Non-invasive Perinatal Postmortem Examination

MPhil Thesis

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In Memory Of My Father

"Abulgasim"

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ABSTRACT

Aim__To evaluate the efficiency of non-invasive perinatal postmortem examination as an alternative option in Middle East countries where full autopsy is denied. The study prospectively compared the results obtained by the non-invasive postmortem techniques with that obtained by full autopsy.

Subjects and methods___ Initially, there was an extensive literature review of the available methods of non-invasive postmortem examination as well as the different causes of fetal and perinatal deaths. The usefulness of these ideas and points were evaluated for the possibility of using them in Middle East Region. Fifty five cases of perinatal and fetal deaths, including cases terminated because of congenital abnormalities, were studied. The efficiency of non-invasive techniques was assessed: these included review of the clinical record, external examination, body weight and measurements, radiological examination, placenta, cord, and membranes examination, as well as microscopic examination (fine needle aspiration, needle biopsy).

Results__In 50 out of 55 (91%) cases, full autopsy did not alter the specific diagnosis made by the non-invasive techniques. In the remaining 5 cases, full autopsy did provide extra information by disclosing further internal malformations and lesions that might have affected genetic counselling. For non-invasive microscopic examination, needle biopsy was superior to fine needle aspiration, and its validity could have been increased by using ultrasound needle biopsy guidance.

Although full autopsy remains the method of choice for evaluating fetal and perinatal deaths, particularly in malformed cases, non-invasive perinatal postmortem techniques are collectively an excellent option, particularly in Middle East societies where full autopsy is restricted.

Conclusion__In Middle East countries, basic steps need to be taken in order to establish and promote a standardised protocol for perinatal postmortem examination. This will facilitate the identification of different causes of fetal and perinatal deaths in this region, so that prevention and proper counselling can be provided for the family.

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ABBREVIATIONS:

AFP	Alfa-fetoprotein
APH	Antepartum haemorrhage
BW	Body Weight
CH	Crown heel
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Crown rump length
FL	Foot length
FNA	Fine needle aspiration
GA	Gestational age
HC	Head circumference
HSV II	Herps simplex type II
IUD	Intrauterine death (including first trimester abortion)
IUFD	Intrauterine fetal death (second trimester abortion & stiilbirth)
IUGR	Intrauterine growth retardation
MRI	magnetic resonance imaging
msAFP	Maternal serum Alfa-fetoprotein
NB	Needle biopsy
NTD	Neural tube defect
PM	Postmortem
SGA	Small for gestational age
TOP	Termination of pregnancy
TTTS	Twin to twin transfusion syndrome
U/S	Ultrasound

OBJECTIVE

The aim of this study was to evaluate different methods of non-invasive postmortem (PM) examination that could be used as part of genetic services in Middle East countries, where religious and cultural traditions strictly limit the performance of full autopsy (invasive PM). The development of non-invasive perinatal PM examination would be helpful in delivering the most benefits to mothers with unfavourable pregnancy outcomes without inflicting damage to these cultural and religious traditions.

In this study we evaluate the validity of non-invasive PM techniques of cases who were delivered after termination of pregnancy (TOP), miscarriage, stillborn or suffered neonatal death within the first week of life. The adequacy and accuracy of the information obtained by these non-invasive methods is compared with that of full autopsy.

The non-invasive PM techniques assessed are:

- clinical record (clinical investigations and antenatal Ultrasound).
- external physical examination and body measurements.
- radiology examination.
- placenta, membranes and cord examination.
- microscopic examination which composes of fine needle aspiration (FNA) and needle biopsy (NB).

FNA and NB are normally regarded as invasive procedures, but as they do not cause any disfiguring to the body, and for the purpose of this study they are considered as non-invasive techniques. The initial proposal also included postmortem ultrasound examination, but this proved not to be possible.

Introduction

Postmortem (PM) is a well known method of investigation of cause of death, and it is an important tool in understanding the different disease processes which occur in different age groups (fetal, neonatal, infant, child, and adult). The term PM is often used synonymously with the words *autopsy* and *necropsy*, to imply a full postmortem examination, which as well as external examination, includes dissection and internal examination of cranial, thoracic, and abdominal cavities. This accepted usage has led to the idea that conducting PM examinations in Middle East countries is not possible. Unfortunately, this puts huge restriction on the services that could be provided for women with an unfavourable pregnancy outcome in those countries. Therefore, there is need to consider that the term PM examination applies to wide range of techniques including non-invasive techniques or performance of full autopsy. This in fact,, another step further in its continuous progress since its early start over 3000 years ago.

Historical background of postmortem examination:

PM seems to have been used as long ago as 1500 BC[1] when knowledge of normal and abnormal anatomy derived from attempts to foretell the future by examination of animall entrails.

Ancient Egyptian records show a good knowledge of anatomy, but disease was attributed tto magic and treated by further magic. Human body dissection was not forbidden and its practice was established in Alexandria in the third century BC. Erasistratus (310-250 BC) noted the effect of disease by observing that the liver of a man who died from dropsy was hard as a stone, while that of a man who died from a snake bite was soft.

In Ancient Greece, autopsy had little place in medicine as they used to explain diseases by humour (which are the four main fluids of the body; Blood, Phlegm, Choler and Melancholy, and according to Galen's theory, these fluids determine a person's physical and mental

qualities[2]) and did not take into consideration the status of solid organs or any pathological changes which may have occurred in them.

In the early years of Christianity, there was no formal prohibition of PM. The general attitude of church leaders towards it was unfavourable, until Pope Sixtus IV (1471-1484) issued a bill allowing studies of human bodies by students[3]. While both Judaism and Islam allow autopsy to be performed on the basis of legally required circumstances, generally, they forbid any disfigurement of the dead body[4]. Furthermore, Islam encourages the carrying out of burial as soon as possible after death which could conflict with the delays resulting from the conducting of autopsy.

In the Renaissance period[1] (14th-17th century) many successful physicians, like Antonio Benivieni and Theophilus Bonetus, did much to promote the performance of PM and the knowledge of pathology by recording their clinical experiences many of which included autopsy. The peak of the pre-modern PM however, was reached by G.B.Morgani (1682-1772), who gathered together masses of pathological data and linked them with clinical observations.

Although Bernord Tornius in the 15th century, made one of the earliest descriptions of PM techniques, they were generally poorly described until the time of Rokitansky (1804 - 1878) and Virchow (1821 - 1902), when the techniques were improved to include detailed studies at cellular level by introducing microscopic examination. The developments made in medicine this century have led to the introduction and usage of many modern therapeutic and diagnostic tools. As result, the role of PM was expanded to include monitoring and evaluation of these new tools, in addition to determining hospital accreditation. It was reasoned that good hospitals had high autopsy rates, while poor hospitals had low rates[1].

However, since the middle of this century, there has been a progressive decline in the adult PM rate. For example, in the USA, the adult hospital necropsy rate (defined as percentage of inpatients who die and on whom the necropsies are performed, and excluding those brought in

dead) had fallen from 60-80% in 1950 to 10% in 1980[5]. Concern about this trend has been raised by some studies[6,7] which have attributed it to many factors including the strong belief in the precision of modern techniques in clinical diagnosis, excessive cost and religious or cultural objections. In contrast to this general pattern, the rates of perinatal PM, which is a subspecialty dealing with pregnancy losses, stillbirths, and infant deaths are relatively high.[8,9,10] In Britain , the perinatal PM examination rate in 1980 was around 60%.[11]

In Middle East countries, modern medicine is being very actively developed but religious conventions restrict the application of full PM. In view of the evolution described above, it is necessary to carefully evaluate the role of PM today, to reach appropriate conclusions for practice in the Middle East. This thesis aimed to evaluate the usefulness of non-invasive perinatal PM in fetal and perinatal practice.

Part I

LITERATURE REVIEW

Chapter I.1

Perinatal Postmortem Examination

1.1 Definitions:

Fetal death: The world health organisation (WHO) defines fetal death as " death prior to the complete expulsion or extraction from its mother of products of conception, irrespective of the duration of pregnancy". The death is indicated by the fact that after such separation, the fetus does not breath or show any other evidence of life such as beating of the heart, pulsation of the umbilical cord or definite movement of voluntary muscle.[12]

Prematurity: Prematurity is defined as delivery of the baby before 37 completed wks of gestation, calculated from the first day of the last menstrual period.

Small for gestational age (SGA): Babies whose birth weight falls below some predetermined cut-off point at a given gestation age (GA), usually two standard deviations below the normal birth weight for that gestation.

The following table summarises definitions of mortality statistics commonly used to study fetal, neonatal and infant deaths.

Table 1.1. Mortality statistical definition.

Mortality statistic	Definition
Stillbirth rate	Fetal deaths delivered after 24* weeks'gestation per 1000 total births.
Perinatal mortality rate (PMR)	Stillbirths and deaths in first week of life per 1000 of total births.
Early neonatal mortality rate	Deaths within 7 days of life per 1000 of live births.
Late neonatal mortality rate	Deaths at age 8-27 completed days of life per 1000 live births.
Neonatal Mortality rate	Deaths in the first 27 completed days of life per 1000 live births.
Infant Mortality rate	Deaths in the first year of life per 1000 live births.

* Variation in defining the starting point of fetal viability by different countries

1.2 Scope of the perinatal postmortem examination:

The perinatal period refers to the period around the time of birth and includes a variable prenatal and postnatal period. There is wide variation from country to country regarding

starting point of the perinatal period or the dividing line between abortion and stillbirth. For example, in England and Wales it has been legally set at 24 wks since October 1992. In the USA and Australia it is set at 20 wks, while in most of other countries the qualifying age is still 28 wks.

The World Health Organisation (WHO) 1977[13] has recommended that for national statistics the term "perinatal death" should include, as well as early neonatal deaths, all fetal deaths that weigh 500 gm or more (or having the gestation age of 22 wks or more, or Crown heel (CH) length of 25 cm or more if weight is unknown). On the other hand, for international statistics, the recommendation is to include only fetal deaths of 1000 gm or more (or having the gestation age of 28 wks or more, or CH length of 35 cm or more if the weight is unknown).

Regardless of this variation in limit, the perinatal PM examination has expanded to include babies and fetuses who may fall beyond these defined limits, including those ex-premature babies who have been treated for months in neonatal intensive care, and fetuses under 20 wks' gestation, mainly those antenatally diagnosed as being abnormal. Moreover, the recent accessibility of first trimester prenatal diagnosis by transvaginal Ultrasound (U/S)[14,15] and DNA analysis for chromosomal error, encourages the perinatal pathologist to expand their area of interest to include such cases and in so doing, has increased the knowledge about the natural history of disease processes in the developing embryo and fetus.[16]

1.3 Value of the perinatal postmortem examination:

Postmortem examination is of paramount importance in confirming, extending or revising the clinical diagnosis, and providing a better understanding of the causes leading to perinatal death, which is imperative in order to achieve a further reduction in perinatal mortality rate.

The information gained from PM is of importance at three different practical levels: the family concerned, the attending clinician and society. For the family, this information helps in the management of guilt and grief as they go through a predictable set of emotions. In western

countries most detected pregnancies undergo a series of U/S examinations, thus building an early fetomaternal bonding, and from there on the fetus is seen as a member of the family. This bonding, coupled with the current tendency towards a smaller family size, accentuates the tragedy for the bereaved family and for them the time following the loss of the infant is extremely difficult. Hence, PM is needed to provide accurate information related to the cause of death, which may alleviate some of the grief that surrounds the family loss and help in their future childbearing management.

Secondly, PM examination is necessary for clinical audit and control of both obstetrics and neonatal care. The major advances seen in the last quarter of this century in the fields of prenatal diagnosis, neonatal intensive care, and clinical genetics, linked with the emergence of fetal medicine as a new sub-speciality, has increased the importance of the role of perinatal PM examination in establishing the precise diagnosis, providing the information needed for obstetric and genetic counselling. Correlating the findings of prenatal U/S and PM is essential for improving the accuracy of prenatal imaging. Once the cause of death is determined or the anomalies are accurately delineated, obstetricians could improve the outcome of the subsequent pregnancy by intensifying the maternal care programme regarding that particular cause. Geneticist could calculate the precise recurrence risk of the anomaly and an appropriate plan for surveillance for the next pregnancy could be implemented.

Porter and Keeling[17], have studied 150 cases of neonatal death cases and 150 stillbirth cases, found that the clinical and pathological diagnosis was different in about 36% of stillbirth cases and 44% of neonatal death cases. Meier et al[18] reported that perinatal PM examination was " the sole means of establishing the cause of death in 26 % of cases". PM has a pivotal role in auditing the effectiveness of new techniques. In the USA a study at Magee Women's Hospital in 1986,[19] revealed that in 46% of fetal anomalies detected by prenatal U/S, PM provided additional information that assisted in making a specific diagnosis, and/or evaluating the severity of an anomaly. Keeling et al[20] suggested that defects which are important for parental

counselling are often not identified on scan. Clayton-Smith et al[21] stated that fetal examination has changed or refined the pre-termination U/S diagnosis in 40% of 133 cases.

PM has a major role in the detection of morphological or histological abnormalities that result from intrauterine exposure to maternal medication. For examples, inhalation anaesthetics are associated with central nervous system (CNS) and musculoskeletal abnormalities and psychtropic drugs can cause cardiovascular abnormalities.[22] Thus, PM data is essential to monitor environmental teratogens.

Sophisticated neonatal intensive care has became more readily available, such as extracorporeal membrane oxygenation, and has resulted in an increase in the incidence and the range of iatrogenic disease in the neonate.[22,23] Careful pathological examination may contribute to the understanding of the development of such diseases.

A variety of Intracranial lesions have been described in babies who received extracorporeal membrane oxygenation[22]. Intracranial haemorrhage occurs in 33% of these babies. PM also showed that 16 out of 19 babies who underwent extracorporeal membrane oxygenation treatment shortly before death, have had thromboemboli, some of which contained aluminium which was thought to originate from extracorporeal membrane oxygenation equipment. Furthermore, basic neonatal intensive care procedures, for example ventilation, can result in bronchopulmonary dysplasia, and umbilical vessel catheterization can lead to umbilical vein thrombosis.

Thirdly, perinatal mortality data is a highly sensitive index of any nation's health. Hence, validation of this information is of utmost importance and would be neither complete nor accurate in the absence of PM examination. Coard et al[24] reported that major congenital malformations account for 8.6% of deaths in perinatal PM examination, compared to 2.6% of non-autopsied cases. Furthermore, PM data provides a good material for research, education, and it is an important contributor to any epidemiological survey.

1.4 Limitation of the perinatal postmortem examination:

Perinatal autopsy is an unpopular examination for all concerned.[25] The reasons for this unpopularity are complex. They include the unwillingness of some parents to give consent for PM based on religious beliefs, cultural concerns or subjective feelings. There are also economic considerations on the part of health authorities.[26]

The dissection and reconstruction method used to facilitate the demonstration of anomalies and the pathological status of each organ may conflict with the need to carry out religious or cultural burials so many parents refuse to give their consent for PM examination. In fact, the consent for the autopsy could be regarded as the main limiting factor[27] and for this reason, a higher rate of PM examination has been attained in non-registerable age births (<20 wks) than that in registerable age births (>20 wks). In the former, births may be considered as surgical specimens and consent may not be requested. As previously mentioned, the increase in both public awareness of the fetus as an entity and the fetomaternal bonding reduces the chance of parental PM consent. This is reflected by the trend of higher PM rates among fetuses terminated as result of congenital anomaly than intrauterine fetal death (IUFD) cases, and an even lower PM rate is observed among live-born babies.[27]

However, the perinatal autopsy rate has generally exceeded that for adults Even so, in many obstetric units, the rates of perinatal PM are still below of the minimum acceptable level of 75% set by the Royal College of Obstetricians and Gynaecologists and Royal College of Pathologists (joint working party 1988). For example, a study carried out in 1980 by Golding[11] showed that in Britain, necropsy was performed in 61% of stillbirth cases and 54% of neonatal death cases. In Wales, the rate of PM examination for perinatal and infant death for 1993 was only 62%.[28] In Scotland, a rate of 65% was recorded in 1991[29] and in the West Midlands, the rate of perinatal PM was 69% in 1987.[30] In University College London Hospitals (UCLH), the PM examination rates of both stillbirth cases and neonatal death cases for different years are

shown in Table 1.2. In fact, even in the presence of a high autopsy rate, the are sometimes problems with the quality of the autopsy itself.[30]

Table 1.2. PM examination rates as percent of perinatal deaths for different years at UCLH.

	1992	1993	1994	1995
Stillbirth cases	61%	63%	73%	59%
Neonatal death cases	36%	59%	76%	58%

The Royal College of Pathologists has designed a scoring system[28] for performing PM examination with a minimal recommended score of 350 (Table 1.3), but many reports show that this minimal score has not been achieved.[28,30]

Table 1.3. The scoring system recommended by Royal College of Pathologists for perinatal autopsy (Standard autopsy)

Category	Score
Body weight (BW)	20
Crown rump (CR), Crown heel (CH)	20
Head circumference (HC)	20
Foot length (FL).	20
Normal values (body measurements)	20
Main organ weight (brain, liver, lungs, kidneys)	40
Organ weight (others)	40
Normal values (organ weight)	20
Histology (main organs)	50
Histology (other organs)	50
Placenta (gross examination)	50
Placenta (histology)	50
Radiology	100
Microbiology	100
Other investigations	100

Chapter I. 2

Non-invasive Perinatal Postmortem Examination

Autopsy, as defined earlier, is the procedure by which full external and internal examination, including the dissection of cranial, thoracic and abdominal cavities, is conducted. Parental unwillingness to have this examination carried out is one of the main obstacles faced by hospitals. Although, in the Western countries it is now an evolving problem, often amenable to proper counselling, this is still not the case for Eastern countries, such as Middle East countries where parental consent would be much more easily obtained if the PM examination was less invasive, or non-invasive.

For non- invasive PM to be productive it should allow external assessment as well as internal examination of different organs in order to provide diagnostic information about the cause of death. External assessment would comprise external physical examination, measurements, and photographic imaging. Information about internal structure would be obtained via radiography, U/S, Magnetic Resonance Imaging (MRI) and microscopic examination of different organs through needle biopsy (NB) or fine needle aspiration (FNA). In addition, placenta, cord and membrane examination would be supportive and in many instances, the main source of diagnostic information. Clinical investigation and antenatal ultrasound are also important sources of information. All the above mentioned methods could be applied individually or collectively to gather data related to cause of death. All of these different methods will be discussed below.

2.1 Clinical investigations:

A review of antenatal care records is essential as it may contain data that helps to identify the cause of fetal death or any predisposing condition. Further antenatal investigations performed on the pregnant mother who has an intrauterine fetal death (IUFD) is essential for discovering the cause of fetal demise (Appendix III, Section II).

Ahlenius et al[31] have extensively investigated 66 fetal death cases in order to find the causes of these deaths. The investigation included; maternal Kleihauer-Betke test; serology analysis for different infectious agents; cervical culture and urine samples and coagulation profile. Fetal autopsy, placental examination, fetal blood investigation and bacterial cultures from fetal external orifices were also performed. The degree of certainty regarding the cause of death was found to be high in the group which were completely investigated.

Wright[32] carried out a study to investigate the prevalence of parvovirus B19 as a cause of fetal death. It was concluded that nearly all cases studied and confirmed histologically as being infected by parvovirus have had a positive maternal serology for this virus.

Most of recent exposure to different viral agents could be detected serologically, mainly by seroconversion of maternal antibodies, but this does not necessary mean that the baby is infected, since the transmission rate differs according to type of agent, time of exposure and other factors.

Fetomaternal haemorrhage is associated with IUFD and accounts for 3-5% of all fetal deaths.[33] This condition can only be diagnosed by the Kleihauer-Betke laboratory test. The contribution of maternal Lupus anticoagulation and anticardiolipin antibodies to IUFD cases will be discussed later. These antibodies are usually diagnosed by studying the maternal coagulation profile.

In the light of all above observations, it is possible to conclude that an extensive and relevant test protocol does provide information needed to determine the cause of death in many IUFD cases.

2.2 Antenatal Ultrasound (U/S):

The role of prenatal U/S in obstetric care is well established. It is the method most often used to verify the correct gestational age (GA), to diagnose multiple pregnancy, and to evaluate the growth status of the fetus. Furthermore U/S plays a pivotal role in enhancing the safety and effectiveness of different invasive diagnostic and therapeutic procedures such as amniocentesis, chorionic villus sampling and fetal blood transfusion.

Since U/S allows examination of both external and internal anatomy of the fetus, its role has been expanded to include screening and diagnosis of congenital anomalies as well as detecting the different signs of chromosomal anomalies.

Classically, autopsy has been the ideal method by which fetal anomalies are detected, but now prenatal U/S has been used for the same purpose. Historically, the first case of fetal malformation detected antenatally by static U/S was in 1972, when Campbell et al diagnosed a case of anencephaly, which consequently was terminated.[34] The development of real time U/S and the use of an advanced high resolution transducer, linked with an increase in diagnostic knowledge and experience of operators had led to an escalation in the diagnosis of an increasing range of fetal structural anomalies.

Several reports have addressed the use of U/S as a screening tool for detecting structural anomaly[35-39], and have demonstrated the ability of scanning to detect 40-85% of fetal abnormalities.[38] The efficiency of prenatal U/S in detecting anomalies is expressed statistically by its sensitivity and the specificity test, defined as.[40]

i) Sensitivity of the test: indicates the proportion of affected individuals having an abnormal result.

ii) Specificity of the test: indicates the proportion of healthy individuals with a normal result.

The sensitivity and specificity of U/S examination for detecting major fetal malformations in low risk group are shown in Table 2.1. in all these studies the anomalies reported at postnatal

examination or autopsy were considered the standard with which ultrasonographic findings were compared.

Table 2.1. The accuracy rates of U/S examination in the low risk population.

	Brocks et al[35] >25 wks (14,297) 1983-89	Rosendahl et al[36] 18 wks±34 wks (9012), 1980-88	Luck[37] 19 wks (8523) 1988-91	Shirley et al[38] 19 wks (6183) 1989-90	Gonçalves et al[39] ≥16 wks 1987-91	Chitty et al[46] <24 wks (8785) 1988-89
Sensitivity	54%	58.1%	85%	60.7%	53%	74.4%
Specificity	99%	99.9%	99.9%	99.8%	99%	99.9%

Figure between parenthesis represent the number of the examined cases.

On the other hand, higher sensitivity in the range of 86-99%, was found for high risk population.[41,42,43] For example, Manchester et al[41] found high rates for both sensitivity and specificity rates of 99% and 91%, respectively. The study was conducted at the University of Colorado (USA) and involved 257 cases who were referred at 12-40 wks' gestation due to suspected anomaly on U/S examination elsewhere. Furthermore, Sabbagha et al[42] have recorded rates of 95% and 99% for the sensitivity and the specificity, respectively when they examined a sample of 615 cases who previously underwent targeted imaging mostly but not all before 24 wks' gestation.

Table 2.2 . Types of anomalies missed by antenatal U/S.

Study	Missed anomalies
Chitty et al[46]	Holoprosencephaly, microphthalmia, cleft lip & palate, pleural effusion, hypoplastic left heart, single atrium, atrioventricular canal defect, tracheoesophageal fistula, duodenal atresia, ectopic vesica, unilateral multicystic kidneys, bilateral renal dysplasia, bilateral tabia and foot deformity, missing digit on one hand, talipes, absent hand, and sacrococcygeal teratoma.
Manchester et al[41]	**Intracranial:** holoprosencephaly, microcephaly, agenesis of the corpus callosum, intracranial calcification, cystic encephalomalacia, aqueductal stenosis, aterio-venous malformation, and brain stem anomalies. **Craniofacial:** cleft lip & palate, nasal cleft, microphthalmia, hypoplastic mandible, microtia. Thoracic: tracheoesophageal fistula, tracheal stenosis, pulmonary hypoplasia, and diaphragmatic hernia. **Cardiac:** Atrial septal defect, ventricular septal defect clonal/truncal, great vessels, hypoplastic left heart, coarctation of the aorta, and myocarditis. **Abdominal:** imperforate anus, intestinal malrotation, intestinal atresia, omphalocele, meconium peritonitis, and Hirschsprung's disease. **Genito-urinary:** obstructive hydronephrosis, adrenal hypoplasia, extrophy of the bladder, ambiguous genitalia, hypospadius, micropenis, and persistent cloaca. **Musculo-skeletal:** arthrogryposis, vertebral/rib anomaly, and limb reduction.
Luck[37]	Distal oesophageal atresia, traceoesophageal fistula, diaphragmatic hernia, cystic adenomatoid malformation of lung, hypoglossia, hypoplastic left heart, hypoplastic right heart, total anomalous of inflow and out flow, ventricular septal defect, atrial solitus, truncus arteriosus, coarcotation of aorta, Fallot tetralogy, transposition of great vessels, and pulmonary atresia
Rosendahl et al[36]	Imperforate anus, omphalocele, diaphragmatic hernia, transposition of great vessels, aortic stenosis, Fallot tetrology, ventricular septal defect, and tracheoesophageal fistula.

It is noteworthy that U/S visualisation is affected by many factors some of which are listed below.

1. Stage of gestation: the age at which the U/S is performed is an important factor. In early scanning many anomalies are not easy to detect because they are either too small to be visible or they are not present at the time of scanning (late presentation of some disorders, such as fetal hydrops, obstruction of urinary tract, and cardiac abnormalities). Thus, the accuracy of U/S examination will generally increase as gestation advances. Rosendahl et al[36] have found that in only 39.4% of malformation cases the diagnosis was made before 24 wks, and the detection rate increased with repeat routine examination from 41.7% at one examination to 63.8% at the second examination. Gonçalves et al[39] have reported that below 20 wks' gestation, the sensitivity of U/S in detecting any anomalies was 47%,

between 20-23 wks was 59% and at 24 wks or more was 68%. However, the introduction of the vaginal U/S probe would allow earlier screening with the same or improved sensitivity and specificity rates.[14]

2. Amniotic fluid status. Congenital abnormalities have been reported to occur in 4-18% of pregnancies with polyhydramnios, and in 9-13% of those with oligohydramnios.[36] However, both of these conditions interfere with proper visualisation.[41]
3. Fetal position: U/S visualisation can be greatly affected by the position of the fetus, but this can be overcome by repeat examination.
4. Maternal obesity: Maternal overweight affects the penetration of U/S waves, thereby reducing the accuracy rate.
5. Malformation type: A high detection rate of congenital anomaly by U/S is found for obvious malformations of the central nervous system (CNS) and the urinary system compared with the other systems. The sensitivity and specificity rates of U/S for detection of Spina bifida in a high risk population are 98% and 100%, respectively.[44] According to Rosendhl and Kivinen[36] the sensitivity and specificity for detection of urinary tract malformation are 84.6% and 99.9%, respectively. In contrast, a low sensitivity rate of 20% for cardiac anomalies has been documented.[35] It is noteworthy that the presence of multiple congenital anomalies is associated with a low detection rate as compared with an isolated anomlies.[19]
6. Fetal outcome: U/S examination on fetuses who died in utero are of limited value because of the lack of fetal activity and presence of oligohydramnios. Therefore, their anomalies could be easily overlooked. The relatively rapid anatomical changes that occur after fetal demise can also result in false positive sonographic diagnosis of congenital anomaly.[45]

Various studies conducted to determine the specificity and sensitivity of antenatal U/S examination. The different types of anomaly missed are summarised in Table 2.2.

2.3 External physical examination:

External physical examination allows observation and documentation of fetal development, fetal nutrition and any evidence of congenital anomaly. It might also permit estimation of the time of death which, could help to explain the death.[47] External measurement is important for evaluating syndromic infants and in determining the state of growth as well as assessing GA.

2.4 Photographic documentation:

Some inherited diseases have special morphological stigmata that help in reaching the proper diagnosis, other abnormalities may form shapes which are difficult to describe. For all such cases, photographic images could be obtained and later used for further assessment. These images could also be sent to obtain consultation and opinion on unusual cases.

2.5 Radiography:

The use of radiography in PM examination goes back as far as 1896.[48] Its value in providing information relevant to the cause of death has been well established. Winter et al[49] in his study of 488 cases reported that plain radiography was a useful diagnostic tool for 40% of cases with external malformation, 100% of dwarfism cases, 9% where there were no external malformation and 16% of all cases. A similar figure of 18% has been published by Foote et al[50] and Griscom et al[51] for 2500 and 488 cases respectively. Kalifa et al[52] studied 400 cases of fetal death in order to assess the value of PM radiography, and found that plain radiography examination was valuable to provide final diagnosis for 13.5 % of all cases. However, additional information was gained by this examination for 34.5% of all cases.

PM radiography is essential for the diagnosis of lethal bone dysplasia. Consequently, the prevalence of the dysplasia can be determined and the genetic implications of an abnormal baby can be estimated. Ryans and Kozlowski[53] 1971 stressed that without radiography the detection of such lethal bony dysplasia can be missed. Radiography also provides clues to the

aetiology of some deformations caused by intrauterine infection, such as the presence of intracranial calcification and celery stalk changes which may indicate rubella or CMV (cytomegalovirus) infection.[54] Rough estimation of GA can be judged through the appearance of ossification centres, although its status is quiet variable as they are affected much by many pathological conditions,[50] such as intrauterine growth retardation (IUGR), and congenital malformation, as well as by sex and race.

Radiography is also necessary for diagnosis of certain condition, such as abnormal collections of gas in the thorax or abdomen (pneumothorax, pneumoperitoneum), abnormal calcification, and evidence of trauma during delivery. In addition, it provides a skeletal survey to detect abnormal ribs, vertebrae, and long bones. Furthermore, radiography is important for diagnosis of the VATER association. Radiography provides a permanent record with objective images available to answer questions about human development and disease as they arise.[51]

The argument about routine radiography has been raised by Griscom et al[51] who studied 340 cases. It was found that radiography was a useful diagnostic tool for 75% of cases of a selected group with a positive family history or where the baby had external malformations, compared with 18% in an unselected group. However, Winter et al[49] have recommended its use in all cases, since they found major skeletal abnormalities in 3 cases where there was no external malformation.

Contrast studies can also be performed to demonstrate CNS, urogenital and vascular anatomy. A PM angiogram has been reported[50] as a method of diagnosing congenital heart disease, but the limitation of this examination is that some functional problems can not be studied for example, direction of blood flow. The chamber size and vessel diameter are also changeable and depend much on the pressure used for the injection as well as the amount and type of the contrast media.

2.6 Image modalities:

Diagnostic images can be used to disclose any gross internal abnormalities. Recently, the use of both U/S and Magnetic Resonance Image (MRI) as tools of non invasive PM has been examined.[55,56,57]

2.6.1 Postmortem ultrasound examination (U/S):

Furness et al[55] have extensively studied the role of U/S in the perinatal PM. They concluded that there is a potential use of U/S in PM by contributing information in specific circumstances. PM U/S can provide detailed information on fetal brain anatomy, such as the status of gyral development and ventricular size even in the presence of maceration. It was suggested that U/S has an important role in auditing antenatal U/S findings by exploring areas of over-diagnosis and under-diagnosis. Furthermore, they suggested that it was possible to supplement PM U/S with contrast studies and NB to confirm the antenatal diagnosis of lower urinary tract obstruction.

This study showed the possibility of using U/S as a supplementary tool in PM. Although U/S has the advantage of being available to most hospitals world-wide, it has the drawbacks of being time consuming and requiring a combination of good quality apparatus and highly skilled and experienced operators familiar with a whole new set of artefacts.

2.6.2 Postmortem Magnetic Resonance Imaging (MRI):

The value of MRI examination in the field of obstetrics has been widely studied.[58,59,60] Powell et al[58] at University Hospital, Nottingham have studied 36 obstetric patients between 10-38 wks to determine the fetal anatomy that could be visualised at different gestations. He found that both fetal brain and lung can be clearly visualised by MRI. In the former, the process of physiological myelination can be studied extensively. Maturity can be assessed by means of

the latter. Generally, MRI is considered superior to U/S examination in delineating CNS abnormalities and soft tissue gastrointestinal abnormalities.[60]

It has been reported that fetal movement has a negative influence on image quality as it produces artefacts. This results in limiting its role in antenatal diagnosis. This view was supported by McCarthy et al[59] at University of California, San Francisco (USA) who recommend that U/S remains the primary choice in the antenatal diagnosis, while MRI is considered to be as a complementary approach in difficult cases.

The efficiency of MRI as a non-invasive method of perinatal PM has been investigated by Ros et al[56] who examined 6 cases including 3 stillbirth cases and 1 neonatal death. It was found that MRI is adequate in detecting gross cranial, pulmonary, abdominal and vascular pathology. It may be even superior to autopsy in detecting the presence of air or fluid in potential spaces of the body, but inferior to autopsy in detecting very small pathology such as microabcesses, petechiae, microinfarction, and focal haemorrhage.

A recent study on the application of MRI in perinatal PM was conducted by Brookes et al[57] and in a sample of 20 cases, autopsy and MRI were in total agreement about detectable abnormalities in 8 cases. MRI was superior in 4 cases, and inferior in the remaining 8 cases, where the autopsy provided more detailed information than MRI examination. In general, there was broad agreement between MRI and autopsy about the diagnostic findings in all cases. Nevertheless, in two cases autopsy provided more important clinical diagnostic information. The first case was diagnosed by autopsy as cystomegaly and bladder wall hypertrophy without upper tract involvement. In the second case, histological examination revealed periaquedactal haemorrhage as the cause of hydrocephaly.

This study also confirmed the ability of MRI examination to detect CNS abnormalities; two CNS anomalies, namely hydrocephaly and bilateral intraventricular haemorrhage were only picked up by MRI examination. This is could be explained to the texture of brain tissue as in many instances it becomes too friable for detailed macroscopic or microscopic examination.

MRI allows examination of the brain in situ without anatomical or hydrostatic disturbance.[57] Additionally, detailed information about certain abnormalities of the musculoskeletal system (acetabular dysplasia) was only available by MRI examination. According to this study, MRI is limited in detecting cardiac anomalies.

Generally, MRI is a time-consuming, relatively inflexible technique, and an expensive tool. It is therefore, unsuitable for application in PM examination in many countries.

2.7 Microscopic examination:

Microscopic examination is of value for detecting the mode and the cause of death, intrauterine stress and presence of intrauterine infection, even in cases of severe maceration.[61] Microscopic examination of the lung and the kidney help in the assessment of GA.

Some studies have indicated routine histological examination is not necessary, and should be confined to cases where gross macroscopic abnormalities are found.[10,26] However, the general attitude amongst pathologists is to consider microscopic examination as a routine procedure in all PM cases. This is due to the fact that histological examination is the only way to diagnose many abnormalities such as myocarditis, congenital hepatic fibrosis, intrauterine pneumonia, hyaline membrane disease, different types of pulmonary hamartomas and perinatal hypoxic brain injury. It is also essential to reach a definitive diagnosis in cases of Potter syndrome, Meckel syndrome, Thanatophoric dysplasia syndrome, polycystic kidneys and other renal abnormalities. Porter & Keeling[17] studied a sample of 150 cases of stillbirth and 150 cases of neonatal death. In their study, histological diagnosis was essential to reach final pathological diagnosis for 20% (18/90) of cases where autopsy findings did not agree with the clinical diagnosis. Histological examination was helpful in almost twice as many normally formed fetuses as in those with abnormalities on gross examination. It is noteworthy that the experience of normal microscopic appearance in organs at different gestations is necessary to

interpret abnormal histology. The Royal College of Pathologists has adopted the histological examination of organs as part of its scoring system for a proper perinatal PM (see Table 1.3). Samples for microscopic examination can be obtained either during the autopsy procedure, or by more recently developed non-invasive approaches, such as fine needle aspiration (FNA) and needle biopsy (NB). Both of these procedures can be applied through intact skin blindly, or under direct imaging. The object of this study is to evaluate the validity and efficiency of this non-invasive approach.

2.7.1 Cytodiagnosis:

Cytodiagnosis is a well accredited technique with a wide range of a clinical applications. Fine needle aspiration (FNA) is now used in many hospitals and clinics throughout the world as a first line of investigation for patients suspected of having malignant diseases. This technique is non-invasive, non disfiguring and gives rapid, but less accurate information than histological diagnosis. Clinically, cytodiagnosis has been used as method of investigation since the 19th[62] century, when attempts were made to describe the microscopic appearance of the cells in blood, pus, ascitic fluid, and urine. Dr. Donaldson (1853) made impression smears from the cut surfaces of tumours.

Regarding the role of cytodiagnosis in PM examination, there have been attempts to explore its validity,[63,64] but its diagnostic value as a non-invasive approach of PM examination has not been fully evaluated. Survarna & Start[63] reported their experience with adult cadavers in diagnosing different neoplastic and infectious conditions. A direct smear was obtained from different organs at full autopsy and stained by the modified Giemsa technique. The aim of his work was to evaluate the possibility of obtaining rapid microscopic confirmation of the initial macroscopic assessment. It was found that cytodiagnosis is a quick and cheap technique as it requires inexpensive and readily available material as well as minimal number of staff. These

make the technique suitable for places where financial resources are limited. It may be used as adjunct to autopsy, to facilitate the generation of necropsy reports[64].

On the other hand, the technique has some limitations, as tissue autolysis may render some samples unsatisfactory for analysis. Furthermore, loss of the architectectural details of the extracted sample makes cytodiagnosis inadequate for diagnosis of pathological conditions that mainly depend on the presence of a disorganised architectecture[63].

Coincidentally, Iwa and Yutani[65] have recently described their experience in cytodiagnosis as they detect parvovirus B19 in the ascitic fluid of a hydrops fetalis case. The procedure was conducted in utero on a fetus of 25 wks' gestation. The aspirated ascitic fluid was centrifuged to prepare smears, which were then stained by the Papanicolaou method. Important cytological findings were found on microscopic examination when a ground-glass appearance of nuclei and perinuclear halo were noted. Subsequently, monoclonal antibodies for parvovirus B19 were applied to the de-stained smear to identify specific antigen in the smears. Positive staining reaction were occurred through out the cytoplasm of infected erythroid cells. This work showed that cytodiagnosis would be valuable in perinatal PM cases, particularly as a non-invasive approach.

2.7.2 Needle biopsy (NB):

The use of core needle biopsy on adult cadavers has been developed mainly, as an alternative approach for cases where full autopsy could not be performed. The idea of using core NB has originated from *Manson-Bahr* in 1950, when its use was suggested for the investigation of yellow fever or Leishmaniasis in South America, as autopsy was not permitted by the indigenous people.[66]

In the 1950s, Terry was the first who described it as needle necropsy.[67] In this study, tissue from various sites was obtained, and a diagnosis was reached in 22 out of 24 needle necropsy.

