wray, 2014). The opening of these channels allows Ca^{2+} to pass through and enter the myocyte, thus further increasing intracellular Ca^{2+} levels. This Ca^{2+} influx in-turn causes more Ca^{2+} to be released from stores in the sarcoplasmic reticulum, again, further increasing intracellular Ca^{2+} levels. This is known as calcium-induced calcium release (Jackson and Boerman, 2018). High levels of Ca^{2+} in the cell then bind to and activate calmodulin (CaM); a calcium dependent protein found in the cytoplasm. CaM binds four Ca^{2+} ions, forming what is known as a Ca^{2+}-CaM complex. This in turn activates the enzyme myosin light chain kinase (MLCK), which goes on to catalyse the transfer of phosphate to the regulatory light chain-20 on myosin (known as MLC20), causing a conformational change to occur on the myosin had and thus activating

the myosin. This now enables cross bridges to form as the activated myosin can now bind to the actin of the thin filaments, and so shortening, or muscle contraction, can commence (Hai and Murphy, 1988). In essence, excitation-contraction coupling begins when OT binding to OTR brings about cross bridge formation and muscle contraction through increased levels of myosin light chain phosphorylation and raised levels intracellular calcium (Ca^{2+}).

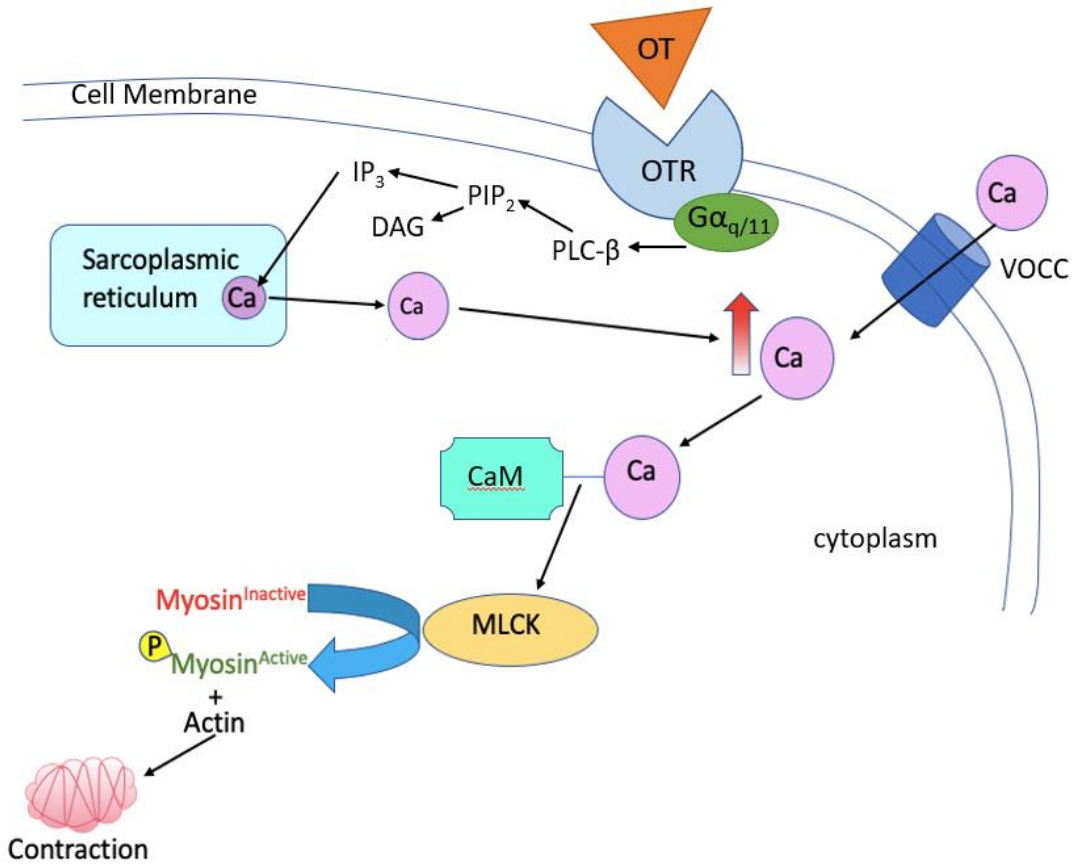


Figure 1 Stylised diagram by the author showing OT binding to OTR, activating $G\alpha_{q/11}$ which in turn activates PLC-β. This causes the hydrolysis of PIP_2 into DAG and IP_3, which enables Ca^{2+} to leave the SR. Diagram also depicts Ca^{2+} entering the cell through open L-type VOCC. This movement of calcium contributes to elevated levels of intracellular Ca^{2+}, which then leads to formation of the calcium- calmodulin complex that activates MLCK. Activation of MLCK then leads to subsequent phosphorylation and activation of MLC20, which enables cross bridges to form with actin and contraction of the smooth muscle may commence (Figure 2 modified from Wray, 2007).

Levels of oxytocin are known to increase towards the end of gestation and during labour. Myometrial OTR concentration has been shown to increase by 12 times, by weeks 37-41 gestation (Fuchs *et al.*, 1984). Regulation of OTRs are increased, causing the uterus to become more sensitive to the contractile effects of the hormone OT (Fuchs *et al.*, 1995). A 2015 systematic review was conducted on 20 articles in an attempt to summarise the narrative around how maternal OT plasma levels change during parturition, and how they may change with synthetic OT infusion (Uvnäs-Moberg *et al.*, 2019). It was found that during pregnancy, levels of OT increase by up to four times the normal basal levels. Pulses of OT levels were shown to become more frequent and larger during labour, to enable more effective uterine contraction in expelling the fetus. The maximal frequency for OT pulses is 3 pulses per ten minutes (Fuchs *et al.*, 1991). The oxytocin pulses also continue after birth and help with the delivery of the placenta and occlude exposed blood vessels to prevent PPH (Wakerley, Poulain and Brown, 1978). During labour and birth, OT levels also increase in the cerebrospinal fluid (CSF), helping to decrease anxiety levels and increase pain tolerance during labour (Moberg, 2014). However, when synthetic oxytocin is given in infusion, it does not cross the blood-brain barrier, thus explaining why women with augmented labour experience more painful uterine contractions (Clark *et al.*, 2009).

Hormonal control is one way in which uterine contractions are stimulated, but human myometrium is described as being myogenic. This means that the myometrium is capable of spontaneous contraction, even without nervous or hormonal stimulation; the coordination of contractile activity comes from within the myocyte itself. This is known as pacemaker activity, although, the exact mechanism of which is still largely unknown (Wray, 1993) . Nonetheless, it has been recently suggested that the source of the pacemaker activity may not be in the myometrial myocytes, but in cervical myocytes instead. Vink et al proposed that myocytes in the cervix may respond to stretch and relay electrical signals (via gap junctions) to the myometrial myocytes to contract, although this novel hypothesis has not yet been tested (Vink, 2020).

All myometrial action potentials result in the triggering of transient increases in intracellular Ca^{2+}, which in turn can cause the myometrium to contract (Burdyga, Wray and Noble, 2007). However, initiation of myometrial action potentials can occur in different ways. Excitation-contraction coupling (ECC) is the term which describes the sequence of events that occur,

from generation of an action potential to the initiation of contraction. The recorded resting membrane potential of myometrial cells was found to be between -35 mV and -80 mV (Sanborn, 2000). In order to reach for the threshold for action potential to be generated, it is thought that spontaneous changes occur in a cell membrane's permeability to ions, particularly sodium, potassium, chloride and calcium ions. Once depolarisation occurs, as described above, the L-type VOCCs (voltage gated L-type Ca^{2+} channels) open, allowing more Ca^{2+} to enter the cell and bind to calmodulin, and thus the rest of the excitation- contraction coupling chain of events ensues. In addition to this, low voltage oscillations of around 10mV, known as slow waves, are thought to help trigger action potentials when the cell membrane is at its least polarised.

The spread of contraction to neighbouring myocytes is facilitated through gap junctions; intercellular channels where excitation- contraction coupling (ECC) is conducted into the next cell, ensuring action potential propagation throughout the myometrium (Garfield, Sims and Daniel, 1977). The permeability and number of gap junctions has been shown to increase as the women approaches full term in her pregnancy (Garfield, Sims and Daniel, 1977). This means that the propagation and speed of ECC is rapidly increased. In addition to this gestational change in excitability, the resting membrane potential of uterine myocytes is also known to increase; from -60mV (non-pregnant) to -45Mv (full term), thus increasing the likelihood of depolarisation (Parkington *et al.*, 1999). The pattern of electrical activity in the myocyte determines the strength and length of uterine contractions, and therefore the characteristics of action potentials change as pregnancy approaches full term. In early gestation, fluctuating Ca^{2+} currents cause simple spike-like action potential as myocytes rhythmically depolarise and repolarise. In contrast, in late gestation, action potentials become plate-like in appearance, with increased periods of cell depolarisation (Kawarabayashi, 1978). These plateaus are reflective of prolonged Ca^{2+} entry and the change in expression of ion channels, such as K^+ channels, for example. In terms of uterine contractility, the plateau action potentials mean that labour contractions are now long and strong enough to deliver the baby.

As briefly mentioned above, myogenic activity is additionally controlled by ion channel expression. In 1993, Khan et al were the first to describe changed in potassium (K^+) ion channel expression, when comparing non-labouring myometrium to labouring myometrium. Calcium-activated potassium channels, known as BK_{ca} channels, are present in myometrial

smooth muscle cells, and are known to suppress contractility during gestation. During labour, however, Khan et al observed that the physiological properties of BK_{ca} channels were markedly altered, or even absent altogether. This means that mechanisms which oppose depolarisation are suppressed, leading to an increased excitability in the myometrial cells during labour (Khan *et al.*, 1993).

Relaxation of the smooth muscle is brought about through the Ca^{2+}-calmodulin-MLCK pathway being reversed. Myosin light chain phosphatase (MLCP) is an enzyme which removes the phosphate from the MLC20, resulting in deactivation of myosin. Intracellular levels of Ca^{2+} also decrease when the voltage gated L- type calcium channels close and Ca^{2+} efflux is enhanced. This results in Ca^{2+} dissociating from calmodulin and MLCK becomes inactive once again (Wray *et al.*, 2001).

Cellular Respiration

Glycolysis is a process that extracts energy from glucose, necessary for cellular metabolism. Simply put, the process of glycolysis sees one molecule of glucose being broken down along the glycolytic pathway where it is converted into two molecules of pyruvate through a series of chemical reactions.

When a glucose molecule first enters a cell, an enzyme (hexokinase) immediately works to trap the glucose within the cell by adding a phosphate group to it through phosphorylation. This phosphate is taken from the hydrolysis of ATP (adenosine triphosphate) and the glucose is now converted to a new sugar called aldose. The aldose sugar is then converted by another enzyme (phosphoglucose isomerase) to form a ketose sugar. Now the ketose is phosphorylated by a third enzyme (phosphofructokinase-1) to form a sugar known as fructose-1, 6-bisphosphate. This is the second and last step in the pathway to use ATP, hence why two ATP molecules are used in total. The enzyme aldolase now cuts the fructose-1, 6-bisphosphate sugar in two, and a triose phosphate isomerase enzyme interconverts the two halves of fructose-1, 6-bisphosphate into two molecules of glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate is then oxidised to produce 1, 3-bisphosphoglycerate where, in this oxidation step, NAD+ (nicotinamide adenine dinucleotide) is reduced to NADH (+ hydrogen). The phosphate groups in both 1, 3-bisphosphoglycerate molecules are then added to two adenosine diphosphate molecules (ADP), thereby generating two ATPs and 1, 3-

bisphosphoglycerate has also now become 3-phosphoglycerate. The newly formed 3-phosphoglycerate undergoes a phosphate group shift (aided by the enzyme phosphoglycerate mutase), from the third carbon atom to the second carbon atom, whereby the molecule is now isomerised to 2-phosphoglycerate. 2-phosphoglycerate is then converted into phosphoenolpyruvate, which in turn undergoes conversion into pyruvate, catalysed by the enzyme pyruvate kinase, and produces 2 ATP molecules. This pyruvate can then be further broken down to release energy during aerobic respiration in the mitochondria by the citric acid cycle.

However, in situations of lowered O_2 levels, where our bodies' demand for energy exceeds its ability to deliver oxygen, our metabolism will become anaerobic. In these conditions, pyruvate gets converted to lactic acid by enzyme lactate dehydrogenase through anaerobic glycolysis. The presence of lactate allows NADH to be converted back into NAD+. The presence of NAD+ is important for our cells as it acts as an oxidising agent in the glycolysis pathway, helping in the production of pyruvate. The two ATP molecules produced per glucose molecule through the glycolysis pathway, as described above, is the means by which energy can be produced in the absence of oxygen. However, in comparison the aerobic respiration, anaerobic respiration is far less efficient in producing ATP. Aerobic respiration sees a net production of 32 ATP molecules per glucose molecule, whereas anaerobic respiration sees a net gain of 2 ATP molecules per glucose molecule (Melkonian and Schury, 2021).

Acidosis and Muscle Contraction

Although it is an essential product of anaerobic respiration, lactic acid is associated with tissue acidosis. At physiological pH (7.4) lactic acid dissociates into lactate and hydrogen ions, and the increased production of H^+ ions lowers the pH of the myocytes, thus causing lactic acidosis. Therefore, lactic acid concentration in the muscles is proportional to activity level and O_2 availability (Melkonian and Schury, 2021). In 2015, a study demonstrated that muscle fatigue from contraction is associated with acidosis, and that the prior intake of sodium bicarbonate improves performance during exercise (Krustrup, Ermidis and Mohr, 2015).

Anaerobic metabolism seems to be used more in the myometrium than in other? striated muscles as the lactate content has found to be double that of skeletal myocytes (Wiberg-Itzel, 2012). Anaerobic respiration in the myometrium is caused by the intermittent periods of

hypoxia during labour contractions. These uterine contractions are known to occlude blood vessels supplying the uterus, hence repeated periods of hypoxia is a normal part of labour (Alotaibi, Arrowsmith and Wray, 2015). In fact, lactate is known to have tocolytic effects which are thought to be a protective mechanism for the fetus by ensuring adequate fetal placental circulation, thus preventing asphyxia (Wray, 2007). However, prolonged periods of hypoxia, as seen in dysfunctional labour, can lead to a build-up of lactic acid and subsequent metabolic acidosis.

Acidosis and Dysfunctional Labour

The results from a 2003 study by Pierce et al suggested that a lowered pH of human myometrium may contribute to dysfunctional labour. Researchers in the study found that intra- and extracellular acidification significantly reduces or abolishes the force of phasic contractions, whether it be spontaneous or oxytocin induced. These depressive effects may be due to the inhibition of L-type VOCC entry and inhibition of Ca^{2+} release from the SR. Pierce et al concluded that decreases in pH significantly decrease calcium signalling and force of contraction in human myometrium and thus is a contributing factor to dysfunctional labour. In the case of alkalinisation, opposite effects were produced. Traces showed that contraction frequency and tension increased and almost coalesced to become tonic when the pH was raised (Pierce *et al.*, 2003).

In 2016 Wiberg-Itzel et al wanted to see if the positive effects of sodium bicarbonate in sports medicine could be used to improve outcomes for labour dystocia. Because the lactate produced by myometrial cells correlates to the lactate in amniotic fluid, changes in amniotic fluid lactate (AFL) levels were measured in women who orally consumed Samarin® (a bicarbonate drink) one hour before oxytocin was administered. Researchers found that maternal pH had increased and pCO_2 had decreased significantly in the bicarbonate group. AFL levels were also reduced and fetal outcome was improved (Wiberg-Itzel, Wray and Åkerud, 2018).

Acidification has always been associated with a decrease in uterine contractility (Wray *et al.*, 1992). An explanation for the decrease in contractility caused by a lowered pH is to do with the calcium ion channels and the calcium levels present in the myometrium. In 1995, Shmigol et al showed a reduction in pH by 0.1 caused a 50% reduction in calcium current. They found

that the inhibition of the sarcoplasmic reticulum had no effect on the calcium changes, which was further supporting evidence for pH alteration affects ion channels - especially L type calcium channels (Shmigol *et al.*, 1995). So myometrial acidification is now widely recognised to be associated with a decrease in uterine contractility (Wray *et al.*, 1992).

A 2007 paper by Wray et al found that the acidification of the uterus found in vitro was caused not only by the biochemical changes of contraction, but also by the biochemical changes of hypoxia. Blood flow to the uterus decreases by 30-40% during contraction. The contraction-relaxation cycle corresponds with a pH cycle too as lactate production is significantly increased during hypoxic conditions (Wray, 2007) .

A 1994 study by Parratt et al set out to identify the cause of labour dystocia. Intracellular pH and force of contraction were recorded simultaneously in human myometrium in-vitro. It was found that acidification abolished uterine contractions. It was thus put forward that the resulting hypoxia from the reduced blood flow to the myometrium during each uterine contraction in labour results in a fall in intracellular pH, and that this acidification may be a main cause for labour dystocia (Parratt, Taggart and Wray, 1994).

Also, in 1994, a study by Larcombe Mcdouall et al. wanted to investigate the effects hypoxia would have on the metabolic and functional activity on smooth muscle in vivo. They focussed their attention on the effect ischaemia had on uterine metabolites, intracellular pH and force of uterine contraction. Researchers simulated the effects of occlusion of the uterine blood supply by placing occluders around the uterine artery. Their research was carried out on 5 rats; 4 out of 5 rats had a force drop in 2 mins and 1 out of 5 had a force fall in 10 minutes after occlusion. In 4 out of 5 rats, recovery increased beyond pre-occlusion levels (down to 75%, then up to 130-140%). Their nuclear magnetic resonance (NMR) data also showed that phosphocreatine and ATP levels in the tissue fell significantly (34% and 28% decrease respectively). The intracellular pH dropped from 7.32 to 7.0 during blood occlusion and was reversed upon reperfusion. It was interesting to note that the changes intracellular pH was closely mirrored by changes in uterine force. Researchers thus concluded that in vivo, the decrease in force of uterine contraction during periods of ischemia was caused by a fall in pH. Findings were particularly significant in relation to labour dystocia, as ischaemia and hypoxia occur in human myometrium too, and that an intracellular fall in pH contributes to a fall in force of contractions in ischaemia (Harrison *et al.*, 1994).

Another key study by Quenby et al obtained blood samples and myometrial strips from women having a caesarean delivery, and it was found that the pH in the uterus of the women suffering from dysfunctional labour was significantly lower, by 0.14 (pH 7.35) than the myometrium of those undergoing an elective caesarean (pH 7.49). The mothers did not have a systemic acidosis, but an acidosis localised to the uterus. Researchers also found women in the dysfunctional labour group to have a lower myometrial capillary blood oxygen saturation and a significantly higher lactate concentration than those in the elective caesarean group. When pH was lowered from 7.5 to 7.3 in vitro, the mean decrease in amplitude was 41% and mean decrease in activity was 44%. Results from this study were sufficient to conclude that the myometrial acid-base balance is associated with dysfunctional labour (Quenby *et al.*, 2004). More recent work has also shown that amniotic fluid lactate levels are also raised in women suffering from dysfunctional labour (Wiberg-Itzel *et al.*, 2008).

In 2015, a study by Hanley sought to investigate the effects of lactate on the myometrium. It was found that lactate significantly decreases spontaneous contractions and inhibits calcium transients in rat myometrium in-vitro. This is because the acidifying qualities of lactate causes an intracellular decrease in pH. If intracellular pH were maintained then lactate would have no effect on contractility. The findings from this study suggest that the accumulation of lactate extracellularly could therefore contribute to dysfunctional labour by dropping intracellular pH (Hanley, Weeks and Wray, 2015).

So far, the current literature has taught us that myometrial contractions during labour occlude blood supply to the uterus, causing intermittent periods of hypoxia. If these periods of hypoxia are prolonged, however, then a build-up of lactic acid in the myometrium may occur. An accumulation of lactic acid is known to decrease the intracellular pH, thus leading to maternal acidosis. Acidotic conditions reduce the level of L-type calcium channel entry, thus reducing Ca^{2+} transients, decreasing muscle contractility and thus decreasing the overall force of contraction. Decreased uterine contractility not only increases incidence of labour dystocia and emergency caesarean rates, but it also increases the risk of a PPH occurring.

It is hypothesised that the weak, uncoordinated contractions of dystocic labour are ineffective in occluding blood flow through the myometrium to the placental site post-partum, therefore it is expected that blood loss is likely to be higher. In order to investigate this hypothesis, data on both PPH and myometrial pH needs to analysed to assess for any correlation. Primary PPH

is the world's leading cause of maternal morbidity and mortality (Goffman, Nathan and Chazotte, 2016). There are several causes of PPH, but it is most commonly caused by the failure of myometrial contractions (uterine atony) to occlude blood vessels of the uterus after birth (Breathnach and Geary, 2009). Although there is a combination of surgical and medical interventions that provide effective treatment for this problem, a number of reports have shown that incidences of PPH are on the rise. For example, a study by Lutomski et al. observed that the overall PPH rate rose from 1.5% in 1999 to 4.1% in 2009, and that atonic PPH increased from 1.0% in 1999 to 3.4% in 2009 (Lutomski *et al.*, 2012). Despite the increase in PPH rates, the surgical and medicinal treatment available for women suffering PPH has seen almost no change over the last 50 years (El-Refaey and Rodeck, 2003). This highlights the need to identify new preventative measures and alternative therapeutics for PPH. In order to do this, the mechanism of causality between uterine atony and PPH needs to be investigated.

As discussed above, the past 30 years have seen various *in vivo* and *in vitro* studies demonstrate a clear association between uterine acidosis and myometrial contractility (Wray *et al.*, 1992; Harrison *et al.*, 1994). Although much of the data from this research have been put towards improving the management of labour dystocia (abnormal labour with weak, irregular contractions) there is also a potential for advances in both the understanding and management of PPH.

Uteroplacental Circulation

By the end of the first trimester of pregnancy, uteroplacental blood flow is fully established through the development of the placenta. The placenta receives two separate blood supplies from both the mother and the fetus, therefore placental circulation can be subdivided into the uteroplacental blood circulation (maternal-placental) and the fetoplacental blood circulation (fetal-placental). These two blood supplies come into very close contact with one another across the placental barrier, but maternal and fetal blood never mix (Wang, 2010). Maternal blood enters the uteroplacental circulation via the uterine artery. The uterine artery branches to form arcuate networks across the circumference of the myometrium (arcuate arteries), which in turn extend into smaller radial arteries, and then eventually into spiral arteries as the vasculature passes through the endometrium and into the intervillous space (D'Errico and Stapleton, 2019).

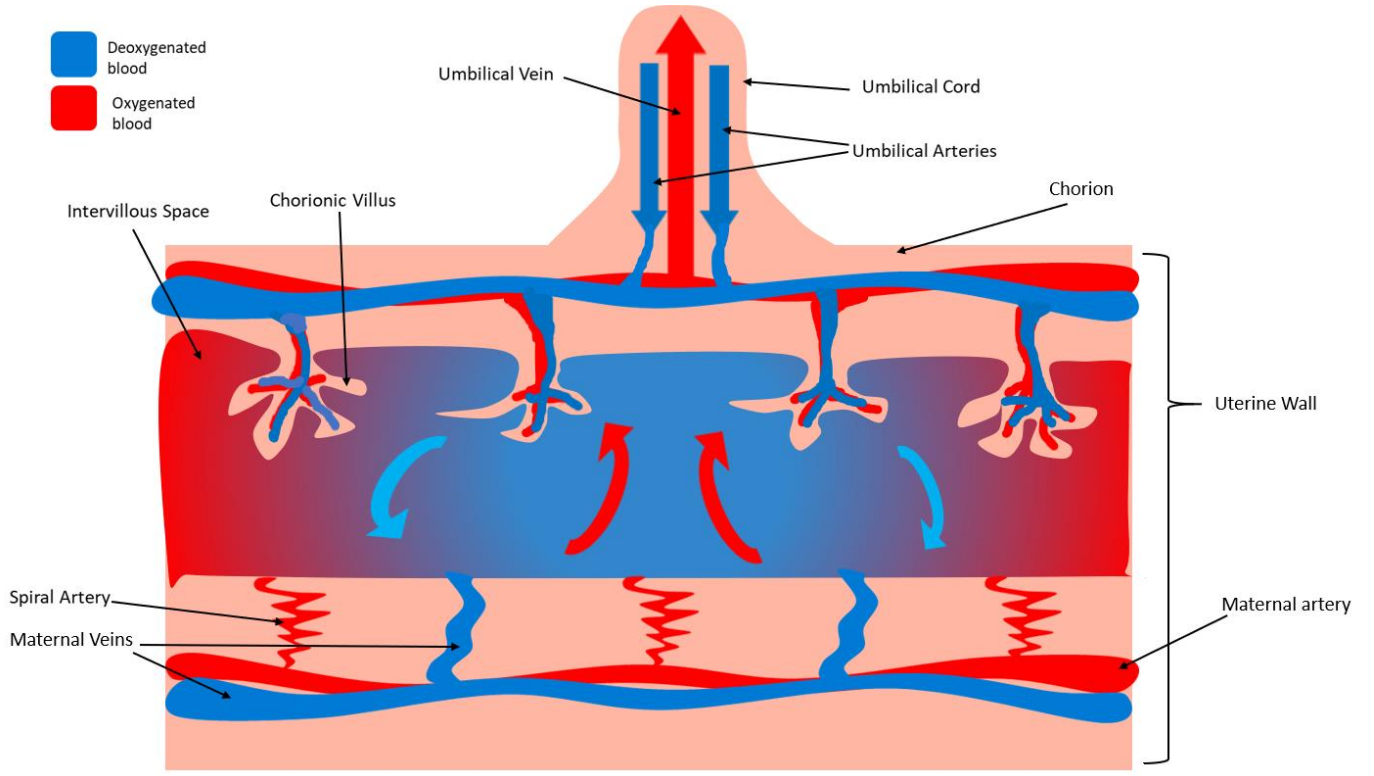


Figure 2 Stylised diagram by the author showing uteroplacental circulation and some of the vasculature within the placenta and the uterine wall. The curved arrows within the intervillous space represent the direction of oxygenated and deoxygenated blood flow. The dispersion of blood ultimately results in a mixed pool of maternal deoxygenated and oxygenated blood within the intervillous space. (Diagram adapted from Fetal Circulation - Embryogenesis and Development - MCAT Biology Review, 2022)

To meet the growing fetus' increased demand for oxygen and nutrients, by the third trimester of pregnancy the spiral arteries are converted into large bore uteroplacental arteries. This essentially creates a 'funnel-like' effect, increasing blood flow and reducing blood pressure in the intervillous space, thus increasing the rate of gas and nutrient transfer across the placental membrane. This process of decidual spiral artery remodelling gives rise to the

description of uteroplacental vasculature being ‘high flow and low resistance’ (James, Chamley and Clark, 2017).

When the oxygenated maternal blood entering the intervillous space reaches the chorionic plate, the blood then spreads laterally, mixing with the blood throughout the intervillous space. The maternal blood entering the intervillous space gradually becomes more deoxygenated as it washes over the back of the chorionic plate and around the branches of the terminal villi. Here, oxygen is exchanged for carbon dioxide, and nutrients are exchanged for waste products from the fetus, across the placental barrier of the chorionic villi. The placental barrier is formed of an outer syncytiotrophoblast cell layer and an inner cytotrophoblast cell layer which are specialised in allowing gas and nutrient exchange between maternal and fetal blood (Gude *et al.*, 2004). It is for this reason that blood in the intervillous space is said to be a mixture of oxygenated blood and deoxygenated blood, after gas exchange has occurred across the terminal chorionic villi.

The now deoxygenated maternal blood then flows back towards the decidua basalis, aided by the force of the in-flowing maternal arterial blood, and drains into the uterine veins, allowing the blood to exit the uteroplacental circulation and re-enter the maternal circulation to be re-oxygenated (Wang, 2010).

On the fetoplacental side of the placental blood supply, two umbilical arteries transport deoxygenated blood from the fetus to the placenta, and one umbilical vein transports the oxygenated blood from the placenta to the fetus. These vessels, encapsulated within a gelatinous connective tissue known as Wharton’s jelly, comprises the umbilical cord (Wang, 2010). The two umbilical arteries enter the placenta in the chorionic plate; each umbilical artery supplying one half of the placenta. From there, the umbilical arteries branch off into a number of smaller chorionic arteries that spread radially across the chorionic plate and then enter the stems of the chorionic villi (D'Errico and Stapleton, 2019).

The structure of chorionic villi can be described as ‘tree-like’ in appearance, with the main stem projecting from the chorionic plate and containing small branches of the umbilical arteries and vein. The chorionic villi also have smaller projections branching off from the main stem known as branch villi, which contain fetal capillary networks, also known as an arterio-capillary-venous system (Gude *et al.*, 2004). This ‘branching’ structure of the chorionic villi is

what provides maximal contact area between the umbilical vessels and maternal blood in the intervillous space, thus enabling efficient gas and nutrient transfer from mother to fetus.

Once gas exchange has occurred, re-oxygenated fetal blood in the capillary network of the terminal villi flows back into the fetus via the umbilical vein (Wang, 2010).

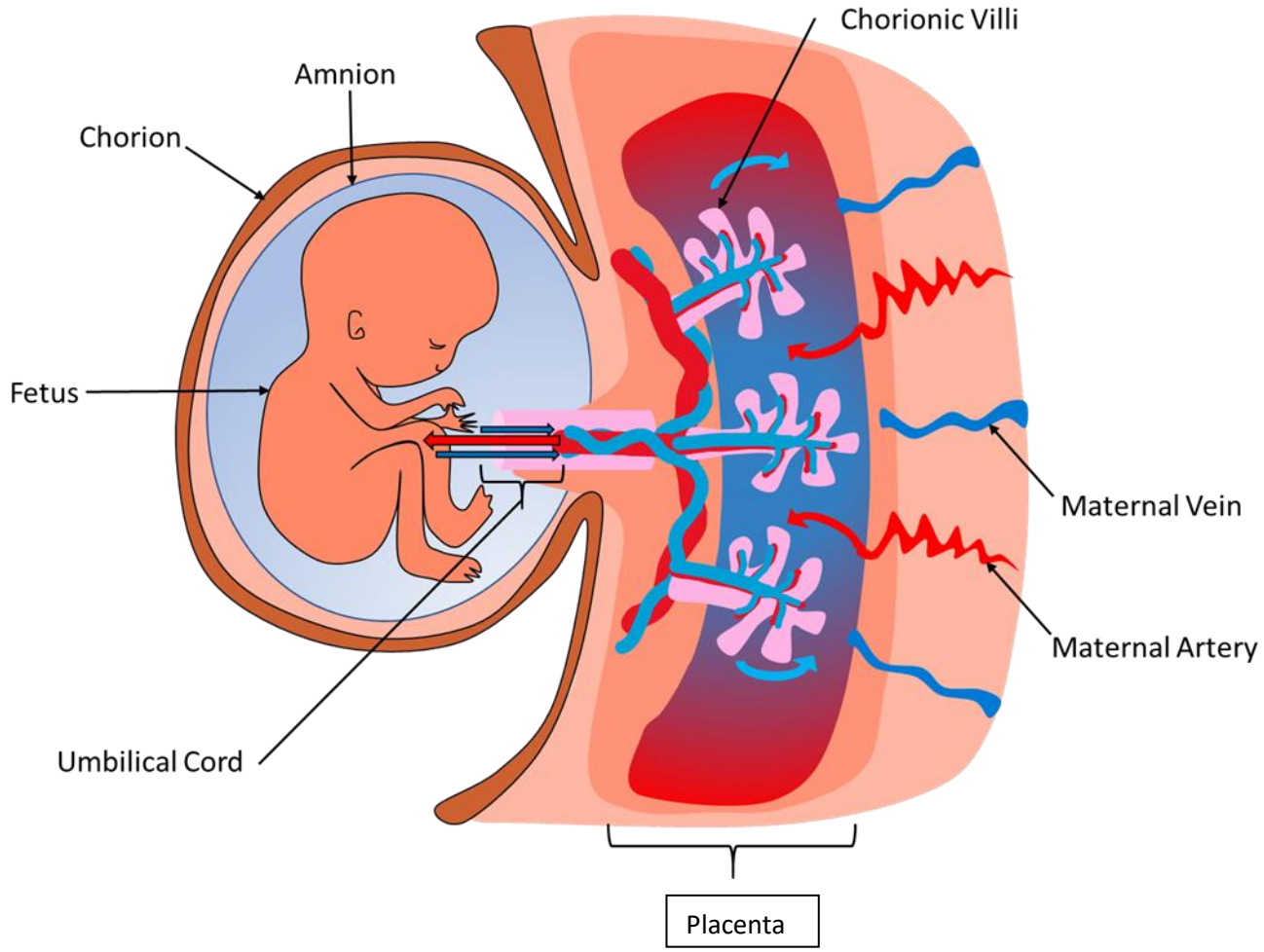


Figure 3 Stylised diagram by the author showing a simplified overview of fetal-placental circulation

MApH and fUVpH relationship

It was discussed, in the 'COVID Disruptions' chapter, that the original protocol for the PhD would have assessed the blood pH from the myometrial capillaries, taken during CS, and compared that with the volume of maternal postpartum blood loss. Myometrial capillary pH was of particular interest to the original study as it was hypothesised that as the myometrium

is the site of lactic acid production, and hence H^+ ion production. Therefore, taking blood from myometrial capillaries would be the truest representative of uterine acidosis.

