# PLACENTAL RELATED FACTORS CONTRIBUTING TO STILLBIRTHS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

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## DECLARATION

I, Nompumelelo Zamokuhle Mtshali, student number 0420087R, declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

**Signature of Student** …… ……..…

**Date**……25 June 2021…….

## DEDICATION:

This study is dedicated to my sister, Thuthukile Mtshali, who passed away during the season of COVID-19 whilst enrolled for her B.Com Honours degree.

## PRESENTATION ARISING FROM THIS RESEARCH

Upon completion of this research, it is intended to be submitted for consideration for presentation at the International Paediatric Pathology Association (IPPA) Advanced Course.

## PUBLICATIONS ARISING FROM THIS RESEARCH

The manuscript is still in the process of submission.
Placental heterogeneity in stillbirth and its relationship to maternal exogenous characteristics

# ABSTRACT

**Objectives:**

To determine the association of placental pathology and stillbirth at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

**Design:**

Retrospective study analysis.

**Setting:**

This study was conducted at CMJAH, a tertiary academic hospital in Gauteng Province, Republic of South Africa (RSA).

**Population:**

We conducted a retrospective study of 122 stillbirths by reviewing placental histology reports at CMJAH. Specifically, our investigation retrieved and reviewed all cases of placentas submitted to pathology following stillbirth during the period January 2016 to July 2018 (19 months). Thereafter, placental histopathology was evaluated, analysed and categorised according to the Amsterdam Placental Workshop Group Consensus Statement.

**Methods:**

This retrospective review study comprised of a total number of 122 placentas from stillbirths more than 28 weeks at CMJAH. Our study only included placentas submitted to the laboratory during the period January 2016 - July 2018 (19 months). The reports and slides were retrieved and reviewed. The maternal data was retrieved from the patients' hospital records and clinical information regarding the foetus was cross-referenced from the maternity registers and hospital records.

The placental pathology was reported using the Amsterdam Placental Workshop Group's consensus criteria. However, placentas of live births and those derived from patients with congenital abnormalities were excluded from the study. Moreover, placentas of patients with incomplete data that prevented accurate classification according to the Amsterdam Placental Workshop Group's consensus criteria were also excluded.

Placental heterogeneity and its relations to maternal characteristics was explored using latent class cluster analysis (LCCA) with Latent Gold 5.1.

**Main Outcome measures:**

The commonest histopathologic condition associated with stillbirth in our setting was ascending intrauterine infection. Furthermore, maternal clinical conditions commonly observed were hypertension (n=43; 35.25%) and HIV infection (n=32; 26.23%).

**Results:**

The most common histological findings associated with stillbirth were acute chorioamnionitis (foetal response) (CA_F) (n=61; 50%), chorioamnionitis (maternal response) (CA_ M) (n=49; 40.16%), foetal vascular malperfusion (FVM) (n=42; 34.43%), maternal vascular malperfusion (MVM) (n=42; 34.43%), retroplacental haemorrhage (RPH) (n=27; 22.13%), APH (n=19; 15.83%) and intrauterine compromise (n=18; 14.75%). However, the least common histopathological abnormalities findings that contributed towards neonatal mortality were delayed villous maturation (DVM) (n=6; 4.92%), maternal floor infarction/ massive perivillous fibrin deposit (MFI/MPFD) (n=5; 4.10%) and meconium-associated vascular necrosis (MAVN) (n=4; 3.28%).

There were five distinct classes that emerged upon performance of the latent class analysis showing heterogeneity in our stillbirth population.

The first class was high in FVM, low in MVM and CAF with placentas near the $10^{th}$ centile. Class 2 was high in MVM, and low CAF CAM, FVM, and VUE. Placental weight was more likely to fall short of the $10^{th}$ decile than any other class. Class 3 had a 99% probability of RPH. CAF, placenta weight, CAM, FVM, MVM, and VUE were low. Class 4 was distinguished by high CAM, while placental weight, FVM, MVM, VUE, RPH were low. Class 5 had high CAF, FVM, MVM, and RPH and low VUE. This cluster was also distinguished by a high HIV probability.

**Conclusion:**

In our setting, chorioamnionitis is the most frequent pathological process observed in stillbirth placentas, particulalry in HIV-positive individuals.

LCCA confirmed heterogeneity in the placentas, confirming heterogeneity in our stillbirth population and highlighting areas which require further investigation.

Clinical history plays a key role to understanding the pathological placental processes, therefore multi-disciplinary communication is vital. Lastly, the macroscopic and

microscopic examination of the placenta should be recorded on a standardized placental template.

**FUNDING:**

None

**KEYWORDS:**

Placenta, histopathology, stillbirth classification systems, Amsterdam Consensus; latent class analyisis, HIV, causes of stillbirth, pregnancy

## ACKNOWLEDGEMENTS

We thank Dr Langanani Mbodi who assisted in accessing clinical data collection.
The authors also thank Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) for affording us access to clinical files for data analysis.
We wish to thank Eric Liebenberg for his assistance in formatting images.
We thank Prof Steven M. Burgess, Tshwane School for Business and Society, Tshwane University of Technology, Private Bag X680, Pretoria 0001 for assisting with statistical analysis.

## NOMENCLATURE

MVM: Maternal Vascular Malperfusion

FVM: Foetal Vascular Malperfusion

VUE: Villitis of Unknown aetiology

RPH: Retroplacental haemorrhage

CA_M: Chorioamnionitis with a maternal response.

CA_F: Chorioamnionitis with a foetal response.

MI/MFPD: Maternal floor infarction/ Massive perivillous fibrin deposit)

MAVN: Meconium-associated vascular necrosis

DVM: Delayed villous maturation

PASS: The Prenatal Alcohol in SIDS and Stillbirth

SCRN: Stillbirth Collaborative Research Network

PI: Primary Investigator

LCCA: Latent Class Cluster Analysis

DM: Diabetes Mellitus

HAEM: Antepartum haemorrhage

HT: hypertension

PROM: premature rupture of membranes

MA: Maternal age

GRAV: Gravidity

PARA: Parity

Ratio 10: Ratio weight relative to $10^{th}$ centile

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

HIV: Human immunodeficiency virus

# CHAPTER ONE-OVERVIEW

## 1.1 INTRODUCTION:

Stillbirth remains a large burden worldwide and is significant in countries with poor health systems (1). South Africa has a double burden of disease driving neonatal mortality; term babies are dying owing to intrapartum-related events, and preterm deaths are linked to multiple complications (2). The antepartum period contributes to about 49.1% of stillbirths globally (1).

The 2,9 million yearly neonatal deaths globally are traceable to three main causes: infections (0,6 million), intrapartum conditions (0,7 million), and preterm birth complications (1,0 million) (3). Boys have a higher biological risk of neonatal death; however, girls often have a higher social risk. Small size at birth due to preterm birth or small-for-gestational-age (SGA), or both, is the biggest risk factor for more than 80% of neonatal deaths and increases risk of post-neonatal mortality, growth failure, and adult-onset non-communicable diseases (3).

Unfortunately, the unreliable systems utilized to record the neonatal mortality in lower-middle-income countries hamper accurate assessment of the death rate. The capturing of every birth and death statistics, in conjunction with accurate identification of the cause of death remains crucial for planning, resource allocation and alleviating the burden of death rates (2).

Although major strides have been made in dealing with the issue of stillbirths, there are unfortunately gaps in knowledge that are a common thread in most countries. These clinical challenges include amongst others, data collection, efficient patient history recording, sampling procedures, vagueness of terminology and racial disparities. In order to accelerate the reduction of stillbirths, these critical factors need to be urgently addressed in both the public and private healthcare facilities.

Unfortunately, the unreliable systems utilized to record the neonatal mortality in lower-middle-income countries hamper accurate assessment of the death rate. The capturing of every birth and death statistics, in conjunction with accurate identification of the cause of death remains crucial for planning, resource allocation and alleviating the burden of death rates (2).

The 2016 Prenatal Problem Identification Programme (PPIP)_data showed that the main causes of all neonatal deaths (i.e. birth weight ≥500 grams) are as a result of complications of prematurity (47.9%); intrapartum-related events, mainly intrauterine

hypoxia (24.3%); and infections (including pneumonia, at 11.6%). Among the top three causes of mortality, foetal distress not detected in labour was one.(2).

Furthermore, the PPIP, documented 2 558 397 births that occurred in the public sector, from a period of 2015 to 2017. Of these recorded births, 10 609 (14.2%) were intrapartum deaths (4) .

Several studies that have been undertaken to investigate and address risk factors associated with stillbirths, however, these are compromised by limited data (5) .

The misclassification of stillbirths is still problematic in other countries and has a negative influence on implementing interventions seeking to improve quality of clinical care to both the mother and neonate (1).

In an attempt to address risk factors associated with adverse pregnancy outcome (5) discovered that racial disparities are key in the contribution of stillbirths. Most large studies that have been undertaken to address these risk factors have had to work with limited clinical data sets.

The placenta may be regarded as the ‘‘black box’’ of pregnancy and detailed examination may afford insight into the foetal and maternal events leading to this tragic outcome (6).

Placentas form a major part of the foetal-maternal interface which makes the placenta a vital organ that when examined using appropriate protocols, can offer invaluable information (7). It is accessible and inexpensive, and a detailed placental examination does not require consent, as is the case with post-mortems.

Unfortunately, neither placental examination nor post-mortems are routinely conducted in South Africa mainly due to expense as well as cultural and religious belief systems. Causes of stillbirth are therefore, normally determined based on clinical history and examination of the fetus at the time of delivery.

Notably, the placenta plays a vital role in assessing adverse neonatal outcome (8) and provides valuable clinical insight pertaining to treatment and preventative strategies for subsequent pregnancies (6).

Accordingly, the objective of this study was to determine the most prevalent placental pathology associated with stillbirth at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a tertiary hospital in the Gauteng Province of the Republic of South Africa.

As the placenta is central to obtaining accurate evaluation for stillbirths, we therefore hypothesize that the causes of stillbirth are more comprehensive and conclusive with the inclusion of a placental histology examination. Therefore, this study will enable enhanced identification of the leading causes of stillbirths at Charlotte Maxeke Johannesburg Academic Hospital and precise classification of placental pathology using the standardized Amsterdam protocol.

The Amsterdam classification criteria was created through a combined effort of 26 pathologists who attended a workshop in Amsterdam in the year 2014 to offer their views on how to evaluate placentas. The fundamental intent was to construct standardized definitions of lesions in the placenta (9).

It's a classification based on a collective international consensus and offers a robust, biological-based, comprehensive system that gives clear guidance in terms of implementation of sampling guidelines as well as description of placental lesions for accurate evaluation (6). It also provides recommendations for placental submission, thus, reducing costs incurred when placentas are submittted for no apparent reasons. Furthermore, it offers valuable information regarding major maternal and foetal vascular processes as well as infectious and idiopathic/immune inflammatory processess (9).

Overall, the Amsterdam classification system incorporates four major pathological placental injuries which includes maternal vascular malperfusion (MVM), foetal vascular malperfusion (FVM), acute chorioamnionitis which can be further categorized into maternal or foetal responses (9).

## 1.2 MATERIALS AND METHODS:

A retrospective review of placental histology reports with a total number of 122 placentas from stillbirths was included in the study. The slides were re-examined by ZM and CW. We retrieved and reviewed all placentas submitted to Charlotte Maxeke Hospital during the period Jan 2016 - July 2018 (19 months) using a SNOMED (Systematized Nomenclature of Medicine search). We screened for cases of stillbirth -defined as foetal death from 28 weeks' gestational age onwards- from the placenta cohort.

The placental pathology was assessed using the Amsterdam Placental Workshop Group consensus criteria (Appendix B, Figure 1).

Placentas of live births as well as congenital abnormalities were excluded from the study. Placentas of patients with incomplete data preventing classification according to the Amsterdam Placental Workshop Group's consensus were also excluded.
The maternal data was retrieved from patients' files / patients' hospital records. The clinical information regarding the foetus was cross-referenced from the maternity registers and hospital records.
Patients eligible for the study had to have a documented clinical diagnosis of stillbirth at more than 28 weeks' gestation in the hospital medical records.

