GROSS PRESENTATION AND HISTOMORPHOLOGICAL CHANGES OF PLACENTAE IN PATIENTS PRESENTING WITH INTRAUTERINE FOETAL DEATH AT KENYATTA NATIONAL HOSPITAL

RESEARCH DISSERTATION SUBMITTED AS PARTIAL FULFILMENT FOR MMED IN OBSTETRICS AND GYNAECOLOGY, UNIVERSITY OF NAIROBI

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DECLARATION

This dissertation is my original work and has not been presented elsewhere. References to work done by others have been clearly indicated

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LIST OF ABBREVIATIONS/ACRONYMS

ANC-antenatal clinic

APH-antepartum haemorrhage

Hb-haemoglobin

HIV-Human Immunodeficiency Syndrome

IUFD-intrauterine foetal death

IUGR-intrauterine growth retardation

KNH-Kenyatta National Hospital

MDGs -Millennium Development Goals

PROM-premature rupture of membranes

SLE-Systemic lupus erythematosus

SPSS- Statistical Package for Social Scientists.

TTTS-twin to twin transfusion syndrome

VDRL-Venereal Disease Research Laboratory

WHO-World Health Organization

DEDICATION

This book is dedicated to my wife Redempter Mwende, my late father Nashon Owino and my mother Angeline Owino. Your love and support has been my strength and I thank you most dearly.

ACKNOWLEDGEMENT

Special thanks go to my supervisors Dr Onesmus Gachuno and Dr Harrison Tamooh for the work they put in to make this research possible. I am also grateful to Dr Emily Rogena for reporting the histology slides.

My gratitude goes to my research assistants Jebet, Dome and Mwaniki for the excellent work they did. I would also like to give thanks to Willis Ochuk for processing the placenta specimen. I am also grateful to my statistician Francis Njiri for working tirelessly to analyse the data collected.

Finally, I would like to thank all my lecturers and colleagues at the University of Nairobi and Kenyatta National Hospital for the support, guidance and encouragement.

ABSTRACT

Background: A stillbirth or intrauterine foetal death (IUFD) is defined as a product of conception weighing ≥500g or with gestational age >22 weeks without evidence of life at birth but other authors regard the lower gestational age as 20 weeks. There are 3.2 million annual stillbirths, at least 98% occur in low-/middle-income countries. Stillbirth rates are below 5 per 1000 in developed countries but approximately 32 per 1000 in developing countries. The rate of abnormal placental pathology in stillbirths range from 20% to 40%. The most useful tests used in the diagnosis of the cause of IUFD are histopathological examination of the placenta, umbilical cord and the foetus. Despite placenta being a source of information as to the cause of death, pathological examination of the placenta is only performed in 25% of stillbirths in developing countries.

Objective: To determine gross presentation and histomorphological changes of placentae in patients presenting with intrauterine foetal death as compared to live births.

Study design: This was a case control study.

Methodology: Clients presenting with IUFD at gestation of 28 weeks and above were recruited after confirmation with ultrasound results. The stillbirth was weighed and examined after delivery then noted whether it was macerated or fresh. The placenta was weighed and then examined grossly together with the umbilical cord and membranes and then immediately fixed in 10% formalin and submitted for histopathology examination. Clients who delivered live births comprised the controls and were matched for age.

Setting: The study was conducted at the Kenyatta National Hospital's labour ward. Kenyatta National Hospital is Kenya's largest referral hospital, located in the capital city Nairobi.

Data: The data was collected using a structured questionnaire. It was then entered into a password protected Ms Access database. Data analysis was performed using Statistical Package for Social Scientists (SPSS Version 17.0).

Measures of outcome: The morphological patterns of placentae from mothers who presented with IUFD were compared to those of mothers who delivered live births.

Results: There was more pathology in the placenta from stillbirths compared to the live births. Reduction of the mass of functioning villi was present in 11.8% of placenta in the stillbirth group compared to 2% in the live birth group (p value-0.002). 16.7% of placenta in the stillbirth group had haematomas and/or thrombi compared to 7.8% in the live birth group (p value-0.017). There was significant presence of other placental abnormalities in the stillbirth group (22.5%) compared to the live birth group (9.8%) (p value-0.002). However, there were no significant differences between the 2 groups involving abnormalities of fetal stem arteries (p value-0.558).

Conclusion: Histological examination of placenta may help in identifying some causes of stillbirths. This knowledge may lead to preventive measures which would lower perinatal mortality.

CHAPTER 1: LITERATURE REVIEW, JUSTIFICATION AND OBJECTIVES

1.1 LITERATURE REVIEW

Burden of stillbirths

A stillbirth or intrauterine foetal death (IUFD) is defined as the complete expulsion from its mother, after at least 22 weeks' of pregnancy (or weighing more than 500 grams if the gestation period is unknown), of a product of conception in which, after such expulsion or extraction, there is no breathing, beating of the heart, pulsation of the umbilical cord or unmistakable movement of voluntary muscle. Stillbirths can be divided into early and late stillbirths. Early stillbirths occur before 28 weeks gestation while late stillbirths occur at or later than 28 weeks gestation [1,2]. Stillbirths can also be classified as those that occur prior to the onset of labour (antepartum stillbirths), and those that occur during labour (intrapartum stillbirths) [3].

Of the world's 3.2 million annual stillbirths, at least 98% occur in low-/middle-income countries, and on average, as many as two-thirds of these stillbirths are thought to occur antenatally, prior to labour. Proportions of antenatal and intrapartum stillbirths may vary in different low- and middle-income country settings depending on the prevalence of risk factors and quality of antenatal and obstetric care [4,5].

While the highest absolute numbers of stillbirths occur in South Asia, driven by the large population size of that region, the incidence rates are highest in sub-Saharan Africa. Wide variations exist: in high-income countries, stillbirth rates are below 5 per 1000 births, compared to approximately 32 per 1000 in South Asia and sub-Saharan Africa [6]. The prevalence of stillbirth at the community level is typically less than 1% in more developed parts of the world but could exceed 3% in less developed areas [7]. The risk of an intrapartum stillbirth in low and middle-income countries is more than 14 times that in high-income countries [3]. A study done at Eldoret District Hospital in 1992 found that the stillbirth rate was 30.5/1000 [8]. According to records at the Kenyatta National Hospital there were 1066 stillbirths out of a total of 17 881 deliveries in 2007 and 2008 combined, which gives a stillbirth rate of 59.6/1000 [9].

Approximately half of stillbirths occur prior to 28 weeks of gestation and about 20 percent are at or near term [10,11]. Intrauterine foetal death (IUFD) occurs in < 1 % of singleton pregnancies. The incidence in twin pregnancies varies between 0.5 and 6.8% [12]. The risk of death in the co-twin of IUFD is greater in monochorionic than dichorionic pregnancies [13]. In addition, structurally normal monochorionic diamniotic twin pregnancies without twin to twin transfusion syndrome (TTTS) or intrauterine growth retardation (IUGR) are complicated by a high rate of unexpected intrauterine death [14,15].

Despite stillbirths being one of the most common adverse pregnancy outcomes [11,16], the condition has not been well studied even though the disease burden approaches that of postnatal deaths [17,18].

Causes of stillbirths

Stillbirths are the largest contributor to perinatal mortality. In many cases it is difficult to be certain of the aetiology of stillbirth. First, many cases are "unexplained," despite intensive investigation of potential causes [19]. Second, more than one condition may contribute to stillbirth in an individual case. It may not be possible to precisely determine which disorder was directly responsible for the loss. Indeed, it is likely that some cases of stillbirth are due to contributions from multiple factors. Finally, conditions may be associated with stillbirth without directly causing them [10].

There are several classification systems for causes of stillbirth. No single classification system is universally accepted and each has strengths and weaknesses. The definition of stillbirth varies among investigators, countries, health organizations, and classification schemes. Many systems are designed to include both stillbirths and neonatal deaths under the blanket heading of perinatal mortality [10].