When this study moved to being a desk-based project, it quickly became clear that literature containing data on myometrial capillary pH was rare to come by. Myometrial capillary pH was not something which was routinely collected in the delivery room as it was logistically complex to do so, and restricted to samples taken during caesarean sections (CS). Therefore, a surrogate measure needed to be used instead if this project was going to be investigated any further. Fortunately, mApH was occasionally measured during delivery, and fUVpH was routinely measured during delivery (more so than mApH) to assess the infant after birth. If the baby is born in poor condition (for example abnormal heart rate or poor breathing) then it is recommended that a paired umbilical cord blood gas analysis is performed (Nunes *et al.*, 2014). After searching the literature, it became apparent that fUVpH and mApH were similarly related, being typically within 0.1 pH units from one another. Therefore, the idea was put forward that in this study, fUVpH would be used as a proxy measure for mApH, and mApH itself is acting as a proxy measure for myometrial capillary pH. Of course, this is several steps removed from the primary hypothesis, that myometrial pH is a marker for PPH, so this will be discussed further as a potential limitation in the discussion chapter. However, in this current chapter, the literature that supports the hypothesis, that fUVpH and mApH are similarly related, is presented in detail.

A 1971 paper first described the relationship between fetal and maternal pH (of fetal scalp blood and maternal arterial blood) and infant Apgar scores at both 1 and 5 minutes. Authors Khazin and Hon found that fetal-maternal pH differences (n=538) could be used to predict neonatal wellbeing because small feto-maternal pH differences were correlated with high Apgar scores (Khazin, Hon and Yeh, 1971). The reason why blood sampling of the umbilical artery is favoured over sampling blood from the umbilical vein is because it primarily reflects fetal metabolism and correlates better with neonatal outcomes, whereas umbilical venous blood reflects a combination of fetal pH, placental function and maternal acid-base status. Reflecting this, the umbilical artery has a higher PCO_2 and a lower pH and PO_2 than umbilical venous blood (Westgate, Garibaldi and Greene, 1994).

It is important to note, however, that when investigating whether fUVpH correlates with maternal pH, the pH values of maternal blood must be taken from her artery. This is because

venous blood has a slightly lower pH than arterial blood due to the increased amount of dissolved carbon dioxide present in deoxygenated blood. A 1992 study has shown uterine venous pH to always be lower than that of the blood from the uterine artery (Pardi *et al.*, 1992). Furthermore, myometrial function will be affected by the incoming arterial blood whilst the composition of the venous blood will reflect both the arterial pH and myometrial muscle aerobic and anaerobic respiration. Therefore, maternal arterial pH should be considered over maternal venous pH when describing the acid-base status of the mother.

Maternal arterial pH is known to reflect the efficiency of the maternal cardio-respiratory system, where in contrast, maternal venous pH (mVpH) reflects the systematic production of hydrogen ions. Ideally, the pH of the myocytes; myometrial capillary blood pH, would be analysed for the purposes of this study. However, myometrial capillary pH is not often collected and therefore is unavailable for analysis. The uterine vein pH does reflect the myometrial pH, but also the maternal blood returning from the placenta. It is acknowledged that myometrial capillary pH is more reflective of myocyte pH, but this is only available at CS, where it can be sampled from the cut section of the myometrium. In contrast, fUVpH is routinely collected as part of fetal intrapartum monitoring, and can be accessed in large datasets for analysis. Admittedly, fUVpH is not perfectly correlated with the blood in the myometrial capillaries, but it is likely to be similar to it in pH as it equilibrates the retroplacental blood.

To supply the fetus with oxygen, maternal blood flow first enters the uteroplacental circulation by flowing through the decidual spiral arteries, and then perfuses the intervillous space, passing around the terminal villi. This in-flow of maternal arterial blood enables nutrients and oxygen to be exchanged with the fetal blood across the placental barrier, while CO_2, urea and other waste metabolites diffuse into the maternal blood and are carried away. The in-flow of arterial blood also helps push the deoxygenated maternal blood into the decidual spiral veins, where it then flows into the uterine veins and eventually back into the mother's systemic circulation. This system means that maternal blood never mixes with fetal blood; instead, the blood is separated by the syncytiotrophoblast of the placenta, which permits the passage of gasses, nutrients, metabolites and hormones between the mother and baby (Wang, 2010). Because maternal blood flowing into the intervillous space is a mixture of

oxygenated blood and deoxygenated blood, it can be assumed that intervillous blood would have a similar blood pH value to maternal capillary blood.

A study by Prystowsky et al (1961) explored the relationship between intervillous space blood pH and umbilical vein blood pH by documenting the feto-maternal pH gradient of 6 women undergoing elective caesarean sections. The intervillous blood pH (taken from the maternal surface of the freshly delivered placenta) and the umbilical vein blood pH was sampled in two groups of women; group one had fetal umbilical blood samples taken up to one minute apart from the intervillous space blood sample (n=4), while group two had an interval of 4-5 minutes in-between the sampling of the umbilical vessels and the intervillous space (n=2). Authors explained the reason for the delay in blood sampling in group 2 was due to 'a difficulty that was encountered in the delivery of the infant's head'. The difference between the pH of the intervillous space blood and umbilical vein blood was found to be 0.04, 0.0, 0.0 and 0.01 in group 1, and the pH difference in group 2 was found to be 0.07 and 0.11. The mean pH difference between intervillous blood and umbilical vein blood, in both groups, was found to be 0.038 pH units, with the pH being lower in the umbilical vein than the intervillous space in four cases, or the same as the intervillous space in two cases (Prystowsky, Hellegers and Bruns, 1961). This would reflect the excess fetal hydrogen ions from the fetal vein transferring across the placenta to the maternal blood. The similarity in the acid-base status between umbilical venous blood and maternal intervillous blood is because blood gasses equilibrate between the two compartments, meaning that umbilical vein pH represents more closely the maternal acid-base status. In contrast, the umbilical arterial cord pH represents the fetal acid-base status.

Another study that compared the blood gasses either side of the placental barrier was conducted in 1996 by Fujikura and Yoshida. The aim of the researchers was to further characterise the gas exchange in the intervillous space by measuring blood gasses from maternal and fetal blood in the placenta and uterine blood in 12 non-labouring, elective caesarean deliveries. Placental blood was collected within three minutes of its delivery and was taken from four sites; the subchorial lake, the marginal lake, the chorionic artery, and chorionic vein. Uterine vein blood was sampled before the transverse uterine incision was made. It would be reasonable to assume that chorionic vein blood pH values would be similar to fUVpH values as the chorionic vein runs from the placental lobes across the surface of the

placenta to feed the umbilical vein. The mean pH value of the blood in the chorionic vein was found to be 7.34 ± 0.07, and the mean pH value of the blood sampled from the uterine vein was found to be 7.36 ± 0.01. This means that there was a difference in mean pH of 0.02 pH units between the maternal blood and the blood which supplies the umbilical vein. Although the published manuscript gave no reference to uterine arterial pH values, the chorionic vein blood (supplying the umbilical vein) still had a minimally lower mean pH value than blood sampled from the uterine vein (Fujikura and Yoshida, 1996).

A later study investigating the maternal-fetal gradient at birth, was carried out on 15 women undergoing elective caesarean sections under general anaesthesia. The maternal-fetal gradient at birth was described as the difference between the maternal (radial) arterial pH (mApH), pCO_2 and bicarbonate levels and that of the blood leaving the placenta via the umbilical vein. The women in the study were subject to an infusion of an acidic salt, ammonium chloride (NH_4Cl) so to induce maternal acidosis. This allowed the researchers to observe the effects of maternal acidosis on the fetus. The women were divided into three groups; 1) a control group (n=9); 2) slow infusion of NH_4Cl (0.8 mEq per minute, n=3); and 3) rapid infusion of NH_4Cl (1.6 mEq per minute, n=3). The mean difference between mApH and fUVpH in the control group was found to be 0.06 pH units (mean mApH being 7.37, and the mean fUVpH being 7.31). For group two, the mean difference in pH was 0.04 pH units and the mean difference was 0.00 pH units for group three. The decreasing difference in pH, as observed in groups two and three, was because the maternal pH was decreased by NH_4Cl but the fetal pH remained relatively unaltered, therefore the transplacental pH gradient was virtually eliminated. This suggests that maternal metabolic acidosis may not necessarily be accompanied by fetal metabolic acidosis. The authors concluded that, based on the current research and previous research on animal studies, the placental barriers are not very permeable to electrically charged HCO_3-, but are more permeable to uncharged CO_2 molecules. These results suggest that if the mother were to become acidotic in labour (which could potentially increase risk of a PPH) the fUVpH would not change. Based on this research, fUVpH values would therefore not accurately detect any maternal acidosis (Blechner *et al.*, 1967).

Despite this, the majority of research contradicts these 1967 findings, hence why the decision to use fUVpH as a surrogate measure for mApH was used in this study. For example, a slightly

larger study published in same year (1967) found that maternal arterial pH values were minimally higher than umbilical venous pH values. Hollmen et al. sought to assess the effects of general anaesthesia and caesarean section on fetal acidosis. Maternal capillary pH and fUVpH were collected at birth as part of this study. The 35 participants were divided into three groups; group A) elective caesarean (no signs of fetal distress of placental insufficiency, n=12), group B) emergency caesarean (suspicion of fetal distress and/ or placental insufficiency, n=12) and group C) spontaneous vaginal delivery (no signs of fetal distress or placental insufficiency, n=11). The maternal blood samples were taken from the fingertip before anaesthesia, and fUVpH was taken no more than 1.5 minutes after birth using a syringe, after the infant had cried and before the cord was clamped. It was found that the difference between mean maternal capillary pH and mean fUVpH in group A was 0.12 pH units, in group B the difference was 0.07 pH units and in group C the difference was 0.08 pH units. In each case, the mean fUVpH was lower than the maternal capillary value at birth. The results from this study could suggest that using fUVpH as a surrogate for mApH might not be as robust as initially thought (Hollmen, Jagerhorn and Pystynen, 1967).

In 1972 Hollmen et al. conducted another maternal-fetal acid base study on 27 women. This time the researchers wanted to investigate whether increased partial pressure of CO_2 in maternal blood during a C-section (under general anaesthesia) improved fetal-acid base parameters. The women in the study were divided into 3 groups; A) healthy women (n=11) B) women with a history that suggested uteroplacental insufficiency (n=10), and C) a control group (n=6). The women in groups A and B inhaled a 10 % carbogen mixture (90 % O_2 + 10 % CO_2) five minutes before administration of anaesthesia. After three minutes of carbogen inhalation, N_2O was added at a ratio of 5:3 for group A (N2O: carbogen) and 4:4 for group B right up until birth, 15 minutes later, in order to increase maternal PCO_2.

The published manuscript provides little data on whether the samples were from elective caesarean section or emergency caesarean section. However, the authors did mention that some women were operated on after the onset of labour, and that 8 of the 10 women in group B already had started labouring. None of the women in group C were labouring and details were not given for group A. Maternal blood samples were taken from the radial artery before inhalation of the carbogen mixture and again at the moment of birth, while the umbilical venous blood sample was simultaneously aspirated into a 2ml syringe “at the

moment of delivery", before the infant's first breath. Blood samples were kept on ice and the pH was analysed within 5 minutes of collection using a Radiometer (PHM) pH meter.

The table below shows maternal and fetal blood pH values (mean ± S.D) in the three groups of women prior to administration of general anaesthesia and then at the time of delivery. The groups in the table are ordered as follows:

Maternal blood samples:

1= maternal arterial pH prior to carbogen mixture inhalation

2= maternal arterial pH at birth (15 minutes after carbogen inhalation)

Fetal blood samples:

3= fetal capillary blood drawn prior to carbogen mixture inhalation

4= umbilical venous at birth

5= Umbilical arterial at birth

Group	No. of cases	pH				
		1	2	3	4	5
A	11	7.47 ± 0.07	7.28 ± 0.08	7.33 ± 0.05	7.27 ± 0.07	7.23 ± 0.08
B	10	7.42 ± 0.05	7.21 ± 0.07	7.31 ± 0.07	7.21 ± 0.07	7.19 ± 0.08
C	6	7.45	7.44		7.31 ± 0.11	7.25 ± 0.11

Table 2: Table from Hollmen et al 1972 with pH values and standard error for maternal blood samples and fetal blood samples.

The difference in mean pH between maternal arterial blood at birth (column 2) and umbilical venous blood at birth (column 4) is 0.01 pH units for group A, 0.0 pH units for group B and 0.13 in group C. Of particular interest is the mean pH difference of the control group (group C) as it most closely reflects normal physiological conditions. Findings from this study provide good evidence that umbilical vein pH is reflective of maternal systemic pH; that with maternal acidosis, fUVpH accurately reflects maternal radial artery pH, whereas the correlation is poorer in physiological conditions. This research also shows how fUVpH is reduced when the mother becomes acidotic (Hollmen and Jagerhorn, 1972).

In a recent prospective-observational cohort study, researchers determined normal values for maternal arterial blood gasses during vaginal deliveries (n=250) and compared them to control values of women having elective caesarean sections (n=58). Blood pH data was collected on both maternal arterial blood and umbilical venous cord blood in both the vaginal delivery group and the planned caesarean group. In the vaginal delivery group, the maternal blood sample was taken at the time of birth from the mother's right radial artery simultaneously with the collection of umbilical cord blood using two millilitre pre-heparinized syringes. In the planned caesarean section group, the anaesthesiologist collected the maternal arterial blood gas sample simultaneously with the sampling of the umbilical cord blood immediately after delivery. In both groups, to ensure optimal quality of analysis, fetal samples were taken immediately after the delivery of the infant, but before the first cry, and all blood samples were analysed within 10 minutes of collection. Researchers found that the maternal arterial pH values correlated significantly with pH values of venous umbilical cord blood ($P < 0.000$) with the correlation coefficient (R^2) being 0.22 (Zaigham *et al.*, 2020).

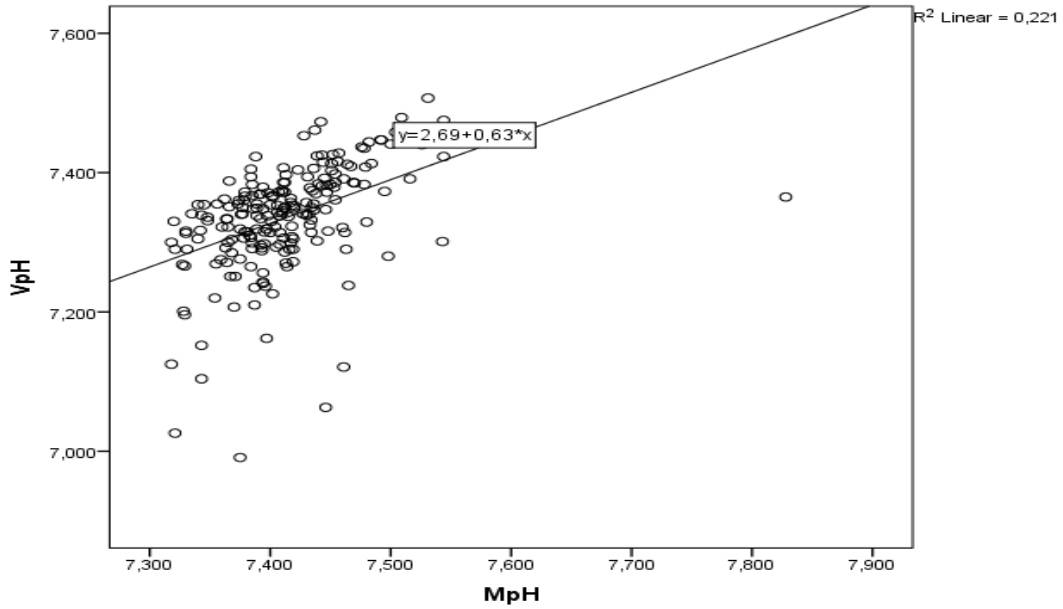


Figure 4 Scatter plot from Zaigham et al., 2020 showing umbilical venous pH plotted against maternal arterial pH. It can be seen that maternal arterial pH values correlate with pH values of venous umbilical cord blood ($P < 0.001$) with $R^2 = 0.22$.

The correlation is r not r^2, taking square root of r^2 gives correlation of 0.47. Also, p value relates to test that correlation coefficient is different from zero and not significance of correlation. This is usually subjective a value greater than or close to 0.5 is reasonable.

7-point diagram

The 7-point diagram below helps explain the theory behind using fUVpH as a surrogate for mApH in a visual format. Point 4, 6 and 7 on the diagram represents the maternal artery (mApH), maternal capillary and maternal vein pH, respectively. Maternal capillaries, point 6, contain a mixture of oxygenated blood coming from point 4, the maternal arteries, and gradually becoming more deoxygenated (as in point 7; the maternal vein) as the blood passes through them. For this same reason, point 5 should also similarly reflect point 6 as it represents maternal oxygenated blood becoming gradually de-oxygenated as it flows over the chorionic plate. Point 3 (the fUVpH) and point 2 should therefore be similar in pH to one another as the fetal blood in the chorionic plate (point 2) is now richly oxygenated and it will leave the placenta via the umbilical vein (point 3) to supply the fetus. Likewise, point 2 and 4 should be similar in pH to one another as they're both carrying richly oxygenated blood, thus making point 3 (fUVpH) also representative of point 4 (mApH).

Point 1, umbilical arterial pH, is less likely to be representative of point 4 as it is deoxygenated, returning from the fetal circulatory system. Fetal umbilical arterial blood is now more acidic, and needs to be re-oxygenated in the uteroplacental circulation in order to restore the fetal-maternal blood pH does.

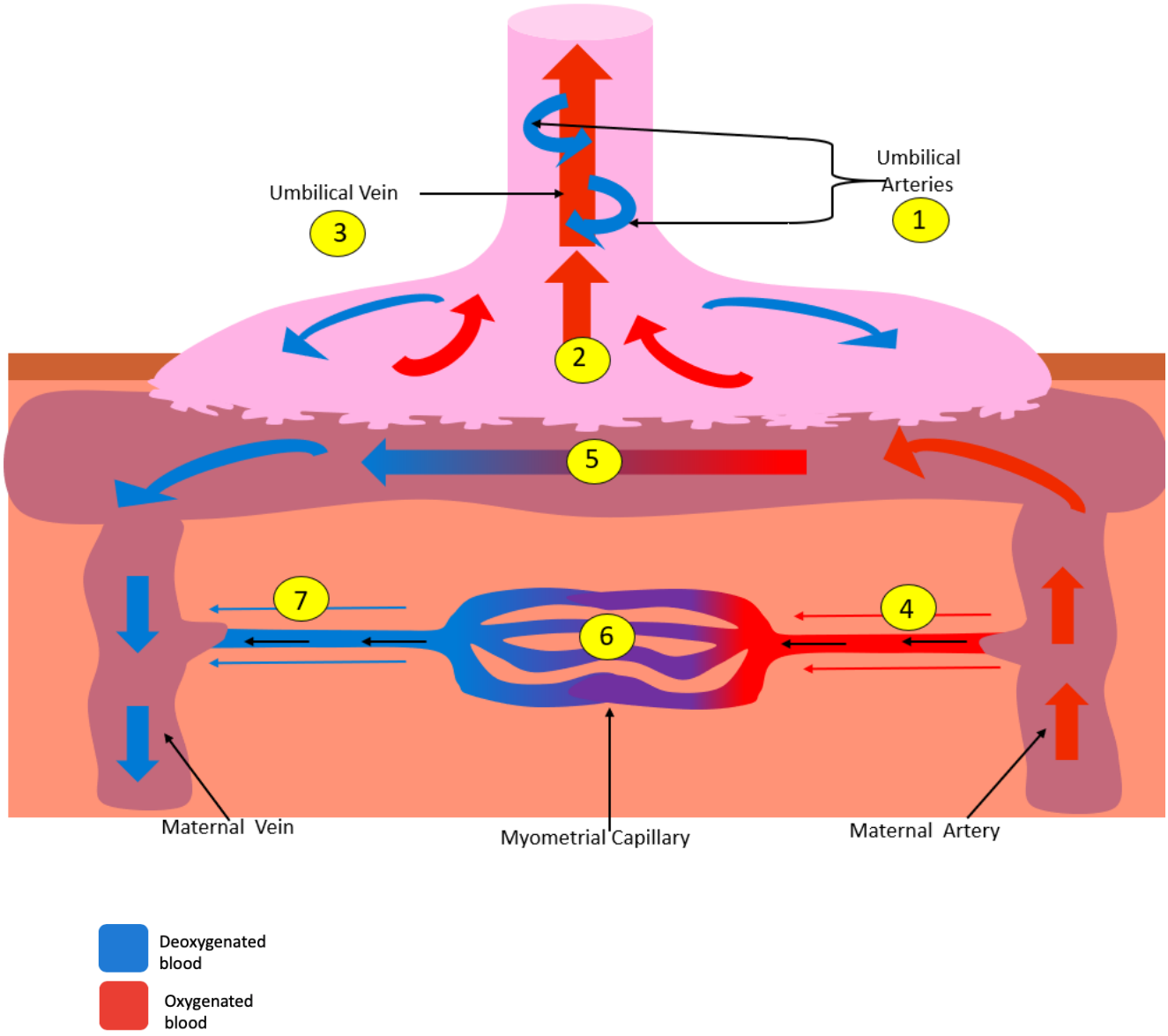


Figure 5 Simplified drawing by the author showing how oxygenated blood becomes more deoxygenated blood as it passes through the uteroplacental circulation. Key: blue represents deoxygenated blood, red represents oxygenated blood.

The above diagram illustrates the various blood pH fluctuations as it passes through the utero-placental circulation. Circled numbers correspond to a blood pH value at each of the seven stages:

1. Fetal blood in the umbilical arteries
2. Fetal blood in the chorionic plate
3. Fetal blood in the umbilical vein
4. Maternal blood from maternal spiral arteries

5. Representation of maternal blood gradually becoming deoxygenated as it washes over the back of the chorionic plate
6. Representation of oxygenated blood becoming deoxygenated blood as it passes through the maternal capillaries.
7. Maternal blood in maternal vein

It is well known that fetal haemoglobin has a higher affinity for O_2 than adult haemoglobin. This difference in affinity can be seen in a left shift on the oxyhaemoglobin dissociation curve, and it ensures that fetal oxygen uptake can occur at a lower pO_2 (partial oxygen pressure) (Pritišanac *et al.*, 2021). In other words, fetal haemoglobin has the ability to bind oxygen more readily from the maternal blood, across the placenta barrier. When relating this knowledge to the 7-point diagram, page 39, point 2 on the fetal side would thus be slightly more oxygenated than point 5 on the maternal side, rather than the oxygen levels both point two and point 5 being in total equilibrium. This also means that point 3, the umbilical vein, would be also be more oxygenated than point 5, hence why it can be said with a degree of confidence that point 3 would be similar in oxygen saturation to point 4; that fUVpH reflects mApH.

However, it must be noted that this does not translate directly when discussing the transfer of H^+ ions. Unlike oxygen, fetal blood is not known to have a higher affinity for H^+ than maternal blood. This means that the spread of H^+ would be equal over the placental barrier. Deoxygenated blood with a lower pH, and thus more H^+, arrives from the umbilical arteries (point 1). These H^+ are then shared with the maternal blood (point 5) and taken away into the maternal circulation, thus ensuring the fetus doesn't become too acidotic. The umbilical vein (point 3), would contain fewer H^+ ions, explaining why umbilical arterial blood is more acidotic than the umbilical vein blood, whereas the blood in the maternal artery (point 4) is relatively deplete in H^+. Because H^+ equilibrium does not behave in the same was as oxygen across the placental barrier, it could pose a problem to the assumption that mApH is similar to myometrial capillary pH, and therefore be another limitation of this study. This will be discussed in more detail in the Discussion chapter.

Defining PPH

It was important to decide on how to define PPH so as to determine what class of PPH (moderate or severe) belonged to the type of delivery (vaginal or caesarean). For this study, a vaginal PPH was defined as a total blood loss of >500mls, a caesarean PPH is a blood loss of >1000mls, and blood loss of >1500ml is a classed as a severe PPH (SPPH), regardless of the mode of delivery. This is the PPH definition specified by the World Health Organisation, and is also the most frequently cited in current literature (WHO, 2012). However, more recently the research community appears to be using 1000ml blood loss as a cut-off for all births as a definition for PPH. In 2014, the American College of Obstetricians and Gynaecologists (ACOG) has sought to redefine the definition of PPH. The updated PPH definition was changed to a cumulative blood loss ≥1000ml with signs hypovolemia within the first 24hours of delivery, regardless of the mode of the mode of delivery (Menard, Main and Currigan, 2014). Therefore, this analysis will be conducted twice; the main analysis will have the traditional definition of PPH being defined as ≥500ml blood loss for vaginal birth ≤1000ml blood loss for CS. A second 'sensitivity analysis' will then be done using ≥1000ml for all births to see if it changes the results**.**

The definitions of PPH were decided upon after searching the literature. At present, there is no single, universal definition of what is classed as a PPH and definitions vary quite widely. There is a need for a consensus in terms of PPH definition, as it would help medical professionals to have a more accurate estimation of the speed and extent of the haemorrhage, and rapidly implement an appropriate action plan (pharmacological and/or surgical treatment).

Visual estimation of blood loss

Most articles cite that PPH is defined as the amount of blood the woman loses post-delivery. Blood loss amount is most commonly estimated visually, which commonly leads to an underestimation of the actual amount of blood lost. A study conducted in Thailand, 2000, set out to evaluate the incidence of PPH in vaginal deliveries, comparing direct measurement of blood loss with visual estimation. The research team found the incidence of PHH (defined as blood loss ≥500ml) to be 27.6% with measured and 5.7% with visual estimation. These findings meant that visual estimation of the incidence of PPH was underestimated by nearly 90% (Prasertcharoensuk, Swadpanich and Lumbiganon, 2000).

Underestimation in blood loss commonly occurs in obstetric practice due to hidden loss in things like the bed linen, in swabs and pads and in the drapes used during caesarean section. Blood loss is also more likely to go un-noticed if it occurs as a slow, steady trickle, rather than a more obvious heavy haemorrhage. In addition to this, other bodily fluids, such as urine and amniotic fluid can contaminate any collected blood loss and cause an inaccurate blood loss measurement (Nelson *et al.*, 1981). In 1981, research was carried out looking into the volume of contaminating fluid in post-partum blood loss. It was found that the amount of contaminating fluids ranged from 4% to 81% of the total volume of fluid collected. Such a large variation in contaminant fluids does highlight how difficult it can be to accurately estimate the total volume of blood loss (Nelson *et al.*, 1981).

However, underestimating blood loss can have severe consequences for the health of the mother. SPPH is believed to be the most common cause of serious maternal morbidity. The medical effects of such a high volume of blood loss can include coagulopathy, renal failure, myocardial ischemia, shock and hysterectomy. Longer term associated morbidities includes anaemia, which can cause serious health problems, particularly in lower income countries (Rath, 2011). In women suffering from severe anaemia, a blood loss of just 250ml could result in the same adverse clinical complications as a woman with a normal haemoglobin value who may lose a much higher volume of blood (Lawson, 1967). Often these classic signs of too much blood loss, do not present until the total amount of blood loss has reached a life-threatening amount. This is why it is so important that diagnosis and initial treatment should happen before any changes in vital signs may occur (Patel *et al.*, 2006).

Blood loss measurements

One method of measuring the amount of blood loss is known as the gravimetric method. This is where the dry weight of the gauze (placed under the labouring women) is subtracted from the blood-soaked weight of the gauze, and then its weight in grams in converted into millilitres of blood loss. This is more accurate that visual estimation. However, the lack of international standardisation of the weight and size of the pads, gauzes and sponges and contamination with liquor produced at the time of birth, means that inaccuracies can also arise from this method of measuring blood loss (Johar and Smith, 1993). Many clinicians use the gravimetric method in combination with other blood loss assessment methods, rather than a stand-alone method, to get a more accurate representation of total blood volume lost.

Another method of measuring blood loss is by using calibrated bags to collect the blood loss (the volumetric method). The calibrated bag, BRASSS-V drape, has been found to be significantly more accurate than visual estimation of blood loss. Patel et al compared drape estimation with visual assessment for estimating PPH. It was found that the drape method was 33% more accurate, with PPH diagnosis occurring 4 times more often with this method than with simple visual estimation (Patel *et al.*, 2006) .

A Drop in Haematocrit

Another way many clinicians define PPH is by a change in haematocrit: this a drop in the ratio of the total volume of blood to the volume of red blood cells. In many hospitals, haematocrit levels are determined routinely where the equipment is available. The American College of Obstetricians and Gynaecologists state that a 10% post-delivery fall in haematocrit concentration, when compared with pre-delivery concentrations, can be used as a secondary definition for PPH (Dunn, Heinrichs and Lynch, 2006).

A case control study, looking at the risk factors of PPH, used a drop in haematocrit of ten points or more, or the need for a red-cell transfusion as an alternative definition for PPH in their research. Combs et al pointed out that defining PPH by a change in haematocrit has several advantages. Benefits include being relatively precise, simple to determine, and that it is routinely measured in postpartum women (Combs, Murphy and Laros Jr, 1991). Although haematocrit changes following a vaginal delivery have been shown to have a negative association with visual estimations of blood loss, haematocrit changes are not always useful as a diagnostic tool in emergency situations. This is because there is a delay, of up to 4 hours or more, during which acute blood loss is not yet reflected by a drop in haematocrit. The peak drop for haematocrit, or haemoglobin levels is often seen up to 2-3 days postpartum (Kodkany, Derman and Sloan, 2006). This means that rapid blood loss could lead to an obstetric emergency before any changes are reflected in haematocrit levels. Furthermore, it is also complicated by the use of intravenous infusion of fluids during a PPH which reduces the haematocrit artificially.

A recent 2020 study evaluated the efficacy of serum lactate being used as a prognostic marker for SPPH. Interestingly, Basil-Kway et al found 2.6mmol/L serum lactate to be a promising predictor of SPPH, and a potential biomarker to identify patients at risk of PPH and its related

complications. Authors used the universally accepted definition of PPH as a blood loss of 500ml or more after a vaginal birth and a loss of 1000ml or more after a CS delivery. In the case of SPPH, authors classified it as a blood loss of 1500ml or more at the end of labour or within 24 hours following delivery (Basil-Kway *et al.*, 2021).

Hemodynamic Instability and Erythrocyte Transfusion

Some clinicians believe that any blood loss that results in hemodynamic instability, or clinical shock, should be considered PPH. Of course, this means that the volume of blood loss that may cause hemodynamic instability would depend on the health condition of the woman. As mentioned before, it is not just about the absolute volume of blood loss that matters in preventing haemorrhage related complications. The health of the mother also greatly depends on her health status at the time of haemorrhage. Studies have shown that women who are healthier and live in higher income countries are generally able to tolerate much higher levels of blood loss without showing any significant hemodynamic issues (Hofmeyr and Mohlala, 2001). It has also been shown that women giving birth in lower income countries have a higher chance of having more comorbidities prior to delivery, such as anaemia, and thus are at a higher risk of having a poorer outcome following a PPH. A 2003 paper by El-Rafeay et al states that a blood loss of just 250ml in a woman with severe anaemia would have the same adverse clinical outcome as a much higher volume of blood loss in a patient with normal haemoglobin levels (El-Refaey and Rodeck, 2003). A consequence of hemodynamic instability is the need for a blood transfusion. Using this to help define PPH as a need for can also cause problems as the practice of blood transfusion varies widely across the world. Blood transfusion can depend upon supply, individual judgement of the clinicians and the acceptance of the patient. The need for blood transfusion isn't therefore useful for defining PPH as the practice of transfusing blood varies widely (Borovac-Pinheiro *et al.*, 2018).

Despite the lack of a consensus on a PPH definition in the current literature, a single, clear definition was necessary for the statistical analysis. An estimated blood loss of more than 500ml for a vaginal birth, and an estimated blood loss of more than 1000ml for a caesarean birth, would be classed as an obstetric haemorrhage. This definition appeared to be the most widely accepted diagnosis for PPH in current obstetric practice. (Green-top-Guidelines, 2016; WHO, 2012)

Causes of PPH

The main causes of primary PPH remain clearly defined in a mnemonic known as 'the 4 T's'. These are; tone (such as uterine atony), trauma (to the genital tract or perineum), tissue (such as retained placenta) and thrombin (relating to blood clotting).

Tone

Uterine atony is the most prevalent cause of PPH and it is estimated that it is responsible for 70% to 80% of PPH cases (Rogers *et al.*, 1998). The main physiologic process that occurs to prevent excessive bleeding from the placental implantation site are strong, effective uterine contractions of the myometrium (Oyelese and Ananth, 2010). Due to the anatomy of the muscle fibres in the myometrium, the longitudinal and circular myometrial fibres contract in a way so as to constrict the spiral arteries and veins that provide blood to the underside of the placenta throughout pregnancy. When the placenta has detached from the uterine wall, but uterine contractions are not strong or regular enough to occlude blood supply, it is known as uterine atony, and PPH is highly likely to ensue as a result (Khan and El-Refaey, 2006).

There are several risk factors associated with uterine atony. A common association with atony is an over extended uterus, which in turn can be caused by polyhydramnios (too much amniotic fluid), multiple gestations (twins, triplets) or even a fetus which is much larger than average (fetal macrosomia). Another risk factor for uterine atony is to do with the nature of the woman's labour. Rapid labour may cause the vigorously contracting uterus to tire too quickly, whereas prolonged labour can also lead to uterine muscle fatigue. A common way to combat uterine atony is to augment or induce labour, either with tocolytics or oxytocin, but these too carry their own risks which may contribute to atonic PPH (Oyelese and Ananth, 2010).