The aim of this study was to review 2 years of placental histology reports at CMJAH in order to determine the most prevalent histologic findings in stillbirths (JAN 2016-JULY 2018) and the corresponding placental histopathological features.
This study also had exploratory components. It is aimed to (1) to classify stillbirth placentas into a small number of relatively homogeneous subgroups (i.e., latent classes) based on pattern similarity using observed diagnostic criteria and (2) examine the associations of these latent classes with relevant maternal characteristics (i.e., covariates), especially maternal HIV.
To achieve these goals, we explored placental heterogeneity in stillbirth and its relations to foetal and maternal exogenous characteristics using latent class cluster analysis (LCCA), a rigorous model-based statistical procedure that can be used to classify placentas into relatively homogenous subgroups based on similarities and differences in diagnostic criteria.
We included exogenous maternal characteristics in the model as covariates that help explain variations in the latent classes, but do not affect the associations between the placental diagnostic criteria directly.
The study approval was obtained from the Committee for Research on Human Subjects, University of the Witwatersrand, Johannesburg (Reference number M180909).
The institutional approval was granted by the chief executive officer of CMJAH.
Patient confidentiality was maintained at all times. No identifying information was included on the datasheet. Patients were identified by a study number. The data was kept in a password protected computer to which only the Primary Investigator had access.

## 1.3 RESULTS

Mean maternal age was 28 years (range 18 - 41 years) and mean gestational age was 34 weeks (range 28 - 42 weeks).

The mean placental weight across 122 cases was 282.50 g (range 77 - 702 g).

Maternal HIV status was reported as seropositive in 32 (26.23 %) cases and negative in 90 (73.77%) cases.

Table 1 summarises the histopathological findings at Charlotte Maxeke Johannesburg Academic Hospital according to Amsterdam Classification Consensus System.

The most common findings were chorioamnionitis (foetal response), chorioamnionitis (maternal response), FVM, MVM, RPH, APH and Intrauterine compromise. The least common histopathological findings were DVM (n=6; 4.92%), MFI/MPFD (n=5; 4.10%) and MAVN (n=4; 3.28%).

Detailed results for the LCCA analyses are presented in the Appendix (Table 2,3,4,5) and Figure 1 (Graph1).

Figure 1 (Graph 1) summarizes results for each latent class, which include the within-class probabilities for the placenta indicator variables and the associated maternal characteristic covariates.

Table 2 reports distinctive characteristics for each latent class, which were determined by examining the within-class probabilities and rescaled class-specific means. These were considered distinctive when near 1.0 or 0.0 (i.e., homogeneous for that class, which was operationalized as $\geq$ .70 or $\leq$ .30 (10).

The analysis identified two large classes comprising 69% of the placentas. At least three placental characteristics were distinctive for each latent class. To make our summary of these results tractable, we refer to high/low within-class probabilities as “high” or low”.

Class 1: FVM (35%)

This class is high in FVM and includes 67% of the placentas showing FVM in the sample. RPH was not observed. MVM and CA_F also are low. Placentas tend to be near the 10$^{th}$ centile. Associated covariates include very low DM, HAEM, HT, and PROM. MA (mean = 25.30) is the youngest of all classes and GRAV, and HIV also are low.

Class 2: MVM (34%)

Class 2 is high in MVM, including 74% of placentas showing MVM in the sample. RPH is absent and CAF very low. Placental weight is more likely to fall short of the 10$^{th}$

centile than any other class. CAM, FVM, and VUE also are low. Associated covariates are very low DM and HAEM and low PROM. Mean MA is 29.7. Although the probability of HIV is 33%, it is noteworthy that the class includes 42% of HIV+ mothers (i.e., 1.24 times class size).

Class 3: RPH (19%)

Placentas in this class have a 99% probability of RPH. CA_F is very low and placenta weight, CA_M, FVM, MVM, and VUE are low. Class 3 includes 81% of placentas with RPH. Associated covariates are high HT, very low DM and PROM, and low PARA and GRAV. Although not distinctive characteristics, 52% show antepartum hemorrhage and 38% with hypertension. This triad traditionally is associated in the literature (11).

Class 4: CA_M (10%)

This smaller class is distinguished by high CA_M. Placental weight, FVM, MVM, VUE, RPH are low. The associated covariates include high GA and MA, PARA and GRAV also are high. There was no maternal HIV seropositivity and DM and PROM were also low. It is known that chorioamnionitis poses a risk to vertical transmission of HIV/AIDS.

Class 5: HIV (3%)

The incremental addition of this small class greatly reduced the level of unacceptable bivariate residuals and significantly improved model fit. CA_F, FVM, MVM, and RPH are very high. CA_M also is high. VUE is very low. However, this cluster appears to be distinguished most by the associated covariate of high HIV (66% probability). PROM, DM, HAEM, and HT are very low. Maternal age, gestational age, parity, GRAV are low.

## 1.4 VALUE OF THIS RESEARCH

Our study will greatly assist in keeping us abreast of the evolving developments made in placental related stillbirth causes. It will also assist in habitualizing placental histopathological examination as a core practice in order to determine the cause of death and making accurate diagnosis.

It brings awareness of the prevalent pathological conditions that are adversely affecting our maternal population, which will then lead to making informed decisions on how best to combat the issue of stillbirth.

The introduction of the use of the standardized Amsterdam classification system in our pathology practice will help incorporate the key clinical comorbidities that will assist the pathological assessment to arrive at an accurate diagnosis. This will assist in counselling of parents who have recurring conditions and also inform clinical care in succeeding pregnancies.

The study opens potential doors to the introduction of further investigations that are considered key to diagnosing probable causes of stillbirth, such as genetic testing (micro-array, karyotype testing etc.).

# CHAPTER TWO – EXTENDED LITERATURE REVIEW

## 2.1 THE PROBLEM OF STILLBIRTH GLOBALLY

The stillbirth definition recommended by WHO for international comparison is a baby born with no signs of life at or after 28 weeks' gestation. The death of a child before birth is a tragedy that can reduced (12).

Lawn *et al.*, (2014) approximately 5.5 million perinatal deaths occur world-wide annually. This large global burden of stillbirths is associated with adverse socioeconomic consequences (13). Many of these perinatal deaths can be averted (3). They are associated with risk factors rather than a specific and exclusive aetiology (14).Socially marginalised women have twice the risk of stillbirth (15).

Poor collection of data has been implicated in bringing complexity to the stillbirth problem (15) . Over 100 countries are able to collect and record stillbirth data rate (SBR) which can significantly assist in investing in the intervention strategies to reduce stillbirths (16). In the same vein, there still remains a significant number of countries without registry data availability, civil registrations and vital statistics systems. Such loopholes in accountability measures create impaired correlation (16).

To efficiently deal with this issue, there needs to be preparedness to perform a substantial number of high quality diagnostic tests in order to significantly reduce the stillbirth burden globally. Perinatal autopsy, placental examination, parvovirus & syphilis serology, toxicology screening, foetal karyotyping, complete blood count, thyroid-stimulating hormone, lupus anti-coagulant and anticardiolipin antibodies are all recommendations for placental evaluation of stillbirth by the American College of Obstetricians and Gynaecologists (14)

In 2014, the Every Newborn Action Plan (ENAP) was approved at the World Health Assembly to come up with strategic plans to reduce the stillbirth number. Its plan of action is to target the low-income and middle-income countries. The ENAP is aiming to reduce stillbirths from 18 to at least 12 per 1000 births by 2030. In order to realize this target, the Global Annual Risk Reduction (ARR) needs to be more than double the present ARR of 2% (17).

The United Nations Millennium Development Goals (MDG) had committed to 8 goals that they had intended to achieve by the year 2015 (18). Amongst the 8 goals, there were health-related goals that needed urgent attention. They observed that about 83% of women had an opportunity to attend antenatal care at least once during their pregnancy between the years of 2007-2014. However, for the recommended minimum of 4 or more visits the figure went down to about 64% (18).

There was also a requirement for skilled health care workers in order to reduce perinatal, neonatal and maternal deaths in the 6 WHO regions. WHO African regions continue to require more coverage as there is still about 51% at the present stage (17). The Sustainable Development Goals (SDG), on the other hand targeted merging within a generation resulting in women and children having an equal opportunity of survival in all countries. However, the equity gaps have proven to be a challenging factor in reaching this goal (19).

In the period of 2009-2014, there were 81 classification systems for stillbirths and neonatal death were in existence according to (Hopkins Leisher *et al.*, 2016). Their review study could not find a single system that had all features of a globally effective system and there was a lack of “ease of use” in all these classifications (Hopkins Leisher *et al.*, 2016).

Stillbirth is a significant world-wide problem. The placenta is a readily available organ, that when carefully examined, can offer valuable information which can assist in pointing to a probable cause of death of the foetus, thus reducing the stillbirth numbers.

## 2.2 STILLBIRTH IN DEVELOPING COUNTRIES WITH PARTICULAR REFERENCE TO SOUTH AFRICA

The approximated number of stillbirths globally is 3 million (16). Sadly, the majority of the stillbirths are in the developing countries, (21). Devastatingly, there remains a significantly high proportion of stillbirth cases that remain idiopathic (22). The sub-Saharan African region has a ten-fold higher rate of neonatal mortality in comparison to the developed countries. Despite these alarming figures, the stillbirths in developing countries have had very little research let alone policy attention (21).

The factors associated with high incidence of stillbirths in developing countries are multifactorial. However, the most pressing issue is under-registration due to the fact that a number of women give birth at home. In the absence of collation of accurate registry data, the potentially preventable conditions cannot be confronted and managed (21). The low-skill health professional system is also another attributing factor (according to the WHO). As a result, improvements in clinical data capturing and analyses will assist in providing targeted interventions for the vast majority of women facing this tragedy (16).

To effectively address stillbirths in an effective manner, programmes addressing stillbirths, mothers and newborns will go a long way in preventing the onset of child mortality (16). Infection-related stillbirths are also an issue of clinical concern as pregnant mothers with malaria and syphilis should receive proper medical attention and no gaps in the health system should be tolerated (16).

South Africa has a double burden of disease driving neonatal mortality; term babies are dying owing to intrapartum-related events, and preterm deaths are linked to related medical complications (2).

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is in the Gauteng province of South Africa. This province together with the KwaZulu-Natal province, collectively recorded the highest number of total birth registrations in 2017 (23). In a study conducted at three academic hospitals in South Africa, placental abruption was the most frequent catastrophic event associated with stillbirths followed by cord prolapse (24).

It has been noted that stillbirth-associated conditions are potentially modifiable and they tend to coexist (16). Therefore, careful attention is urgently needed to offer adequate training of the caregivers as well as to identify and address societal challenges that may contribute to child death. These include amongst others, identification of patients from a low socio-economic background, nutritional challenges as well as provision of extensive medical care to expectant mothers with advanced age. Improvement of appropriate skills in obstetric care and accessibility to such are essential requirements (16). In addition, a standardized accessible classification system will assist in categorizing the disease processes accounting for perpetual stillbirths in developing countries (21).

Excellent leadership and management are critical components in closing the equity gaps (16) and provision of critical medical interventions, thus, decreasing the incidence of neonatal deaths in various clinical settings.

## 2.3 REVIEW OF STILLBIRTH IN HIV POSITIVE PATIENTS

Maternal and infant death statistics were shown to be high in maternal HIV infection in South Africa (25) . HIV/AIDS remains a global problem claiming many lives. Sub-Saharan Africa carries a burden of approximately 70% of the world's HIV and AIDS burden (26). An estimated 6.3million people in South Africa are infected with HIV and AIDS (26).

In this geographical setting, HIV infection is complicated by an increasing number of patients presenting with HIV and tuberculosis (TB) co-infections. Although combination therapy is applied as an effective strategy to combat both microbial infections, an estimated 38 million people in South Africa were living with HIV in 2019 (27) and 10 million with TB during the same time frame (28).

Previously, an interesting observation was made whereby a decline of HIV was noted in women between the ages of 15-29, while women at the ages above 30 have shown a rise in the statistics (26).

A definite link between HIV infection and stillbirth is suggested by a global literature review (29). Encouragingly, there have been attempts to improve mother-to-child-transmission of HIV, and these have significantly reduced HIV related stillbirths (30).

A possibility that HIV and antiretroviral treatment have an impact on the placental development during pregnancy, has also been considered.

The main route of transmission of HIV to infected children is vertical (31). In this study there was an observation that the presence of chorioamnionitis further increases the risk of vertical transmission. There are other known risk factors for perinatal HIV transmission, over and above chorioamnionitis, such as advanced disease process, prolonged rupture of membranes, low CD4 count and high viral load (32).

In the study by Baurakiades et al., 2011, smaller villi were also noted histologically, in HIV infected patients. The pathophysiology involves the placental trophoblast which are infected through cell-cell transmission because of their proximity to the maternal blood vessels (31). The interesting feature observed in this study was the presence of a high number of CD8+ T-cells which led to a hypothesis that there is an existence of HIV-specific villitis which is not related to classical VUE. (31).

In a study performed by Kalk (33) the most prevalent pathological condition associated with HIV was maternal vascular malperfusion.

In an audit performed in Tshwane, South Africa, stillbirth and neonatal mortality rates were noted to be high in HIV-positive women (25). This finding was also a feature in a study that was performed in Cape Town, South Africa where neonatal encephalopathy was prevalent in neonates born to HIV-positive mothers (29).