In a study of 50 adult cases published in 1957, West[67] found that autopsy findings differed from NB findings in about 52% of all cases. The discrepancy was attributed to inadequacy of NB, due to the limited accessibility of the needle to certain sites and organs, blindness of the procedure, or samples being unrepresentative of the whole organ or system. On the other hand, Baumgart et al[68] carried out NB on series of 16 cases who died from the Acquired immunodeficiency syndrome (AIDS). The degree of certainty regarding the cause of death was increased from 44% on clinical ground alone to 93.8% after NB. The technique was recommended for cases which have high risk of infection, such as Acquired Immunodeficiency syndrome (AIDS). It is also the indicated procedure for obtaining tissue needed for special investigations soon after death. However, with NB, it is not possible to assess any organ in situ and describe their gross appearance. It may be ideal for detecting conditions that cause diffuse changes in organs rather than focal pathology. In general terms, NB is most likely to be successful when there is an obvious mass or an enlarged organ. This implies that diseases which produce a palpable mass are more likely to be successfully diagnosed by this method.

2.8 Placenta, membranes, and cord examination:

The placenta is a unique organ of limited life. It manufactures some of the essential hormones required for maintaining the pregnancy and acts as respiratory and alimentary organ. By interposing between two separate individuals (mother & fetus), it acts as a site for maternofetal and fetomaternal exchange.

Both maternal and fetus disorders could affect the placental status. In fact, some genetic or metabolic errors, infections, and immunological disorders could be detected by macroscopic or microscopic placental examination. Placental abnormalities are found in association with a number of maternal disorders, such as diabetes mellitus, pre-eclampsia, and antiphospholipid disorders.

The placenta can be viable for many days after delivery hence, its microscopic examination is entirely reliable up to one week and is considerably useful for up to 4 wks.[69]

The importance of placental examination was studied by Rayburn et al[69] (1979-1983) at University of Michigans women's Hospital. The study included 89 cases of fetal death beyond 20 wks' gestation. Almost all cases (98%) had abnormal placental histological findings, while the percentage of abnormalities detected by clinical examination and autopsy were 87% and 70%, respectively. Furthermore, placenta examination was supportive to both clinical and autopsy examination in 77% of cases.

Curry[70] has studied 378 cases of pregnancy loss and found that fetal death could be attributed directly to placental or cord abnormalities in 15.6% of cases. In a further 6.3%, ascending infection was noted on placental examination.

Placental examination can produce vital information related to several clinical presentations of fetal and perinatal deaths, such as twins, prematurity, Intrauterine growth retardation (IUGR), chromosomal abnormalities, metabolic error and intrauterine fetal infection. This can be of importance in countries with limited financial resources as such information could be reached without the need for the cytogenetic studies and sophisticated biochemical laboratory tests.

In twin pregnancies a monochorial placenta is associated with an excessive fetal mortality rate, due to placental vascular connections resulting in a net imbalance of blood flow which in turn is presented as classical twin-to-twin transfusion syndrome (TTTS) either chronic or acute. Chronic TTTS is more frequent, and occurs during mid gestation rather than around the time of labour. Classically, chronic TTTS manifests itself as a low birth weight, anaemic donor and a heavy, plethoric, and sometimes hydropic recipient. These features may be accompanied by oligohydramnios in the sac of the donor and polyhydramnios in that of the recipient. These changes are the result of a gradual antepartum transfusion through deep artery-to-vein anastomoses.

By contrast, the acute form has been attributed to rapid transfer of blood through superficial artery-to-artery or vein-to-vein anastomoses during labour, resulting in a hypovolaemic, anaemic donor twin and a hypervolaemic, plethoric recipient twin of a similar weight.

While, the monochorial placenta can be confirmed by examining the membranes, in particular the septum between the two amniotic cavities, the vascular connection is better assessed by injection of the vascular tree of the placenta with a contrast medium. However, it can be difficult to identify the vascular connection, as the clinically most significant anastomoses (unidirectional arteriovenous shunts) pass through the parenchymal villous tissue (third circulation) and are quite small and hard to demonstrate. TTTS has traditionally been diagnosed or confirmed after birth by the discovery of a haemoglobin difference between twins of more than 5 gm/dl and birth weight difference of more than 20%, but controversy still exist as to the best diagnostic parameters for this syndrome.[71]

In prematurity cases the most frequent finding in the placenta of spontaneous deliveries between 20 and 30 wks of gestations is chorioamnionitis. There is good evidence indicating that ascending placental infection, which causes labour, is frequently present before rupture of the membranes and may have a devastating impact on fetal well-being. Ascending infection leads to inflammation of the membranes and cord (funisitis), but the villi are rarely involved.

Inflammation of membranes and cord are readily apparent by placental examination. The loss of membrane translucency and foul smelling of placenta could be noted on macroscopic examination. In early infection, the infiltration of polymorphonuclear leucocytes of maternal origin can be detected microscopically in the membranes nearest to the internal cervical os. Thereafter, maternal leucocytes accumulate beneath the chorionic plate and become embedded in fibrin. The infection is then extended into the adjacent intervillous space. At first, polymorphs are maternal in origin, but later a fetal response also develops which is first evident as a vasculitis in the chorionic plate.

Cases of IUGR may have a specific placental pathology that is also best identified by placental study. Histopathologic features of reduced placental perfusion include non-marginal infarcts, shrunken placental villi, increased syncytial knots, and increased perivillous fibrin. When the degree of reduction in uteroplacental blood flow is severe, the fetal response manifests itself in

the form of increased fetal nucleated red blood cells, thrombi in fetal blood vessels and haemorrhagic vasculopathy.[72] Placental histopathology of antiphospholipid disorders is indistinguishable from that of pre-eclampsia, as both conditions are associated with low uteroplacental blood flow and IUGR babies.

Placentas of infants with autosomal trisomy are often small. Histologically, findings, such as cystic and dysmorphic villi with stromal trophoblastic inclusions suggest chromosomal anomalies.[69] In Turner's syndrome, hydropic villi, mineralization of trophoblast basement membranes, and massive subchorial thrombosis are also described.[73,74] Occasionally, the connection between the malformation and placental finding is obscure. For example, vacuolation of the amniotic epithelium correlates with the diagnosis of gastroschisis, but not with omphalocele and has been determined to be lipid but, its origin is not yet known.[73] Many complex malformations are associated with absence of one of the umbilical arteries. Sirenomelia sequence and VATER association, trisomy 13, trisomy 18 and Zellweger syndromes are typical examples of this association.[74] The incidence of single umbilical artery is from 0.2% to 1.1% of all births and increases up to 50% in malformed cases.[74]

It is noteworthy that the cause of some congenital malformations can be clarified only by placental findings. The classical example is the bizarre constellation referred to as an early amnion rupture sequence which can only be diagnosed and confirmed by the histological demonstration of a chronic rupture of amnion. In such cases, the chorial plate is mostly devoid of amnion

and lined only by vascularized chorion.[70] In cases of inborn errors of metabolism, mainly lysosomal storage disorders, vacuolation of syncytiotrophoblast can occur, for example in the placenta of a fetus with GM1 gangliosidosis.[74]

Nevertheless, there are certain placental lesions, in which the causes are often undetermined, but they clearly indicate some antenatal disturbance that may have significance for fetal development.

Characteristically, they are recurrent, and may contribute to repeated mid and late pregnancy loss.[73] Examples of these lesions are Villitis, Haemorrhagic endovasculopathy and Maternal floor infarction.

Villitis:

Villitis refers to an inflammation of the chorionic villi. Some patterns are associated with blood born infection, particularly viral ones, but in 98% of cases no organism is isolated and serological investigation results are negative.[74] This is known as chronic villitis of unknown aetiology. There has been considerable debate regarding its cause[72,74], some attribute it to a state of immunological aberration in the form of graft-versus-host disease, others regard it as being the result of undiagnosed infection.

Villitis is commonly prevalent in the placentas of IUGR babies.[74] It may be diffuse in distribution throughout the parenchyma or confined to the parabasal area close to the decidual surface. The type of inflammatory cells are usually lymphocytic or histocytic.

Maternal floor infarction:

Maternal floor infarction refers to fibrin deposits in the intervillous space of the placenta in the basal region. It presents itself grossly as a pale thickening of the maternal surface of the placental disc. The etiopathogenesis is not fully understood, but it can be associated with fetal growth retardation and intrauterine death.[73] The reason for its recurrence is unknown, as the cause has not yet been determined.

Haemorrhagic endovasculopathy:

Haemorrhagic endovasculopathy is a lesion of unclear pathogenesis and has been associated with fetal growth retardation, fetal death and perinatal distress, but it has also been seen with apparently normal infants.[73] Unless it is extensive, the lesion is not easily identifiable, and is usually found only on microscopic examination. The lesion may involve any segment of fetal circulation beyond the umbilical cord, but is most common in medium- sized villi. It consists of a loss of definition in the walls of the vessels.

Chapter I. 3

Classification and Causes of Fetal and Neonatal deaths

In this chapter, the pattern of causes of fetal and perinatal death will be reviewed in order to assess the reliability of non-invasive perinatal PM examination in achieving a valid diagnosis of the different categories of disease.

3.1 Trends:

A dramatic and universal fall in neonatal deaths and fetal deaths has been achieved in the last three decades. In the UK, perinatal mortality rate in 1958 was 30.5/1000,[75] compared to 8.8/1000 in 1986.[76] In Finland perinatal mortality rate fell from 19.9/1000 in 1968 to 7.4/1000 in 1982.[77] This reduction, as out lined below, is attributed to several factors, such as improvements in antenatal, intrapartum and neonatal care, as well as changes in social and biological attitudes of the family.

i) Antenatal care

- The use of tocolytic drugs and corticosteroids as well as control of urinary tract infection during pregnancy, has led to some reduction in the incidence of prematurity or its complications, which are critical contributors in neonatal death cases. On the other hand, improvement in antenatal care combined with advances in neonatal intensive care offer better chances to mothers with chronic illness to have a healthy baby.
- The availability of preconception care has reduced the representation of some maternal disease, such as diabetes mellitus, which is known to be responsible for many perinatal deaths.
- Prenatal screening is readily accessible for many infectious agents such as TORCH screening, leading to proper management for infected cases.

- Administering anti D immunoglobulin has had a dramatic effect in diminishing hydrops fetalis (immune type) cases.
- Mass vaccination of all children to eliminate endemic rubella has led to a marked decline in its contribution to perinatal mortality rate.
- The accessibility of prenatal diagnosis for congenital anomalies has some influence on perinatal mortality, since early detection and termination of such cases before fetal viability has led to a decrease in the fetal and neonatal malformation element in perinatal deaths.[78]

ii) Intrapartum care:

Intrapartum deaths have been remarkably reduced due to;

- availability of intrapartum fetal monitoring system
- active management of labour
- increase in the rate of Caesarean sections.

iii) Neonatal care:

The rapid advances in neonatal intensive care have had a major dramatic effect on reducing neonatal deaths. In particular, low birth weight infants have shown a significant beneficial effect.[79] In Australia neonatal deaths have dropped by 55% from 12.9/1000 in 1971 to 5.9/1000 in 1981, while stillbirth rate has dropped only by 29% from 12.24/100 to 8.65/1000 for the same period.[80] A similar pattern has been demonstrated by Morrison[81] in his series of perinatal deaths (1977-1981) in Manitoba (Canada), when he found that the representation of neonatal deaths in the perinatal mortality rates has dropped from 53% in 1977 to 40% in 1981.

iv) Social and biological factors:

Changes in maternal age group and parity accounted for a quarter of the overall improvement in perinatal mortality.[80,82]

3.2 Prediction of risk for fetal and neonatal deaths:

- **Maternal age**

- Maternal age generally is represented as a U-shaped curve in relation to the risk of perinatal deaths; very young and older mothers having the highest rates. For the former group, this could be explained by poor development of the uterus and its blood supply. Whereas for the latter group, the reason could be the prevalence of diseases, such as diabetes mellitus and hypertension, that cause generalised vasculopathy including uterine vessels. In addition, they have a relative high birth rate of infant with chromosomal disorders.

- **Parity**

Parity is measured as the number of previous pregnancies that ended with live births or stillbirths. Second or third pregnancies are considered to be at the lowest risk of perinatal death, beyond that there is a constant increase of the risk with higher parity due to an increased frequency of antepartum haemorrhage (APH), hypertension, malpresentations and umbilical cord complications.

- **Past obstetric history**

There is an increased risk of recurrence of perinatal death following an affected pregnancy. This recurrence could be due to a similar combination of genes in the conceptuses, but more often to continuous exposure to the same biological and environmental hazards. Therefore, the outcome of the preceding pregnancy is considered to be the most important factor in estimating the risk of the current pregnancy. In Britain, the risk of perinatal loss in the current pregnancy rises by 200% if there has been a previous history of perinatal death.[13] This risk increases further if the

perinatal loss happened in the immediately preceding pregnancy. The fetal death rate has been reportedly increased with prolonged intervals between pregnancies. Conversely, a shorter interval carries an increased risk of neonatal death[13]

- **Ethnic group**

In the USA the perinatal mortality rate among the black population exceeds the rate for white. In the UK immigrants have a higher perinatal mortality rate than amongst indigenous population; while socio-economic factors play a role in this difference, an increase in incidence of malformations among some group of immigrants, as result of traditional consanguineous marriage, is considered to be an additional factor.[76,83]

- **Bleeding**

A history of bleeding in pregnancy is associated with an elevated risk of perinatal death. Placental abruption is associated with a high mortality rate, but statistically more deaths are actually related to non specific APH or an early bleeding in the first two trimesters.[13] Moreover, an association between first trimester bleeding and an increase in the incidence of malformations has been reported by Hovatta et al.[84]

- **Birth weight.**

There is an inverse relationship between perinatal mortality rate and birth weight up to 3500 gm, where this relationship is inverted to an upturn, with heavier infants being slightly more prone to perinatal death.

- **Alphafetoprotein test:**

Alphafetoprotein (AFP) is the major protein produced during early fetal life by the yolk sac and then by the fetal liver. AFP reaches the amniotic fluid early in gestation by diffusion from the yolk sac and through the non keratinized fetal skin; then later, once the kidneys start to function, in the fetal urine. The AFP level in the amniotic fluid is about 2% of that in fetal serum.[85]

AFP crosses the placenta and membranes to reach the maternal circulation, where its level is about one ten-thousandth of the level in fetal serum. The maternal serum AFP (msAFP) level continues to rise until 32 wks thereafter remains constant till the term. Table 3.1 shows the different situations associated with increase in msAFP or amniotic fluid AFP.[85] In contrast, low level of msAFP has been noted to be associated with Down's syndrome. More complex combined msAFP tests such as the "triple test" is becoming widespread. This test consists of measurements of the level of the following in maternal blood:

1. msAFP. (Low in Down syndrome)
2. Unconjugated oestriol. (Low in Down syndrome)
3. Human chorionic gonadotrophin (HCG). (raised in Down syndrome)

The results of two or all of these assays can be integrated mathematically with maternal age and U/S dating to produce a statistical risk for each pregnancy that the fetus has Downs syndrome.

Moreover, possible relationship between an unexplained high second trimester msAFP level and risk of subsequent fetal death, neonatal death and low birth weight has been raised by several studies.[86,87,88] Burton[87] has studied the outcome of pregnancy in 350 obstetric patients with unexplained elevation of msAFP. Their outcome data ranged from fetal death in 14 cases (4%), low birth weight (<2.5kg) in 49 cases (15%), neonatal death in 7 cases (2.1%) and congenital anomalies in 26 cases (7.4%).

Table 3.1. The main causes of high AFP in maternal serum or amniotic fluid in the second trimester[85].

Elevation AFP	Pathogenesis	Association
Maternal serum	Physiological: increased production	Multiple pregnancy
	Pathological: loss of placental surface integrity	Abdominal pregnancy Fetomaternal haemorrhage Placental angioma
Maternal serum & amniotic fluid	Interruption of cutaneous integrity	Neural tube defect Omphalocoele Gastroschisis Amnion rupture sequence Meckel' syndrome Scalp defect
	Altered skin permeability	Missed abortion
	Increased urine production	Monoamniotic twins
	Defective renal function	Congenital nephrotic syndrome Polycystic kidneys
	Concentration because of oligohydramnios	Renal agenesis
	Large/abnormal placenta	Triploidy

3.3 Classification of perinatal deaths:

Perinatal deaths have been classified in different ways by Epidemiologists, Obstetricians, Neonatologists and Pathologists, each having their own approach. For obstetricians, the documentation of the primary incident or primary factor that initiated the chain of the events leading to each death is the first priority. Such classification described why the death has happened, but not how it happened and was first adopted by Baird et al (Aberdeen classification 1954) and later was developed by Baird & Thomson (1969).[76] Recently, two attempts have been carried out by Cole et al (1986)[89] and Whitfield et al (1986)[90] in order to expand and revise this classification (Table 3.2).

On the other hand, the clinical features of the perinate are the basis of the well known clinico-pathological classification (Wigglesworth classification 1980)[91] which consists of 5 groups:

1. Normally formed macerated stillbirth, including antepartum asphyxia.

2. Congenital malformation (stillbirth or neonatal death).

3. Asphyxial condition developed in labour (Fresh stillbirth or neonatal death).

4. Condition associated with prematurity (neonatal death).

5. Specific condition other than above (stillborn or neonatal death).

This classification is simple. It also has the advantage of being easily interpretable. An excess of one category could indicate an area of inadequacy in health care. For example, many deaths in the congenital abnormality group suggests that more attention should be paid to preconception care and prenatal diagnosis. However, there have been attempts to modify and clarify this classification (Table 3.3). [92,-94]

In fact, in order to obtain a set of data with a high degree of accuracy and consistency PM is needed, since clinical diagnosis is not always 100% accurate. These data then allow the regional perinatal mortality committees to study and monitor the pattern of fetal and neonatal deaths. However, the aim of this study is to asses the efficiency of non-invasive methods of perinatal PM examination in comparison to the full autopsy, in providing information related to the cause of death.

Table 3.2. The original Aberdeen classification and the other two modified classifications.

Aberdeen classification	Baird & Thomson classification	Cole et al classification
Premature unknown	Malformation	Congenital anomaly
Mature unknown	Serological incompatibility	Isoimmunization
Mechanical	Toxaemia	Pre-eclampsia
Toxaemia	APH	APH
Maternal disease	Mechanical	Mechanical
APH	Maternal disease	Maternal disorder
Deformity	Infection of the fetus or infant@	Miscellaneous
Blood group incompatibility	Miscellaneous	Unexplained ≥2.5 kg
Others	Uncertain. Mature or Premature	Unexplained < 2.5 kg
-	Unclassifiable	Unclassifiable

Abbreviation: @ = This category has been included to either Maternal disorder or Miscellaneous group in Cole et al classification.

Table 3.3. Extended Wigglesworth classification

1. **Congenital defect/malformation (lethal or severe):** Multiple minor anomalies that form a complex and they had died before labour, should be included.
2. **Unexplained antepartum fetal death:** Most early losses should be coded here. Any baby dies later because of problems during the antepartum period, code this as "specific causes". **2A-Placental abruption:** Before onset of labour.
3. **Death from intrapartum "asphyxia", "anoxia" or "trauma":** This group covers any baby who would have survived but for catastrophe occurring during labour . This means, all the fetal death of whatever weight, without malformation or specific condition and provided that death occurred during labour (Fresh stillbirth). Live -born infants weighing over 1000g, who died at less than 4 hours of age should be included in this category (neonatal death). Any infant surviving longer than 4 hours, for whom there was evidence of cerebral birth trauma or asphyxia should be also included.
4. **Immaturity:** Include only live births of under 37 wks' gestation (neonatal death),who subsequently die from problems of prematurity. Infants weighing less than 1000g should be presumed to belong to this group irrespective of the time of death.
5. **Infection:** Where there is clear evidence of infection before, during, or after delivery that could have caused death (stillborn or neonatal death).
6. **Due to other specific causes:** This include all specific recognisable fetal, neonatal or paediatric condition not covered under the earlier categories. Examples include:

1-fetal conditions: Twin to Twin Transfusion syndrome (TTTS) and hydrops fetalis;

2-neonatal conditions: pulmonary haemorrhage and pulmonary hypoplasia due to prolonged spontaneous rupture of membranes.

3-paediatric conditions: malignancy and acute abdominal catastrophe.

7. **Due to accident or non intrapartum trauma.**
8. **Sudden infant death, cause unknown:** this will include all infants in whom the cause is unknown or unsuspected at the time of death.
9. **Unclassifiable:** to be used as a last resort.

3.4 Causes of fetal death:

Fetal death can be divided in to two groups, depending on GA, previable fetal death, which starts from 11 wks' gestation up to 24 wks or 20 wks in some countries and late fetal death (stillbirth). In the previable fetal death, which is also known as mid trimester abortion, causes such as the presence of anatomical defects of the uterus or cervix and an intrauterine infection are common. In contrast to the mid trimester abortion, chromosomal abnormality is considered

to be the most important cause of losses in the first trimester, and was accounted for as many as 61.5% of 1498 cases.[16] The vast majority of such errors arise denovo and are not liable to recur in subsequent pregnancies.

Late fetal death occurs in 0.7-1.0 % of all deliveries,[95] and when it occurs during labour (intrapartum death), the stillborn usually has an intact integument (fresh stillbirth). In contrast, death preceding labour (antepartum death), usually demonstrates cutaneous and visceral changes (collectively known as maceration), accounts for two thirds of stillbirth cases.[96-98] Fetal death prior to the onset of labour is difficult to explain, as the direct cause of death is found in only half of such cases, and in the rest, multiple pathological findings may exist, which may be considered as of less significance.[61]

Several studies have evaluated the frequencies of various causes of fetal death which may be related to maternal or placental or fetal conditions.[75,84,96,98,99,100] Table 3.4 shows the frequency of certain causes which have contributed to fetal death in selected series. In these studies, PM was conducted with varying rates ranging from 50 -100%, so the extent and the contribution of each cause is considered to be reliable. For reasons mentioned earlier, it is not always possible to conduct full autopsy, so the use of non-invasive methods may be useful when full autopsy is ruled out.

Table 3.4. The frequencies of the main causes of fetal death *per 1000* according to various studies.

Cause	Machin[75] ϕ 1970-73 (346), 12.1/1000*	Hovatta[8] # 1974-79 (243) 6.2/1000*	Magani[98] ϕ 1972-82 (325) 12/1000*	Brans[96] © 1978-82 (320) 10.4/1000*	Morrison[99] © 1977-82 (765)	Cartlidge[100] δ 1993 (221) 6/1000*
Congenital anomaly	210	169	200	80	100	58
Placental abrubtion	-	140	160	90	150	-
Cord accident	-	119	80	45	80	-
Infection	-	-	-	60	-	54
IUA	380	380	380	210	430	-
UFD	610	9o	110	320	190	782

Abbreviation: ϕ = stillborn ≥28 wks, # = stillborn ≥26 wks, © = stillborn ≥20 wks,
δ = stillborn ≥ 24 wks, * = fetal death rate, IUA = intra uterine asphyxia,
UFD = unexplained fetal death..
The values in parentheses are the number of stillbirth cases.

3.4.1 Maternal factors:

- Maternal pathological condition such as pregnancy induced hypertension is associated with 5-22% of fetal deaths.[84,96,99] On other hand, diabetes mellitus contributes to an increase in the incidence of congenital anomalies. Women with insulin dependent diabetes have been reported to be at about 6% risk of conceiving a malformed baby in each pregnancy.[101] Moreover, placental blood flow is reduced in diabetic patient by 35%-45% and this was noted, particularly in insulin-dependant diabetic mothers.[61] Diabetes mellitus has been reported to be a cause of fetal death in 1.2-5% of stillbirth cases.[84,96,99] Mothers with urinary tract infection reportedly have an increased risk of fetal death. Magani et al[98] reported it's prevalence in about 2.4% of 375 stillbirth cases, while Hovatta et al[84] revealed a significantly higher rate (9.1% of 243 stillbirth cases).
- Lupus anticoagulant and anticardiolipin antibodies (IgG, IgM) are associated with a high incidence of fetal death in the second as well as in the third trimesters of pregnancy. In fact, Lupus anticoagulant and anticardiolipin antibodies comprise a rather heterogeneous group of poorly defined antibodies directed against negatively charged phospholipids. these interfere with the coagulation pathway, leading to thrombosis and thrombocytopenia. As a consequence, utero-placental vascular damage occurs, resulting in significant placental infarction. These antibodies are frequently found in patients with Lupus erythematosus and other autoimmune diseases, but they may also exist without apparent disease in 1-2% of healthy pregnant women.[31] These antibodies are also found to be prevalent in 15% of women with recurrent miscarriage compared with 2% in women with no history of previous miscarriage.[102] A different mechanism which may contribute to a small proportion of fetal deaths to mothers with connective tissue disorders is the development of complete heart block in the fetus. Anticardiolipin antibodies are usually detected by solid-phase immunoassays, whereas the Lupus anticoagulant is measured as an activity that prolongs

lipid-dependent clotting reactions (activated partial thromboplastin time and kaolin clotting time).

- Fetomaternal haemorrhage is clinically significant when 20% of the fetal blood volume is lost,[103] and has been found in 10-15% of otherwise unexplained fetal deaths, and in 3-5% of all fetal deaths.[33] The standard method of detection of fetal erythrocytes in the maternal circulation is Kleihauer-Betke staining of a peripheral blood smear. This test is based on the different acid elution characteristics of fetal and adult haemoglobin.

3.4.2 Fetal and placental factors:

- Multiple births occur in 1 in 80 deliveries, the majority of these births being twins. Most twins are dizygotic, resulting from coincident ovulation of two ova with dichorionic placentation. In contrast, monozygotic twins have different types of placentation, reflecting the difference in the timing of fission after fertilisation (dichorionic diamniotic, monochorionic diamniotic, monochorionic monoamniotic). Monochorial placenta is associated with an increase in fetal death rate due to the communication between the two circulations which, may result in acute or chronic twin to twin transfusion syndrome (TTTS), and later on in fetal demise. In fact, these anastomoses can be identified in up to 98% 0f monochorionic placentas, while TTTS is only reported to occur in less than 35% of such cases.[104] This is due to a state of imbalance of blood flow across the twins' chorionic circulation. Fetal death amongst monochorionic-monoamniotic twins is higher than in (monochorionic diamniotic) twins, since the incidence of cord accidents and cord abnormalities are higher leading to an increase in mortality. In general, the mortality in multiple births is about five times higher than for singletons, and twins were three times as common amongst macerated stillbirths.[61] The antepartum death of one of the twins occurs in 2.5-3.8% of twins cases in the last trimester,[105] which eventually puts both mother and co-twin at risk of intravascular coagulation as a result of the release of thromboplastin from

dying tissue. Renal cortical necrosis and cerebral haemorrhage have been described as a sequence of co-twin death.

- Of all fetal deaths, 20-43% are attributed to intrauterine fetal asphyxia,[84,96,98,99] which is characterised by the presence of **visceral petechial haemorrhage** identified in thoracic organs, with or without squamous debris in air ways, **splanchnic haemorrhagic necrosis** in the liver, spleen, kidneys and adrenals, **cerebral subependymal haemorrhage and intraventricular haemorrhage.** Asphyxial lesions themselves do not define the cause of fetal demise, but do implicate a pathophysiological mechanism of ischaemia. Asphyxia can occur broadly in association with intrauterine infection, placental infarction, placenta abrubtion, IUGR and cord accidents.[99,106] However, not all the causes of fetal asphyxia are clearly understood, Hovatta et al[84] gave a figure of 3.3% of fetal deaths where the cause of the asphyxia was unexplained. It is fairly commonly invoked as a cause of fetal death, accounting for rates of 50-360 fetal deaths per 1000 births in selected groups, such as post-date deliveries or in fetuses of diabetic mothers.[107]
- Placental abruption implies a premature separation of the placenta from the maternal surface with decidual haemorrhage, and is considered to be the cause of death in 13-21% of fetal deaths.[84,90,98,108] It has a high prevalence in the second trimester (20 -28 wks), where it is the most common known cause of fetal death.[90,108]
- A substantial proportion of fetal deaths occur due to cord accident; true knot and cord prolapse, amounting to 8-18% of all cases of fetal deaths.[84,98,99,109] In Morrison's[99] series of 755 of fetal deaths, cord accidents accounted for 8% of all stillbirth cases. About 47% of these cord accidents occurred in fetal weight group ≥2.5 Kg. At PM, a pale groove in the neck or plethora of the face and scalp are fair evidence of cord around neck. Furthermore, the recognition of thrombus formation in the umbilical vessels and iron-laden macrophages in Wharton's jelly would indicate antemortem formation of the true knot.

- 10-30% of fetal deaths are associated with poor uteroplacental blood flow, leading to numerous large placenta infarcts. It is generally accepted that infarction occupying less than 10% of the parenchyma is insignificant.[74] However, Burke[110] considers that "any size of placental infarcts can be significant, but that their importance may be modified by other factors such as gestational age (GA) and placental reserve function".
- The rate of malformation among stillbirth cases is higher than that among the general population, most of which occur without prior indication of risk. However, congenital anomalies are more prevalent amongst babies of low birth weight.[98,99,105] This view has been supported by Whitfield,[90] who found that the highest number of malformed cases was amongst stillbirth cases weighing less than 1500 gm (39/78), and 78% of stillbirth cases with lethal anomalies occurred in infants weighing less than 2.5 kg. In fact, the association between malformation and IUGR is well established. Malformation generally occurs in 6-23% of all fetal deaths.[75,84,90,98,106,108,111] The increased availability of the antenatal diagnosis for lethal abnormalities, isolated hydrocephaly, and neural tubal defect (NTD), followed by pregnancy termination has led to a significant fall in the contribution of congenital anomalies to the perinatal mortality rate. This was clearly disclosed in the survey conducted by Northern Regional Health Authority[78] where the percentage of perinatal deaths due to congenital anomalies in 1982 was 23% compared with only 14% in 1990. Lethally malformed babies have high frequency of chromosomal error, amounting up to 49%.[112] These chromosomal anomalies are mainly confined to those cases in which the anomalies do not primarily involve the CNS.[112,113] Chromosomal abnormality was diagnosed in the British Colombia Hospital study[106] 1984-1988, in 7.2% of 817 fetal deaths (from 11 wks' gestation onward) versus 4.9% of early neonatal deaths. According to this study, the most common karyotypic abnormality was trisomy followed by monsomy X. In fact, the prevalence of chromosomal anomalies has been reported to be higher in

macerated stillbirths than in the fresh stillbirths (Table 3.5) and it rises up to 100% in malformed macerated babies (stillborn & missed abortion).[112]

Table 3.5. The frequency of chromosomal abnormalities in stillbirth cases ≥ 20 wks in different studies.

Category	Machin[114]*	Sutherland[112*]	Angell[115]	Bauld[116]
Macerated stillbirth	9%	19%	9%	33%
Fresh stillbirth	4%	11%	1%	3%

Abbreviations: The values in parentheses are the number of the studied cases, * = Stillborn ≥28 wks.

Fetal karyotyping has variable yield when applied for all cases of fetal death. For example, macerated stillbirth has a lower yield than fresh stillbirth, which indicates that prolonged death in utero is a major limiting factor in the success rate of tissue culture.[117] However, the highest figure of 67% in macerated stillbirths has been reported by Angell et al[115] (Table 3.6).

Table 3.6. The success rate of tissue culture of stillbirth cases in different series

Category	Machin[114]	Sutherland[112]	Angell[115]	Bauld[116]	Smith[118]
Fresh stillbirth	76.2%	95%	92%	82%	69%
Macerated stillbirth	18%	54%	67%	21%	0%

It is noteworthy that aneuploidy in the placenta (confined placental mosaicism) is associated with an elevated risk of fetal death.[119]

- Infection is an established cause of fetal death. It can occur either by the ascending route, the transplacental route, or by both routes. Ascending infection may be either primary, with intrauterine infection of a live fetus, or secondary, arising after intrauterine fetal death. Bacterial infection (anaerobic, gram-negative aerobic, and gram-positive aerobic) usually

occurs via the ascending rout predominantly in the second trimester with either intact or ruptured membranes.[106,111] Pathologic characteristics of this type of infection include both maternal and fetal inflammatory response. The maternal response is characterised by acute inflammation of dependant membranes, which could be found in 2-53% of all cases of fetal death,[84,109] though this higher rates may not reflect the actual rate of fetal infection. In Driscoll's series of 100 stillbirth cases,[97] as many as 67 cases had chorioamnionitis, but only 20 fetuses had infection. Of these 14 cases also had intrauterine pneumonia. Fetal response then develops in the form of vasculitis in the chorionic plate. Similar changes may be seen in the umbilical cord causing funisitis, which indicates fetal infection. Polymorphonuclear leucocytes are first seen in the arterial walls, then in the vein, and finally extend to Wharton's jelly. The presence of polymorphonuclear leucocytes in the alveolar ducts (congenital pneumonia) and in the stomach are good evidence of fetal infection. Although, there was debate whether these leucocytes have maternal or fetal origin, a recent work[120] using an in situ hybridization technique, identifies that the actual source of these leucocytes is the fetus. In fact, the causative organisms are usually identified with difficulty since after delivery mixed isolates are common. Infection with *group B-haemolytic Streptococci* is one of the most serious problems of perinatal life. Not only because of its devastating consequence in postnatal life, but because inflammation of umbilical vessels induced by bacterial toxins may cause their contraction and thus reduce placental venous return.[121] This is also likely to occur in cases infected by *Escherichia coli (E. coli)*. The endotoxin secreted by this bacterium may affect the fetal brain and placenta, as it associated with periventricular leucomalacia formation in the former, and intervillous fibrin deposition in the latter.[72] These actions are thought to be a result of direct toxic neural damage as well as indirect damage via the placental Shwartzman reaction. According to Altshuler,[72] placental fibrin deposition is commonly found in cases where there is a history of maternal urinary tract infection (mainly with *E. coli* bacteria).

In the British Colombia Hospital study 1984-1988,[106] 19% of infectious cases had had placental abrubtion, Naeye[109] suggested that infection plays the primary role by damaging decidual blood vessels.

Most viruses and some bacteria and protozoa use the transplacental route. From a numerical point of view, the most important virus causes fetal wastage is rubella, cytomegalovirus (CMV), parvovirus, and herpes simplex (HSV). The pathology is extremely variable depending on the agent, the time of exposure, and other factors. It may include IUGR, malformation as in rubella, CMV and HSV or hydrops fetalis as in parvovirus and CMV. The risk of fetal demise from parvovirus infection is 9%, and there is a high rate of transmission of 30%.[122] Characteristically, parvovirus has a predilection for progenitor erythroid cells, leading to inhibition of cell maturation and then to progressive anaemia and heart failure. Fetal death could be the anticipated result in severely affected infants. Parvovirus infection occurs in epidemics. hydrops fetalis has been considered to be a useful marker for such infection. However, a study carried out by Wright et al[32] revealed that this is not a constant feature, since only 3 out of 11 cases of parvovirus infection showed hydropic changes. It was explained that, in larger fetuses, the greater haematological reserve does allow time for the development of hydropic changes, while smaller fetuses might die relatively quicker.

Fetal CMV infection can follow primary or recurrent maternal infection, but an excess in fetal death and congenital anomalies has been noted following primary maternal infection.[122,123] The main lesions are CNS abnormality, fetal hydrops or congenital fetal heart block, which eventually leads to fetal death.[124]

Fetal wastage and CNS malformations as a consequence of HSV II infection have been reported.[125,126] Both primary and recurrent maternal infections can lead to intrauterine transmission of the virus to the fetus. However, recurrent infection is far more common

compared with primary infection in pregnant women. Intrauterine transmission is acquired by a combination of transplacental and ascending routes. Ascending infection from maternal genital HSV causes latent infection in the endometrial or decidual cells. Eventually, infection disseminates through the placenta to the fetus. Transmission occurs throughout the pregnancy and is not restricted to the first trimester.

Infection with *listeria monocytogenes* is characterised by nercotising villitis and microabscess formation in the placenta, followed by fetal septicaemia, and finally fetal death, typically in early third trimester. The principal route is presumed to be transplacental, although ascending infection can occasionally take place.

Infection by syphilis usually occurs late in the pregnancy and is usually detected on the basis of maternal serology. It has been estimated that around 34% of infants with congenital syphilis are stillborn.[122] Protozoal infection such as toxoplasmosis may also cause fetal demise. Although the severity of fetal infection is greater when the infection is acquired in the first or second trimester,[127] the chances of infection is reported to be higher if the mother is infected in the third trimester, with 59% transmission rate compared to 14-29% in the first and second trimesters.[122]

- Prior to the wide use of rhesus-immune globulin prophylaxis, blood group incompatibility with severe anaemia, was formerly a common cause of death, accounting for 4.4% of fetal deaths in the UK (1958 British perinatal mortality survey) and 9 per 10,000 births.[75] However it's occurrence has now remarkably diminished, Fretts et al[111] have published that number of Rhesus deaths in Montreal (Canada) for 1960s and 1980s as 4.3 & 0.7 per 10,000 births, respectively. By simple calculation this indicates that Rhesus deaths have dropped from 3.8 % of fetal deaths (13/335) in 1960s to 1.25 % of fetal deaths (2/160) in 1980s.