For women at risk of uterine atony, active management of the third stage of labour is frequently recommended as a prophylactic measure against PPH. The three main components of active management of the third stage of labour are; administration of oxytocin or a uterotonic agent, early cord clamping and controlled traction of the umbilical cord (Breathnach and Geary, 2009). Another way in which clinicians can stimulate uterine contraction is through uterine massage and bimanual compression (BMC). BMC is where a single clinician places one hand one the woman's abdomen (above the uterine fundus),

applying pressure, while the other hand is placed inside the vagina and pressure is applied internally against the body of the uterus. This technique is intended to compress the haemorrhaging vessels within the uterus, and it is hoped that the flow of blood will eventually cease (Andreatta *et al.*, 2011). Although BMC is known to be highly effective in stopping PPH, without an epidural already in place the manoeuvre can be quite painful for the mother, as well as tiring for the clinician to sustain long enough to achieve haemostasis. Fortunately, a low-cost, novel medical device, known as the PPH butterfly (PPHB), has been recently designed which is intended to be less invasive than BMC, but just as effective in bleeding cessation. Furthermore, the ergonomic efficiency of the PPHB design means that users are able to use their weight to stabilise the device against the bed, as well as putting sustained pressure on the uterus. This allows clinicians to use this device for longer without tiring as quickly as with BMC. It is hoped that this device will allow PPH treatment to be more widely practiced in low resource settings, where most of the PPH related deaths occur. The PPHB design is based on a combination of a shelf pessary (used to treat uterine prolapse) and a speculum, providing a surface in which the uterus can be compressed against from external pressure on the abdomen (Cunningham *et al.*, 2017). In 2021, 57 women were recruited to trial the efficacy and safety of the PPHB. Study participants were suffering a PPH following a vaginal birth and were unresponsive to oxytocin administration. Afterwards, participants, birth partners and attending medical professionals were interviewed about their experience of PPHB. Overall, it was found that the device was viewed positively, and that PPHB was useful in both stopping and diagnosing the source of the bleeding (Weeks *et al.*, 2021).

Trauma

Another common obstetric complication which can lead to a PPH is obstetric trauma, often caused by genital tract, uterine and perineal lacerations which occur during operative vaginal births, spontaneous vaginal births and Caesarean deliveries. This is the most likely cause of obstetric haemorrhage if uterine atony is not responsible.

During delivery, the genital tract is likely to be subject to physical trauma which may be iatrogenic in nature (caused by medical intervention). This includes lacerations from instrumental delivery (vacuum or forceps), episiotomy or caesarean section. Al-Zirqi et al. found that elective caesarean sections doubled the risk of SPPH and emergency caesarean deliveries tripled the risk of SPPH (blood loss >1500 mL) compared with that of vaginal births

(Al-Zirqi *et al.*, 2008). Genital tract trauma may also be caused by spontaneous delivery, such as perineal tear, uterine rupture or uterine inversion. Either of these causes of trauma can be responsible for a large amount of blood loss, thus increasing the likelihood of PPH (Chandraharan and Arulkumaran, 2008).

Tissue

The final 'T' in the PPH pneumonic stands for tissue, and is it associated with the retention of products of conception. Retained placenta, the failed separation of the placenta from the uterus within 1 hour after birth (WHO, 1990), may increase PPH risk by preventing the uterus from contracting strongly enough to occlude blood vessels. On average, the time between delivery of the fetus to delivery of the placenta is between 8 and 9 minutes (Magann *et al.*, 2005). However, time intervals which exceed this are known to be associated with a higher risk of PPH. In a 2005 study, researchers showed that where PPH rates double after ten minutes (OR 2.1, 95% CI 1.6-2.6). At 20 minutes the risk doubled again, (OR 4.3, 95% CI 3.3-5.5) and at 30 minutes, the risk of a PPH were 6 time that of delivering within the optimum time frame (OR 6.2, 95% CI 4.6-8.2) (Magann *et al.*, 2005). Retained placenta is known to occur in 0.5 to 3% of vaginal births, resulting in a mortality rate between 3-6% in more remote areas (Weeks and Mirembe, 2002).

Often the first line of management for this obstetric complication is to inject the umbilical vein with an oxytocin solution, in the hope that it will improve uterine tone (Nardin, Weeks and Carroli, 2011). However, if this fails, physicians may proceed with manual removal of the placenta. This can be done using the Brandt-Andrews manoeuvre, where the physician applies firm traction to the umbilical cord, while simultaneously applying counterpressure pressure externally on the suprapubic region of the mother(Anderson and Etches, 2007). If this manoeuvre still fails to extract the placenta then a more invasive, surgical route may be considered.

Thrombin

Another important physiologic process that helps prevent PPH is blood clot formation. In terms of the body's defence mechanisms against PPH, coagulation is only secondary to uterine contraction, and therefore a PPH may still occur if the woman is suffering from uterine atony but has a healthy blood coagulation system. The PPH's that are primarily caused by a

coagulation defect are most common in late PPH (>24 hours after delivery). Coagulation defects may be inherited, such as von Willebrand disease, or they may be acquired during pregnancy and birth. For example, certain pregnancy complications can increase a woman's risk of disseminated intravascular coagulopathy (DIC), which in turn increases the risk of placental abruption (where the placenta prematurely separates from the uterus) and this can then lead to obstetric haemorrhage (Tikkanen, 2011). A 2007 study showed that coagulation defects increase PPH risk. James and Jamison were able to show that women with Von Willebrand disease were significantly more likely to have a PPH and 5 times more likely to receive a blood transfusion after birth than those without the disease (James and Jamison, 2007)

Coagulopathies are most often identified before delivery so to allow for advanced preparation for PPH prevention. Doctors may even consider administrating blood clot promoting medication (such as tranexamic acid, TA) to reduce haemorrhage risk (Alamia and Meyer, 1999). In 2011, a RCT trail set out to assess the efficacy of a high dose of TA in reducing blood loss due to PPH. It was found that in the TA group (median 173ml, n=72), blood loss was significantly less than the control group (median 221ml, n=72) (P = 0.041) (Ducloy-Bouthors *et al.*, 2011).

Risk Factors for PPH

As mentioned earlier, this study is a secondary analysis of previously collected obstetric data from the Lausanne University Hospital, which was collected over a ten-year period. It is a privilege to have permission to re-analyse data on over 33,000 women. However, it is important to provide a justification as to which predictor variables were considered for the multivariate analysis by researching as much of the available literature as possible. Some confounding variables that are known to be associated with increased risk of PPH may not be included in the multivariate analysis. This is either because there is not enough data on these variables in the original database, or their association with PPH is too weak.

Using the current literature, a brief justification is provided for each of the predictor variables that are considered for the multivariate analysis.

Previous PPH

History of previous PPH is an important risk factor for recurring PPH. A 2010 population-based study from Australia also demonstrated that prior PPH is an important risk factor for recurring PPH. The study used birth and discharge records from 125,295 women to observe the occurrence and recurrence of PPH. Of the total 5.8% women who had a PPH in their first pregnancy, 14.8% of those women had a PPH again in their second pregnancy. This meant that the risk of a PPH in the second pregnancy, following a PPH in their first pregnancy, was three times higher for these women (relative risk, 3.3; 95% CI, 3.1-3.5). Authors Oyelese et al also noted that for those women who had a PPH in both their first and second pregnancy then had a 21.7% chance of having a PPH again in their third pregnancy. Women who had a PPH in their first pregnancy, but did not suffer a PPH in their second pregnancy still had an 10.2% increased risk of having a PPH in their third pregnancy (Ford *et al.*, 2007).

Coagulation Abnormalities

Another important physiologic process that helps prevent PPH is blood clot formation. In terms of the body's defence mechanisms against PPH, coagulation is only secondary to uterine contraction, and therefore a PPH may still occur if the woman is suffering from uterine atony but has a healthy blood coagulation system. The PPHs that are primarily caused by a coagulation defect are most common in late PPH (>24 hours after delivery) (Oyelese and Ananth, 2010).

Coagulation defects may be inherited, such as von Willebrand disease, or they may be acquired during pregnancy and birth. For example, pregnancy complications can increase a woman's risk of placental abruption (where the placenta prematurely separates from the uterus) which can then lead to disseminated intravascular coagulopathy (DIC), which in turn increases the risk of obstetric haemorrhage (Tikkanen, 2011). It is also common for DIC to complicate a PPH once the blood loss reaches about 1500mls, which thus exacerbates the problem.

A 2007 study showed that coagulation defects increase PPH risk. Von Willebrand disease is known as one of the most common coagulation disorders. James and Jamison were able to show that women with this disorder were significantly more likely to have a PPH and 5 times more likely to receive a blood transfusion after birth (James and Jamison, 2007).The fact that

a patient may not have a PPH despite having a major clotting disorder demonstrates how effective the physiological ligature is in preventing PPH. Most of the blood loss that is associated with coagulation defects is likely to come from uterine and genital tract trauma, where there is no physiological ligature to stop the blood loss.

It is interesting to note that significant a body of research has demonstrated that PPH can lead to rapid decrease in fibrinogen, a blood plasma clotting coagulation protein, and therefore fibrinogen levels in the blood can be used as a marker for severity of PPH.

It is known that towards the end of healthy pregnancy, several blood anticoagulant factors decrease and coagulant factors increase. This results in the mother's blood being in a hypercoagulable state, providing some protection against reducing blood loss in childbirth (Bremme, 2003). The breakdown of fibrinogen is reduced at this time, thus resulting in its rise in blood plasma levels. However, a study in 2007 showed that a drop in fibrinogen plasma levels during PPH may actually be responsible for the perpetuation of PPH. Over a 24 period, researchers compared changes in coagulation markers, including fibrinogen, amongst two groups of women suffering from PPH; severe PPH (n=50) and non-severe PPH (n=78). It was found that in the first hour of study enrolment, each $1gL^{-1}$ decrease of fibrinogen resulted in a 2.63 fold increase in SPPH risk. Authors concluded that measuring the blood concentration of fibrinogen could be a method for determining risk of PPH developing into SPPH in a patient (Charbit *et al.*, 2007).

A 2012 study also showed that a decrease in fibrinogen levels during PPH could be used as a biological marker for predicting the risk of severity of PPH. A secondary analysis of a population-based study examined fibrinogen levels in 738 women with PPH after vaginal birth. Authors compared fibrinogen concentrations in women whose PPH became severe, and with those whose PPH did not worsen. PPH was defined as blood loss >500ml in 24 hours after delivery. In the non-severe group at diagnosis, it was found that fibrinogen blood concentration was 4.2 g $litre^{-1}$, whereas it was found to be 3.4 g $litre^{-1}$ among patients whose PPH became severe. In addition to these findings, authors also showed that fibrinogen concentration was related to severity of PPH independently of other factors. Cortet et al concluded that fibrinogen blood concentration at diagnosis of PPH should be used as a marker to alert clinicians of the risk that the bleeding may become severe (Cortet *et al.*, 2012).

Placental Abnormalities

Retained placenta is known to be associated with excessive bleeding after birth. Retained placenta refers to the failure of the whole placenta to deliver after the birth, or the retention of placental fragments after most of the placenta has been expelled. This 'retained products of conception' where fragments of the placenta are still attached to the inside of the uterus, can lead to excessive blood loss in the days or weeks after birth. This is because retained placental fragments can prevent efficient myometrial contraction, thus failing to properly occlude blood supply - much in the same way that uterine atony works to cause PPH (Perlman and Carusi, 2019)

Placenta accreta is an obstetric condition where the placenta invades too deeply into the myometrium. This results in the placenta not being able to properly detach from the uterus after delivery. Any manual attempts to remove the placenta are likely to cause the placenta to rip and tear, leaving placental fragments act as a further cause of heavy bleeding (Oyelese and Ananth, 2010)

A Canadian retrospective cohort study collected obstetric data from 570,637 deliveries in an attempt to quantify how placenta accreta contributes to PPH and SPPH rates. Placenta accreta was defined as any placental adhesion to the surrounding organs and musculature, as well as adhesion to the uterine wall. SPPH was defined as a need for hysterectomy, blood transfusion, suturing, ligation or artery embolization. Logistic regression analysis showed there to be a strong association between placenta accreta and PPH (rate ratio 8.3, 95% CI 7.7–8.9). Although authors accepted that the frequency of association was low, a strong association was observed between placenta accreta and PPH with hysterectomy (rate ratio 286, 95% CI 226–361). Mehrabadi et al conclude that placenta accreta has a substantial contribution to the proportion of PPH with hysterectomy (Mehrabadi *et al.*, 2015).

Similarly, placenta previa can also increase risk of excessive bleeding. Placenta previa is where the placenta inserts itself too low down in the uterus, in the lower segment where the uterine wall is largely derived from cervical tissue and therefore has few myometrial fibres. This means that the strong contraction necessary for blood vessel occlusion after delivery of the placenta is much less likely to occur, thus leading to increased risk of haemorrhage (Oyelese and Ananth, 2010).

Placenta previa has been shown to be associated with adverse maternal outcomes such as PPH. Prospective data from women with a diagnosis of placenta previa were gathered from 10 Austrian hospitals between 1993 and 2012. Of the 328 patients analysed in the study, maternal morbidity was considered high, with PPH, defined as blood loss >2000ml, occurring in 7.1% of cases (Kollmann *et al.*, 2016).

Augmentation of Labour and Oxytocin Use

It is widely understood that increased risk of PPH is a common complication associated with induction of labour and augmentation of labour (administering oxytocin, for example).

In 1978, Brinsden and Clark retrospectively compared the incidence of PPH after induced labour with that of spontaneous labour, using labour records of 1000 consecutive deliveries. The authors discovered a higher incidence of PPH in the induced group, so they conducted further analysis on 3674 deliveries. From this, they concluded that PPH incidence was higher in women who had their labour induced. In addition to these findings, the PPH risk was almost doubled in comparison to the spontaneous birth group for primiparous women (women who have been pregnant and given birth once) (Brinsden and Clark, 1978). However, it is worth noting that the association between induction and PPH may not necessarily be causal. This is much like the way in which travel in an ambulance is closely associated with high death rates, but it does not necessarily mean that the ambulance is causing this association. It is therefore likely that the underlying cause of the PPH is not the induction itself, but the underlying reason for the induction. For example, PPH has been closely associated with pre-eclampsia, multiple pregnancy and increased maternal age, all of which are common reasons for induction.

In Professor Baud's 2013 paper; 'Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labour at term,' it was found that induction of labour was linked to increased PPH rates. Coincidentally, this is also the same paper in which the data for this MPhil analysis was initially sourced from. This was a retrospective cohort study which sought to compare adverse maternal and neonatal outcomes in two groups of patients; those elected for labour induction and those who were medically indicated for labour induction. PPH was defined as blood loss >500ml. Baud et al found that when compared to spontaneous labour (n=8,881), women who underwent elective induction of labour (n= 5,090) were at a

significantly higher risk of PPH (OR 1.75, 95% CI 1.55–1.99). Authors concluded elective induction of labour carried the same amount of neonatal and maternal risks as medically indicated induction of labour, and therefore should be discouraged as much as possible (Baud *et al.*, 2013).

Maternal Age

It is known that older maternal age is associated with an increased risk of many complications, such as PPH. An extensive study, looking at maternal age as a risk factor for blood loss during parturition, was carried out in 2003. The multivariate analysis included a total of 10,053 women delivering a single infant. It was found that a maternal age of more than or equal to 35 years was an independent risk factor for PPH, regardless of the mode of delivery (Ohkuchi *et al.*, 2003).

A later study conducted in 2008 also sought to investigate the effect of advanced maternal age on obstetric outcome. 15 727 pregnancies were included in the analysis, where authors compared maternal age at delivery among multiparous and nulliparous women in an over 40 year age group and an under 40 years age group. Results showed there to be an increased risk of obstetric complications, such as PPH, in the over 40 group, for both nulliparous and multiparous women (Chan and Lao, 2008).

A retrospective cohort study analysed the birth records of first-time mothers at three hospitals, from July 1986 to June 1990. The objective was to compare pregnancy and delivery complications in women aged 35 years and older, with women aged 25-29 years old (Oboro and Dare, 2006). In this study, authors found rates of PPH to be higher in the ->35-year group, at 1.1% 9(n=890), whereas rates of PPH in the 25-29 years age group was 0.2% (n=1054).

Gestational Age

Several studies report a nonlinear (or J-shaped) association to exist between gestational age at birth and the risks of PPH. Typically, it is women who deliver after 41 weeks gestation and those who deliver before 37 weeks are at the greatest risk. women who gave birth between 2014-2017 in Sweden and women who gave birth in California between 2011-2015 were included in population-based retrospective cohort study to assess the relationship between PPH and gestational age at delivery. In both Sweden and California, the incidence of PPH was highest for deliveries between 41- 42 weeks' gestation (7,186/75,539 [9.5%] and

8,921/160,267 [5.6%], respectively). In both cohorts, a gestational age of 39 weeks was observed to have the lowest risk of a PPH (Butwick *et al.*, 2021).

Arrowsmith et al conducted contractility experiments on human myometrium from both term pregnancies and post- term pregnancies. The data collected from these in-vitro studies showed there to be reduced contractility of the myometrium from women with post-term pregnancies compared to term pregnancies. This suggests that post-term women may be at a greater risk for atonic haemorrhage (Arrowsmith *et al.*, 2012).

A retrospective cohort study of 119,254 low risk women who delivered beyond 37 weeks, found that rates of PPH were increased at 41 weeks gestation (OR 1.21, 95% CI (1.10, 1.32), compared to women who deliver closer to term (Caughey *et al.*, 2007).

In 2014 the World Health Organization conducted a secondary analysis of cross-sectional data on a total of 352 health facilities in 28 countries. The multivariate logistic regression, on 274,985 women, revealed that women who had a gestational age of less than 37 weeks and more than 41 weeks, were at a higher chance of suffering a PPH, as compared with women who had a gestational age between 37 weeks and 41 weeks (ORs were 2.63; 95% CI 2.28–3.04 and 1.56; 95% CI 1.02–2.38, respectively) (Sheldon *et al.*, 2014)

Second Phase Length

In 2009, Lu et al conducted a retrospective case-control study to assess the relationship between prolonged second stage of labour and PPH. 91 cases of PPH, as well as 323 control cases were included from four Southern California hospitals in 2003, and the relationship was examined using bivariate and multivariate analyses. It was found that those women who had a prolonged second stage. were more than three times more likely to be at risk of a PPH than the controls (OR = 3.35; 95% CI 1.22-9.19). Authors concluded that close supervision by clinical staff was needed for women experiencing a prolonged second stage of labour, as it was considered to be an important risk factor for PPH (Lu *et al.*, 2009).

In 1952, Hellman and Prystowsky conducted a retrospective cohort study to assess any increased risk of PPH with prolonged second stage of labour. Uncomplicated singleton deliveries were included, and it was found that significant increases in PPH were associated with a second stage lasting longer than 2 hours (Hellman and Prystowsky, 1952).

Another retrospective cohort study, on 15,759 women, also reported a significant association between PPH and prolonged second stage. Research showed that the frequency of PPH increased from 7.1% during the 0- to 1-hour period of the second stage to 30.9% when second stage progressed beyond 4 hours (Cheng, Hopkins and Caughey, 2004).

The underlying reason for the increase is not known. However, randomised trials of epidural use or routine augmentation with oxytocin have not found an increase in PPH (Costley and East, 2012), suggesting that these are not underlying causes. The use of instrumental birth is closely associated with a prolonged third stage of labour, and is also widely recognised to cause vaginal lacerations and thus PPH, and so may be the causal factor.

Childbirth Total Length

A 2009 review from the International PPH Collaborative Group has recommended that further research is needed classifying prolonged labour as a potential risk factor for PPH (Knight *et al.*, 2009).

In 2017, the association between duration of active labour and SPPH was investigated. In the case control study, the effect of the total duration of active labour, the effect of each stage of active labour, and the gradient effect of duration of labour on SPPH was examined. In those women who experienced SPPH, a significantly longer mean duration of labour was observed, compared with controls (5.4 versus 3.8 hours, $p<0.001$). Compared with controls, women with a SPPH were also noted to have a longer duration of all stages of active labour, and they were more likely to have a prolonged labour exceeding 12 hours (adjusted odds ratio = 2.44, 95% confidence interval: 1.69–3.53, $p< 0.001$) (Nyfløt *et al.*, 2017).

Neonatal Weight and Macrosomia

Macrosomia is defined by a birthweight of an infant exceeding 4 kg at birth, or more accurately, a birth weight ≥90th percentile for a given gestational age. (Chandrasekaran, 2021). To identify demographic risk factors for macrosomia and to quantify the obstetric risks, data gathered from 1988-1997 on 350,311 singleton pregnancies were analysed using logistic regression. In 2003, Jolly et al found that women who delivered a macrosomic baby were at increased risk of PPH (OR 2.01; CI 1.93, 2.10). Authors added that this was presumably due to a greater chance of perineal trauma and increased uterine distension, which makes the mother more likely to develop uterine atony (Jolly *et al.*, 2004).

A 2016 case control study found that one of the most common obstetric complications associated with delivering a macrosomic infant was PPH, which occurred in 17.5% of the study group. In addition to this, authors reported that mothers delivering macrosomic babies were at a 5-fold increased risk of developing PPH than the mothers in the control group. Direct causes of PPH were found to be uterine atony, perineal tears and uterine rupture (Said and Manji, 2016).

Multivariate analysis conducted to investigate the relationship between large for gestational age infants (LGA) and PPH. Results showed that LGA births were significantly associated with PPH (adjusted OR 2.34, 95% CI 1.71-3.19) and SPPH (adjusted OR 2.43, 95% CI 1.21-4.86). Authors concluded that large for gestational age babies should be considered an independent risk factor for PPH caused by uterine atony (Yumeno, 2014).

Obesity

A cohort study was conducted in 2012 which aimed to determine whether overweight and obesity should be considered independent risk factors for SPPH (≥1000ml blood loss). Women included in the study were nulliparous and delivering at term, and gave birth vaginally or by a caesarean section. It was found that overall, the rates of SPPH were increased in the women who were classed as overweight or obese, compared with that of normal-weight women (n=255 [9.7%], n=233 [15.6%]), n=524 [7.2%], p <.001) respectively. Conclusions from the study stated that being obese should be considered an important risk factor for SPPH, following both caesarean and vaginal delivery. (Fyfe *et al.*, 2012)

Another study that investigated maternal BMI and risk of PPH (≥1000ml blood loss) also found increasing BMI to be associated with increased risk of PPH. This Swedish cohort study (n=1,114,071) included women with singleton pregnancies who gave birth between 1997-2008. The women were divided into 6 BMI classes, where obese women were included in class 1-3. Blomberg et al found that there was an increased risk of PPH for women with a BMI of more than 40 (5.2%) after normal delivery (OR 1.23, 95% CI 1.04–1.45]) compared with women of normal weight (4.4%). This increased risk was found to be even more pronounced (13.6%) after instrumental delivery (OR 1.69, 95% CI 1.22–2.34) compared with normal-weight women (8.8%) (Blomberg, 2011).

A cohort study collected obstetric data from women who gave birth in a Californian hospital between 2008 and 2012. Butwick et al examined the relationship between BMI and haemorrhage using multilevel regression. Atonic haemorrhage and SPPH were the secondary outcomes, and analysis was stratified according to mode of delivery. Authors found that the odds of an overweight women and women in obesity class 1 (BMI 30- 34.9) suffering a haemorrhage and atonic haemorrhage were moderately increased in comparison to women with a normal BMI. The odds of overweight and obese women having an atonic haemorrhage were increased up to 19% in vaginal birth, whereas the odds decreased to 14% after caesarean birth. Authors thus concluded that although maternal obesity has a modest effect on PPH rates, rates are also influenced by mode of birth (Butwick *et al.*, 2018)

Mode of Birth

It can be argued that the mode of delivery can influence the cause of PPH. Caesarean delivery causes surgical blood loss from incisions and tears, and can also predispose the mother to increased rates of intra-abdominal adhesions and placenta accreta spectrum disorders in future pregnancies. In addition to this, instrumental deliveries such as episiotomy, forceps and ventouse can predispose a patients to increased genital bleeding and tissue trauma , which may also increase PPH rates (Ngene *et al.*, 2021).

In a Danish retrospective cohort study, researchers included the data from women giving birth from 2001 to 2008 so they could examine any link between intended mode of delivery and SPPH. Holm et al compared the use of blood transfusion by mode of delivery in low-risk, nulliparous women and in women who had a previous CS. Red blood cell (RBC) transfusion within 7 days of giving birth was the primary outcome measure and variables such as maternal age, BMI, birthweight, smoking, parity, multiple births and previous caesarean delivery were adjusted for. The definition of intended vaginal delivery included all intended vaginal deliveries and all women who underwent emergency CS during labour. The definition for intended caesarean delivery included CS conducted both before labour and during labour. The term ‘intended’ refers to the procedure being scheduled >8 hours prior to delivery. Researchers found that the risk of a RBC transfusion in women who had an intended caesarean delivery was significantly lower than in women with an intended vaginal delivery (odds ratio 0.82; 95% CI 0.73–0.92; P<0.01). This led authors to conclude that rates of SPPH were lower for women with a planned caesarean delivery, compared with intended vaginal

delivery. This was most likely due to the fact that intended vaginal delivery included women who underwent emergency CS during labour; the mode of delivery with the highest risk for PPH (Holm *et al.*, 2012).

However, 2010 retrospective cohort study found that the risk of SPPH (blood loss >1000ml) was twice as high after caesarean delivery, compared with the risk associated with vaginal delivery (5.9%; 95% CI 5.3-6.6 vs. 2.8%; 95% CI 2.6-2.9). A Norwegian study (n=41,365) collected obstetric data from 1998 to 2007, were included in this study. Over this ten-year period, authors also observed that frequency of obstetric interventions increased. Rates of elective caesarean sections increased from 2.4 to 4.9%, emergency caesarean sections increased from 5.5 to 8.9% and operative vaginal deliveries increased from 9.3 to 12.5%. This lead Rossen et al to speculate that the increase in SPPH rates may be linked to the increase in frequency of obstetric intervention during childbirth (Rossen *et al.*, 2010).

Perineal Damage and Episiotomy (trauma)

It has been commonly believed that an episiotomy (a surgical cut to the vagina and/or the perineum during vaginal delivery) prevents spontaneous tears, especially third degree tears. However, there is a growing amount of published data that indicates that routine use of episiotomy may increase the risk of perineal trauma, thus increasing the mother's risk of haemorrhage. An episiotomy certainly guarantees perineal trauma and the need for sutures, whereas a perineal tear may not always occur during a spontaneous vaginal birth.

A 2009 Cochrane highlights that there has been a trend in obstetric care to move away from routine use of episiotomy in place of a more restrictive use of episiotomy. Authors reviewed 8 studies, which included 5541 women, and divided subjects into a routine episiotomy group and a restrictive episiotomy group. Cochrane reviewers revealed that in the restrictive episiotomy group there was less suturing, less trauma and less healing complications. There was also no difference in severe perineal/vaginal trauma, and no difference in analgesic use. However, restrictive episiotomy did increase risk of anterior perineal trauma (Carroli and Mignini, 2009).

A 2017 update of the 2009 Cochrane review included 12 randomised controlled trials (6177 women). Again, reviewers compared risk factors associated with selective episiotomy use versus routine use of episiotomy. Authors also found that there were fewer women with

severe perineal/ vaginal trauma who were treated under a selective episiotomy policy. Authors conclude that there is currently is not enough evidence to justify the belief that routine episiotomy reduces perineal and vaginal trauma, and the associated complications such as PPH (Jiang *et al.*, 2017).

A 2014 study looked at identifying risk factors associated with mediolateral episiotomy. The study included data on singleton, term, vaginal deliveries, collected between 2007 and 2014. Nulliparous and multiparous women were compared separately, as well as spontaneous and operative vaginal deliveries. Amongst the 41,347 women who gave birth spontaneously, 12,585 (30.4%) were nulliparous. In this group, episiotomy was shown to be associated with PPH (adjusted OR 1.49, 95% CI 1.19–1.87, $p = 0.001$). Authors concluded that episiotomy does not protect women from adverse events such as PPH, and that a more conservative use of episiotomies should be adopted (Shmueli *et al.*, 2017).

Preeclampsia

Studies have shown that preeclampsia is positively associated with PPH. One such study was conducted in 2009 and included data on 315,085 women (collected between 1999-2004) who gave birth to a single infant after 21 weeks gestation. Researchers focused on assessing the relationship between factors associated with preeclampsia and excessive bleeding after delivery. It was found that in cases of preeclampsia, PPH (>1500ml blood loss) occurred in 3.0% (399/13,166) of cases, compared with 1.4% (4,223/301,919) of cases where mothers did not suffer from preeclampsia ($p<0.01$). Authors also noted that moderate postpartum bleeding (blood loss >500ml) was more common in women with preeclampsia, occurring in 22.9% preeclampsia cases compared with 13.9% of cases with no preeclampsia (Eskild and Vatten, 2009).

A 2013 prospective cohort study also identified a positive association with preeclampsia and PPH. Obstetric records, collected from 2000-2008, included women who gave birth >19 weeks gestation. The data from 1,457,576 women was analysed using univariate and multivariate logistic regression and PPH was defined as blood loss of ≥1000 ml in the first 24 hours after delivery. After adjusting for confounding factors, authors revealed that women who had pre-eclampsia were at 1.53 fold increased risk of PPH (adjusted odds ratio 1.53; 95% CI 1.46 to 1.60) (von Schmidt auf Altenstadt *et al.*, 2013).

Previous CS and Scarred Uterus

It is known that caesarean sections leave behind significant uterine scars in the women who deliver in this way. It is known that uterine scares are related to increases in placenta accreta rates, which in turn are a risk factor for PPH. In addition to this, the myometrium is thinner at the site of the incision, and can thus lead to weakened uterine contractions at the site of the scar, thus increasing chances of uterine atony. In addition to this, chances of excessive placental implantation are increased at the incision site as the placental villi is more able to invade the uterine muscle, which can also cause severe bleeding (Kollmann *et al.*, 2016). Caesarean scars are also known to increase incidences of uterine rupture, thus leading to haemorrhage if over-stretched (Mastrolia *et al.*, 2016).

The results from a retrospective cohort study from China supports the correlation between uterine scars and a higher incidence of PPH. Between January 2013 and December 2017, 3,722 women who delivered in a north China teaching hospital were enrolled in the study. The main aim of the study was to develop a tool, known as a nomogram, to predict the risk a woman has (singleton pregnancy and a scarred uterus) of suffering a PPH during caesarean section. Of the 3,722 women with a scarred uterus, 6.53% (n= 243) of those women suffered a PPH, defined as blood loss ≥1000ml. The incidence of PPH in this group was high, and authors stated that a scarred uterus could be accounted for this increased risk (Chen *et al.*, 2020).

Multiple Gestations

It is known that PPH occurs more frequently in women with multiple gestations than in those with singleton gestations. Multiple fetuses in the womb cause increased uterine distention, which in turn can weaken myometrial contractions and increase risk of uterine atony (Blitz *et al.*, 2020)

From 1999 to 2008, authors Kramer et al analysed over 8.5 million deliveries in the US Nationwide Inpatient Sample in order to identify risk factors and temporal trends in SPPH. It was found that SPPH complicated 25,906 of those deliveries. Multiple gestation was amongst several significant risk factors (aOR, 2.8; 95% CI, 2.6–3.0) (Kramer *et al.*, 2013).

Another study, by Conde-Agudelo et al, demonstrated that multiple gestations could increase the risk of PPH and maternal death. Authors analysed 885,338 records from the perinatal

database (from their hospital in Uruguay) using multiple logistic regression models and adjusting for confounding factors. This was done to assess the association between adverse maternal outcomes and multiple gestation. They found that multiple gestations double the risk of death during delivery in comparison with singleton gestations [adjusted RR 2.1; 95% confidence interval (CI) 1.1, 3.9]. In addition to this, women with a multiple gestation had adjusted RRs of 2.0 (95% CI, 1.9, 2.0) for PPH (Conde-Agudelo, Belizán and Lindmark, 2000)

Parity

To test whether multiparity increases the risk of PPH, Durmaz et al conducted a meta-analysis that included 6 studies and with a total of 1,118,490 women. using the Mantel–Haenszel method to analyse the fixed effects model, analysis showed that primiparous mothers had a higher risk of PPH than multiparous mothers (*OR* = 1.37, 95% CI [1.35, 1.40], $p < .001$) (Durmaz and Komurcu, 2018).

Background Conclusion

This background chapter has covered the relevant aspects of scientific theory and research, helping to explain the motive behind this studies objective. From the physiology of myometrial contraction to how acidosis may influence PPH rates. The PPH risk factors used in the analysis were explored, and the main causes of PPH were explained. Literature supporting the hypothesis that fUVpH could be used as a surrogate measure for myometrial pH, or mApH was also demonstrated, hopefully providing one with an understanding behind this surrogate-measure theory in relation to uteroplacental circulation.

So, to reiterate the aims of this study; this will be a secondary analysis obstetrical data that was collected on 2000 live, cephalic- presenting, singleton, term pregnancies, which was originally used in the study 'Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labour at term,' (Baud et al., 2013). This dataset contains details of both maternal postpartum blood loss and on umbilical vein blood pH. Therefore, it is appropriate to conduct a multiple logistic regression on the data, where the primary study objective is to determine whether patients with maternal acidosis have higher rates of PPH, compared to patients without maternal acidosis. The secondary study objective was to determine whether fUVpH was an independent risk factor for PPH. There are many other predictors for PPH (e.g. type of delivery, prolonged labour, fetal birth weight, parity, multiple

pregnancy), and therefore their impact on blood loss needed to be investigated alongside the effects fUVpH may have on blood loss. To validate the predictive properties of this model, ROC curves and sensitivity against specificity curves will be produced, as well as negative and positive predictive values being calculated.

Chapter 2. Methodology

Finding Data to Analyse

In order to assess the relationship between mApH and PPH, a suitable database first needed to be identified in order to conduct a secondary analysis. After searching the literature, it became apparent that data on mApH was hard to come by as it was rarely collected. It was noted, however, that there was plenty of data on fUVpH, as fetal blood pH is routinely collected in many places for assessment of the new-born's health. Blood that enters the placenta from the umbilical vein is in close contact with maternal blood on the mother's side of the placenta. Therefore, there is a lot of evidence that suggests umbilical vein blood pH mirrors that of maternal blood pH. Another search of the literature suggested that this may well be the case. Fetal umbilical vein pH data was now of particular relevance to the proposed research, as it could be used to provide evidence for a correlation between maternal acidosis and PPH.