The Tshwane study also revealed a high propensity for postpartum haemorrhage as well as perinatal deaths in HIV-positive patients (25).

The rigorous identification of placental patterns and their relations to maternal and foetal characteristics at stillbirths can be a critical step to widening our understanding of the role of placentas in stillbirths and our understanding of the impact of HIV.

A small placenta was also a feature noted in placentas from HIV positive mothers (33). In this study, the authors speculate that the disruption of placentation could be secondary to the recovery of the immune system following ART which then leads to MVM.

The PROMISE (Promoting Maternal and Infant Survival Everywhere) trial demonstrated significant advantages of triple-drug ART in a sense that all three regimens showed an HIV transmission rate of less than 2%. However, these ART regimens had adverse maternal events and adverse pregnancy outcomes (34).

They emphasized a need for further research in order to ensure healthier outcomes for the infants who are HIV negative born to HIV positive mothers.

## 2.4. CLASSIFICATION SYSTEMS IN STILLBIRTH

There have been many classification systems for both placental pathology and for stillbirth over the decades. Initially, a total of 81 different placental classification systems were noted by Flenady and colleagues.These classifications were used to report causes of stillbirths between 2009 and 2014 (15). There have been many classification systems for both placental pathology and for stillbirth over the decades. Initially, a total of 81 different placental classification systems were noted by Flenady and colleagues.These classifications were used to report causes of stillbirths between 2009 and 2014 (15).

In the review reported by (20), they proposed that the lack of alignment and consistency in classification systems for causes of perinatal death may very well contribute to the inability to combat the death rates. It also explains the rapid developments of new and modified systems at the rate of ten a year in a space of 5-

year period. This further leads to impediment in the widespread acceptance of the proposed classification systems (20)

The PASS Network (The Prenatal Alcohol in SIDS and Stillbirth) Classification system was established prior to the Amsterdam Classification in 2017 (Fig 2). Its approach was to assess for potential primary sites of origin of stillbirth-related pathology i.e. Foetal, Placental, Maternal, External/Environmental, or Undetermined origin (35). The PASS is advantageous in the sense that it is simple to use, it also has mechanistic formulations and consolidates a compact clinicopathological correlation (35). It also has a simplistic, non-laborious and feasible approach (35). It incorporates the recurrence risk category and can be employed with limited data (35).

In many instances the cause of stillbirths cannot be explained (15). Conveniently, the PASS makes provision for such in that it has an undetermined category which implies that certain conditions necessitate further supplementary research.

The PASS classification is then further subdivided into mechanisms implicated in each category. It has both the cause of death as well as the recurrent risk carried in each category (35).

It has a foetal category which is further subdivided into intrinsic foetal disorder and infections. The foetal category encompasses congenital diseases, which is critical to know when placental specimens are submitted.

The placental category addresses the umbilical cord pathology and the perfusion failure, both acute and non-acute. The external factors include subcategory like mechanical forces which include termination of pregnancy. Lastly, it also embraces the iatrogenic category where the cause or mechanism of death may not be known (35). The additional advantages of the PASS classification system are that, it includes the mechanism of demise as well as the aetiology (35). The PASS has wider applicability for use by perinatal mortality review boards for evaluation of stillbirth (36). It involves a broader range of spectrum of investigations such as autopsy, placental evaluation, clinical obstetric records etc.

The Stillbirth Collaborative Research Network (SCRN) consists of a multidisciplinary team of investigators whose intent is to determine a workable classification system for data collection for evaluation of stillbirth (7). Their study included wide variety of tests such as toxicology, cytogenetics as well as post-mortems.

The SCRN study discovered that about 64.6% of cases were attributable to placental pathology thus placing further greater emphasis on the need for investigating stillbirths by Pathologists (14). It enables pathologists to establish what placental findings are associated with stillbirth, according to gestational age (7).

Studies have shown that the aetiology of stillbirths is heterogeneous in nature. As a consequence, there is no exclusive entity entirely responsible for all child mortalities. There is therefore, a need for an accessible classification that has information routinely collected at the clinical level, which also takes into consideration the importance of placental histology that can help in the evaluation of stillbirths (Madhi *et al.*, 2019).

## 2.5 CLASSIFICATION SYSTEMS IN PLACENTAL PATHOLOGY

The examination of the placenta is highly dependent on the classification system used (22) . For this reason, there needs to be a unified approach in terminology and sampling criteria for placentas that can be accessible in tertiary hospitals and community hospitals (37).

A sequence of unprecedented work has been performed in attempting to accurately classify stillbirth pathological process in the placenta. In previous studies, the assessment of placental pathology placed much emphasis on the macroscopic description of placental abnormalities such as marginal and velamentous cord insertions and succenturiate lobe (11). In essence, the gross examination of the placenta plays a vital role in evaluating the viability of the foetus. As such, the examination of the gross abnormalities of the placenta requires some basic knowledge by a clinician as most abnormalities come to light during delivery.

To achieve effective prevention strategies of stillbriths in our country, a detailed user-friendly placental histology examination remains the gold standard for investigating the cause of stillbirths. This examination offers insights to the cause of deaths and points the clinician to treatment options in subsequent pregancies (6).

The varying definitions of placental lesions and under sampling of placental lesions pose a challenge pertaining to correct interpretation of the overall placental pathology. The placental terminology has evolved and this requires a need to adapt to a standardized, consistent and current reporting which will offer the clinicians a clear call to action and inform future research into stillbirth.

In order to allow the maximum utility of the placental report, we need to institutionalize classifications such as the Amsterdam Classification system in tertiary referral centres and make them readily available worldwide. We also need to craft ways to disseminate the standardized classification so that it can be readily available and be understood by the professional attendants.

The Amsterdam classification criteria was created through a combined effort of 26 pathologists who attended a workshop in Amsterdam in the year 2014 to offer their views on how to evaluate placentas. The fundamental intent was to construct standardized definitions of lesions in the placenta (9).

The value of the Amsterdam classification system is that, it is based on a collective international consensus and offers a robust, biological-based, comprehensive system that gives clear guidance in terms of implementation of sampling guidelines as well as description of placental lesions for accurate evaluation (6). It also provides recommendations for placental submission, thus, reducing costs incurred when placentas are submittted for no apparent reasons. Therefore, this classification facilitates indepth studying of pathogenesis, diagnosis and treatment of obstetric disorders (9).

Furthermore, it offers valuable information regarding major maternal and foetal vascular processes as well as infectious and idiopathic/immune inflammatory processess (9).

The recommended standards for grading and staging of lesions such as chronic histiocytic intervillositis, high-grade villitis of unknown aetiology, and maternal floor infarction are critical as these lesions tend to have a high recurrence rate and are generally associated with adverse pregnancy outcomes (9).

Accordingly, the Amsterdam classification system addresses the critical gross descriptions of the placenta that are routinely encountered at put through in both public and private medical institutions (38). The correct gross assessment can be highly informative even before the specimen gets processed for histology. These include methods on how to record placental weight, umbilical cord and membrane description and placental disc description. The classification also recommends submission of a minimum of four blocks, which include two cross-sections of the umbilical cord, a roll of extraplacental membranes and three full-thickness sections taken from the central two thirds of the disc as well as one adjacent to the umbilical cord insertion site (38).

There was an agreement that a placenta should be trimmed before weighing and a detailed description of disorders of the basal plate should be documented (9). It is known that the placental weight plays a vital role in placental function (39).

The weight modifies the effect of foetal growth as well as the birthweight, which plays a vital role in the health and growth of the foetus (39).

It has been recommended that the cord description should include its diameter, length, site of insertion and the presence or absence of strictures. These are helpful in the clinical context as they may be associated with adverse clinical outcomes (9).

The membrane description must include the colour/opacity and completeness of the placenta in question.

Overall, the Amsterdam classification system incorporates four major pathological placental injuries which includes maternal vascular malperfusion (MVM), foetal vascular malperfusion (FVM), acute chorioamnionitis which can be further categorized into maternal or foetal responses (9). However, these are not the only components of the classification system. In our study there were a total of 42 (34.4%) cases of MVM, 42 (34.4%) of FVM, acute chorioamnionitis with a maternal response 49(40.1%) and 61 (50%) acute chorioamnionitis with a foetal response. The least common histopathological findings were DVM (n=6; 4.92%), MFI/MPFD (n=5; 4.10%) and MAVN (n=4; 3.28%), respectively.

Maternal vascular malperfusion is typically characterized **macroscopically** by placental hypoplasia, non-peripheral infarction (including infarction haematoma) and retroplacental haemorrhage (9) (Please refer to figures 3A and B).

On **microscopy**, the MVM features include distal villous hypoplasia, accelerated villous maturation and decidual arteriopathy (9).

The ascending intrauterine infection was discussed and the key factor to note is that this entity is not equivalent to clinical chorioamnionitis. Notably, the topography and constituents of inflammation should also be reported. The inflammation is recorded as acute chorioamnionitis with/without secondary inflammation in either of the following places, namely the chorionic vessels, umbilical vein or umbilical artery. Please refer to figures 3D, E and F.

The retroplacental haemorrhage (RPH) is another key component of the Amsterdam classification which can sometimes be confused with a clinical diagnosis of abruption. The RPH is characterized by indentation of the placenta with an associated haemorrhage at the maternal surface. Histologically, there is blood beneath or dissecting through the decidua which can be accompanied by villous agglutination, congestion and /or intravillous haemorrhage (Khong *et al.*, 2016).

It was also noted that the following lesions required further studying and that their presence did not necessarily provide sufficient evidence for MVM. These lesions are: increased in fibrin with extravillous trophoblast, pseudocyst of the placenta, membranous decidual necrosis as well as pseudocyst of the chorionic laeve.

Conversely, FVM is associated with impaired blood flow to the foetus. Please refer to Fig 3H. It is characterized histologically by the presence of thrombosis (which is a premortem occurrence), avascular villi, villous stromal-vascular karyorrhexis, vascular intramural fibrin deposition, stem vessel obliteration/fibromuscular sclerosis as well as

vascular ectasia. It was further decided that the FVM lesion should be further categorized into a high or low grade pattern (9).

The Amsterdam classification also includes villitis of unknown aetiology (VUE) (9) (Please refer to Figure 3G). VUE is defined as a lymphohistiocytic inflammatory infiltrate of the villi which can occasionally show plasma cells, of which no known cause has been identified. The grading schema for these lesions is either high or low grade and the low grade lesions are further divided into focal or multifocal.

There are other pathological entities that tend to be associated with villitis which should be recognized. These are eosinophilic/T-cell chorionic vasculitis, chronic deciduitis and chronic histiocytic intervillositis. Of interest is that eosinophilic/T-cell chorionic vasculitis is a rare form of chorionic vasculitis characterized by an infiltrate composed predominantly of $CD3^+$ T cells and eosinophils with thrombosis (40). Chronic histiocytic intervillositis has a recurrence rate of 65 to 80% and therefore is essential to be identified within the placenta (11).

The participatory group did acknowledge that a number of lesions may evolve in the future and that further research was still required to build up on the current placental classification criteria.