- Ischaemic lesions in the brain in the form of intraventricular haemorrhage and periventricular leucomalacia have been described in fetuses that died in utero before the onset of labour. Approximately 6% of stillbirth cases are found to have intraventricular haemorrhage at autopsy.[128] A recent study by Squier & Keeling[129] revealed the presence of intracranial ischaemia and haemorrhage in 40% of stillbirth cases. Characteristically, these abnormalities were only detected in the brains of fetuses of more than 27 wk's gestation. Furthermore, an association between this cerebral pathology both with chorioamnionitis and with placental infarcts has been described. Chorioamnionitis was found in 36% (9/25) of cases with intrauterine brain ischaemia,[128] while placenta infarcts were documented in 55% (39/70) of such cases.[110]
- Intrapartum death of normally formed babies as result of fetal asphyxia, trauma or a combination of both, accounted for 40% of stillbirth cases in the UK (1958 British perinatal mortality survey)[75]. However, a figure of 31% of stillbirth cases was recorded in a survey in South-east London (1970-73).[75] An even lower incidence of intrapartum death was recorded in 1993 in a small hospital based study in the UK, giving a figure of only 5% of all stillbirth cases.[100]
- Unexplained fetal death comprises the largest group in most perinatal mortality studies, mostly asphyxial intrauterine deaths without a known underlying cause, macerated fetal death accounting for a considerable portion of this group. The risk of an imminent unexplained stillbirth however, seems to be increased in the third trimester, particularly near the term.[31,130] It is notable that with the dramatic fall in perinatal mortality rates, there has been a relative increase in the representation of unexplained deaths (Table 4), increasing from 9% of cases in 1974-1979[75] to 78% of cases in 1993.[100] Massive fetomaternal haemorrhage, maternal lupus anticoagulant and anticardiolipin may account for a significant percentage of previously unexplained deaths. Recent studies have documented the association of confined placental mosaicism with unexplained fetal death.[119]

3.5 Causes of neonatal deaths:

The neonatal period is the most important period of infancy and childhood during which the highest mortality rate occurs. It has been estimated that approximately 80% of all neonatal deaths, 49% of all infant deaths and 32% of all childhood deaths occur in the first week of life.[131]

Prematurity and its complications still account for high percentage of neonatal deaths . For example, in USA 6-10% of all new-born deaths are attributed to prematurity.[132] Premature labour can occur spontaneously (premature onset of labour) or electively for fetal or maternal well-being. Premature onset of labour occurs in association with premature rupture of membranes, cervical incompetence, polyhydramnios, multiple pregnancy, placental abrubtion, maternal infection, and congenital uterine anomalies. However, in more than half the cases, no apparent cause is found.[79]

There have been reports of a reduction in the number of births weighing ≤2.5 Kg during the 1970s.[80,133] Stanley[80] pointed out that the rate fell from 7.55/1000 in 1971 to 2.86/1000 live births in 1981. Furthermore, Gordon[133] revealed a noticeable reduction in the number of very light live born deliveries during the 1960s-1970s, and attributed this to the management in the prevention of premature onset of labour. Overall, as demonstrated in Table 3.7, there was a reduction in the number of very low birth weight infants, as well as a remarkable decline in the associated mortality rate.[96,134]

However, the developments in neonatal intensive care since the mid 1970s, linked with the ability of obstetricians to assess fetal well-being, have encouraged Obstetricians to deliver a mother prematurely when the prognosis for conservative management is poor. This now accounts for 33-40% of premature deliveries.[79] Moreover, the recent reports have shown an increase in the prevalence and survival rates of very low birthweight infants.[135,136]

Neonatal intensive care has greater effect on the management of hyaline membrane disease than on intracranial haemorrhage. In the former years, most cases have died due to hyaline membrane disease especially among infants weighing between 1-2.5 Kg,[134] but due to the influence of neonatal intensive care, Intracranial haemorrhage became the leading cause of death.[137] In fact, the introduction of a different therapeutic regime either in antenatal or postnatal life has also great influenced the management of hyaline membrane disease. For example, the administration of tocolytics to delay labour and corticosteroid to enhance lung maturity has resulted in a reduction of hyaline membrane disease cases, Similarly, the introduction of artificial surfactant is a major step forward in preventing hyaline membrane disease and pulmonary interstitial emphysema.

Table 3.7. The percentage of mortality for specific birth weight group in two different series.

Birth weight specific groups	Valdes-Dapena[134] (1960-1966) 26/1000* live births, 19,900#	Brans[96] (1978-1982) 10/1000* live births, 30,608#
Live births weighing (1Kg) as percentage of total live births	1.4%	0.7%
Neonatal mortality as percentage of live births of specific group (1 Kg)	91%	71%
Live births weighing (1.5Kg) as percentage of total live births	-	1.4%
Neonatal mortality as percentage of live births of specific group (1.5 Kg)	-	38%
Live births weighing (2.5 Kg) as percentage of total live births	13%	8.3%
Neonatal mortality as percentage of live births of specific group (1.5 Kg)	8%	10%

Abbreviations: *= Neonatal mortality rate, # = Total number of live births.

3.6 Impaired fetal growth:

Impaired fetal growth is one of the causes of fetal and perinatal death. It is also a condition where it can be readily identified by non-invasive PM techniques, namely external physical

examination, body weight (BW) & measurements, and placenta, membranes, and cord examination.

3.6.1 Definitions:

Impaired fetal growth is defined in terms of the amount of deviation from the range of expected fetal growth at any given stage of gestation.[106] Clinically, impaired fetal growth refers to two different states of growth; small for gestational age (SGA) and intrauterine growth retardation (IUGR). Although, these terms are still used as synonyms, the separate definition of each is delineated as follows:

i) SGA applies to babies with birth weight below a certain centile limit for the given gestational age (GA). The centile cut-off could be the 3rd, or 5th, but is most commonly the 10th centile.

ii) IUGR refers to a baby in whom evidence of fetal growth restriction is observed, usually by serial antenatal U/S examination, even if that growth is still within the normal range for the given GA.

3.6.2 Classification of impaired fetal growth:

Impaired fetal growth has been classified in to three types.

i) Symmetrical IUGR (Type I), in which head size and trunk are reduced simultaneously.

ii) Intermediate IUGR, produces a variable appearance depending on the onset of insult.

iii) Asymmetrical IUGR (type II), in which the abdominal girth and the fat stores are reduced more than the head, due to a brain-sparing effect.

This classification reflects the timing of the onset of the insult with reference to the three different phases of fetal cellular growth.[138]

A) Cellular hyperplasia phase:

This phase is confined to the first 16 wks of embryonic and fetal life when the increase in cell population is at its maximum rate. Hence, any fetal insult during this phase results in symmetrical IUGR (type I).

B) Combined cellular hyperplasia and hypertrophy phase:

This phase usually comprises a period from 16 wks to 32 wks, during which, a progressive decline in the rate of cellular hyperplasia is combined with a marked increase in cell size. Therefore, a fetal insult during this phase usually produces a mixed or intermediate type of IUGR, with features resembling type I if the insult occurs earlier, and type II if the insult occurs in the later stage of this phase.

C) Cellular hypertrophy phase:

This phase extends from 32 wks to term during which cell size remarkably increases. Any insult during this period usually produces type II (asymmetrical IUGR). It is noteworthy that uteroplacental insufficiency secondary to maternal disorder is considered to be the main underlying cause of this type of IUGR during this stage of pregnancy.

However, there is an alternative approach for classifying the impaired fetal growth and is based on factors that suppress fetal growth potential. These factors could be extrinsic or intrinsic in nature (Table 4.8)[138]

Table 3.8. Summary of the causes of impaired fetal growth.

	Intrinsic	Combined Intrinsic & Extrinsic	Extrinsic	Idiopathic
incidence	10% to 20%	5% to 10%	30% to 35%	40%
timing of insult	<16 wks	16 to 24 wks	>24 wks	>24 wks
Type of IUGR	Symmetrical	Intermediate	Asymmetrical	Asymmetrical
Causes	Genetic	Sever malnutrition	Uteroplacental insufficiency	Unknown
	Fetal infection	Drugs	Maternal disorder	
	Environmental	Smoking		
		Alcohol		
		Placental pathology		

i) Extrinsic IUGR (type II).

Extrinsic growth retardation is attributed to different maternal diseases or placental disorders that mainly restrict the transportation of oxygen and nutrients to the fetus.

- Maternal nutritional status.

  Any reduction in the nutrient supply to the fetus can affect adversely the state of fetal growth. Chronic nutritional deprivation produces a symmetrical reduction in both weight and length (symmetrical IUGR) while acute maternal deprivation has a much greater effect on weight than length (asymmetrical IUGR).

- Smoking.

  Smoking reduces fetal weight by an average of 200 gm.[139] The different ingredients contained in tobacco smoke, namely are nicotine, carbon monoxide and cyanide all of which act both directly on fetal enzymes and indirectly on uterine blood flow.

- Maternal disease.

  Fetal growth may be restricted by many maternal diseases mostly resulting from maternal systemic hypoxaemia. [139] Mothers suffering from cardiac disease or asthma, particularly in the active stage, are at risk of delivering a SGA baby. Pregnancy induced hypertension is frequently accompanied by growth retardation and the mean birth weight is reduced by 300-500 gm in such instances. 30-40% of babies of mothers who suffer from sickle-cell anaemia are growth retarded.

- Placental factors.

  Blood supply to the fetal side of the placenta is naturally reduced by multiple infarcts and recurrent placental abruption. There is also an association between a single umbilical artery and SGA though this could be explained by the increased incidence fetal abnormalities in

babies with single umbilical artery.[139] The association of large chorioangiomas and circumvallate placentation with impaired fetal growth is well documented.[139]

ii) Intrinsic IUGR (type I):

This type of IUGR are mainly caused by genetic and congenital anomalies. Moreover, some extrinsic factors such as intrauterine infection can induce such type of IUGR.

- Intrauterine infection, particularly the type resulting from viral infection such as rubella and CMV may reduce fetal weight and length. This is attributed to defective organogenesis caused by an early infectious insult. Other infectious agents namely, toxoplasmosis, listeriosis, congenital syphilis and malaria may also be associated with SGA babies. Most of these infectious agents reach the fetus through the hematogenous route.

- Genetic. It is a well known fact that both parent's genes have a major influence on the state of intrauterine fetal growth. A male infant is usually heavier than a female infant. This may be simply reflect the paternal contribution of the Y chromosome or the higher level of the testosterone hormone secreted by the male fetus in the second half of pregnancy.[138] Chromosomal and single-gene anomalies can alter the state of fetal growth. Growth retardation is typical feature of Triploidy, trisomy 13, trisomy 18 and trisomy 21. In overall, 38% of chromosomal abnormal infants are SGA babies.[139] It is noteworthy that even in the chromosomally-normal fetus the cause of IUGR may be related to the confined placental mosaicism (CPM).[119] There are many genetic syndromes notably, Smith-Lemli-Optiz syndrome, Russell-Silver syndrome, and dwarfing syndromes that manifest themselves at birth with IUGR. Many non-chromosomal anomalies are associated with impaired fetal growth. For example, congenital cardiac malformations (with the exception of transposition of the great vessels and tetralogy of Fallot) are associated with increased incidence of IUGR.[138]

- Environmental factors such as chemicals and drugs have teratogenic effects leading to a wide spectrum of malformation syndromes, and IUGR is a feature of these syndromes. These include folic acid antagonists, anticonvulsant drugs, warfarin and heavy metals.

iii) Idiopathic IUGR

- This type of IUGR accounts for 40% of IUGR cases and usually presents after 24 wks, thus producing asymmetrical IUGR. The causes of this type of IUGR is still unknown, though in nearly half these cases abnormal placental changes are exist notably, in the spiral arteries.[138]

3.6.3 Diagnostic criteria of impaired fetal growth:

Clinical criteria:

- the risk of delivering IUGR babies increases with the existence of certain factors such as a previous SGA baby, history of vaginal bleeding and maternal disease, particularly pregnancy induced hypertension. Routine clinical palpation can detect some IUGR cases. Serial antenatal U/S examination however, is best used to predict and identify any deviation in fetal growth. Numerous parameters, include doppler waveform indices, abdominal circumference (AC), head circumference/abdominal circumference ratio (HC/AC) and estimating fetal weight (EFW) are considered to be good indicators of SGA at birth. Both abdominal circumference and estimated fetal weight detect around 80% of SGA infants with a false positive rate of 25%.[139] Serial U/S measurements clearly have the capability to detect abnormalities of fetal growth when compared with the standard U/S fetal growth curves. An establishment of an individual fetal growth curve using mathematical modelling is another proposal. The subsequent measurements are then compared with expected values, a consistent deviation from the curve suggest IUGR. This method requires at least two U/S scans before 26 wks.[139]

- birth weight is used to determine any abnormal growth in the prenatal or postnatal period. Birth weight is plotted against the calculated GA on Standard growth curves. The 10th centile has commonly been chosen as cut-off value. Different growth curves such as the Denver growth curve, the California curves, and the Baltimore curves are used in practice. The Denver growth curves were developed by Lubchenco et al[140] at University of Colorado Medical Centre and were published in 1963. Lubchenco compiled birth weight and GA data for 51635 live-born Caucasian infants aged between 24 and 42 wks and produced arithmetically smoothed graphs for 10th, 25th, 50th, 75th and 90th C. The California curves were developed by Williams et al[141] and based on data extracted form vital records of over 2 million singleton births between 1970-1976. The Baltimore curves were developed by Gruenwald.[142] The data was collected from indigent black population at sea level and many of them were perinatal deaths.
- Morphometric measurements are used to assess the neonatal growth. For example, weight-height ratio, skin fold thickness and Ponderal index (PI). The latter consist of [birth weight (gm) x 100/ length $(cm)^3$], produces a measure for body proportionality.[143] In symmetrical IUGR, all body organ weights and dimensions (body weight and length) tend to be proportionally reduced and Ponderal index found to be normal in this type of IUGR. By contrast, in the asymmetrical type the body weight and some organ weights are more affected, and Ponderal index is below the 10th centile. The mean values increase from 2.25 at 30 wks to 2.55 at 38 wks, beyond this age, there are no further changes. The 10th centile values for 30 wks and 38 wks are 2 and 2.2, respectively. However, the reliability of Ponderal index is a debatable as an abnormal Ponderal index (below 10th centile) was found in nearly 50% of cases in both types of IUGR.[144] The ratio of mid arm circumference (MAC) and head circumference (HC) has been described by Sasanow et al,[145] when 204 infants of 25 to 42 wks were examined, and a Standard chart was introduced for the practical use of assessing muscle, fat stores, and body proportionality.

Pathological criteria:

- Assessment of body weight (BW) and different body measurements; head circumference (HC), crown rump (CR), crown heel (CH), and foot length (FL) versus GA are useful for identifying any deviation in fetal growth. In fact, many standard charts have been published and are used by pathologists.[146,147,148] Thus, any deviation from these charts need to be studied and explained. For example, normal length, but reduced body weight may indicate late nutritional type II IUGR (asymmetrical) whereas reduction of both weight and length may manifest type I IUGR (symmetrical) or problems with dating the pregnancy. In macerated fetal death cases where the BW and CH are altered by artefacts, FL is the least affected and is considered to be the most reproducible measurement. The body measurements in fetal anomaly cases are also liable for artefacts, so FL again is the least altered.[149,150] Keeling[105] used BW of less than 2.3rd centile for gestation and an inappropriate low FL as parameters for identification of IUGR cases when she studied 253 cases of macerated stillbirth of which 33 cases were diagnosed as IUGR.
- Nearly all organ weights in babies with uteroplacental insufficiency are affected. For example, heart weight is moderately increased while the weight of liver and lungs are decreased and weight of the brain is relatively spared.[151] This view is supported by Larroch et al[152] who found that the brain of such infants is larger and heavier than expected for the body weight, but smaller and lighter than expected for the actual GA. Consequently, the ratio of brain weight to liver weight was established and used as marker of growth retardation mainly due to uteroplacental insufficiency. The normal ratio is between 2:1 and 4:1 while a ratio over 4 suggests IUGR. Additionally, the ratio of liver weight to heart weight was used for the same purpose.[153] The normal ratio is between 4:1 to 7:1. A ratio below 4 almost always indicates malnutrition.
- Placental examination is of paramount importance in identifying the different pathological circumstances that lead to fetal and neonatal loss. Brown and Veall (1953) have stated that "Growth retardation is a result of reduced maternal blood flow to the placenta".[74] Recently, this reduction in the blood flow has been investigated and attributed to a state of

vasculopathy occurring in the spiral arteries that lacks an adequate response to the increase of the maternal blood flow throughout the pregnancy. It was found that under normal circumstances, certain physiological changes do take place in the decidual portion of the spiral arteries rendering them flexible and flaccid, in order to tolerate the increase of maternal blood flow. These changes are initiated by the invasion of the spiral arteries by the extravillous cytotrophoblast. This results in destroying the medial musculoelastic tissue, and the arteries become flaccid, thin and tube like structures. Failure of cytotrophoblastic invasion results in endothelial disruption, intimal thickening and formation of atheromatous like lesions, and leads to partial occlusion of these vessels. It was found that over 50% of normotensive placentas of women delivering SGA babies (idiopathic IUGR) exhibit these pathophysiological changes in spiral arteries.[139] It is noteworthy that spiral artery vasculopathy also exists in placentas of women destined to develop pregnancy induced hypertension.

Conclusion:

Many of these causes of fetal and perinatal deaths, such as intrauterine infection, fetomaternal haemorrhage and IUGR can be identified by applying non-invasive perinatal PM techniques. *Maternal clinical investigations*, such as blood sugar, maternal Kleihauer-Betke test, msAFP, Combes test, TORCH screening, and haematological profile for maternal autoantibodies can provide information about the cause of death. *Placental, membranes, and cord examination* is important to identify intrauterine infection, cord accident and placental infarction. It can provide important information related to several clinical presentations of fetal and perinatal death, such as IUGR, APH, and twins, particularly in TTTS. Furthermore, placenta is a source of tissue for cytogenetic studies. *External assessment and measurements* have role in identifying IUGR and malformed cases. *Antenatal Ultrasound* detects many internal malformations, while *Postmortem Ultrasound* may detect the other missing anomalies.

Chapter I. 4

Perinatal Mortality in Middle East Countries

Information regarding the causes of perinatal deaths in Middle East countries is limited as only few and scattered data is available. This might be due to the lack of well organised bodies for collecting of this type of statistical information.

Abu-Heija al at[154] Princess Basma Teaching Hospital (Jordan) investigated the causes of perinatal deaths during the period June 1991- May 1992. The study consisted of 124 stillbirth cases and 126 neonatal death cases with perinatal mortality rate of 30.6/1000. The two main identifiable causes of stillbirth cases were congenital anomalies and placental abruption accounting for 20 % and 14.6 % of the total stillbirth cases, respectively. Pre-eclampsia and cord prolapse were responsible for 5.6 % and 4.8 % of total stillbirth cases, respectively. The highest proportion of stillbirth cases of 47.6 % of total stillbirth cases however, was recorded as an unexplained IUFD. The author related this high percentage to the lack of PM examination, as the local law in Jordan, which is not untypical for that region, prohibits full autopsy except for criminal cases.

The main causes of early neonatal deaths were prematurity, congenital anomalies and neonatal asphyxia accounting for 54%, 20%, and 7% of total early neonatal death cases, receptively. The highest perinatal mortality rate of 647/1000 was recorded in the group weighing less than 1 Kg and the lowest rate of 5.8/1000 was reported in babies weighing between 3.5 and 3.99 Kg. The mortality rate increased again up to 11/1000 for babies weighing over 4 Kg.

Table 4.1 demonstrates the comparison between the percentage of specific birth weight group of this study and a recent European study.[100] The highest difference was in the group weighing < 2.5 Kg. In Jordan the perinatal mortality rate of this group is as high as 3 times of

that in Europe. This is could be a reflection of the difference in the quality of neonatal care offered to babies in Middle East countries and Western countries.

Table 4.1. The percentage of mortality for specific birth weight group in comparison with Western study.

	Abu-Hejja[154] Jordan (1991-1992)		Cartlidge[100] UK (1993).	
No of births	8146		36783	
	Birth weight < 1 Kg	Birth weight < 2.5 Kg	Birth weight < 1 Kg	Birth weight < 2.5 Kg
Birth weight as percentage of total birth	0.2%	6.7%	0.5%	6.5%
Perinatal mortality as percentage of total birth of each specific group	64.7%	27.5%	58.8%	8%

Congenital anomalies are important cause of perinatal mortality in Middle East countries as in Western countries. For example, in Abu-Heija's study, congenital anomalies were present in 20% (50/250) of total perinatal deaths. Most recorded anomalies were neural tubal defect (NTD) (18/50) and hydrocephaly (15/50).

Table 4.2 presents the contribution of congenital anomaly to perinatal mortality rate as recorded by different studies in some Middle East countries. We can note that for example, in Riyadh, the contribution of congenital anomaly in perinatal mortality rate has increased from 16.6% in 1979-80[155] to 35.6% in 1983-87[157] though its contribution to total birth has decreased from 6.7/1000 to 4.7/1000. This could be explained by the concomitant reduction in the contribution of asphyxia in perinatal mortality rate from 14.7% to 11.3% for the same period. In fact, this could be due to an improvement in obstetric care. The studies give a figure of 3-6/1000 births for congenital anomalies which is notably higher than the 2.5/1000 typical for Western countries.[101] However, the lack of PM examination and unavailability of cytogenetic diagnostic services in many Middle East countries make the reported figure of 3-

6/1000 serious under estimate, as in most such cases external examination is the usual way to determine whether the baby is malformed or not.

Table 4.2. The contribution of congenital anomalies to perinatal mortality rate in different local studies.

	Year of study	PMR/10000	Perinatal deaths due to congenital anomaly	
			Per 1000 of total birth	% perinatal deaths
Maternity and Children Hospital, Riyadh[155]	1979-1980	39.8	6.7	16.6
King Khalid University Hospital, Riyadh[156]	1986	14.5	3.3	23
Armed Forces Hospital, Riyadh[157]	1983-7	13.2	4.7	35.6
Misurata Teaching Hospital, Libya[158]	1992-5	22.5	3.6	-
Princess Basma Teaching Hospital, Jordan[154]	1991-2	30.6	6.1	20

Abbreviation: PMR= perinatal mortality rate

The low contribution of congenital anomalies to perinatal mortality rate in Western countries could be partly explained by the availability of prenatal diagnosis and selective abortion and neonatal surgical interventions supported by well equipped neonatal intensive care. Many fetal anomalies are detected at early stages of fetal life and many affected pregnancies are terminated. Some fetal anomalies are liable to be surgically corrected either during intrauterine life or sooner after delivery. At the other end of the scale, high figures for congenital anomalies in Middle East countries could be attributed to several factors including[101]:

- High incidence of traditional consanguineous marriage resulting in increased frequency of congenital anomalies due to autosomal recessive inheritance.
- Relatively high birth rate of infants with chromosomal disorders related to advanced maternal age.

- Relatively high birth rate of infants with malformation due to new dominant mutations, related to advanced paternal age.
- Inadequate health care during and prior to pregnancy, leading to increased frequency of;

-Congenital infections including rubella and CMV.

-Inadequate dietary intake of folate and other vitamins before and during the pregnancy.

-Unsupervised intake of drugs and folk remedies during early stages of pregnancy.

-Uncontrolled maternal diabetes mellitus.

It seems likely that many cases of congenital anomalies could be avoided in this region by promotion of health education, family planning, and proper genetic counselling.

The annual statistics for 1992-5 in Obstetric & Gynaecology Department at Misurata Teaching Hospital (Libya)[158] are summarised in Tables 4.3 & 4.4. Data regarding the causes of perinatal mortality are not available, but the incidence of different fetal and maternal complications in pregnancy or during the labour, which might contribute to many perinatal deaths, can be evaluated.

Table 4.3. Total number of births, stillbirth rate and early neonatal death rate for the years 1992-5 according to annual statistics of Misurata Teaching Hospital.[158]

Year	1992		1993		1994		1995	
Total birth	7249		7543		7598		7323	
	No	rate	No	Rate	No	rate	No	rate
Stillbirth*	116	16	101	13.3	114	15	92	12.5
Early neonatal death	107	15	110	14.7	122	16.3	118	16.3

* = >28 wks.

As Table 4.4 demonstrates that in Libya, antepartum haemorrhage (APH), Pre-eclampsia and diabetes mellitus are common complications during pregnancy and at labour. These complications however, could be related to high parity and advanced maternal age which are common presentation in this region.

Diabetes mellitus is increasingly known medical problem in Middle East countries. It is known contributor to fetal deaths since, it reduces the blood flow in placenta by 35%-45%[61] as well as in perinatal deaths due to the high chance of birth asphyxia associated with shoulder dystocia in infants (big baby) of diabetic mothers. Diabetes mellitus also contributes to an increase in the incidence of congenital anomalies. Women with insulin dependent diabetes reportedly have about 6% risk of conceiving a malformed baby in each pregnancy.[101] Alwan and Modell[101] have reported that in Libya, 13.8% of the infants of mothers with insulin-dependent diabetes mellitus had easily recognisable congenital anomalies compared with 3% in a non-diabetic population. Furthermore, there are other factors, such as unsupervised drug intake, inadequate dietary intake of folate, and other vitamins which exist in this region that might increase the risk of conceiving a malformed baby.

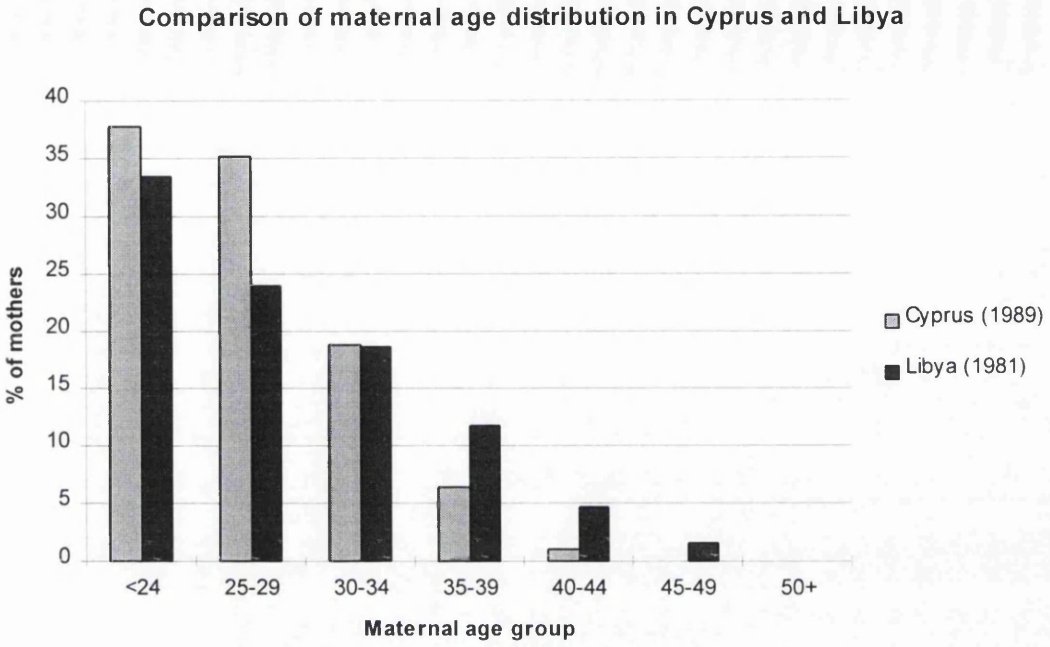
Table 4.4. The annual statistics of the Obstetrics & Gynaecology Department for the years 1992-5.[158]

	1992 (7249)		1993 (7543)		1994 (7598)		1995 (7323)	
	No	/1000*	No	/1000*	No	/1000*	No	/1000*
Prematurity	355	48.9	391	51.8	418	55	267	36.4
Maternal age (35 Ys)	-	-	-	-	1038	136.6	1049	143
Diabetes mellitus	65	8.9	58	7.8	104	13.6	77	10.5
Pre-eclampsia	80	11	125	16.5	111	14.6	132	18
APH	123	16.9	135	17.8	127	16.7	97	13.2
Twins	94	12.9	91	12	92	12.1	76	10.3
Isoimmunization	-	-	-	-	9	1.1	7	0.9
birth weight ≤ 1.5 Kg	-	-	-	-	84	11	56	7.6
birth weight ≤ 2.5 Kg	-	-	-	-	468	61.5	351	47.9
Gross malformations	43	5.9	29	3.8	16	2.1	21	2.8

Abbreviations; Ys = years, * = calculated from total number of deliveries, The value in parenthesis represents the total number of births

Much information, related to the contribution of different causes of fetal and perinatal mortality, is not available due of lack of reliable diagnostic resources. Data on chromosomal anomalies is not available due to the lack of advanced cytogenetic diagnostic services. However, there is an established association between advanced maternal age and the prevalence of chromosomal anomalies in the conceived baby. As stated earlier, an advanced maternal age is common in Libya and this means that an increased proportion of babies with these anomalies would be likely to exist. Figure 1 compares the maternal age distribution in Libya in 1981 (the most recent year available in United Nations statistics) with Cyprus in 1989.[101] The latter is one of the most westernised countries in the Region.

Data regarding perinatal infection is not available mainly due to the scarcity of relevant laboratory tests especially for viral infection. Isoimmunization is still considered a problem resulting in many perinatal deaths as well as many congenital abnormality cases and in Misurata, Libya[158] (1994-1995) it accounted for 10/10,000 total births, but its contribution to mortality and handicap is not known. In Libya, in 1994 -1995, the rate of babies weighing ≤2.5 kg was 5.4% of total number of births, which is nearly same figure recorded in Jordan[154] (6.7%) and in UK[100] (6.5%). Statistics at present not good enough to assess perinatal mortality in this group and would expect it to be more than in Jordan.[154]



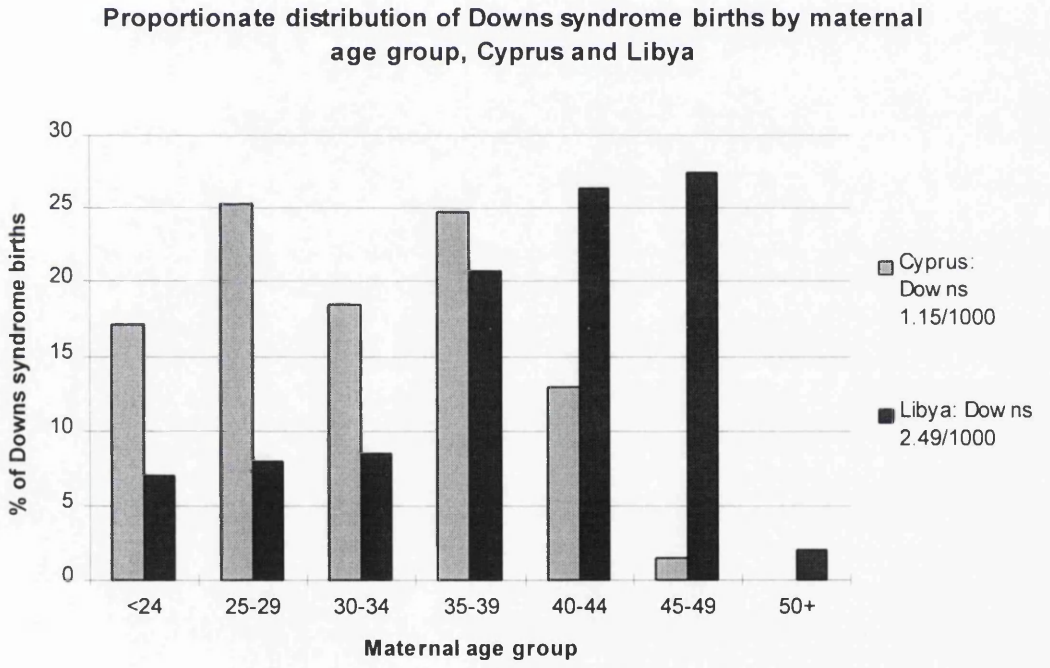


Figure 1. Comparison of maternal age distribution, estimated prevalence of Downs syndrome births, and their distribution by maternal age, in Libya and Cyprus. The figure is based on data in reference.[101] .

Part II

THE RESEARCH STUDY

Chapter II. 1

Materials and Methods

The purpose of this study was to examine the data and information obtained from the application of non-invasive PM techniques on TOP cases, miscarriage cases, stillbirth cases, and early neonatal death cases. The degree of adequacy and accuracy of these techniques will be compared with that of full autopsy.

In other wards, the study is to examine the validity and reliability of non-invasive PM in identifying and detecting the causes of deaths among the above mentioned groups. The material and the techniques used to collect and derive the results will be outlined in this chapter.

This prospective multidisciplinary study was carried out during the period from June 1995 to December 1996 at University College London Medical School (UCLMS) under the supervision of Prof. B Modell (Professor of Community Genetics, UCLMS) and Dr. V Sams (Senior Lecturer in Histopathology, UCLMS). Dr. R Scott (Senior Lecturer in Histopathology, UCLMS) took over from Dr Sams at the beginning of 1997.

1.1 Population set:

Fifty five cases are included. All were prospectively examined at the perinatal pathology unit at UCLMS, which is a referral unit for the North-West London area. There were 44 Caucasian cases, 3 Afro-Caribbean cases, 3 mixed cases, 4 Asian cases, and 1 Oriental case. All fell into one of the four categories shown in Table 1.1. There were 7 twin pregnancies including 1 pair of twins (case No 9 and 10). The studied sample also included 4 cases of hydrops fetalis.

Table 1.1. The four categories, and the number of cases in each category.

Category	Number of cases	Average gestational age, and range (wks)
Cases terminated due to antenatally diagnosed fetal malformation or chromosomal anomalies. TOP group.	24	21.5 (14 - 30)
Mid-trimester abortions of GA 14 - 23 wks (missed, spontaneous). Miscarriage group.	10	19.5 (15 - 23)
Fetal deaths with GA of 24 completed wks or more. Stillbirth group.	11	31 (24 - 41)
Neonatal deaths (death within the first wk of life). Neonatal death group.	10	-

The gestational age was assessed using menstrual age (dated from the first day of the last menstrual period) and U/S examination performed before 24 wks. In case of uncertainty or discrepancy, assessment of GA was based on the greatest concordance of the all available data, such as appearance of the baby, morphometric measurements, and microscopic assessment of kidney and lung development. To estimate the GA of macerated fetal death cases (No=15). In addition to the above, the latest U/S examination performed while the baby was alive and the last recorded fetal movement were both traced, and an assessment of degree of maceration was performed. The information shown below was used as rough guide to determined the interval between fetal death and delivery (Table 1.2).

Table 1.2. External changes in relation to interval between fetal death and delivery.

External changes	Estimated fetal death-delivery interval
Massive sloughing of skin[159]	More than 72 hours
Colour of the skin gradually fades and changes to yellowish-brown[54]	More than one wk
Mummification of fetal body[47]	Two wks or more

For every case both non-invasive techniques as well as full autopsy were carried after parental permission was obtained in a special form (see consent form, Appendix I, Section II). The dead babies' bodies were stored at 3-5 degree centigrade for an interval period between birth to PM,

which varied from 1 to 10 days for IUFD and TOP cases, and 1 to 5 days for neonatal death cases.

1.2 Information collection:

The basic information about the circumstances of death, maternal age, medical obstetric and family history was usually supplied on the request form of PM examination (see Appendix I, Section I). Further information was obtained from the discussions in perinatal mortality meetings or from the hospital computer database. The following data was obtained from the full autopsy report;

- Data on histological examination of placenta, membranes and cord.
- Data on the microscopic examination of different internal organs.

Antenatal U/S reports were also reviewed, as most of these cases have had U/S examination at Obstetric Hospital at UCLMS.

The scan was designed to confirm the fetal viability, check the number of fetuses, estimate GA, determine placenta location and amniotic fluid status as well as examining the fetal anatomy. The anatomical checks that are made routinely are listed below.

- Placental appearance and location.
- Assessment of amniotic fluid volume.
- Body measurements [Biparietal diameter (BPD), Occipitofrontal diameter (OFD), Transverse cerebellum diameter (TCD), Cisterna Magnum (CM), Head circumference(HC), Abdominal circumference (AC), Femur length (FL), Hemisphere (Hem) and ratios [BPD/OFD, HC/AC, anterior ventricular horn/hemisphere (Va/H) and posterior ventricular horn/hemisphere (Vp/H)].
- Visualise of any anomalies in the following fetal parts: Head, Face, Brain, Spine, Heart, Thorax, Kidneys, Bladder, and all four limbs.

For the purpose of this study all the above information was entered in to a computer database (Microsoft office access database® version 2), and multiple queries were designed to examine particular issues further.

1.3 Methods:

In this study, I am responsible about collecting all the available data and performing fine needle aspiration as well as needle biopsy. However, the histology slides of the NB were interpreted by the perinatal Pathologist and analysed by me (comparison to standard histology findings). FNA slides were performed and interpreted by me after initial training by the Cytologist Dr. G Kocjan (Senior Lecturer in Histopathology, UCLMS).

1.3.1 External physical examination, Body weight & measurements:

Each case was assessed to determine the nutritional and maturity status,

and general observation was made for any external anomalies or evidence of maceration.

The following measurements were recorded:

(i) Body weight (BW), measured to the nearest gm on an electronic balance.

(ii) Head circumference (HC), defined as the largest occpitofrontal circumference in cm. It was obtained by placing a string around the head and then read off against a ruler.

(iii) Crown rump length (CR) which the distance in cm between the crown of the head to the most dependant part of the trunk with the neck and back in straight line and the legs are bent to 90 degree at hip joint. It was measured by the use of baby body measuring ruler.

(iv) Crown heel length (CH) which is defined as the distance in cm between crown of the head to the posterior surface of the calcaneum with the neck and back are in straight line. It was also measured by the use of baby body measuring ruler.

(v) Foot length (FL), is defined as the distance in mm between the tip of the longest toe to the posterior surface of calcaneum.

These body measurements were then tabulated according to the category of case (TOP cases, miscarriage cases, stillbirth cases, neonatal death cases) and assessed against its related gestational age (GA) data extracted from standard curves designed by Guihard-Costa and Larroche[160] (see Appendix II). These charts contain curves of percentiles for each measurement, with 95th and 5th percentile corresponds corresponding approximately to the mean plus or minus 2 standard deviations, respectively. They were chosen because they are recent, cover all the above fetal measurements and are based on French study of 476 cases ranging from early mid-trimester to third-trimester fetal death cases, so comparison with our the studied sample should be more compatible.

In this study, all the cases in the neonatal death category are premature babies and died within the first week of life (delivered before completing 37 wks GA and died during the first postnatal wk). The above mentioned standard curves were also applied for these cases, as there is no published standard pathological chart for this particular age group.

1.3.2 Photography:

All the studied cases had photography of whole body view with face forwards and close-up face forwards and face to side. In addition, focal macroscopic abnormalities were photographed as required.

Photographic instruments:

i) Nikon F70 fitted on measurement stand with auto-focus micro Nikkor lens (60 mm).

ii) Fluorescent light X2 kAISER rb 5000 (2x2).

iii) Fujichrome provia coloured film (100ASA) (36 exp) day light.

iv) Ruler, with supporting block.

1.3.3 Radiography:

For all cases the whole body radiography in antero-posterior (AP) and lateral views were taken with optimal positioning, such as extending the lower limbs in the antro-posterior view to allow better assessment of possible leg bowing and extending the neck to allow a good view of the cervical spine. Radiography of large babies and the occasional mammography film were carried out in radiography department whereas all other cases were X rayed in the autopsy room.

Instruments:

Enclosed X rays system Faxitron (Hewlett Packard) was used with range of 30-50 KVP for 30 second, depending on GA of the baby and the BW of the baby. X ray film is Kodak-Ektascan NB with the cassette size of 20.3x25.4 cm. Kodak M35-Mxomat processor was used for developing the X rays film.

The X rays films were reviewed and organised in different sets according to GA in order to facilitate the study by comparing the X rays of the same GA and detect any abnormalities. Radiographic reports were not available

1.3.4 Microscopic examination:

This examination was divided into two categories, one was the study of the baby's tissue cytology via fine needle aspiration (FNA), and the other was the study of the baby's tissue histology via needle biopsy (NB). Both techniques were performed immediately prior to dissection on certain chosen organs; the right lung, liver and kidneys. These organs were selected because of their accessibility for percutaneous blind procedures and they are the organs where critical pathology might be observed. Right lung was chosen rather than left lung in order to avoid any accidental biopsy of other organs (heart).

i) Fine needle aspiration (FNA):

FNA was carried out to investigate lung and liver for 54 cases, and kidneys for 44 cases.