The known causes of stillbirth include genetics (25-35% of stillborn infants undergoing autopsy have genetic abnormalities), infections (10-15% stillbirths have been associated

with bacterial, protozoal and viral infections) and fetomaternal hemorrhage (3-14% of all stillbirths) [4,10].

Infection may cause stillbirth by several mechanisms, including direct infection, placental damage and severe maternal illness [20]. Histological chorioamnionitis can be associated with stillbirth [21]. In a study done in Mozambique, the presence of vasculitis in one fifth of the stillborns indicated that the foetus was alive at the onset of infection [22].

Maternal conditions associated with stillbirth include diabetes mellitus, antiphospholipid syndrome, thyroid disease, cardiovascular disease, asthma, kidney disease, SLE and familial thrombophilias. However, there is a poor correlation between thrombophilia state and placental pathological changes in women with adverse pregnancy outcome. Other factors that are associated with increased stillbirth rates include maternal exposure to cigarette smoke and alcohol. Umbilical cord accidents also contribute to the number of stillbirths [4,10,23]. Occupational exposure to pesticides during the first two months of gestation is positively associated with stillbirths due to congenital anomalies [24].

Obstetric complications associated with IUFD include pre-eclampsia, premature rupture of membranes (PROM), preterm labour, abruptio placenta, placenta previa and vasa previa [4,10]. It is believed that a need for increased placental surface area due to such factors as extensive endometrial scarring is probably the driving force for the formation of placenta previa. Therefore, placenta previa not only results in the reduced supply of oxygen and nutrients to the foetus due to decreased placental surface area, suboptimal uterine location, and bleeding, but the presence of the condition also indicates reduced uteroplacental oxygen and delivery of nutrients. In the case of placental abruption, the premature separation of the normally implanted placenta will result in a decreased surface area for maternal-foetal blood exchange, thus leading to a reduced supply of oxygen and essential nutrients [25]. Pre-eclampsia complicated by premature placental separation is associated with more serious abnormalities both of the decidua and chorionic villi [26].

Other conditions associated with foetal death include trauma, obstructed labour, intrauterine growth restriction and hydrops [26].

Placental, foetal membrane, umbilical cord and gross foetal pathology

According to a study done in Italy, abnormal placental pathology in stillbirths ranged from 20.7% to 39.6% depending on the classification system used [16]. Another study found that 22% of causes of IUFD were placental [27] while in a study conducted in Germany in 2003 and another one conducted in the Netherlands in 2007, placental causes formed the largest group [28,29]. In a study conducted in Brazil in 2000, primary placental diseases were responsible for 30% of the deaths in early stillbirths and 40% in late stillbirths [30].

The sub-groups of placental cause of death are: (1) Placental bed pathology which is due to inadequate spiral artery remodeling and/or spiral artery pathology leading to utero-placental insufficiency such as infarction and abruptio placentae; (2) Placental pathology which originates during development of the placenta itself (e.g. placenta circumvallate, vasa praevia, villus immaturity and placental hypoplasia), abnormalities in the parenchyma (e.g. foetal thrombotic vasculopathy, maternal floor infarct, villitis of unknown origin, massive peri-villous fibrin deposition and fetomaternal haemorrhage without obvious cause) or localization of the placenta (e.g. placenta praevia); (3) umbilical cord complications include lesions interfering with the blood flow in the cord e.g. true knot [28,31]. There may also be cord lesions that by themselves are not necessarily of serious consequence but which are frequently associated with more sinister conditions e.g. single umbilical artery. Other cord lesions such as tumours of the cord are of unpredictable clinical significance. Funicitis is usually seen in sepsis. [31]

The most common histological abnormalities are: infarction, decreased villous vascularity, peri-villous fibrin deposition and leukocyte infiltration [32]. Villous dysmaturity as well as villous and uteroplacental vascular pathology may result in chronic and acute placental insufficiency which represents the cause for intrauterine death [29,33].

Abnormality of the foetal membranes may also point to the causes of stillbirths. Presence of amnion nodosum dominates the morphological picture of the amniotic surface in most cases of oligohydramnios. Chorioamnionitis is an inflammation of the amnion and chorion due to infection. It is reliably diagnosed only by microcopy. Clinical evident amniotic infection occurs far less frequently than histological chorioamnionitis. The consequences of such infections include maternal septicaemia, preterm premature rupture of membranes and premature delivery but occasionally a serious neonatal infection can supervene and may lead to foetal demise. Amnionitic strands may lead to constriction of the umbilical cord as well as causing malformations such as neck constriction which can lead to foetal death [31].

In a study of 120 stillbirths between gestation of 23 and 40 weeks, it was found that among the placental causes of death, 51% of the cases were direct cause or major contributor to death in the aetiology of maternal vascular supply abnormalities, 26% of the cases in the aetiology of foetal vascular supply abnormalities and 12% in the aetiology of inflammatory lesions. Maternal vascular supply abnormalities were more common in preterm stillbirths and foetal vascular supply abnormalities were more common among term stillbirths [35]. Placental inflammatory disorders represent a diverse and important category of pathological processes leading to foetal and neonatal morbidity and mortality. These processes can be divided into two broad subcategories, those caused by micro-organisms and those caused by host immune responses to non-replicating antigens [36].

Gross foetal abnormalities such as anencephaly, spina bifida or omphalocele will be obvious on examination but some abnormalities readily identifiable in the full-term foetus are not identifiable in the second trimester e.g. mongoloid facies. Macerated foetuses present particular problems. Abnormalities may be erroneously diagnosed if it is not appreciated that after foetal death the resorption of amniotic fluid results in compression and distortion of the head and limbs. However, gross external malformations should be readily identifiable [31].

Importance of placental histology

The most useful test towards a diagnosis after stillbirth is pathological examination of the placenta and the foetus [7,10,37]. However, one study in Italy on stillbirths and deaths in infancy showed that the placenta was examined in only 44% of intrapartum stillbirths [7]. In a study to evaluate classification systems for stillbirths, it was found that the placenta was an important source of information in just under one quarter of stillbirths in developing countries compared with 72% in the developed country cohorts as it was not often examined in developing countries [34]. As placental pathology is an important finding in many stillbirths, further definition of the placental causes of stillbirth is needed and further research to investigate the clinical manifestations of these placental causes of stillbirth is important in the prevention of these deaths [34]. In many high income countries, foetal autopsies and placental histological examination, in addition to the medical history, have been evaluated together to designate cause of death [11].

It is recommend that histological analysis of placental tissue be offered in all cases of stillbirth even when full infant post-mortem is declined, as useful information may be obtained. Examination of the placenta reduces the proportion of 'unexplained' stillbirths [32]. Detection of causes of stillbirths is important to identify deficiencies in the provision of care, to focus attention on areas in which improvements are possible, and to indicate areas in which new developments or knowledge may be expected to lead to preventive measures to lower perinatal mortality [16]. In the field of litigation process, placental morphology may be very important for the demonstration that, for example, brain damage or intrauterine death resulted from placental or umbilical cord pathology and not from physician failure [29]. Importantly, routine histologic examination of the placenta after a recent foetal death provides helpful information in counselling the parents and in planning any future childbearing [38,39]. However, the pathophysiology of IUFD is complex and involves maternal, foetal as well as placental entities. In order to assign a cause of death these entities should be addressed together [28].

This study was designed to determine the gross and histological morphology of placentae, membranes and umbilical cords from mothers who presented with IUFD and compared with those mothers who delivered live babies.

1.2 STUDY JUSTIFICATION

Analysis of placental tissue in cases of stillbirth offers useful information even when full infant post-mortem is not done. In the field of litigation process, placental morphology may be important for demonstration that intrauterine death resulted from placental or umbilical pathology. The large contribution of stillbirth to overall perinatal deaths combined with static or increasing rates over the past decade clearly demonstrates that stillbirth is a major public health problem. While the world's neonatal deaths have received increasing global attention in recent years, stillbirths have remained virtually invisible to policy-makers and funding agencies despite the fact that stillbirths have many common risk factors with neonatal deaths and maternal deaths, both of which are centrally placed in the Millennium Development Goals (MDGs). Classification of cause of death is needed for the individual patient in the process of mourning, for the purpose of genetic counselling and prevention and for medico-legal purposes. Despite the high numbers of IUFDs at KNH, no evaluation has previously been done to determine their causes. Moreover, there is little research in this area at the hospital in particular and the whole country in general. Hence the need to conduct this study.