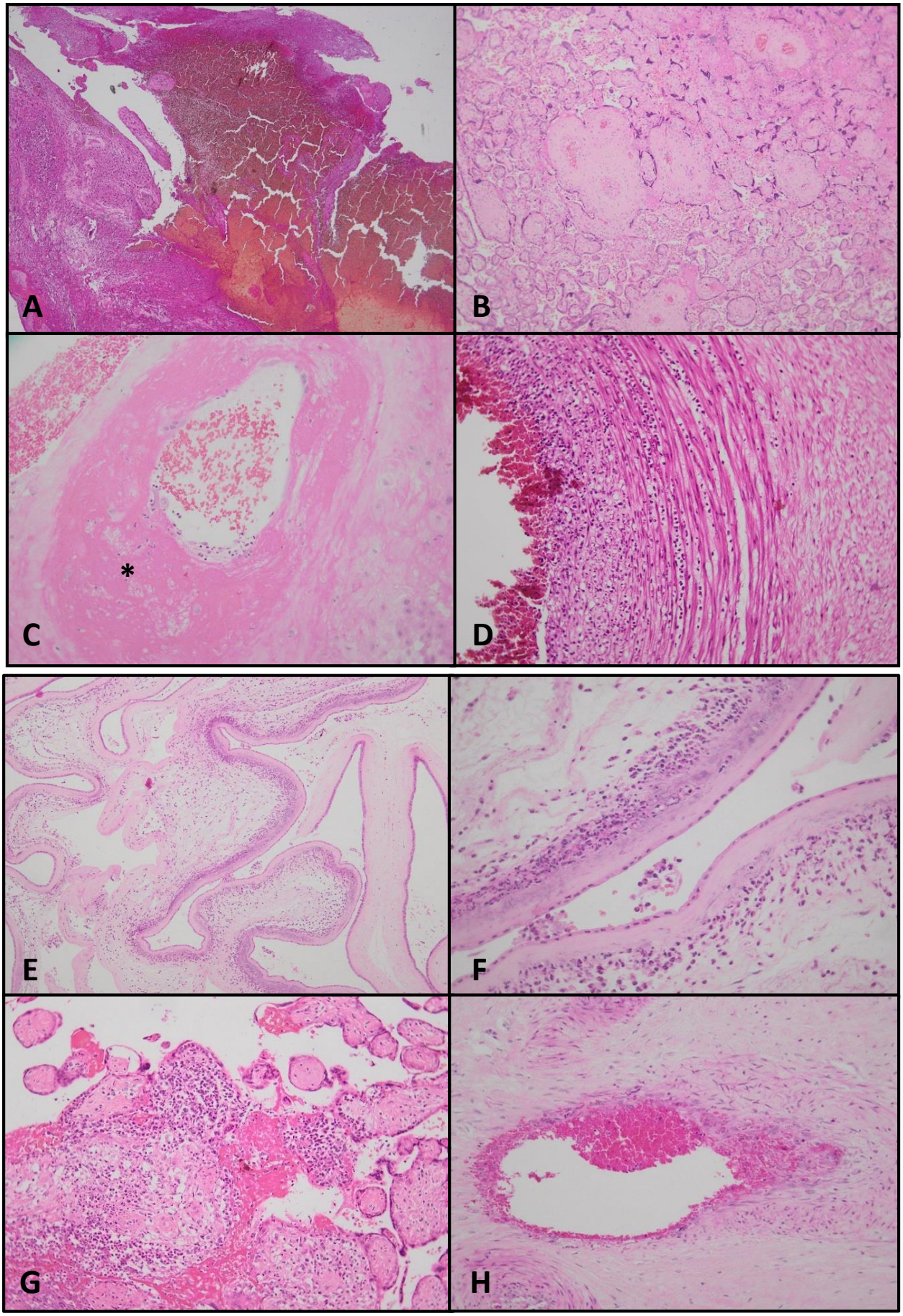
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**Figure 3**: Image A, Retroplacental haemorrhage (hematoxylin-eosin, original magnification x 4); B, Infarction seen in the context of MVM (hematoxylin-eosin, original magnification x 10); C, Decidual arteriopathy showing fibrinoid necrosis (haematoxylin-eosin, original magnification x 40); D, Funisitis (foetal inflammatory response) (haematoxylin-eosin, original magnification x 10); E, Chorioamnionitis (maternal response) (hematoxylin-eosin, original magnification x 10); F, Chorioamnionitis (maternal response) (haematoxylin-eosin original magnification x 40); G, VUE with villous lysis and avascular villi (haematoxylin-eosin, original magnification x 20); H, FVM showing a thrombus and stromal vascular karyorrhexhis (haematoxylin-eosin, original magnification x 20.

## 2.6 THE VALUE OF PLACENTAL PATHOLOGY VS AUTOPSY IN STILLBIRTH

Examination of the placenta offers much value to the understanding of the causation of stillbirths. The placenta has been termed as “diary of gestational life” (41) as it provides critical cellular, physiological and immune information pertaining to the mother and neonate during the gestation period.

Physiologically, the placenta plays a vital role in providing transportation of nutrients, oxygen and fluid from mother to foetus. Additionally, this vital organ also plays a crucial role in the removal of waste products (41) and forms a protective barrier to shield the the foetus from bacterial and other microbial infections (6).More importantly, the entire intra-uterine environment is reflected by the placenta.

The placenta remains available long after the stillbirth has occurred. Since this organ reflects a number of pathological processes that may have led to stillbirth, comprehensive investigation and analysis of the placenta may yield valuable insights relating to the maternal and neonatal conditions that may have contributed to foetal mortality. This is crucially important for decreasing and preventing neonatal deaths in both public and private medical institutions, particularly in underdeveloped countries where patients may not afford expensive medical costs generally associated with private hospitals.

The assessments required to arrive at a diagnosis include detailed placental examination, autopsy and foetal chromosomal analysis.

Unfortunately, cultural, religious and personal values can prohibit patients from giving consent for placental examination and autopsy. The other barrier to the performance of autopsies is the mind-set that the investigation will not “bring the child back” or make any difference (14). The inability to provide autopsy denies parents an opportunity to ascertain the precise aetiology of their baby’s death, perpetuate the number of inexplicable stillbirths and hinder the effectiveness of subsequent audits (15). Nonetheless, the value of placental examination is witnessed in instances such as neurologic injury as well as some unforseen maternal disorders such as maternal vascular diseases (41). Additionally, the placenta can also offer insight in genetic, chromosomal or hematologic disorders (41).

The clinicians may be hesitant to even suggest this investigational study. The idea of performing a full autopsy may be overwhelming as opposed to performing a lesser detailed autopsy. To alleviate this anxiety, an alternative adjunct option can be offered.

For example, a partial autopsy can be of aid in providing useful information that can assist in determining a possible cause of stillbirth (42).

Apart from autopsies, other useful medical procedures that can provide valuable clinical data include radiographs and post-mortem magnetic resonance (14). These however may be costly and/or unavailable.

A number of litigations have arisen againts obstetricians, neonatologists and anaesthesiologists because of the claims that neurological disabilities or stillbirths have emerged as a result of delays in intervention or failure in offering precise diagnosis and a correct disease management (43).

In light of the above, the clinicians should be encouraged to be even more vigilant in submitting the placentas for pathological consultation. This should be accompanied with a detailed relevant clinical history that can assist in coming to a conclusion after placental examination. This practice may greatly reduce litigation and offer appropriate management.

A study was performed to determine the profitability of performing post-mortems to determine the cause of stillbirths. (44) Their conclusive finding was that it is beneficial to at least examine the placenta using a recommended classification system, even if the clinician fails to obtain permission to perform a post-mortem from the parents. In the same investigation, the advantage of performing placental examination included the discovery that infarction and chorioamnionitis were the predominant causes of neonatal mortality in the studied samples.

An attempt to evaluate aetiology in the causation of stillbirth also requires careful clinicopathological correlation. In the setting where there is paucity of clinical history, it becomes difficult to assess the placenta, let alone attaining an accurate diagnosis that can help establish the probable cause of stillbirth. Therefore, open and continuous multi-disciplinary communication lines with the clinicians should remain open, in order to avoid such loopholes.

To achieve effective prevention strategies of stillbirth, a detailed examination of placental histological characteristics remains the gold standard in establishing the cause of stillbirths.

The inspection of the placenta offers insights to the cause of deaths and directs the clinician to potential treatment options in subsequent pregancies (6).

For this reason, placental examination plays a vital role not only in diagnosing pathology that affects the mother and the foetus, however, it is also useful in identifying conditions that are likely to recur in subsequent pregnancies (11)

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# CHAPTER THREE – SUBMISSIBLE FORMAT

**Title:** Placental heterogeneity in stillbirth and its relationship to maternal exogenous characteristics

**Running title:** Placental heterogeneity in stillbirth

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Abstract word count: 64

**Abstract**

The goals of this exploratory study were to classify 122 stillbirth placentas from a tertiary academic hospital in Gauteng Province into latent classes, based on similarity in patterns of observed diagnostic criteria, and to examine the associations of these latent classes with maternal characteristics, especially maternal HIV. Five distinct classes emerged, showing heterogeneity in our stillbirth population and highlighting areas which require further investigation.

*Keywords*: Placenta, stillbirth classification systems, Amsterdam Consensus, latent class analysis

## Introduction

The important role of the placenta in stillbirth is becoming clearer. Recent progress is due, at least in part, to programmatic research initiatives, such as the Stillbirth Collaborative Research Network and the Prenatal Alcohol in SIDS and Stillbirth (PASS) Research Network. Collaborative international networks are encouraging interdisciplinarity and convergence and sharpening the focus on longstanding obstacles to progress. The increasing research rigor and synthesis [ 1, 2, 3], and development of new standardized approaches [e.g., stillbirth classification systems, 1, 4, 5] are important examples of the fruit of this work. A striking and consistent finding in this recent body of research concerns the heterogenous nature of stillbirth. This is particularly true of the patterns of multiple concurrent placental lesions that can have adverse effects in stillbirth. This heterogeneity remains an unexplored but important consideration, making placental heterogeneity an important research topic in its own right [5]

The goals of this exploratory study are two-fold: (1) to classify stillbirth placentas into an ideally small number of relatively homogeneous subgroups (i.e., latent classes) based on pattern similarity in observed diagnostic criteria and (2) examine the associations of these latent classes with relevant maternal characteristics (i.e., covariates), especially maternal HIV. To achieve these goals, we explore placental heterogeneity in stillbirth and its relations to fetal and maternal exogenous characteristics using latent class cluster analysis (LCCA), a rigorous model-based statistical procedure that can be used to classify placentas into relatively homogenous subgroups based on similarities and differences in diagnostic criteria. We include exogenous maternal characteristics in the model as covariates that help explain variations in the latent classes, but do not affect the associations between the placental diagnostic criteria directly.

The research makes theoretical, empirical, and practical contributions. *Theoretically*, heterogeneity in patterns of concurrent placental lesions implies the varied presence/absence of possibly associated placental diagnostic criteria. Placental pathology in stillbirth research is at an early stage. Thus, identifying patterns in placental diagnostic criteria and relating these patterns to exogenous maternal characteristics can yield important information for theory development. *Empirically*, there remains an important need to understand the impact of HIV/AIDS on stillbirth. We collect data in South Africa, a country with one of the world's highest HIV infection

rates. The results reveal important new insights into the relations of HIV with other placental diagnostic criteria. *Practically*, many healthcare professionals struggle to explain stillbirth to grieving parents and other interested parties. The knowledge of associated patterns of diagnostic criteria in stillbirth can be very helpful in this regard.

## Methods

### Placentas

This was a retrospective assessment of placentas from stillbirths delivering at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a tertiary academic hospital in Gauteng Province South Africa for the period January 2016 - July 2018. Ethical clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee (HREC). Medical and institutional approval was granted by CMJAH. We retrieved data and slides from women who had a confirmed stillbirth and a gestational age of 28 weeks or more. All slides were reviewed by two pathologists (ZM and CW) and reclassisfied into the Amsterdam Consensus Classification System[5]. Live births and records with incomplete data that made it difficult to classify the pathology were excluded. Maternal data was extracted from the hospital records and clinical information regarding the fetus was cross-referenced from the attending clinician and hospital records, but this was minimal and was not included in the data analyzed.

### Data analysis

Placental heterogeneity and its relations to maternal characteristics was explored using latent class cluster analysis (LCCA). LCCA flexibly accommodates exploratory models with covariates and mixed mode data (i.e. nominal and ratio scaled data), as in the current research [6, 7]. Our LCCA model is a model in which the observed stillbirth placental variables, *viz*. weight placenta as a proportion of $10^{th}$ centile (Ratio10), chorioamnionitis maternal response (CAM,) chorioamnionitis foetal response CAF, foetal vascular malperfusion (FVM), maternal vascular malperfusion (MVM), villitis of unknown etiology (VUE), retroplacental hemorrhage (RPH), and delayed villous maturation (DVM), are assumed to be indicators of a single unobserved categorical latent variable that has an unknown number of relatively homogeneous, mutually-exclusive, and exhaustive latent classes. Local independence was assumed for the placental indicator variables conditional on class

membership and no local dependencies were allowed. Thus, a placenta is a member of one class and class membership explains the associations among the observed variables for that placenta. Ten exogenous stillbirth maternal characteristics (viz., maternal age (MA), gestational age (GA), parity (Para), gravidity (Grav), maternal HIV status (HIV), premature rupture membranes (PROM), diabetes mellitus (DM), antepartum hemorrhage (HAEM), and hypertension (HT) are included as covariates in the model. The covariates have direct effects on the latent variable but no direct effect on the endogenous placental diagnostic indicator variables, the associations of which are explained by the latent variable.

The LCCA models were fit to the raw dataset with no missing values in Latent Gold 5.1 [8]. Beginning with a 1-class model, the number of latent classes was increased incrementally to determine the best fitting model. Since latent class models can converge at suboptimal local maxima, the analysis began from 250 random starting values, which ran for 200 iterations. Of these, the best 10% were run for an additional two iterations and the best solution was selected as the starting point for the current analysis. All analyses converged normally without warning messages or boundary condition warnings. There was no missing data, and no local dependencies were allowed.