Procedure site:

Two different sites (Table 1.3) for both right lung and liver were chosen with the baby in the supine position. For the kidneys only one site was considered and renal angle was chosen to be the land mark. This site was achieved by turning the baby to prone position with a sponge placed underneath the abdomen in order to push and support the kidneys to the back of the baby. the fingers (index and thumb) were used, where necessary to locate and hold the kidneys in place.

Table 1.3. The different sites of FNA for the right lung and liver.

Organ	Site I	Site II
Right lung	8th intercostal space MAL	5th intercostal space MCL
Liver	11th intercostal space MAL	Just below the costal margin MCL

Abbreviations: MCL= mid clavicular line, MAL= mid axillary line.

Instruments:

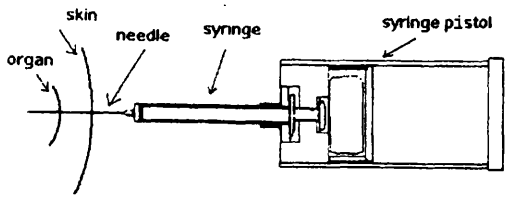
- Disposable needles of 23 gauge (23G x 1", 0.6 x 25) and 25 gauge (25G x 1", 0.5 x 25).
- Disposable 20 ml plastic syringes.
- Syringe pistol [E&I (SELSDON) Ltd. Surrey, UK. Catalogue No 100220].

These were assembled together as shown in Figure 3, (end of this chapter).

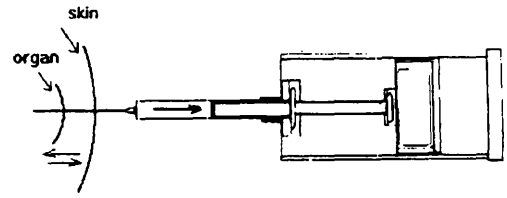
Technique:

- The syringe was placed in the gun and the needle then firmly attached to the syringe with the plunger at the bottom of the syringe in a resting position.
- The apparatus was held with the needle perpendicular to the skin before the needle was gently inserted into the organ (Figure 2.a).

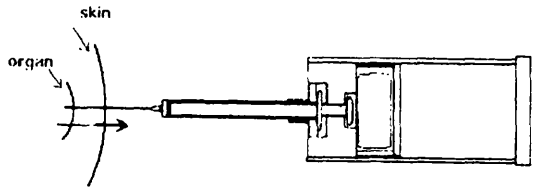
- A negative pressure was then applied with forward and backward movement of the needle inside the organ, so that maximal number of cells were aspirated (Figure 3.b).
- Before withdrawing of the needle, negative pressure was released to avoid the cells from entering the syringe (Figure 2.c).
- The needle was separated from the syringe and the syringe was filled with air (Figure 2.d).
- The needle was then reconnected to the syringe and directed onto the upper end of pre-labelled microscopic slides. The plunger was gently pressed to expel the contents of the needle (Figure 2.e).
- This material was evenly spread on the slide by applying light pressure using an identical type of slide in horizontal direction (Figure 2.f).
- All the specimens were air dried fixed and then stained with May- Grunwald/Giemsa stain.
- During autopsy, tissue imprint and direct FNA was performed for each case for the lung, the liver, and the kidneys in order to obtain material for comparison with the percutaneous FNA and for use as reference material. This is needed because published comparable data could not be found in the literature.



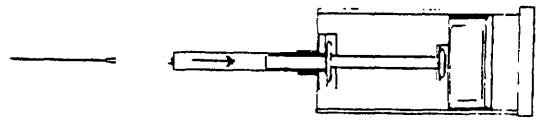
(2.a) Needle insertion



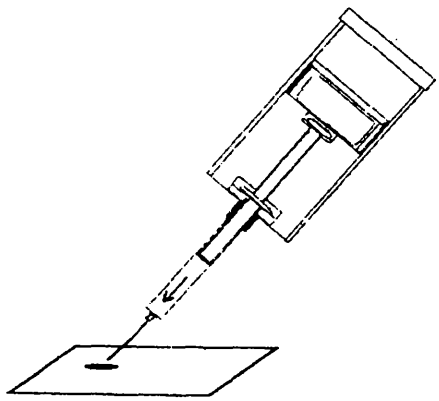
(2.b) Aspiration with negative pressure, forward & backward movement



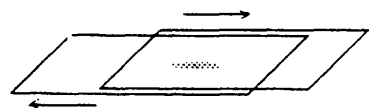
(2.c) Release negative pressure before needle withdrawal



(2.d) Disconnect needle & fill syringe with air



(2.e) Expel needle content into microscopic slide



(2.f) Spread material with applying light pressure

Figure 2. Steps of FNA Technique

ii) Needle biopsy (NB):

At the beginning of this study a Tru-cut needle biopsy was not available, so an alternative intravenous catheter (Jeko intravenous catheter unit 14 gauge) (Figure 4, at the end of this chapter), described by Campbell et al[161] was used as follows:

- the plastic sheath was shortened to the length of 10 mm connected to 10 ml syringe which was partially filled by formal saline.
- A small cut was made in the skin covering the liver in 11th intercostal space mid axillary line.
- The obturator was introduced, through the shortened plastic sheath, until the organ was in contact with obturator.
- The plastic sheath was then pushed gently following the obturator track. The obturator was extracted slowly while the plastic sheath was still being pushed forward.
- The partially filled syringe was then fixed to the plastic sheath and negative pressure (suction) was applied in order to extract the tissue sample .
- The plastic sheath was withdrawn as the negative pressure was maintained in order to increase the chance of obtaining a sample.

This technique was tried initially in right lung and liver as direct approach during autopsy procedure and only liver tissue was successfully obtained (case No 10). Afterward, it was tried through percutaneous approach on intact body after a cut with a blade was made on the surface of the liver (case No 20). A core of tissue was obtained from the liver. This technique was proved to be of limited application and time-consuming, so it was abandoned.

A Tru-cut needle biopsy was then used with 14 gauge TW x 4.5" (11.4cm) Baxter Health care corporation. Deerfield, Illinois 60015 USA with 20 mm specimen notch (Figure 5, at the end of this chapter). It was applied to right lung in 25 cases, the liver in 26 cases, right kidney in 21 cases and left kidney in 19 cases. All four organs were sampled in 18 cases. Due to the risk of

causing damage to the baby's internal structures, which might affect autopsy findings, cases were carefully selected for this procedure, and only one application per organ was performed. The sites of Tru-cut NB for the right lung and liver are shown in Table 1.3, for the kidneys, the renal angle was again chosen to be the land mark. As per FNA, the baby's lying position was supine for both right lung and liver. For the kidneys, the baby was turned to prone position and a sponge pad was used for support.

Table 1.4. The chosen sites for Needle biopsy

Organ	Site
Right lung	11th intercostal space MAL.
Liver	8th intercostal space MAL

Abbreviation: MAL= mid axillary line.

Technique:

In all the studied cases the tissue core was obtained percutaneously.

- The Tru-cut needle was inserted with obturator retracted to cover the specimen notch.
- On contact with the organ to be sampled the obturator was advanced followed by the cannula, till the latter locked on the former.

The Tru-cut needle was then withdrawn as whole and the tissue core was removed from the specimen notch.

Tissue cores were processed routinely. The section were stained with haematoxylin and eosin and any other histological stains as were felt to be necessary to make a diagnosis.

1.3.5 Full autopsy:

The full autopsy was performed for all 55 cases by a perinatal pathologist and according to the Royal College of Pathologists score system (Table 1.3, Chapter I. 1) each case was recording a score of 500 or higher. Full autopsy included external physical assessment, body dimensions

measurements (BW, HC, CR, CH, and FL), photography, radiography, and placenta, membranes, and cord examination. In addition, internal examination, dissection, histological examination, and ancillary studies were performed. The ancillary studies included microbiological (bacteriology and virology) and/or chromosomal studies. Bacteriological examination was conducted in cases with a history of prolonged or premature spontaneous rupture of membranes or evidence of maternal infection. Samples for virological investigation were obtained from the baby organs (lung, liver, and/or spleen) in cases of hydrops fetalis (non-immune type) or IUGR cases not explained by maternal disease or/and where other fetal abnormalities were suggestive of intrauterine infection. Chromosomal analysis however, was conducted in cases of unexplained IUGR, dysmorphic and hydrops fetalis (non immune type) cases. The samples were usually taken from skin and/or placenta.

The invasive technique composed of the following;

i) Internal examination and dissection. In this examination thymus, heart, lungs, liver, kidneys, adrenals, spleen, and brain were weighed and the results were compared with standard chart.

ii) Histological examination of all weighed organs and in addition gonads, thyroid, larynx, stomach, pancreas and any other organs if indicated.

The instruments used in non-invasive microscopic PM examination

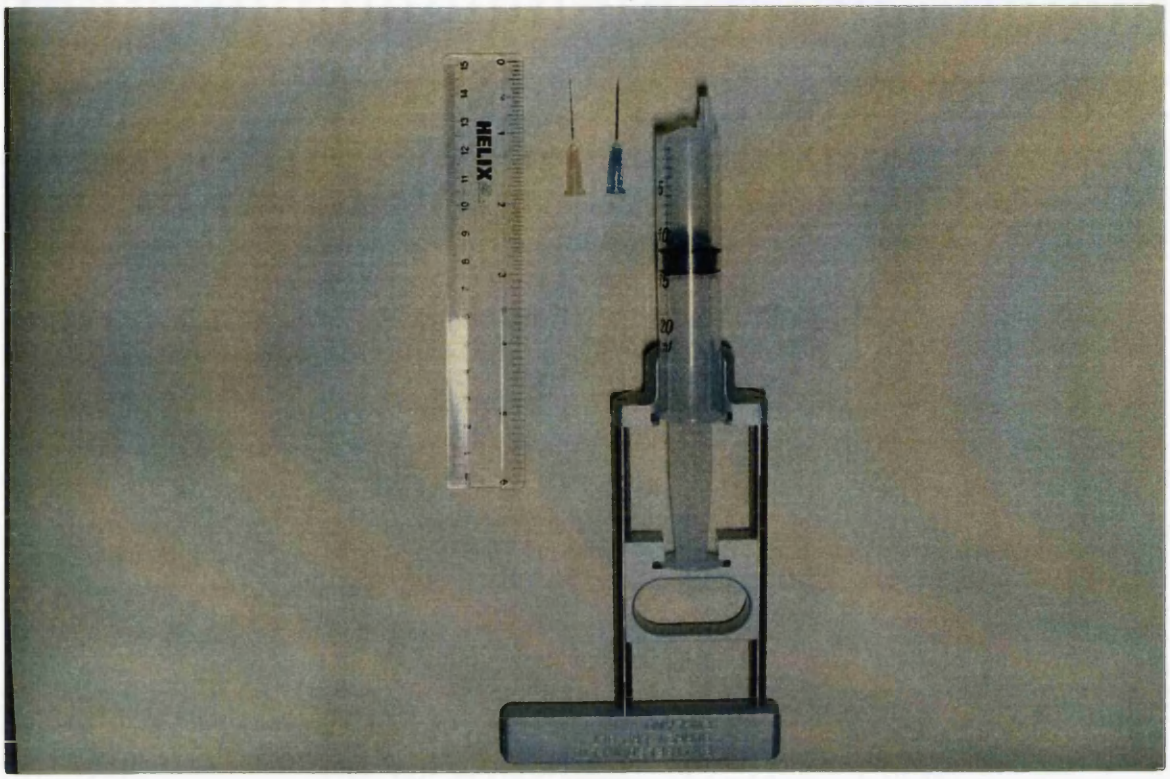


Figure 3. Fine needle aspiration (FNA) instruments.

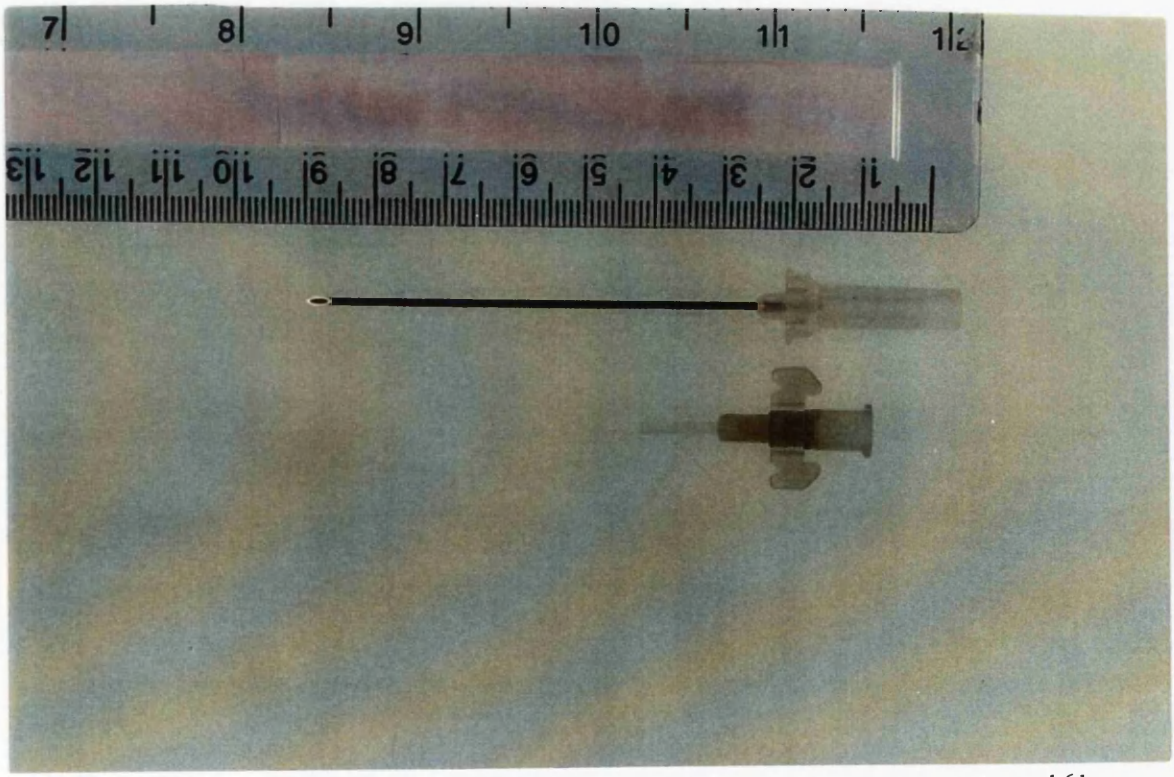


Figure 4. Needle biopsy (NB) instrument as described by Campbell et al [161]

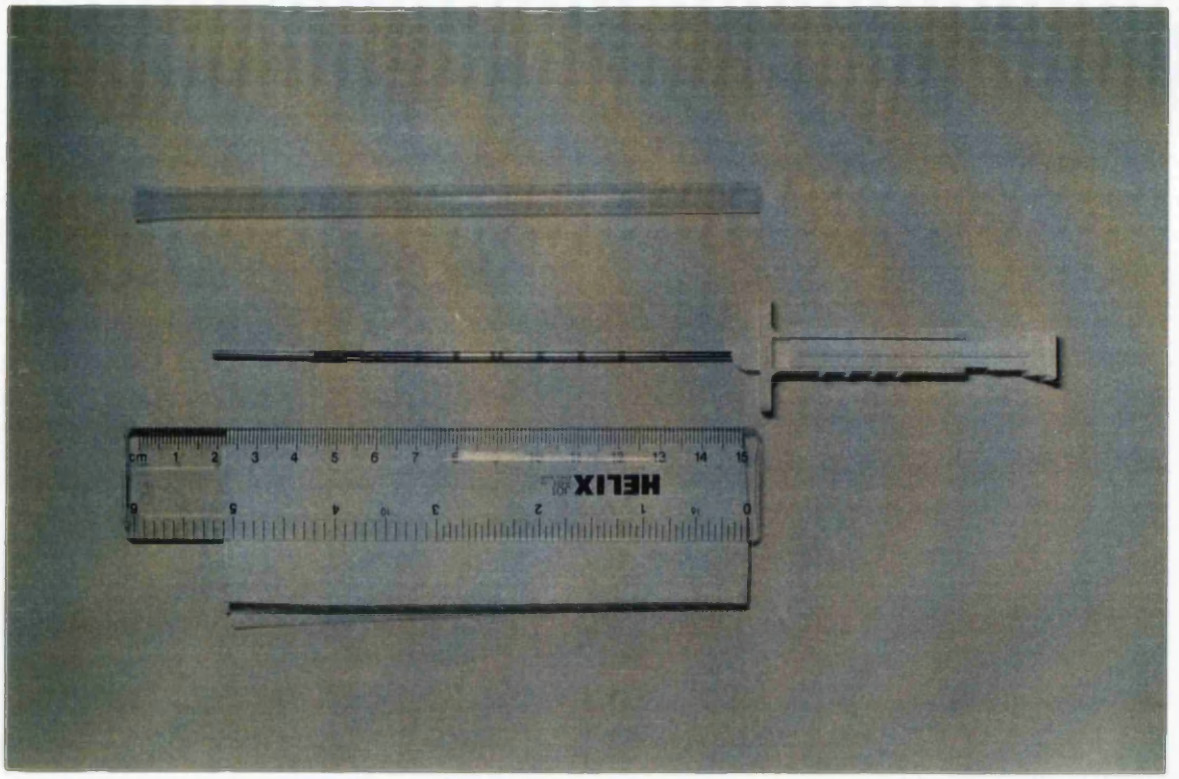


Figure 5. Tru-cut needle biopsy.

Chapter II. 2

Results of The Techniques

The complete data for individual cases is presented in the next chapter. This chapter summerises technical results as well as evaluates the used method. The first section is devoted to growth status by analysing the results obtained from the body physical measurements. Charts designed by *Guihard-Costa and Larroche*[160] (Appendix II) will be used as standard charts for comparison throughout this study. In the second section, the results obtained by the application of the non-invasive microscopic examination (FNA and NB) on four organs (right lung, liver and both kidneys) are shown.

2.1 Body measurements and growth assessment:

The use of anthropometeric measurements for clinical objective and pathological assessment of infant growth is well established. The recorded physical measurements BW, HC, CR, CH and FL for each case per individual category are shown in Tables 2.1, 2.2, 2.3. & 2.4. One case (case No 8) was at 14 wks gestation; its physical measurements are not included as it is thought to be too small for this analysis. The criterion adopted in this study to define IUGR is the combination of BW and FL being at or below 5th centile on the standard charts derived by *Guihard-Costa and Larroche*,[160] (Figures 6 & 7). When this criterion was applied 13 out of 54 cases were diagnosed as IUGR cases, and their measurements are shown in Table 2.5.

Table 2.5. IUGR cases with their measurements

Case No	Category	GA (wks)	BW (gm)	FL (mm)
1	Stillbirth	28	583	46
7	Miscarriage	18	133.1	20
10	Neonatal death	25	510	42
11	TOP	20	191.3	28
13	TOP	21	113.7	23
19	Miscarriage	23	226	34
29	Stillbirth	26	604	45
42	TOP	15	21.8	12
44	TOP	17	29.7	11
45	TOP	23	396	37
46	Stillbirth	28	495	39
48	TOP	30	1050	53
50	Neonatal death	25	482	40

As can be seen in Table 2.6, the identification of IUGR in these cases was further supported by the following;

(i) The existence of many clinical factors which are known to predispose to IUGR.

(ii) Antenatal U/S growth assessment indicated IUGR in 7 cases of the 13 cases and failed to identify it in 3 cases. An U/S report was not available in 1 case, and growth was difficult to assess in 2 cases due to spontaneous rupture of membranes.

(iii) Placenta, membranes, and cord examination was performed in 12 out of 13 cases, and 5 cases showed additional abnormalities relevant to IUGR status.

Table 2.6. The predisposing factors, U/S and PCM examination findings in the 13 IUGR cases.

Case No	Clinical history	U/S growth assessment	PMC examination
1	Maternal hypertension	SGA	Small infarcts 5-10%
7	Maternal hypertension, spontaneous rupture of membranes	ND	NR
10	twin pregnancy	Normal	Velamentous insertion of cord
11	maternal hypertension, spontaneous rupture of membranes	ND	Normal
13	malformation, hydrops fetalis	-	Normal
19	unexplained ↑ msAFP	SGA	↑syncytial knots & perivillous fibrin deposition
29	maternal hypertension	SGA	Infarcts < 15% of variable age, ↑syncytial knots & perivillous fibrin deposition
42	chromosomal anomaly	SGA	Normal
44	chromosomal anomaly	SGA	Normal
45	Malformation	Normal	Normal
46	Unknown	SGA	Old infarcts < 10%, nucleated fetal RBCs are prominent
48	Malformation	Normal	NR
50*	unexplained ↑msAFP	SGA	ND

Abbreviations: PMC= placenta, membranes, and cord, ND = Not done,
NR = Non relevant, - =not available, ↑= increased, * = Alloimmune thrombocytopenia under predinsolone treatment.

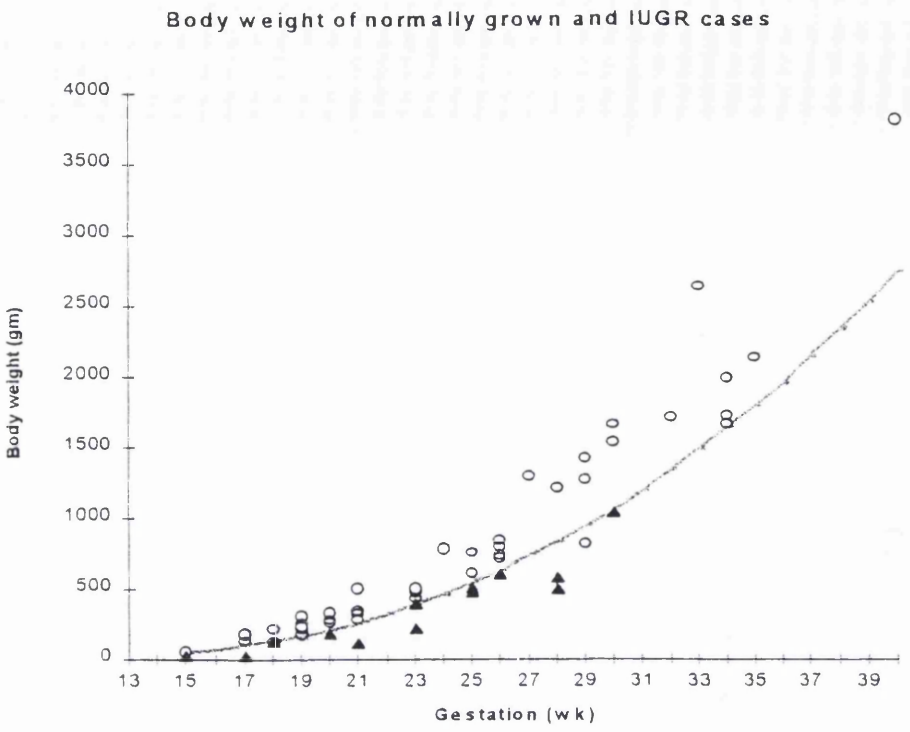


Figure 6. The curve represents the 5th centile of the standard Guihard-Costa and Larroche chart for body weight. Open circles represent normally-grown cases, black triangles represent IUGR cases.

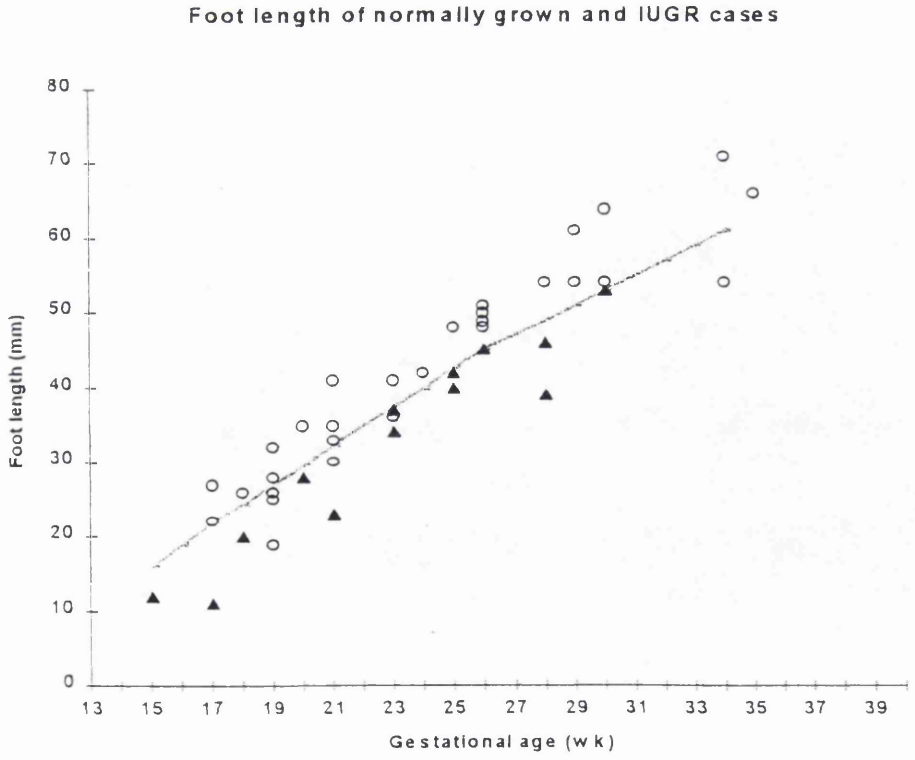


Figure 7. The curve represents the 5th centile of the standard Guihard-Costa and Larroche chart for foot length. Open circles represent normally-grown cases, black triangles represent IUGR cases.

Method Evaluation:

Body weight (BW) is the most used parameter for determining baby's growth, but in many cases, it is affected by artefacts. Macerated babies are usually lighter than expected and cases with hydrops fetalis are usually heavier than expected. This problem could be minimised by the use of Foot length (FL) parameter as it is less liable to artefacts.[149,150] In this study, both BW and FL were used to assess growth and an IUGR case is defined when both these parameters are at or below the 5th centile on the Standard charts derived by *Guihard-Costa and Larroche*.[160] This resulted in diagnosing 13 IUGR cases out of the 54 studied sample. It is noteworthy that growth is affected by many factors, such as race, economic status, and environmental conditions, therefore a specific charts should be drown for each specific population.

Early fetal growth retardation still remains a difficult field of study.[162] Artefacts is one problem with growth assessment, the other major problem is the estimation of fetal age. This arises as many babies are dead for some time before delivery. For such instance, it is necessary to relate the pattern of growth and maturation of the fetus to the time of fetal death rather than the time of delivery. Estimation of death timing is not an easy task. Gross external changes of maceration could be used to estimate the fetal death-delivery interval (Table 1.2, Chapter II. 1) It is noteworthy that several factors other than intrauterine retention can affect the gross external appearance of macerated fetuses, such as the gestation of the infant and the underlying pathological condition. For this reason these external changes should be used as rough guid.[54,159] Wigglesworth[54] has noted that the process of maceration is accelerated in immature growth-retarded infants and in hydropic infants.

2.2 Microscopic examination:

This examination of right lung, liver, and kidneys was limited to two categories, one is to study the baby's organ cytology via FNA, while the other category is concerned with the study of the baby's organ histology via NB.

2.2.1 Fine needle aspiration (FNA):

This technique was carried out to examine lung, liver and kidneys.

i) LUNG FNA:

The right lung was investigated by FNA in 54 cases. As mentioned in Chapter II. 1, two different sites were chosen and both needle size (25 G, 23 G) were used for each site.

Table 2.7. The success rate of FNA lung in each site and with each needle size.

	Lung	Site I	Site II	Site I		Site II	
				25 G	23 G	25 G	23 G
No of cases	54	53	51	44	41	45	42
No of cellular samples	24	21	15	16	10	11	10
Success rate	44.4%	39.6%	29.4%	36.3%	24.3%	24.4%	23.8%

As demonstrated in Table 2.7, site I (mid axillary line) produced more cellular samples than site II (mid clavicular line). This could be attributed to the special shape of the lung, since at site I the possible area of contact is larger than it is for the site II.

Another factor that may affect the sampling outcome is the GA. In this study the rate of successful sampling for cases aged 25 wks or less was higher by 33% than those cases aged more than 25 wks (in cases dated 25 wks or less, the success rate was 48% compared with a rate of 32% recorded in cases dated more than 25 wks). This variation could be explained by the fact that lung texture below 25 wks has more mesenchymal tissue which then reduces as the GA progresses and the lungs transform to a sponge like structure.

At the early stages of conducting this study, there were 2 cases (case No 4, cases No 7) diagnosed as having bronchopneumonia. In case No 4, a cellular sample was obtained and microscopic examination revealed the presence of rod-like structures (see Figure 8, at the end

of this chapter). In fact, standard histology of the lung revealed bronchopneumonia with features consistent with gram-negative causative agent (bacteria were not seen) and the lung tissue culture revealed a pseudomonas bacteria. In case No 7, no cellular sample was obtained. FNA of the lung, as in case No 4, could have a beneficial value especially if it is accompanied by microbiological culture for the sample. This however, would not be the cases for picking up viral infection. For example, in case No 29 viral inclusion for CMV was detected on standard histological examination of most of fetal organs. Lung FNA was acellular, and direct as well as imprint smears of the lung did not detect the presence of viral inclusions.

ii) LIVER FNA:

Liver FNA was performed in 54 cases. At two different sites, site I (mid axillary line) and site II (mid clavicular line), (Table 2.8).

Table 2.8. The success rate of FNA liver in each site and with each needle size.

	Total	Site I	Site II	Site I		Site II	
				25 G	23 G	25 G	23 G
No of cases	54	52	53	45	38	41	40
No of cellular samples	51	46	37	38	25	24	24
Success rate	94.4%	88.4%	69.81	84.4%	65.7%	58.5%	60%

This high yield from FNA of liver could be attributed to the consistency of liver tissue, since the ideal aspirate should have a high cell content in a small amount of fluid. A creamy residue that remains within the lumen of the needle is commonly seen in liver aspirate, but rarely seen in lung aspirate. In fact, the 5.6% failure rate (3/54) could be explained partly by the state of liver itself, since in 2 out of 3 cases, liver was autolysed which has caused an increase in fluidity of the sample.

In case No 36, HSV II was detected in hepatic cells on standard histological examination. Cellular sampling was obtained in this case at site I, 25 G needle size and site II, 23 G needle,

but cells were too necrotic and virus particles could not be identified. The spare slides were acellular so a specific immunohistochemistry stain did not show any findings. On gross examination, the liver showed multiple pale and necrotic areas covering most of the surface.

iii) KIDNEYS FNA:

FNA of the kidneys was performed for a total of 44 cases. The needle was applied at the renal angle and both needle size 25 G and 23 G were used in most cases. However, the kidney FNA produced the lowest success rate as compared with lung (44.4%) and liver (94.4%) (Table 2.9).

Table 2.9. The success rate (% of cases) of FNA kidneys with each needle size.

	R Kidney	L Kidney	R Kidney		L Kidney	
			25 G	23 G	25 G	23 G
No of cases	44	41	34	31	33	29
No of cellular samples	16	12	10	9	8	7
Success rate	36.3%	29.2%	29.4%	29%	24.2%	24.1%

abbreviations: R= right, L = left.

This low success rate is mainly due to the size, mobility, and inaccessibility of the kidneys. An attempt to locate and hold the kidneys in place (case No 19 onward) by using fingers (index and thumb) was not always successful as the rate raised from 20.5% (8/39) to 29.5% (26/88) of the total number of sampling attempts.

It was noted that the mobility of the kidneys increased with the loss of tone in the abdominal and diaphragmatic musculature which occurs naturally in dead bodies. This could have been further accentuated by the placement of the body in the prone position and the use of a supporting sponge (as described in Chapter II. 1) which was designed to push the kidneys towards fetus' back. The loss of the muscle tone however, caused an upward movement of the kidneys towards the thorax. There was no notable relationship between the GA of the baby and successful sampling. Of 44 studied cases there were 40 cases with normal kidneys and 3 cases

of cystic dysplastic kidney and 1 case of CMV were diagnosed on standard histology of the kidneys. For the two cases of cystic dysplastic kidney (case No 8, 21) FNA sampling was acellular in both kidneys, which might be expected since the kidneys sometimes become fibrotic and are not susceptible for aspiration. In the third case (case No 34) the sample was cellular, but no diagnostic finding was detected. This might be expected since pathological diagnosis in this condition requires a study of the kidney architecture which was not possible on FNA sampling.

In case No 30 standard histology of the kidneys showed viral inclusions for CMV, FNA was performed and was acellular in both kidneys. Retrospectively, direct FNA and imprint smears were studied and viral inclusions were not detected.

Method Evaluation:

Fine needle aspiration involves obtaining a sample without any disfigurement to the dead body, so it was approached with high hopes that some results could be achieved by using this methods. Four organs, right lung, liver, and both kidneys, were chosen because of their accessibility for percutaneous procedure. However, successful sampling rate of those organs varied from 44.4% for the right lung, 94,4% for the liver, 36.3% for the right kidney, and 29.2% for the left kidney. This variability could be explained by the high cellular content of the aspirate as well as tissue consistency of the liver.

Overall, the cellular yield was improving as the study progressed.

Generally, two fundamental requirements on which the success of FNA depend are the representative nature of the sample and the quality of the preparation. The lowest success rate was recorded with kidneys sampling is in fact, due to the size, mobility, and inaccessibility of these organs in dead fetus and new-born.

FNA of lung showed positive indication in identifying bacterial agents as in case No 4 when rod-like structures (pseudomonas species) were detected on FNA lung. In cases of viral

infection, it may be less possible to identify the virus due to tissue necrosis that could interfere with viral identification as in case No 29 (CMV case) and case No 36 (HSV II case).

For FNA kidneys, cases of some pathology, such as cystic dysplastic kidney, the tissue sometimes become fibrotic and are not susceptible for aspiration. Nevertheless, even in the presence of aspirated material, the diagnostic findings could not be detected, since pathological diagnosis in cystic dysplastic requires a study of the kidney architecture which was not possible on FNA sampling.

In this study, FNA was of very limited value as diagnostic method, this could partly be a result of lack in experience and reference material in this field. The descriptions of fetal and neonatal cytology were rarely found in the literature.[163] These factors limit the outcome of FNA at perinatal PM in this study.

2.2.2 Needle biopsy (NB):

Needle biopsy was performed for the right lung in 25 cases, the liver in 26 cases, right kidney in 21 case, and left kidney in 19 cases. The four organs were all sampled in 18 cases. Initially, an intravenous catheter was used for NB, as described in Chapter II. 1, in two cases. In the first case (case No 10) NB was performed on direct approach after body was dissected to sample the liver and the right lung, but only liver was successfully sampled. For the second case (case No 20) NB was applied percutaneously to the liver after the skin was cut with a blade and a core of tissue was again successfully obtained. However, this proved to be time-consuming procedure and could not be practically used for the lung and kidneys. Therefore, it was abandoned and replaced by the more practical Tru-cut needle biopsy.

i) LUNG NB:

Lung NB was successfully obtained in 13 out of 25 (52%) cases and all sampling was undertaken from mid axillary line. The relationship of successful sampling with GA was not

clear as the number of cases are so small. This low success rate compared with the liver could be explained by the tissue consistency of the lung.

Among the 25 cases where lung sampling was performed, 11 pathological conditions were picked up by standard histology (40% of the cases). This high percentage of diagnostic lesions in lung sections reflects the importance of lung as site for many pathological conditions in fetal and neonatal life.

However, as demonstrated in Tables 2.10 & 2.15 only 4 out of these 11 cases had a successful NB. In only one of these 4 cases, a significant pathology was picked up on NB sample (case No 50).

Table 2.10. Cases with abnormal histology of lung and the result of NB in 11 cases.

Case No	GA (wks)	Sd histology	NB	Autopsy diagnosis
23	30	Congestion, extensive interstitial haemorrhage	-	Neonatal death. Imperforated nasopharynx, cleft palate, abnormal tongue, absent uvula, micrognathia, bicornuate uterus, 2 (cervix, vagina).
24	29	Intra-alveolar fetal squames	-	Neonatal death. TTTS-recipient, congestive heart failure, infarction (cerebral, liver).
28	40	Intra-alveolar fetal squames	-	Macerated stillbirth. Unexplained.
29	26	Intra-alveolar fetal squames	Normal	Fresh stillbirth. IUGR, placental infarction
39	32	Intra-alveolar fetal squames	Normal	Macerated stillbirth. Unexplained.
46	28	Intra-alveolar fetal squames	Normal	Macerated stillbirth. IUGR, chronic uteroplacental insufficiency
30	21	CMV inclusions	-	Missed abortion. CMV infection, hydrops fetalis
37	24	Pulmonary hypoplasia	-	TOP. Bilateral renal agenesis, large heart, pulmonary hypoplasia
35	19	Pulmonary hypoplasia	-	TOP. Pulmonary hypoplasia, cystic dysplastic kidney, bladder outlet obstruction
48	30	Pulmonary hypoplasia	-	TOP. Microcephaly, hydrops fetalis, HC, cerebral ischaemia, pulmonary hypoplasia
50	25	Hyaline membrane disease, emphysematous changes, alveolar haemorrhage	Hyaline membrane disease, alveolar haemorrhage	Neonatal death. Prematurity, bilateral intraventricular haemorrhage, subependymal haemorrhage, hyaline membrane disease, IUGR

Abbreviation: Sd= standard, - = unsuccessful sampling.

ii) LIVER NB:

NB sampling was successful in 18 out of 26 (69.2%) cases and the chosen site for all the samples was mid axillary line. Although, no relationship could be found between the success of sampling and GA, the macroscopic status of the liver (liquefied, non liquefied) had an influence on the sample outcome. This can be seen in Tables 2.11 & 2.12, where in 5 cases out of the 8 cases with failed sampling, the liver was liquefied and none of the 18 successful sampling had a liquefied liver.

Table 2.11. The macroscopic status of liver in the unsuccessful NB sampling.

Case No	GA (wks)	Macroscopic status	Category
23	30	Normal	Neonatal death
28	40	Liquefied	Macerated stillbirth
29	26	Normal	Fresh stillbirth
35	19	Normal	TOP
39	32	Liquefied	Macerated stillbirth
43	20	Liquefied	Missed abortion
49	23	Liquefied	TOP
54	23	Liquefied	Macerated stillbirth

From the 26 cases where liver sampling was performed, standard histology of the liver were normal for 19 cases, but it revealed a pathological condition for the rest of 7 cases (26.9%). When the result of standard histology of these 7 cases compared with NB findings, 4 cases showed an agreement, in 2 cases the pathology was not picked up by NB as there were localised lesions, and in one case the liver was not successfully sampled. On the basis of final standard autopsy findings, only 2 cases had important findings on NB (Tables 2.13 & 2.15). There are case No 24 (diagnosed as having liver infarction) and case No 38 (diagnosed as being infected with HSV II, see Figure 9 at the end of this chapter). This was detected when specific immunohistochemistery stain was applied for HSV II in NB sample.