After deciding fUVpH would be used as surrogate for mApH, several weeks of conducting an extensive literature search produced a collection of suitable databases, compiled into one table **(See Appendix 2).** Possible papers whose authors may or may not have access to a relevant obstetric database were selected and compared, and so the decision was made to reach out to Professor David Baud and request a collaboration.

In Baud's 2013 paper ('*Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labour at term'*) it appeared that Baud et al had gathered data on more than 33,000 women, over a ten-year period, from the Lausanne University Hospital in Switzerland. As mentioned previously, it was found that estimated blood loss and fUVpH were both recorded. We then wrote to Professor Baud to ask if he would be interested in working together to explore this possible relationship, and fortunately Professor Baud accepted our request. It was agreed that Professor Baud would be included in the authorship of the thesis, as the work would be a collaboration between him, myself, and my supervisory team.

Data Available for Analysis

As previously mentioned, this MPhil is centred around conducting a secondary analysis of data that was collected from Lausanne CHUV over a ten-year period. COVID-19 pandemic travel restrictions meant that I could not travel to the Lausanne to conduct the analysis on the full

33,000 patient database. Instead, a sample of 2000, anonymised, patient records was permitted to be sent to me for analyse.

The 2000 patient dataset has been analysed using STATA 14 (StataCorp, College Station, Texas), with the help of statistician Dr Steven Lane. STATA commands, known as .do files, have been written and used to organise and clean the data, before conducting a univariate and multivariate analysis on the dataset. The .do commands have also been written to demonstrate data distribution, in the form of histograms, scatter graphs and box plots **(See Appendix 3-7).** The STATA files containing the .do commands (known as the 'data cleaning' .do files) were then sent via email to Professor Baud in Switzerland. There, he ran the data cleaning .do files on the original dataset, allowing him to replicate the data cleaning on the 33,000 patient records. The results from this analysis would not conflict with any data protection laws, and therefore could be sent back to the UK for use in this MPhil project.

As mentioned in the 'COVID Disruptions' chapter, after data cleaning was complete, the rest of the analysis was conducted on the 2000 patient sample. Writing .do files on STATA for multiple regression requires each variable to be added and subsequently removed if and when they become non-significant. Therefore, this could have only worked if I were able to work on the 33,000-patient dataset in real-time. Professor Baud could not conduct the multiple regression for me. Fortunately, 2000 obstetric records were sufficient for the purposes of this MPhil thesis.

This methodology is thus for the complete analysis on the 2000 patient sample dataset, where PPH is defined as >500ml blood loss for vaginal birth and >1000ml blood loss for CS. A second 'sensitivity analysis' will be also be conducted using >1000ml blood loss for all births to see if it changes the results at all. These definitions for PPH were decided upon after searching the literature for studies on the quantification of obstetric blood loss. The PPH definitions used in this thesis still appear to be the most widely used method of defining PPH to this day (see page 41).

In CHUV Hospital, Lausanne, STATA is the statistical software of choice for many publications that have arisen from the original dataset. It was for this reason, and also on request of Professor Baud, that STATA v14 is used in the secondary analysis of this project. Choosing to work using STATA meant that collaboration with Prof Baud would run smoothly, as he was

proficient with using this software, and has had success with it on previous publications. **See table 3, below, for a summary of the data that was available for analysis.**

Table 3: Data available in Lausanne database for analysis

variable	Type	Description	Categories / units
1. age_mat	Continuous	Maternal age	Years
2. Ag2	continuous	Gestational age at delivery	Weeks
3. t2phas	Time	Second phase length	Minutes
4. synto	Dichotomous	Did we use oxytocin?	Yes / No
5. t_synto	Continuous	For how long did we use oxytocin?	Minutes
6. actd_cs	Dichotomous	Scarred uterus (CS during a previous delivery)	1= Yes/ 0= no
7. Kg_nn	Continuous	Neonatal weight	Grams
8. pHa	Continuous	Umbilical Arterial pH	pH
9. pHv	Continuous	Umbilical Venous pH	pH
10. BMI	continuous	Body Mass index	Numerical
11. Obesity	Dichotomous	Was the body mass index ≥30?	1= Yes/ 0= no
12. Pe500	Dichotomous	Was the blood loss ≤500ml?	1= Yes/ 0= no
13. Pe500-1000	Dichotomous	Was the blood loss >500ml but ≤1000ml?	1= Yes/ 0= no
14. Pe1000	Dichotomous	Was the blood loss >1000ml?	1= Yes/ 0= no
15. Emergency_CS	Dichotomous	Did she have an emergency CS?	1= Yes/ 0= no
16. Elective_CS	Dichotomous	Did she have an elective CS?	1= Yes/ 0= no

17. Forceps	Dichotomous	Were forceps used in her delivery?	1= Yes/ 0= no
18. ventouse	Dichotomous	Was a ventouse used in her delivery?	1= Yes/ 0= no
19. Labour_induced	Dichotomous	Was her labour induced?	1= Yes/ 0= no
20. Perineal_tear	Dichotomous	Did she have any perineal tears?	1= Yes/ 0= no
21. episiotomy	Dichotomous	Did she have an episiotomy?	1= Yes/ 0= no
22. Perineal_trauma	Dichotomous	Did she sustain any perineal tears or require the need for an episiotomy?	1= Yes/ 0= no
23. Coagulation_abnormalities	Dichotomous	Did she have any coagulation defects that increased bleeding?	1= Yes/ 0= no
24. preeclampsia	Dichotomous	Did she have preeclampsia?	1= Yes/ 0= no
25. Previous_CS	Dichotomous	Did she have a CS in the past?	1= Yes/ 0= no
26. Oxytocin_infusion_in_labour	Dichotomous	Did she receive an oxytocin infusion during labour?	1= Yes/ 0= no
27. Multiple_gestation	Dichotomous	Did she have a multiple pregnancy?	1= Yes/ 0= no
28. Parous	Dichotomous	Has she previously given birth to at least one fetus with a gestational age of 24 weeks or more?	1= Yes/ 0= no
29. Low_inserted_placenta	Dichotomous	Did she have a low-lying placenta? (i.e placenta praevia?)	1= Yes/ 0= no
30. Retained_placenta	Dichotomous	Did she have a retained placenta requiring manual removal?	1= Yes/ 0= no
31. Operative_vaginal_birth	Dichotomous	Were any instruments (forceps/ ventouse) used in her delivery?	1= Yes/ 0= no
32. Vaginal_delivery	Dichotomous	Did she have a vaginal delivery?	1= Yes/ 0= no
33. Total_mins	Continuous	Total childbirth length	Minutes
34. Blood_loss	Categorical	Category of blood lost during delivery	1= ≤500ml 2=>500ml - ≤1000ml

			3= >1000ml
35. Birth_mode	categorical	Mode of delivery	0= spontaneous vaginal 1= operative vaginal 3= emergency_CS
36. PPH_yes_no		Did she have a PPH defined as blood loss ≥1000ml for CS and blood loss ≥500ml for vaginal birth	1= Yes/ 0= no

Key

- White: Other possible predictor variables
- Blue: Main predictor variable
- Red: primary outcome variable

Study Objectives

This project is exploring the relationship between maternal acidosis and postpartum haemorrhage (PPH). A direct analysis was conducted which looked at whether the umbilical vein pH differs in women who had a PPH and those that did not have a PPH. A logistic regression was then conducted to see whether any relationship was independent of other factors or not.

The primary study objective was to determine whether patients with maternal acidosis have higher rates of PPH, compared to patients without maternal acidosis, using fUVpH as a proxy measure for mApH. We looked at whether fUVpH can predict maternal postpartum blood loss.

The secondary study objective was to determine whether fUVpH was an independent risk factor for PPH. There are many other predictors for PPH (e.g. type of delivery, prolonged labour, fetal birth weight, parity, multiple pregnancy), and therefore their impact on blood loss needed to be investigated alongside the effects fUVpH may have on blood loss. **The working hypothesis of this study is that low umbilical venous pH predicts maternal PPH. The null hypothesis is that low umbilical venous pH has no impact on PPH rates.**

The traditional definition of a PPH is a blood loss of >500ml for a vaginal birth and >1000ml blood loss for a caesarean birth (Basil-Kway *et al.*, 2021; WHO, 2012). However, more recently the research community appears to be using 1000ml blood loss as a cut-off for all births as a definition for PPH. In 2014, the American College of Obstetricians and Gynaecologists (ACOG) has sought to redefine the definition of PPH. The updated PPH definition was changed to a cumulative blood loss ≥1000ml with signs hypovolemia within the first 24hours of delivery, regardless of the mode of the mode of delivery (Menard, Main and Currigan, 2014)

Therefore, this analysis will be conducted twice; the main analysis will have the traditional definition of PPH being defined as ≥500ml blood loss for vaginal birth ≤1000ml blood loss for CS. A second 'sensitivity analysis' will then be done using ≥1000ml for all births to see if it changes the results**.**

Research Setting

The Centre Hospitalier University Vaudois (CHUV) is a maternity hospital based in Lausanne, Switzerland. As of 2020, the hospital operated with 1,546 beds and 48,227 hospitalized

patients. The number of births that take place in the hospital has been gradually increasing over the past decade, with 2,654 births recorded in 2011, through to 3,180 births recorded in 2021. (Le CHUV en Chiffres, 2022).

The data used in this study was collected at the CHUV between 1997 and 2007. The data included information on general patient data (date of birth, insurance, marital status and ethnicity), as well as details on labour and delivery, and details on both neonatal and maternal outcome (Baud *et al.*, 2013).

Data Cleaning

Once a suitable database had been found and a clear definition of a PPH had been decided upon, the next task was to translate the database as it was written in French. Translation of the database was conducted using translation software. As this has to be done by hand, the process of translation took a considerable amount of time and effort.

Then, once the database had been translated from French to English, the data was 'cleaned'. Cleaning data is the process of correcting or filling in any missing values, or removing those values completely. For the univariate analysis to be successfully carried out on the dataset, the data first needed to be correctly formatted and have any incorrect values removed. Redundant variables, duplicates, missing values, and outliers, were all dealt with so as not to warp the distributions, medians and means of the data. The next step was to remove all elective CS from the analysis as it is highly unlikely the woman has laboured. In addition, all arterial pH <7.1 was removed as it would have indicated that the acidosis was coming from the fetus and not the mother.

Outliers were shown on a box plot as anything more than 3 standard deviations away from the median. Some impossible outliers were deleted as a mistake (e.g. pHv = 70). Outliers that were consistent with possible physiological values, however, were retained.

We aimed to deal with missing data by:

1. Removing any participant for whom there was no blood loss recorded
2. Removing any participant for whom there is no umbilical vein pH recorded
3. All remaining participants' data remained in the database without imputation

In addition, we refined the data by removing those women whose data would skew the data analysis:

1. Participants for whom the umbilical artery pH is 7.1 or less (2SD below the mean) were removed. This is because these are hypoxic babies – an acidotic umbilical vein pH is therefore likely to be as a result of the fetal state rather than the myometrium.
2. Any women who underwent an elective CS was removed. This is because…

There were originally 166 separate variables containing patient data in the 2000 patient dataset. However, once irrelevant variables were removed, 36 predictor variables remained to go forward in the univariate analysis. The decision on which confounding factors to include and which to exclude were based on the RCOG Green Top guidelines on PPH (Green-top-Guidelines, 2016).

Over 300 .do files were individually written to clean the data. The .do files were then sent to Professor Baud so that he could replicate the data cleaning on the full dataset. Data cleaning was a considerably time-consuming portion of the full analysis, taking several weeks to complete.

See Appendix 3 for all STATA .do files for data cleaning

Histograms

Once data had been cleaned and missing values had been removed, the next step was to check the main continuous variables for normality. This was done for both umbilical arterial pH and umbilical venous pH in a two-way histogram. For this, a two-way histogram was created using STATA where both umbilical venous blood and umbilical arterial blood pH values were superimposed onto the same histogram. This gave a side by side demonstration of the two pH distributions (see figure 6).

Scatterplots

For data visualisation, a scatterplot for pH values was constructed. Umbilical arterial pH (pHa) was plotted on the y axis and umbilical venous pH (pHv) was plotted on the x axis. This was done so to give a visual indication on how the values stray from the line of perfect correlation (see figure 7).

Box and Whisker Plots

Box and whisker plots were constructed for all continuous variables against PPH_Yes_No, where 0= no PPH (<500ml blood loss via vaginal birth and <1000ml blood loss via CS) and 1 = Yes PPH (≥500ml blood loss via vaginal birth and ≥1000ml blood loss via CS). This was done to visualise the data distribution by showing the data averages and percentiles.

See Appendix 4 for STATA .do files on all descriptive statistics.

Demographics and Univariate Analysis

Demographics on all explanatory variables by those patients who had a PPH and those who did not have a PPH were reported. Continuous variables were reported using the mean (standard deviation) or median (IQR) if not normally distributed. Categorical variables were reported as number n (percentage) for each category within each blood loss category (see table 4).

A univariate analysis was then carried out on predictor variables against PPH, known as PPH_Yes_No. The p values from univariate analysis were also included in the demographics table, and the significance level was 5% ($p<0.05$). Logistic regression was used because PPH is a binary variable (yes/no) and logistic regression was the equivalent to the T-test for continuous data and the Chi-squared test for categorical data.

The variables that were identified as having a statistically significant relationship with the outcome variable (PPH) went forward into the multivariate analysis, those that were identified as not being significantly related to the outcome were excluded, unless there was a clinical reason for retaining them (such as fUVpH).

See Appendix 5 for STATA .do files on the univariate analysis.

Multivariate Analysis

The model for the multivariate analysis was built by adding one significant variable, from the univariate analysis, at a time. This was done in order of decreasing significance. Significant variables were retained and any non-significant variables were excluded. Excluding non-significant variables does not lose information, it just demonstrates that two explanatory variables are explaining the same variability in the outcome variable.

See Appendix 6 for STATA .do files on the multivariate analysis.

Validation of Predictive Properties of Model

Sensitivity is the proportion of cases that are correctly identified (women who have a PPH), specificity is the proportion of non-cases that are correctly identified (women who do not have a PPH).

A ROC curve was produced to show that the multivariate model can discriminate between women who are likely to have a PPH and women who are not likely to have a PPH. The ROC curve is a plot of sensitivity against 1 - specificity. The closer the area under the curve is to one, the higher the discrimination of the model. A value of 0.5 or lower would indicate that predicting outcomes would be no better than leaving it to chance, or tossing a coin.

Risk scores comes from the logistic regression model, constructed in STATA. A risk score is a value between 0 and 1, and the closer the woman's value is to 1, the higher the risk of experiencing a PPH. The more risk factors the woman has, the higher their risk score is expected to be.

Sensitivity and specificity values were then plotted on the same graph for a range of possible risk scores, the objective is to identify the optimum risk score (cut off value) that optimises both sensitivity and specificity. This is the risk score value at the point where the sensitivity and specificity curves intercept. This gives us a cut-off value for the risk score, which will classify a patient at being at risk of a PPH (if above the risk score) or not at risk of PPH (if below the risk score). If women have a risk score above the cut-off value then clinicians may administer preventative drugs, or maybe even 'group and save' in advance in case a blood transfusion is needed.

See Appendix 7 for STATA .do files on the ROC curve.

Sensitivity Analysis

As mentioned at the beginning of the data management plan, a second 'sensitivity analysis' was conducted using >1000mls as a definition of PPH for all births to see if it changes the results. Every step that has been taken for the data analysis will be repeated a second time, using the <1000ml blood loss definition of PPH.

Chapter 3. Results

As a reminder, the abbreviations for the two definitions of PPH used throughout the analysis are as follows:

- **PPH_Yes_No:** where PPH is defined as a blood loss exceeding 500ml for vaginal or instrumental vaginal birth, and as a blood loss exceeding 1000ml for an emergency caesarean section.
- **Pe1000:** where PPH is defined as blood loss exceeding 1000ml, regardless of whether the mode of birth was vaginal, instrumental vaginal or emergency CS.

Obstetric data on 33,601 women was included in the full database (that I could not access for analysis). Of those women, there were 4724 women with blood loss at ≥500-1000ml (14.06%) and 781 women with blood loss ≥1000ml (2.32%)

For the data that I could access, 2000 women were included in the analysis. These women gave birth in Lausanne between from 1997-2011. Of the 2000 patient data sample, data on postpartum blood loss was collected from 906 women. 123 of the 906 women (13.58%) suffered a PPH as defined as blood loss >500ml for vaginal delivery, or >1000ml for EM CS delivery. For a PPH defined as blood loss >1000ml, regardless of mode of delivery, 21 of the 906 women (2.32%) experienced this.

Normality Checks

Both umbilical arterial pH and umbilical venous pH were visually checked for normality using a two-way histogram. (figure 6).

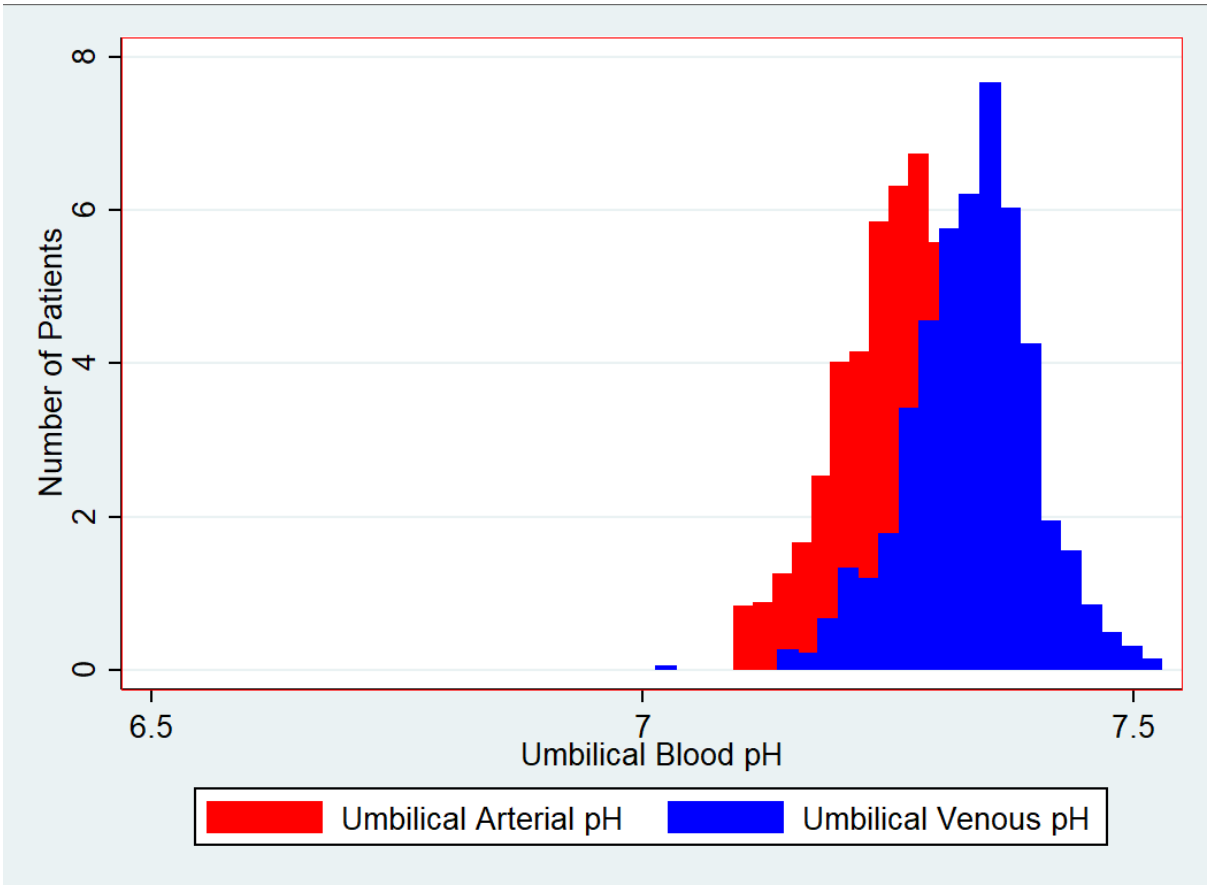


Figure 6 Above: Two-way histogram demonstrating the normal distribution of the umbilical venous pH, superimposed onto the umbilical arterial pH. Note that the data is censored so as to remove any umbilical artery pH under 7.1 (venous pH values were not censored).

Both umbilical venous (blue) and umbilical arterial (red) blood pH values have been superimposed onto the same histogram so as to give a side by side demonstration of the two pH distributions. Both umbilical arterial and venous blood pH are normally distributed, with umbilical arterial blood lying slightly to the left of umbilical venous blood as it has been deoxygenated by fetal circulation and thus more acidotic, with lower pH values (figure 6).

Correlation

For data visualisation, a scatterplot is provided for pH values, which shows how values may stray from the line of perfect correlation. It can be seen that umbilical arterial pH is positively correlated with umbilical venous pH.

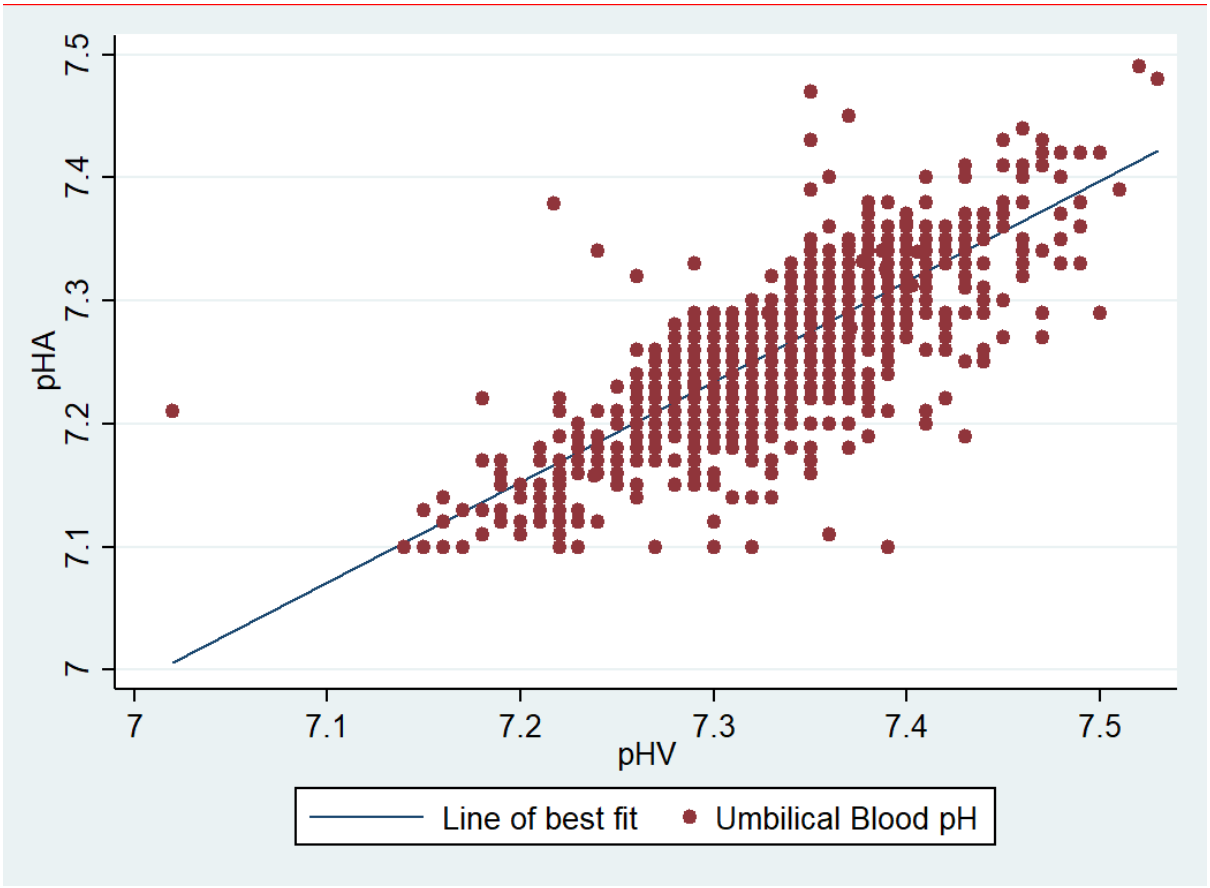


Figure 7 Scatter graph showing the correlation between umbilical venous Ph (pHv) on the horizontal axis against umbilical arterial pH (pHa)on the vertical axis.

Data Visualisation

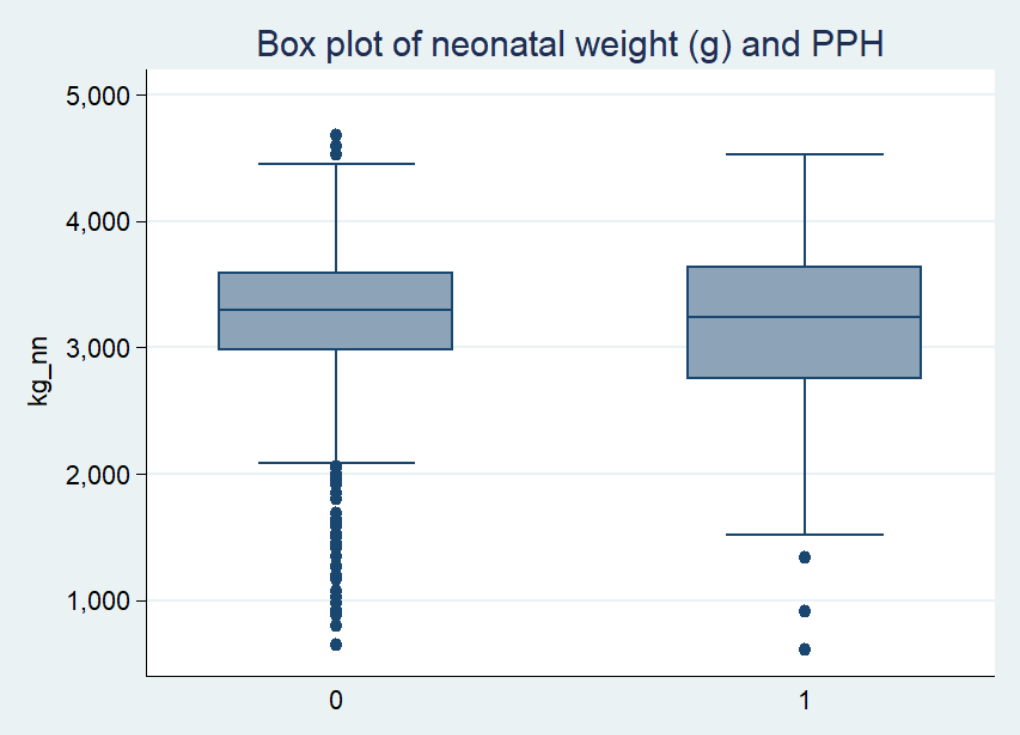
Box plots were produced for all continuous variables stratified by PPH_Yes_No, so to give a visual indication of the skew and distribution of data and skewness. The continuous variables are:

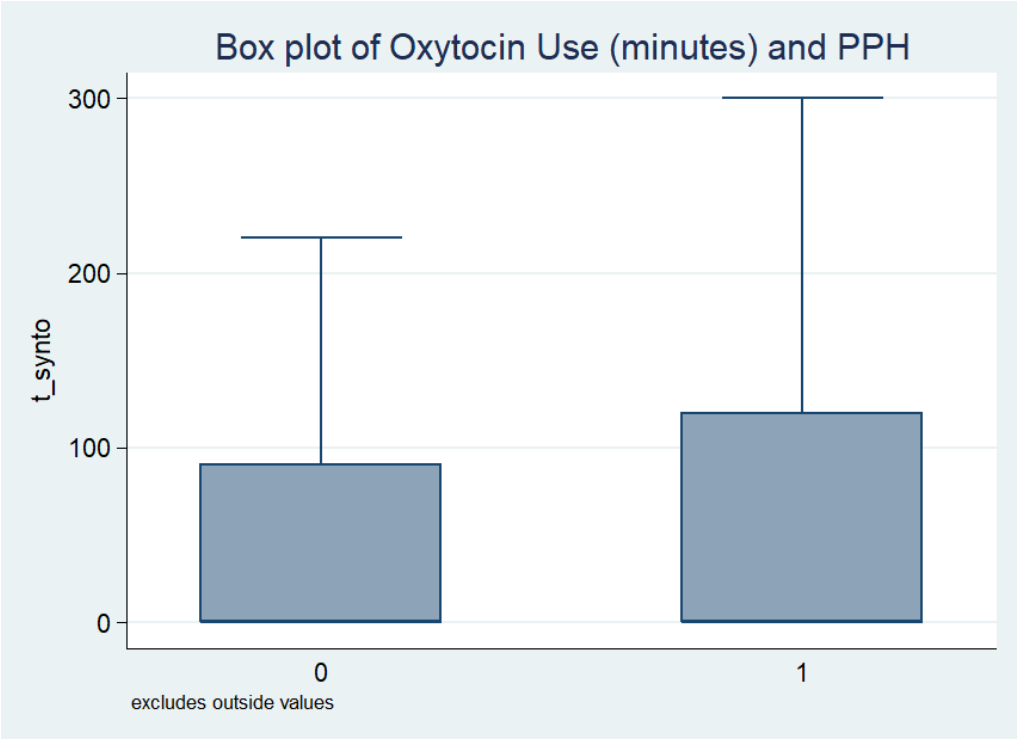
1. neonatal weight (g)
2. oxytocin use (minutes)
3. childbirth second phase length (minutes)
4. gestational age at delivery (weeks)
5. total childbirth length (minutes)
6. maternal age (years)
7. BMI