1.3 RESEARCH QUESTION

Are there differences in morphological patterns of the placenta, umbilical cord and membranes in cases of intrauterine foetal death compared to live births?

1.4 OBJECTIVES

1.4.1 Broad objective

To determine gross presentation and histomorphological changes of placentae in patients with intrauterine foetal death as compared to those with live births.

1.4.2 Specific objectives

1. To compare the medical and obstetric profiles of mothers presenting with IUFD with those who have live births.
2. To compare gross features of the stillbirths with those of live births.
3. To compare differences in gross morphology in the placentae, umbilical cords and foetal membranes of stillbirths and live births.
4. To compare differences in histomorphology of placentae, umbilical cords and foetal membranes of stillbirths and live births

CHAPTER 2: STUDY DESIGN AND METHODOLOGY

2.1 STUDY DESIGN

Case control study. This was a hospital-based study.

2.2 STUDY SITE

The study was conducted at the Kenyatta National Hospital's labour ward and the Department of Human Pathology, University of Nairobi. Kenyatta National Hospital is Kenya's largest referral hospital, located in the capital city Nairobi. It also serves as a primary hospital serving residents of Nairobi. The hospital also serves as a teaching hospital for the University of Nairobi and the Kenya Medical Training College. The labour ward at KNH admits many referral patients who mostly have complications. Patients who are being followed at the antenatal clinic also deliver at this hospital's labour. This site was chosen because of the many cases of IUFD seen there and because of the availability of ultrasound services which confirms the foetal demise. The pathology laboratory of the University of Nairobi is also located within the same building as the labour ward and this ensured that specimen was delivered promptly.

2.3 STUDY POPULATION

Mothers who presented with IUFD at a gestation of 28 weeks and above constituted the cases. These mothers were chosen after confirmation of IUFD by ultrasound. The next step was to select those who were above 18 years old since this the legal age at which consent can be given as an adult. Comparative group of mothers who delivered live babies at the hospital were matched for age.

Inclusion criteria

Cases: Mothers who presented with IUFD at a gestation of 28 weeks and above.
18 years old and above.
Mothers who gave consent.

Controls: Mothers who delivered live babies at a gestation of 28 weeks and above.

18 years old and above.

Mothers who gave consent.

Exclusion criteria

Mothers who presented with abdominal pregnancies.

IUFD below 28 weeks.

2.4 SAMPLE SIZE CALCULATION AND SAMPLING PROCEDURE

2.4.1 Sample size calculation

According to literature, abnormal placental pathology is present in between 20% and 40% of IUFD [16,27,28,29,30]. Assumption was made that the proportion of women with abnormal placental histology were (p_1=35%) in the group with intra-uterine foetal death and (p_2=10%) in the group with live births. At 80% power (β=0.2) and 95% (*z=1.96*) confidence interval using the formula below (Fleiss JL 1981) for comparing proportions, 51 participants in each group were included.

For unequal groups of size n_1 and n_2, where $r = n_2/n_1$, is

$$n_1' = \frac{\left\{z_{\alpha/2}\sqrt{(r+1)\bar{p}\,\bar{q}} + z_{\beta}\sqrt{rp_1q_1 + p_2q_2}\right\}^2}{rd^2}$$

where $\bar{p} = \frac{p_1 + rp_2}{r+1}$ and $n_2 = rn_1$.

For small samples, employ a "continuity correction"

$$n_1 = \frac{n_1'}{4}\left(1 + \sqrt{1 + \frac{2(r+1)}{n_1' r|d|}}\right)^2.$$

n is the number of subjects

p is the proportions in each category

d= p_1- p_2

p_1. (bar) = 1 - p_1

p_2. (bar) = 1 - p_2

z=value of standard level for 95% confidence interval

2.4.2 Sampling procedure

Every woman presenting with IUFD at labour ward during the study duration and meeting the inclusion criteria was recruited after confirmation of fetal demise by ultrasound. The recruitment was done until the desired sample size was achieved. A similar number of women with live births was also recruited and matched for age.

2.4.3 Data collection

At KNH, a diagnosis of IUFD is made after ultrasound confirmation. Mothers who present with IUFD are admitted to the labour ward for delivery. Prostaglandins are used to induce labour if the patient is not in labour at the time of admission. Labour is then monitored using the partogram.

The principal investigator and the assistants (midwives) approached a client who presented with IUFD (or a client with live foetus in case of controls) at the labour ward and who met the inclusion criteria. The purpose and nature as well as the benefits of the study were explained to the client by the principal investigator and/or the assistants. An informed consent was sought. Once informed consent was given, information was obtained from the client and the ANC card if available.

After delivery, the baby was examined. The placenta, the foetal membranes and umbilical cord were collected, weighed and put in a container with 10% formalin at a volume ratio of placenta to formalin of 1:10. The information from the client, ANC card, baby, placenta, foetal membranes and umbilical cord was entered into a pre-formatted questionnaire. The placenta, foetal membranes and umbilical cord were then taken to the histology laboratory with a request form which had a corresponding serial number as the questionnaire of the client from whom the placenta was obtained.

The placenta, foetal membranes and umbilical cord were examined as soon as they are delivered to the laboratory. The descriptions and sections followed standard procedures (Appendices 4 and 5). The histological processing, sectioning, staining and mounting also followed standard procedures (Appendix 6). The slides were examined by a pathologist

and quality control was carried out by review of randomly selected slides by a blinded pathologist. The findings were entered into the questionnaire.

All reagents were prepared and assessed for quality following a standard operating procedure (Appendix 6). The handling of tissue and fixation also followed standard operating procedure as well as processing, staining and mounting of tissues. Random review of every 10^{th} case was carried out by a blinded pathologist.

The study instrument was a pre-formatted questionnaire which consisted of eight sections covering socio-demographic data, past obstetric history, history of index pregnancy, medical history, gross examination of foetus, examination of umbilical cord, examination of placenta.

2.5 DATA MANAGEMENT AND STATISTICAL ANALYSIS

2.5.1 Data Management

All participant data did not bear the names of the participant but rather a serial number. Data forms were kept in a secure lockable cabinet only accessible by the principal investigator and the statistician. Data was entered into a password protected Ms Access database prepared by the statistician. The investigator upon completion of data entry checked all the entered data against the hard copy forms.

2.5.2 Statistical analysis

Data analysis was performed using Statistical Package for Social Scientists (SPSS Version 17.0). The proportion of women presenting with IUFD and those with abnormal placental histology was estimated using simple frequencies. Comparison between women presenting with IUFD with those with live births with regard to placental histology was done using Chi-squared statistics and Fishers exact statistics. At the univariate analysis level, correlates associated with these factors were performed using chi-squared and Fishers statistics for nominal variables and t-tests for continuous variables. Multivariate analysis was performed using logistic regression to determine independent factors associated with abnormal placental histology.

2.6 ETHICAL CONSIDERATIONS

There were no serious ethical issues given that the product of interest is usually disposed of. However, confidentiality of the results was paramount and was maintained.

This study was approved by the Kenyatta National Hospital Ethics and Research Committee. Informed consent was obtained from the client before being recruited. This involved signing a consent form after an explanation by the investigator about the details of the study. This included the facts and basis of the study, the risks and benefits anticipated as well as confidentiality and voluntary nature of the study.

The contact address of the investigator was given to the client in case she may have required further details about the study or may have wished to withdraw from the study. The information was communicated both verbally and in writing (appendices 1 and 2). Refusal to participate in the study did not deny the patient the appropriate management. The client did not bear the cost of the histology examination done on the placenta and the histology report was communicated to the client and the clinical team that managed the client.

2.7 STUDY LIMITATIONS

Determination of causes of IUFD would have been more complete if autopsy of the stillbirth would have been performed in addition to the placental pathology. Autopsy of the stillbirth was not performed in this study. This was not possible because of financial constraints.