Table 2.12. The macroscopic status of liver in the successful NB sampling.

Case No	GA (wks)	Macroscopic status	Category
22	34	Enlarged	Fresh stillbirth
24	29	Enlarged, congested liver	Neonatal death
25	21	Normal	TOP
26	26	Normal	TOP
27	26	Normal	Fresh stillbirth
36	21	Normal	TOP
37	4	Pale areas on surface	TOP
38	30	Enlarged, pale areas	Macerated stillbirth
41	23	Normal	TOP
45	23	Normal	TOP
46	28	Normal	Macerated stillbirth
47	21	Normal	Macerated stillbirth
48	30	Enlarged, congestion	TOP
50	25	Localised haematoma	Neonatal death
51	26	Normal	TOP
52	26	Normal	TOP
53	19	Normal	TOP
55	17	Normal	TOP

Table 2.13. Cases with abnormal histology of liver and the result of NB in these cases.

Case No	GA (wks)	Sd histology	NB	Autopsy diagnosis
23	30	Congestion	-	Neonatal death. Imperforated nasopharynx, cleft palate, abnormal tongue, absent uvula, micrognathia, bicornuate uterus, 2 (cervix, vagina).
24	29	Infarction in Subcapsular area.	Infarction in Subcapsular area.	Neonatal death. TTTS-recipient, infarction (cerebral, liver), congestive heart failure..
27	26	Congestion	Congestion	Fresh stillbirth. Spontaneous rupture of membranes, chorioamnionitis, funisitis recurrent .APH.
37	24	Infarction	Normal	TOP. Bilateral renal agenesis, pulmonary hypoplasia, large heart.
38	30	+ve immunohistoche-mical reaction for HSV II	+ve immunohistoche-mical reaction for HSV II	Macerated stillbirth. Disseminated HSV II infection, hydrocephaly
41	23	Extramedullary erythropoiesis	Extramedullary erythropoiesis	TOP. Hydrocephaly with abnormal anterior horn.
50	25	Subcapsulare haematoma	Normal	Neonatal death. Prematurity, bilateral intraventricular haemorrhage, subependymal haemorrhage, hyaline membrane disease

Abbreviations: Sd= standard , + = positive, - = unsuccessful sampling.

iii) KIDNEYS NB:

Needle biopsy was performed for 21 cases on right kidney and in 19 cases on left kidney. Renal angle was used as land mark with locating and fixing the kidney in position by fingers. A sample was obtained in 8 out of 21 (38%) cases in right kidney and in 9 out of 19 (47.3%) cases in left kidney. These low rates as compared with liver (69.2%) and lung (52%) was attributed to the size and the mobility of the organ. The use of a supporting pad was not helpful, as it caused the kidney to be displaced towards the thorax. Moreover, no relationship was found between successful sampling and GA.

Of this small number of cases only 3 pathological conditions were detected on standard histology of kidneys (Tables 2.14 & 2.15) one of them was cystic dysplastic kidney (case No 35). In this case NB kidney (left) showed dysplastic tissue and proved to be more reproducible than FNA in detecting abnormal kidney. However, cystic lesions were not seen on NB in this case. Dr. S Knowles[164] (personal communication) has noted this fact and ascribes it to the loss of kidney architecture which was maintained partly by the internal pressure of the cyst content.

Table 2.14. Cases with abnormal histology of kidneys and the result of NB in 3 cases

Case No	GA (wks)	Right kidney		Left kidney		Sd autopsy
		Sd histology	NB	Sd histoloy	NB	
26	26	Congestion	-	Congestion	-	TOP. Holoprosencephaly (semilobar).
29	26	Congestion	Normal	Congestion	-	Fresh stillbirth. IUGR, Placental infarction
35	19	Cystic dysplastic kidney	-	Cystic dysplastic kidney	Dysplastic kidney	TOP. Pulmonary hypoplasia, bladder outlet obstruction.

Abbreviations: Sd= standard, - = unsuccessful sampling.

Method Evaluation:

Needle biopsy as PM examination method has been developed in adult cadavers, since 1950s, mainly as alternative to autopsy.[66,67,68,165,166,167] However, an extensive English Medline search failed to trace any reports on the use of NB in *perinatal PM examination.*

Needle biopsy as Fine needle aspiration has no or very small disfigurement and its success would be of importance in developing of non-invasive PM approach. The following table summarises the results of NB in all four organs. As can be noted, the highest success rate was obtained in the liver (69.2%). This could be attributed to the accessibility of this organ for percutaneous procedure partly because of size, surface anatomy, and tissue consistency. On the other hand, the low success rate in kidneys could be explained by the small size and great mobility of this organ in the dead fetus and new-born (Table 2.15).

Table 2.15. The results of NB for all four organs

Organ*	Successfully sampled		Pathology detected by standard histology		Pathology detected by NB	
	Number	Percent	LP or SP	NSP	LP or SP	NSP
Right lung	13/25	52	6	5	1	0
Liver	18/26	69.2	2	5	2	2
Right Kidney	8/21	38	1	2	0	0
Left Kidney	9/19	47.3	1	2	1	0

Abbreviations; * = in all these cases Tru-cut needle was used in the sampling, LP = lethal pathology, SP = significant pathology, NSP = non significant pathology.

In general, the tissue recovery in the this study was less than that found in an adult cadaver (Table 2.16). This could be explained by the small size of the organs in perinates and the kind of the pathology usually found in adult cadaver. In the latter group, tumours and infectious agent are usually found at PM examination. This sort of pathology causes diffuse changes in organs, an obvious mass or an enlarged organ, so making the organ more likely to be successfully sampled. Moreover, full autopsy was always needed for all cases involved in this study and the fear from damaging and disturbing the babies' internal structures by the needle was raised. So, only one application of NB was performed for all cases. However, repeating the procedure for one organ from the same puncture with different angles, might have resulted in more successful sampling. Anyhow, NB was proved to be more useful as non-invasive method of perinatal PM than FNA in detecting various pathology. This is clearly demonstrated in NB findings of case No 35 (left kidney), case No 24 (liver), case No 38 (liver), case No 50

(lung). Furthermore, NB would permit a wide range of investigations including microbiology, immunological studies, frozen section, and electron microscopy studies. However, postmortem U/S imaging seems to have the potential to improve the NB both in terms of tissue collection and providing some anatomical information.

Table 2.16. The technical success rates for organs sampled of adult cadavers in different studies.

Study	No of cases	Needle type	Technical success rates for organs sampled		
			lung %	liver %	kidneys %
Wellmann[165] (1969)	357	Vim-Silverman	45.7	92.2	33.6
Baumgart et al[68] (1994)	16	Tru-cut	94	100	44
Fooudi et al[166] (1995)	21	Tru-cut	76	100	9.5
Huston et al[167] (1996)	20	Microvasive ASAP biopsy system	90	100	80
This study	26	Tru-cut	52	69.2	42.6

Abbreviation: The value in parenthesis represents the year of publication.

The External measurements of the studied cases

Table 2.1. The BW and different measurements in TOP cases.

Case No	GA (wks)	BW (gm)	HC (cm)	CR (cm)	CH (cm)	FL (mm)
5	20	275	14.5	16.8	25	31
6	20	340	17.5	17.3	26.6	35
11	20	191.3	15.8	15	21.3	28
13	21	113.7	12.5	13.5	19.4	23
14	18	223.7	14.3	14.5	21	25
17	19	188.1	14	14	25	25
18	29	1279	27.5	26.8	38.9	53
25	21	512	21	21	30.5	41
26	26	720	20.5	22	34	51
32	19	261	16	17.5	22.3	28
35	19	314	17.2	16	24	32
36	21	284	17	15	21.5	33
37	24	790	23	21	32	42
41	23	486.5	20	20	29.5	41
42	15	21.8	6.4	8.5	12	12
44	17	29.7	9.5	7.9	14.5	11
45	23	396	19.3	16.5	24.7	37
48	30	1050	26.5	24	35.3	53
49	23	435	17.3	19.3	26.5	36
51	26	844	25	25.8	36.5	50
52	26	739	20	23.9	34.6	48
53	19	230	15.4	15.5	22	28
55	17	187.2	13.9	15	22	27

Abbreviations: GA= gestational age, BW= body weight, HC= head circumference, CR= crown rump CH= crown heel

Table 2.2. The BW and different measurements in miscarriage cases

Case No	GA (wks)	BW (gm)	HC (cm)	CR (cm)	CH (cm)	FL (mm)
3	17	122.4	12.5	12.2	17.5	22
7	18	133.1	12.5	13.6	19.2	20
19	23	226	14	17.1	24.4	34
30	21	352.5	15.3	18.5	27	30
31	19	226.6	16.5	17	24	30
34	15	54.5	9.7	9.5	13.5	15
40	18	132	15	14	21	26
43	20	270	17.2	17.5	25.2	31
47	21	324.5	18.5	20	28.5	35
54	23	512	20	23	31	42

Abbreviations: GA= gestational age, BW= body weight, HC= head circumference, CR= crown rump, CH= crown heel,

Table 2.3. The BW and different measurements in Stillbirth cases.

Case No	GA (wks)	BW (gm)	HC (cm)	CR (cm)	CH (cm)	FL (mm)
1	28	583	22	22.5	33.5	46
12	29	1430	27.5	28	41	61
15	35	2143	31.5	33	46.4	66
16	34	1738	31.7	28.5	43.8	54
22	34	1675	26.5	29	42.3	64
27	26	800	21.5	23.5	34	49
28	40	3830	34.5	37	53	86
29	26	604	21	22	32	45
38	30	1542	28	28.5	41.5	58
39	32	1719	30	31	44	65
46	28	495	19.9	19.7	28.5	39

Abbreviations: GA= gestational age, BW= body weight, HC= head circumference CR= crown rump CH= crown heel,

Table 2.4. The BW and different measurements in neonatal deaths

Case No	GA (wks)	BW (gm)	HC (cm)	CR (cm)	CH (cm)	FL (mm)
2	27	1302	26.3	27	40	55
4	25	760	21.5	22	32.5	48
9	25	615	20	21	31	40
10	25	510	21.5	20.5	30.5	42
20	28	1220	26.5	25.5	36.5	54
21	34	2000.1	31	29.7	45.7	71
23	30	1664	29.9	29	43.7	64
24	29	825	25.8	25.6	36.9	54
33	36	2638	33.3	33	48	75
50	25	482	20.5	20	30.5	40

Abbreviations: GA= gestational age, BW= body weight, HC= head circumference, CR= crown rump, CH= crown heel.

Cases with significant non-invasive microscopic PM results

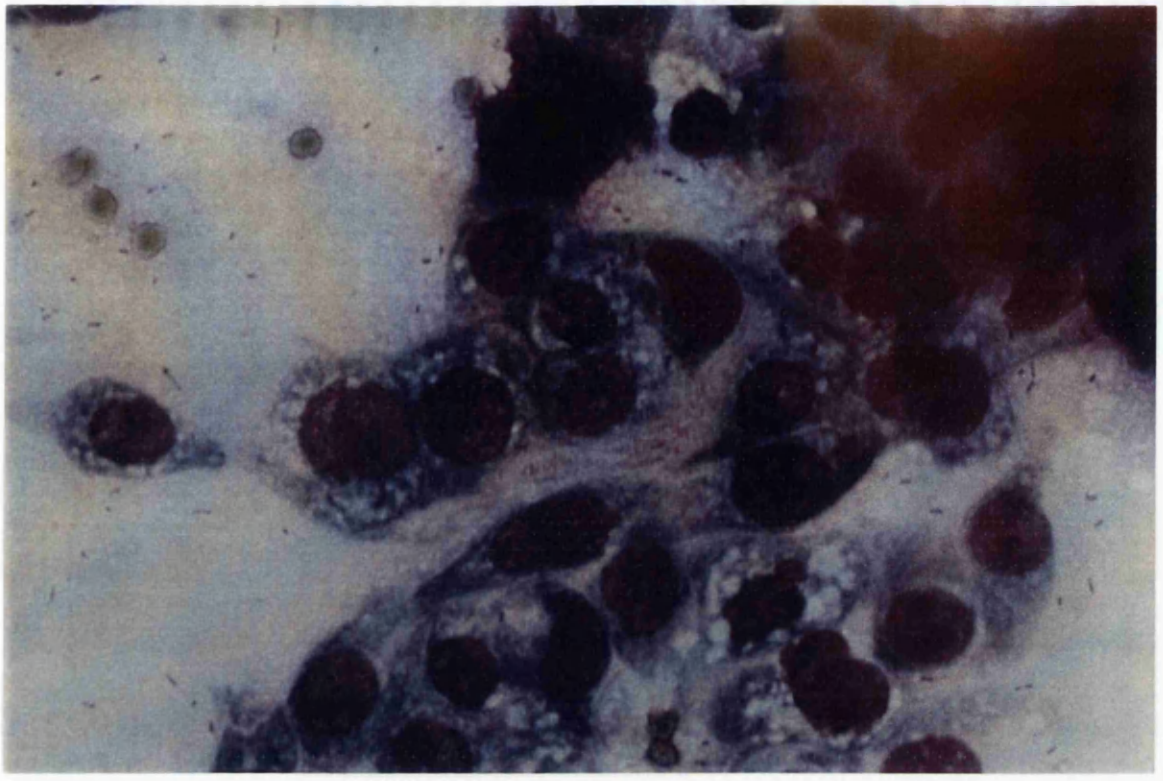


Figure 8. FNA smear from lung (case No 4) containing rod-like structures consistent with bronchopneumonia found on standard histology (*Pseudomonas aeruginosa)*

Figure 9. Biopsy specimen (case No 38): liver showing positive immunochemistry stain for HSV II

Chapter II. 3

Case Description

In this chapter, the results obtained from application of the whole set of non-invasive methods (clinical investigations, U/S examination, physical examination and body measurements, X rays, placenta, membranes, and cord examination, and microscopic examination) are analysed for each case by category (TOP, miscarriage, stillbirth and neonatal death), and compared with the yield of the full autopsy. In order to facilitate comparison, each case was classified into one of three following classes: (Tables 3.1, 3.3, 3.5, 3.7, 3.9);

Class A: Full autopsy confirmed the non-invasive techniques findings without adding any extra information. For convenient this group within tables is referred as *(FA unnecessary)*.

Class B: Full autopsy provided additional information, but did not change the overall diagnosis or the cause of death. For convenient this group within tables is referred as *(FA "helpful")*.

Class C: Full autopsy provided important information that determined the cause of death or affected genetic counselling or diagnosed a specific syndrome. For convenient this group within tables is referred as *(FA essential)*.

In some cases where certain non-invasive methods could not be used, a discussion is presented of the likelihood of non-invasive techniques having reached the same diagnosis as the definitive autopsy

Table 3.1. Classification of the study cases.

Category (No of cases)	Total No of cases (%) class A (FA unnecessary)	Total No of cases (%) class B (FA"helpful")	Total No of cases (%) class C (FA essential)
TOP (24)	11 (46%)	10 (41%)	3 (13%)
Miscarriage (10)	6 (60%)	3 (30%)	1 (10%)
Stillbirth (11)	8 (72%)	3 (27%)	0 (0%)
Neonatal death (10)	2 (20%)	7 (70%)	1 (10%)
Total (55 cases)	28 (51%)	22 (40%)	5 (9%)

3.1 Outcome categories:

Here, the comparison between invasive and non-invasive techniques was studied in detail for the four categories of pregnancy outcome (TOP, miscarriage, stillbirth, and neonatal death). If a technique contributes to the final diagnosis, that technique is considered to be of a significant value. The clinical findings, non-invasive PM techniques as well as the invasive PM technique are described for each individual case. A brief discussion is conducted and a general comment on some pathogenesis is made in most cases. The results for each category are summarised and tabulated at the end of the relevant section (Tables 3.3, 3.5, 3.7, & 3.9).

3.1.1 Termination of pregnancy (TOP) category

There were 24 cases which were terminated because of malformation or chromosomal abnormalities. The clinical presentation of each case is shown in Table 3.2, which includes, maternal obstetric history, antenatal diagnosis, and data on antenatal U/S.

The results of the comparison of the non-invasive approach with full autopsy are summarised in Table 3.3, page 148.

Table 3.2. The clinical presentation of TOP cases.

Case No	GA (wks)	Maternal obstetric history	Antenatal U/S	Antenatal diagnosis
5	20	IUFD. Vaginal bleeding	Microcephaly, alobar holoprosencephaly, nasal proboscis, hypotelorism, single atrium heart, Bil dysplastic kidneys, polydactyly.	Trisomy 13
6	20	1 miscarriage, history of baby with cleft lip	Bil cleft lip & palate.	Bil cleft lip & palate
8	14	1 abortion, history of neonatal death (polydactyly), vaginal bleeding	Polydactyly, ABN posture of lower limbs, encephalocele.	Multiple congenital anomaly (?Meckels Syndrome)
11	20	Vaginal bleeding, ↑BP on M-dopa, Nefedipin, spontaneous rupture of membranes at 14 wks	Normal → spontaneous rupture of membranes	Prolonged spontaneous rupture of membranes
13	21	history of neonatal death with Truncus arteriosus.	Solitary arterial trunk astride, ventricular septal defect.	Truncus arteriosus
14	18	1 miscarriage	Tense bladder, bright kidneys, HU. Pericardial effusion, ascites.	Bladder outlet obstruction
17	19	Ovarian cyst.	Nuchal fluid	Triploidy
18	29	-	Brachycephaly, intracranial calcification, hydrocephaly, pericardial effusion, hyperechogenic bowel.	Hydrocephaly
25	21	-	Hydrocephaly	Unilateral hydrocephaly
26	26	Neonatal death.	Holoprosencephaly (semilobar), microcephaly, globular heart.	Microcephaly. Holoprosencephaly.
32	19	Vaginal bleeding, 3 IUD.	Craniostenosis, cysts (post fossa), nuchal oedema, small bright kidneys, absent (bladder), ascites, small chest, short bones (4 limbs).	Short ribs polydactyly syndrome, ? Elejaide Syndrome
35	19	3 IUD.	Ascites, dilated bladder, nuchal oedema, HU, large bright kidneys, hydronephrosis, talipes.	Bladder outlet obstruction

Table 3.2. The clinical presentation of TOP cases (continue).

Case No	GA (wks)	Maternal obstetric history	Antenatal U/S	Antenatal diagnosis
36	21	1 IUD.	Spina bifida, absent cerebellum, talipes, ABN heart (LV). SGA.	NTD. IUGR
37	24	Vaginal bleeding	Dolichocephaly, nuchal oedema, pericardial effusion, enlarged right atrium, small kidneys.	Multiple congenital anomaly
41	23	Fetal anaemia	Hydrocephaly, porencephalic cyst, brachycephaly. Tortuous umbilical artery	Hydrocephaly. Chronic fetal anaemia
42	15	1 IUD.	Strawberry shaped head, hydrops fetalis, IUGR, cystic hygroma. SGA.	Ttisomy 18
44	17	Vaginal bleeding.	Strawberry shaped head, hyperechogenic bowel. SGA.	Trisomy 18
45	23	-	Hypoplastic aorta and LV, nuchal oedema, hemivertebra, kyphoscoliosis, bright large kidneys, syndactyly.	Multiple congenital anomaly
48	30	Polyhydramnios.	Hydrops fetalis, hydrocephaly, microcephaly, flexion deformity of the limbs. Pericardial, pleural effusion.	Fetal anomalies, Hydrops fetalis.
49	23	-	Encephalocele.	Encephalocele (NTD)
51	26	-	Hydrocephaly	Hydrocephaly
52	26	1 IUD, epileptic on Valproate	Spina bifida, hydrocephaly.	Spina bifida (NTD)
53	19	1 IUD, vaginal bleeding.	Normal	Trisomy 21
55	17	4 IUD, previous pregnancy complicated by holoprosencephaly.	Microcephaly, similobar holoprosencephaly, midline facial cleft.	Holoprosencephaly.

Abbreviations; Bil= bilateral, ABN=abnormal, ↑BP= high blood pressure, HU=hydroureter, - = not available, LV= left ventricle.

:

Case No 5:

Clinical findings; A 42-year-old Caucasian woman who had an anomaly fetal scan showed holoprosencephaly, nasal proboscis, hypotelorism, polydactyly and single atrium. All these findings pointed towards trisomy 13 which was later confirmed by fetal karyotyping. Termination was performed at 20 wks GA.

Non-invasive PM findings; the female fetus was dysmorphic with holoprosencephaly, nasal proboscis, hypotelorism and polydactyly in all four limbs as well as the presence of an overriding digits of the upper and lower limbs. The X rays showed holoprosencephaly and polydactyly. Placenta, membranes, and cord examination was normal. NB was not performed in this case.

Invasive PM findings; Internal examination showed features of alobar holoprosencephaly and the presence of atrial septal defect which was seen by antenatal U/S as single atrium. Brain sections were too disintegrated for performing histological examination.

Classification: class B (FA "helpful").

Trisomy 13 is caused by full non-disjunction for about 80% of cases, the other 20% of cases are due to unbalanced Robertsonian translocations and mosaics.[113] There is an association between trisomy 13 and an increased maternal age. Holoprosencephaly has risk of recurrence of 1% if chromosomal abnormality is present. This was established by the non-invasive techniques and was not altered by full autopsy.

Case No 6:

Clinical findings; A 39-old- year Caucasian woman who have had one previous baby with cleft lip. Fetal anomaly scan in this current pregnancy showed bilateral cleft lip and palate. Termination was performed at 20 wks GA. The result of the clinical investigations were not available, but fetal karyotyping was normal.

Non-invasive PM findings; External examination and X ray findings have confirmed the antenatal U/S finding. Placenta, membranes, and cord examination was normal.

Invasive PM findings; Internal examination and standard histology were normal.

Classification: class A (FA unnecessary).

There is a marked tendency for the prevalence of cleft lip & palate to rise with maternal age,[168] which is existed in this case as the mother aged 39-years-old. Cleft lip & palate may have a genetic, teratogenic or unknown basis. It occurs commonly with chromosomal error especially trisomy 13 and trisomy 18 and may form part of the spectrum of teratogenic effects of hydantoin derivatives, valproate, and trimethadione. Some facial and palatal clefts are associated with amniotic bands.

Case No 8:

Clinical findings; A 25-year-old Asian woman who had fetal anomaly scan showed polydactyly and encephalocele. The mother's origin is from Gujarati population where the Meckel syndrome is common, and had previous neonatal death with polydactyly. So in the light of this information, the fetus was diagnosed as having Meckel syndrome and terminated at 14 wks GA. Clinical investigations including karyotyping were normal.

Non-invasive PM findings; On external examination, the appearance was difficult to assess due to the early gestation (14 wks), but there was an obvious abnormal head, the brain appeared to be covered by partially cartilaginous membrane. The eyes were prominent. X rays findings were difficult to assess. Placenta, membranes, and cord examination revealed that the cord had velamentous insertion.

Invasive PM findings; Internal examination revealed cystic changes in the kidneys. Standard histology confirmed the presence of cystic change in the kidneys, revealed an increase in the fibrous tissue in the portal tracts of the liver and confirmed the presence of glial tissue in the brain. All these findings indicate meckel syndrome.

Classification: class B (FA "helpful").

Meckel syndrome, transmitted as an autosomal recessive trait, was first described in 1822[169] and the anomalies include CNS malformation mostly in occipital encephalocele, eye anomalies, cleft lip or cleft palate, cystic dysplastic kidney, postaxial polydactyly, congenital heart disease, and genital anomalies. In 1981, Fraser and Lytwyn defined Meckel syndrome as " ... cystic kidney dysplasia plus at least two other defects including relevant anomalies of the brain (usually), heart, liver, genitalia, spleen, urinary system or lip". This case as it was terminated at an early gestation (14 wks GA), it was difficult to delineate the exact cranial defect. Some of features are suggestive of anencephaly (prominent eyes and Skull vault defect) while the presence of covering membrane (skin and cartilage) and glial tissue are in favour of encephalocele. In any case, the definition of Meckel syndrome includes both

defects. Portal-biliary dysgenesis has been described in Meckel syndrome cases. In this case, there was an increase in the fibrous tissue at the portal tracts which could be regarded as a form of Portal-biliary dysgenesis. The classical ductal plate malformation was not seen and it is not expected to be seen at this stage of pregnancy on standard histology.

Case No 11:

Clinical finding; This pregnancy was terminated at 20 wks GA as antenatal U/S revealed no amniotic fluid. This confirmed the given maternal history of spontaneous rupture of membranes at 14 wks. There was also history of essential hypertension which was treated by Methyl-dopa and Nifedipine. The results of the clinical investigations were normal.

Non-invasive PM findings; The baby was well formed, but considered to be growth retarded as the BW and FL were below 5th centile. The presence of bilateral talipes equinovarous was confirmed by both external examination and X rays. Placenta, membranes, and cord examination was normal. Internal examination showed small lungs.

Invasive PM findings; The standard histology of the lung showed a reduction of the branches of the bronchi, a feature in keeping with pulmonary hypoplasia. This finding can be expected as result of prolonged spontaneous rupture of membranes.

Classification: class A (FA unnecessary).

In this case, the status of the kidneys was not assessed antenatally (U/S), but the given history of leaking, which could be confirmed clinically would indicate the cause of oligohydramnios. Antenatal U/S could have picked pulmonary hypoplasia in such cases by examining the chest circumference/abdominal circumference ratio in cases where the abdomen is not altered. This ratio is constant through out the gestation at 0.89 ± 0.12, value up to 0.77 are consistent with pulmonary hypoplasia. In fetus whose abdomen is altered in size the chest

circumference/femur length ratio is used for diagnosing pulmonary hypoplasia. The 5th centile value for this ratio are 4.0 and 3.4 for 18-24 and 25-40 wks, respectively.[170]

Case No 13:

Clinical findings; This pregnancy was terminated at 21 wks because of Truncus arteriosus diagnosed on antenatal U/S examination. There was a previous history of a baby born with the same cardiac defect. The results of the clinical investigations were not available.

Non-invasive PM findings; The external development was normal, but the baby according to the external measurements (low BW and FL) was considered as IUGR. X rays and placenta, membranes, and cord examination were normal.

Invasive PM findings; Internal examination confirmed the presence of truncus arteriosus, and standard histology showed no additional lesions.

Classification: class A (FA unnecessary).

Truncus arteriosus is attributed to the failure of septation of bulbus cordis. It accounts for 1.4% of congenital cardiac anomalies and has multifactorial inheritance, which can result from teratogens, such as thalidomide. In this case, there is a family history of previous baby with same cardiac defect. In fact, this could pointed towards the presence of inherited deletion of chromosome 22q11, which could be detected by using fluorescence in situ hybridization technique (FISH).[171]

A strong association have been also found to exist between truncus arteriosus and overt maternal diabetes,[172] which does not exist in this case. High incidence of extra cardiac congenital anomalies has been reported up to 50% of truncus arteriosus cases, including absence or hypoplasia of one of kidney, absent gallbladder, hypoplastic lungs and cleft palate or bony abnormalities,[173] but this does not also exist in this case. In general, the causes of

congenital heart disease are identified in only 10-15% of cases including chromosomal abnormalities, single gene defect, multifactorial, and teratogens. Single gene defect causing congenital malformation which accounts for 3-4% of congenital anomaly cases and are usually part of a syndrome, as in Noonan syndrome. In fact, most of these heart anomalies have multifactorial inheritance which refers to an undefined genetic predisposition acting with unknown environmental influences. The average empirical risk for recurrence has been reported to be 2-5%.[172]

Case No 14:

Clinical findings; This pregnancy was terminated at 18 wks after antenatal U/S showed enlarged bladder and hydronephrosis (bladder outlet obstruction). Subsequent scan showed possible ruptured bladder and ascites. Clinical investigations including karyotyping were normal.

Non-invasive PM findings; Externally, there was abdominal distension, but the infant was otherwise normally developmed. Plain X ray was normal. Placenta, membranes, and cord examination showed occasional oedematous villi and single umbilical artery.

Invasive PM findings; Internal examination revealed distended bladder with hypertrophied wall and right hydroureter. Prior to dissection, a contrast medium was injected directly into the bladder and an X ray showed retrograde flow to the left kidney, but no flow into the right ureter. A small amount of contrast traversed the length of the urethra, but this was less than would be expected. On standard histology, the kidneys showed normal architecture.

Classification: class B (FA "helpful").

In this case the diagnosis given by antenatal U/S was confirmed by standard histology. A successful NB of the kidneys might have provided this information on the kidneys' lesion. The status of the urethra could be evaluated non-invasivally through injection of contrast media into bladder under U/S guidance. The findings found here by contrast medium study

are most consistent with posterior urethral valves causing sub-total obstruction and mild hydronephrosis. In fact, posterior urethral valves are often difficult to detect by anatomical dissection, hence the importance of carrying out contrast medium study on such cases. In this case, the presence of single umbilical artery, which has an association with an increase in the incidence of renal and urinary tract abnormalities,[159] is consistent with the antenatal U/S diagnosis, but single umbilical artery is often found in the absence of other lesions.
Bladder outlet obstruction comprise obstructive anomalies in the lower urinary tract, and malformation consequent to obstruction in the upper urinary tract. Most cases have urethral atresia, stenosis or posterior valves as in this case. Kidneys are always involved and show variable cystic to hypoplastic dysplasia. Most cases of Bladder outlet obstruction are sporadic, though familial cases have been reported and it also recognised in trisomy 21, trisomy 18 and trisomy 13.

Case No 17:

Clinical findings; The case was terminated at 19 wks because of abnormal karyotyping (triploidy). Antenatal U/S examination revealed a normal fetus , but maternal enlarged cystic ovaries. Clinical investigation results revealed high maternal HCG and AFP.
Non-invasive PM; On external examination, the baby was of average size with a small jaw and somewhat flattened nose. There was overlapping of the third left toe by the 2nd and 4th. The genitalia were male with labioscrotal fusion and penis with mild hypospadias. X ray was normal. Placenta, membranes, and cord examination revealed that a few chorionic villi appeared to be oedematous and there was focal trophoblastic proliferation with indentation of villi. All of these features are suggestive of a partial mole.
Invasive PM findings; Internal examination reveal a structurally normal fetus. Standard histological examination of the testes showed dysgenetic features.
Classification: class B (FA "helpful").

Triploidy indicates the presence of an extra haploid set of chromosomes. This occurs in approximately 1% of all recognised human pregnancies that ends as Spontaneous abortion, usually in the embryonic period. It arises from a fertilisation error either by two haploid sperms or a diploid sperm fertilising a haploid ovum or through a diploid ovum being fertilised by a haploid sperm.[113] The aetiology of triploidy is different from other chromosomal anomalies in that it does not arise through meiotic recombination or non-disjunction. Therefore, there is no known increased recurrence risk in a patient who had a triploid fetus. In triploid conceptuses that have an extra paternal set of chromosome show cystic chorionic villi with trophoblastic hyperplasia (as in this case) whereas those of maternal origin are usually non-hydropic. The source of the extra set of chromosomes has also an influence on the phenotype, in that where the extra haploid set of chromosomes is of paternal origin, there is relatively normal fetal growth, while where the extra set is of maternal origin there is a classical phenotype with sever IUGR, relative macrocephaly, and a small non-cystic placenta. In this case, the source of the extra haploid set of chromosomes seems to be of paternal origin.

Case No 18:

Clinical findings; This pregnancy was terminated at 29 wks GA because of antenatal U/S finding of hydrocephaly. Intracranial calcification and pericardial effusion were also detected on U/S examination. The results of clinical investigations including karyotyping were normal.

Non-invasive PM findings; The baby was of normal formed appearance and of average size male baby. X rays and placenta, membranes, and cord examination were normal.

Invasive PM findings; Internal examination of the brain confirmed the presence of hydrocephaly. Standard histology of the brain revealed the presence of haemosiderin deposition around the aqueduct, indicating previous intracranial haemorrhage.

Classification: class C (FA essential).

Hydrocephaly is a congenital defect producing enlarged or abnormal shaped cerebral ventricles due to an increase of the free cerebrospinal fluid in the cranial cavity. This is mostly due to obstructive lesions rather than to an increase in the production of cerebrospinal fluid. Obstructive hydrocephaly is commonly caused by narrowing or occlusion of the cerebral aqueduct and up to 25% of cases of aqueductal obstruction in males may be the result of an X-linked recessive inheritance.[159, 174] Hence, the importance of keeping fetal sample for DNA storage in this case. Other causes of obstructive hydrocephaly could be midline brain tumours, vein of Galen aneurysems, subdural haematoma of posterior fossa and Dandy walker malformation. Hydrocephaly can be associated with structural anomaly in brain, such as holoprosencephaly and hydranencephaly. It has also been described in genetic syndromes, such as Apert syndrome and Roberts syndrome. An association of hydrocephaly with trisomy 18 has been observed. Infectious agents, such as toxoplasmosis and CMV can result in hydrocephaly.

In this case, the presence of haemosiderin deposition around the aqueduct, indicating previous intracranial haemorrhage. In fact, one of the main causes of intracranial haemorrhage, at this stage of pregnancy, is a germinal eminence haemorrhage, which may occur any time from the beginning of the second trimester to 32-34 wks. The germinal eminence is a small mound of primitive neurodermal cells lying between the ependyma and caudate nucleus. It is programmed to melt away after about 32 to 34 wks. It contains fragile capillaries which are vulnerable to hypoxic-ischaemic damage induced by any fluctuation in cerebral blood flow.[169]

Case No 25:

Clinical findings; The pregnancy was terminated at 21 wks GA on the basis of antenatal U/S findings of right-sided hydrocephaly with slight displacement of midbrain to the left side. Other clinical investigations were normal.

Non-invasive PM findings; Externally, the infant was of normal growth and development. X ray was normal. Placenta, membranes, and cord examination revealed multiple small fresh clots adherent to the maternal surface.

Invasive PM findings; Internal examination revealed under development of arachnoidal vasculature on the right side of the brain. The presence of hydrocephaly was confirmed by internal examination and standard histology.

Classification: class B (FA "helpful").

Case No 26:

Clinical findings; This pregnancy was terminated at 26 wks GA because of anomaly scan showed semilobar holoprosencephaly and microcephaly. The clinical investigations including karyotyping were normal.

Non-invasive PM findings; On external examination the baby was a well-grown. There was abnormal head size and shape which was also seen on X rays. Placenta, membranes and cord examination was normal.

Invasive PM findings; Internal examination revealed abnormal brain with rudimentary hemispheric lobe and incomplete inter-hemispheric fissure, especially anteriorly. Olfactory bulbs were not present. These findings were confirmed by standard histology of the brain.

Classification: class A (FA unnecessary).

Holoprosencephaly implies a failure of normal septation of prosencephalon into cerebral hemisphere. There is an association of holoprosencephaly with trisomy 13. Familial cases have been reported showing both autosomal dominant and recessive inheritance, therefore a sample from the fetus for DNA storage is recommended in this case. Holoprosencephaly has

also been described in the infants of diabetic mothers and with other teratogens including alcohol.

Case No 32:

Clinical findings; Detailed antenatal scan of this case showed small lungs and short ribs and limbs, in addition to polydactyly, bright kidneys, ascites, posterior fossa cyst and possible craniostenosis. On the light of these findings, the case was diagnosed as short-ribbed polydactyly syndrome or probably Elejalde syndrome and terminated at 19 wks GA. The results of other clinical investigations were not available.

Non-invasive PM findings; On external examination, the baby was of normal BW and had dysmorphic features with probable hypertelorism. The ears appear abnormal and the chin appears micrognathic, in addition to posterior mid-line cleft palate. Postaxial polydactyly was present on the upper limbs. There was no evidence of craniostenosis and the anterior fontanelle appeared to be large. The X rays confirmed the presence of short ribs and limbs and polydactyly. There was no radiological evidence of craniostenosis. Placenta, membranes, and cord examination was normal.

Invasive PM findings; Internal examination revealed small lungs, gastropancreatic fusion and normal kidneys. Standard histology of kidneys was normal. Other organs showed no gross histopathological abnormalities, in particular no excess of connective tissue or perivascular neural proliferation.

Classification: class B (FA "helpful").

In this case, NB was not performed, but on hypothetical basis the absence of dysplastic changes of the kidney could have been defined by NB of the kidneys and absence of the connective tissue hyperplasia could have been found by NB of the liver. If this had happened, the case would have been classified as class A, but as it stands the classification is class B. In

this case the external examination, radiological examination and histological examination pointed towards short-ribbed polydactyly syndrome and excluded Eljelad syndrome. Eligalde syndrome is bizarre syndrome characterised by large BW, over-growth of subcutaneous tissue giving rise to pseudo-hydropic appearance, craniostenosis, polydactyly, exomphalos and cystic/dysplastic kidneys.[159]

Case No 35:

Clinical findings; Bladder outlet obstruction was diagnosed in this case by antenatal U/S and the pregnancy was terminated at 19 wks GA. The diagnosis was based on U/S findings of severe oligohydramnios, ascites, dilated bladder, hydroureter, hydronephrosis, and bright kidneys. Clinical investigation results including karyotyping were normal.

Non-invasive PM findings; on external examination, abdomen was distended. Nuchal oedema and bilateral talipes were noted. X rays and placenta, membranes, and cord examination were normal. NB was attempted in this case for liver and lung and kidneys and was only successfully sampled in left kidney, where the sample showed dysplastic changes.

Invasive PM findings; Internally, there were small lungs, enlarged kidneys with multicystic cortical cut surface and hydronephrosis. All these findings were confirmed histologically. There was also dilated bladder and hydroureter. Contrast medium study was not performed in this case.

Classification: class A (FA unnecessary).

This case was diagnosed antenataly as being a case of bladder outlet obstruction. Furness et al[55] have demonstrated the capability of non-invasive PM techniques to delineate the exact anomalies in such cases by the use of contrast medium and U/S guided NB of the kidneys. In the British Colombia Hospital study[175] (1980-89), of 13 cases of bladder outlet obstruction consisting of 11 males and 2 females, with the GA varying from 9-33 wks, was performed.

The most common pathological finding in the urethra was atresia (9/13). Other findings included, posterior valve (2/13) and urethral stenosis (2/13).

The upper urinary tract pathological findings were cystic dysplastic in 10/13 cases, hydronephrosis in 2/13 cases. Other associated anomalies were anal atresia in 1 case and abnormal genitalia in another case.

Case No 36:

Clinical findings; This pregnancy was terminated at 21 wks GA as antenatal U/S examination revealed Spina bifida, absent cerebellum, talipes, abnormal heart and SGA. The result of clinical investigations revealed high msAFP.

Non-invasive PM findings; On external examination, the growth was regarded to be within normal range (BW and FL). The presence of spina bifida at the lumbar region was confirmed as well as the presence of talipes and flexion deformity of both wrists. These findings were also seen on radiological examination. Placenta, membranes, and cord examination was normal. NB was successfully performed for liver and right lung.