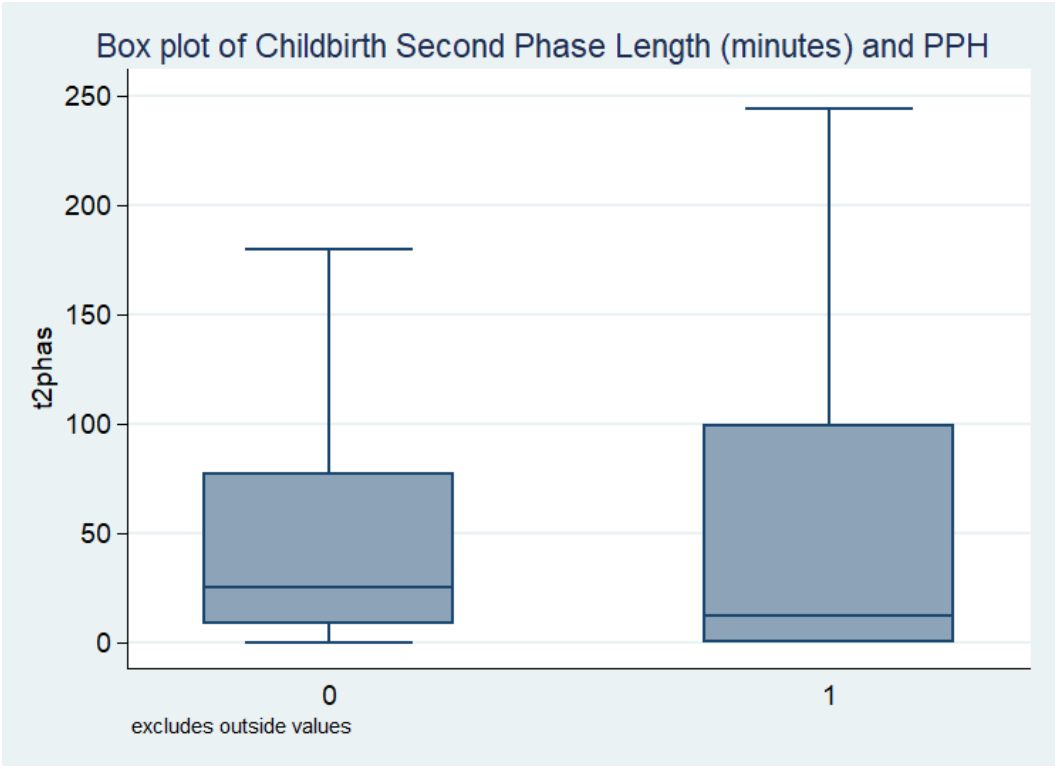
8. umbilical arterial pH

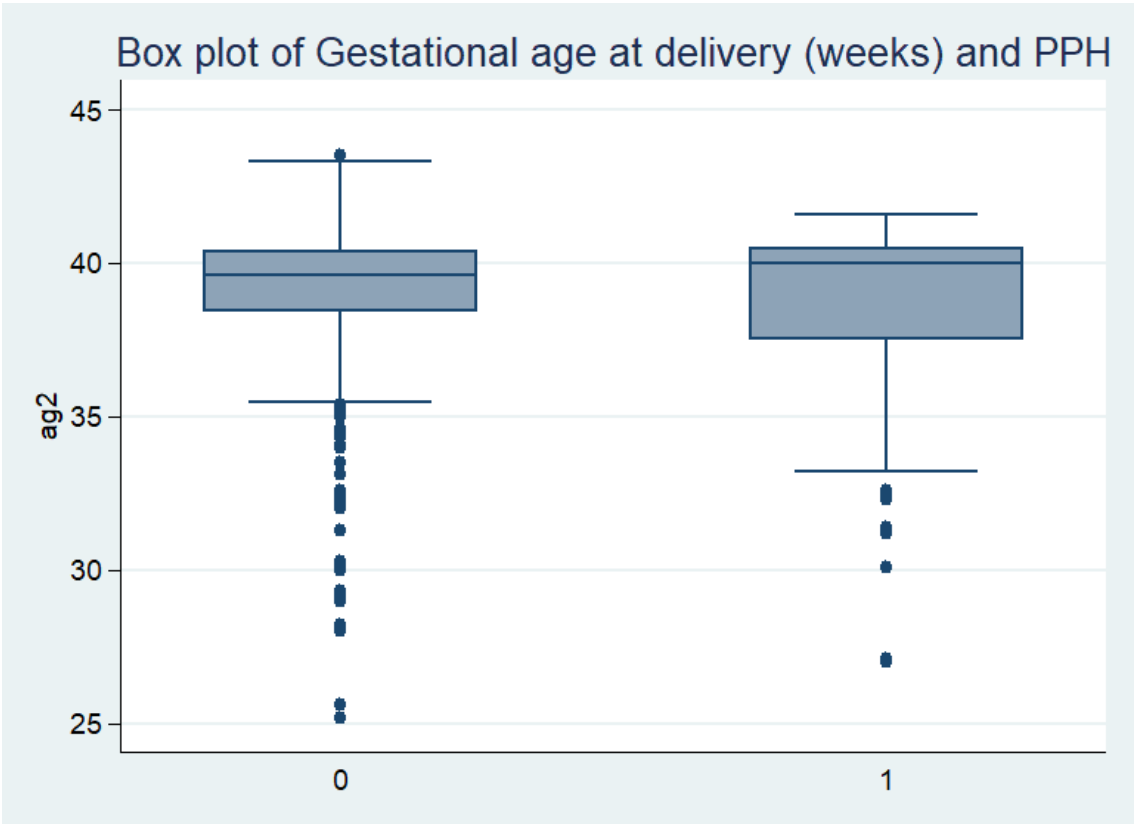
9. umbilical venous pH

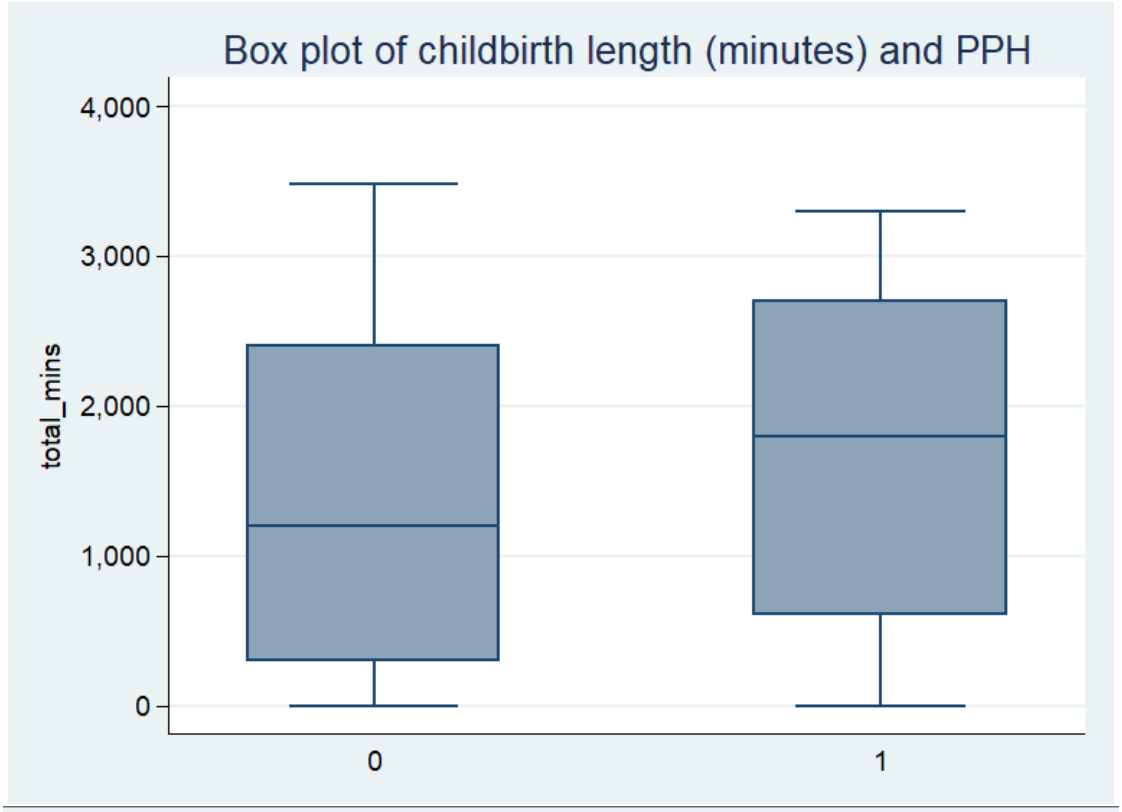
Note on horizonal axis: 0= No PPH, 1= PPH

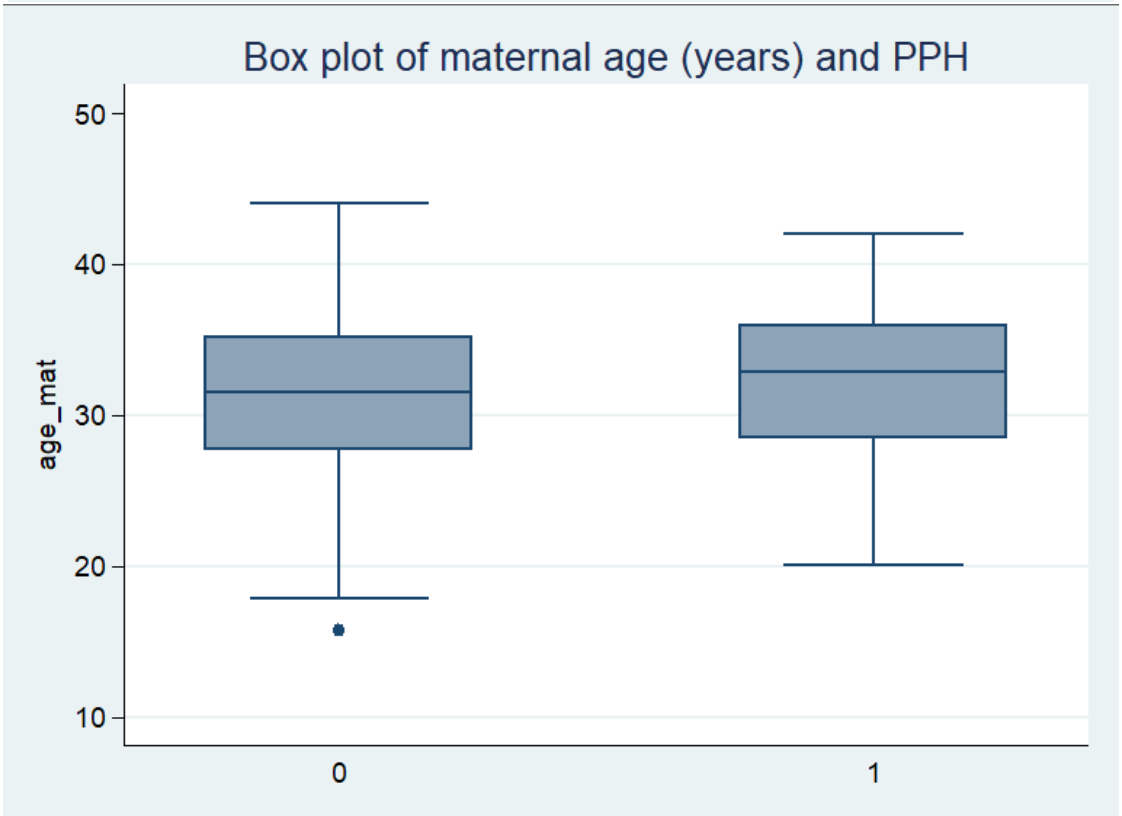


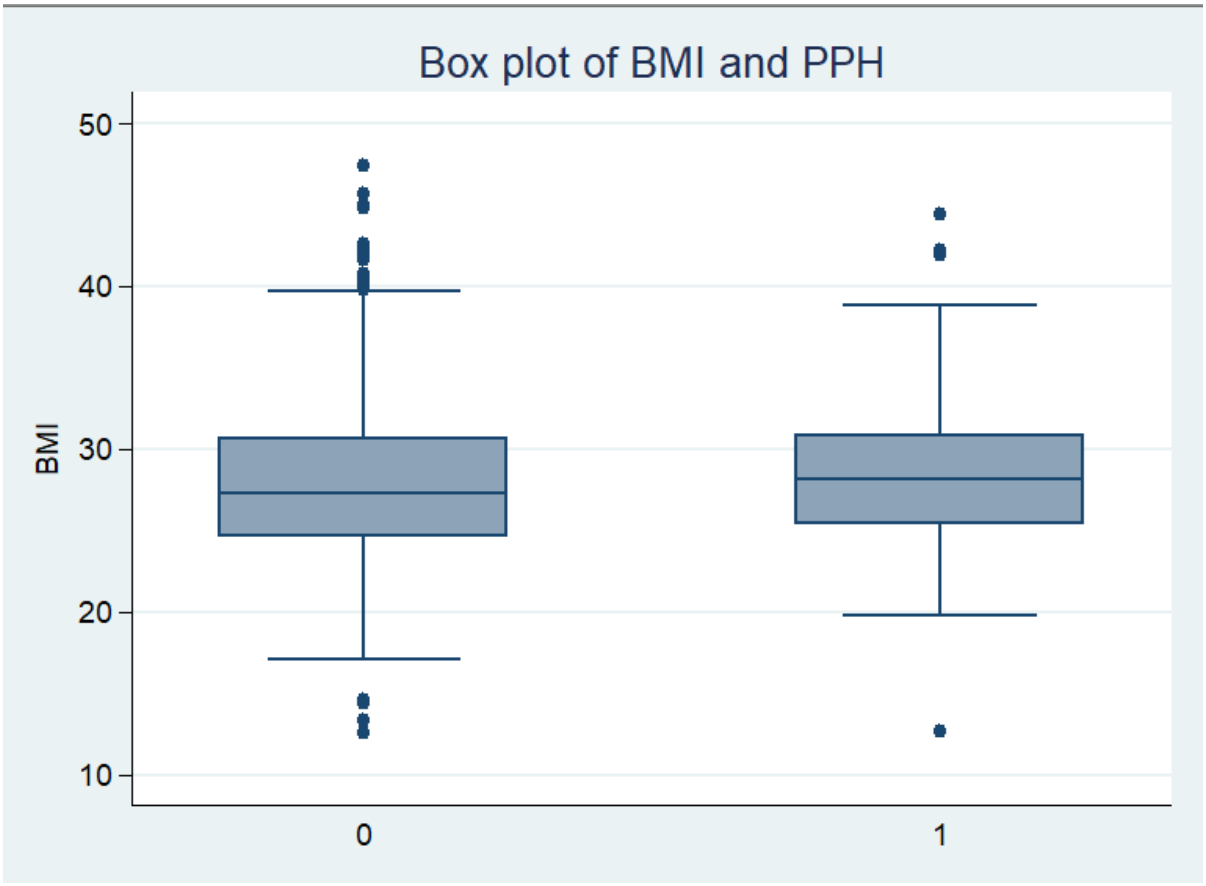


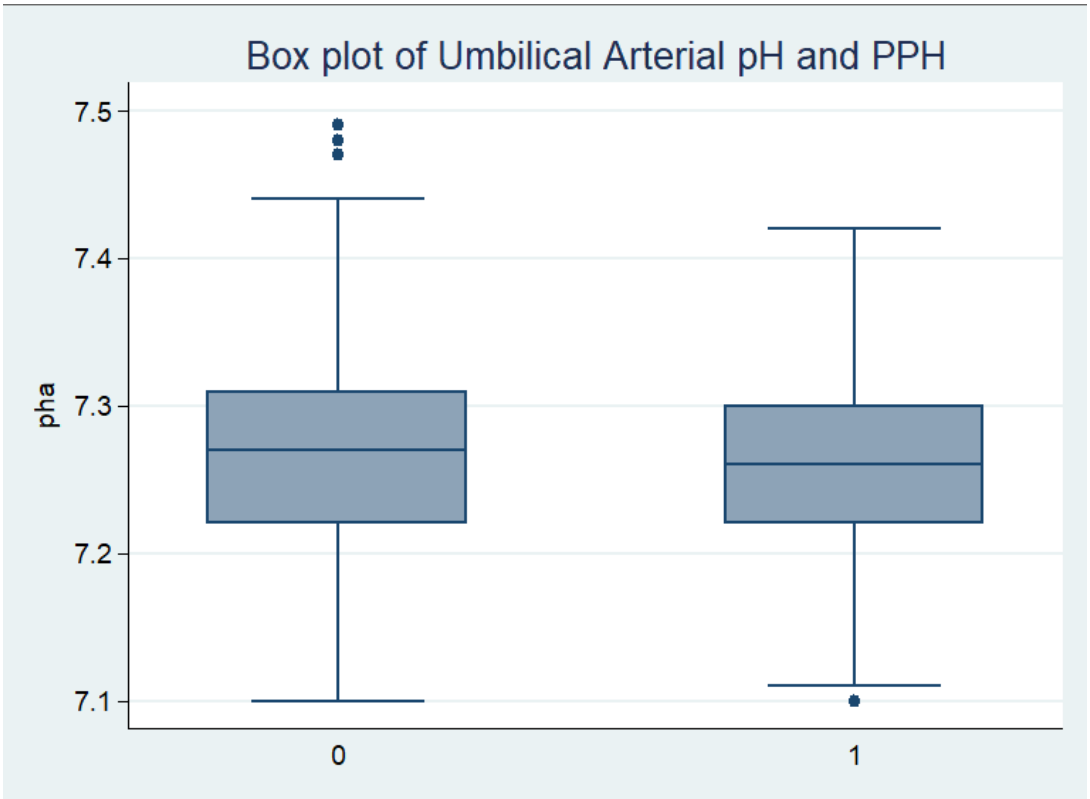

Box plot of Childbirth Second Phase Length (minutes) and PPH
t2phas
250
200
150
100
50
0
0
1
excludes outside values


Box plot of Gestational age at delivery (weeks) and PPH
ag2
45
40
35
30
25
0
1


Box plot of childbirth length (minutes) and PPH
total_mins
4,000
3,000
2,000
1,000
0
0
1


Box plot of maternal age (years) and PPH
age_mat
50
40
30
20
10
0
1


Box plot of BMI and PPH
BMI
50
40
30
20
10
0
1


Box plot of Umbilical Arterial pH and PPH
pha
7.5
7.4
7.3
7.2
7.1
0
1

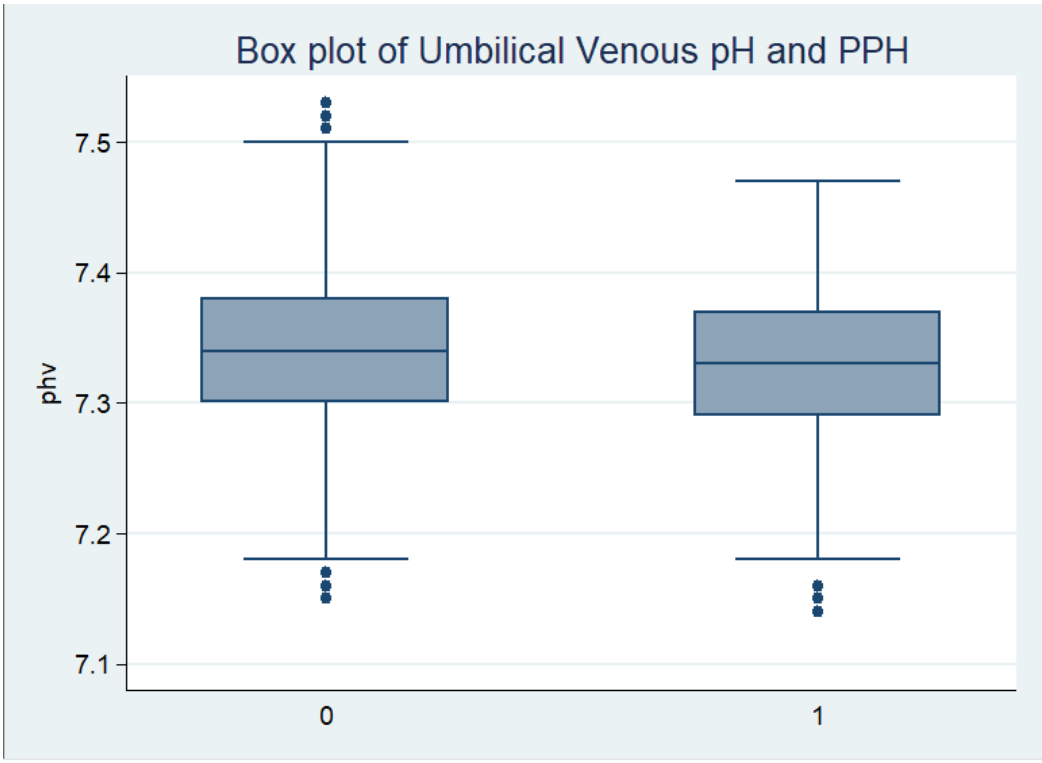


Figure 8 Box plots of all continuous variable against PPH_Yes_No: Neonatal weight (g), oxytocin use (minutes), childbirth second phase length (minutes), gestational age at delivery (weeks), total childbirth length (minutes) maternal age (years), BMI, umbilical arterial pH and umbilical venous pH. Note on x-axis: 0= No PPH, 1= PPH

Demographics and Univariate Analysis

Demographics have been reported on all explanatory variables stratified by those patients who had a PPH and those who did not have a PPH (according to the PPH_Yes_No definition). Continuous variables have been reported using the mean (standard deviation) or median (IQR) if they are not normally distributed. Categorical variables, such as blood loss, have been reported as number n (percentage) for each category within each blood loss category. Logistic regression was used for the univariate analysis and to build the multivariate model, so the demographics table also contains the p values from the univariate analysis.

The variables that show statistical significance then go forward into multivariate analysis. Others variables can be excluded unless there is a clinical reason for retaining them.

Table 4: Patient demographics for women included in study (PPH_Yes_No outcome variable) and Univariate Analysis

Variable		**Women who didn't have a PPH**	**Women who had PPH**	**Univariate analysis significance (p_value)**
N(%)		783 (86.42)	123 (13.5)	
Maternal age Mean (St.dev.)		31.35 (5.36)	32.11 (5.12)	0.14
Gestational age at delivery Mean (St.dev)		39.12 (2.33)	38.82 (2.81)	0.20
Second phase length Mean (St.dev)		51.83 (66.27)	52.85 (68.34)	0.88
Neonatal weight (g) Mean (St.dev)		3221.64 (597.35)	3139.67 (712.45)	0.17
Umbilical Arterial pH Mean (St.dev)		7.27 (0.06)	7.26 (0.07)	0.33
Umbilical Venous pH Mean (St.dev)		7.34 (0.06)	7.32 (0.06)	**0.04**
BMI Mean (St.dev)		27.98 (4.84)	28.50 (4.71)	0.27
Childbirth total length (minutes) Mean (St.dev)		1428.31 (115.39)	1653.20 (1159.41)	0.21
How long Oxytocin was used for (minutes) Mean (St.dev)		60.58 (99.95)	80.24 (132.70)	0.06
Obesity Yes?n(%)		225 (28.74)	39 (31.71)	0.50
Forceps yes? n(%)		65 (77.38)	19 (15.45)	**0.01**
Ventouse yes? n(%)		7 (8.30)	1 (0.81)	0.93
Induced labour Yes? n(%)		336 (42.91)	60 (48.78)	0.22
Perineal tear yes? n(%)		101 (12.90)	8 (6.50)	**0.05**
Perineal trauma yes? n(%)		382 (48.79)	52 (42.28)	0.18
Episiotomy yes? n(%)		286 (36.53)	44 (35.77)	0.87
Coagulation abnormalities that increase bleeding n(%)		0 (0.00)	1 (100)	Predicts success perfectly
Preeclampsia n(%)		14 (1.79)	4 (22.22)	0.29
Previous CS n(%)		79 (10.09)	21 (21.00)	**0.02**
Was oxytocin infused in labour, yes? n(%)		346 (44.19)	58 (14.36)	0.25
Multiple gestation >1 n(%)		20 (2.55)	9 (31.03)	**0.008**
Low inserted placenta yes? n(%)		2 (0.26)	0 (0.00)	predicts failure perfectly
Retained placenta yes? n(%)		15 (1.92)	25 (62.50)	**<0.001**
Parous? >0 n(%)		439(56.10)	57.(11.49)	**0.05**
Birth mode:	0= spontaneous vaginal birth n(%)	596 (76.18)	58 (8.87)	Reference

	1= operative vaginal birth (forceps/ ventouse) n(%)	72 (9.20)	20 (21.74)	**<0.001**
	2= emergency CS n(%)	115 (14.69)	45 (28.13)	**<0.001**

Multivariate Analysis

The table below reports the odds ratio, 95% confidence interval and the significance level (p value). Variables that remained significant (p<0.05) were operative vaginal birth, emergency CS and retained placenta, and these were the only variables retained in the final model

Table 5: Multivariate model for PPH_yes_no

Variable	**Odds ratio**	**95% confidence interval**	**Multivariate analysis significance (p_value)**
Birth mode			
Spontaneous vaginal	Reference		
Operative vaginal	3.43	(1.88, 6.29)	**<0.001**
Emergency CS	5.30	(3.31, 8.50)	**<0.001**
Retained placenta			
No	Reference		
Yes	19.35	(9.45, 39.63)	**<0.001**
Multiple gestation			
No	Reference		
Yes	2.12	(0.85, 5.21)	0.11
Forceps			
No	Reference		
Yes	1.71	(0.20, 14.88)	0.63
Previous_CS			
No	Reference		
Yes	1.27	(0.71, 2.27)	0.42
Umbilical Venous pH	0.26	(0.01, 9.07)	0.46
Parous >0			
No	Reference		
Yes	0.97	(0.63, 1.50)	0.90
Perineal Tear			
No	Reference		
Yes	0.79	(0.35, 1.77)	0.57

Risk Score

Each risk factor is not equally valued which is why the regression model was used in this analysis; to allow for higher weighting for some variables compared to other variables.

The significant variables (operative vaginal birth, emergency CS and retained placenta) were derived from the logistic regression model. Because there are only two explanatory variables; mode of delivery (spontaneous vaginal birth, operative vaginal birth and CS) and retained

placenta (yes/no), there are only 6 possible combinations of risks. In this example, operative vaginal birth and CS are recorded as one or the other, so a patient cannot have both. The possible combinations are; 1) spontaneous vaginal birth and no retained placenta (no risk factors), 2) spontaneous vaginal birth and retained placenta, 3) operative vaginal birth and no retained placenta, 4) operative vaginal birth and retained placenta, 5) CS and no retained placenta and 6) CS and retained placenta. Therefore, there are only 6 possible risk scores that can come from this model, and they are on a scale from 0 to 1 (the closer to 1, the higher risk of PPH). **See table below for risk scores.** For ease of use, rather than using the actual risk, it is usual to determine an optimal cut-off value for classifying women as either being at risk or not at risk.

Emergency CS	**Operative Vaginal Birth**	**Retained Placenta**	**Risk score**
Yes	No	**Yes**	0.87
No	**Yes**	**Yes**	0.82
No	No	**Yes**	0.57
Yes	No	No	0.27
No	**Yes**	No	0.19
No	No	No	0.06

Table 6 Calculated risk scores for each possible outcome

As it can be seen from table 6 above, all factors increase the risk of PPH, especially when combined with retained placenta. The ROC scores suggest that a cut-off of 0.19 is optimal (see figures 9 and 10), so any retained placenta with operative birth, or retained placenta or emergency CS alone all are predictive of PPH and should prompt precautionary measures.
The risk score is the same as chance of getting a PPH. So, a risk score of 0.87 is the same as 87% chance of getting a PPH.

Validation of Predictive Properties of Model

The generated STATA output showed that the lower value of the confidence 95% interval is 0.71 (0.71-0.80). The lower value will be taken as the value for the area under the curve as this is lowest level of discrimination. The diagonal line on the ROC curve represents AUC=0.5, which would be the same as tossing a coin, or 50-50 chance. The current model can discriminate between a woman who is likely to have a PPH and a woman that not likely to have a PPH as the AUC is equal to 0.71.

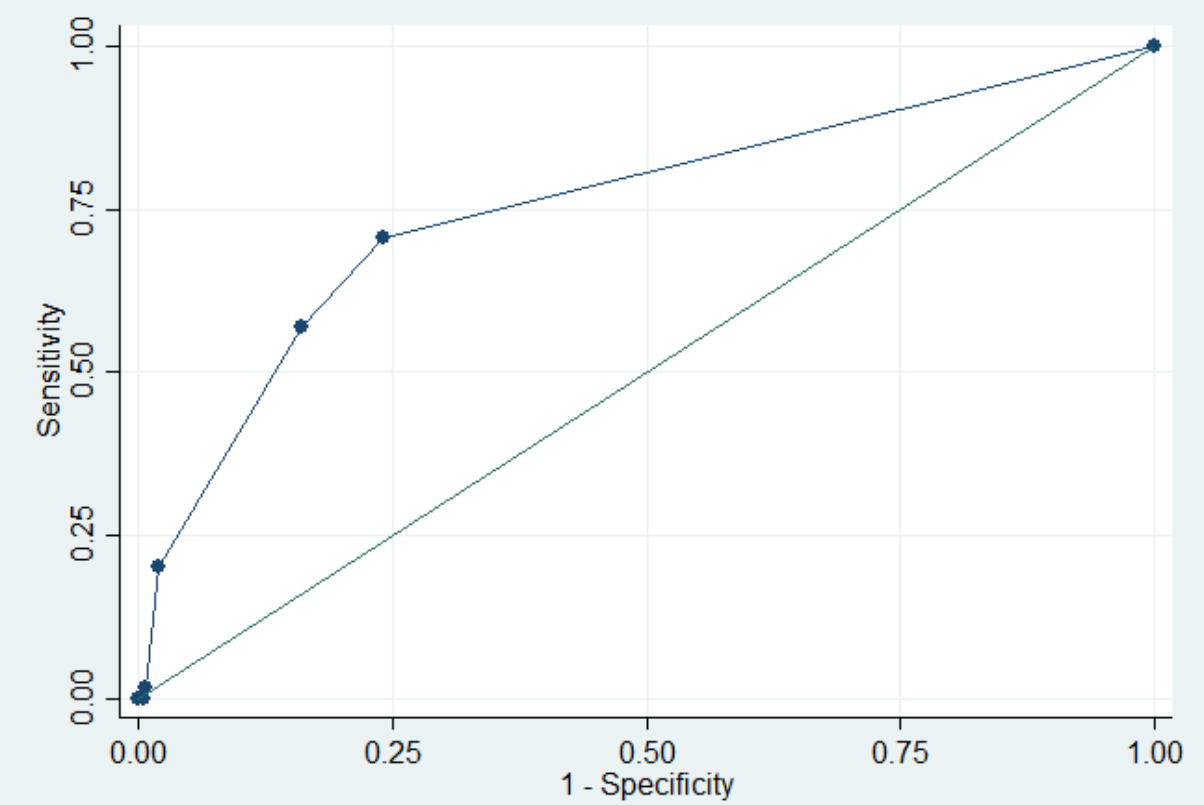


Figure 9 ROC curve showing cut points plotted against sensitivity and specificity. AUC= 0.71

Determining an Optimal Cut-Off

Although the optimal cut-off or the risk score can be determined by visualising the ROC curve (figure 9) and seeing which value is closest to the top left-hand corner of the graph, the value can be determined more accurately through the creation of a sensitivity and specificity curves. In Figure 10, the cut off values for sensitivity and specificity have both been plotted on the same graph for a range of cut-off values. The cut-off point would be then used to classify whether a woman would be at risk or not at risk of a PPH.

From figure 9, the optimum cut-off, in terms of optimising sensitivity and specificity, appears to be 0.19 (sensitivity 71.54%, specificity 74.97%). Figure 10 confirms that 0.19 is a reasonable cut-off point by the position at which the two curves cross.

This means that if a woman has a risk score of 0.19 or more, then she can be classified as being at risk of a PPH, with a 71.54% accuracy of diagnosis. If the woman has a risk score of less than 0.19 then she is classified as not being at a risk of PPH, with 74.97% accuracy. For clinical use, determining the cut off values may determine whether clinicians have blood prepared for the woman for when she goes into labour, for example. Cut-off values may also determine whether the patient is given additional drugs to prevent PPH, or whether a certain medical professional of superiority is assigned to that patient as they may be experienced with dealing with PPH, or whether steps have been taken to prepare blood for transfusion.

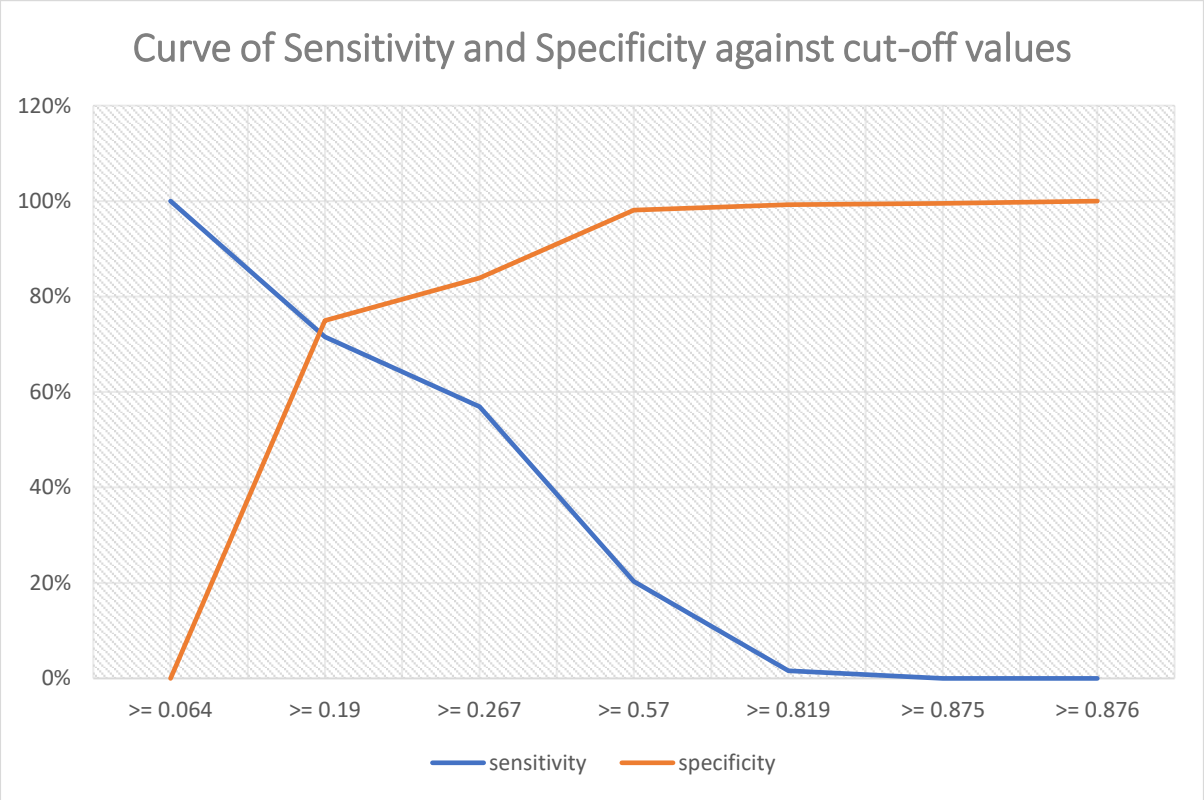


Figure 10 curve of sensitivity and specificity both plotted against cut-off values for risk scores.

Positive and Negative Predictive Values

A Positive predictive value (PPV) is the probability that a woman, predicted to be at risk of a PPH, will genuinely have a PPH. **A negative predictive value (NPV)** is the probability that a woman, predicted to not be at risk of a PPH, will genuinely not have a PPH.

Below is the 2x2 table used to calculate the positive predictive values (PPV) and negative predictive values (NPV).

Risk Score	**PPH: Yes/ No**		**Total**
	No	Yes	
0	587	35	622
1	196	88	284
Total	**783**	**123**	**906**

Table 7 2x2 table used to calculate PPV and NPV.

To calculate the positive predictive value and negative predictive value:

PPV: (88/284) *100 = 30.99%

NPV: (587/622) *100 = 94.37%

This means that if a woman is identified as being at risk of a PPH by the model, then there is a 31% chance she will experience a PPH. If a woman is identified at not being at risk of a PPH by the model, then there is a 94% chance she will not experience a PPH.

Sensitivity Analysis Results

This second section of the results chapter contains the results from the sensitivity analysis. The definition for PPH has been changed to a blood loss of ≥1000ml for all modes of birth, to determine if the change in definition would make a significant difference to the outcomes of the multivariate analysis and other subsequent statistical measures, such as, positive/negative predictive values. Box plots were produced for all continuous variables against pe1000, but have not been shown. However, this data is available on request.

Results of the sensitivity analysis differed slightly to that of the main analysis. There were far fewer patients who had a PPH with blood loss of ≥1000ml. 21 women, 2.32%, had a PPH according to the pe1000 definition. 885 women, 97.68%, did not have a PPH according to the

pe1000 definition. In comparison, the PPH_Yes_No definition (n=123,13.5%) resulted in 5.86 times more patients to be classified as having a PPH than the pe1000 definition.

The smaller sample size has not only meant that 95% confidence intervals are much wider, but the significant variables from the univariate and multivariate analysis have differed from the main analysis too. Logistic regression in the sensitivity analysis showed retained placenta and induced labour to be significant predictor variables. Logistic regression in the main analysis also showed retained placenta to be a significant variable, but it also showed emergency CS and operative vaginal birth to be significant predictor variables.

Demographics and Univariate Analysis for pe1000

Demographics have been reported on all explanatory variables by those patients who had a PPH and those who did not have a PPH (according to the pe1000 definition).