No bacterial cultures or special stains were performed on the placenta, therefore, micro-organisms could not be identified in cases where there were histological signs of infection.

CHAPTER 3: RESULTS

This study was carried out over 4 months between June 2010 and September 2010. During this period 102 mothers were enrolled in the study. There were 51 mothers in the live births group and 51 mothers in the IUFD group.

Table 1: Socio-demographic characteristics of the participants by pregnancy outcome

Characteristic	Pregnancy outcome						P value
	Live		Stillbirth		Total		
	No	%	No	%	No	%	
Religion							
1 Catholic	19	(37.3)	15	(29.4)	34	(33.3)	0.465
2 Protestant	31	(60.8)	33	(64.7)	64	(62.7)	
3 Muslim	1	(2.0)	3	(5.9)	4	(3.9)	
Education Level							
1 None	2	(3.9)	3	(5.9)	5	(9.8)	0.117
2 Primary	7	(13.7)	17	(33.3)	24	(23.5)	
3 Secondary	23	(45.1)	21	(41.2)	44	(43.1)	
4 College/University	19	(37.3)	10	(19.6)	29	(28.4)	
Employment status							
1 Salaried job	13	(25.5)	10	(19.6)	23	(22.5)	0.098
2 Self-employed	13	(25.5)	6	(11.8)	19	(18.6)	
3 Unemployed	25	(49.0)	35	(68.6)	60	(58.8)	
Marital status							
1 Single	4	(7.8)	5	(9.8)	9	(8.8)	0.547
2 Married	46	(90.2)	45	(88.2)	91	(89.2)	
4 Divorced	0	(0)	1	(2.0)	1	(1.0)	
5 Separated	1	(2.0)	0	(0)	1	(1.0)	
Current smoker							
None	51	(100.0)	51	(100.0)	102	(100.0)	-
Alcohol consumption							
No	51	(100.0)	50	(98.0)	101	(99.0)	0.315
Yes	0	(0)	1	(2.0)	1	(1.0)	

Table 1 shows that the two groups were comparable in their socio-demographic characteristics.

Table 2: Past obstetric history of the participants by pregnancy outcome

Past obstetric history		Pregnancy Outcome						P value
		Live		Stillbirth		Total		
		No	%	No	%	No	%	
No of pregnancies	1	16	(50.0)	14	(50.0)	30	(50.0)	0.897
	2	9	(28.1)	9	(32.1)	18	(30.0)	
	3	4	(12.5)	3	(10.7)	7	(11.7)	
	4	3	(9.4)	1	(3.6)	4	(6.7)	
	7	0	(0)	1	(3.6)	1	(1.7)	
No of abortions	1	2	(100.0)	2	(40.0)	4	(57.1)	0.195
	2	0	(0)	2	(40.0)	2	(28.6)	
	3	0	(0)	1	(20.0)	1	(14.3)	
Still births	1	3	(75.0)	3	(75.0)	6	(75.0)	0.850
	2	0	(0)	1	(25.0)	1	(12.5)	
	4	1	(25.0)	0	(0)	1	(12.5)	
Live births preterm		1	(100.0)	2	(100.0)	3	(100.0)	1.000
Live births term	1	17	(60.7)	9	(47.4)	26	(55.3)	0.570
	2	6	(21.4)	8	(42.1)	14	(29.8)	
	3	4	(14.3)	1	(5.3)	5	(10.6)	
	4	1	(3.6)	0	(0)	1	(2.1)	
	7	0	(0)	1	(5.3)	1	(2.1)	
Previous CS								
	No	48	(94.1)	48	(94.1)	96	(94.1)	1.000
	Yes	3	(5.9)	3	(5.9)	6	(5.9)	

Table 2 shows that most of the participants in both live birth and stillbirth group had one previous live baby.

Table 3: Gestation at delivery by pregnancy outcome

Pregnancy outcome	Gestation (weeks) at delivery				P value
	Mean	Std. Deviation	Minimum	Maximum	
Live (N=51)	38.64	2.388	28	42	<0.001
Stillbirth (N=51)	34.59	4.681	28	43	
Total (N-102)	36.64	4.210	28	43	

From table 3, the mean gestation at delivery in the live birth group was 38.64 weeks while that in the stillbirth group was 34.59 weeks and this was statistically significant.

Table 4: Medical and obstetric complications present in the index pregnancy by pregnancy outcome

Medical and obstetric complications	Pregnancy Outcome						P value
	Live		Stillbirth		Total		
	No	%	No	%	No	%	
HIV status							
Negative	44	(86.3)	40	(78.4)	84	(82.4)	0.295
Positive	0	(0)	2	(3.9)	2	(2.0)	
Untested	7	(13.7)	9	(17.6)	16	(15.7)	
Anaemia in the current pregnancy							
No	51	(100.0)	48	(94.1)	99	(97.1)	0.079
Yes	0	(0)	3	(5.9)	3	(2.9)	
APH							
No	51	(100.0)	46	(90.2)	97	(95.1)	0.022
Yes	0	(0)	5	(9.8)	5	(4.9)	
PROM							
No	45	(88.2)	43	(84.3)	88	(86.3)	0.565
Yes	6	(11.8)	8	(15.7)	14	(13.7)	
Diabetes							-
No	51	100.0)	51	(100.0)	102	(100.0)	
Pre eclampsia							
No	50	(98.0)	40	(78.4)	90	(88.2)	0.002
Yes	1	(2.0)	11	(21.6)	12	(11.8)	

From table 4, 9.8% of the participants in the stillbirth group had APH as opposed to none in the live birth group (p value-0.022). In the stillbirth group, 21.6% of the participants had pre-eclampsia as compared with 2% in the live birth group (p value-0.002). Both these findings were statistically significant.

Table 5: Medical history of the mothers by pregnancy outcome

Medical history	**Pregnancy Outcome**						
	Live		**Stillbirth**		**Total**		**P value**
	No	**%**	**No**	**%**	**No**	**%**	
History of chronic hypertension							
No	51	(100.0)	51	(100.0)	102	(100.0)	-
History of cardiac disease							
No	51	(100.0)	50	(98.0)	101	(99.0)	0.315
Yes	0	(0)	1	(2.00	1	(1.0)	
History of other illness							
No	51	(100.0)	49	(96.1)	100	(98.0)	0.153
Yes	0	(0)	2	(3.9)	2	(2.0)	
On long term medications							
No	51	(100.0)	51	(100.0)	102	(100.0)	-

Table 5 shows that the medical history was comparable between the two groups.

Table 6: Gross examination of foetus by pregnancy outcome

Gross examination of the foetus	Pregnancy Outcome						
	Live		Stillbirth		Total		
	No	%	No	%	No	%	P Value
Sex of foetus							
Female	26	(50.9)	22	(43.1)	48	(47.1)	0.713
Male	25	(49.1)	29	(56.9)	54	(52.9)	
Condition of foetus							
Fresh	0	(0)	12	(23.5)	12	(11.8)	<0.001
Live	51	(100.0)	0	(0)	51	(50.0)	
Macerated	0	(0)	39	(76.5)	39	(38.2)	
Foetus outcome							
Live	51	(100.0)	0	(0)	51	(50.0)	<0.001
Still Birth	0	(0)	51	(100.0)	51	(50.0)	
Gross foetal malformations (spina bifida)							
No	50	(98.0)	50	(98.0)	100	(98.0)	1.000
Yes	1	(2.0)	1	(2.0)	2	(2.0)	

Table 6 shows that, in the stillbirth group, there were more babies who were macerated (38% of the total) compared to those who fresh (11.8%). This was statistically significant.