Comparative model fit was assessed using the bootstrap likelihood ratio tests (500 iterations). The Akaike Information Criterion (AIC), and the Bayesian Information Criterion (BIC) statistics, and bivariate residuals (BVRs) also were examined. Based on the results summarized in the Appendix Table A.1, the 5-class result was chosen. Of 84 BVR test results, only two exceeded the critical value (i.e., > 3.84), suggesting that the final model may not explain the association of Ratio10 with HT and VUE with PROM completely. *Class separation* was confirmed by significant Wald test results for all indicator variables across the five latent classes ($p \leq 0.006$ in all cases, see Table A.2 in the Appendix for details). Wald statistic tests of paired class comparisons also supported separation ($p \leq 0.05$ in 83% of indicator comparisons). Thus, the results show that the model distinguishes between classes well. Overall, these results suggest that the model fits these data well.

## Results

### Sample

The sample comprised 122 placentas. The mean maternal age was 28 years (range 18 - 41 years) and the average gestational age was 34 weeks (56-2370 g). The mean placental weight across 122 cases was 282.5 g (range 77-702 g). As most placentas were below the $10^{th}$ centile for gestational age, this was expressed as relative to the $10^{th}$ centile (Ratio 10). Maternal HIV status was positive in 32 (26.23 %) cases.

### Latent class cluster analysis

Detailed results and technical details for the 5-class LCCA model are reported in the Appendix. Figure 1 summarizes results for each latent class, which include the within-class probabilities for the placenta indicator variables and the associated maternal characteristic covariates. For criteria not measured on a binary scale (present/absent) scale (viz. Ratio10, MA, GA, Para, Grav), Figure 1 reports class-specific means re-scaled to lie within the 0-1 range[8]. Table 1 reports distinctive characteristics for each latent class, which were determined by examining the within-class probabilities and rescaled class-specific means. These were considered distinctive when near 1.0 or 0.0 (i.e., homogeneous for that class, which was operationalized as $\geq$ .70 or $\leq$ .30 [6]).

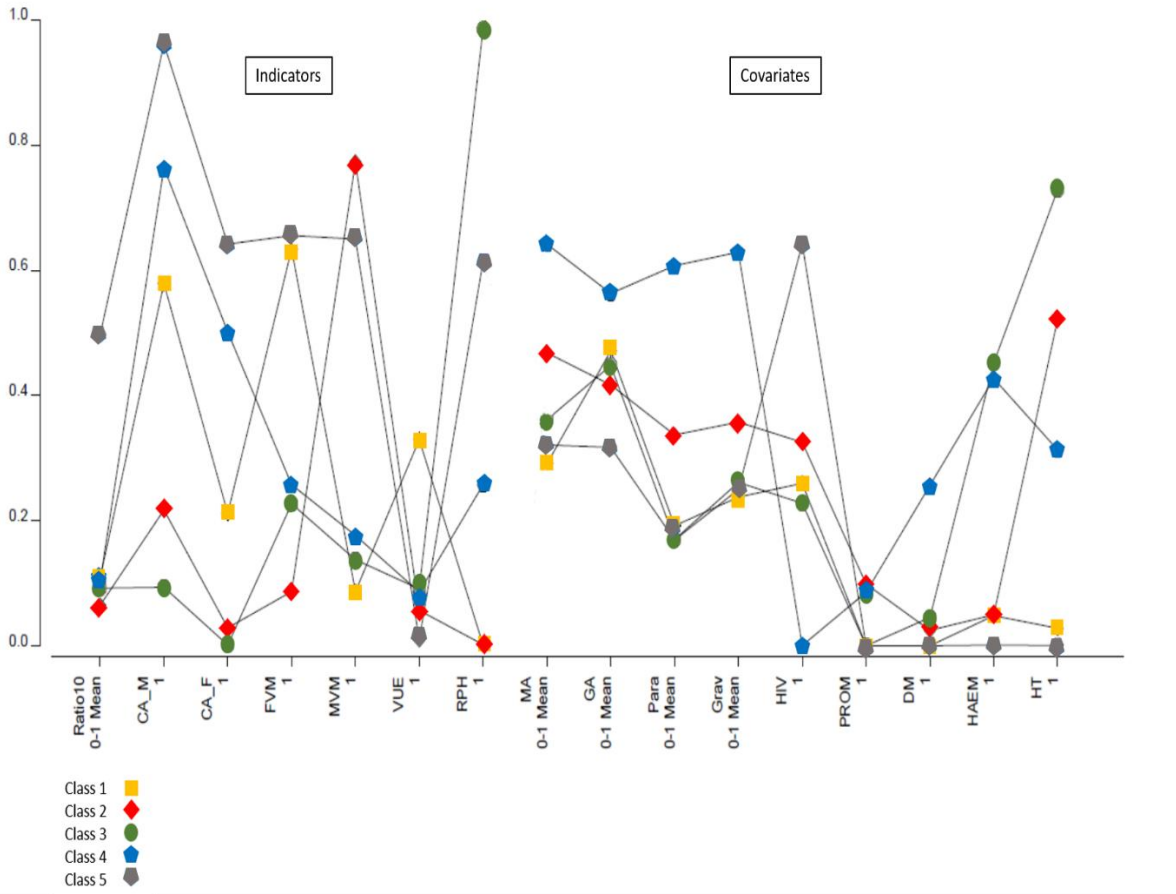


***Figure 1:*** *A latent class profile plot including the within-class probabilities on the Y-axis for the specific placental pathologies (viz. Ratio10, CA_M, CA_F, FVM, MVM, VUE, RPH, and DVM) as indicators and the associated maternal characteristic (viz., MA, GA, Para, Grav, HIV, PROM, DM, HAEM, and HT) as covariates on the X-axis. The latent classes will differ in size and within-class conditional probabilities concerning the placental characteristics.*

**Table 1:** The characteristics and size of each latent class based on the expression of the stillbirth placental criteria (viz. Ratio10, CA_M, CA_F, FVM, MVM, VUE, RPH, and DVM) as indicators of a single unobserved latent variable, with subpopulations or latent classes. Nine stillbirth maternal characteristics (viz., MA, GA, Para, Grav, HIV, PROM, DM, HAEM, and HT) are included in the model as covariates

| | Distinctive latent class characteristics | | | |
|---|---|---|---|---|
| Class size | Indicator variables | | Covariates | |
| | High | Low | High | Low |
| Class 1: FVM (35%) | *FVM* | *RPH,* RATIO10, CA_F, MVM | | *DM*, *HAEM*, *HT*, *PROM*, MA, GRAV, HIV |
| Class 2: MVM (34%) | MVM | *CA_F*, *RPH*, RATIO10, CA_M, FVM, VUE | | *DM*, *HAEM*, PROM |
| Class 3: RPH/HT (19%) | RPH | *CA_F*, RATIO10, CA_M, FVM, MVM, VUE | HT | PARA, GRAV, HIV, *PROM*, *DM* |
| Class 4: CA_M (10%) | CA_M | RATIO10, FVM, MVM, VUE, RPH | MA, *GA*, PARA, GRAV | *HIV*, PROM, DM |
| Class 5: HIV (3%) | *CA_F*, *FVM*, *MVM*, *RPH*, CA_M | *VUE* | *HIV* | *PROM*, *DM*, *HAEM*, *HT*, MA, GA, PARA, GRAV |

Note: See Table A.3 for detailed results. Abbreviations defined in text. High refers to within-class probabilities exceeding 0.70 except when in italics, which exceed 0.60. Low refers to within-class probabilities below 0.30 except when in bold, which indicate values below 0.05.

## Distinctive class characteristics

The analysis identified two large classes comprising 69% of the placentas. At least three placental characteristics were distinctive for each latent class. To make our summary of these results tractable, we refer to high/low within-class probabilities as “high” or ”low”

**Class 1: FVM (35%)**

This class is high in FVM and includes 67% of the placentas showing FVM in the sample. RPH was not observed. MVM and CA_F also are low. Placentas tend to be near the 10$^{th}$ centile. Associated covariates include very low DM, HAEM, HT, and PROM. MA (mean = 25.3) is the youngest of all classes and GRAV, and HIV also are low.

**Class 2: MVM (34%)**

Class 2 is high in MVM, including 74% of placentas showing MVM in the sample. RPH is absent and CAF very low. Placental weight is more likely to fall short of the 10$^{th}$ decile than any other class. CA_M, FVM, and VUE also are low. Associated covariates are very low DM and HAEM and low PROM. Mean MA is 29.7. Although the probability of HIV is 33%, it is noteworthy that the class includes 42% of HIV+ mothers (i.e., 1.24 times class size).

**Class 3: RPH (19%)**

Placentas in this class have a 99% probability of RPH. CA_F is very low and placenta weight, CA_M, FVM, MVM, and VUE are low. Class 3 includes 81% of placentas with RPH. Associated covariates are high HT, very low DM and PROM, and low PARA and GRAV. Although not distinctive characteristics, 52% show antepartum hemorrhage and 38% with hypertension. This triad traditionally is associated in the literature [9]

**Class 4: CA_M (10%)**

This smaller class is distinguished by high CA_M. Placental weight, FVM, MVM, VUE, RPH are low. The associated covariates include high GA and MA, PARA and GRAV also are high. No mothers were HIV+ and DM and PROM also are low.

**Class 5: HIV (3%)**

The incremental addition of this small class greatly reduced the level of unacceptable bivariate residuals and significantly improved model fit. CA_F, FVM, MVM, and RPH are very high. CA_M also is high. VUE is very low. However, this cluster appears to be distinguished most by the associated covariate of high HIV (66% probability). PROM, DM, HAEM, and HT are very low and MA, GA, PARA, GRAV are low.

## Discussion

The goals of this exploratory study were to classify stillbirth placentas into an ideally small number of relatively homogeneous latent classes based on similarity in patterns of observed diagnostic criteria and (2) examine the associations of these latent classes with relevant maternal characteristics (i.e., covariates), especially maternal HIV. The placental coding procedure was compatible with the Amsterdam Consensus system [5] and the latent class analyses in accordance with accepted best practice [6]. The 5-class LCCA distinguished among the endogenous effects of placental diagnostic criteria on placental heterogeneity and the effects of maternal covariates and provided a good fit to the data.

Our research potentially makes theoretical, practical, and empirical contributions. *Theoretically*, we identify the size and content of five placental classes in stillbirth. Three classes (MVM, FVM, and CA_M) bear similarity to the generalized patterns of placental injury identified by Redline et al.[5]. A notable difference in our results is the emergence of two classes distinguished by high placental RPH and maternal HIV, respectively, and the non-emergence of a class distinguished by VUE. The size and content of the latent classes and their similarity/dissimilarity to the more generalized patterns identified by Redline et al.[5] suggest potential new avenues for investigation and theory development concerning the role of the placenta in stillbirth.

*Empirically*, we collect data in an emerging African country with high HIV infection rates. The emergence of a distinct 5$^{th}$ class is a potentially important finding that may be replicated in countries with high HIV infection rates focusing on unanswered questions around the impact of HIV in stillbirth.

*Practically*, the identification of placental classes and their relations to exogenous maternal characteristics provides clinicians with new guidance that can help explain adverse outcomes and predict possible recurrences. In this vein, there is an increasing awareness of the role placental pathology can play in understanding stillbirth. In the SCRN study[4], a specific placental cause was identified as the leading cause of antepartum stillbirth (25%). Roescher et al.'s 2014 review of 135 papers examining placental lesions and perinatal mortality found that placental lesions cause stillbirths in 12–65% of cases[10].

More generally, we note that two-way communication with clinicians is pivotal. In practice, an accurate histopathological diagnosis may be greatly improved by clinical

information. Gestational age is essential and maternal comorbidities may contribute to an understanding of the pathological processes present in the placenta. Communication can be improved by a specific request form for placentas. Also, the macroscopic and microscopic examination of the placenta should be recorded on a standardized placental template. These communication tools help ensure the obstetric and maternal disease processes and the placental pathology diagnoses are seamlessly integrated and the reports are standardized[9]. This makes it easier for the clinician to communicate more effectively with parents.

We close with a recommendation for future research. There is a need for more research that implements structured approaches, such as in this study. The rigorous identification of patterns in placental lesions and their relations to maternal and foetal characteristics in stillbirth can be an important next step in expanding our understanding of the role of the placenta in stillbirth and our understanding of the impact of HIV.

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# CHAPTER FOUR – CONCLUSION AND RECOMMENDATIONS

We close with a recommendation for standardization and adaptation of a stillbirth classification system that can add value to the understanding of the pathophysiology of stillbirth. We also affirm the hypothesis that examination of placentas, render useful and great insight to the understanding of the aetiology of stillbirth. High quality investigations such as autopsy and genetic testing should also be considered, particularly in academic institutions.