Table 8 Patient demographics for women included in study (pe1000 outcome variable) and Univariate Analysis

Variable	**Women who didn't have a PPH**	**Women who had PPH**	**Univariate analysis significance (p_value)**
N(%)	885 (97.68)	21(2.32)	
Maternal age Mean (St.dev.)	31.43 (5.35)	32.71(4.61)	0.29
Gestational age at delivery Mean (St.dev)	39.08(2.40)	38.98(2.33)	0.84
Second phase length Mean (St.dev)	51.67(68.19)	64.81(74.80)	0.38
Neonatal weight (g) Mean (St.dev)	3210.30(613.21)	3219.52(680.74)	0.95
Umbilical Arterial pH Mean (St.dev)	7.26(0.06)	7.28(0.06)	0.16
Umbilical Venous pH Mean (St.dev)	7.33(0.06)	7.35(0.06)	0.24
BMI Mean (St.dev)	28.07(4.85)	27.31(3.26)	0.47
Childbirth total length (minutes) Mean (St.dev)	1448.22(1126.17)	1730.46(1033.04)	0.38
How long Oxytocin was used for (minutes) Mean (St.dev)	62.20(101.85)	107.38(198.58)	0.06
Obesity Yes?n(%)	260 (29.38)	4(19.04)	0.31

Forceps yes? n(%)		82 (9.27)	2(9.52)	0.97
Ventouse yes? n(%)		8(0.90)	0(0.00)	predicts failure perfectly
Induced labour Yes?n(%)		382(43.16)	14(66.67)	**0.04**
Perineal tear yes? n(%)		109(12.31)	0(0.00)	predicts failure perfectly
Perineal trauma yes? n(%)		428(48.36)	6(28.57)	0.08
Episiotomy yes? n(%)		324 (36.61)	6(28.57)	0.45
Coagulation abnormalities that increase bleeding n(%)		1(0.11)	0(0.00)	predicts failure perfectly
Preeclampsia n(%)		17(1.92)	1(4.76)	0.37
Previous CS n(%)		97(10.96)	3(14.29)	0.63
Was oxytocin infused in labour, yes? n(%)		395(44.63)	9(42.86)	0.78
Multiple gestation >1 n(%)		27(3.05)	2 (9.52)	0.12
Low inserted placenta yes? n(%)		2(0.23)	0(0.00)	predicts failure perfectly
Retained placenta yes? n(%)		30(3.39)	10(47.62)	**<0.001**
Parous >0 n(%)		484(54.69)	12(57.14)	0.82
Birth mode:	0= spontaneous vaginal birth n(%)	638(72.09)	16(76.19)	Reference
	1= operative vaginal birth (forceps/ ventouse) n(%)	90(10.17)	2(9.52)	0.87
	2= emergency CS n(%)	157(17.74)	3(14.29)	0.67

Multivariate Analysis for pe1000

The table below reports the odds ratio, 95% confidence interval and the significance level (p value) taken from the multivariate analysis.

Table 9 Multivariate model for pe1000

Variable	**Odds ratio**	**95% confidence interval**	**Multivariate analysis significance (p_value)**
Retained placenta No yes	 Reference 30.90	 (11.66, 81.88)	 **<0.001**
Induced labour No Yes	 Reference 3.47	 (1.28, 9.37)	 **0.01**

Validation of Predictive Properties of Model

A ROC curve was produced to show that the current model can discriminate between women who are likely to have a PPH and women who are not likely to have a PPH.

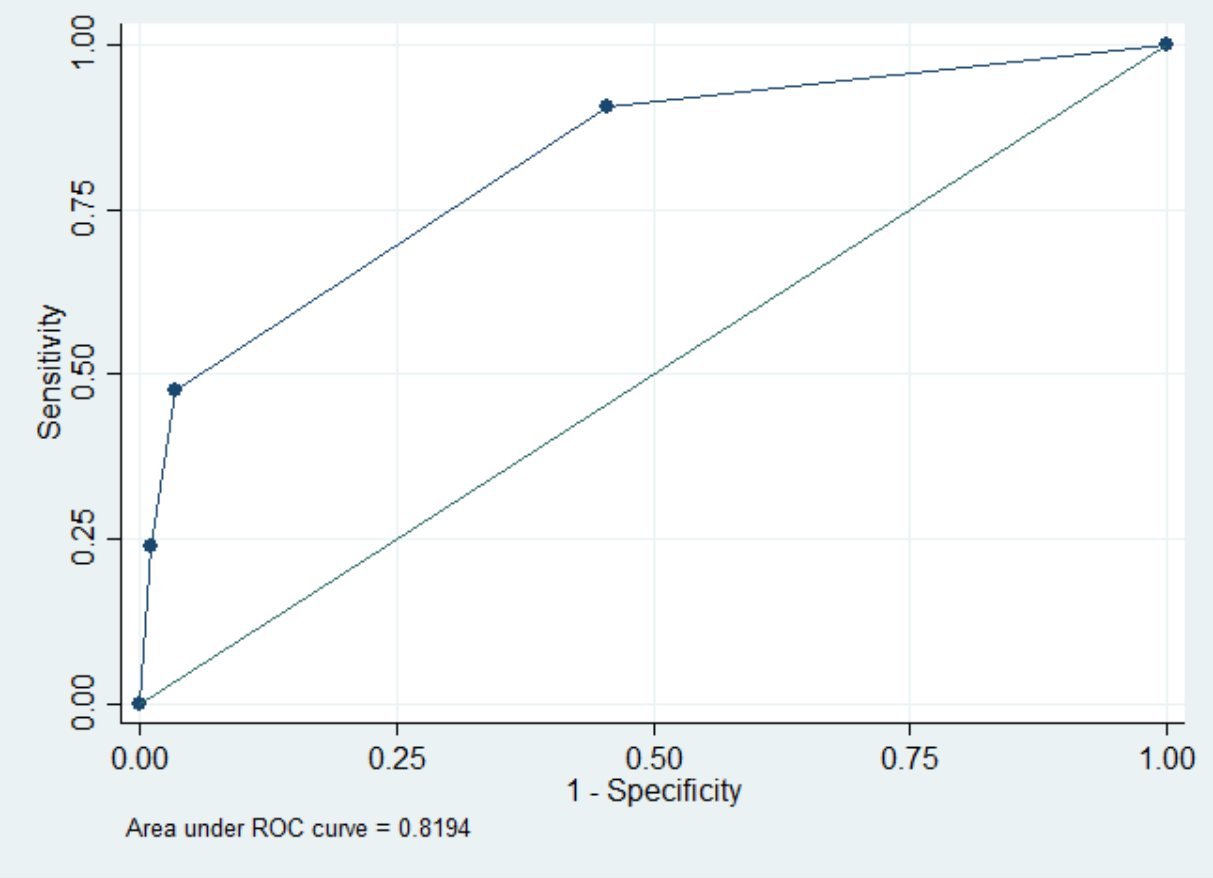


Figure 11 ROC curve for pe1000 showing cut points plotted against sensitivity and specificity. AUC= 0.73

The ROC curve for the sensitivity analysis shows that the current model can discriminate between women who are likely to have a PPH and women who are not likely to have a PPH.

The generated STATA output showed that the lower value of the 95% confidence interval is 0.73 (0.73-0.91) which will be taken as the value for the area under the curve. Therefore, the model for the sensitivity analysis can discriminate between a woman who is likely to have a PPH and a woman that not likely to have a PPH as AUC= 0.73.

Determining an Optimal Cut-Off

Cut off values for the sensitivity curve and the specificity curve have both been plotted on the same graph (figure 12).

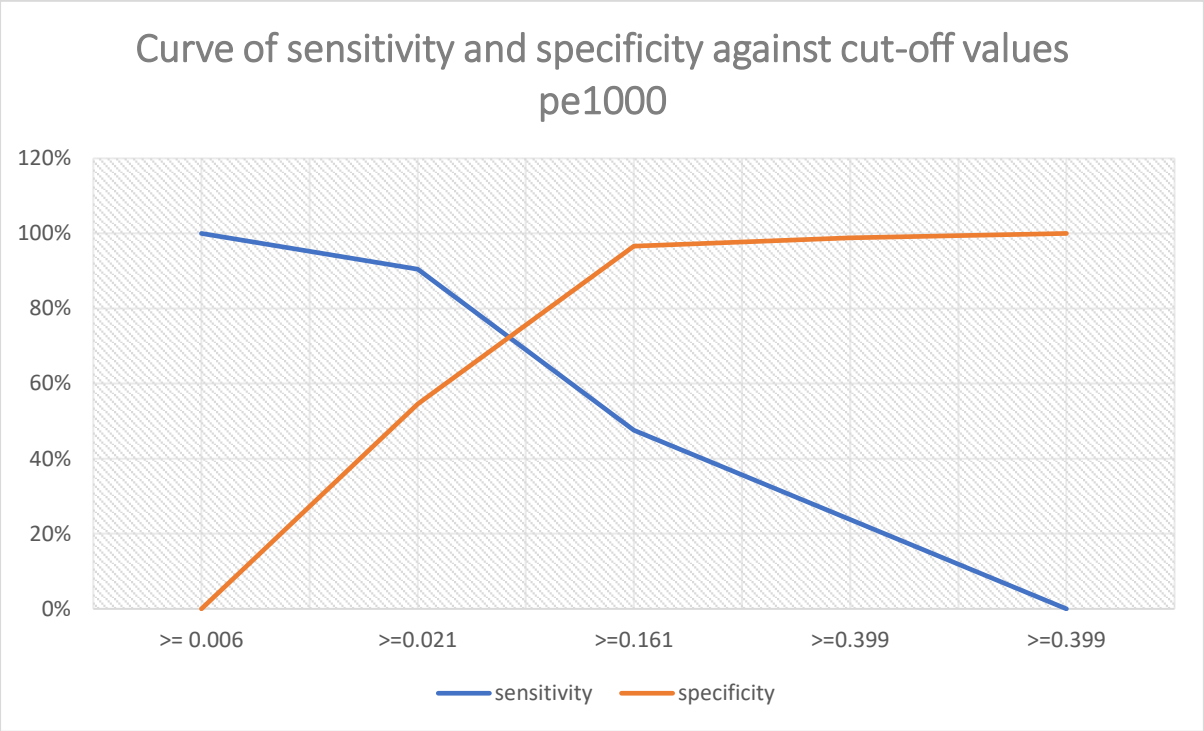


Figure 12 curve of sensitivity and specificity both plotted against cut-off values for risk scores.

From the ROC curve (figure 11) the optimum cut-off, in terms of optimising sensitivity and specificity, appears to be 0.021 (sensitivity 90.48%, specificity 54.58%). Figure 12 confirms that 0.021 is a reasonable cut-off point by the position at which the two curves cross.

This means that if a woman has a risk score of 0.021 or more, then she can be classified as being at risk of a PPH, with a 90.48% accuracy of diagnosis. If the woman has a risk score of <0.021 then she is classified as not being at a risk of PPH, with a 54.58% accuracy of diagnosis.

Positive and Negative Predictive Values for pe1000

Below is the 2x2 table used to calculate the positive predictive values (PPV) and negative predictive values (NPV).

Risk Score	PPH: Yes/ No		Total
	No	Yes	
0	483	2	485
1	402	19	421
Total	**885**	**21**	**906**

Table 10 2x2 table used to calculate PPV and NPV for the sensitivity analysis

To calculate the positive predictive value and negative predictive value:

PPV: (19/421) *100 = 4.51%

NPV: (483/485) *100 = 99.59%

This means that if a woman is identified as being at risk of a PPH, then there is a 4.51% chance she will suffer a PPH. If a woman is identified at not being at risk of a PPH, then there is a 99.59% chance she will not suffer a PPH.

Chapter 4. Discussion

Summary of Findings

The main aim of this thesis was to report on an association, if any, between maternal acidosis and increased PPH rates. The study also reported on predictors of PPH rates and incidence of PPH, using two different definitions of PPH.

Although results from the univariate model showed that low fUVpH had a correlation with PPH incidence (p=0.04), results from the multivariate model showed that fUVpH was not independently associated with PPH incidence when controlling for other variables, regardless of definition used for PPH. The data revealed that when PPH is defined as >500ml blood loss for a vaginal birth and >1000ml blood loss for a caesarean birth, the predictor variables that significantly increased PPH risk were; an operative vaginal birth, an emergency CS and a retained placenta.

The predictive properties of the multivariate model were validated using ROC curves. It was found that the model could discriminate between a woman who is likely to have a PPH and a woman who is not likely to have a PPH. The optimum cut-off value for classifying women as being at risk of a PPH was 0.19, with a sensitivity of 71.54% and specificity of 74.97%. PPV and NPV was also calculated to be 31% and 94% respectively.

To reiterate, 0.19, or the optimum cut-off value, is the value of the screening tool that optimises accuracy, in terms of correctly identifying cases and non-cases (sensitivity and specificity). It is this value that is then used to classify patients of being either a case or non-case. However, some clinical judgement can be used in this instance. For example, if someone is screening for a high-risk cancer, then one might choose a cut-off that increases the detection of cases but also trade off some of the accuracy in detecting non-cases.

Screening tools such as this may often have very low positive predictive values, but very high negative predictive values. This is still clinically useful as it gives reassurance that if a patient is showing up as not being at risk of poor clinical outcome, then clinicians can be confident that the patient is unlikely to get a PPH.

It was found that results differed during the sensitivity analysis. When the definition for PPH was changed to >1000ml blood loss for all modes of birth, retained placenta remained a

significant predictive factor for PPH. However, the only other predictive factor was found to be induced labour. The differences in the analyses are of clinical interest as it highlights the importance of having a robust PPH definition. Results show how one needs to be careful with how PPH is defined, because changes in definition resulted in changes in the explanatory variables that are associated with PPH. For example, when the definition of ≥1000ml EBL (estimated blood loss) for all birth modes was used, it meant that emergency CS stopped becoming a significant predictor variable. When the PPH definition of ≥500ml-≤1000ml EBL for vaginal births and ≥1000ml EBL for emergency CS births is used, then emergency CS is a significant predictor variable.

The ROC curve also showed that the model for the sensitivity analysis, using 1000mls for all births could discriminate between a woman who is likely to have a PPH and a woman that not likely to have a PPH. The optimum cut-off value was 0.021, with a sensitivity of 90.48%, and specificity of 54.58%. PPV and NPV was also calculated to be 4.51% and 99.59% respectively.

As mentioned in the 'Covid Disruptions' chapter, data protection laws and being unable to travel to Switzerland prevented me from having access to the 33,000-patient dataset. 6% of the total dataset, 2000 patient records, was anonymised and sent to me. Fortunately, this sample dataset was large enough to be used for the purposes of this MPhil thesis, allowing me to build up the model and show proof of concept. Once pandemic travel restrictions are fully lifted, Professor David Baud has agreed that I may travel to Switzerland and run the STATA files on the full 33,000 myself, with the aim of yielding more robust results.

Similar Studies

It is believed that this study is the first to specifically look at the relationship between blood pH and PPH rates. Furthermore, this study is certainly the only one of its kind to use umbilical vein pH as a surrogate measure for myometrial capillary pH. However, several similar studies have previously looked into using lactate, rather than direct blood pH, as an indicator for the occurrence of PPH and the need for subsequent blood transfusion.

One of the first retrospective analyses that directly assessed the association between maternal blood lactate and massive blood transfusion for PPH was conducted by Sohn et al in 2015. From a total of 302 patients, 101 (33.4%) suffered a PPH and required a massive blood transfusion. Of these patients, blood Lactate concentration was found to have an

independent association with the need for blood transfusion, OR 1.56, 95% CI 1.31-1.87, p=<0.01. Area under the receiver operating curve (ROC) was found to be 0.788 (95% CI: 0.736–0.840; P<0.01). Specificity was found to be 86.1% for lactate levels above 4.0 mM L−1. These elevated lactate levels also had a positive predictive value (PPV) of 59% (Sohn *et al.*, 2018).

A Japanese retrospective cohort study, at the Osaka City General Hospital, aimed to identify more predictive factors for the need for massive blood transfusion in PPH cases. The analysis cohort consisted of 31 patients who were transferred to the emergency department with PPH, between April 2012 and March 2016. The primary outcome was the requirement for massive blood transfusion as (≥10 units/ per 24 hours of packed red blood cells). Okada et al calculated the odds ratio for lactate concentration to be 1.62 (mmol/L) (95% CI, 1.08–3.02). Area under the curve for the ROC was 0.734, and 4 mmol/L was found to be the optimal cut-off concentration value. Authors concluded that their findings suggest that blood lactate concentration could be used as an effective predictive factor for the need for massive blood transfusion in cases of PPH (Okada *et al.*, 2020).

A very similar study, conducted between August 2018 and June 2020, also aimed to identify predictive factors in the need for blood transfusion in PPH cases. Authors Attali et al defined PPH as an EBL of >500 ml after vaginal delivery and >1000 ml after CS. The study included 94 patients in the final analysis who had a PPH, and who also had data on both postpartum lactate and haemoglobin concentrations. In addition, the shock Index (SI: heart rate divided over systolic blood pressure) was calculated for each woman. For women who received a blood transfusion, the mean lactate concentration was found to be significantly higher (3.2 ± 1. mmol/L, p = 0.016), however, significance was lost in multivariate analysis. The overall findings of this study were that the combination of lactate and haemoglobin concentration as well as SI, had a good predictive power for the need for blood transfusion (Attali *et al.*, 2021).

In 2020, Basil-Kway et al evaluated the efficacy of serum lactate being used as a prognostic marker for SPPH. Authors revealed that 2.6mmol/L serum lactate could be a promising predictor of SPPH, and a potential marker to identify patients at risk of PPH and PPH related complications. In this study, PPH was defined as a blood loss of ≥500ml after a vaginal birth

and a loss of ≥1000ml after a CS delivery. Authors classified SPPH it as a blood loss of ≥1500ml at the end of labour, or within 24 hours following delivery (Basil-Kway *et al.*, 2021).

While these studies do not directly look at the effects of low blood pH as an indicator for PPH, an accumulation of lactic acid is known to decrease the intracellular pH, thus leading to myometrial acidosis. Acidotic conditions are known to decrease muscle contractility (see Chapter 1; Acidosis and Dysfunctional Labour) which in turn increases the risk of an atonic PPH occurring. Therefore, the findings of this study are still relevant to the existing body of research around acidosis and PPH. If low blood pH in muscle is known to decrease force of contraction, and a major cause of PPH is known to be uterine atony, then it makes sense to investigate what role low blood pH may have on PPH rates. As mentioned above, the univariate model showed that low fUVpH had a correlation with PPH incidence (p=0.04), suggesting that there may be some strength to the argument that low pH reduces contractility, which in turn leads to atonic haemorrhage. Regardless, it is clear that this area of research is still relevant in understanding the pathophysiology of PPH, and thus needs further investigation.

Comparison with Other Literature

The results of this analysis did show that several predictive factors, other than fUVpH, had a significant association with PPH. The positive predictive factors for PPH were; retained placenta, emergency CS, operative vaginal and labour induction. These predictive factors are also well recognised within the current literature to be associated with PPH. Below are a few examples of papers that support our findings on significant PPH risk factors.

A cohort study conducted in wales, 2020, reported the incidence of PPH over a 1-year period using quantitative blood loss measurement. With PPH being defined as blood loss >1000ml, Bell et al found that the incidence of PPH varied according to the mode of delivery. The mean incidence of PPH was highest in women who underwent emergency caesarean section, with a 95% confidence interval (CI) of 19.8 (18.6–21.0). Instrumental vaginal deliveries had the second highest PPH incidence rate, with a 95% CI of 18.4 (17.1–19.8). For unassisted vaginal deliveries, the mean PPH incidence was lowest, with 95% CI of 4.9% (4.6–5.2) (Bell *et al.*, 2020).

In 2010, a Norwegian retrospective cohort study by Rossen et al showed the risk of SPPH (blood loss >1000ml) to be twice as high after caesarean delivery, compared with that of vaginal delivery (5.9%; 95% CI 5.3-6.6 vs. 2.8%; 95% CI 2.6-2.9) (Rossen *et al.*, 2010). Additionally, caesarean delivery is known to increase risk of infection at the surgical site, intra-abdominal adhesion and placenta accreta spectrum disorders, which all can in turn increase PPH rates.

Another Norwegian population-based study, by Al-Zirq et al revealed that emergency caesarean sections could increase the risk of SPPH (defined as blood loss >1500 mL) by more than three times the normal amount (OR 3.61). This 2008 study also identified other significant obstetric risk factors, which included vacuum delivery, OR 1.83 95% CI 1.56–2.07), forceps delivery (OR 1.87, 95% CI 1.40–2.42), and induction of labour (OR 1.60, 95% CI 1.46–1.75) (Al-Zirqi *et al.*, 2008).

In 1988, Kamani et al, conducted a study that analysed obstetric blood transfusion rates. Authors found that although the rates of caesarean delivery were only 21% in their study group, 67% of the blood transfusion units were administered to women who underwent a c-section delivery. On top of this, Kamani et al identified that uterine atony and retained placenta were among the major indicators for blood transfusions (27% and 17% of units of blood transfused, respectively) (Kamani, McMorland and Wadsworth, 1988).

Combs et al conducted a case control study that analysed risk factors for PPH among vaginal deliveries in 1991. Researchers defined PPH as a drop in haematocrit by ≥10 points or by a need for blood transfusion. PPH was found to occur in 374 of 9598 vaginal deliveries (3.9%). Interestingly, factors found to have an association with PPH included vacuum and forceps delivery (OR 1.66) (Combs, Murphy and Laros Jr, 1991).

Retained placenta has been shown many times that it may significantly increase a mother's risk of PPH. A paper published in Israel, 2005, acknowledges such association. From 1988-2002, obstetric data from singleton deliveries (n = 154 312) was collected from the Soroka medical centre, and multivariable analysis of PPH risk factors was conducted. PPH was defined as blood loss >500 ml, occurring within 24 hours of delivery. Interestingly, Sheiner et al found that retained placenta more than tripled the risk of PPH (OR, 3.5; 95% CI, 2.1-5.8) (Sheiner *et al.*, 2005).

Another prospective cohort study that found retained placenta to be a significant risk factor for PPH was conducted in Latin America by Sosa et al. The primary outcome of this study was moderate PPH, defined as blood loss ≥ 500 ml Secondary outcomes were SPPH (≥ 1000 ml blood loss) and the need for blood transfusion. In this study, blood loss was measured by collecting the blood in a plastic drape placed under the buttocks. It was found that the incidence of retained placenta for moderate PPH was 33.3% (OR 6.02, 95% CI 3.50-10.36), and incidence of retained placenta in SPPH was found to be 17.1% (OR 16.04, 95% CI 7.15-35.99). It is also worth noting that this study also found induced labour to be a significant factor for SPPH; 3.5% (OR 2.00, 95% CI 1.30-3.09) (Sosa *et al.*, 2009).

Similarly, a Dutch population-based cohort study set out to determine risk factors associated with moderate (≥500ml blood loss) and SPPH(≥1000 ml blood loss), in the Zaanstreek district, Netherlands. A multiple logistic analysis of PPH risk factors was conducted on 3464 nulliparous women. Authors found that the most significant risk factors for both moderate and SPPH was third stage of labour ≥30 min and retained placenta, OR 14.1, 95% CI 10.4–19.1) (Bais *et al.*, 2004).

In a review and analysis of 1000 labour records, Brinsden and Clark identified that labour induction almost doubled the rate of PPH in primiparous deliveries, compared with that of PPH rates in spontaneous vaginal deliveries (39 of 1000 women (8.4%) had induced labour and PPH). Authors hypothesised that labour induction may be increasing PPH rates because the oxytocic drugs administered to induce labour have the potential to make the uterus work harder over a shorter period of time. This means that by the time the fetus is born, the myometrial muscle of the uterus is fatigued and may become atonic. In addition to this, oxytocic drugs then administered to stimulate postpartum uterine contraction (to prevent haemorrhage) may have little effect if the uterus has had prior exposure to high levels of the same drugs for a significant period of time (Brinsden and Clark, 1978).

As mentioned above, the significant predictive factors found in this study are also widely reported in the current literature to have the same significant association with PPH, thus adding weight to the validity of this study. Retained placenta, emergency CS, instrumental vaginal delivery labour induction are all well known risk factors for PPH. Clinicians, faced with a patient who is presenting with one (or several) of these variables, knows to treat the patient accordingly, and are on increased alert for signs and symptoms of PPH.

Strengths and Limitations

Although there are many caveats and limitations associated with this study, it can be argued that work in this area of research is still meaningful and clinically useful. There is a significant amount of literature that points towards acidosis having a decreased effect on uterine contractility. There is also plenty of research which proves how decreased uterine contractility is associated with increased PPH rates. Therefore, this piece of research, in essence, attempts to link the two bodies of research and describe what (if any) association there is between acidosis and PPH rates. To this day, PPH remains the world's leading cause of maternal deaths, claiming over 127,000 lives each year (WHO, 2007). The need to better understand the underlying pathophysiology behind this obstetric complication is undeniably important; for discovering new therapeutics, for improving treatment and ultimately, in saving lives.

Admittedly, the covid-19 pandemic dictated much of the direction of this study, and ultimately forced us into using a weaker methodology than originally planned. One of the most notable limitations of which is the fact that much of this analysis relies on the assumption that there is perfect hydrogen ion transfer between fetal and maternal blood across the placenta. Maternal uterine venous pH is assumed to be the same as the fetal umbilical venous pH, and therefore can be used as a surrogate measure for maternal pH in this study. Umbilical venous pH is easy to acquire and is often routinely collected as part of fetal monitoring procedures during labour, whereas maternal arterial pH is assessed far less frequently. Although an extensive literature search concluded that maternal uterine venous pH mirrored that of fetal umbilical venous pH, suggesting that it could be used as a surrogate measure, not all available literature was found to support this hypothesis. For example, a 1967 study by Blechner et al sought to investigate the maternal-fetal gradient at birth on 15 women undergoing elective CS under general anaesthesia. The women in the study were subject to an infusion of ammonium chloride (NH4Cl) so to induce maternal acidosis, and the effects of maternal acidosis on the fetus were observed. In the control group (n=9), where women had no NH4Cl infusion, the mean difference between mApH and fUVpH was found to be 0.06 pH units. However, in those who had NH4Cl infused (n=6) the transplacental pH gradient between mApH and fUVpH values was eventually eliminated, meaning that the maternal pH was decreased by NH_4Cl but the fetal pH remained relatively unaltered.

Researchers thus hypothesised that maternal metabolic acidosis may not necessarily be accompanied by fetal metabolic acidosis, and that if the mother were to become acidotic in labour (which could potentially increase risk of a PPH), the fUVpH would not change. This research therefore discredits the validity of fUVpH being used as a surrogate for myometrial acidosis It must be noted though, with a study group of 15, the sample size of this study could may have been too small to draw any significant conclusions form, and hence why the decision went ahead to use fUVpH as a surrogate measure, regardless (Blechner *et al.*, 1967).

A much more recent study, 2021, showed there to be a significant difference between concentrations of umbilical vein lactate and myometrial blood lactate, whilst also showing that there were smaller differences between umbilical vein lactate and maternal venous blood lactate concentrations. All women in this study had obstructed labour in a Ugandan delivery suite. Focusing on the control arm of the study, Musaba et al measured myometrial capillary and umbilical venous blood lactate levels at one hour after administration of 0.9% sodium chloride solution, n=239 (the intervention group received of 8.4% sodium bicarbonate, n=238). The mean umbilical venous lactate was found to be 7.9 mmol/L (95% CI 5.0,12.2), while myometrial blood lactate was found to be 4.8 mmol/L (95% CI 3.3,7.4). However, the maternal venous blood lactate concentration was also measured and found to be similar to the umbilical venous lactate concentration at 7.5 mmol/L (95% CI 4.0,15.9) (this was taken at one hour after administration of 0.9% sodium chloride solution as placebo). These results suggest that pH levels in both the maternal and umbilical vein blood may be much lower, with a higher lactate concentration, than the pH of the myometrial capillary blood, which has a lower lactate concentration (difference of 3.1 mmol/L) (Musaba *et al.*, 2021) .

Referring to Figure 5 (7-Point Diagram on page 39), the acidity of the maternal vein, in comparison to that of the maternal artery, pH levels are lower because lactate levels are higher due to lactate being produced in the myometrial tissue. As was initially intended for the original PhD study **(see Appendix 1)** a sample of myometrial capillary blood from the cut uterine surface (during CS delivery) would be taken for pH analysis as it was believed that myometrial capillaries would most closely reflect myometrial acidosis. However, the findings from Musaba's recent 2021 study suggest that because the capillary blood would be a mixture of retroplacental blood and capillary blood, which in turn is a mixture of arterial and venous

blood, then lactate levels would be lower and the pH would be higher than in the maternal vein. This may be because the maternal vein carries deoxygenated blood, and thus more H+ ions, from any site of acidosis. In the case of the labouring woman, the contracting myometrium would be the site of acidosis and lactate production. Therefore, the maternal vein pH may even be a better measure than myometrial capillary pH as capillaries contain a mixture of arterial and venous blood. In addition to this, maternal vein pH will give a general measure of systemic pH, to which the uterine activity contributes. mApH was unfortunately not measured in the 2021 study, as it would have provided yet more insight as to whether fUVpH is an accurate surrogate measure for mApH.

A second speculative theory behind why there were lower lactate levels (and a higher pH) observed in the myometrial capillary blood than the maternal venous blood could be down to the forced hyperventilation during the active second stage of labour. This can cause a decrease in alveolar pCO_2 and result in a respiratory alkalosis. Respiratory alkalosis is described as increase in blood pH above 7.45. In the human body exists a pH buffer system, which serves to maintain homeostasis and prevent blood pH from venturing outside of the normal range (7.35-7.45). Bicarbonate (HCO_3-) and carbon dioxide (CO_2) levels are maintained at equilibrium, where HCO_3- acts an alkaline substance and CO_2 acts as an acidic substance. H^+ ions combine with HCO_3- to produce carbonic acid (H_2CO_3), which is in equilibrium with CO_2 and water (H_2O), which also combine to form H_2CO_3. Changes in concentration of substances either of the equation results in a shift in equilibrium until the balance is restored and pH is brought back to its normal range. Increases in HCO_3- (metabolic alkalosis) or a decrease in CO_2 (respiratory alkalosis) will make the blood more alkaline, raising the pH. This is why hyperventilation during labour can cause pCO2 levels to drop as too much CO_2 is expelled from the blood stream, and thus blood pH rises. Normally HCO_3- buffering prevents significant changes in maternal blood pH , however, if the buffering system is compromised then alkalosis may occur (Omo-Aghoja, 2014). Furthermore, the Musaba et al study's focus is on obstructed labour and not women who had atonic PPH. Therefore, the lower average blood pH that one may expect from an atonic PPH cohort is not reflected in the average lactate blood concentrations observed in the 2021 study.

The way in which this project differs from the original PhD protocol must also be discussed as a limitation. The original study sought to investigate what affect myometrial blood pH had on

PPH rates. When this work moved to a desk-based project, it became clear that myometrial capillary blood samples were rarely collected, and so there was very little data in the literature on myometrial pH. However, it was found that there were more data on mApH, as it is sometimes collected to assess the acid-base status of the mother during delivery. In addition to this, it is known that fUVpH is routinely collected, and so there were a lot of data available. This is why fUVpH was used as a proxy measure for mApH, where mApH itself is acting as a proxy measure for myometrial capillary pH. Admittedly this isn't the ideal study set-up, as it is several steps removed from the original PhD protocol to assess myometrial capillary pH and PPH association. Indeed, a key question which needs to be addressed in future work on this project would be; 'How much does fUVpH reflect myometrial pH?' The current literature suggests that fUVpH reflects mApH well, but does mApH reflect the true pH of myometrial capillary blood? Maternal arterial pH gives a reflection of systemic maternal acidosis; mApH is a function of how well the mother's heart and lungs are working, whereas the original PhD hypothesis relates to how acidotic the uterus is, relating to localised acidosis.

To reiterate, the recent study by Milton et al is particularly interesting as the data was gathered on the hard-to-come by myometrial capillary pH. The fact that the results showed that lactate levels were lower in the myometrium than in the uterine vein should be noted, as it suggests that perhaps the protocol for the original PhD could be amended slightly. As mentioned above, instead of looking at myometrial capillary pH as the truest measure for uterine acidosis, perhaps blood pH measurements should be taken from the uterine vein instead.

Another limitation of this study lies in the quality of the data, especially that blood loss measurements were estimated, not measured. As mentioned previously, visual estimation of blood loss can be quite inaccurate. Clinicians frequently underestimate total blood loss, thus putting mothers at increased risk of PPH-related complications. A major reason for this blood loss underestimation comes down to hidden blood loss in things like bed linen, in swabs and pads and in the drapes used during caesarean section. In addition to this, if the blood loss occurs in a slow, steady trickle then it can often go unnoticed for some time. Visual estimations of blood loss are also likely to vary according to the experience of the clinician doing the estimating. It is widely cited that a more accurate way of measuring volume of postpartum blood loss is by using the gravimetric method. This method of measuring blood

loss is done by placing gauze pads under the labouring women before she delivers, and then the dry weight of the gauze is subtracted from the blood-soaked gauze post-delivery. The weight difference of the gauze is then converted to millimetres to give a measured volume of blood loss (Johar and Smith, 1993). Although the lack of international standardisation of the weight and size of the pads, gauzes and sponges means that inaccuracies can arise from this method of measuring blood loss, the gravimetric method is still a more accurate method than visual estimation of blood loss. In 2000, Prasertcharoensuk et al directly compared the incidence of PPH by visual estimation versus direct measurement of blood loss in women undergoing vaginal deliveries. It was found that with visual estimation, the incidence of PPH was underestimated by 88.88%. The study also revealed that the mean blood loss volume by visual estimation was 100ml less than the mean measured blood loss volume. Authors cautioned that obstetricians should be aware of other signs and symptoms of PPH, as visual estimation of blood loss is insensitive to detecting primary PPH (Prasertcharoensuk, Swadpanich and Lumbiganon, 2000).