Table 7: Examination of the umbilical cord by pregnancy outcome

Examination of the umbilical cord	Pregnancy Outcome						P value
	Live		Stillbirth		Total		
	No	%	No	%	No	%	
Attachment of cord to placenta							
Central insertion	47	(92.2)	44	(86.3)	91	(89.2)	0.473
Marginal insertion	4	(7.8)	6	(11.8)	10	(9.8)	
Velamentous insertion	0	(0)	1	(2.0)	1	(1.0)	
Presence of umbilical knots							
No	51	(100.0)	50	(98.0)	101	(99.0)	0.315
Yes	0	(0)	1	(2.0)	1	(1.0)	
No of umbilical cord veins							
1	51	(100.0)	51	(100.0)	102	(100.0)	-
No of umbilical cord arteries							
2	51	(100.0)	51	(100.0)	102	(100.0)	-

From table 7, there were no significant differences in attachment of the cord between the two groups.

Table 8: Gross and microscopic examination of the foetal membranes by pregnancy outcome

Gross and microscopic examination of foetal membranes	Pregnancy Outcome						P value
	Live		Stillbirth		Total		
	No	%	No	%	No	%	
Gross examination of membranes							
Presence of pus	0	(0)	3	(5.9)	3	(2.9)	0.198
Meconium stained	10	(19.6)	8	(15.7)	18	(17.6)	
Fresh	41	(80.4)	40	(78.4)	81	(79.4)	
Microscopic examination of membranes							
Presence of amnion nodosum							
No	48	(94.1)	51	(100.0)	99	(97.1)	0.079
Yes	3	(5.9)	0	(.0)	3	(2.9)	
Presence of amniotic strands							
No	51	(100.0)	51	(100.0)	102	(100.0)	-
Presence of histological chorioamnionitis							
No	36	(70.6)	25	(49.0)	61	(59.8)	0.026
Yes	15	(29.4)	26	(51.0)	41	(40.2)	

From table 8 above, histological chorioamnionitis was more marked in the stillbirth group compared to the live birth group. There was chorioamnionitis in 51% of the membranes in the stillbirth group compared to 29.4% in live birth group (p value-0.026).

Table 9: Weight and diameter of the placenta by pregnancy outcome

Gross examination of placenta	Mean	Std. Deviation	Minimum	Maximum	p values
Weight of placenta (gms)					
Live (N=51)	486.27	119.114	180	750	0.001
Still Birth (N=51)	390.90	155.600	100	700	
Total (N=102)	438.59	145.966	100	750	
Diameter of placenta (cm)					
Live (N=51)	14.31	3.530	8	30	0.004
Still Birth (N=51)	12.41	2.968	8	20	
Total (N=102)	13.36	3.383	8	30	

From table 9, there was a statistically significant difference in the mean weight (p value-0.001) and diameter (p value-0.004) of the placenta in the live birth group compared to the stillbirth group.

Table 10: Microscopic examination of placenta by pregnancy outcome

Microscopic examination of placenta	Pregnancy Outcome						P value
	Live		Stillbirth		Total		
	No	%	No	%	No	%	
Presence of developmental abnormalities							
No	51	(100.0)	51	(100.0)	102	(100.0)	-
Presence of lesions which reduce the mass of functioning villi							
No	50	(98.0)	40	(78.4)	90	(88.2)	0.002
Yes	1	(2.0)	11	(21.6)	12	(11.8)	
Presence of haematomas and thrombi							
No	47	(92.2)	38	(74.5)	85	(83.3)	0.017
Yes	4	(7.8%)	13	(25.5)	17	(16.7)	
Presence of villous abnormalities							
No	47	(92.2)	25	(49.0)	72	(70.6)	<0.001
Yes	4	(7.8)	26	(51.0)	30	(29.4)	
Presence of abnormalities of fetal stem arteries							
No	50	(98.0)	49	(96.1)	99	(97.1)	0.558
Yes	1	(2.0)	2	(3.9)	3	(2.9)	
Presence of other placental abnormalities							
No	46	(90.2)	33	(64.7)	79	(77.5)	0.002
Yes	5	(9.8)	18	(35.3)	23	(22.5)	

Table 10 shows that there was more pathology in the placenta from stillbirths compared to the live births. Reduction in the mass of functioning villi was present in 11.8% of placenta in the stillbirth group compared to 2% in the live birth group (p value-0.002). In the stillbirth group, 16.7% of placenta had haematomas and/or thrombi compared to 7.8% in the live birth group (p value-0.017). There was significant presence of other placental abnormalities in the stillbirth group (22.5%) compared to the live birth group (9.8%) (p value-0.002). These were mainly placental infarcts. However, there were no significant differences between the 2 groups involving abnormalities of fetal stem arteries (p value-0.558).

CHAPTER 4: DISCUSSION

Histological changes were present significantly in the placentae from IUFD compared with those from live births.

In this study there was a significant difference between mothers who presented with pre-eclampsia and had stillbirth as compared to mothers who had live births. Pre-eclampsia is one of the leading obstetric complications associated with IUFD [4,10]. It may be complicated by premature placental separation which can lead to serious abnormalities both of the decidua and chorionic villi [26]. Preeclampsia also affects villous maturity and placental function. Villous dysmaturity as well as villous and uteroplacental vascular pathology may result in chronic and acute placental insufficiency which represents the cause for intrauterine [29].

A significant proportion of mothers in the IUFD group presented with APH. Placenta previa and abruption results in reduced supply of oxygen and nutrients to the foetus. This occurs due to decreased placental surface area, suboptimal uterine location, and bleeding [25]. There was no distinction made between the two conditions in this study. According to studies, fetomaternal hemorrhage has been shown to contribute to 3-14% of all stillbirths [4,10].

Premature rupture of membranes (PROM) is associated with stillbirths [4,10]. However, in this study there was no significant difference between mothers who had live births compared with those who had stillbirths.

Although maternal diabetes is one of the conditions associated with IUFD [4], this finding was not found in this study. Women with pre-gestational (type I and type II) diabetes mellitus (DM) have an increased risk of second and third trimester stillbirth compared to women without diabetes [10]. Even with modern obstetric care and diabetes management, stillbirth rates in women with type II DM have been reported to be 2.5-fold higher than non-diabetic women [10]. The factors that account for the increased risk of late pregnancy fetal loss in diabetic women are not fully understood [10]. Although an

increased likelihood of fetal anomalies contributes to the increased risk, the stillbirth rate for non-anomalous fetuses is also higher in diabetic women than in non-diabetics [10].

In this study there was no significant presence of amnion nodosum in the foetal membranes. Studies elsewhere have shown that the presence of amnion nodosum dominates the morphological picture of the amniotic surface in most cases of oligohydramnios [31].

This study showed significant presence of histological chorioamnionitis among membranes from stillbirths. A majority of these were acute chorioamnionitis with abscesses which is suggestive of bacterial infection. Where the cause of stillbirth is known, 10-15% of the causes are associated with infections [4,10]. Chorioamnionitis is caused by more than 50 different organisms, and among the most common are *Ureaplasma urealyticum*, *Mycoplasma hominis, Escherichia coli*, and Group B streptococcus [11]. Infection may cause stillbirth by several mechanisms, including direct infection to the foetus, placental damage and severe maternal illness [20]. Inflammatory lesions occur 2.6 times more often in stillbirths [29].

This study did not show any significance in babies who had gross congenital anomalies. A number of life threatening congential malformations can cause IUFD [31]. Some of these would have required autopsy to discover. However, autopsies were not carried out in this study hence limiting interpretation on the prevalence.

Abnormal placental pathology in stillbirths range from 20.7% to 39.6% depending on the classification system used [16,27,28,29,30]. There were significant differences in the placental pathology between the 2 groups. All the different aspects of abnormal placental pathology were significantly present in the IUFD group compared to the live birth group. The most common histological abnormalities are: infarction decreased villous vascularity, peri-villous fibrin deposition and leukocyte infiltration [32]. Villous dysmaturity as well as villous and uteroplacental vascular pathology may result in

chronic and acute placental insufficiency which represents the cause for intrauterine death [29,33].