There is a need for more research that implements structured approaches, such as in this study. The identification of patterns in placental lesions and their relations to maternal and foetal characteristics in stillbirth can be an important next step in expanding our understanding of the role of the placenta in stillbirth which has remained elusive for a long time. It will also offer valuable insight to the understanding and management of preventable diseases.

The definitions offered by placental classifications systems need to be expanded, and offer a clinical relevance in the causation of stillbirths. The better the clinicians understand the relevance of these classifications within their clinical context, the more placental submission criteria can be refined, which will bring down costs in the health sector (11).

Research is also needed to further refine guidelines regarding placental submission for histology. This will help design a cost-effective process of adressing the stillbirth burden.

There remains much work to be done in order to circulate the current information to the academic institutions so that everyone is well equipped with information that will help take us forward in our attempt to reduce the burden of stillbirth in our country.

Furthermore, this study has shown that the LCCA statistical procedure, can be used to classify heterogeneous populations into relatively homogenous subgroups.

# CHAPTER FIVE – APPENDICES

## APPENDIX A – RESEARCH PROTOCOL

### THE PLACENTAL RELATED FACTORS CONTRIBUTING TO STILLBIRTHS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

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**CONTENTS**

**THE CONTRIBUTION OF PLACENTAL PATHOLOGY TO THE UNDERSTANDING OF THE CAUSATION OF STILL BIRTH AT CHARLOTTE MAXEKE HOSPITAL**

## 1. BACKGROUND

According to Lawn *et al.*, (2014) approximately 5.5 million perinatal deaths occur world-wide annually. Most of the perinatal deaths can be averted (3). Perinatal death incorporates stillbirths as well as neonatal deaths. The perinatal period age of viability as defined by the World Health Organization (WHO) commences at 22 weeks of pregnancy for stillbirths and ends within the first seven days of life for early neonatal deaths (WHO, 2006). Internationally, stillbirth is defined as foetal death from 28 weeks' gestation onwards (Lawn *et al*.,2016). Neonatal deaths is defined as death within the first 28 days of life (19).

The aetiology of death is any disease, abnormality or injury with an harmful effect on life (45).

The placenta forms a functional unit between the mother and the foetus. The placenta is also an easily available specimen that has the potential to supply information on the causes of stillbirth, preterm delivery, intrauterine growth restriction (IUGR), and neurodevelopmental impairment. Placental pathology accounts for a significant number of stillbirths and therefore careful histopathological examination is essential in determining the aetiology of perinatal deaths.

Following the Amsterdam Placental Workshop meeting, a new classification of placental lesions was adopted (9). It was noted that classification of placental lesions has evolved from being a purely descriptive exercise of major pathophysiological processes such as disorders of maternal implantation and the amniotic fluid infection syndrome were first described, to a comprehensive classification system that includes all of the major maternal and foetal vascular and infectious and idiopathic/immune inflammatory processes (Redline *et al*., 2015). The implementation of this system with reproducible grading and staging should assist in constructing informative guidance for placental submission and accelerate headway studying the pathogenesis, diagnosis, and treatment of obstetric disorders with an underlying placental diseases (Redline *et al*., 2015).

Accurate interpretation of placental pathology is crucial as it informs the both the mother and the clinicians of how to implement preventative measures for the potential recurrent adverse foetal outcome/stillbirth. In addition, maternal conditions with the risk of recurrence can be identified leading to preventative measures during subsequent pregnancies.

According to Flenady *et al*. (2016) placental histopathology, autopsy, and genetic analysis was valuable in adressing the casues of stillbirths. The inability to provide autopsy denied parents an opportunity to ascertain the precise aetiology of their baby's death, perpetuated the numbers of inexplicable stillbirths and hindered the effectiveness of subsequent audits (15). Fundamental to the prevention of perinatal deaths, is precise comprehension of their causes of death within a given population.

A systematic review came to a conclusion that pathology of the placenta, cord, or membranes is attributed as a cause or contributory to stillbirth in 11% to 65% of cases in various classifications, depending on the classification used (9). The 2,9 million yearly neonatal deaths globally are traceable to three main causes: infections (0,6 million), intrapartum conditions (0,7 million), and preterm birth complications (1,0 million) (Lawn *et al*.,2014). Boys have a higher biological risk of neonatal death, however girls often have a higher social risk. Small size at birth due to preterm birth or small-for-gestational-age (SGA), or both, is the biggest risk factor for more than 80% of neonatal deaths and increases risk of post-neonatal mortality, growth failure, and adult-onset non-communicable diseases (3).
Placental lesions can be classified into "maternal malperfusion" or "foetal vascular abnormalities". Maternal malperfusion lesions include the maternal circulation, such as abnormal maternal vasculature, parenchymal infarcts or thrombus and intervillous/ perivillous lesions. Foetal vascular abnormalities reflect lesions on the foetal side of the placenta, including abnormal development or thrombus/infarct of the foetal vasculature within the placenta (46).

Despite the global burden of perinatal deaths, there are multiple, contrasting systems used to classify causes of perinatal deaths. However, none of them appear to be globally-acceptable. These inconsistencies hinder accurate estimates of causes of death and impede effective prevention strategies. World Health Organisation (WHO)

is developing a globally-acceptable classification approach for perinatal deaths (47). Recently the PASS Research network developed a classification system based on clinical information as well as placental pathology and autopsy in a high risk cohort of 12000 maternal foetal deaths which is user friendly, simple and allows for an undetermined category (Boyd *et al.*, 2017).

Peri-natal death classification systems aim to meet this need by allowing analysis of the factors leading to stillbirth within a population, but the use of multiple, incongruent systems hampers comparison of the underlying causes of perinatal death in different regions of the world (15).

Globally in 2012, complications from preterm birth (1·03 million, 36%), intrapartum-related conditions (previously called birth asphyxia; 0·66 million, 23%), and infections (notably sepsis, meningitis, and pneumonia; 0·66 million, 23%) were the main causes of neonatal deaths. Intrapartum-related conditions (27%) and preterm birth (41%) dominated in the early neonatal period, and infections (48%) were common in the late period (3).

In Roescher *et al* (2014) study the results of placental examinations were only reported back to the obstetrician instead of being communicated to the pediatrician. This was viewed as a missed opportunity. Information on placental lesions can often be helpful towards explaining an abnormal neonatal outcome and might have consequences for treatment (48).

## 2. AIMS

The aim of this study is to review 2 years of placental histology reports at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in order to determine the histological findings in stillbirths (JAN 2016 - JULY 2018) and the contribution of placental causes of stillbirths.

## 3. OBJECTIVES

a) To illustrate the value of placental pathology in determining the cause of stillbirths at Charlotte Maxeke Johannesburg Academic Hospital

b) To determine the most prevalent placental pathology associated with stillbirth at Charlotte Maxeke Johannesburg Academic Hospital

## 4. HYPOTHESIS

Though placental examination is expected to be done after delivery, examination of the placenta is not routinely done in South Africa. Causes of stillbirth are normally determined based on the history of clinical examination of the mother and the stillbirth at the time of delivery. A high percentatge of stilbirths are classified as having an unknown cause of death. We hypothesize that the causes of stillbirth are more conclusive with the inclusion of a placental histology examination. This study will enable us to classify leading causes of stillbirths at Charlotte Maxeke Johannesburg Academic Hospital with more accuracy.

## 5. METHODOLOGY

### a) Study design

This is a cross sectional retrospective study using placental histology reports to determine placental causes of stillbirths. The study will be conducted at Charlotte Maxeke Johannesburg Academic Hospital. All cases of placentas from stillbirths at Charlotte Maxeke Johannesburg Academic Hospital during the period Jan 2016 -July 2018 will be retrieved; followed by a review of clinical data, history and pathological diagnoses from pathology reports.

### b) Study location

Charlotte Maxeke Johannesburg Academic Hospital, National Health Laboratory Service (NHLS).

### c) Case collection

All placentas submitted to the Division of Anatomical Pathology from deliveries at Charlotte Maxeke Johannesburg Academic Hospital during the period Jan 2016 - July 2018 will be included in the study. The histopathology was performed and reported using a standardised reporting template (Table 1).

### d) Inclusion Criteria

All placentas with a clinical history of stillbirth

**e) Exclusion Criteria**

Live birth placentas

**f) Data extraction from pathology reports**

The reports will be reviewed by Dr Mtshali. If inadequate information is present, the case will be reviewed by Dr Mtshali and Prof Wright.

The following data will be extracted from pathology reports and tabulated (see Table 1 in Appendix):

a) Age of the patient
b) Patient's obstetric history
c) Patient's clinical history
d) Gestational age
e) Pathological diagnosis

From the extracted data, the most prevalent pathology associated with stillbirth at Charlotte Maxeke Johannesburg Academic Hospital will be determined and possible contributing factors will be recorded.

## 6. ETHICAL CONSIDERATIONS

Permission to conduct the study will be obtained from Wits Human Ethics Research Committee. Permission will be sought from NHLS and CEO of CMJAH. Patient confidentiality will be maintained at all times. No identifying information will be included on the datasheet. Patients will be identified by a study number. This data will be kept in a password protected computer to which only the PI has access.

## 7. BUDGET

There will be no funds required to conduct this research.

## 8. DATA ANALYSIS

The collected data will be compiled via a SNOMED (Systematised Nomenclature of Medicine) system and descriptive statistics will be utilised to describe the data using STATA 14**.** Continuous variables (e.g.: age and placental weight) will be summarised as means and categorical variables (e.g. infarction, infection) will be summarised as proportions. Descriptive analysis will be done using a T-test for continuous variables and Pearson chi-square test for categorical variables.

## 9. TIMELINES

| | Aug 2018 | Sept 2018 | Oct 2018 | Nov 2018 | Dec 2018 | Jan 2019 | Feb 2019 | Mar 2019 | Apr 2019 | May 2019 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Protocol presentation** | | | | | | | | | | |
| **Ethics submission** | | | | | | | | | | |
| **Data collection** | | | | | | | | | | |
| **Data analysis** | | | | | | | | | | |
| **Report write up and submission** | | | | | | | | | | |

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## 11. APPENDIX

### Table 1: Data collection tool

| Case Identifier | Age | Obstetric history | | | | Clinical History | | Gestational age (weeks) | Pathology diagnosis |
|---|---|---|---|---|---|---|---|---|---|
| | | Primi | Para | Gravida | Miscarriage | Intrauterine death (IUD) | Stillbirth | | |
| | | | | | | | | | |
| | | | | | | | | | |

## APPENDIX B - FIGURE 1: AMSTERDAM PLACENTAL WORKSHOP GROUP'S CONSENSUS

**Placental classification (incorporating the 2014 Amsterdam Placental Workshop Group criteria)**

1. Placental vascular processes
   - a. Maternal stromal-vascular lesions
     - Developmental
       - Superficial implantation/decidual arteriopathy
       - Increased immature extravillous trophoblast
     - Malperfusion
       - Global/partial
         - Early: distal villous hypoplasia
         - Late: accelerated villous maturation
       - Segmental/complete
         - Villous infarct(s)
     - Loss of integrity
       - Abruptio placenta (arterial)
       - Marginal abruption (venous)
         - Acute
         - Chronic
   - b. Fetal stromal-vascular lesions
     - Developmental
       - Villous capillary lesions
       - Delayed villous maturation (maturation defect)
       - Dysmorphic villi
     - Malperfusion
       - Global/partial
         - Obstructive lesions of umbilical cord
         - Recent intramural fibrin in large fetoplacental vessels
         - Small foci of avascular or karyorhectic villi
       - Segmental/complete
         - Chorionic plate or stem villous thrombi
         - Large foci of avascular or karyorhectic villi
     - Loss of integrity
       - Large vessel rupture (fetal hemorrhage)
       - Small vessel rupture (fetomaternal hemorrhage)
       - Villous edema
2. Placental inflammatory-immune processes
   - a. Infectious inflammatory lesions
     - Acute
       - Maternal inflammatory response: chorioamnionitis, subchorionitis
       - Fetal inflammatory response: chorionic/umbilical vasculitis
     - Chronic
       - Villitis (CMV, others)
       - Intervillositis (malaria, others)
   - b. Immune/idiopathic inflammatory lesions
     - Villitis of unknown etiology and related/associated lesions
       - Chronic villitis
       - Chronic chorioamnionitis
       - Lymphoplasmacytic deciduitis
       - Eosinophil T-cell fetal vasculitis
     - Chronic histiocytic intervillositis
3. Other placental processes
   - Massive perivillous fibrin(oid) deposition (maternal floor infarction)
   - Abnormal placental shape or umbilical insertion site
   - Morbidly adherent placentas (accreta)
   - Meconium-associated changes
   - Increased circulating nucleated red blood cells

# APPENDIX C - FIGURE 2: PASS CLASSIFICATION

**PASS STILLBIRTH CLASSIFICATION**

**Instructions:**
**I. Choose the cause of death**
**II. Choose a predisposing or coincident condition if applicable**
**III. Indicate whether potentially recurrent (R)**
**IV. Indicate data available**