To add to this, the visual estimation of blood loss was then categorised, thus weakening the methodology further. Ideally, blood loss and umbilical pH would have both been continuous sets of data. This would have allowed for a relatively straight forward correlation between the two variables, giving a detailed picture of how the two variables may fluctuate with one another. Instead, blood loss estimations were divided into one of the three blood loss categories; 0-500ml, 500-1000ml and >1000ml. This categorisation of blood loss forced the analysis down the route of conducting a logistic regression analysis. While this method of analysis can ultimately produce adequate results, it must be recognised that some of the detail has been lost through this process. Furthermore, as a clinician responsible for estimating blood loss, knowing that blood loss is to be fitted into one of three categories increases the incidence of rounding blood loss estimations, thus increasing the likelihood of having a different estimated blood loss to actual blood loss.

A final criticism of this research is that the dataset was quite small. Although the original data was collected on 33,000 women, COVID travel restrictions meant we only had access to data on 2000 women. Then only 906 of these 2000 women had both blood loss and umbilical vein pH data recorded. From this sample size of 906, 123 women experienced a PPH, according to our definition of EBL ≥500ml for vaginal birth and ≥1000ml for caesarean birth. This small

cohort size means that statistical power to detect significant differences in observations is very low. If we had access to the much larger 33,000 patient dataset then it would have allowed for smaller differences to be detected with a higher statistical power. The larger the sample size, the smaller the confidence intervals and the more confident one can be that results are accurate.

Relevance of Findings

As mentioned earlier, findings from the univariate analysis showed low fUVpH to be a predictor of PPH. However, when other predictors of PPH were included in the multivariate analysis then fUVpH became statistically non-significant, showing no relationship between acidosis and PPH. Notably, retained placenta remained highly significant in both the multivariate analysis for both PPH definitions. One may therefore theorize that retained placenta may be the associated factor that was so highly significant to push umbilical vein pH out of the multivariate analysis.

Work carried out by Musaba et al also suggests that there may not be a relationship between uterine acidosis and PPH. It was hypothesised that administration of 50ml bolus of 8.4% sodium bicarbonate (n=238) would significantly reduce PPH rates, in comparison with the control group, who received 50ml of 0.9% sodium chloride as placebo (n=239). However, authors found that there was no significant difference between the two study groups. The rates of primary PPH between control and intervention group was found to be 12.2% and 10% respectively, with a risk ratio of 0.62, 95% CI (0.29,1.3) Blood pH differences were also non-significant. The median difference in maternal venous lactate between the intervention group and the control group, at one hour after drug administration, was 1.2 mmol/L, p-value = 0.087. The median difference in fetal umbilical venous blood lactate was 0.6 mmol/L, p-value = 0.466 (Musaba *et al.*, 2021).

This current study shows that the process of conducting a secondary analysis, using multivariate regression works in so far that a large database can be successfully interrogated and valid comparisons can be made from the data. It is from this point that it must be noted that the database is also adequately robust enough to be used in this kind of analysis. There is only a minor association between pH and PPH, and this disappears in the multivariate analysis. However, as previously mentioned, this may be because it is related to the positive

predicted factors found in the multivariate analysis; retained placenta, emergency CS, operative vaginal (and induced labour for the sensitivity analysis, p=0.01).

Future Work

The most straight forward way in which this research could be continued would be to conduct the analysis on the full, 33,000 patient dataset. As mentioned previously, once pandemic travel restrictions permit travel to Switzerland, the STATA files could be run on the entire dataset, which may hopefully yield more robust results. Having a much larger cohort size would increase statistical power, therefore potentially reducing the confidence intervals and allowing one to be more confident that results are accurate.

As this study was a secondary analysis of pre-collected data, it would be a good idea to collect primary data in order to investigate this analysis further. Much like the original plan for the PhD (**see Appendix 1**) the relationship between uterine atony and maternal acidosis (both systemic and myometrial) could be observed in greater detail in women having emergency caesarean sections. Ideally, this would be a prospective clinical cohort study to assess any correlation between maternal and myometrial pH to atonic PPH, conducted in a clinical setting. The desired patients to participate in such study would be women undergoing emergency caesarean section for failure to progress; dysfunctional active labour. This would give the 'truest' reading for any uterine acidosis, as mother would have experienced uterine contractions for some time, which is important in the build-up of lactate. The original protocol stated that, with consent, pH readings from capillary samples of myometrial and cutaneous blood would be taken during labour. However, findings from Musaba et al 2021 suggest that the maternal vein may better represent acidosis, over myometrial capillary pH. Vaginal blood loss would be measured during caesarean section, through the gravimetric method of weighing swabs and gauzes, or through visual estimation of blood loss. Data would also be collected on whether any additional uterotonic therapy was used during the caesarean section, along with a clinician's assessment of uterine tone, would have also been recorded as part of this study. To further enhance this research, data from contractility assays on myometrial biopsies taken from emergency caesarean sections would be collected too. Tissue bank myometrium samples collected from women who underwent elective-c sections would have acted as a control, as these women would have never laboured. Collecting

contractility data would be extremely useful as it could revel what effect tissue acidosis has on the force, frequency and duration of uterine contraction.

Future work in this area could also look into scientifically testing the hypothesis that umbilical venous blood pH could be used as a surrogate measure for maternal arterial pH. pH readings from a maternal artery, taken during active labour, could be compared directly with umbilical venous cord blood samples to see if the data is positively correlated. Although several previous publications have collected both maternal blood pH and umbilical cord pH readings form the same patient as part of the same study, none have specifically focussed on the detailing umbilical vein and maternal arterial pH fluctuations during labour.

Continuing on from this theme of hypothesis testing, it may be reasonable to further explore the concept that capillary pH is actually a useful surrogate for myometrial pH. Results from studies by Musaba et.al indeed suggest that perhaps uterine vein pH may be a better representative for myometrial tissue pH than capillary pH (Musaba *et al.*, 2021). This is because the blood pH of the uterine vein represents the output of blood from the uterine muscle, rather than the mixed input and output of blood in the myometrial capillary. Referring to Figure 5 (7-Point Diagram on page 39), the blood behind the placenta is shown to have not passed into the myometrium, demonstrating that it may not be a good marker for myometrial pH. Perhaps, better still, a maternal venous blood pH sample might be the best option, as it would represent a maternal systemic acidosis, but it would also be easier to access from a clinician's point of view (e.g. ulnar vein in the arm as opposed to uterine vein). Regardless, it would be worthwhile testing this hypothesis through the collection of primary data; taking pH readings from myometrial capillary blood and comparing them with uterine vein samples, and then comparing results again with maternal ulnar vein samples, to see which readings correlate most closely with one another. Although no scientific research has directly explored this, findings from an extensive literature search supported this hypothesis. It would be reasonable to suggest that a clinical study, collecting primary data, could be conducted. This would compare blood acidity in both the myometrial blood and the umbilical vein blood, drawing up such correlations, if any.

Conclusion

There is still no clear answer to whether uterine acidosis can increase PPH risk. Indeed, the results from this current research shows little association between low umbilical venous pH and volume of blood lost. However, much of the surrounding literature on this topic does suggest an association between blood lactate concentration and haemorrhage rates. This is of particular relevance to this study as blood lactate levels are very closely linked with blood pH levels during labour. As previously discussed, it is known that the strong, prolonged uterine contractions that occur during labour may decrease blood supply and subsequent oxygenation to the uterus. This reduced oxygen supply is then compensated by an increase in anaerobic respiration, which results in the accumulation of the biproduct lactate. Under normal conditions, lactate production is met with physiological bicarbonate and protein buffering systems to neutralise excess H+ ions, and to reduce the negative effects of acidosis. However, if labour significantly limits oxygen supply and lactate levels accumulate, the buffering systems can become depleted, thus lactic acid accumulation and metabolic acidosis can ensue. Not only is uterine acidosis responsible for dystocic labour, but acidosis of the myometrium is also known to compromise the efficiency and strength of the uterine contractions which occur after the delivery of the placenta (Wray, 2007). If the blood vessels that once supplied the placenta are not adequately occluded then they will continue to bleed, which will result in an atonic PPH.

It is clear that the physiological theory behind our myometrial acidosis and PPH association hypothesis is well documented and extensively studied in the current literature. The current methodology of this project is ultimately quite weak and contains many areas in need for improvement. The issues with this study could even possibly explain the non-significant findings. There is still the potential that an improved, more scientifically robust analysis on myometrial blood pH and post-partum blood loss volume could show a positive correlation.

Despite this, it must be said that the findings from this current research, as well as all previous research in this area, still gives an overall unclear picture on what association uterine acidosis has with PPH. It can be argued that ultimately, the jury is still out on whether uterine acidosis may increase the risk of PPH.

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Appendix

Appendix 1

Condensed PhD Protocol Draft

Assessing the relationship between postpartum hemorrhage and maternal acidosis

GLOSSARY OF ABBREVIATIONS

HRA	Health Research Authority
REC	Research Ethics Committee
CS	Caesarean section
EM	emergency
PPH	Postpartum haemorrhage
UVpH	Umbilical vein pH
UApH	Umbilical artery pH
MapH	Maternal arterial pH

KEYWORDS

- PPH
- Uterus
- Myometrium
- Womb
- contraction
- Acidosis
- Lactic Acid
- Lactate
- pH

TITLE

Assessing the relationship between postpartum haemorrhage and maternal acidosis

DESIGN

The assessment of the relationship between postpartum haemorrhage and maternal acidosis will be done using data gathered from a pre-existing database on neonatal venous cord pH values and estimates maternal postpartum blood loss. Neonatal umbilical vein blood pH will be used as a surrogate for maternal arterial pH.

AIMS

To determine the correlation, if any, between low umbilical venous pH and increased estimated maternal blood loss.

OUTCOME MEASURES

maternal PPH / blood loss

POPULATION ELIGIBILITY

Predictor variables to be:

Elective CS

Emergency CS

fUApH <7.1

Placenta praevia or abruption

Induction of labour

Prolonged labour (>12 hours)

Operative vaginal birth with forceps or ventouse

Episiotomy or perineal tear

First birth

Multiple pregnancy

Maternal hypertension / pre-eclampsia

RATIONALE FOR CURRENT STUDY

The hypothesis for this study is that prolonged labour results in systemic and myometrial maternal acidosis, which in turns causes myometrial atony and PPH. If this is the case, then we would envisage that, in future, women having emergency caesarean section (CS) might be able to have their acid-base status assessed through a capillary lactate sample before a CS, so as to correct any acidosis and prevent PPH.

This PhD project will therefore assess the relationship between uterine atony and maternal acidosis (both systemic and myometrial) in women having emergency caesarean sections. Our specific research questions will include:

In women undergoing emergency caesarean sections, is there a difference in myometrial pH between those who require additional uterotonic therapy for atony (clinician decided), and those who don't?

How does myometrial pH correlate with myometrial lactate?

How does myometrial pH correlate with cutaneous pH (skin stab capillary pH)?

How does myometrial pH affect vaginal blood loss during CS?

In addition to the tissue bank myometrium samples, work will be carried out on capillary samples of myometrial and cutaneous blood, weighing of vaginal blood loss during caesarean section and data on whether additional uterotonic therapy was used during the Caesarean section.

The null hypothesis is that uterine acidosis has no impact on rates of post-partum haemorrhage

STUDY OBJECTIVES

Primary Study Objectives:

To examine whether patients with in myometrial tissue acidosis have higher rates of intrapartum vaginal blood loss compared to patients without uterine acidosis

Secondary Study Objectives:

To explore which method of measuring acidosis correlates best with myometrial capillary blood acidosis levels.

To measure how clinicians' assessment of uterine contractility at 10 minutes postnatally are impacted by acidosis.

STUDY DESIGN

This will be a cohort study carried out at Liverpool Women's Hospital. We will recruit patients undergoing emergency caesarean section for Failure to Progress (dysfunctional active labour), foetal Distress/ Bradycardia/Breech (and Failed Induction of labour (never in active labour). We will also collect samples from women who are undergoing Elective-c sections (never laboured) to act as a control.

The number of patients needed to be recruited will be calculated using a power calculation, but based on the literature, this study will need at least 50 samples.

Once patients are identified, they will be supplied with an information leaflet and a consent form specific to this study will be filled.

This study will run for 12 months

PICO framework:

Population= Women undergoing an emergency C-section.

Intervention= Amount of PPH/ blood loss, acidosis levels, myometrial capillary pH, maternal venous or capillary pH, lactate, myometrial contractility and ex-vivo contractility measurements of force, amplitude, duration and AUC.

Compared with= Women undergoing an elective C-section

Outcome of interests = Blood loss, acidosis levels, contractility.

My study will be a continuous, outcome superiority observational study (Sealed Envelope Ltd. 2012) a power calculation will be made based on the assumption that significance level is <5%, and the power level will be a minimum of 80%. The estimate a difference in myometrial capillary pH between women undergoing emergency C-section and those undergoing an elective C-section with an approximate precision of 10% in both groups, at least ____ women (___ emergency c-section and ___ elective c-section) shall be recruited to make the results of this study statistically significant.

For reference, similar study by Quenby et al. 2004 used a total of 52 participants; 23 non-labouring women and 29 labouring women. The 2004 study was observing the relationship between labour dystocia and myometrial lactic acidosis. Because my study is also observing a relationship between two factors (myometrial lactic acidosis and PPH rates) I would expect the power of my study to be similar of that of Quenby et al. 2004.

STUDY OUTCOME MEASURES

Amount of PPH/ blood loss:

Measured intrapartum vaginal blood loss using the gravimetric method (Inco pads and vaginal swabs to be weighed after delivery of the placenta and the dry weight of each subtracted)

Acidosis Levels:

Myometrial capillary pH at caesarean section. Sample to be immediately analysed in the blood gas analyser (Bayer) on delivery suite.

Maternal venous or capillary pH and lactate at time of CS.

Myometrial Contractility:

Uterine tone to be determined by clinician as a rating from 1-10 at 10 minutes following birth.

Requirement for additional therapeutic uterotonics.

Ex-vivo contractility measurements of force, amplitude, duration and AUC (area under curve).

PARTICIPANT ENTRY

Patients will need pre-registration evaluations, a patient information leaflet, a consent form and a data extraction form on arrival to theatre; and a venous or capillary lactate and pH (venous if possible, but capillary if routine venous sample not being taken)

PRE-REGISTRATION EVALUATIONS

Patient Information Leaflet, consent form and data extraction form.

Patients will need on arrival to theatre

Venous lactate

Venous pH

INCLUDION CRITERIA

>37 weeks pregnant

>18 years of age

Undergoing an EM CS (defined as having painful contractions for over 6 hours, or in labor with an epidural, or having received an oxytocin infusion for at least 6 hours)

Singleton pregnancy

EXCLUSION CRITERIA

Foetal abnormalities

Twin pregnancies

Category 1 CS (i.e. to be performed within 30 minutes)

Inability to understand the study because of language, educational or emotional constraints

WITHDRAWL CRITEREA

Patients have the right to withdraw at any point during the study without it affecting their quality of care. A withdrawal consent form needs to be completed which discloses what information the patient is happy for the researcher to continue to use. Any information the patient does not wish to be stored or used must be destroyed.

If a patient withdraws from the study, all research activity must from that point on, must stop. However, data gathered using the biopsy up until that point can still be used in the study.

Any patient withdrawing from the study must be notified to the Chief Investigator and a Case report form (CRF) needs to be filled. Withdrawal of a patient from a trial must be clearly documented in the notes. Patients may also be discontinued from the study at the discretion of the Investigator if patients are found to have a foetal malformation.

Of the women who are eligible to take part in this study, they will be grouped into the following categories:

Failed induction of labour

Normally contracting (e.g caesarean delivery for foetal distress)

Normally contracting on oxytocin

Dysfunctional labour

ADVERSE EVENTS

Senior consultants (both Obstetrics and gynaecology and Anaesthesiology) and midwives have provided feedback in the development of this project. Additionally a PPI committee has been approached and comments have been taken on board from patients in the development of this project.

Any adverse events must be reported to the chief investigator.

A Case report Form must be completed for any

ASSESSMENT AND FOLLOW-UP

Patients will be followed up for a 24 hours following delivery. Gravimetric measurement of blood loss will be conducted. Midwives to fill out PPH recording study diary. Catheters to remain in for 24 hours to ensure correct blood loss measurement

STATISTICS AND DATA ANALYSIS

The primary analysis will involve SPSS software. Continuous data will be recorded and a Shapiro Wilks test will assess the distribution of the data. Following this ANOVA or Kruskal Wallis (depending on distribution) models will be used to detect significant difference among the groups. A significance of $P<0.05$ will be used with IQR ranges too.

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and will abide by the EU General Data Protection Regulation 2016 and Data Protection Act 2018.

Data will be stored in a password encrypted Excel spreadsheet with limited access to only study investigators. Bar Hospital Number- no other patient identifiers e.g. name and address will be recorded in the spreadsheet. This data will be uploaded to the SPSS software and from here analysis will be done. Security of record will be assessed and reviewed annually. Following end of study- encrypted password will e changed and only known by chief investigator.

STUDY MANAGEMENT

Additionally staff champions will be involved from the obstetrics, anaesthetic, midwifery team. Overall running of the study will be overseen by Chief investigator and Trial manager.

END OF STUDY

The study will run for a period of 9 months in total. Start Date dependent on formal Ethics approval but hoping to be March 2020.

PUBLICATION POLICY

Consent forms for patients will ask about consent for use of data for publication on University and affiliated websites, Conferences and Journals. Data presented will be anonymised with no patient identifiers utilised.

Appendix 2

Table of 20 Papers; Sourcing Data to Analyse

Key:

Text left as normal: probably not useful

Text in italics: might be useful

Text in bold: should be useful

<u>Text underlined: from a database</u>

	Paper	Date	Number of participants	pH data	PPH data	Comments	Reference
1	Placental passage of the oxytocin antagonist atosiban	1995	1 with PPH (8 in total)	Cord blood gas analysis	One woman in the study had a PPH	Too small to use.	Valenzuela, G., Craig, J., Bernhardt, M. and Holland, M., 1995. Placental passage of the oxytocin antagonist atosiban. *American Journal of Obstetrics and Gynecology*, 172(4), pp.1304-1306.
2	**Effects of delayed compared with early umbilical cord clamping on maternal postpartum hemorrhage and cord blood gas sampling: a randomized trial**	**2012**	**382**	**Blood gas analysis from umbilical vein**	**Blood loss estimated by midwife**	**This could be useful**	**Andersson, O., Hellström-Westas, L., Andersson, D., Clausen, J. and Domellöf, M., 2012. Effects of delayed compared with early umbilical cord clamping on maternal postpartum hemorrhage and cord blood gas sampling: a randomized trial. *Acta Obstetricia et Gynecologica Scandinavica*, 92(5), pp.567-574.**
3	Uterine Artery Embolization before Delivery to Prevent Postpartum Hemorrhage	2016	50	Cord blood test	Need for blood transfusion? unsure	This might be too small	Niola, R., Giurazza, F., Nazzaro, G., Silvestre, M., Nasti, G., Di Pasquale, M., Albano, G., Valentino, L., Sirimarco, F. and Maglione, F., 2016. Uterine Artery Embolization before Delivery to Prevent Postpartum Hemorrhage. *Journal of Vascular and Interventional Radiology*, 27(3), pp.376-382.
4	Effect of delayed umbilical cord clamping on blood gas analysis	2012	60	They obtained paired blood samples from the umbilical artery and vein of 60 term new borns	-	I found no reference to PPH in this paper, but as the study was carried out on 60 women, there's a change there might be some data on postpartum blood loss. Regardless, this study is too small	Valero, J., Desantes, D., Perales-Puchalt, A., Rubio, J., Diago Almela, V. and Perales, A., 2012. Effect of delayed umbilical cord clamping on blood gas analysis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 162(1), pp.21-23.
5	Umbilical cord blood acid–base and gas analysis after early versus	2011	158	Blood gas analysis of umbilical cord vein	-	I can't access the full text, but from reading the abstract I can see that they have data on cord pH of 158	De Paco, C., Florido, J., Garrido, M.C. et al. Umbilical cord blood acid–base and gas analysis after early versus delayed cord clamping in neonates at term. Arch Gynecol

	delayed cord clamping in neonates at term					mothers, again the potential is there for data on PPH… but the study might be too small	Obstet 283, 1011–1014 (2011). https://doi.org/10.1007/s00404-010-1516-z
6	Effects of delayed cord clamping on the third stage of labour, maternal haematological parameters and acid–base status in foetuses at term	2016	95 (50 ECC and 45 DCC)	blood acid–base and gas assessment in the umbilical vein.	A blood test at 48 h post-delivery in each woman was also carried out to evaluate postpartum blood loss	I read this paper, and although I could not see any specific data on the volume of blood loss (despite reference to PPH) the author's may still have this information, which may be useful for my study, but with 95 participants, it might be too small	De Paco, C., Herrera, J., Garcia, C., Corbalán, S., Arteaga, A., Pertegal, M., Checa, R., Prieto, M., Nieto, A. and Delgado, J., 2016. Effects of delayed cord clamping on the third stage of labour, maternal haematological parameters and acid–base status in foetuses at term. European Journal of Obstetrics & Gynecology and Reproductive Biology, 207, pp.153-1
7	Effects Of Early Versus Delayed Umbilical Cord Clamping During Antepartum Lower Segment Caesarean Section On Placental Delivery And Postoperative Haemorrhage: A Randomised Controlled Trial.	2017	156	-	postoperative blood loss was calculated by Volume of blood in sucker bottle = percentage of packed cells in the 10ml sample × total volume in sucker bottle ÷ preoperative hematocrit	I am not sure about this one… I couldn't see anywhere a reference to cord blood testing, but as they're clamping the cord they may have taken a blood pH test. 156 paricipants may be too small though	Withanathantrige, M. and Goonewardene, I., 2017. Effects Of Early Versus Delayed Umbilical Cord Clamping During Antepartum Lower Segment Caesarean Section On Placental Delivery And Postoperative Haemorrhage: A Randomised Controlled Trial.
8	Delayed umbilical cord clamping at birth has effects on arterial and venous blood gases and lactate concentrations.	2008	70	Umbilical cord arterial and venous blood was sampled repeatedly every 45 seconds until the cord pulsations spontaneously ceased	-	This paper has a lot of information on cord blood pH and neonatal outcomes, but little is written about maternal outcomes. However, the study was carried out on 70 neonates, so there's a chance that there might be data on postpartum blood loss. Regardless, this study is probably too small	Wiberg N, Ka¨lle´n K, Olofsson P. Delayed umbilical cord clamping at birth has effects on arterial and venous blood gases and lactateconcentrations. BJOG 2008;115:697–703.
9	Timing of Umbilical Cord Occlusion in Premature Babies(<33 w). Delayed vs Early	2016	150	Umbilical cord blood pH 0-15 minutes after delivery	Maternal PPH incidence within 24 hours after birth	From Clinicaltrials.gov. Recruitment status unknown. Contact Melchor Carbonell, MD, 0034626470597 xormd11@gmail.com	Socias, M., Gregoraci, A., Canino, M. and Linde, M., 2016. Timing Of Umbilical Cord Occlusion In Premature Babies(<33 W). Delayed Vs Early. Clinicaltrials.Gov.
10	Introducing Fetal Scalp Stimulation as an Adjunct to Intermittent	2016	550	Predicted fetal acidosis from cord blood pH (at thresholds of cord blood	Doesn't specify	From Clinicaltrials.gov PPH not a primary/ secondary outcome, just collected as comorbidity data	Schmitt, J., 2016. Introducing Fetal Scalp Stimulation As An Adjunct To Intermittent Auscultation In Low-Resource Settings. - Clinicaltrials.Gov.

	Auscultation in Low-Resource Settings.			pH<7.0, 7.1, and 7.2) [Time Frame: Day 1]			
11	*Obstetric Characteristics for a Prolonged Third Stage of Labour and Risk for Postpartum Hemorrhage', Gynecologic and Obstetric Investigation*	*2008*	*1607*	-	-	*This might have the data I need, but I can't access it. The study is large enough though.*	*Magann, P, Doherty, D, Briery, CM, Niederhauser, A, Chauhan, SP & Morrison, JC 2008, 'Obstetric Characteristics for a Prolonged Third Stage of Labor and Risk for Postpartum Hemorrhage', Gynecologic and Obstetric Investigation, vol. 65, no. 3, pp. 201-205. https://doi.org/10.1159/000112227*
12	*The impact of migration background on maternal near miss*	*2019*	*6767 total-near-miss events occurred in 141 women.*	*the ORs ranged from 2.15 for an arterial umbilical cord pH value < 7.1–2.47 for premature delivery. (not sure what OR means, also not sure if this would be useful because I'm looking for venous cord pH)*	*> 1000 ml Blood loss*	*Need to access full paper. Also, it talks about arterial cord pH in the abstract, not venous, but the study is large*	*David, M., Razum, O., Henrich, W. et al. The impact of migration background on maternal near miss. Arch Gynecol Obstet **300,** 285–292 (2019). https://doi.org/10.1007/s00404-019-05179-9*
13	**Buccal misoprostol to decrease blood loss after vaginal delivery: a randomized trial.**	**2004**	**848**	**States that umbilical cord pH was recorded**	**Postpartum hemorrhage was defined as blood loss exceeding 500 mL. occurred in 3 to 5% of women**	**This study could be useful**	**Bhullar, A., Carlan, S., Hamm, J., Lamberty, N., White, L. and Richichi, K., 2004. Buccal Misoprostol to Decrease Blood Loss After Vaginal Delivery: A Randomized Trial. *Obstetrics & Gynecology*, 104(6), pp.1282-1288.**
14	Effects of Pushing Techniques in Birth on Mother and Fetus: A Randomized Study	2008	100	umbilical artery pH, Po2 (mmHg), and Pco2 (mmHg) levels at 1 and 5 minutes were evaluated in newborns	1 hourly pad controls	May be too small, also arterial cord blood, not venous, was mentioned so this might not be useful	Yildirim, G. and Beji, N., 2008. Effects of Pushing Techniques in Birth on Mother and Fetus: A Randomized Study. *Birth*, 35(1), pp.25-30.
15	*Is There an Obstetric July Phenomenon?*	*2003*	*7814*	*Umbilical cord gases were determined. low scores were defined as <7), umbilical artery pH (low cut offs evaluated included <7.00 and <7.20),*	*postpartum haemorrhage (defined as >500 mL for a vaginal delivery and >1000 mL for caesarean)*	*This paper mentions arterial pH, not venous… don't know if this means it won't be useful, but there are a lot of participants*	*MYLES, T., 2003. Is there an obstetric July phenomenon?. Obstetrics & Gynecology, 102(5), pp.1080-1084.*
16	*Adverse obstetrical and neonatal outcomes in elective and*	*2013*	*13 971*	*>500 ml blood loss*	*arterial umbilical cord pH<7.1*	*Can't access the full paper, but it might not be useful as it has data on arterial pH, not venous pH.*	*Baud, D., Rouiller, S., Hohlfeld, P., Tolsa, J. and Vial, Y., 2013. Adverse obstetrical and neonatal outcomes in elective and medically indicated inductions of labor at term. The*

	medically indicated inductions of labor at term					*Despite this, this study is huge*	*Journal of Maternal-Fetal & Neonatal Medicine, 26(16), pp.1595-1601.*
17	*Acupuncture as Pain Relief During Delivery: A Randomized Controlled Trial*	*2009*	*607*	*No other detail was given in the paper other than PPH being a secondary obstetric outcome*	*umbilical cord pH value were significantly higher among infants in the acupuncture group compared with infants in the other groups. Was noted in the paper as a median cord blood pH value (and range)*	*This paper doesn't specify whether the cord blood was venous or arterial. Large participant number though.*	*Borup, L., Wurlitzer, W., Hedegaard, M., Kesmodel, U. and Hvidman, L., 2009. Acupuncture as Pain Relief During Delivery: A Randomized Controlled Trial. Birth, 36(1), pp.5-12.*
18	*Selective vaginal breech delivery at term - still an option.*	*2012*	*1008*	*Mothers in planned c-section group suffered more from massive bleeding and needed blood transfusions.* *51 mothers in the study had a blood loss of >1500ml*	*Umbilical cord artery pH as a secondary outcome. More infants in the vaginal delivery control group had a cord pH <7.05*	*This study could be useful but again, I'm not sure about arterial cord pH being recorded and not venous pH.*	*TOIVONEN, E., PALOMÄKI, O., HUHTALA, H. and UOTILA, J., 2012. Selective vaginal breech delivery at term - still an option. Acta Obstetricia et Gynecologica Scandinavica, 91(10), pp.1177-1183.*
19	*Neonatal advantage of epidural anesthesia in elective and emergency cesarean sections: a report of 531 cases*	*1986*	*531 in total but only 114 babies had blood gas analysis*	*Little detail was given about maternal bleeding, just that 22.7% women had 'maternal bleeding' in the epidural group and 30.3% had bleeding in the general anaesthetic group (n=531)*	*Umbilical venous blood was analysed in a blood gas analyser no later than 10 minutes after delivery*	*Little detail was given about maternal bleeding, plus this paper is really old so unsure if the authors would still have access to the data?*	*Gale, R., Slater, P. and Zalkinder-Luboshitz, I., 1986. Neonatal advantage of epidural anesthesia in elective and emergency cesarean sections: a report of 531 cases. European Journal of Obstetrics & Gynecology and Reproductive Biology, 23(5-6), pp.369-377.*
20	**Comparison between general, spinal, epidural, and combined spinal-epidural anesthesia for cesarean delivery: a network meta-analysis**	**2019**	**46 randomized control trials, with a total of 3689 women**	**maternal estimated blood loss as a secondary outcome. There is blood loss data on 566 women. General anesthesia was associated with greater blood loss, but not require-ment for transfusion**	**umbilical arterial and venous pH <7.2 as a secondary outcome. Umbilical venous pH with epidural anaesthesia was significantly higher than that with general anesthesia or with spinal anesthesia. This data is on 1257 women.**	**This was a systematic review and meta-analysis. This review stated that umbilical cord pH was lower (than epidural) with general anaesthesia, and that general anaesthesia was associated with a greater maternal blood loss… so that could be my answer**	**Kim, W., Hur, M., Park, S., Yoo, S., Lim, T., Yoon, H., Kim, J. and Bahk, J., 2019. Comparison between general, spinal, epidural, and combined spinal-epidural anesthesia for cesarean delivery: a network meta-analysis. *International Journal of Obstetric Anesthesia*, 37, pp.5-15.**

21	Effects of Early Vs Delayed Umbilical Cord Clamping During Antepartum Lower Segment Caesarean Section on Post Operative Blood Loss	2018	120	Amount of blood loss was measured by measuring postoperative haematocrit levels	-	No mention of cord blood pH, but the authors could have access to this… however with a sample size of 120, this study could be too small	sohail saqib chatha , mahwish farooq , amna farooq , maham farooq , umar farooq , abdullah zakaullah; effects of early vs delayed umbilical cord clamping during antepartum lower segment caesarean section on post operative blood loss; p j m h s vol. 12, no. 3, jul – sep 2018
22	A multicentre randomized trial comparing delivery with a silicone rubber cup and rigid metal vacuum extractor cups	1989	258	Estimated blood loss	Mean umbilical vein pH	Only 16 patients were estimated to have a blood loss of 500ml or more, meaning this study is probably too small	COHN, M., BARCLAY, C., FRASER, R., ZAKLAMA, M., JOHANSON, R., ANDERSON, D. and WALKER, C., 1989. A multicentre randomized trial comparing delivery with a silicone rubber cup and rigid metal vacuum extractor cups. *BJOG: An International Journal of Obstetrics and Gynaecology*, 96(5), pp.545-551.
23	Effect of spontaneous pushing versus Valsalva pushing in the second stage of labour on mother and fetus: a systematic review of randomised trials	2011	425	Estimated maternal blood loss from 105 women	Umbilical venous blood gas levels from 32 women	Despite this systematic review covering 425 participants, after further reading I have found that there is umbilical vein pH from only 32 participants	Prins, M., Boxem, J., Lucas, C. and Hutton, E., 2011. Effect of spontaneous pushing versus Valsalva pushing in the second stage of labour on mother and fetus: a systematic review of randomised trials. *BJOG: An International Journal of Obstetrics & Gynaecology*, 118(6), pp.662-670.
24	Randomized, Double-Masked Comparison of Oxytocin Dosage in Induction and Augmentation of Labor.	1999	1307 in the study in total	45 women had a PPH	There were 37 neonates with an umbilical vein pH of less than or equal to 7.2	Although this is a large study, there might not be enough data within it on PPH and umbilical vein pH	MERRILL, D. and ZLATNIK, F., 1999. Randomized, Double-Masked Comparison of Oxytocin Dosage in Induction and Augmentation of Labor. *Obstetrics & Gynecology*, 94(3), pp.455-463.
25	*The Effect of Maternal Position on Maternal, Fetal and Neonatal Outcomes: A Systematic Review*	*2020*	*4848*	*Mean blood loss volume <1000ml*	*Umbilical venous pH*	*It's a systematic review, not sure if it could be useful?*	*Mirzakhani K, Karimi FZ, Mohamadzadeh Vatanchi A, Zaidi FF, Mirzaii Najmabadi KH. The Effect of Maternal Position on Maternal, Fetal and Neonatal Outcomes: A Systematic Review. Journal of Midwifery and Reproductive Health. 2020; 8(1): 1988-2004. DOI: 10.22038/jmrh.2019.38133.1423*