Invasive PM findings; Internal examination revealed normal heart, and presence of cerebellum. Standard histology showed no additional lesions.

Classification: class C (FA essential.

Neural tubal defect (NTD) has multifactorial inheritance since, for most of cases both genetics and environmental factors are involved in the aetiology of this defect. In a few cases, chromosomal abnormality and a major gene defect are implicated. Maternal ingestion of teratogens, such as antifolates, dietary deficiency of folic acid and other vitamins are considered as risk factors.[159]

For almost all cases of spina bifida, there is an existence of Arnold-Chiari type II malformation. This malformation is indicated by the displacement of the cerebellum and distal brain stem through the foramen magnum into upper cervical canal. It is visualised on

U/S as a lemon shaped head " lemon sign" and a banana shaped cerebellum " banana sign".[170]

Case No 37:

Clinical findings; This pregnancy was terminated at 24 wks because of antenatal U/S findings of reduced liquor, small kidneys, nuchal oedema and enlarged right atrium. Clinical investigations revealed normal karyotype and a high msAFP.

Non-invasive PM findings; On external examination, the baby was morphologically normal. X rays and placenta, membranes, and cord examination were normal. NB was attempted for liver and lung, but was only successful in liver.

Invasive PM findings; On internal examination, the heart was enlarged and the aortic knuckle was prominent. There was bilateral renal agenesis with a small bladder and small lungs. There were multiple small pale areas on the liver indicating infarction which was confirmed histologically.

Classification: class C (FA essential).

In this case, the NB sample of the liver was normal and did not pick up the foci of infarction which were revealed by standard histology. On the other hand, NB of kidneys under U/S guidance could reveal bilateral renal agenesis as sampling of the apparent kidney mass would reveal adrenal tissue.[55]

Bilateral renal agenesis occurs in approximately 1:6000 live born babies.[175] In almost half of infants with bilateral renal agenesis, there are no another malformations except those associated with oligohydramnios. These cases are thought to be sporadic. Bilateral renal agenesis may be a part of VATER association, Sirenomelia sequence, and Caudal regression sequence. Occasionally, familial cases do exist and may include members with agenesis of both kidneys, unilateral agenesis and sever dysplasia of the other kidney or severe dysplasia of both kidneys. The risk for recurrence of bilateral renal agenesis or sever dysplasia is 15-20%, compared with the 3.5% empirical recurrence risk for non-familial renal agenesis.[175] Renal

agenesis pathogenesis is the result of failure of development of ureteric bud from the mesonephros or of its early degeneration: absence of the ureteric bud leads to failure of the metanephric blastema to develop. According to Sabbagha[170] only 50% of fetuses with renal agenesis appear abnormal on the U/S. The reasons for this poor sensitivity include oligohydramnios and enlargement of adrenal glands , which fill the renal fossa and mimic a kidney.

Case No 41:

Clinical findings; The pregnancy was terminated at 23 wks because of fetal anomaly scan revealed hydrocephaly, porencephalic cyst around ant horn and tortuous umbilical artery (right). Fetal karyotyping was normal. Anti-platelet antibody and tests for parvovirus & TORCH infection were negative. However, fetal blood sampling showed chronic fetal anaemia with a normal platelet count.

Non-invasive PM findings; on external examination, the baby was of normal appearance for GA. There was no external stigma of disease. X ray was normal. Placenta, membranes, and cord examination showed single umbilical artery which was dilated and tortuous within the abdomen and some nucleated fetal red blood cells were noted in the placental vessels. NB was attempted for the all four organs, but was only successfully obtained in liver which showed normal histology.

Invasive PM findings; Internal examination of the brain showed the presence of hydrocephaly, petechial haemorrhage on the parietal cortex, and periventricular yellow areas. Histologically, brain sections confirmed the hydrocephaly, showed areas of destruction in the right frontal cortex, and evidence of old haemorrhage within the white and gray matter. There was evidence of chronic fetal stress (an increase in fat accumulation in the adrenals and foci of extra-medullary erythropoiesis in the liver and spleen).

Classification: class A (FA unnecessary).

Chronic fetal anaemia could be a result of an increase in blood destruction caused by immune-mediated (Rh antibodies and others) or by non- immune mediated defects of the fetal red cells or by bone marrow suppression due to parvovirus infection. Moreover, fetal haemorrhage and fetomaternal haemorrhage cause fetal blood loss. In this case, the CNS abnormalities were confirmed, the porencephalic cyst represents a local destructive processes, presumably secondary to fetal anaemia. However, the exact aetiology of the fetal anaemia in this case is obscure. An extensive haematological study and evaluation of haemostasis in the next pregnancy is essential in such cases.

Case No 42:

Clinical findings: An antenatal U/S examination of this case showed skin oedema, cystic hygroma, SGA, and a strawberry-shaped head which were considered as markers of aneuploidy. These suggestion was confirmed by a fetal karyotyping result of trisomy 18. The pregnancy was terminated at 15 wks GA.

Non-invasive PM findings: On external examination, facial dysmorphism was difficult to assess due to the maceration. There was a possible micrognathia. The BW and FL were below 5th centile and the baby was considered to be growth retarded. X rays and placenta, membranes, and cord examination were normal. NB was not performed for this case.

Invasive PM findings: Internal examination and standard histology showed no additional lesions.

Classification: class A (FA unnecessary).

Trisomy 18 occurs in 0.18% of recognised pregnancies and 0.015% of live-born infants.[113] It is reported to be second only to trisomy 21 in the frequency of multiple malformation syndromes, but it is more lethal than trisomy 21. As with most other trisomies, an increase in maternal age is a risk factor, and the origin of the extra chromosome is maternal in 96% of cases in whom the chromosome origin could be determined. The characteristic stigmata of trisomy 18 include, IUGR, small size face, micrognathia, abnormal ears, and facial cleft. The

upper extremities will often have the hand clenched into a fist with the index finger overlapping the third, and the fifth finger overlapping the fourth. The feet may have a prominent heel a "Rocker-bottom" appearance.

Case No 44:

Clinical findings: This pregnancy was terminated at 17 wks GA because of antenatal U/S findings of strawberry-shaped head, SGA and hyperechogenic bowel, all which suggest aneuploidy. Fetal karyotyping showed Triploidy. There was also an increase in the maternal serum HCG.

Non-invasive PM findings: On external examination, the body measurements (BW and FL) were consistent with IUGR. There was micrognathia and a small defect in the midline between anterior and posterior fontanelles suggesting encephalocele. X rays revealed abnormal thoracic vertebrae and the cranial defect. Placenta, membranes, and cord examination was normal. NB was not carried out in this case.

Invasive PM findings: Internal examination revealed intestinal malrotation, otherwise normally structure fetus. Standard histology revealed no further lesions.

Classification: class B (FA "helpful").

Case No 45:

Clinical findings: This pregnancy was terminated at 23 wks after fetal anomaly scan revealed hypoplastic left heart, hemivertebra, dysplastic kidneys, nuchal oedema and syndactyly. Clinical investigations including karyotyping were normal.

Non-invasive PM findings: On external examination, the baby was short with measurements (BW and FL) consistent with IUGR status. There were nuchal oedema and rudimentary ears. The hands were abnormal and showed a prominent 'V' shaped cleft between index and middle fingers. The feet showed overlapping of the third and fourth toes. X rays findings were

significant (Figure 10), as it shows a vertebral anomalies with many hemivertebrae in the thoracolumbar region. The ribs appear fan-like or crab-like and they are fused anteriorly. Placenta, membranes, and cord examination was normal. NB was attempted for liver, lung, and kidneys, but was successful only in liver.

Invasive PM findings: Internal examination confirmed the presence of hypoplastic left heart, but showed normal structure right kidney and left renal agenesis. Standard histology showed no additional lesions.

Classification: class B (FA "helpful").

On the light of above findings the baby was suspected of having Jarcho-Levin syndrome [176] which is a severe form of Spondylocostal dysplasia, and is associated with other anomalies including congenital heart disease and urogenital anomalies. The syndrome has autosomal recessive mode of inheritance. On this basis, the standard autopsy only add some findings, but the main diagnosis was reached by non-invasive techniques.

Hypoplastic left heart, which was found in this case, is a heterogeneous condition characterised by a variable degree of hypoplasia of the left ventricular cavity and its inflow and outflow tracts.[177] This defect can be associated with chromosomal anomalies including, Turner's syndrome, trisomy 18, and trisomy 21 and other extracardiac anomalies including, two-vessel cord, craniofacial, gastrointestinal, and genitourinary anomalies. In fact, the presence of these extracardial anomalies ranges from 11% to 40% of cases.[178] Furthermore, these anomalies could represent a genetic syndrome, the list includes Ellis-van Creveld syndrome, Saldino-Noonan syndrome and Smith-Lemli-Opitz syndrome which have autosomal recessive inheritance and Holt-Oram syndrome and Apert syndrome which have autosomal dominant inheritance.[179]

Retrospectively, if NB was carried out for the kidneys under U/S guidance, the presence of normal right kidney and the absence of left kidney, might have been detected and the case could have been considered as class A.

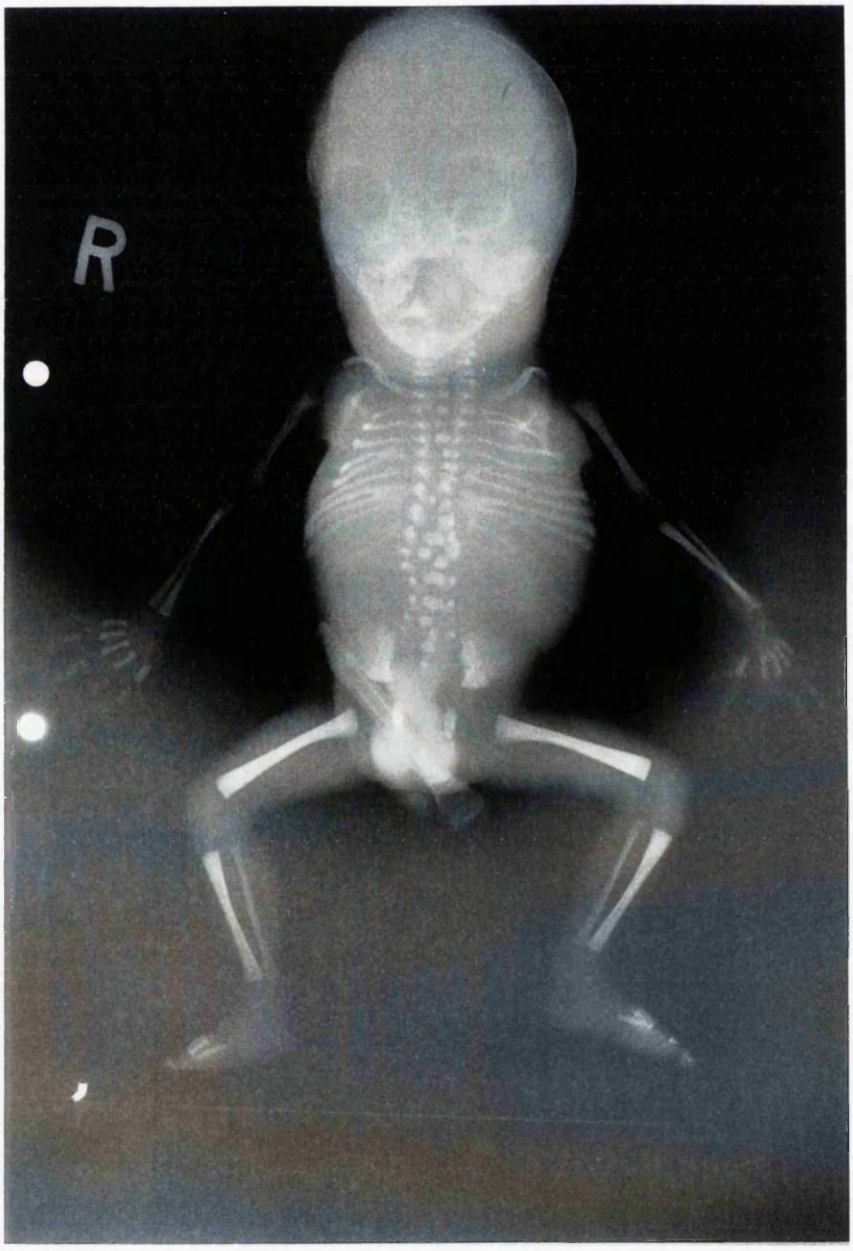


Figure 10. Radiography of case No 45 showing vertebral anomalies with many hemivertebrae in the thoracolumbar region and ribs anomalies characteristic of having Jarcho-Levin syndrome.

Case No 48:

Clinical findings; This pregnancy was terminated at 30 wks GA because of U/S findings of hydrops fetalis, hydrocephaly, microcephaly, and flexion deformity of the limbs. Clinical investigations include karyotyping were normal.

Non-invasive PM findings: On external examination, there were nuchal oedema, micrognathia, and flexion deformity of the limbs with clenched fingers. This was also seen on X rays, in addition to truncal oedema (upper half of the body). Placenta, membranes, and cord examination showed that the placenta had variable oedema. NB was attempted in all four organs and was successful in liver and right kidney.

Invasive PM findings: Internal examination revealed an enlarged heart and small lungs. Standard histology of the thymus showed a " starry sky" cortical appearance, fetal adrenal cortex was thin with evidence of fat accumulation. Both of these features suggest chronic intrauterine stress. Sections of the brain showed hydrocephaly and evidence of old ischaemic injury.

Classification: class B (FA "helpful").

The findings of the non-invasive techniques; hydrocephaly, nuchal oedema, flexion deformity of the limbs, and clenched fingers may all be accounted for by the ischaemic damage found by internal examination of the brain. The exact aetiology of the hydrops in this case was not so apparent though the lack of limb movement (hypomobility) and diminished respiratory movement may result in the accumulation of the subcutaneous fluid and lymphoedema.[180]

Case No 49:

Clinical findings: This pregnancy was terminated at 23 wks because of U/S findings of large encephalocele containing most of the cerebellum. Clinical investigations include karyotyping were normal.

Non-invasive PM findings; On external examination, the posterior occipital protuberance which was partially skin covered was found. This was also seen on X rays. There was no other external abnormalities and placenta, membranes, and cord examination was normal.

Invasive PM findings: Internal examination revealed normal formed cerebral hemispheres, and the mid-brain appeared to protrude into the occipital bony defect. The cerebellum appeared hypoplastic. Standard histology showed no additional lesions.

Classification: class B (FA "helpful").

Encephalocele is a herniation of brain tissue outside the cranial cavity. The vast majority of its cases are at in the occipital region. It may occur as an isolated defect or in association with other anomalies as a part of various inherited syndromes, such as Meckel-Gruber syndrome. Hence, it is important to examine kidneys histologically. In this case, NB of right kidney was performed and revealed normal structure. Isolated encephalocele has multifactorial inheritance with recurrence risk of 2% raising to 25% if it is associated with Meckel-Gruber syndrome.

Case No 51:

Clinical findings; This pregnancy was terminated at 26 wks GA on the basis of antenatal U/S finding of hydrocephaly and fetal karyotyping finding of a balanced denovo translocation, involving chromosomes 3, 6 and 10.

Non-invasive PM findings; On external examination, there were small ears and high arched palate, otherwise a normally formed baby. X rays and placenta, membranes, and cord examination were normal. NB was successfully performed for all four organs.

Invasive PM findings; On internal examination, the presence of hydrocephaly was confirmed and corpus callosum was not apparent. Standard histology of the brain was limited due to disintegration of the brain slices.

Classification: class B (FA "helpful").

Corpus callosum refers to the fibres that cross from side to side on a glial sling. This can be presented as primary agenesis due to an early embryonic defect of lamina terminalis. It occurs sporadically as an isolated anomaly, without apparent clinical effect. Secondary agenesis however, originates in the later part of fetal life as a sequel of inflammation or of porencephalic or hydrocephaly processes. Furthermore, agenesis of corpus collosum has been described in cases of trisomy 13 and trisomy 18.

Case No 52:

Clinical findings; This pregnancy was terminated at 26 Wks GA after U/S finding of spina bifida and hydrocephaly. The results of the clinical investigations were not available, but the mother was an epileptic and on Valproate medication.

Non-invasive PM findings; On external examination, there was a defect at lumbosacral region which was partly skin and partly membrane covered. The feet were abnormally flexed. These findings were also seen on X rays. Placenta, membranes, and cord examination was normal. NB was attempted for all the four organs, but failed in left kidney.

Invasive PM findings; Internal examination revealed a normally formed fetus. However, the brain was very soft so the presence of hydrocephaly could not be confirmed. Standard histology for the rest of organs were normal.

Classification: class A (FA unnecessary).

In this case there was a maternal history of Valproate medication, which is known to increase the incidence of spina bifida in babies.[168]

Case No 53:

Clinical findings; A 36-old-years women who had abnormal serum biochemistry (↓AFP) and abnormal fetal karyotyping with trisomy 21. Antenatal U/S examination revealed normally

formed fetus. The pregnancy was terminated at 19 wks GA.

Non-invasive PM findings; External examination and X rays showed normally formed fetus. Placenta, membranes, and cord was normal. NB was attempted for all the four organs, but failed in right kidney.

Invasive PM findings; Internal examination and standard histology revealed no further lesions.

Classification: class A (FA unnecessary).

Case No 55:

Clinical findings; This pregnancy was terminated at 17 wks GA because of U/S findings of semi-lobar holoprosencephaly, microcephaly, and midline facial cleft. Clinical investigation results were not available but there was a significant family history of a previous pregnancy complicated by holoprosencephaly which could indicate a familial inheritance condition.

Non-invasive PM findings; On external examination there was microcephaly as well as oro-nasal midline cleft, which communicated anteriorly with left nares. This was confirmed by X rays. Placenta, membranes, and cord examination was normal.

Invasive PM findings; Internal examination of the brain confirmed the U/S findings. Standard histology showed no additional lesions.

Classification: class A (FA unnecessary).

In this case, there is family history of previous baby with the same defect. This could point towards the presence of autosomal dominant mode of inheritance with incomplete penetrance and variable expression.[177] Therefore, a sample from the fetus for DNA storage is recommended.

Summary of TOP cases:

As can be seen in Table 3.3 Significant findings were revealed by external examination in 19 cases, by placenta, membranes, and cord examination in 4 and by X ray in 12 cases, including 2 where X ray was of diagnostic value (case No 32 & 45).

In 3 of the 24 TOP cases (13%) full autopsy was essential to disclose either the underlying pathogenesis, or the actual anomaly that might affect genetic counselling. In one case (No 36) internal examination delineated the exact anomaly from a "complex anomaly" to an ordinary NTD, in one case (No 37) internal examination disclosed the actual anomaly (renal agenesis), and in one case (No 18) standard histology identified the underlying pathogenesis of hydrocephaly as a haemosiderin deposition around the aqueduct, indicating previous intracranial haemorrhage.

Table 3.3. The value of non-invasive techniques and invasive technique for TOP cases.

Case No	Clinical investigations	Antenatal U/S	Ext. examination	X rays	PMC	Int. examination	Sd histology	Sd autopsy findings	IClassification
5	S	S	S	S	NS	S	NS	Holoprosencephaly, atrial septal defect, polydactyly, hypotelorism, nasal proboscis. (Trisomy 13)	B
6	S	S	S	S	NS	NS	NS	Bilateral cleft lip & palate	A
8	S	S	S	NS	NS	S	S	Rostral NTD, polydactyly, cystic dysplastic kidneys. (Meckle syndrome)	B
11	NS	S	S	S	NS	S	S	Spontaneous rupture of membranes, pulmonary hypoplasia.	A
13	-	S	S	NS	NS	S	NS	Truncus arteriosus, ventricular septal defect, IUGR.	A
14	S	S	S	NS	S	S	S	Bladder outlet obstruction (subtotal), single umbilical artery.	B
17	S	NS	S	NS	S	NS	S	Hypospadia, dysgenetic testis, partial mole. (Triploidy)	B
18	S	S	NS	NS	NS	S	S	Bilateral hydrocephaly, aqueduct stenosis, atrial septal defect.	C
25	NS	S	NS	NS	NS	S	S	Hydrocephaly, abnormal arachnoidal vasculature (right).	B
26	S	S	S	S	NS	S	S	Holoprosencephaly (semilobar)	A
32	-	S	S	S	NS	S	S	Posterior midline cleft palate, pulmonay hypoplasia, polydactyly. (Short-ribbed polydactyly syndrome),	B
35	S	S	S	NS	NS	S	S	Bladder outlet obstruction, cystic dysplastic kidney.	A
36	S	S	S	S	NS	S	NS	Spina bifida.	C
37	S	S	NS	NS	NS	S	NS	Bilateral renal agenesis, large heart, pulmonary hypoplasia.	C
41	S	S	NS	NS	S	S	S	Hydrocephaly, abnormal anterior horn, single umbilical artery.	A
42	S	S	S	NS	NS	NS	NS	Trisomy 18, IUGR.	A
44	S	S	S	S	NS	NS	NS	Encephalocele, abnormal vertebrae, intestinal malrotation, (Triploidy).	B
45	S	S	S	S	NS	S	NS	Hypoplastic left heart, renal & skeletal anomalies, (? Jarcho -Levin syndrome).	B
48	S	S	S	S	S	S	S	Hydrops fetalis, microcephaly, hydrocephaly, cerebral ischaemia, pulmonary hypoplasia.	B
49	S	S	S	S	NS	S	NS	Occipital encephalocele	B
51	S	S	S	NS	NS	S	NS	Hydrocephaly, atrial septal defect, absent corpus callosum, chromosomal abnormality (balanced denovo translocation of 3,6, and 10 chromosomes)	B
52	-	S	S	S	NS	NS	NS	Spina bifida	A
53	S	NS	NS	NS	NS	NS	NS	Trisomy 21, normally structure fetus.	A
55	-	S	S	S	NS	S	NS	Holoprosencephaly, microcephaly, midline facial cleft.	A

Abbreviations: Ext= exteral, PMC= placenta, membranes, and cord examination, Int= internal, Sd= standard, S = significant, NS = Non significant, - = not available.

3.1.2 Miscarriage (spontaneous/missed) Category

There were 10 miscarriage cases. The clinical presentation of each case is shown in Table 3.4, which includes, maternal obstetric history, antenatal diagnosis and data on antenatal U/S. The results of the comparison of the non-invasive approach with full autopsy are summarised in Table 3.5, page 162.

Table 3.4. The clinical presentation of miscarriage cases.

Case No	GA (wks)	Maternal obstetric history	Antenatal U/S	Antenatal Diagnosis
3	17	NS	-	Spontaneous abortion
7	18	Vaginal bleeding, ↑BP on Methyl-dopa, spontaneous rupture of membranes at 14 wks	Spontaneous rupture of membranes	Intrauterine infection
19	23	1 IUFD.	SGA	IUGR, IUFD
30	21	1 IUD at 21 wks	Nuchal oedema, general oedema, ascites, short femur, small chest.	Multiple pterygium syndrome.
31	19	Vaginal bleeding, 1 IUFD, 1 miscarriage.	IUFD, tallipes. Refereed as case of ?spina bifida & meningocele.	IUFD. Multiple congenital anomaly, IUGR.
34	15	Vaginal bleeding, spontaneous rupture of membranes.	-	Spontaneous abortion
40	18	-	IUFD	Missed abortion
43	20	NS	Normal → IUFD	Missed abortion
47	21	2 IUD	Hydrops fetalis, pericardial effusion, ventricular disproportion.	Hydrops fetalis, minor heart anomalies
54	23	-	Arachnoid cyst, small cerebellum, hydrocephaly, echogenic cordae tendinae	Multiple congenital anomaly. IUFD.

Abbreviations; NS= non significant, - = not available, ↑BP= high blood pressure.

Case No 3:

Clinical Findings; A mother of unknown age who have had no prenatal care was presented with a painless labour. The fetus was spontaneously delivered at 17 wks GA. Clinical investigations were normal.

Non-invasive PM findings; An external and radiological examination of the baby were normal. Placenta, membranes, and cord examination revealed mild chorioamnionitis and amnion nodosum. NB was not performed.

Invasive PM findings; Internal examination and standard histology revealed no further lesions.

Classification: class A (FA unnecessary).

Although, the presence of amnion nodosum suggests that the membranes may have been ruptured for some time, there was no evidence of pulmonary hypoplasia. The chorioamnionitis caused by ascending infection may have been the reason for this spontaneous abortion. NB was not performed in this case, but had this technique been carried out, mainly for the lung, there was a possibility that the presence or exclusion of pneumonia would have been reached. Furthermore, NB sample could be microbiologically studied.

Case No 7:

Clinical findings; A 32-year-old woman who had hypertension discovered at first trimester and treated with Methyl-dopa. There was a history of spontaneous rupture of membranes at 14 wks GA. Antenatal U/S at 17 wks GA revealed no liquor. Spontaneous onset of labour and delivery occurred at 18 wks GA.

Non-invasive PM findings; Anthropometric measurements revealed low BW and FL so, the baby was considered to be IUGR. The presence of talipes and mild jaw recession indicated a prolonged rupture of membranes. X rays showed talipes. Placenta, membranes, and cord examination showed chorioamnionitis, and bacteriological culture revealed Pepto-streptococcus species (non pathogenic). NB was not carried out.

Invasive PM findings; Internal examination showed normal structures. Standard histology of the lung showed neutrophil polymorphs within bronchioles and distal air spaces which indicate early intrauterine bronchopneumonia. Tissue from lung and liver were handled for bacteriological culture, revealed no growth in the former, *E. coli* and *Pseudomonas stutzeri* in the latter.

Classification: class B (FA "helpful").

The clinical data (spontaneous rupture of membranes) and pathological data (neutrophils in the bronchioles and chorioamnionitis) suggest that the cause of the spontaneous abortion in this case is infection, though tissue culture was negative (lung) or indicated contamination (placenta and liver).

NB was not performed in this case, but with the wisdom of hindsight, NB of lung could have detected bronchopneumonia and a sample for bacteriological culture could have been obtained. On this basis, autopsy would not have added any significant findings.

Case No 19:

Clinical findings; A 28-year-old mother who had had previous history of IUFD. Anomaly scan was performed at 18 wks GA, because of abnormal maternal serum biochemistry (↑msAFP), revealed normal structure fetus. On rescanning at 22^{+4} wks GA, the fetus found to be SGA with normal amniotic fluid volume in addition to an absent end diastolic flow in the umbilical artery, and increased flow in the middle cerebral artery. These findings suggest a uteroplacental insufficiency case. Thereafter, the labour was induced and the baby was delivered at 24 wks GA, after being found to have died in utero.

Non-invasive PM findings; The body was macerated with the presence of mummification, but was normally formed. According to the given history and external examination, the estimated GA at time of fetal death thought to be 23 wks GA. However, FL and BW measurements are found to be below 5th centile for 23 wks gestation, so the baby was considered to be IUGR. X ray was normal. Placenta, membranes, and cord revealed minimal syncytial knot with perivillous fibrin deposition. NB was not performed in this case.

Invasive PM findings; Internal examination and standard histology revealed no additional lesions.

Classification: class A (FA unnecessary).

In this case the cause of IUGR and fetal death are unexplained. Several reports[86,87,88] have suggested an association between unexplained high second trimester msAFP level, which was found in this case and risk of subsequent fetal death, low birth weight baby and neonatal death.

There is possibility that the unexplained fetal death in this case could be related to placental aneuploidy (confined placenta mosaicism). Confined placenta mosaicism is reportedly associated with an elevated risk of fetal death, and IUGR.[119] In this case, chromosomal study for the fetus and placenta would be of value to exclude confined placenta mosaicism. Unfortunately, this was not performed.

Case No 30:

Clinical findings; A 36-old-year woman was referred for investigation of hydrops fetalis found on U/S at 21 wks GA. A detailed fetal anomaly scan performed at 22 wks GA revealed the fetus had died. Generalised oedema and hydrops in association with increased nuchal thickening and oligohydramnios were noted. Hence, the possibility of a genetic syndrome namely, multiple pterygium syndrome was raised. Clinical investigations showed normal fetal karyotyping, but low msAFP. Additionally, the mother had positive IgM for CMV at 14 wks GA, but changed to IgM negative at 22 wks GA. The patient was induced and delivered at 23 wks GA.

Non-invasive PM findings; The presence of nuchal oedema and hydrops fetalis were confirmed by the external examination. However, comment on the growth status was difficult (BW was at 25th centile and FL below 5th centile) since, hydrops as well as the maceration found in this case can make BW measurement unreliable. X ray was normal. Placenta, membranes, and cord examination showed villitis with focal necrotic villi. Viral inclusions consistent with CMV were present. NB was attempted only in lung but was not successful.

Invasive PM findings; Internal examination was normal. Standard histology revealed viral inclusions in most organs except the ribs. Moreover, standard histology of lung and kidneys showed maturation consistent with less than 20 wks gestation.

Classification: class A (FA unnecessary).

CMV is the most common cause of congenital infection, which can result from primary infection or reactivation of latent maternal infection. In the latter case, the acquired fetal disease usually has a milder form than that one resulting from primary CMV.[123] Up to one third of seropositive women experience reactivation of latent CMV infection during pregnancy, resulting in congenital infection in 1-2% of babies.[181] Moreover, It has been reported that following primary maternal infection, 18% of these babies will be symptomatic at birth, and by further follow up, up to 25% of babies will have long-term complications (intellectual impairment and/or deafness).[123] Specific IgM is concordant with a recent infection with CMV, but the period for which it exists varies.

Gestational CMV infection is associated with a wide variety of placental changes, ranging from an absence of abnormalities, to massive villous destruction with inflammation. CMV can penetrate placental villi when there is maternal viraemia, usually in a woman with primary infection.

The relationship between the GA and fetal infection is uncertain, but evidence suggests that the risk of congenital infection is probably higher when the fetal infection is acquired during the first half of pregnancy.[127] Gross fetal effects of CMV namely, oligohydramnios, IUGR, hydrops fetalis and CNS abnormalities could be detected antenatally by U/S.

In this case, the clinical investigation (seropositive for CMV) and the U/S findings (oligohydramnios, hydrops fetalis) pointed towards fetal infection with CMV. This is substantially supported by the impressive findings on placenta, membranes, and cord examination.

Case No 31:

Clinical findings; A 21-year-old woman was referred for further evaluation as U/S examination at 19 wks GA revealed spina bifida/meningocele and talipes. A repeat scan at 21 wks GA showed that the fetus had died, there was talipes, but it was difficult to confirm the rest of the U/S findings. Clinical investigations, including maternal serum biochemistry and fetal karyotyping were normal. The labour was induced at 21 wks GA.

Non-invasive PM findings; On external examination, the baby was macerated fetus with mummification. The morphometric data suggested the fetus was more the size of 19 wks GA. It also showed bilateral talipes, an intact omphalocele, and abnormal perineum with single midline orifice. X rays showed talipes and abnormalities of lower lumber and sacral spine. Placenta, membranes, and cord examination was normal. NB was not performed for this case.

Invasive PM findings; Internal examination revealed the presence of microcolon, normal left kidney with tortuous left ureter, but absence of right kidney. There were a small bladder, and the continuity between left ureter and bladder was difficult to establish. The genital organs consisted of structures resembling hemi-uterus, ovary and fallopian tube present only on the left side. Pubic rami were not identified on dissection of the anterior pelvis. Standard histology confirmed the presence of microcolon and normally developed left kidney. It also revealed that the mesenteric gut was ganglionic type and the bladder lumen was absent.

Classification: class C (FA essential).

The exact diagnosis of this case is still obscure though, the findings showed on PM examination (non-invasive and invasive techniques) could point towards 1 of the 4 possible PM diagnosis;

1) Agenesis of cloacal membrane[182]

This consists of combination of defects leads to absence of anal, genital, and urinary orifices. The morphological findings are determined as a primary malformation, secondary

deformations, and different types of other malformations of uncertain pathogenetic relationship to the primary malformation. There are three categories of findings.

a) Malformation of genitourinary structures and hindgut leading to complete bladder and bowel obstruction

b) Deformations due to an external fetal compression or combined external fetal compression and internal distension of hollow viscera with deformation of contiguous structures

c) Different types of anomalies involving cardiovascular, gastrointestinal, and other systems.

The exact pathogenesis of this syndrome is not clear, but the preliminary data suggest a teratogenic cause.

2) The megacystis-microcolon-intestinal hypoperstalsis syndrome (autosomal recessive inheritance), presents in new-born babies with abdominal distension, dilatation of the urinary bladder and ureters, and microcolon.[159] In this syndrome, microcolon is attributed to hypoperistalsis. Notably, the enteric plexi and smooth muscle are usually normal on histological examination.

However, in this case, the abdomen was not distended which could be explained by the earlier presentation (19 wks). The bladder was small which is not consistent with the typical finding of this syndrome.

3) In this case the presence of localised multiple malformation (absent right gonad, absent right kidney, microcolon, omphalocele, absent pubic remi) could suggest amnion rupture sequence as the aetiology for the multiple anomalies found on full autopsy. Nevertheless, the presence of chronic rupture of the amnion (diagnostic for this sequence) was not identified on placenta, membranes, and cord examination.

4) Lubinsky 1980 (uncertain inheritance)[183]

This is the association of Prune belly syndrome secondary to urethral agenesis, vertebral anomaly, atresia of distal hindgut and abnormal external genitalia. There is an enlarged sacular mass in the perineal region, sometimes with an apparent glans (pseudo

hermaphroditism). Diagnostic features also include renal anomalies (cystic or agenesis), atresia/short vagina, large bladder, and omphalocele.

Case No 34:

Clinical findings; This fetus was spontaneously delivered at 15 wks GA. There was maternal history of vaginal bleeding a few wks earlier. Clinical investigations revealed no further information.

Non-invasive PM findings; External examination showed normally formed developed fetus. X ray was normal. Placenta, membranes, and cord examination revealed a prominent intervillous haemorrhage in relation to the maternal surface. NB was not performed in this case.

Invasive PM findings; Internal examination and standard histology showed no additional lesions.

Classification; class A (FA unnecessary).

Case No 40:

Clinical findings; This case was delivered as missed abortion and labour was induced at 23 wks GA (by date) and 19^{+} wks GA (U/S), as fetal scan showed the fetus is dead in utero. Clinical investigations were normal.

Non-invasive PM findings; The body was macerated with the presence of mummification, but normally formed. The measurements were consistent with an 18 wks GA. X ray was normal. Placenta, membranes, and cord examination showed a small retroplacental haemorrhage. NB was not performed for this case.

Invasive PM findings; Internal examination revealed normal structure. Standard histology showed fat accumulation in adrenals and a thin thymic cortex, both of which suggest chronic intrauterine stress. Lung section revealed intra-alveolar squames indicating intrauterine gasping.

Classification: class B (FA "helpful").

In this case, retropaclental haemorrhage found on placenta, membranes, and cord examination could be the cause of fetal hypoxia which is picked up by standard histology of adrenals and thymus. In fact, it is one of the commonest cause of intrauterine asphyxia.

Case No 43:

Clinical findings; A Mother of 27-year-old with a non contributory medical and family history, delivered as missed abortion at 21 wks GA. The labour was induced because of antenatal U/S finding of IUFD. Clinical investigations were normal.

Non-invasive PM findings; The body was macerated with massive sloughing of skin, but normally formed. According to the appearance and the measurements, GA was estimated to be 20 wks gestation. X ray was normal. Placenta, membranes, and cord examination revealed prominent nucleated fetal RBCs in the villi which may indicate intrauterine hypoxia. NB was attempted for all four organs and sampling was success in kidneys.

Invasive PM findings; Internal examination did not revealed any abnormalities. Standard histology of adrenals showed a possible fatty changes, but for the rest of other internal organs were too autolysed for further assessment.

Classification: class A (FA unnecessary).

Case No 47:

Clinical findings; A 44-year-old woman who had fetal anomaly scan revealed ventricular disproportion and mild pericardial effusion. Additionally, there was a conflict between menstrual age (20 wks) and scan dating (18 wks) as the former was higher than the latter by 2^{+3} wks. The baby was rescanned at 22 wks (U/S), after a history of no fetal movement 5 days earlier, and found to be died with gross oedema of the abdomen and thorax, and bilateral pleural effusion. All these findings were thought to be antemortem changes. The delivery of

this missed abortion was induced at 22 wks GA (U/S). Clinical investigations including fetal karyotyping were normal.

Non-invasive PM findings; The body was a macerated fetus with massive sloughing of skin. There were features suggestive of truncal oedema on X ray. The external measurements (BW and FL) suggested that the GA at fetal death was 21 wks gestation, consistent with fetal movement history and degree of maceration. Placenta, membranes, and cord examination revealed pale and hydropic placenta and single umbilical artery. NB was performed successfully for the all four organs (right lung, liver, and kidneys).

Invasive PM findings; Internal examination revealed a small ventricular septal defect, both right and left ventricular walls appeared thick with little post valvular dilatation. Standard histology of adrenals and thymus showed features consistent with chronic intrauterine stress.

Classification: class B (FA "helpful").

This fetus appeared to be structurally normal, a part from the small ventricular septal defect identified on internal examination. Although, the specific aetiology for the hydrops have not been identified, it could traced back to the increased heart wall thickness which might have raised the systemic venous pressure.

Case No 54:

Clinical findings; A 29-year-old mother referred for confirmatory U/S scan at 23 wks GA, the scan showed an arachniod cyst containing an echogenic focus and a small cerebellum with dilated 3rd ventricle. On rescanning at 24 wks GA, the fetus was found to be dead, and induction of labour was then performed. Clinical investigation results were not available, but fetal karyotyping was normal.

Non-invasive PM findings; The fetus was macerated and there were no dysmorphic features. X rays and Placenta, membranes, and cord examination were normal. NB was unsuccessfully attempted for all the four organs.

Invasive PM Findings; Internal examination of the brain revealed a cystic anomaly of the falx with haemorrhage in the left side of the cyst wall. Bilateral posterior fossa haematoma was also found. The cerebellum was not identified partially because of the fragmented and soft brain. Standard histology of the brain was not performed due to the severely autolysed brain. The rest of the organs were unremarkable.

Classification: class A (FA unnecessary).

The exact aetiology for these vascular lesions found in the CNS is obscure.

Summary of miscarriage cases:

Table 3.5 shows that significant findings were obtained by external examination in 5 cases, by X ray in 3 cases, and by placenta, membranes, and cord examination in 8 cases. Internal examination was of significant value in 3 cases; in 1 it supported the diagnosis made by non-invasive techniques, in 1 it altered the anatomical diagnosis made by non-invasive technique (No 31), and in 1 it provided additional information. Standard histology provided significant findings in 4 cases, in 1 it supported the diagnosis given by non-invasive techniques, in 2 it supplied further information, and in 1 (No 31) it altered the diagnosis, though a precise diagnosis of the syndrome could not be achieved even by full autopsy.