**I and II (Use classification below; subcategorize to the extent known)**
**I. CAUSE OF DEATH**
Numeral. __
Capital letter. __
Numeral) __
Lowercase letter. __
Lowercase Roman numeral) __

**II. PREDISPOSING/COINCIDENT CONDITION**
Numeral. __
Capital letter. __
Numeral) __
Lowercase letter. __
Lowercase Roman numeral) __

1. FETAL
A. Intrinsic fetal disorder
    1) Lethal congenital malformation(s) (R)
    2) Lethal congenital tumor
    3) Lethal chromosomal or genetic disorder without malformations (R)
    4) Significant fetal growth restriction in the absence of a recognized cause (R)
    5) Other
B. Infection
    1) Hematogenous
        a. Bacterial
        b. Viral
        c. Protozoal
        d. Spirochetal
    2) Ascending
        a. Bacterial
            i) Cervical incompetence (R)
        b. Fungal
    C. Anemia
        1) Ruptured vasa previa / velamentous vessel
        2) Fetal-maternal hemorrhage
        3) Twin-twin transfusion
        4) Alloimmunization (R)
        5) Non-immune hydrops (R)
        6) Other

    2. PLACENTAL
    A. Umbilical cord
        1) Acute fatal flow restriction
            a. Cord prolapse
            b. Cord rupture
            c. Cord hematoma
            d. Other

2) Non-acute fatal flow restriction
    a. Nuchal or body-wrapped cord
    b. True knot
    c. Velamentous / marginal cord
    d. Hypercoiling
    e. Torsion
    f. Oligohydramnios
    g. Unknown cause

B. Placental perfusion failure
1) Acute
    a. Abruptio
        i) Pre-eclampsia / eclampsia (R)
        ii) Chronic hypertension (R)
        iii) Autoimmunity (R)
        iv) IDDM (R)
        v) Uterine anomaly (R)
        vi) Unknown cause , not traumatic (R)

2) Non-acute
    a. Intervillous/villous interface
        i) Chronic histiocytic intervillositis (R)
        ii) Maternal floor infarction / Perivillous fibrin deposition (R)
        iii) Chronic villitis (R)
        iv) Other
    b. Uteroplacental malperfusion
        i) Pre-eclampsia / eclampsia (R)
        ii) Chronic hypertension (R)
        iii) Autoimmunity (R)
        iv) IDDM (R)
        v) Uterine anomaly (R)
        vi) Unknown cause (R)
    c. Chronic abruption/oligohydramnios sequence

3. MATERNAL
A. Systemic disease, not listed under Placental Perfusion Failure
1) Cardiovascular (R)
2) Pulmonary (R)
3) Gastrointestinal (R)
4) Hepatic (R)
5) Endocrine (R)
6) Renal (R)
7) Hematologic (R)
8) Neoplastic (R)
9) Other (R)

B. Acute vascular derangement
1) Maternal hypotension (e.g. shock, DIC)
2) Maternal vasoconstriction (e.g. hyperstimulation)
3) Uterine rupture (R)
4) Other

C. Structural anomalies
1) Cervical incompetence
2) Other

4. EXTERNAL
A. External toxic/metabolic
    1) Environmental exposure (R)
    2) Maternal intake (R)
    3) Other
B. Mechanical forces
    1) Iatrogenic trauma
        a) Termination of pregnancy
            i) Fetal indications
            ii) Maternal indications
        b) Other
    2) Non-iatrogenic trauma

5. UNDETERMINED
A. No cause identified
B. No direct cause identified; associated predisposing condition(s) present
C. Insufficient information available

**III. RECURRENCE RISK**
(1=yes, 0=no, X=unknown)
R __

**IV. DATA AVAILABLE**
(1=yes, 0=no, X=unknown)
C=Clinical history
P=Placenta
A=Autopsy
C __P __A __

## APPENDIX D- TABLE 1: PREVALENT HISTOPATHOLOGICAL FINDINGS AT CMJAH

Table 1 summarises the histopathological findings at Charlotte Maxeke Johannesburg Academic Hospital according to Amsterdam Classification System. The most common findings were chorioamnionitis (foetal response) (n=61; 50%), chorioamnionitis (maternal response) (n=49; 40.16%), FVM (n=42; 34.43%), MVM (n=42; 34.43%), RPH (n=27; 22.13%), APH (n=19; 15.83%) and Intrauterine compromise (n=18; 14.75%). The least common histopathological findings were DVM (n=6; 4.92%), MFI/MPFD (n=5; 4.10%) and MAVN (n=4; 3.28%).

**Table 1: Prevalent histopathological findings at Charlotte Maxeke Johannesburg Academic Hospital according to Amsterdam Classification System**

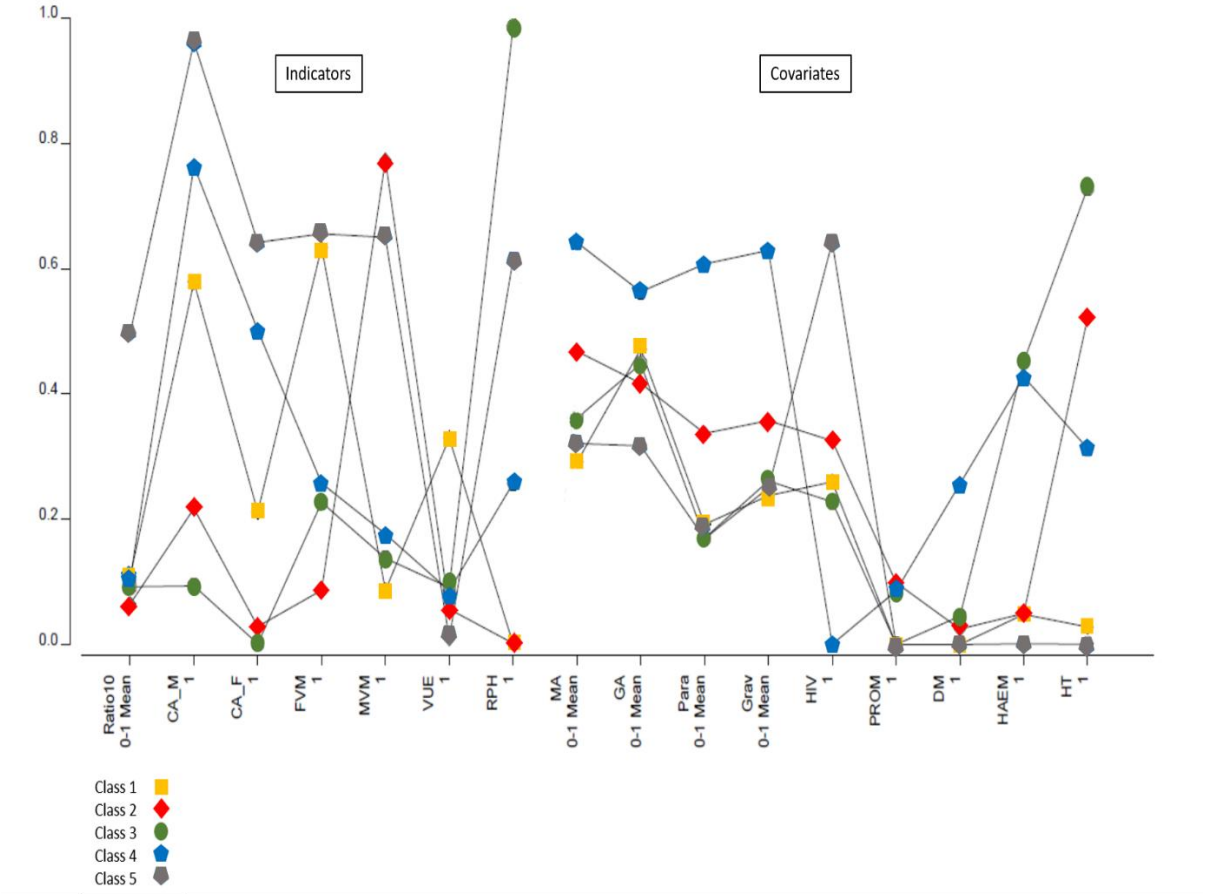
| **Histopathological finding** | **N (%)** |
|---|---|
| APH (anti-partum haemorrhage) | 19 (15.83) |
| Chorioamnionitis (maternal response) | 49 (40.16) |
| Chorioamnionitis (foetal response) | 61 (50) |
| FVM (foetal vascular malperfusion) | 42 (34.43) |
| MVM (maternal vascular malperfusion) | 42 (34.43) |
| VUE (villitis of unknown etiology) | 19 (15.57) |
| RPH (Retroplacental haemorrhage) | 27(22.13) |
| DVM (Delayed Villous Maturation) | 6 (4.92) |
| MFI/MPFD (Maternal floor infarction/ Massive perivillous fibrin deposit) | 5 (4.10) |
| MAVN (Meconium-associated vascular necrosis) | 4 (3.28) |
| Intrauterine compromise | 18 (14.75) |

## APPENDIX E - TABLE 2 DISTINCTIVE LATENT CLASS CHARACTERISTICS

| Class size | Distinctive latent class characteristics | | | |
|---|---|---|---|---|
| | Indicator variables | | Covariates | |
| | High | Low | High | Low |
| Class 1: FVM (35%) | *FVM* | *RPH,* RATIO10, CA_F, MVM | | *DM*, *HAEM*, *HT*, *PROM*, MA, GRAV, HIV |
| Class 2: MVM (34%) | MVM | *CA_F*, *RPH*, RATIO10, CA_M, FVM, VUE | | *DM*, *HAEM*, PROM |
| Class 3: RPH/HT (19%) | RPH | *CA_F*, RATIO10, CA_M, FVM, MVM, VUE | HT | PARA, GRAV, HIV, *PROM*, *DM* |
| Class 4: CA_M (10%) | CA_M | RATIO10, FVM, MVM, VUE, RPH | MA, *GA*, PARA, GRAV | *HIV*, PROM, DM |
| Class 5: HIV (3%) | *CA_F*, *FVM*, *MVM*, *RPH*, CA_M | *VUE* | *HIV* | *PROM*, *DM*, *HAEM*, *HT*, MA, GA, PARA, GRAV |

High refers to within-class probabilities exceeding 0.70 except when in italics, which exceed 0.60. Low refers to within-class probabilities below 0.30 except when in bold, which indicate values below 0.05.

# APPENDIX F - GRAPH 1: LATENT CLASS PROFILE PLOT



## APPENDIX G – TABLE 3: TECHNICAL DETAILS ANALYSIS

LCCA models incorporating covariates and mixed mode data model were fit to the raw data in Latent Gold 2.1, increasing the number of latent classes incrementally to determine the best fit. There were no missing data. For each incremental increase, the analysis began from 250 random starting values, which ran for 200 iterations. Of these, the best 10% were run for an additional two iterations and the final model chosen. The final model converged normally after nine iterations. There were no warning messages or other indications of boundary conditions.

### Model fit

Model fit was assessed by examining the results of likelihood bootstrap estimates of incremental model fit (500 iterations), the Bayesian Information Criterion (BIC), and Akaike Information Criterion (AIC). The prevalence of bivariate residuals exceeding the critical value (i.e., > 3.84) also was examined. Based on the results summarized in Table 2**,** the 5-class result was chosen.

### TABLE 3: MODEL FIT INDICES

| Model | LL | BIC(LL) | AIC(LL) | Npar | Class.Err. | Δ -2LL | Bootstrap p-value | Entropy $R^2$ | Standard $R^2$ | Bivariate residuals |
|---|---|---|---|---|---|---|---|---|---|---|
| 1-Class | -538.48 | 1115.18 | 1092.95 | 8 | 0 | | | 1.00 | 1.00 | 10% |
| 2-Class | -451.94 | 1028.14 | 955.89 | 26 | 0.0006 | 173.06 | 0.000 | 0.96 | 0.97 | 10% |
| 3-Class | -417.15 | 1044.59 | 922.31 | 44 | 0.0055 | 69.58 | 0.006 | 0.96 | 0.97 | 9% |
| 4-Class | -385.24 | 1066.78 | 894.47 | 62 | 0.0342 | 63.83 | 0.002 | 0.90 | 0.91 | 4% |
| **5-Class** | **-362.57** | **1107.47** | **885.14** | **80** | **0.0285** | **45.33** | **0.032** | **0.93** | **0.94** | **2%** |
| 6-Class | -337.19 | 1142.74 | **870.38** | 98 | 0.0166 | 50.756 | 0.000 | 0.97 | 0.97 | 3% |

Note: Reported are Entropy $R^2$ and Standard $R^2$ for the measurement model. Δ -2LL reports results of likelihood bootstrap estimates of incremental model improvement (see Vermunt & Magidson, 2016).