The sub-groups of placental cause of death are: (a) Placental bed pathology which is due to inadequate spiral artery remodeling and/or spiral artery pathology leading to utero-placental insufficiency such as infarction and abruptio placentae; (b) Placental pathology which originates during development of the placenta itself (e.g. placenta circumvallate, vasa praevia, villus immaturity and placental hypoplasia), abnormalities in the parenchyma (e.g. foetal thrombotic vasculopathy, maternal floor infarct, villitis of unknown origin, massive peri-villous fibrin deposition and fetomaternal haemorrhage without obvious cause); (c) umbilical cord complications include lesions interfering with the blood flow in the cord e.g. true knot [28,31]. There may also be cord lesions that by themselves are not necessarily of serious consequence but which are frequently associated with more sinister conditions e.g. single umbilical artery. Funicitis is usually seen in sepsis [31].

Conclusion

1. Abnormal placental pathology is significantly present in placentae from stillbirths. Therefore histological examination of the placenta in cases of IUFD is important since useful information can be obtained.
2. Histological examination of placenta may help in identifying some causes of stillbirths. This knowledge may lead to preventive measures which would lower perinatal mortality.

Recommendations

1. All stillbirths should have their placentae examined histologically since this provides useful information as to the cause of death.
2. There should be more research on causes of IUFD in Kenya as the data available is deficient.

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BUDGET

ITEM DESCRIPTION	AMOUNT (Ksh)
1. Research assistants per diem	46 000
2. Stationary	5 000
3. Statistician	25 000
4. Laboratory material and reagents	37 470
TOTAL	**113 470**

The study was self-funded by the principal investigator.

APPENDIX 1: PATIENT INFORMATION AND CONSENT

The study is on correlation of morphological patterns of placenta from stillbirths and live births at KNH.

Principal investigator: Allan E. Owino, MBChB, MMed student in the Department of Obstetrics and Gynecology, University of Nairobi .Tel. No. 0722656610

Chairperson KNH-ERC: Prof. K. M Bhatt, 0202726300.

Introduction

The purpose of this consent form is to give you information about the study on examination of placenta in the laboratory. This information will help you decide whether to be in the study or not. Please read the form carefully (If clients cannot read the explanation will be read to them or interpreted in a language they understand best). You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer and anything else about the research or this form that is not clear. When we have answered all questions, you can decide if you want to be on the study or not. This process is called informed consent. If you wish, we will give you a copy of this form for your records.

Reasons for research

The purpose of this study is to analyze the placenta from stillbirths in the laboratory. We do know that information obtained when the placenta is examined in the laboratory is a source of information as to the cause of stillbirth. Stillbirths cause anxiety and often the cause is unknown. I therefore intend to lay baseline information that will be used in future to help reduce the number of stillbirths and improve pregnancy outcomes. For this to be achieved, co-operation between health care giver and recipient is required.

Benefits

By participating in the study you will be able to understand circumstances that cause stillbirths. Understanding causes of IUFD will help in prevention in those cases that are

preventable for you and others. In some cases, a definite cause of death will be established. There will be no monetary gain.

Possible risks

There are no risks associated with the study because there is no invasive procedure to be performed on you as part of the study.

Confidentiality

The information given to researchers will be kept in strict confidence. This information will be part of your clinical records. However, no information by which your identity can be revealed will be released or published.

Participant's agreement.

I voluntarily agree to participate in the study on the comparison of placenta in the laboratory obtained from stillbirths and live births at KNH labour ward. I understand that participation in the study does not entail financial benefit. I have been informed that the information obtained will be treated with utmost confidentiality and my treatment will not be compromised if I decline participation or withdraw from the study. I have had a chance to ask questions, if I have questions later about the research I can ask the researcher. If I have questions about my rights as a research subject, I can call the ethical review committee at Kenyatta National Hospital on telephone number 726300 ext.44102

…… ……...

Signature of subject Date

……... ……

Witness Date

I certify that the nature and purpose, potential benefits, possible risks associated with participating in this study have been explained to the above participant.

…… ……

Signature of principal investigator Date

Dr Allan Owino
Principal Investigator
Tel No: 0722656610

APPENDIX 2: MAELEZO KWA MGONJWA NA RUHUSA

Utafiti ni kuhusu uhusiano wa jinsi kondo la nyuma ya mtoto (placenta) lilivyo baina ya mtoto aliyezaliwa hai na yule anayezaliwa mfu katika hospitali kuu ya Kenyatta.

Mtafiti mkuu: Allan Owino MBChB, Mwanafunzi katika chuo kikuu cha Nairobi. Nambari ya simu 0722656610

Mwenyekiti KNH-ERC: Prof. K. M Bhatt, 0202726300.

Utangulizi

Madhumuni ya fomu hii ni kukupatia maelezo kuhusu utafiti utakaofanywa maabara kwa kondo la nyuma ya mtoto. Maelezo haya yatakusaidia kuamua kuhusika au kutohusika katika utafiti huu. Tafadhali soma fomu hii kwa makini (wale ambao hawawezi kusoma wataelezwa). Unaweza kuuliza maswali kuhusu madhumuni ya utafiti huu, jinsi utakavyo husika, faida na madhara ya utafiti huu, haki yako kama mhusika na maswali mengine yeyote yanayohusiana na utafiti au fomu hii ambayo hujayaelewa vizuri. Ukiridhika na majibu yetu, utaamua kama utakuwa mhusika au la. Maelezo haya ni ya kukusaidia kuamua kuhusika bila kushurutishwa. Ukipenda tutakupatia fomu hii kwa rekodi zako.

Madhumuni ya utafiti

Madhumuni ya utafiti huu ni kuchunguza katika maabara kondo la nyuma ya mtoto waliozaliwa wafu. Tunajua ya kwamba uchunguzi wa kondo la nyuma ya mtoto inaweza kutoa maelezo muhimu kuhusu sababu ya kifo cha mtoto kabla ya kuzaliwa. Mtoto anayezaliwa mfu husababisha wasiwasi kwa wazazi na mara nyingi chanzo cha kifo hakijulikani. Kwa sababu hiyo natarajia kutoa maelezo kutokana na utafiti huu ambayo yatakumika baadaye kusaidia kupunguza nambari ya watoto ambao huzaliwa wafu na kusaidia kuleta matokeo mazuri ya mimba. Kufaulu kwa utafiti huu kutategemea ushirikiano baina ya daktari na mhusika.

Faida

Utafiti huu utasaidia kukujulisha hali ambazo zinazochangia kusababisha kuzaliwa kwa watoto wafu. Kwa baadhi ya watoto waliozaliwa wafu, chanzo halisi cha kifo kinaweza kujulikana. Hakuna faida yoyote ya pesa.

Madhara

Hakuna madhara yeyote yanayohusiana na utafiti huu kwa sababu hakuna uchunguzi wa undani unaofanywa kwa mama ila tu kwa kondo la nyuma ya mtoto baada ya kuzaliwa.

Usiri

Maelezo utakayowapa watafiti yatatunzwa kwa usiri. Maelezo haya yatakuwa moja wapo ya rekodi zako katika jalada lako la hospitali. Hata hivyo, maelezo yanayoweza kubainisha utambulisho wako hayatatolewa au kuchapishwa.

Ruhusa ya aliyejitolea kuhusika kwa utafiti

Mimi nakubali kwa hiari kushiriki katika utafiti huu ya kondo la nyuma ya mtoto waliozaliwa wafu na waliohai katika hospitali kuu ya Kenyatta katika wodi ya wazazi. Naelewa ya kwamba kushiriki katika utafiti huu hakutaleta faida ya pesa. Nimeelezwa pia kuwa maelezo nitakayotoa yatakuwa siri baina yangu na watafiti na pia nikiamua kutoshiriki katika utafiti huu basi kutibiwa kwangu katika hospitali hii hakutaathirika vyovyote vile. Nimepewa pia nafasi ya kuuliza maswali na nikiwa na maswali kuhusiana na utafiti huu baadaye, naweza kumuuliza mtafiti. Nikiwa na maswali kuhusiana na haki yangu kama mhusika niliyejitolea, naweza kupiga simu kwa afisi kuu ya utafiti hospitali kuu ya Kenyatta nambari ya simu 726300 ext 44102

…… ……...

Sahihi ya mhusika Tarehe

……... ……

Shahidi Tarehe

Nathibitisha ya kwamba nimeeleza kikamilifu kuhusu jinsi utafiti utakavyo fanywa, madhumuni ya utafiti, faida na madhara ya utafiti kwa mhusika aliyejitolea kushiriki katika utafiti huu.