Table 3.5. The value of non-invasive techniques and invasive technique for miscarriage cases.

Case No	Clinical investigations	Antenatal U/S	Ext. examination	X rays	PMC	Int. examination	Sd histology	Sd autopsy findings	Classification
3	NS	-	NS	NS	S	NS	NS	Spontaneous abortion. Mild chorioamnionitis, amnion nodosum.	A
7	NS	S	S	S	S	NS	S	Spontaneous abortion. Spontaneous rupture of membranes chorioamnionitis, intrauterine bronchopneumonia, IUGR.	B
19	S	S	S	NS	S	NS	NS	Missed abortion. IUGR.	A
30	S	S	S	NS	S	NS	S	Missed abortion. CMV infection, hydrops fetalis.	A
31	S	NS	S	S	NS	S	S	Missed abortion. Anal malformation, absent right gonad, renal agenesis (right), omphalocoele, abnormal lumboscral vertebrae, ?Absent pubic bone, microcolon. (? syndrome).	C
34	NS	-	NS	NS	S	NS	NS	Spontaneous abortion (unexplained).	A
40	NS	NS	NS	NS	S	NS	S	Missed abortion. APH.	B
43	NS	NS	NS	NS	S	NS	NS	Missed abortion (unexplained)	A
47	S	S	S	S	S	S	NS	Missed abortion. Hydrops fetalis, ventricular septal defect, single umbilical artery.	B
54	S	S	NS	NS	NS	S	NS	Missed abortion. Cystic anomaly of falx, posterior fossa haematoma, absent cerebellum.	A

Abbreviations: Ext= external, Int= internal, Sd= standard, S = significant findings,
NS = Non significant findings, - = not available.

3.1.3 Stillbirth Category

There were 11 stillbirth cases, defined as any fetal death with GA of 24 completed wks or more. The clinical presentation of each case is shown in Table 3.6, which includes, maternal obstetric history, antenatal diagnosis and data on antenatal U/S.

The results of the comparison of the non-invasive approach with Full autopsy are summarised in Table 3.7, page 177.

Table 3.6. The clinical presentation of Stillbirth cases.

Case No	GA (wks)	Maternal obstetric history	Antenatal U/S	Antenatal Diagnosis
1	28	Conceived on Progestogen, ↑BP on Labetolol	Dilated heart, hyperechogenic bowel. SGA.	Uteroplacental insufficiency., IUGR
12	29	1 IUFD, pyrexia during labour, APH.	Normal	Maternal sepsis, APH
15	35	NS	Normal →IUFD	IUFD
16	34	NS	Normal Twins → IUFD	IUFD
22	34	Maternal asthma on antihistamine & Salbutamol.	Small chest, Bil. Hydronephrosis , Bil. HU, thick-walled bladder, small dysplastic kidneys, dolichocephaly, hydrocephaly.	Bladder outlet obstruction, cystic dysplastic kidneys, hydrocephaly.
27	26	Recurrent APH, spontaneous rupture of membranes.	Spontaneous rupture of membranes	APH, IUFD
28	40	NS	Normal	IUFD
29	25	↑BP, Cerebral vascular accident on Warfarin.	SGA	IUGR. Uteroplacental insufficiency
38	30	History of HSV II infection.	Absent corpus collosum, hydrocephaly, micrognathia, collapsed stomach, ascites.	Multiple congenital anomaly.
39	32	Placental abruption, anaemia.	Normal	IUFD, APH
46	28	2 IUD.	Transient pyelectasia, hyperechogenic bowel, SGA.	IUGR , IUFD

Abbreviations; ↑Bp= high blood pressure, NS= non significant, Bil.=bilateral, HU=hydroureter.

Case No 1:

Clinical findings; A 30-year-old woman who had medical history of hypertension treated with Labetalol. Antenatal U/S scan revealed SGA fetus and oigohydramnios, a picture suggesting uteroplacental insufficiency. The baby was rescanned at 28 wks GA and found to be dead. Induction of labour was carried out. Clinical investigations showed an increase in maternal blood urate.

Non-invasive PM findings; The body was that of macerated and normally-formed baby. External measurements (low BW and FL) suggested that the baby is IUGR. X ray was normal. Placenta, membranes, and cord examination showed multiple small infarcts of varying ages occupying 5-10% of placenta surface. NB was not performed in this case.

Invasive PM findings; Internal examination showed a normally formed fetus. On Standard histology, the lungs were full of squames in the peripheral air spaces, and the adrenals showed fatty changes.

Classification: class A (FA unnecessary).

There is evidence of uteroplacental insufficiency, presumably secondary to the known maternal hypertension.

Case No 12:

Clinical findings; A 34-year-old mother presented with fever and recurrent vaginal bleeding. Antenatal U/S was then performed at 29 wks GA and revealed IUFD. Thereafter, induction of labour was carried out at 29 wks GA. Clinical investigations were significant since maternal blood culture revealed streptococcus group B.

Non-invasive PM findings; The body was a normally formed and developed fetus. X ray was normal. Placenta, membranes, and cord examination showed no evidence of clot, or of surface depression to indicate retroplacental haemorrhage. Histologically, there was focal villitis and

chorionitis with more focal amnionitis. However, cultures of placenta showed a heavy growth of streptococcus organisms. NB was not performed in this case.

Invasive PM findings; Internal examination and standard histology were normal, but the tissue cultured from lung grew Streptococcus group B organisms.

Classification: class A (FA unnecessary).

Fetal death due to Streptococcus group B is well documented.[184] Infection with this organism may progress so rapidly that fetal death proceeds before an inflammatory response is apparent.[185] In this case, there is histological evidence of sepsis in the placenta corresponding to the heavy growth of streptococcus organisms from tissue sent to bacteriology. This is further supported by the history of maternal sepsis 12 hours prior to delivery. The presence of focal villitis suggests that haematogenous spread had occurred as a result of this maternal bacteraemia. Moreover, culture from the maternal cervical swab revealed no evidence of these organisms.

In this case, placenta, membranes, and cord examination could not confirm or exclude APH as there was no clot or surface depression on the maternal side, but this does not preclude a sizeable acute retroplacental haemorrhage which may leave very little morphological evidence of its existence.[74]

Case No 15:

Clinical findings; A 41 year-old mother with a non contributory medical and family history who had induction of labour at 36 wks GA after the fetus diagnosed as IUFD with presence of Spalding's sign. Clinical investigations including karyotyping were normal.

Non-invasive PM findings; The body was macerated with massive skin sloughing, but normally formed. The time of the baby's death was estimated to be at 35 wks GA. X rays and placenta, membranes, and cord examination were normal. NB was not performed in this case.

Invasive PM findings; Internal examination of the heart revealed an atria sepal defect. Standard histology of the lung showed many keratinized squames within alveolar spaces.

Classification: class B (FA "helpful").

The cause of death is not explained, so the case could be considered as unexplained stillbirth. The atria sepal defect found on internal examination is in fact, an incidental finding.

Cases No 16:

Clinical findings; This is twin pregnancy (twin II) which was reportedly uncomplicated (normal grown twins with normal amniotic fluid) until 34 wks GAB, when U/S identified that this fetus had died.

Caesarean section was performed and this case was delivered as stillborn with birth weigh of 1840 gm and the other CO-twin (twin I) was delivered with birth weight 2630 gm and Agar scores of 8/10. Clinical investigations were normal.

Non-invasive PM findings; The body was of normal developmental appearance. All the measurements indicate a baby of normal size for 34 GAB. X ray was normal. Placenta, membranes, and cord examination revealed monochorionic diamniotic placenta without vascular overlap. NB was not conducted in this case.

Invasive PM findings; Internal examination and standard histology revealed no additional lesions.

Classification: class A (FA unnecessary).

This pregnancy was interrupted at 34 wks (Caesarean section) because of fear from the sequence that follows the death of one twin, as renal cortical necrosis and cerebral haemorrhage have been described in the surviving co-twin as a result of a sudden loss of blood pressure in a shared circulation. The cause of fetal demise in this case is unexplained, although the discrepancy in size could suggest chronic TTTS.

Case No 22:

Clinical findings; A 28-year-old mother who had history of asthma on antihistamine and Salbutamol treatment. A routine fetal scan at 18 wks GA showed a reduction in the amniotic fluid, bilateral choroid plexus cyst and dilated bladder. A further scan at 20 wks, 23 wks, 28 wks, 33 wks, revealed lower urinary tract obstruction with small dysplastic kidneys and absent amniotic fluid. However, at one stage (U/S at 23 wks and 28 wks) hydrocephaly was also identified. Finally, the baby was delivered spontaneously at 34 wks GA as fresh stillbirth. Clinical investigations including fetal karyotyping were normal.

Non-invasive PM findings; The fetus was of appropriate size for the 34 wks GA. There was no obvious developmental anomaly. X ray was normal. Placenta, membranes, and cord examination showed amnion nodosum, consistent with the history of amniotic fluid absence. NB was performed successfully for the liver.

Invasive PM findings; Internal examination confirmed the U/S findings namely, small lungs, abnormal kidneys, hydronephrosis, bilateral hydroureter, hypertrophic bladder wall, urethral stenosis. Standard histology confirmed the above findings. Additionally, the presence of cystic dysplastic changes in the kidney were identified. However, hydrocephaly was not identified on either internal or histological examination of the brain.

Classification: class B (FA "helpful").

In this case, a histological examination of the kidneys was needed to identify the cystic dysplastic changes. NB of the kidneys might have revealed the dysplastic changes. In one stage of this pregnancy hydrocephaly was identified however, gross and histological examination of the brain showed no evidence of this lesion. In fact, Furness et al[55] have demonstrated the ability of postmortem U/S examination in picking up such anomaly in a mid-trimester fetus.

Case No 27:

Clinical findings; This pregnancy was complicated with spontaneous rupture of membranes at 25 wks GA and a recurrent vaginal bleeding. Later on, IUFD was diagnosed, and induction of labour was performed at 26 wks GA resulting in fresh stillbirth. Clinical investigation was significant as the urine culture of the mother revealed growth of *Pseudomonas aeruginosa* (gram negative bacilli).

Non-invasive PM findings; The body was of that normally formed baby with measurements consistent with 26 wks GA. There were asphyxial petechiae over the skin of upper half of the body. X ray was normal. On placenta, membranes, and cord examination, the maternal surface of the placenta showed a small amount of a fresh blood. There were marked funisitis and chorioamnionitis, and a pseudomonas species was cultured from the membranes. NB was performed only for liver and was normal.

Invasive PM findings; On internal examination, many structures namely, pleura, adrenals and thymus showed petechiae. Standard histology showed congestion in many organs. Standard histology of the lung did not show any evidence of inflammation though, the pseudomonas species was grown from the lung.

Classification: class A (FA unnecessary).

Pseudomonads are commonly isolated from nosocomial infections. *Pseudomonas aeruginosa*, the commonest pathogenic species, produces an exotoxin that inhibits polymorphonuclear phagocytosis. Pseudomonas aeruginosa has rarely been reported as a pathogen responsible for intrauterine infection and fetal death.[186]

In this case the detection of this bacterium in the maternal urine, fetal membranes and fetal lung at once suggests an intrauterine fetal infection. Furthermore, this suggestion is supported by the presence of chorioamnionitis and funisitis, the latter is considered to be a good indicator of intrauterine fetal infection. The ascending route is the most probable. The absence of an inflammatory reaction on lung histological examination is in fact, typical for

pseudomonas infection. It produces a typical picture of a pneumonia with foci of necrosis and haemorrhage due to vasculitis and infarction rather than neutrophilic infiltration of bronchioles and air spaces.[185]

The association between ascending infection and vaginal bleeding is well documented.[109] It is a debatable issue whether the deciduitis caused by ascending infection might lead to the rupture of retroplacental vessels, or whether the loss of cervical plug following the bleeding allowed the ascending infection.[73]

Case No 28:

Clinical findings; A 27-year-old mother with a non contributory medical and family history, presented at 40 wks GA with IUFD. Antenatal U/S scan at 18 wks revealed normal pregnancy. Induction of labour was performed resulting in Macerated stillbirth. Clinical investigations were normal.

Non-invasive PM findings; The body was of a well-grown and normally developed baby with measurements consistent with the given GA. There were multiple petechial haemorrhages over the skin of upper half of the body. X ray was normal. Placenta, membranes, and cord examination revealed long cord (82 cm), greenish colour membranes, and hyperplastic amnion with pigment in macrophages. Placenta was cultured and showed mixed growth. NB was unsuccessfully attempted for all four organs.

Invasive PM findings; Internal examination revealed surface petechial haemorrhages on lung and thymus. On standard histology, the lung section showed congestion and intra-alveolar squames and the brain sections revealed some neural damage of an ischaemic nature.

Classification: class B (FA "helpful").

This was a well-grown and developed baby with a gross asphyxial petechia. The cause of this asphyxia is unexplained, although an excess in cord length found in this case could suggest

cord accident, but there was no skin grooving around the baby's neck and no umbilical cord lesions.

Case No 29:

Clinical findings; A 20-year-old woman who had a history of thrombosis and warfarin medication, presented at 25 wks GA with reduced fetal movement, ankle oedema, and high blood pressure. Her previous antenatal U/S at 19 wks GA was normal. However, a fetal scan at 25^{+} wks showed a SGA fetus without structural anomaly, and increased resistance in both uterine arteries with bilateral notching. These findings were in keeping with uteroplacental insufficiency. The case was rescanned after 2 days and the baby was found to be dead. Induction of labour was performed at 26 wks GA.

Non-invasive PM findings; The body was that of well-formed fresh stillbirth with measurement indicating a state of IUGR (BW below 5th centile and FL at 5th centile). X rays was normal. On placenta, membranes, and cord examination, the serial sections of the placenta showed areas of dark infarction occupying less than 15% of placenta volume. Histologically, there was an increase in syncytial knot formation and perivillous fibrin deposition as well as foci of infarction of variable ages. NB was attempted for the all four organs, but succeeded in lung and right kidney only.

Invasive PM findings; Internal examination revealed normal structure. Standard histology showed squames in distal airways of the lung.

Classification: class A (FA unnecessary).

In this case both of the history and the U/S findings at 25 wks GA were suggestive a case of uteroplacental insufficiency. This was further supported by anthropometeric measurements and the findings found on placental examination. In this case.

There is history of warfarin medication, which is known human teratogen. It has been reported that the risk of teratogenic injury is about 25-35% if this medication is given through

6th to 9th wk of pregnancy. [187] Furthermore, the maternal thrombosis recorded in this case could be related to a deficiency in one of haemostaic regulators, namely protein C, protein S, and anti-thrombin III. In fact, fetal death has been reported in such cases, which are associated with generalized fetal and placental thrombosis.[188] Therfore, here consideration should be given to the testing of parents for protein C and other similar deficiency.

Case No 38:

Clinical findings; A 16-year-old mother who was referred at 30 wks GA for confirmatory fetal anomaly scan. At this time she also presented with a reduction in fetal movement and her scan showed increased liquor volume, collapsed stomach, absent corpus callosum, hydrocephaly and micrognathia. A further scan after 5 days revealed IUFD and labour was then induced. There was a maternal history of genital herps. Clinical investigation was significant as HSV II antibodies were found in the mother's serum.

Non-invasive PM findings; The body was macerated stillbirth which, in addition appeared to have multiple skin ulcers, many of which had prominent white edges. The body measurements were consistent with the stated GA. X ray was normal. On placenta, membranes, and cord examination, the placenta was thinned in about 25% of its area. The were multiple dark areas with a fewer pale areas. Histolgically, there was chorioamnionitis and membranes were positive immunohistochemically with antibodies to HSV II. NB was successfully performed for the all four organs and liver sample stained positively with HSV II antibodies.

Invasive PM findings; Internal examination revealed an enlarged liver with multiple pale areas, and hydrocephaly. On standard histology, sections of skin, adrenals, and brain showed areas of necrosis which were positive by immunohistochemistry with antibodies to HSV II.

Classification: class A (FA unnecessary).

The baby's antenatal presentation and demise were the result of disseminated HSV II infection acquired in utero as result of reactivation of a primary lesion. The type of the

inflammation (chorioamnionitis) noted on placenta, membranes, and cord examination suggested that the route of infection was ascending rather than haematogenous, as villitis was not detected. Furthermore, the histological examination of the membranes and NB of the liver confirmed that the baby was infected in utero.

It is noteworthy that recurrent genital HSV II infection is far more common than primary infection in pregnancy since recurrent genital infections have been noted to occur in about 85% of pregnant women with past history of genital HSV infection.[126] The transmission occurs at all times during gestation and is not limited to early first trimester.

Case No 39:

Clinical findings; A 25-year-old who was diagnosed as having anaemia and treated with oral iron. Additionally, she had a history of vaginal bleeding at 23 wks GA, but antenatal U/S at 19 wks and 24 wks GA did not reveal any abnormality. However, IUFD was diagnosed on U/S at 32 wks GA and induction of labour was performed. Clinical investigations were normal apart from low maternal haemoglobin (8.5 gm/dl).

Non-invasive PM findings; The body of a macerated stillbirth with skin sloughing involving parts of abdomen and legs, but the baby was of appropriate size and development. X ray and placenta, membranes, and cord examination were normal. NB was attempted for all four organs and succeeded in right lung and kidneys.

Invasive PM findings; Internal examination revealed normal anatomy. Standard histology of the lung showed prominent intra-alveolar squames suggestive of intrauterine gasping.

Classification: class A (FA unnecessary).

This appeared to be a normally grown and structurally normal fetus. The cause of death in this case was not clear though. there was evidence of acute stress (intrauterine gasping), but not chronic intrauterine stress.

Case No 46:

Clinical findings; A 39-year-old who had history of pervious 2 IUFD at 15-27 wks GA. She was followed up more closely, as pyelectasia (transient) was found on U/S at 19 wks and 21 wks GA. Poor fetal growth and abnormal doppler findings were noted at 21 wks, 24 wks, 25 wks, 26 wks, and 28 wks GA. These were accompanied by reduced liquor at 25 wks GA onward. At 28^{+3} wks GA, there was a 2-day history of reduced fetal movement and by 28^{+5} wks GA, the baby was found to be dead so the labour was induced resulting in macerated stillbirth. Fetal karyotyping was not performed as it was refused by the parents, but the other clinical investigations were normal.

Non-invasive PM findings; The body of a normally formed baby with measurements (BW and FL) were below the 5th centile so the baby was growth retarded. X ray was normal. Placenta, membranes, and cord examination revealed multiple old infarcts of varying age seen occupying <10% of total surface area. An increase in nucleated fetal RBCs in chorionic vessels and an increase in the fetal vascularity were also detected on histological examination of the placenta. NB was attempted for all organs, but only succeeded in liver and lung.

Invasive PM findings; Internal examination revealed no abnormalities. On Standard histology, sections of adrenals showed fat accumulation, and sections of thymus showed cortical depletion, both of which are features consistent with chronic intrauterine stress. Lung sections however, revealed distal air ways squames.

Classification: class A (FA unnecessary).

In this case, the doppler abnormalities, poor fetal growth and the abnormalities detected on placenta examination are more consistent with chronic uteroplacental insufficiency. The nucleated fetal RBCs found in the placenta of this case is in fact, a feature of severely anoxic growth retarded fetus.

Summary of stillbirth cases:

Table 3.7, shows that significant findings were obtained by external examination in 5 cases, by placenta, membranes, and cord examination in 8 cases. X ray was not significant in any case. Internal examination revealed significant information in 2 cases, in one it supported the diagnosis made by non-invasive techniques, and in the other case it provided extra information. Standard histology provided significant findings in 4 cases; in 2 it supplied further information, and in the other 2 it supported the diagnosis made by non-invasive techniques.

Table 3.7. The value of non-invasive techniques and invasive technique for stillbirth cases.

Case No	Clinical investigations	Antenatal U/S	Ext. examination	X rays	PMC	Int. examination	Sd histology	Sd autopsy findings	Classification
1	S	S	S	NS	S	NS	NS	Macerated stillbirth. IUGR, placental infarction (uteroplacental insufficiency).	A
12	S	NS	NS	NS	S	NS	NS	Macerated stillbirth. Maternal sepsis, chorioamnionitis, villitis.	A
15	NS	NS	NS	NS	NS	NS	NS	Macerated stillbirth (unexplained), atrial septal defect.	B
16	NS	NS	NS	NS	S	NS	NS	Macerated stillbirth (unexplained)	A
22	S	S	NS	NS	NS	S	S	Fresh stillbirth. Balder outlet obstruction, bilateral hydroureter, cystic dysplastic kidney, pulmonary hypoplasia.	B
27	S	NS	S	NS	S	NS	NS	Fresh stillbirth. Spontaneous rupture of membranes, chorioamnionitis, funisitis, recurrent APH.	A
28	NS	NS	S	NS	S	NS	S	Macerated stillbirth (unexplained)	B
29	-	S	NS	NS	S	NS	NS	Fresh stillbirth. IUGR, placental infarction (uteroplacental insufficiency).	A
38	S	S	S	NS	S	S	S	Macerated stillbirth. Disseminated (HSV II), hydrocephaly.	A
39	NS	NS	NS	NS	NS	NS	NS	Macerated stillbirth (unexplained)	A
46	NS	S	S	NS	S	NS	S	.Macerated stillbirth. IUGR, placental infarction (chronic uteroplacental insufficiency).	A

Abbreviations: Ext= external, Int= internal, S = significant findings,
NS = Non significant findings, - = not available.

3.1.4 Neonatal death Category

There were 10 early neonatal deaths. The clinical presentation of each case is shown in Table 3.8, which includes, maternal obstetric history, antenatal diagnosis, and data on antenatal U/S as well as, postnatal cranial U/S. The results of the comparison of the non-invasive approach with full autopsy are summarised in Table 3.9, page 197.

Table 3.8. The clinical presentation of neonatal death cases.

Case No	GA (wks)	Postnatal age	Maternal Obstetric history	U/S fetal anomalies	Antenatal Diagnosis	Postnatal U/S
2	27	6 hours	Polycystic ovary, conceive on Clomiphene citrate, , spontaneous rupture of membranes at 23 wks	Normal	Intrauterine infection	ND
4	25	4 days	Shirodkar Suture, 2 abortions. Vaginal bleeding	Normal	Sepsis. Intracranial haemorrhage	Bil subependymal haemorrhage
9	25	4 days	Asthma on Salbutamol, Antihistamine	Ascites. Large bowel obstruction, TTTS-donor, SGA	TTTS-donor. IUGR, distended bowel	Bil intraventricular haemorrhage
10	25	7 days	Asthma on Salbutamol, Antihistamine.	TTTS-recipient, Bil. hydronephrosis, dilated & tense bladder	TTTS-recipient, megacystis, Bil mild hydronephrosis	Resolved intraventricular haemorrhage
20	28	1 day	1 abortion, polyhydramnios.	Cardiomegaly, ascites	TTTS-recipient, Hydrops fetalis	Hydrocephaly. Posterior periventricular flare
21	34	8 hours	-	Diaphragmatic hernia (left), , no mediastinal shift	?Fryn's Syndrome.	-
23	30	1 hours	-	Transient pyelectasis, micrognathia, obstructed large bowel.	Multiple congenital anomaly	ND
24	29	7 hours	1 abortion, polyhdramnios.	TTTS-recipient	TTTS-recipient	Multiple cystic lesion in brain (left hemisphere), atrophy of right hemisphere.

Tabe 3.8. The clinical presentation of neonatal death cases (continue).

Case No	GA (wks)	Postnatal age	Maternal Obstetric history	U/S fetal anomalies	Antenatal Diagnosis	Postnatal U/S
33	36	38 hours	NS	Normal	Birth asphyxia	-
50	25	4 days	2 IUD, 1TOP (CNS anomaly). Anti platelet antibodies on Prednisolone, intrauterine fetal platelet transfusion. Ritodrine, dexamethason, vaginal bleeding.	IUGR	Premature onset of labour	Bil. intraventricular haemorrhage, hydrocephaly (right).

Abbeviations; ND= not done, Bil.=bilateral, - = not available, NS= non significant.

Case No 2:

Clinical findings; This was a 27 wk GA baby who was delivered spontaneously as twin I of a setof twins with an Apgar scores of 8/10. He received endotracheal surfactant, but his cordition deteriorated with persistent metabolic acidosis. Pneumothorax developed which was treated with a chest drain. Coliforms (*E. coli*) were cultured from gastric aspirate. The baby diel at the postnatal age of 6 hours.

During antenatal life there was a history of spontaneous rupture of membranes at 23 wks. Anenatal U/S performed at 26 wks GA revealed twin pregnancy with normal growth and amniotic fluid. Additionally, Dexamethasone was given prior to delivery.

Non-invasive PM findings; The body was a normally formed baby with measurements consistent with the given GA. Placenta, membranes, and cord examinations revealed monochorionic diamniotic placenta without vascular anastomoses. Histological sections showed chorioamnionitis and funisitis in both sacs. Coliforms (*E. coli*) were also cultured from theplacenta. NB was not performed in this case.

Invasive PM findings; Internal examination showed a collapsed left lung and petechial haemorrhages in both lungs. Standard histology showed congestion in most organs. Futhermore, lung sections showed a neutrophil exudate in small bronchioles, hyaline

membrane disease, and focally dilated lymphatics. These features were suggestive of acute pneumonia.

Classification: class B (FA "helpful").

The above findings (spontaneous rupture of membranes, cultured coliforms from gastric aspiration and placenta, chorioamnionitis, and funisitis) are in consistent with ascending infection and congenital pneumonia following premature and prolonged rupture of membranes. NB was not performed in this case, but had this technique been carried out, mainly for the lung, there was a possibility that the presence of bronchopneumonia would have been reached. Furthermore, NB sample could be microbiologically studied.

Case No 4:

Clinical findings; This a 25 wks GA baby who was delivered spontaneously as T*win II* of a set of twins with Apgar scores of 8/10 and haemoglobin of 20 gm/dl. During labour, *Twin II's* membranes were ruptured and liquor was green, but twin I delivered with intact membranes. *Twin II* initially cried, but rapidly became cyanotic and bradycardiac. *Twin II* transferred to neonatal unit, where she developed hypoglycaemia and hypotension, and colloid and inotropes were administered. Hyaline membrane disease then developed requiring endotracheal surfactant and intravenous antibiotic treatment. An umbilical artery catheterization was performed at one stage, but subsequently removed due to blockage and malposition and reinserted under radiological guidance. *Twin II's* condition deteriorated and on cranial U/S at day 4 bilateral subependymal haemorrhage and bilateral intraventricular haemorrhage were found. Blood cultures were taken and have subsequently grown *Pseudomonas aeruginosa.* The baby eventually died at the postnatal age of 4 days.

Antenatal clinical history revealed that the mother had two previous mid-trimester abortion hence, in this pregnancy cerclage was performed at 15 wks GA. Intravenous Salbutamol was administered, but subsequently discontinued as result of vaginal bleeding. Dexamethasone was

given to enhance lung maturity and mother was transferred to a hospital where the delivery occurred, Clinical investigation results on the mother were not available.

Non-invasive PM findings; The body was normally formed and normally grown. There were multiple punctures, flared nostrils and puffy eyelids, all of which were consistent with clinical history. X ray was normal. Placenta, membranes, and cord examination showed monochorionic diamniotic placenta without vascular connection. There were two cords attached to the placenta. One of them presumably related to *Twin II* (this case) had marginal insertion. The other cord possibly related to *Twin I* had velamentous insertion. Histologically, the cord of *twin II* showed funisitis. NB was not performed for this case.

Invasive PM findings; Internal examination revealed thrombus formation in both internal iliac arteries, an enlarged and dark liver, and haemorrhagic consolidation of both lungs, and bilateral subependymal haemorrhage as well as bilateral intraventricular haemorrhage in brain. Cultures from liver and lung tissue grew *Pseudomonas aeruginosa.* On standard histology, sections of lungs showed features consistent with partially resolved hyaline membrane disease in addition to bronchopneumonia with features consistent with gram-negative agents.

Classification: class A (FA unnecessary).

In this case, both of clinical history and the findings found on Placenta, membranes, and cord examination suggest intrauterine infection. Pseudomonase species commonly occur as nosocomil infection, but it can cause chorioamnionitis and congenital infection as in this case. The bacteria was not identified on standard histology of lung and liver, but FNA smears of these organs showed the presence of rod like structures (pseudomonas species).

Case No 9 and 10:

Clinical findings; This a set of twins of 25 wks GA diagnosed at 11 wks GA. A scan at 20 wks GA revealed a thin septum with the possibility of single placenta. The mother was referred for further investigation. *Twin I* (case No 9) was assessed antenatally as having IUGR,

polyhydramnios, ascites, obstructed large bowel, large stomach and was thought to be a donor in TTTS, while *Twin II* (case No 10) was assessed as having polyhydramnios, bilateral hydronephrosis, dilated tense bladder, and was though to be a recipient in TTTS. Decompression amniocenteses required for maternal distress and in total of three litres of liquor were drained from both twins, but this did not prevent the onset of labour. *Twin I* was delivered spontaneously with Apgar scores of 4/9 and haemoglobin of 16 gm/dl. *Twin II* delivered spontaneously, 15 minutes after *Twin I* with Apgar scores of 1/3 and haemoglobin of 15.7 gm/dl. For both twins, antibiotics, and endotracheal surfactant were administered and umbilical artery catheterization was performed. *Twin I's* condition continued to deteriorate as hypotension, hypoglycaemia, jaundice, thrombocytopaenia and anuria developed. Pneumothorax developed for which a chest drain was inserted. Cranial U/S however, showed bilateral intraventricular haemorrhage and despite all active measurements, the baby died at a postnatal age of 4 days. *Twin II's* condition also deteriorated as hypotension was persistent and jaundice, and anuria developed. Cranial U/S however, showed small bilateral intraventricular haemorrhage which gradually seemed to resolve. Despite all active measurements, *Twin II* died at a postnatal age of 7 days. There was a maternal history of asthma treated by Salbutamol. *Non-invasive PM findings; Twin I* was a normally formed baby whose abdomen appeared distended. There were marks on the body indicating postnatal procedures (chest drain, umbilical artery catheterization, and intravenous line). Growth parameters showed normal BW, but low FL (below 5th centile). *Twin II* was a well formed baby whose growth status appeared to be IUGR (low BW and FL). There were focal skin puncture sites consistent with previous neonatal unit blood sampling. X rays was normal in *twin I*, but *Twin II* showed air bullae in the right upper lobe of lung. Placenta, membranes, and cord examination revealed monochorionic diamniotic placenta, that *Twin II's* cord had a velamentous insertion and was thicker in diameter (1.5 cm compared to 1 cm of twin I). NB was not performed for these cases.

Invasive PM findings; Internal examination of *Twin I* showed that lungs were dark in colour. The abdomen contained clear ascites and the terminal half of the ileum appeared to be partially looped and obstructed in relation to a band. Liver showed extensive areas of pallor throughout the organ. Brain showed bilateral subependymal haemorrhage. Internal examination of *Twin II* revealed that the heart was larger than *Twin I's* heart (5.6 gm compared to 3.9 gm). Right lung showed an interstitial emphysema at the upper lobe. The ureters appeared slightly dilated, but not tortuous, but the bladder was normal.

The standard histology revealed that T*win I*'s lungs showed an extensive alveolar haemorrhage and features of early hyaline membrane disease with interstitial emphysema. Sections of liver showed extensive necrosis with an appearance consistent with hypoperfusion injury. Focal calcification was noted and bile ducts appeared increased in number. Section of kidneys showed a deficiency of proximal convoluted tubules. Sections of adrenals and thymus showed fatty changes in the former and cortical depletion in the latter. Sections of the abnormal ileum revealed ischaemic damage in ganglionic bowel. T*win II*'s lungs showed interstitial emphysema and organised hyaline membrane disease. There was patchy of alveolar haemorrhage, yellow hyaline membranes and bronchopneumonia. Squames were also present in many peripheral air spaces .

Classification of twin I: class B (FA "helpful").

Classification of twin II: class B (FA "helpful").

Twin II was diagnosed antenatally as TTTS-recipient on the basis of the presence of excessive polyhydramnios, and bilateral hydronephrosis and a tense dilated bladder. At birth Twin II weighed less than Twin I (495 gm compared to 528 gm) and the haemoglobin was 15.7 gm/dl compared to 16 gm/dl in Twin I. However, growth discrepancy is not considered a reliable criterion by many investigators. Features such as discordant heart size[189,190] and increased fetal micturation are considered to be more reliable.[189] In this case (Twin II) heart weight was

5.6 gm compared to 3.9 gm in the Twin I and kidneys weight were 6.3 gm compared to 3.7 gm in Twin I.

In Twin II case a significant observation is that the cord had a velamentous insertion. It is well known that this type of insertion is common in TTTS pregnancies and may contribute to the development of profound discrepancy. This type of insertion is also thought to be related to the smaller twin of a twin pregnancy. This may be explained by the fact that any compression of the velamentous vessels by folding or direct pressure on the membranes supporting the cord could obstruct both arterial and venous blood flow. This could encourage the establishment of a circuit that exacerbated the imbalance in a shared twin circulation. This may further accentuated by development of polyhydramnios of the recipient twin which in-turn exerts a haemodynamically significant pressure on the velamentously inserted cord.[191]

TTTS has classically been diagnosed after birth by the presence of a haemoglobin difference >5 gm/dl between twins and birth weight difference >20%, providing evidence of the chronic form of the syndrome. However, Danskin and Neilson[192] have studied the reliability of these diagnostic criteria for TTTS and found that dichorionic twins without anatomic basis for transfusion often have differences in weight and hematocrit of the same order as monochorionic twins. They concluded that these standards still cannot be used reliably to assess whether TTTS has been taking place.

In these cases, it is difficult to know the exact sequence of events in this twin pregnancy, in particularly the exact contribution of any TTTS, but it would appear that *Twin I* suffered at some point a significant hypoperfusion injury which has notably and significantly affected the kidneys, liver, and probably brain. The additional presence of obstructed bowel may well have contributed to the severe polyhydramnios of this twin. The morphometric measurements indicate that *Twin I* was larger than *Twin II.*

Case No 20:

Clinical findings; This a 28 wk GA female baby who was delivered by Caesarean section, as *Twin I* of a set of twins. Antenatally, TTTS was suspected at 22 wks GA based on the presence of a discrepancy in the growth and amniotic fluid volume between two twins. This baby was though to be TTTS-recipient. Amniodrainage was carried out from the sac of this twin at 23 wks, 25 wks and lastly, at 26 wks GA. At that time (26 wks GA) ascites was noted, and on a further scan cardiac enlargement was detected.

At birth the baby was blue with Apgar scores 4/6 and haemoglobin of 15.2 gm/dl. She was intubated and ascitic fluid was drained. Subsequently, she developed left pneumothorax which was treated by a chest drain. Cranial U/S examination showed bilateral mild ventricular dilatation and bilateral posterior periventricular flares. As poor peripheral perfusion was maintained, the condition continued to deteriorate and she died at postnatal age of 1 day.

Non-invasive PM findings; The body was that of normally formed baby with normal growth parameters. There were abdominal distension, marks of active neonatal procedures (chest drain, venous cannula and umbilical artery catheterization), and bruises over venepuncture sites. X ray was normal. On Placenta, membranes, and cord examination, the placenta was very fragmented and assessment of the number of amniotic sacs at the junction was not possible so only the chorionicity was determined as being monochorionic placenta. The thicker cord was related to this twin (*Twin I*). NB was not performed for this case.

Invasive PM findings; Internally, there was an enlarged heart with biventricular hypertrophy and firmness of the lungs. On standard histology, the lungs were congested and type II pneumocytes were prominent. There were dilated respiratory bronchioles with hyaline membrane formation. Brain section showed germinal matrix haemorrhage.

Classification: class B (FA "helpful").

Case No 21:

Clinical findings; This baby was transferred in-utero after antenatal U/S showed a left diaphragmatic hernia with mediastinal shift and polyhydramnios. Fetal karyotyping was normal. At 34 wks GA, labour was augmented with syntocinon, and delivery was assisted by forceps. The baby was floppy and pale at birth with Apgar scores of 1/3 and her condition was deteriorating till she died at postnatal age of 8 hours.

Non-invasive PM findings; On external examination, there was a dysmorphic face with low set ears and a flat nasal bridge. X rays showed asymmetry of ribs number, 11 (right) and 12 (left). Placenta, membranes, and cord examination showed prominent perivillous fibrin deposition and syncytial knot formation. The amnion was hyperplastic. NB was performed in liver.

Invasive PM findings; Internal examination showed diaphragmatic hernia on the left side with mediastinal shift resulting in the right main bronchus being longer than the left. The left hemi-diaphragm was mostly absent. It also revealed that stomach, spleen, pancreas, and a portion of liver were present in the left pleural cavity. The appearance of the cut section of brain suggested an acute ischaemic damage. On standard histology, the lung had patchy dilatation of respiratory bronchioles with coalescing and adjacent collapse, together with hyaline membrane disease. These changes were patchy in the lung right, but more uniform and severe in the left lung, in which there were some interstitial leaks. Thymus sections showed starry sky appearance suggestive of acute intrauterine stress. Adrenals sections revealed medullar interstitial haemorrhage. Both ovaries sections showed an accelerated development of the primary follicles with some macroscopic cystic appearance on the left side. Liver sections showed very prominent haematopoiesis.

Classification: class B (FA "helpful").

Diaphragmatic hernia often occurs in the left posterolateral portion. As result of this defect, abdominal viscera are displaced into thorax. The postnatal survival rate is low: 11% if polyhydramnios coexists, and only 55% in the absence of polyhydramnios.[193] Most cases have

a sporadic occurrence in which the recurrence risk for the sibs is 1-2%. However, familial cases (apparently autosomal recessive) do occur. For example, Familial congenital diaphragmatic hernia which characterised by the presence of unilateral agenesis of the diaphragm,[193] and Fryn's syndrome which characterised by the presence of cloudy cornea, hirsutism, absent or hypoplastic finger nails and distal phalanges and internal malformations. Absence or eventration of the diaphragm, bicornuate uterus and a degree of holoprosencephaly are often exist.[159] Diaphragmatic hernia could be associated with chromosomal anomalies, commonly with trisomy 18.[193].

In this case, it is not clear whether to consider it as Fryn's syndrome. though, the absence of cloudy cornea and absent or hypoplastic finger nails and distal phalanges, could be explained by the occurrence of variable experessivity in this syndrome, which sometimes does not result in a full phenotype.[194]

The lung abnormalities which were detected by standard histology are the sequelae of diaphragmatic hernia and mediastinal shift and might be picked up non-invasively by NB.

Case No 23:

Clinical findings; This baby was transferred in-utero after antenatal U/S showed abnormalities of the fetal brain and gastro-intestinal tract. The labour was spontaneous and the baby was born in good condition at 30 wks GA. The baby then developed respiratory problems and intubation could not be performed. The baby died 1 hour after birth.