Another important indicator of model fit was the separation of classes, which would be indicated by at least one class differing across classes. We assessed separation by testing the difference of model parameters across classes using the Wald est. The classes differ in respect of every indicator variables, according to the Wald tests ($p \leq .01$).

The results for five covariates (i.e., HIV, PROM, DM, HAEM, and HT) also were significant at the same level, with PARA significant at $p \leq .10$.

## APPENDIX H - TABLE 4: PARAMETER ESTIMATES

| Indicators | | Class1 | Class2 | Class3 | Class4 | Class5 | Wald | p-value | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| **Ratio10** | | | | | | | | | |
| | | -0.52 | -0.87 | -0.65 | -0.56 | 2.59 | 18.29 | 0.001 | 0.505 |
| **CA_M** | | | | | | | | | |
| | **0** | -0.04 | 0.76 | 1.25 | -0.47 | -1.49 | 30.71 | 0.000 | 0.258 |
| | **1** | 0.04 | -0.76 | -1.25 | 0.47 | 1.49 | | | |
| **CA_F** | | | | | | | | | |
| | **0** | -0.43 | 0.67 | 2.22 | -1.09 | -1.38 | 69.34 | 0.000 | 0.229 |
| | **1** | 0.43 | -0.67 | -2.22 | 1.09 | 1.38 | | | |
| **FVM** | | | | | | | | | |
| | **0** | -0.62 | 0.83 | 0.27 | 0.19 | -0.67 | 22.34 | 0.000 | 0.258 |
| | **1** | 0.62 | -0.83 | -0.27 | -0.19 | 0.67 | | | |
| **MVM** | | | | | | | | | |
| | **0** | 0.79 | -1.00 | 0.53 | 0.38 | -0.70 | 16.50 | 0.002 | 0.431 |
| | **1** | -0.79 | 1.00 | -0.53 | -0.38 | 0.70 | | | |
| **VUE** | | | | | | | | | |
| | **0** | -0.93 | 0.13 | -0.13 | -0.10 | 1.03 | 14.51 | 0.006 | 0.117 |
| | **1** | **0.93** | -0.13 | 0.13 | 0.10 | -1.03 | | | |
| **RPH** | | | | | | | | | |
| | **0** | 2.40 | 2.18 | -3.03 | -0.39 | -1.16 | 210.52 | 0.000 | 0.833 |
| | **1** | -2.40 | -2.18 | 3.03 | 0.39 | 1.16 | | | |

| Intercepts | | Overall | Wald | p-value |
|---|---|---|---|---|
| **Ratio10** | | | | |
| | | 1.56 | 22.11 | 0.000 |
| **CA_M** | | | | |
| | **0** | -0.11 | 0.30 | 0.580 |
| | **1** | 0.11 | | |
| **CA_F** | | | | |
| | **0** | 1.09 | 31.59 | 0.000 |
| | **1** | -1.09 | | |
| **FVM** | | | | |
| | **0** | 0.34 | 4.14 | 0.042 |
| | **1** | -0.34 | | |
| **MVM** | | | | |
| | **0** | 0.39 | 5.11 | 0.024 |
| | **1** | -0.39 | | |
| **VUE** | | | | |
| | **0** | 1.28 | 35.92 | 0.000 |
| | **1** | -1.28 | | |
| **RPH** | | | | |
| | **0** | 0.92 | 27.38 | 0.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | -0.92 | | | | | | |

| **Error Variances** | **Class1** | **Class2** | **Class3** | **Class4** | **Class5** | | |
|---|---|---|---|---|---|---|---|
| **Ratio10** | 0.14 | 0.10 | 0.08 | 0.04 | 7.59 | | |

| **Intercept** | **Class1** | **Class2** | **Class3** | **Class4** | **Class5** | **Wald** | **p-value** |
|---|---|---|---|---|---|---|---|
| | -1.34 | 6.22 | 4.90 | -12.11 | 2.32 | 5.57 | 0.230 |

| **Covariates** | **Class1** | **Class2** | **Class3** | **Class4** | **Class5** | **Wald** | **p-value** |
|---|---|---|---|---|---|---|---|
| **MA** | | | | | | | |
| | -0.17 | -0.08 | -0.13 | 0.50 | -0.11 | 3.01 | 0.560 |
| **GA** | | | | | | | |
| | 0.04 | -0.01 | -0.03 | 0.13 | -0.13 | 1.21 | 0.880 |
| **Para** | | | | | | | |
| | -1.21 | -0.14 | -2.91 | 6.13 | -1.87 | 8.53 | 0.074 |
| **Grav** | | | | | | | |
| | 0.77 | 0.36 | 2.09 | -4.20 | 0.98 | 2.56 | 0.630 |
| **HIV** | | | | | | | |
| **0** | -0.80 | -1.05 | -0.76 | 4.23 | -1.62 | 23.27 | 0.000 |
| **1** | 0.80 | 1.05 | 0.76 | -4.23 | 1.62 | | |
| **PROM** | | | | | | | |
| **0** | 2.74 | -0.78 | 1.08 | -4.62 | 1.57 | 18.64 | 0.001 |
| **1** | -2.74 | 0.78 | -1.08 | 4.62 | -1.57 | | |
| **DM** | | | | | | | |
| **0** | 3.47 | 0.00 | 0.70 | -6.07 | 1.90 | 48.28 | 0.000 |
| **1** | -3.47 | 0.00 | -0.70 | 6.07 | -1.90 | | |
| **HAEM** | | | | | | | |
| **0** | 1.46 | 0.99 | -0.85 | -4.14 | 2.53 | 47.52 | 0.000 |
| **1** | -1.46 | -0.99 | 0.85 | 4.14 | -2.53 | | |
| **HT** | | | | | | | |
| **0** | 1.36 | -0.63 | -1.58 | -0.73 | 1.58 | 35.86 | 0.000 |
| **1** | -1.36 | 0.63 | 1.58 | 0.73 | -1.58 | | |

## APPENDIX I - TABLE 5: PROFILES

### Profile

| | Class1 | Class2 | Class3 | Class4 | Class5 | Overall |
|---|---|---|---|---|---|---|
| **Cluster Size** | 0.35 | 0.34 | 0.19 | 0.10 | 0.03 | |
| **Indicators** | | | | | | |
| **Ratio10** | | | | | | |
| **Mean** | 1.05 | **0.70** | 0.91 | 1.01 | **4.15** | 0.99 |
| **CA_M** | | | | | | |
| **Absent** | 0.42 | **0.78** | **0.91** | 0.24 | 0.04 | 0.61 |
| **Present** | 0.58 | 0.22 | 0.09 | **0.76** | **0.96** | 0.40 |
| **CA_F** | | | | | | |
| **Absent** | **0.79** | **0.97** | **1.00** | 0.50 | 0.36 | **0.85** |
| **Present** | 0.21 | 0.03 | 0.00 | 0.50 | 0.64 | 0.15 |
| **FVM** | | | | | | |
| **Absent** | 0.37 | **0.91** | **0.77** | **0.74** | 0.35 | 0.66 |
| **Present** | 0.63 | 0.09 | 0.23 | 0.26 | 0.66 | 0.34 |
| **MVM** | | | | | | |
| **Absent** | **0.91** | 0.23 | **0.86** | **0.82** | 0.35 | 0.65 |
| **Present** | 0.09 | **0.77** | 0.14 | 0.18 | 0.65 | 0.35 |
| **VUE** | | | | | | |
| **Absent** | 0.67 | **0.94** | **0.91** | **0.91** | **0.99** | **0.84** |
| **Present** | 0.33 | 0.06 | 0.09 | 0.09 | 0.01 | 0.16 |
| **RPH** | | | | | | |
| **Absent** | **1.00** | **1.00** | 0.01 | **0.74** | 0.39 | **0.77** |
| **Present** | 0.00 | 0.00 | **0.99** | 0.26 | 0.61 | 0.23 |
| **Covariates** | | | | | | |
| **MA** | | | | | | |
| **5-Jan** | 0.34 | 0.14 | 0.18 | 0.00 | 0.32 | 0.21 |
| **8-Jun** | 0.26 | 0.08 | 0.27 | 0.09 | 0.32 | 0.19 |
| **11-Sep** | 0.14 | 0.25 | 0.23 | 0.08 | 0.03 | 0.18 |
| **16-Dec** | 0.22 | 0.22 | 0.19 | 0.34 | 0.32 | 0.23 |
| **17 - 25** | 0.05 | 0.30 | 0.14 | 0.49 | 0.00 | 0.19 |
| **Mean** | 25.27 | 29.70 | 27.02 | 34.04 | 26.02 | 27.99 |
| **GA** | | | | | | |
| **3-Jan** | 0.16 | 0.23 | 0.27 | 0.09 | 0.33 | 0.20 |
| **6-Apr** | 0.21 | 0.26 | 0.23 | 0.09 | 0.32 | 0.22 |
| **8-Jul** | 0.22 | 0.14 | 0.05 | 0.34 | 0.00 | 0.17 |
| **11-Sep** | 0.23 | 0.26 | 0.23 | 0.31 | 0.35 | 0.25 |
| **15-Dec** | 0.18 | 0.11 | 0.23 | 0.17 | 0.00 | 0.16 |
| **Mean** | 34.60 | 33.83 | 34.27 | 35.88 | 32.44 | 34.34 |
| **Para** | | | | | | |
| **0** | 0.47 | 0.20 | 0.50 | 0.00 | 0.65 | 0.34 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | 0.31 | 0.37 | 0.32 | 0.17 | 0.03 | 0.31 |
| | **2** | 0.19 | 0.33 | 0.18 | 0.40 | 0.32 | 0.26 |
| | **3** | 0.02 | 0.07 | 0.00 | 0.26 | 0.00 | 0.06 |
| | **4** | 0.00 | 0.02 | 0.00 | 0.17 | 0.00 | 0.03 |
| **Mean** | | **0.77** | 1.35 | 0.68 | **2.43** | **0.68** | 1.11 |
| **Grav** | | | | | | | |
| | **1** | 0.38 | 0.17 | 0.37 | 0.00 | 0.64 | 0.28 |
| | **2** | 0.38 | 0.40 | 0.23 | 0.17 | 0.03 | 0.33 |
| | **3** | 0.14 | 0.31 | 0.41 | 0.31 | 0.00 | 0.26 |
| | **4** | 0.10 | 0.07 | 0.00 | 0.34 | 0.32 | 0.10 |
| | **5** | 0.00 | 0.05 | 0.00 | 0.17 | 0.00 | 0.03 |
| **Mean** | | 1.95 | 2.43 | 2.04 | **3.51** | 2.00 | 2.29 |
| **HIV** | | | | | | | |
| | **Absent** | **0.74** | 0.67 | **0.77** | **1.00** | 0.36 | **0.74** |
| | **Present** | 0.26 | 0.33 | 0.23 | 0.00 | 0.64 | 0.26 |
| **PROM** | | | | | | | |
| | **Absent** | **1.00** | **0.90** | **1.00** | **0.91** | **1.00** | **0.96** |
| | **Present** | 0.00 | 0.10 | 0.00 | 0.09 | 0.00 | 0.04 |
| **DM** | | | | | | | |
| | **Absent** | **1.00** | **0.98** | **0.95** | **0.74** | **1.00** | **0.96** |
| | **Present** | 0.00 | 0.02 | 0.05 | 0.26 | 0.00 | 0.04 |
| **HAEM** | | | | | | | |
| | **Absent** | **0.95** | **0.95** | 0.55 | 0.57 | **1.00** | **0.84** |
| | **Present** | 0.05 | 0.05 | 0.45 | 0.43 | 0.00 | 0.16 |
| **HT** | | | | | | | |
| | **Absent** | **0.97** | 0.48 | 0.27 | 0.69 | **1.00** | 0.65 |
| | **Present** | 0.03 | 0.52 | **0.73** | 0.31 | 0.00 | 0.35 |

# APPENDIX J - ETHICS CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Nompumelelo Mtshali

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

## CLEARANCE CERTIFICATE NO. M180909

| | |
|---|---|
| **NAME: (Principal Investigator)** | Dr Nompumelelo Mtshali |
| **DEPARTMENT:** | Anathomical Pathology<br>National Health Laboratory Service<br>Charlotte Maxeke Johannesburg Academic Hospital |
| **PROJECT TITLE:** | Placental related factors contributing to stillbirths at Charlotte Maxeke Johanneburg Academic Hospital (CMJAH) |
| **DATE CONSIDERED:** | 28/09/2018 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Prof Colleen Wright and Dr Salome Maswime |
| **APPROVED BY:** | Dr C Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 05/10/2018 |

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**