…… ……

Sahihi ya mtafiti mkuu Tarehe

Dr Allan Owino
Mtafiti mkuu
Nambari ya simu: 0722656610

APPENDIX 3: DATA COLLECTION SHEET

Section A: Socio-Demographic Data

1. Serial Number ☐☐☐
2. Hospital Number ☐☐☐☐☐☐☐
3. Age (Years) ☐☐
4. What is your religion?
    1. Catholic ☐
    2. Protestant ☐
    3. Muslim ☐
    4. Others ☐ Specify_____
5. What is the highest education level you have completed?
    1. None ☐
    2. Primary ☐
    3. Secondary ☐
    4. College/University ☐
    5. Don't know ☐
6. What is your employment status?
    1. Salaried job ☐
    2. Self- employed ☐
    3. Unemployed ☐
7. What is your marital status?
    1. Single ☐
    2. Married (monogamous) ☐
    3. Married (polygamous) ☐
    4. Divorced ☐
    5. Separated ☐
    6. Widowed ☐

8. Do you currently smoke cigarettes or use traditional tobacco?
    1. Yes ☐
    2. No ☐

9. If yes to question 8 indicate the quantity _____(no. of sticks per day)

10. Do you currently drink alcohol?
    1. Yes ☐
    2. No ☐

11. If yes to question 10 indicate the quantity_____(specify the quantity per week)

Section B: Past Obstetric History

12. How many pregnancies have you ever had? ☐☐

13. What was the outcome of the pregnancies? (indicate number)
    1. Abortions ☐☐
    2. Stillbirths ☐☐
    3. Live births (preterm) ☐☐
    4. Live births (term) ☐☐

14. When was your last delivery? (indicate year) ☐☐☐☐

15. History of previous Caesarean section
    1. Yes ☐
    2. No ☐

Section C: History of Index pregnancy

16. Gestation at the time of delivery (weeks) ☐☐
17. Antenatal profile
    1. HB ☐☐
    2. Blood group ☐☐
    3. HIV status: Positive ☐
       Negative ☐
       Not tested ☐
    4. VDRL Positive ☐
       Negative ☐
       Not tested ☐

18. Has there been anaemia in the current pregnancy
    1. Yes ☐
    2. No ☐

19. If yes in 18 what was the level of Hb ☐☐

20. Has there been APH in the current pregnancy
    1. Yes ☐
    2. No ☐

21. History of PROM
    1 Yes ☐
    2 No ☐

22. History of diabetes mellitus
    1. Yes ☐
    2. No ☐

23. History of pre-eclampsia
    1. Yes ☐
    2. No ☐

Section D: Medical History

24. History of chronic hypertension

1. Yes ☐
2. No ☐

25. History of cardiac disease

1 Yes ☐
2 No ☐

26. History of other illnesses

1 Yes ☐ Specify_____
2 No ☐

27. Are you currently on long-term medication

1. Yes ☐
2. No ☐

Section E: Gross Examination of Foetus

28. Sex of the foetus

1. Male ☐
2. Female ☐

29. Condition of the foetus

1. Fresh ☐
2. Macerated ☐
3. Live ☐

30. Presence of gross foetal malformations

1. Yes ☐ Specify_____
2. No ☐

31. Foetal weight (in grams): ☐☐☐☐

Section F: Examination of the Umbilical Cord

32. Was there cord presentation or prolapse during labour

    1. Yes ☐
    2. No ☐

33. Length of the cord ☐ ☐ ☐ cm

34. Diameter of the cord ☐ ☐ ☐ cm

35. Attachment of the cord to the placenta

    1. Central insertion ☐
    2. Marginal insertion ☐
    3. Velamentous insertion ☐

36. Presence of umbilical knots

    1. Yes ☐
    2. No ☐

37. Number of umbilical cord blood vessels

    1. Veins ☐
    2. Arteries ☐

Section G: Examination of the foetal membranes

38. Condition of the membranes

    1. Presence of pus ☐
    2. Meconeum-stained ☐
    3. Fresh ☐

39. Presence of amnion nodosum

    1. Yes ☐
    2. No ☐

40. Presence of amniotic strands

    1. Yes ☐
    2. No ☐

41. Presence of histological chorioamnionitis

    1. Yes ☐
    2. No ☐

Section H: Examination of Placenta

42. Weight of placenta (grams) ☐☐☐☐

43. Diameter of placenta (cm) ☐☐☐

44. Presence of developmental abnormalities

1. Yes ☐ Specify_____
2. No ☐

45. Presence of lesions which reduce the mass of functioning villi

1. Yes ☐ Specify_____
2. No ☐

46. Presence of haematomas and thrombi

1. Yes ☐ Specify_____
2. No ☐

47. Presence of villous abnormalities

1. Yes ☐ Specify_____
2. No ☐

48. Presence of abnormalities of the foetal stem arteries

1. Yes ☐ Specify_____
2. No ☐

49. Presence of other placental abnormalities

1. Yes ☐ Specify_____
2. No ☐

APPENDIX 4: EXAMINATION OF THE FOETUS, UMBILICAL CORD AND FOETAL MEMBRANES

Description

1 Sex; weight; crown-rump or crown-heel length or foot length (see accompany drawings)
2 Approximate length of gestation (see table, p. 2658, and figure below)
3 General condition: well preserved? macerated?
4 External and internal anomalies and other changes
5 Umbilical cord: appearance, number of vessels
6 Placental tissues accompanying fetuses:
  a Weight
  b Membranes: insertion, color, transparency, completeness; extramembranous pregnancy? marginal or membranous hemorrhage?
  c Umbilical cord: insertion, color, focal changes
  d Chorionic plate: vascular pattern, vessel caliber, color; subchorionic hemorrhage?
  e Villi: hydatidiform change? (note proportion of involved villi and size of cysts); focal lesions?

Sections for histology

1 Small embryos: submit whole embryo or one half, depending on size.
2 Large fetuses: submit one section from lungs, stomach (including gastric contents), kidneys, and other organs, as indicated.
3 Placental tissues:
  a Extraplacental membranes (one section)
  b Umbilical cord (one section)
  c Chorionic plate (one section)
  d Villi from chorion frondosum, including maternal surface (one section)

Table App H-1 Relations of age, size, and weight in the human embryo

Age of embryo	Crown-rump (CR) length (mm)	Crown-heel length (mm)	External diameter of chorionic sac (mm)	Weight in grams	Amount of increase each month when value at start of month equals unity	
					CR length	Weight
One week	0.1	—	0.2			
Two weeks	0.2	—	3			
Three weeks	2.0	—	10			
Four weeks	5.0	—	10	.02	49.0	40000.00
Five weeks	8.0	—	25			
Six weeks	12.0	—	30			
Seven weeks	17.0	19.0	40			
Two lunar months	25.0	30.0	50	1	3.6	49.00
Three lunar months	56.0	73.0	—	14	1.4	13.00
Four lunar months	112.0	157.0	—	105	1.0	6.50
Five lunar months	160.0	239.0	—	310	0.43	1.95
Six lunar months	203.0	296.0	—	640	0.26	1.07
Seven lunar months	242.0	355.0	—	1080	0.14	0.69
Eight lunar months	277.0	409.0	—	1670	0.14	0.55
Nine lunar months	313.0	458.0	—	2400	0.13	0.43
Full term (38 weeks)	350.0	500.0	—	3300	0.12	0.38