Appendix 3

STATA .do Files for Data Cleaning

```
generate BMI = kg_m/(cm_m/100)^2

replace BMI = 999 if (BMI ==.)

drop if BMI>60

drop if BMI<10

generate obesity =.

replace obesity = 1 if (BMI>30)&(BMI<999)

replace obesity = 0 if (BMI <30)

generate pe500 = .

replace pe500 = 1 if (pe == "< 500 ml")

replace pe500 = 0 if (pe == "500 ml à 1000 ml" )

replace pe500 = 0 if (pe == "> 1000 ml" )

generate pe500_1000 =.

replace pe500_1000 = 1 if (pe == "500 ml à 1000 ml")

replace pe500_1000 = 0 if (pe == "< 500 ml")

replace pe500_1000 = 0 if (pe == "> 1000 ml" )

generate pe1000 = .

replace pe1000 = 0 if (pe == "< 500 ml")

replace pe1000 = 1 if (pe == "> 1000 ml")

replace pe1000 = 0 if (pe == "500 ml à 1000 ml" )

generate emergency_CS = 0

replace emergency_CS = 1 if (act1 == "césarienne d'urgence")

replace emergency_CS = 1 if (act2 == "césarienne d'urgence")

replace emergency_CS = 1 if (act3 == "césarienne d'urgence")

replace emergency_CS = 1 if (act4 == "césarienne d'urgence")

replace emergency_CS = 1 if (act5 == "césarienne d'urgence")

replace emergency_CS = 1 if (act6 == "césarienne d'urgence")

replace emergency_CS = 1 if (ind_cs == "Césarienne en urgence")

replace emergency_CS = 1 if (ind_cs2 == "Césarienne en urgence")

replace emergency_CS = 1 if (ind_cs == "Césarienne pour DPPNI")
```

```
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour DPPNI")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne HELLP syndrome")
replace emergency_CS = 1 if (ind_cs == "Césarienne HELLP syndrome")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour bradycardie d'expulsion")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour bradycardie d'expulsion")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour disproportion foeto-pelvienne")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour disproportion foeto-pelvienne")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour hydramnios")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour hydramnios")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour hypertension maternelle")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour hypertension maternelle")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour hypotrophie")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour hypotrophie")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour infection maternelle")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour infection maternelle")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour laparoschisis")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour laparoschisis")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour métrorragies")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour métrorragies")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour non dilatation")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour non dilatation")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour non engagement")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour non engagement")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour non progression")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour non progression")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour pathologie foetale avant acc.")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour pathologie foetale avant acc.")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour placenta praevia")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour placenta praevia")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour prématurité")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour prématurité")
```

```
replace emergency_CS = 1 if (ind_cs == "Césarienne pour présentation du front")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour présentation du front")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour prééclampsie")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour prééclampsie")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour rupture utérine")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour rupture utérine")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour souffrance foetale")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour souffrance foetale")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour stagnation de la dilatation")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour stagnation de la dilatation")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour échec de forceps")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour échec de forceps")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour échec épreuve du travail")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour échec épreuve du travail")
replace emergency_CS = 1 if (ind_cs == "Césarienne pour éclampsie")
replace emergency_CS = 1 if (ind_cs2 == "Césarienne pour éclampsie")
replace emergency_CS = 1 if (motif_hosp == "césarienne d'urgence")
replace emergency_CS = 1 if (typ_acc == "Césarienne en urgence")
replace emergency_CS = 1 if (typ_acc == "césarienne d'urgence")
generate elective_CS = 0
replace elective_CS = 1 if (act1 == "césarienne élective")
replace elective_CS = 1 if (act2 == "césarienne élective")
replace elective_CS = 1 if (act3 == "césarienne élective")
replace elective_CS = 1 if (act4 == "césarienne élective")
replace elective_CS = 1 if (act5 == "césarienne élective")
replace elective_CS = 1 if (act6 == "césarienne élective")
replace elective_CS = 1 if (ind_cs == "Césarienne demande maternelle")
replace elective_CS = 1 if (ind_cs2 == "Césarienne demande maternelle")
replace elective_CS = 1 if (ind_cs == "Césarienne iterative")
replace elective_CS = 1 if (ind_cs2 == "Césarienne iterative")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour anamnios")
```

```
replace elective_CS = 1 if (ind_cs == "Césarienne pour anamnios")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour anamnios")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour bassin pathologique")
replace elective_CS = 1 if (ind_cs == "Césarienne pour bassin pathologique")
replace elective_CS = 1 if (ind_cs == "Césarienne pour malformation foetale")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour malformation foetale")
replace elective_CS = 1 if (ind_cs == "Césarienne pour omphalocèle")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour omphalocèle")
replace elective_CS = 1 if (ind_cs == "Césarienne pour présentation podalique")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour présentation podalique")
replace elective_CS = 1 if (ind_cs == "Césarienne pour présentation transversale")
replace elective_CS = 1 if (ind_cs2 == "Césarienne pour présentation transversale")
replace elective_CS = 1 if (ind_cs == "Césarienne prophylactique")
replace elective_CS = 1 if (ind_cs2 == "Césarienne prophylactique")
replace elective_CS = 1 if (ind_cs == "Césarienne prophylactique")
replace elective_CS = 1 if (ind_cs2 == "Césarienne prophylactique")
replace elective_CS = 1 if (ind_cs == "Césarienne sur utérus cicatriciel")
replace elective_CS = 1 if (ind_cs2 == "Césarienne sur utérus cicatriciel")
replace elective_CS = 1 if (ind_hosp == "césarienne élective")
replace elective_CS = 1 if (ind_hosp2 == "césarienne élective")
replace elective_CS = 1 if (ind_hosp3 == "césarienne élective")
replace elective_CS = 1 if (ind_hosp4 == "césarienne élective")
replace elective_CS = 1 if (ind_hosp5 == "césarienne élective")
replace elective_CS = 1 if (motif_hosp == "césarienne élective")
replace elective_CS = 1 if (motif_hosp == "césarienne élective / bilan gestose")
replace elective_CS = 1 if (motif_hosp == "césarienne élective / cerclage")
replace elective_CS = 1 if (motif_hosp == "césarienne élective / douleur abdominale")
replace elective_CS = 1 if (motif_hosp == "césarienne élective / maturation pulmonaire")
replace elective_CS = 1 if (motif_hosp == "césarienne élective / maturation pulmonaire /
matur")
```

```
replace elective_CS = 1 if (motif_hosp == "césarienne élective / maturation pulmonaire / menac")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / menace d'accouchement prématu")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / métrorragies / métrorragies")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / pathologies du liquide amniot")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / placenta bas inséré / menace")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / placenta bas inséré / placent")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / prééclampsie")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / problème psychosocial")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / rupture de la poche des eaux")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / traumatisme / cerclage")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / version par manoeuvre externe")

replace elective_CS = 1 if (motif_hosp == "césarienne élective / vomissements")

generate forceps = 0

replace forceps = 1 if (act1 == "extraction par forceps")

replace forceps = 1 if (act2 == "extraction par forceps")

replace forceps = 1 if (act3 == "extraction par forceps")

replace forceps = 1 if (act4 == "extraction par forceps")

replace forceps = 1 if (act5 == "extraction par forceps")

replace forceps = 1 if (act6 == "extraction par forceps")

replace forceps = 1 if (act1 == "extraction par forceps moyen")

replace forceps = 1 if (act2 == "extraction par forceps moyen")

replace forceps = 1 if (act3 == "extraction par forceps moyen")

replace forceps = 1 if (act4 == "extraction par forceps moyen")

replace forceps = 1 if (act5 == "extraction par forceps moyen")

replace forceps = 1 if (act6 == "extraction par forceps moyen")
```

```
replace forceps = 1 if (typ_acc == "extraction par forceps")
replace forceps = 1 if (typ_acc == "extraction par forceps bas")
replace forceps = 1 if (typ_acc == "extraction par forceps moyen")
generate ventouse = 0
replace ventouse = 1 if (act1 == "application de ventouse obstétricale")
replace ventouse = 1 if (act2 == "application de ventouse obstétricale")
replace ventouse = 1 if (act3 == "application de ventouse obstétricale")
replace ventouse = 1 if (act4 == "application de ventouse obstétricale")
replace ventouse = 1 if (act5 == "application de ventouse obstétricale")
replace ventouse = 1 if (act6 == "application de ventouse obstétricale")
replace ventouse = 1 if (typ_acc == "application de ventouse obstétricale")
replace ventouse = 1 if (typ_acc == "extraction par ventouse")
generate labour_induced = 0
replace labour_induced = 1 if (decl_trav == "pas de précision")
replace labour_induced = 1 if (decl_trav == "provoqué pour autre motif")
replace labour_induced = 1 if (decl_trav == "provoqué pour convenance personnelle")
replace labour_induced = 1 if (decl_trav == "provoqué pour grossesse prolongée")
replace labour_induced = 1 if (decl_trav == "provoqué pour pathologie foetale")
replace labour_induced = 1 if (decl_trav == "provoqué pour pathologie maternelle")
replace labour_induced = 1 if (decl_trav == "provoqué pour pathologie materno-foetale")
replace labour_induced = 1 if (decl_trav == "provoqué pour rupture prématurée des
membranes")
replace labour_induced = 1 if (decl_trav == "échec de tocolyse")
replace labour_induced = 1 if (motif_hosp == "/ déclenchement")
replace labour_induced = 1 if (motif_hosp == "déclenchement")
replace labour_induced = 1 if (motif_hosp == "déclenchement / ?????????")
replace labour_induced = 1 if (motif_hosp == "déclenchement / bilan gestose")
replace labour_induced = 1 if (motif_hosp == "déclenchement / bilan gestose /
déclenchement / abs")
replace labour_induced = 1 if (motif_hosp == "déclenchement / bilan gestose /
prééclampsie")
replace labour_induced = 1 if (motif_hosp == "déclenchement / cardiopathie")
```

```
replace labour_induced = 1 if (motif_hosp == "déclenchement / césarienne élective / pathologies f")

replace labour_induced = 1 if (motif_hosp == "déclenchement / diabète")

replace labour_induced = 1 if (motif_hosp == "déclenchement / douleur abdominale / menace d'accou")

replace labour_induced = 1 if (motif_hosp == "déclenchement / douleur abdominale / repos")

replace labour_induced = 1 if (motif_hosp == "déclenchement / déclenchement")

replace labour_induced = 1 if (motif_hosp == "déclenchement / dépassement de terme")

replace labour_induced = 1 if (motif_hosp == "déclenchement / dépassement de terme / menace d'acc")

replace labour_induced = 1 if (motif_hosp == "déclenchement / dépassement de terme / métrorragies")

replace labour_induced = 1 if (motif_hosp == "déclenchement / dépassement de terme / pathologies")

replace labour_induced = 1 if (motif_hosp == "déclenchement / dépassement de terme / rupture de l")

replace labour_induced = 1 if (motif_hosp == "déclenchement / maturation pulmonaire")

replace labour_induced = 1 if (motif_hosp == "déclenchement / maturation pulmonaire / cerclage")

replace labour_induced = 1 if (motif_hosp == "déclenchement / maturation pulmonaire / maturation")

replace labour_induced = 1 if (motif_hosp == "déclenchement / maturation pulmonaire / menace d'ac")

replace labour_induced = 1 if (motif_hosp == "déclenchement / menace d'accouchement prématuré")

replace labour_induced = 1 if (motif_hosp == "déclenchement / menace d'accouchement prématuré / m")

replace labour_induced = 1 if (motif_hosp == "déclenchement / menace d'accouchement prématuré / p")

replace labour_induced = 1 if (motif_hosp == "déclenchement / métrorragies")

replace labour_induced = 1 if (motif_hosp == "déclenchement / métrorragies / métrorragies")

replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologie infectieuse et grossesse")
```

```
replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologie infectieuse et grossess")

replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologies du liquide amniotique")

replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologies du liquide amniotique /")

replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologies foetales")

replace labour_induced = 1 if (motif_hosp == "déclenchement / pathologies foetales / maturation p")

replace labour_induced = 1 if (motif_hosp == "déclenchement / prurit - bilan hépatique")

replace labour_induced = 1 if (motif_hosp == "déclenchement / prurit - bilan hépatique / maturati")

replace labour_induced = 1 if (motif_hosp == "déclenchement / prééclampsie")

replace labour_induced = 1 if (motif_hosp == "déclenchement / prééclampsie / métrorragies")

replace labour_induced = 1 if (motif_hosp == "déclenchement / prééclampsie / retard de croissance")

replace labour_induced = 1 if (motif_hosp == "déclenchement / retard de croissance intra-utérin")

replace labour_induced = 1 if (motif_hosp == "déclenchement / retard de croissance intra-utérin /")

replace labour_induced = 1 if (motif_hosp == "déclenchement / surveillance de fin de grossesse")

replace labour_induced = 1 if (motif_hosp == "déclenchement / traumatisme")

replace labour_induced = 1 if (motif_hosp == "déclenchement / version par manoeuvre externe")

replace labour_induced = 1 if (motif_hosp == "déclenchement / vomissements / repos")

replace labour_induced = 1 if (motif_hosp == "dépassement de terme / déclenchement")

replace labour_induced = 1 if (motif_hosp == "rupture de la poche des eaux / déclenchement")

replace labour_induced = 1 if (motif_hosp == "faux travail / déclenchement")

replace labour_induced = 1 if (motif_hosp == "faux travail / déclenchement / dépassement de terme")

replace labour_induced = 1 if (motif_hosp == "pathologies foetales / déclenchement")

replace labour_induced = 1 if (motif_hosp == "prééclampsie / déclenchement")
```

```
replace labour_induced = 1 if (motif_hosp == "retard de croissance intra-utérin / déclenchement")
replace labour_induced = 1 if (motif_hosp == "retard de croissance intra-utérin / déclenchement /")
replace labour_induced = 1 if (motif_hosp == "rupture de la poche des eaux / déclenchement")
replace labour_induced = 1 if (motif_hosp == "version par manoeuvre externe / déclenchement")
replace labour_induced = 1 if (ind_hosp == "déclenchement")
replace labour_induced = 1 if (ind_hosp == "déclenchement lors de gestose")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour RCIU")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour dépassement")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour hydramnios")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour malformation")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour oligoamnios")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour pathologie m")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour rupture des")
replace labour_induced = 1 if (ind_hosp == "déclenchement pour souffrance f")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement lors de gestose")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour RCIU")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour dépassement")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour hydramnios")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour malformation")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour oligoamnios")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour pathologie m")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour rupture des")
replace labour_induced = 1 if (ind_hosp2 == "déclenchement pour souffrance f")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement lors de gestose")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour RCIU")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour dépassement")
```

```
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour hydramnios")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour malformation")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour oligoamnios")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour pathologie m")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour rupture des")
replace labour_induced = 1 if (ind_hosp3 == "déclenchement pour souffrance f")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement lors de gestose")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour RCIU")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour dépassement")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour hydramnios")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour malformation")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour oligoamnios")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour pathologie m")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour rupture des")
replace labour_induced = 1 if (ind_hosp4 == "déclenchement pour souffrance f")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement lors de gestose")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour RCIU")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour dépassement")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour hydramnios")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour malformation")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour oligoamnios")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour pathologie m")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour rupture des")
replace labour_induced = 1 if (ind_hosp5 == "déclenchement pour souffrance f")
generate perineal_tear = 0
replace perineal_tear = 1 if (act1 == "suture d'une déchirure du 2° degré")
replace perineal_tear = 1 if (act1 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act1 == "suture d'une déchirure du 4° degré")
replace perineal_tear = 1 if (act2 == "suture d'une déchirure du 2° degré")
```

```
replace perineal_tear = 1 if (act2 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act2 == "suture d'une déchirure du 4° degré")
replace perineal_tear = 1 if (act3 == "suture d'une déchirure du 2° degré")
replace perineal_tear = 1 if (act3 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act3 == "suture d'une déchirure du 4° degré")
replace perineal_tear = 1 if (act4 == "suture d'une déchirure du 2° degré")
replace perineal_tear = 1 if (act4 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act4 == "suture d'une déchirure du 4° degré")
replace perineal_tear = 1 if (act5 == "suture d'une déchirure du 2° degré")
replace perineal_tear = 1 if (act5 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act5 == "suture d'une déchirure du 4° degré")
replace perineal_tear = 1 if (act6 == "suture d'une déchirure du 2° degré")
replace perineal_tear = 1 if (act6 == "suture d'une déchirure du 3° degré")
replace perineal_tear = 1 if (act6 == "suture d'une déchirure du 4° degré")
generate episiotomy = 0
replace episiotomy = 1 if (act1 == "épisiotomie + suture")
replace episiotomy = 1 if (act2 == "épisiotomie + suture")
replace episiotomy = 1 if (act3 == "épisiotomie + suture")
replace episiotomy = 1 if (act4 == "épisiotomie + suture")
replace episiotomy = 1 if (act5 == "épisiotomie + suture")
replace episiotomy = 1 if (act6 == "épisiotomie + suture")
replace episiotomy = 1 if (act1 == "pratique d'un accouchement normal avec épisiotomie")
replace episiotomy = 1 if (act2 == "pratique d'un accouchement normal avec épisiotomie")
replace episiotomy = 1 if (act3 == "pratique d'un accouchement normal avec épisiotomie")
replace episiotomy = 1 if (act4 == "pratique d'un accouchement normal avec épisiotomie")
replace episiotomy = 1 if (act5 == "pratique d'un accouchement normal avec épisiotomie")
replace episiotomy = 1 if (act6 == "pratique d'un accouchement normal avec épisiotomie")
generate perineal_trauma = 0
replace perineal_trauma = 1 if (perineal_tear == 1)
replace perineal_trauma = 1 if (episiotomy == 1)
generate coagulation_abnormalities = 0
```

```
replace coagulation_abnormalities = 1 if (comorb == "Autres thrombopénies primaires")
replace coagulation_abnormalities = 1 if (comorb == "Maladie de von Willebrand")
replace coagulation_abnormalities = 1 if (comorb2 == "Autres thrombopénies primaires")
replace coagulation_abnormalities = 1 if (comorb2 == "Maladie de von Willebrand")
replace coagulation_abnormalities = 1 if (comorb3 == "Autres thrombopénies primaires")
replace coagulation_abnormalities = 1 if (comorb3 == "Maladie de von Willebrand")
replace coagulation_abnormalities = 1 if (comorb4 == "Autres thrombopénies primaires")
replace coagulation_abnormalities = 1 if (comorb4 == "Maladie de von Willebrand")
replace coagulation_abnormalities = 1 if (comorb5 == "Autres thrombopénies primaires")
replace coagulation_abnormalities = 1 if (comorb5 == "Maladie de von Willebrand")
generate preeclampsia = 0
replace preeclampsia = 1 if (ind_cs == "Césarienne pour prééclampsie")
replace preeclampsia = 1 if (ind_cs2 == "Césarienne pour prééclampsie")
replace preeclampsia = 1 if (motif_hosp == "prééclampsie")
replace preeclampsia = 1 if (motif_hosp == "bilan gestose / prééclampsie / maturation
pulmonair")
replace preeclampsia = 1 if (motif_hosp == "césarienne élective / prééclampsie")
replace preeclampsia = 1 if (motif_hosp == "déclenchement / bilan gestose / prééclampsie")
replace preeclampsia = 1 if (motif_hosp == "déclenchement / prééclampsie")
replace preeclampsia = 1 if (motif_hosp == "déclenchement / prééclampsie / métrorragies")
replace preeclampsia = 1 if (motif_hosp == "déclenchement / prééclampsie / retard de
croissance")
replace preeclampsia = 1 if (motif_hosp == "prééclampsie / bilan gestose")
replace preeclampsia = 1 if (motif_hosp == "prééclampsie / déclenchement")
replace preeclampsia = 1 if (motif_hosp == "prééclampsie / traumatisme")
generate previous_CS = 0
replace previous_CS = 1 if (atcd_cs == "1")
replace previous_CS = 1 if (atcd_cs == "2")
replace previous_CS = 1 if (atcd_cs == "3")
replace previous_CS = 1 if (atcd_cs == "4")
replace previous_CS = 1 if (atcd_cs == ">4")
```

```
generate oxytocin_infusion_in_labour = .

replace oxytocin_infusion_in_labour = 1 if (synto == "oui")

replace oxytocin_infusion_in_labour = 0 if (synto == "non")

generate multiple_gestation = 0

replace multiple_gestation = 1 if (typ_gros == "gémellaire")

replace multiple_gestation = 1 if (typ_gros == "triple")

replace multiple_gestation = 1 if (typ_gros == "quadruple")

generate parity = 0

replace parity = 1 if (par>0)

generate low_inserted_placenta = 0

replace low_inserted_placenta = 1 if (motif_hosp == "césarienne élective / placenta bas
inséré / menace")

replace low_inserted_placenta = 1 if (motif_hosp == "césarienne élective / placenta bas
inséré / placent")

replace low_inserted_placenta = 1 if (motif_hosp == "métrorragies / placenta bas inséré")

generate retained_placenta = 0

replace retained_placenta = 1 if (act1 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act2 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act3 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act4 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act5 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act6 == "délivrance artificielle, extraction manuelle du
placenta complet (inclus : révisi")

replace retained_placenta = 1 if (act1 == "révision manuelle isolée de la cavité utérine (après
délivrance naturelle, complè")

replace retained_placenta = 1 if (act2 == "révision manuelle isolée de la cavité utérine (après
délivrance naturelle, complè")

replace retained_placenta = 1 if (act3 == "révision manuelle isolée de la cavité utérine (après
délivrance naturelle, complè")
```

```
replace retained_placenta = 1 if (act4 == "révision manuelle isolée de la cavité utérine (après délivrance naturelle, complè")

replace retained_placenta = 1 if (act5 == "révision manuelle isolée de la cavité utérine (après délivrance naturelle, complè")

replace retained_placenta = 1 if (act6 == "révision manuelle isolée de la cavité utérine (après délivrance naturelle, complè")

replace retained_placenta = 1 if (anom_pc == "placenta incomplet")

generate operative_vaginal_birth = 0

replace operative_vaginal_birth = 1 if (forceps== 1)

replace operative_vaginal_birth = 1 if (ventouse== 1)

generate vaginal_delivery = 0

replace vaginal_delivery = 1 if (typ_acc == "accouchement normal")

replace vaginal_delivery = 1 if (act1 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act2 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act3 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act4 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act5 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act6 == "délivrance naturelle")

replace vaginal_delivery = 1 if (act1 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act2 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act3 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act4 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act5 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act6 == "épisiotomie + suture")

replace vaginal_delivery = 1 if (act1 == "pratique d'un accouchement normal avec épisiotomie")

replace vaginal_delivery = 1 if (act2 == "pratique d'un accouchement normal avec épisiotomie")

replace vaginal_delivery = 1 if (act3 == "pratique d'un accouchement normal avec épisiotomie")

replace vaginal_delivery = 1 if (act4 == "pratique d'un accouchement normal avec épisiotomie")
```

```
replace vaginal_delivery = 1 if (act5 == "pratique d'un accouchement normal avec épisiotomie")

replace vaginal_delivery = 1 if (act6 == "pratique d'un accouchement normal avec épisiotomie")

replace vaginal_delivery = 1 if (act1 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act2 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act3 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act4 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act5 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act6 == "pratique d'un accouchement normal avec ou sans épisiotomie")

replace vaginal_delivery = 1 if (act1 == "pratique d'un accouchement normal sans épisiotomie")

replace vaginal_delivery = 1 if (act2 == "pratique d'un accouchement normal sans épisiotomie")

replace vaginal_delivery = 1 if (act3 == "pratique d'un accouchement normal sans épisiotomie")

replace vaginal_delivery = 1 if (act4 == "pratique d'un accouchement normal sans épisiotomie")

replace vaginal_delivery = 1 if (act5 == "pratique d'un accouchement normal sans épisiotomie")

replace vaginal_delivery = 1 if (act6 == "pratique d'un accouchement normal sans épisiotomie")

generate PPH_Yes_No = .

replace PPH_Yes_No = 1 if (emergency_CS ==1) & (pe1000== 1)

replace PPH_Yes_No = 0 if (emergency_CS ==1) & (pe500_1000== 1)

replace PPH_Yes_No = 0 if (emergency_CS ==1) & (pe500== 1)

replace PPH_Yes_No = 1 if (elective_CS ==1) & (pe1000== 1)

replace PPH_Yes_No = 0 if (elective_CS ==1) & (pe500_1000== 1)

replace PPH_Yes_No = 0 if (elective_CS ==1) & (pe500== 1)

replace PPH_Yes_No = 1 if (operative_vaginal_birth ==1) & (pe1000== 1)
```

```
replace PPH_Yes_No = 1 if (operative_vaginal_birth ==1) & (pe500_1000== 1)
replace PPH_Yes_No = 0 if (operative_vaginal_birth ==1) & (pe500== 1)
replace PPH_Yes_No = 1 if (vaginal_delivery ==1) & (pe1000== 1)
replace PPH_Yes_No = 1 if (vaginal_delivery ==1) & (pe500_1000== 1)
replace PPH_Yes_No = 0 if (vaginal_delivery ==1) & (pe500== 1)
generate str11 ntime = variable(td_dt)
generate nhours = substr(ntime, 4,2)
generate hours =real(nhours)
generate nmins= substr(ntime, 7,2)
generate mins = real(nmins)
generate total_mins = ((60*hours) + mins)
drop if elective_CS >= 1
drop if pha>7.8
drop if phv>7.8
drop if pha<7.1
generate blood_loss = 0
replace blood_loss = 1 if (pe == "< 500 ml")
replace blood_loss = 2 if (pe == "500 ml à 1000 ml")
replace blood_loss = 3 if (pe == "> 1000 ml")
generate birth_mode = .
replace birth_mode = 0 if (vaginal_delivery == 1)
replace birth_mode = 1 if (operative_vaginal_birth == 1)
replace birth_mode = 2 if (emergency_CS == 1)
```

Appendix 4

STATA .do Files for Descriptive Statistics; main analysis and sensitivity analysis

```
graph twoway (lfit pha phv) (scatter pha phv)

histogram pha, bin(20) frequency ytitle(Number of Patients) xtitle(Umbilical Arterial Blood pH) name(pha_Histogram)

histogram phv, bin(20) frequency ytitle(Number of Patients) xtitle(Umbilical Venous Blood pH) name(phv_Histogram)

twoway (histogram pha, start(6.5) bin(50) color(red) ytitle(Number of Patients)) (histogram phv, start(6.5) bin(50) color(blue)xtitle(Umbilical Arterial Blood pH))

graph box phv, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of Umbilical Venous pH and PPH)

graph box pha, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of Umbilical Arterial pH and PPH)

graph box BMI, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of BMI and PPH)

graph box age_mat, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of maternal age (years) and PPH)

graph box total_mins, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of childbirth length (minutes) and PPH)

graph box ag2, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of Gestational age at delivery (weeks) and PPH)

graph box t2phas, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of Childbirth Second Phase Length (minutes) and PPH) nooutsides

graph box t_synto, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of Oxytocin Use (minutes) and PPH) nooutsides

graph box kg_nn, over(PPH_Yes_No) ylabel(, angle(horizontal)) title(Box plot of neonatal weight (g) and PPH)

tab PPH_Yes_No

tab PPH (PPH_Yes_No), summarize (age_mat)

tab PPH (PPH_Yes_No), summarize (ag2)

tab PPH (PPH_Yes_No), summarize (t2phas)

tab PPH (PPH_Yes_No), summarize (kg_nn)

tab PPH (PPH_Yes_No), summarize (pha)

tab PPH (PPH_Yes_No), summarize (phv)

tab PPH (PPH_Yes_No), summarize (BMI)

tab PPH (PPH_Yes_No), summarize (total_mins)
```

tab PPH (PPH_Yes_No), summarize (t_synto)

tab PPH obesity, column

tab PPH forceps, column

tab PPH ventouse, column

tab PPH labour_induced, column

tab PPH perineal_tear, column

tab PPH perineal_trauma, column

tab PPH episiotomy, column

tab PPH coagulation_abnormalities, column

tab PPH preeclampsia, column

tab PPH previous_CS, column

tab PPH oxytocin_infusion_in_labour, column

tab PPH multiple_gestation, column

tab PPH low_inserted_placenta, column

tab PPH retained_placenta, column

tab PPH parity, column

tab PPH birth_mode, column

---

graph box phv, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of Umbilical Venous pH and PPH (>1000ml))

graph box pha, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of Umbilical Arterial pH and PPH (>1000ml))

graph box BMI, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of BMI and PPH (>1000ml))

graph box age_mat, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of maternal age (years) and PPH (>1000ml))

graph box total_mins, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of childbirth length (minutes) and PPH (>1000ml))

graph box ag2, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of Gestational age at delivery (weeks) and PPH (>1000ml))

graph box t2phas, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of Childbirth Second Phase Length (minutes) and PPH (>1000ml))nooutsides

```
graph box t_synto, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of Oxytocin Use (minutes) and PPH (>1000ml)) nooutsides

graph box kg_nn, over(pe1000) ylabel(, angle(horizontal)) title(Box plot of neonatal weight (g) and PPH (>1000ml)) nooutsides

tab pe1000

tab pe1000, summarize (age_mat)

tab pe1000, summarize (ag2)

tab pe1000, summarize (t2phas)

tab pe1000, summarize (kg_nn)

tab pe1000, summarize (pha)

tab pe1000, summarize (phv)

tab pe1000, summarize (BMI)

tab pe1000, summarize (total_mins)

tab pe1000, summarize (t_synto)

tab pe1000 obesity, column

tab pe1000 forceps, column

tab pe1000 ventouse, column

tab pe1000 labour_induced, column

tab pe1000 perineal_tear, column

tab pe1000 perineal_trauma, column

tab pe1000 episiotomy, column

tab pe1000 coagulation_abnormalities, column

tab pe1000 preeclampsia, column

tab pe1000 previous_CS, column

tab pe1000 oxytocin_infusion_in_labour, column

tab pe1000 multiple_gestation, column

tab pe1000 low_inserted_placenta, column

tab pe1000 retained_placenta, column

tab pe1000 parity, column

tab pe1000 birth_mode, column
```

Appendix 5

STATA .do Files for Univariate Analysis; main analysis and sensitivity analysis

```
logit PPH_Yes_No age_mat

logit PPH_Yes_No ag2

logit PPH_Yes_No t2phas

logit PPH_Yes_No total_mins

logit PPH_Yes_No kg_nn

logit PPH_Yes_No pha

logit PPH_Yes_No phv

logit PPH_Yes_No BMI

logit PPH_Yes_No obesity

logit PPH_Yes_No forceps

logit PPH_Yes_No ventouse

logit PPH_Yes_No labour_induced

logit PPH_Yes_No perineal_tear

logit PPH_Yes_No episiotomy

logit PPH_Yes_No perineal_trauma

logit PPH_Yes_No coagulation_abnormalities

logit PPH_Yes_No preeclampsia

logit PPH_Yes_No previous_CS

logit PPH_Yes_No oxytocin_infusion_in_labour

logit PPH_Yes_No t_synto

logit PPH_Yes_No multiple_gestation

logit PPH_Yes_No parity

logit PPH_Yes_No low_inserted_placenta

logit PPH_Yes_No retained_placenta

logit PPH_Yes_No i.birth_mode
```

```
logit pe1000 age_mat

logit pe1000 ag2

logit pe1000 t2phas
```

```
logit pe1000 total_mins
logit pe1000 kg_nn
logit pe1000 pha
logit pe1000 phv
logit pe1000 BMI
logit pe1000 obesity
logit pe1000 forceps
logit pe1000 ventouse
logit pe1000 labour_induced
logit pe1000 perineal_tear
logit pe1000 episiotomy
logit pe1000 perineal_trauma
logit pe1000 coagulation_abnormalities
logit pe1000 preeclampsia
logit pe1000 previous_CS
logit pe1000 oxytocin_infusion_in_labour
logit pe1000 t_synto
logit pe1000 multiple_gestation
logit pe1000 parity
logit pe1000 low_inserted_placenta
logit pe1000 retained_placenta
logit pe1000 i.birth_mode
```

Appendix 6

STATA .do Files for Multivariate Analysis; main analysis and sensitivity analysis

logistic PPH_Yes_No i.birth_mode retained_placenta

logistic PPH_Yes_No i.birth_mode retained_placenta multiple_gestation

logistic PPH_Yes_No i.birth_mode retained_placenta forceps

logistic PPH_Yes_No i.birth_mode retained_placenta previous_CS

logistic PPH_Yes_No i.birth_mode retained_placenta phv

logistic PPH_Yes_No i.birth_mode retained_placenta parity

logistic PPH_Yes_No i.birth_mode retained_placenta perineal_tear

logit PPH_Yes_No i.birth_mode retained_placenta

---

logistic pe1000 retained_placenta labour_induced

logit pe1000 retained_placenta labour_induced

Appendix 7

STATA. Do Files for ROC Curve; main analysis and sensitivity analysis

```
generate B_M1 = 0

replace B_M1 = 1 if (birth_mode ==1)

generate B_M2 = 0

replace B_M2 = 1 if (birth_mode ==2)

generate PPH_Yes_No_risk = 1/(1+exp(2.68-(1.23*B_M1)-(1.67*B_M2)-
(2.96*retained_placenta)))

roctab PPH_Yes_No PPH_Yes_No_risk, detail graph

generate risk_score = .

replace risk_score = 1 if (PPH_Yes_No_risk >= 0.1900)

replace risk_score = 0 if (PPH_Yes_No_risk < 0.1900)

tab risk_score PPH_Yes_No
```

```
generate PPH_Yes_No_risk = 1/(1+exp(5.08-(3.43*retained_placenta)-
(1.24*labour_induced)))

roctab pe1000 PPH_Yes_No_risk, detail graph

generate risk_score = .

replace risk_score = 1 if (PPH_Yes_No_risk >= 0.0210)

replace risk_score = 0 if (PPH_Yes_No_risk < 0.0210)

tab risk_score pe1000
```