APPENDIX 5: EXAMINATION OF THE PLACENTA

PLACENTA—SINGLETON

Procedure and description

1 Examine as soon as possible after delivery in the fresh state; handle the specimen with great care, avoiding lacerations.
2 Note the amount of blood and clots in the container and search for separate pieces of membranes, cord, or placenta.
3 Examine in this order: membranes, cord, fetal surface, and maternal surface.
4 Measure the distance from the placental margin to the nearest point of rupture (zero: marginal placenta previa).
5 Examine membranes for completeness (if a portion is missing, notify the obstetrician), insertion, decidual necrosis, edema, extra-amniotic pregnancy, retromembranous hemorrhage, meconium staining, color, and transparency.
6 Take a long, 2- to 3-cm wide section of membranes beginning with the point of rupture and extending to and including a small portion of placental margin. Roll the specimen with amniotic surface inward, fix for 24 hours, take a 3-mm section from the center (taking care not to strip the amnion off), and submit for histology. Take a second section including amnion, chorion, and decidua from the rim of the site rupture (in vaginal deliveries).
7 Trim the remaining membranes from the placental margin.
8 Measure the length of the cord and the shortest distance from the cord insertion to the placental margin.
9 Examine the cord: insertion (nonmembranous or membranous; if latter, are vessels intact?), number of umbilical vessels (by sectioning the cord transversely at two or more points), color, true knots, torsion, stricture, hematoma, thrombosis.
10 Remove the cord from the placenta 3 cm proximal to the insertion, and take a 2 - to 4-cm segment from its midpoint; fix this segment for 24 hours, take a 3-mm section, and submit for histology.
11 Examine the fetal surface: color, opacity, subchorionic fibrin, cysts (number and size), amnion nodosum, squamous metaplasia, thrombosis of fetal surface vessels, chorangioma.
12 Examine the maternal surface: completeness, normal fissures, laceration (extent), depressed areas, retroplacental hemorrhage (size and distance from margin).
13 Measure the maximum diameter, thickness in the center, weight (after trimming cord and membranes), shape.
14 Hold the placenta gently with one hand, maternal side up on a flat surface, and make parallel sections with a large sharp knife at 10-cm intervals. The fetal surface will not be cut through and will hold the specimen together.
15 Remove four 2-cm pieces that include the fetal surface and intact maternal surface, selecting tissues of the placenta (within 2 cm of placental margins). Take the piece so that the fetal surface vessels are cut at right angles to their long axis; fix for 24 hours, trim a 3-mm section (through and through), and submit for histology. One section should include the chorionic plate in an area with minimal subchorionic fibrin. The other sections should include the maternal surface. Submit similar sections of any lesions present.
16 Examine all cross sections for infarcts (location, size, number); intervillous thrombi (number); laminate, perivillous fibrin deposition, pallor, consistency, calcification (extent), cysts, tumors. Describe lesion location (central, lateral, or marginal), depth (parabasal, intermediate, or subchorionic), and age (recent or old).

Sections for histology

1 Placenta (as indicated previously plus abnormal areas, if present)
2 Membranes
3 Cord

PLACENTA—TWIN

Procedure and description

1 If placentas are separate (nonfused): examine each placenta as a singleton.

2 If placentas are fused:

a Note whether the two cords are labeled twin A and twin B. If not, label them arbitrarily and make a statement to that effect.

b Determine the presence and type of dividing membranes:

(1) If absent (monochorionic-monoamniotic), so state.

(2) If present:

(a) Remove a square of the dividing membrane, roll it, fix it for 24 hours, take a 3-mm section, and submit for histology.

(b) Attempt to determine grossly whether the dividing membrane has chorion or not, according to the accompanying tables.

(c) Record the kind and number of vascular anastomoses in monochorionic-diamniotic placentas: artery-to-artery, vein-to-vein, artery-to-vein (arteriovenous shunts). The latter can be better demonstrated by injecting the artery of one twin along the plane of fusion of the placenta with 30 to 50 ml of saline solution containing a dye and noting whether the fluid emerges from the vein of the other twin through one or more common villous lobules. The placenta must be intact to perform this test. Arteries always run over veins.

c Divide the fused twin placenta along the "vascular equator" (rather than through the base of the dividing membrane).

d Examine each half as a singleton placenta.

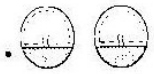
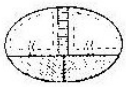
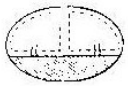
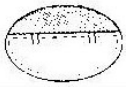
Sections for histology

1 Placenta from twin A
2 Membranes from twin A
3 Cord from twin A
4 Placenta from twin B
5 Membranes from twin B
6 Cord from twin B
7 Dividing membrane, if present

Table App H-3 Dividing membrane in twin placentas

Features	Dichorionic-diamniotic (fused)	Monochorionic-diamniotic
Appearance	Thick and opaque	Thin and transparent
Separation of membranes by stripping	Difficult	Easy
Point of attachment to fetal surface	Ridge or tearing of chorion	Smooth and continuous, without ridge
Vascular anastomoses	Very rare	Numerous

Table App H-4 Types of twin placentas

Type	Incidence	Gross	Twin type
Dichorionic-diamniotic (separate)	35%		Monozygotic or dizygotic
Dichorionic-diamniotic (fused)	34%		Monozygotic or dizygotic
Monochorionic-diamniotic	30%		Monozygotic
Monochorionic-monoamniotic	1%		Monozygotic

APPENDIX 6: TISSUE PROCESSING AND STAINING

Fixation

This is the preservation of tissue elements in as life-like manner or condition as possible. This is done to prevent autolysis and putrefaction of tissue. It also renders tissue resistant to damage by osmotic pressure.

10% formalin

This is the preferred fixative.

Requirements for preparation: -formaldehyde
-distilled water/tap water
-sodium chloride

Haematoxyline and eosin staining method

1. Bring sections to water
2. Stain in haematoxyline and eosin for 7 minutes
3. Rinse in water
4. Differentiate in 1% acid alcohol for 3 seconds
5. Rinse in water
6. Blue in Scotts tap water-3 dips
7. Rinse in water
8. Stain in eosin 0.5% for 5 minutes
9. Dehydrate in ascending grades of alcohol
10. Clear in 3 changes of xylene
11. Mount using DPX
12. Microscopy
    - Expected results: -nucleus-blue
      -cytoplasm-pink

Tissue processing

1. Trimming-cutting of tissue into small sizes that can fit in standard size cassette.
2. Fixation-fix tissues in 10% formal saline to preserve tissue elements
3. Dehydration-removal of water from tissues to allow complete processing. This is achieved by taking tissues through ascending grades of alcohol-30%, 50%, 75%, 95%, absolute
4. Clearing-removal of alcohol from tissues by use of clearing agents and the preferred agent is chloroform-pass the tissue in about 3 changes of chloroform
5. Impregnation-saturation of tissues with molten paraffin wax to remove the clearing agent.
6. Embedding-process of giving tissues supporting medium to allow microtomy

Microtomy

The slicing of tissues to recommendable sizes to allow staining with H/E or any other stain.

Summary of automatic processing

1. Fix tissues
2. *Select pieces of tissue for cutting/trimming*
3. *Dehydrate up to 2-5mm thick*
    a. *50% alcohol-2 hours*
    b. *70% alcohol-2 hours*
    c. *90% alcohol-2 hours*
    d. *100% alcohol-2 hours*
    e. *100% alcohol-overnight*
    f. *Chloroform- 1.5 hours*
    g. *Chloroform-1.5 hours*
    h. *56^{o}-molten paraffin wax 1.5 hours*
    i. *56^{o}-molten paraffin wax 1.5 hours*
    j. *56^{o}-molten paraffin wax 1.5 hours*

4. Embedding-in molten paraffin wax
5. Microtomy
6. Staining

NB: in italics-standard automated tissue processing schedule

APPENDIX 7: ETHICAL APPROVAL

KENYATTA NATIONAL HOSPITAL
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Ref: KNH-ERC/ A/456

15th April 2010

Dr. Allan E. Owino
Dept.of Obs/Gynae
School of Medicine
University of Nairobi

Dear Dr. Owino

RESEARCH PROPOSAL: "CORRELATION OF THE CLINICAL PICTURE AND MORPHOLOGICAL PATTERNS OF PLACENTAE, FOETAL MEMBRANES AND UMBILICAL CORDS FROM MOTHERS PRESENTING WITH INTRAUTERINE FOETAL DEATH AT KENYATTA NATIONALHOSPITAL" (P337/12/2009)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 15th April 2010 to 14th April 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
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