Antenatally, a scan at 20 wks GA, showed abnormalities of brain (hydrocephaly), neck and gastro-intestinal. The baby was rescanned at 22 wks GA, which showed multiple anomalies including, hydrocephaly, transient pyeloctasis in the right kidney, cardiac enlargement, and liquor volume at the upper limit of normal. At 28 wks GA U/S revealed marked increase in liquor volume and some views suggested micrognathia. The scan suggested that the bowel was dilated. A week later, there was a tense polyhydramnios of which 2.5 litres were drained and

the possibility of oesophageal atresia or tracheo-esophageal fistula was raised. There was no significant maternal medical history, and the results of the clinical investigations including fetal karyotyping were normal.

Non-invasive PM findings; On external examination, the measurements were in consistent with the given GA and the face was dysmorphic with micrognathia. The soft palate was clefted. The nares were obstructed. The floor of the mouth was abnormal, as the tongue was not attached to the frenulum and no uvula was identified. X ray was normal. Placenta, membranes, and cord examination revealed some perivillous fibrin deposition and syncytial knot formation. NB was unsuccessfully attempted for the liver and lung.

Invasive PM findings; Internal examination showed imperforate nasopharynx which appeared to be due to soft tissue rather than bone. There was also a bicornuate uterus and double cervix and vagina. On standard histology, congestion was seen in many organs and lung sections showed extensive interstitial haemorrhage and haemosiderin deposits.

Classification: class C (FA essential).

In this case, the imperforate nasopharynx had led to obstructed flow of the air and fluid and accounted for the polyhydramnios and the difficulty encountered on attempting to intubate after birth. The exact pathogenesis for these multiple anomalies is not known.

Case No 24:

Clinical findings; The baby was the second of twins delivered spontaneously at 29 wks GA with Apgar scores of 2/5. The haemoglobin value was not available, but haemoglobin of the co-twin was low (12.6 gm/dl). Cranial U/S showed an extensive multicystic lesions, particularly in the left hemisphere, with marked atrophy of the right hemisphere. The baby's condition was deteriorating and the baby died at a postnatal age of 7 hours. Antenatally, the baby was diagnosed at 20 wks GA as being TTTS-recipient with polyhydramnois, requiring repeated drainage.

Non-invasive PM findings; The body was that of normally formed baby with peripheral cyanosis. A growth parameters showed low BW, but normal FL. X ray was normal. Placenta, membranes, and cord examination showed monochorionic monoamniotic placenta. The two cords had the same thickness, but one of them had a velamentous insertion. Histologically, the placenta had a mild increase in thrombus formation, syncytial knots and calcification. NB was attempted in three organs (liver, lung, right kidney). sample was successfully obtained in liver and showed infarction.

Invasive PM findings; Internally, there was cardiomegaly and an enlarged liver with subcapsular infarction. Brain macroscopic examination showed extensive left-sided cortical necrosis. Standard histology showed a subcapsular area of necrosis in liver and the presence of intra-alveolar squames in lung.

Classification: class A (FA unnecessary).

This a well developed baby with cardiomegaly consistent with the TTTS-recipient. The infarction found in brain and liver could be result of the polycythaemia which is typically found in TTTS-recipient. This is further supported by the presence of an increase in thrombus formation on placenta examination.

Case No 33:

Clinical findings; A 36 wk GA baby who delivered by emergency Caesarean section because of decreased fetal movements, and an abnormality found on fetal monitoring (Cardiotocograph). The baby was born without heart-beat or breathing. The baby was pale, and the haemoglobin was found to be low (7 gm/dl). Abnormal movement and rhythmical gasps were then developed, indicating a poor prognosis. On cranial U/S, the ventricles were difficult to visualise suggesting an element of cerebral oedema. In the view of these findings, intensive care was withdrawn and the baby died at postnatal age of 38 hours. A clinical investigations were significant as the maternal Kleihauer-Betke test was strongly positive indicating a significant

fetomaternal haemorrhage. Antenatally, this pregnancy was uncomplicated and fetal scan was normal.

Non-invasive PM findings; Externally, the body was that of normally formed and well grown baby, body measurements were in consistent with the stated 36 wks GA. X rays and Placenta, membranes, and cord examination were normal. NB was not performed in this case.

Invasive PM findings; On internal examination, the brain cut sections showed that the ventricules were small because of oedema, but no definite periventricular lesions. A small depressed pale scar was found on the left lobe of the liver. On standard histology, lung sections showed congestion, patchy atelectasia and intra-alveolar squames. The scar noted grossly on liver is a hypocellular area of hepatocytes and ducts embedded in fibrous stroma. Kidney sections showed tubular damage with occasional glomerular cyst. Brain sections showed no evidence of gross ischaemic injury.

Classification: class B (FA "helpful").

The association between massive fetomaternal haemorrhage and poor perinatal outcome is well established.[103] In this case the definitive diagnosis was achieved by the clinical investigation namely, maternal Kleihauer-Betke test. This emphasis the importance of including certain diagnostic measures as a part of the evaluation protocol for perinatal death cases.

Case No 50:

Clinical findings; The mother had history of a termination for hydrocephaly followed by two IUFD at 15-27 wks GA. She was known to have antiplatelet antibodies, in this pregnancy, she was on prednisolone treatment and she had episodes of vaginal bleeding. Between 16-24 wks GA, six fetal intraperitoneal and intravascular platelet transfusions were performed. During the last one a bradycardic episode occurred. Nevertheless, fetal monitoring was normal in the following 24 hours. Four days latter, the mother presented with intermittent contraction, for which intravenous ritodrine and intramuscular dexamethasone were administered. Following

spontaneous rupture of membranes, the mother was transferred to a referral hospital where emergency Caesarean section was performed at 25 wks GA. A baby was born without any respiratory effort. The baby was intubated and ventilated and endotracheal surfactant was given. At birth, he was noted to be bruising easily and his blood investigations revealed low haemoglobin (7.8 gm/dl), and low platelet count (16 x 10^9/l). Umbilical vessel catheterization was performed and blood transfusion was administered, in addition to antibiotics. However, his condition continued to deteriorate and cranial U/S showed bilateral intraventricular haemorrhage with hydrocephaly on the right and parenchymal extension on the left. The baby died at a postnatal age of 4 days.

Antenatally, the baby was scanned several times and his growth parameters were along the 5th centile, and amniotic fluid volume was reduced. The baby was diagnosed as having alloimmune thrombocytopenia.

Non-invasive PM findings; The body that of well formed baby with growth parameters (low BW, and FL) consistent with the IUGR state. X rays showed the presence of air bullae in the lung fields and dystrophic calcification in the abdomen. Placenta, membranes, and cord examination was not performed as placenta and its appendages were not available. NB was attempted for all four organs and failed in kidneys. NB of the lung showed the presence of hyaline membrane disease.

Invasive PM findings; On internal examination, the lungs were heavy and haemorrhagic with multiple emphysematous cysts. Adhesions were found in the peritoneal cavity with focal calcification. There was also a dilated loop of a small bowel. On the liver, there was a small red dark area at the antero-inferior surface representing a subcapsular haematoma. The brain showed an extensive haemorrhage around the brain stem and over the surface of the cerebellum. On slicing, there was dilatation in the 3rd ventricle in which blood was found as well as in the 4th ventricle. Additionally, bilateral intraventricular haemorrhage with left-sided parenchymal extension were found.

On standard histology, the lung showed hyaline membrane disease, pulmonary interstitial emphysema and alveolar haemorrhage. Liver sections confirmed the presence of a small subcapsular haematoma. Adrenals sections revealed congestion and focal haemorrhages. Thymus was atrophic on standard histology. Standard histology of brain confirmed the presence of bilateral intraventricular haemorrhage and subependymal haemorrhage. There were also features of early periventricular parenchymal ischaemic damage.

Classification: class B (FA "helpful").

In this case, the baby was diagnosed to have an alloimmune thrombocytopaenia. This is a result of maternal exposure to a paternal platelet antigen that is also present on fetal platelets. As a consequence of the maternal production of platelet-specific antibodies (IgG) that cross the placenta and bind to fetal platelets, causing severe fetal thrombocytopaenia in the presence of a normal maternal platelet count. Alloimmune neonatal thrombocytopaenia is more severe than that one associated with maternal immune thrombocytopaenia (autoimmune thrombocytopaenia), with a mortality up to 14% from intracranial haemorrhage.[195] An affected infant in the first pregnancy occurs in up to 50% of cases and in the following pregnancy in up to 75%.[195,196]

In these cases the baby usually presents with unexplained thrombocytopaenic purpura at birth or within two days of delivery. The clinical presentation may also include the development of intracranial haemorrhage that results in death or development of neurological sequelae in up to 25% of cases.[196]

The IUGR found in this case could be explained by the prolonged use of prednisolone or could reflect a disease of the placenta that affected its function. In this case, the placenta and its appendages were not available for examination, but this examination could be of a significant value especially if the mother had history of vaginal bleeding as in this case. To my knowledge, placenta pathology associated with this disorder (alloimmune thrompocytopaenia) has not been

described. Nevertheless, a variable degree of placental infarction has been reported with autoimmune thrombocytopaenia.[73]

The presence of intraperitoneal calcification and adhesions as well as a small subcapsular haematoma are compatible with the history of multiple intraperitoneal fetal blood transfusion. Thus, the findings could be considered as an iatrogenic pathology.

Summary of neonatal death cases:

Table 3.9 shows that significant findings were revealed by external examination in 4 cases, by X rays in 3 cases, and by placenta, membranes, and cord examination in 6 cases. Internal examination revealed significant information in all 10 neonatal death cases. In 4 cases it supported the diagnosis made by non-invasive techniques, in 5 cases it provided extra information, and in 1 case it altered the anatomical diagnosis made by non-invasive techniques, though the exact pathogenesis for these multiple anomalies picked on full autopsy is not known. Similarly, standard histology provided significant findings in all neonatal death cases; in 7 it supplied further information, and in 3 it supported the diagnosis made by non-invasive techniques.

Table 3.9. The value of the non-invasive techniques and invasive technique for neonatal death cases.

Case No	Clinical investigations	Antenatal U/S	Cranial U/S	Ext. examination	X rays	PMC	Int. examination	Sd histology	Sd autopsy findings	Classification
2	S	NS	-	NS	-	S	S	S	Prematurity, spontaneous rupture of membranes, chorioamnionitis, funisitis, bronchopneumonia, hyaline membrane disease, pulmonary hypoplasia.	B
4	S	NS	S	NS	NS	S	S	S	Prematurity, hyaline membrane disease, bronchopneumonia, Pseudomonas septicaemia, funisitis, bilateral intraventricular haemorrhage, Internal Iliac thrombosis	A
9	NS	S	S	NS	NS	S	S	S	Prematurity, hyaline membrane disease, ascites, small bowel obstruction, ischaemic liver, bilateral intraventricular haemorrhage & subependymal haemorrhage, renal tubal dysgenesis, interstitial emphysema.	B
10	NS	S	S	S	S	S	S	S	Prematurity, hyaline membrane disease, interstitial emphysema, bronchopneumonia, IUGR.	B
20	NS	S	S	NS	NS	S	S	S	Dysmorphic face. nuchal thickening. Hyaline membrane disease. diaphragmatic hernia, pulmonary hypoplasia., (?Fryn's syndrome)	B
21	S	S	-	S	S	NS	S	S	TTTS, hydrops fetalis, cardiomegaly, hyaline membrane disease, subependymal haemorrhage.	B
23	S	S	-	S	NS	NS	S	S	Micrognathia , imperforated naso-pharynx, cleft palate, abnormal tongue. Absent uvula , bicornuate uterus , 2 Cervix, 2 Vagina (MCA of unknown aetiology)	C
24	-	S	S	NS	NS	S	S	S	TTTS , infarction (cerebral, liver), congested heart failure.	A
33	S	NS	S	NS	NS	NS	S	S	Fetal maternal haemorrhage	B
50	S	S	S	S	S	-	S	S	Prematurity, bilateral intraventricular haemorrhage, subependymal haemorrhage, hydrocephaly, hyaline membrane disease, IUGR.	B

Abbreviations: PMC= placenta, membranes, and cord examination, AN U/S= antenatal U/S, Ext= external, Int= internal, S = significant findings, NS= non significant findings, - = not available, MCA= multiple congenital anomaly

3.2 Analysis:

From the total 55 cases, 28 cases were classified as *class A (Full autopsy unnecessary)*, 22 cases as *class B (Full autopsy "helpful),* and 5 cases as *class C (Full autopsy essential).* Classes B & C cases (where new information is provided by full autopsy) include fewer stillbirth cases (27%) and miscarriage cases (40%) than neonatal death cases (80%), (Table 3.1). This could be due to the considerable number of severely macerated babies (8 cases) in stillbirth and miscarriage cases, which is not the case for neonatal deaths. For TOP category about half the cases (54%0), full autopsy did proved new information (classes B & C). When TOP cases are excluded, the causes of fetal and perinatal deaths for the all 31 cases (miscarriage , stillbirth, neonatal death categories) were examined and showed that the principle cause of death determined by the non-invasive techniques was not changed after full autopsy in any case (Table 3.10). However, in two cases a specific diagnosis was reached after performing full autopsy, mainly due to the discovery of unsuspected internal malformation. This was important for genetic counselling.

Table 3.10. The causes of death in 31 cases of fetal and perinatal deaths.

Cause of death	class A *(FA unnecessary)*	Class B *(FA "helpful")*	Class C *(FA essential)*	Total
Congenital abnormalities	1	2	2	5
Unexplained antepartum death	5	2	0	7
APH	0	1	0	1
Uteroplacental insufficiency	3	0	0	3
Infection (including congenital infection)	5	3	0	8
TTTS	1	3	0	4
Fetomaternal haemorrhage	0	I	0	1
Hydrops	0	1	0	1
Alloimmune thrombocytopaenia	0	1	0	1

Abbreviation: FA= full autopsy.

Table 3.11. The significance of each specific technique per each category.

Category (No of cases)	Cases with significant Ext. examination result. N0 (%)	Cases with significant X rays result No (%)	Cases with significant PMC result No (%)	Cases with significant Int. examination result No (%)	Cases with significant Sd histology result No (%)
TOP (24)	19 (79%)	12 (50%)	4 (17%)	18 (75%)	11 (46%)
Miscarriage (10)	5 (50%)	3 (30%)	8 (80%)	3 (30%)	4 (40%)
Stillbirth (11)	5 (45%)	0 (0%)	8 (73%)	2 (18%)	4 (36%)
Neonatal death (10)	4 (40%)	3 (33%)*	6 (66%)*	10 (100%)	10 (100%)
Total (55)	33 (60%)	18 (33%)	26 (48%)	33 (60%)	29 (53%)

Abbreviations: Ext.=external, PMC=placenta, membranes, and cord,
Int.= internal, Sd= standard, * = technique was performed in 9 cases.

As can be seen in Table 3.11, the external examination and X rays were found to be more reproducible in TOP cases than other cases. The yield of placenta, membranes, and cord examination was best for miscarriage, stillbirth, and neonatal death cases where infection and twin pregnancies were common pathology.

In this study, there were 29 cases with a congenital abnormally, including chromosomal anomalies, and excluding cases with congenital infection. 24 of which were terminated. In the rest there was either a miscarriage (2 cases), a stillbirth (1 cases) or a neonatal death (2 cases). In these cases, different types of malformation and chromosomal defect were found on PM examination (Table 3.12).

Table 3.12. The spectrum of anomaly in congenital anomaly cases .

Type of anomaly	No of cases	Classification		
		A (FA unnecessary)	B (FA "helpful")	C (FA essential)
CNS	10	5	3	2
Urogenital/Oligohydramnios	5	2	2	1
Cardiac	1	1	0	0
Facial	1	1	0	0
Hypoxia/hydrops	1	0	1	0
Syndromes/Multiple congenital anomaly	6	0	4	2
Chromosomal defects	5	2	3	0
Total	29	11	13	5

In these group, 11 cases (38%) were classified as *class A* (full autopsy did not change or add any information), 13 cases (45%) were classified as *class B* (full autopsy was not needed for auditing the final diagnosis given by non-invasive technques), but in 5 cases (17%) were classified as *class C* (full autopsy could be needed for proper diagnosis and genetic counselling).

Table 3.13. The congenital anomaly cases and the significant result of each non-invasive technique

Non-invasive techniques	No of cases examined	No of cases with significant result (%)
Clinical investigations	25	23 (92%)
Antenatal U/S	29	26 (90%)
Ext. examination	29	22 (76%)
X rays	29	14 (48%)
PMC examination	29	4 (14%)

Abbreviations: Ext = external, PMC= placenta, membranes, and cord

For most these cases, special clinical investigations, such as fetal karyotyping and maternal serum biochemistry were performed either because of maternal age, previous history of malformed baby or to exclude chromosomal defects. The results of these clinical investigations available for 25 cases, showed significant finding in 23 cases (92%). Non-invasive techniques were performed for all 29 cases with the results are shown in Table 3.13. Antenatal U/S as well as external examination gave significant results in 4 cases out of 5 cases of aneuploidy, and placenta, membranes and cord examination was significant in one case of the 5 cases, (Table 3.14).

Table 3.14. The details of structural anomalies detected by non-invasive methods in cases with abnormal karyotype.

Karyotype	Significant anomalies detected by U/S	Significant anomaly detected by Ext. examination	Significant anomaly detected by PMC examination	Significant anomalies detected by invasive techniques (autopsy)
Trisomy 13 (case No 5)	Holoprosen-cephaly Polydactyly Hypoteloris-m	Holoprosencephaly, nasal proboscis, polydactyly, hypotelorism, overriding digits.	None	Atrial septal defect
Triploidy (case No 17)	Nuchal fluid	Overlapping of the 3rd left toe by the 2and and 4th, labioscrotal fusion, mild hypospadias.	Partial mole	Dysgenetic testis
Trisomy 18 (case No 42)	Strawberry shaped head Skin oedema Cystic hygroma, SGA	Dysmorphic face, IUGR.	None	None
Triploidy (case No 44)	Strawberry shaped head, hyperechoge-nic bowel, SGA.	Micrognathia, encephalocele, IUGR	None	Intestinal malrotation
Trisomy 21 (case No 53)	None	None	None	None

PMC= placenta, membranes, and cord.

Chapter II. 4

General Discussion

In this chapter, the results of non-invasive PM investigations (clinical investigations, antenatal U/S, external examination, X rays, non-invasive microscopic examination, and placenta, membranes, and cord examination) are evaluated and compared with the full autopsy findings. The limitations of the present study are discussed and the extent to which these results might apply in a Middle Eastern setting are considered. The chapter concludes with a proposed protocol for non-invasive PM examination in Middle East countries.

Here, the most convenient way to summarise the results of the study is to reintroduce table 3.1. Of the total 55 cases, 28 were classified as *class A (full autopsy unnecessary)*, 22 as *class B (full autopsy "helpful")*, and 5 as *class C (full autopsy essential).*

Table 3.1. Classification of the study cases.

Category (No of cases)	Total No of cases (%) class A (FA unnecessary)	Total No of cases (%) class B (FA"helpful")	Total No of cases (%) class C (FA essential)
TOP (24)	11 (46%)	10 (41%)	3 (13%)
Miscarriage (10)	6 (60%)	3 (30%)	1 (10%)
Stillbirth (11)	8 (72%)	3 (27%)	0 (0%)
Neonatal death (10)	2 (20%)	7 (70%)	1 (10%)
Total (55 cases)	28 (51%)	22 (40%)	5 (9%)

Abbreviation: FA= full autopsy

Broadly speaking in this study, the principle cause of death determined by the non-invasive techniques was not changed after full autopsy in any case, but a specific diagnosis was reached after performing full autopsy, mainly due to the discovery of unsuspected internal malformation or lesion. This was important for genetic counselling.

Standard histology was significant (in the sense of confirming the findings with non-invasive techniques, or adding more information, or changing the diagnosis) in 29/55 cases (53%) and specifically was very significant in neonatal death cases (100%).

This finding is supported by a previous study [17] as the histological diagnosis was *essential* to reach final pathological diagnosis for 20% (18/90) of cases and was helpful in almost twice as many normally-formed fetuses as in those with abnormalities on external examination.

Additionally, it is well documented that histological examination is the only way to diagnose many abnormalities such as myocarditis, congenital hepatic fibrosis, intrauterine pneumonia, hyaline membrane disease, different types of pulmonary hamartomas and perinatal hypoxic brain injury. It is also essential to reach a definitive diagnosis in cases of Potter syndrome, Meckel syndrome, Thanatophoric dysplasia syndrome, polycystic kidneys and other renal abnormalities. In this study, we therefore attempted to evaluate the efficiency of non-invasive microscopic examination by the means of fine needle aspiration (FNA) and needle biopsy (NB).

Fine needle aspiration was performed for four organs, right lung, liver, and both kidneys. They were chosen because of their importance in fetal and perinatal life and their accessibility for the blind percutaneous procedure. The successful sampling rate of those organs varied from 44.4% for the right lung, 94,4% for the liver, 36.3% for the right kidney, and 29.2% for the left kidney. FNA of lung was useful in identifying bacterial agents, but its value was limited in cases of viral infection, which may not be detected because of tissue necrosis could interfere with viral identification. In kidneys, FNA of cases with certain pathology, such as cystic dysplastic kidney, could not detect any abnormalities, since the diagnosis requires a study of the kidney architecture which was not preserved on FNA sampling.

The very limited value of FNA in this study could partly be due to lack of experience, and of reference material in this field. Full descriptions of fetal and neonatal cytology were rarely found in the literature.[161] The field needs further exploration by experts. Cytological techniques may also be of value when fluid is present.[65]

Needle biopsy, like FNA causes no or very small disfigurement and would be important in developing a non-invasive PM approach. The highest success rate was obtained in the liver (69.2%) and the lowest success rate was obtained in kidneys (42.6%). However, only one application of NB was performed for all cases because full autopsy was always needed subsequently. There was concern not to damage or disturb the babies' internal structures by the needle. Repeating the procedure from the same puncture site using different angles might have produced a higher success rate. From my experience of conducting this procedure, I think postmortem U/S imaging would improve the NB results both in terms of tissue collection and providing some anatomical information, and I plan to investigate this in future.

In conclusion, NB was certainly a more useful non-invasive method than FNA in detecting various pathology, such as viral infection, dysplastic kidneys, and hyaline membrane disease of the lung. NB also permits a wide range of investigations including microbiology, immunological studies, frozen section, electron microscopy studies, and investigation for metabolic diseases.

4.1 Potential of non-invasive PM in Middle East countries:

The data obtained from conducting full autopsies in a Western country can not be extrapolated to Middle East countries, as the spectrum of causes of fetal and perinatal deaths is different. Therefore, the initial aim is to establish a standardised and acceptable form of PM examination, in order to start the process of collecting data on the causes of fetal and perinatal mortality in this region.

In Middle East countries, intrapartum perinatal deaths and fetal and perinatal deaths due maternal illness still account for a considerable number of deaths. In Algwiser's study[155] APH accounted for 18/292 cases (16%). Intrapartum asphyxia and mechanical factors accounted for 30/292 cases (10%) of perinatal deaths. Maternal diseases accounted for 14/292 cases (4.7%) and mainly, diabetes mellitus came on the top of this list (10/14). Pre-eclamptic toxaemia was

attributed to 10/292 cases (3.4%). Isoimmunization accounted for 4/292 (1.3%) of perinatal deaths.

However, few Middle East countries yet have adequate standard demographic data on frequency and causes of fetal and perinatal mortality. From my own experience in working in Libya, which is fairly typical for the region, perinatal infection seems to be an important cause of pregnancy loss and neonatal death, but because of the paucity of laboratory techniques, most of the causative organisms can not be diagnosed. Toxoplasmosis is endemic in Middle East countries, and is one cause of congenital infection, which could be avoided if the infection is diagnosed and treated. In a study, carried out in Saudi Arabia,[191] of randomly collected sera from 386 pregnant women, the prevalence of toxoplasma antibodies varied between 25.4%-36%. In Libya[192] 47% of 369 pregnant women were found to have been infected by toxoplasmosis. Rubella also accounts for many pregnancy losses, mainly as result of congenital anomaly. In most Middle East countries most women of child-bearing age (85%-90% of cases) have acquired natural immunity by that time,[193, 194] but this still leaves a large number of non-immune women at high risk.

On a theoretical basis, it could be postulated that all the above-mentioned causes of perinatal deaths in Middle East countries might be clarified by non-invasive perinatal PM examination. For example, for deaths due to intrapartum asphyxia and mechanical factors including cord accidents, medical history, external examination, and X ray will be of value. Deaths due to maternal illness could be explained by reviewing the maternal medical records, as well as external examination and placenta, membranes, and cord examination. This also applies to deaths due to Rh Isoimmunization. For fetal and perinatal deaths due to infection, the placenta, membranes, and cord examination and culture, external examination, maternal clinical record, and NB of lung could be of value.

However, in order to gain an approximate idea of the potential of these method in the Middle East for diagnosis of congenital anomalies, we compared the results of this study with a published sample of 104 perinatal deaths due to congenital abnormalities in Saudi Arabia[155] in

1983-87. This group of cases matches the 29 congenital abnormality cases in this study. Based on the percentage of success in my study, the number of Saudi cases for which the non-invasive PM techniques would have been of benefit is listed in Table 4.1.

Table 4.1. The potential of non-invasive PM techniques in Middle East countries

Non-invasive PM technique	Percentage of cases with significant result in this study	The postulated number of cases with beneficial result in the examined Saudi Arabia population.[155]
Clinical investigations	92%	96
Antenatal U/S	90%	94
External examination	76%	79
X rays	48%	50
PMC examination	14%	15

Abbreviation: PMC = placenta, membranes and cord

In our study sample 11 congenital abnormality cases (38%) were classified as *class A* (full autopsy did not change or add any information), 13 (45%) were classified as *class B* (full autopsy was not needed for auditing the final diagnosis given by non-invasive technques), and 5 (17%) were classified as *class C* (full autopsy could be needed for proper diagnosis and genetic counselling). If the same classification is applied for these Saudi Arabian cases[155] it could be postulated that for 86 /104 congenital abnormalities cases the genetic counselling and clinical management would not be changed (classes A & B), and in particular, for 40 out of these 86 cases conducting the autopsy would not be of any benefit (class A). In this particular group only 18 cases would have benefited from conducting full autopsy, as the genetic counselling might have changed.

Non-invasive techniques, as shown in Chapter II. 3, are much more effective in certain types of diseases. For example, congenital infection (bacteria or virus) cases give the best results from non-invasive PM. On the other hand, cases with syndromes or multiple congenital anomaly are liable to be missed if full autopsy is not performed, as the presence of internal minor malformation could alter the diagnosis from an isolated malformation to one which is syndromic.

In this study, chromosomal defects are mainly diagnosed antenatally by fetal karyotyping, which would apply for only a small proportion in the Middle East countries. Full autopsy confirms that the anatomical abnormality corresponded to the abnormality known to arise from the chromosomal defects, or in many cases adds additional information, but does not change the diagnosis. Antenatal U/S does detect certain signs that suggest the presence of chromosomal anomalies (Table 4.2). In fact, in this study, Antenatal U/S as well as external examination did give significant results the cases of aneuploidy (Table 3.15, Chapter 3. II). Placenta, membranes, and cord examination can also reveal significant findings in those cases (Section 2.8, Chapter 2. I), but due to small numbers, placenta, membranes, and cord examination did not provide significant findings in this study. Therefore, antenatal U/S, external examination, and placenta, membranes, and cord examination could be highly valuable in Middle East countries, where the facility of cytogenetics is not always available.

Table 4.2. The different signs of chromosomal anomalies.

Fetal anomalies	Possible karyotype
Nuchal Oedema	Trisomy 21, Turners
Bilateral facial cleft① other stigma	Trisomy 18
Duodenal atersia	Trisomy 21
Omphalocele	Trisomy 13, 18
Pleural effusion	Trisomy 21
Diaphragmatic hernia	Trisomy 18
Abnormal fetal heart	Trisomy 13, 18, 21
Pyelectasis	Trisomy 13, 21
Choroid plexus cysts	Trisomy 18, 21
Hydrocephalus	Trisomy 13, 18, 21

4.1.1 Antenatal U/S:

This is the method of choice for detecting structural anomalies in the fetus. It is an non-invasive examination, which has the advantages of being relatively simple and is increasingly available in most Middle East hospitals. However it is still an imaging modality, with false positive and false negative rates, which depend on many factors, mainly the type of the

machine used and the experience of the operator. Normally in Western countries full autopsy is used to audit the diagnosis made by antenatal U/S and provide feedback to the operator. In Middle East countries, however auditing must rely on postmortem external assessment, X rays, and possibly postmortem U/S. If the antenatal diagnosis would develop to a stage where it would detect most malformations, it seems likely that more genetic abortion would be carried out. This would promote the performance of full autopsy, as it is believed that parents of such babies are emotionally more prepared to allow full autopsy.

In fact, this study showed that full autopsy is essential for cases with malformation detected either by antenatal U/S or external examination, and its findings could affect genetic counselling and future childbearing management. Moreover, Antenatal U/S examination would help in providing a basis for further investigation to study each case. For example, any diagnosed renal anomalies on U/S indicates to investigate the case by needle biopsy (NB). Also the presence of any signs of chromosomal anomalies (Table 4.3) could alert to the importance of performing cytogenetic studies. All these conditions are expected to be seen in Middle East countries, and non-invasive PM examination could to some extent deal with the diagnosis of such cases.

In this study the congenital anomalies detected by the antenatal U/S were compared with those observed at pathological examination (invasive and non-invasive PM techniques) and vice versa (Table 4.3) . In total, 68 congenital anomalies were diagnosed by antenatal U/S examination. In 66% (No=45), the congenital anomalies detected by U/S were confirmed by pathological examination. A further 26 additional anomalies, which could have been but were not diagnosed by U/S were discovered at pathological examination.

Table 4.3. The spectrum of antenatal U/S findings and pathological examination findings.

Prenatal anomaly (U/S)	Total	Postnatal diagnosis anomaly (full autopsy)			
		AND confirmed	**AND modified**	**AND absent (other diagnosis)**	**Additional anomaly**
CNS anomalies:	29	20	-	9	2+(1)
Spina bifida/Myelomeningocel	3	2	-	1	-
Encephalocele	2	2	-	-	1
Hydrocephaly	8	6	-	2*	-
Microcephaly	4	4	-	-	-
Holoprosencephaly	3	3	-	-	-
Intracranial calcification	1	-	-	1	-
Intracranial cyst	3	1	-	2	-
Craniostenosis	1	-	-	1	-
Small/absent cerebellum	2	-	1	1	1
Absent corpus collosum	2	1	-	1	-
Cardiac anomalies:	8	5	2	1	(3)
Hypoplastic left heart	1	1	-	-	-
Cardiomegaly	2	2	-	-	-
Truncus arteriosus	1	1	-	-	-
Abnormal heart	1	-	-	1	-
Ventricular disproportion	1	-	-	-	-
Ventricular septal defect	1	1	1⊕	-	-
Uniatrial	1	-	-	-	-
Urogenital anomalies:	17	10	1	6	1+ (5)
Dysplastic kidneys	5	2	-	3	1
Tense bladder	4	3	-	1	-
Absent bladder	1	-	-	1	-
Hydronephrosis	3	2	-	1	-
Hydroureter	3	3	-	-	-
Small kidneys	1	-	1♦	-	-
Abdominal defects:	4	2	-	2	(5)
Bowel obstruction	2	1	-	1	-
Small stomach	1	-	-	1	-
Diaphragmatic hernia	1	1	-	-	-
Facial anomalies:	6	5	-	1	6
Nasal proboscis	1	1	-	-	-
Hypotelorism	1	1	-	-	-
Cleft lip & palate	1	1	-	-	-
Midline facial cleft	1	1	-	-	2#
Micrognathia	2	1	-	1	4
Limb defects:	3	3	-	-	3
Polydactyly	1	1	-	-	2
Sandactyly	1	1	-	-	1
Short limbs	1	1	-	-	-
Total	67	45	3	19	26

Abbreviation: AND = Antenatal diagnosis, * = very soft brain so histology could not be performed on those cases, ⊕ = ventricular septal defect, • = bilateral renal agenesis, # = one case diagnosed as midline cleft palate (soft) and the other as midline cleft palate (hard)

The values in parenthesis included anomalies not covered by this table anomalies spectrum.

It is worth mentioning here that most of the missed anomalies by U/S could be picked up by application of non-invasive PM techniques, namely external assessment, X rays, NB, and possibly postmortem U/S. Table 4.4 showed the missed anomalies by antenatal U/S and the status of the amniotic fluid which were only detected by dissection and internal examination and are not liable to be detected by the non-invasive PM techniques. Defects consistently missed in most series include atrial septal defect and small ventricular septal defect (see Table 2.2 Chapter 2. I). The presence of these minor malformations is important, in that they may alter the diagnosis from isolated malformation to one which is syndromic.

Table 4.4 .The False negative diagnosis of U/S and the additional finding at internal examination and dissection

Autopsy findings (invasive technique)	Prenatal U/S finding	Status of amniotic fluid
1) CNS	Small cerebellum	↓
Absent cerebellum	None	Normal
Small cerebellum	None	Normal
Abnormal arachnoid vasculature		
2) CVS	Single atrium	Normal
Atrial septal defect	IUFD	↓
Atrial septal defect	None	Normal
Atrial septal defect	None	Normal
Atrial septal defect	Ventricular disproportion	Normal
Ventricular septal defect		
3) GIT	Bowel obstruction	↑
Imperforated naso-pharynex	None	Normal
intestinal malrotation	None	Normal
Microcolon	Large bowel obstruction	Normal
Small bowel obstruction		
4) UGT	Gestational trophoblastic disease	Normal
Dysgenetic testes		

Abreviations: CNS= central nervous system, CVS= cardiovscular system, GIT= gastointestinal tract, UGT= urogenital tract.

The first column lists, by the organ systems, the individual major or minor anomalies that were not detected prenatally. The second column describes the prenatal diagnosis that were made in each case. The third column describes the state of amniotic fluid during U/S examination.

4.1.2 Postmortem U/S & needle biopsy:

Postmortem U/S has been shown to be useful for detecting urogenital anomalies, some intra-abdominal pathologies, and major intracranial problems[162] For the latter, histology is needed to reveal the underlying pathogenesis in the cases with normal karyotype, but fetal and perinatal brain is not attainable for NB due to its higher water content. On the other hand, renal agenesis can not be definitely confirmed or excluded antenatally (U/S), but performing NB under U/S guidance could improve the detection rate of such anomaly by the non-invasive PM technique. Though it appears to have limited application as a diagnostic technique, postmortem U/S has considerable potential as a guide to NB.

In fact in this study, one of the main problems encountered with NB was that full autopsy had to be conducted after the application of non-invasive methods, so NB sampling had to be limited to only one application. This problem would not be encountered in the Middle East countries, as full autopsy would not be conducted, so multiple tissue sampling from the same puncture with different angles would be more feasible, and success could be enhanced by U/S guidance. Both of these would increase the chance of obtaining more useful sampling by NB than that achieved in this study.

4.1.3 A standard protocol for non-invasive PM examination:

All the above predictions are still to be tested in the Middle Eastern environment and the non-invasive PM examination is still to be organised. The first building block would be to produce and test an appropriate protocol for implementing the PM examination, in Misurata Teaching Hospital (Libya) a multidisplinary team consisted of Obstetrician, Paediatrician, Pathologist, and Microbiologist should be organised, together ensuring a proper diagnosing for the causes of pregnancy loss or perinatal deaths.

Appendix III contains a suggested protocol, which is in three sections and designed to give all the required information for fetal and perinatal deaths. Section I deals with the obstetric history for each case, Section II contains the clinical investigation that should be performed for the

mother who had a pregnancy loss, and in the third section there is a check-list of external examination that should be followed. Section IV contains a flow-chart using the non-invasive PM methods to classify each case, according to the extended Wigglesworth classification (Table 3.3, Chapter I.3).

Finally, it is worth mentioning here again that the yield of applying of this non-invasive approach is expected to be less than that obtained by conducting full autopsy, as it does not provide detailed anatomic evaluation. This particularly so for malformed cases. After careful consideration of the benefits and limitations of non-invasive perinatal PM examination, we thought that some information is better than nothing at all.

Chapter II. 5

Conclusion

Postmortem examination is of paramount importance to understand the different diseases process that occur in different age groups (fetal, neonate, infant, child, and adult). It is an important tool for education and research, evaluating the efficacy of any new diagnostic technique and medical therapy as well as validating national statistics on the causes of perinatal and infant deaths and overall effectiveness of the health care system. Furthermore perinatal PM examination has the advantage of being useful in genetic counselling. Therefore, on the basis of the PM findings, the geneticist could calculate the recurrence risk of the anomaly and the obstetrician could implement a plan to improve the outcome of the next pregnancy.

Despite these advantages, the perinatal PM rates are still low, and even in the UK have rarely reached the minimum target level recommended by the Royal College of Pathologists. This is due to many reasons, which include personal, cultural and religious beliefs of family members of the deceased baby regarding autopsy.

This study attempted to demonstrate the value and limitations of non-invasive methods of perinatal PM. A sample of 55 cases was studied and it was found that 50/55 cases (91%), full autopsy did not change the specific diagnosis based on the non-invasive techniques. In the remaining 5 cases full autopsy was needed to provide information that might have affected the genetic counselling.

However, it was found that certain components proved to be more productive in certain circumstances. For example, the clinical investigation, antenatal U/S, X rays and external assessment are of paramount value to study malformed cases, while placenta, membranes, and cord examination and microscopic examination are needed to study miscarriage or stillbirth cases.

Broadly speaking, standard autopsy remains the study of choice for evaluating causes of fetal and perinatal death, mainly in malformed babies. However, when this is not possible as in Middle East countries, the applications of the different non-invasive techniques of PM is the alternative method to provide benefits in terms of diagnosis and prognosis, without doing damage to the cultural and religion traditions. While non-invasive PM is unlikely to turn up any new descriptions of disease or new syndromes, full autopsy is still needed to supply malformation database with different and new types of malformation syndromes. It could be concluded that any non-invasive procedure depends on finding external correlation with internal diseases, based on the comprehensive body of knowledge gained through conducting full autopsy.

It was also concluded that in Middle East countries a standardised non-invasive perinatal PM examination protocol would help to identify causes of fetal and perinatal death, and to give a proper counselling to avoid its recurrence in the next pregnancy. The following conclusions were reached.

- There is a need to improve the diagnostic laboratories, set-up regional specialist laboratory services including cytogenetic diagnostic services.
- There is a need to promote the use of antenatal U/S, as well as to improve its practice and skills.

- Further studies are needed to assess the role of postmortem U/S, especially in guiding needle biopsy procedures.
- Organised training programmes are needed for Obstetricians, Neonatologists, perinatal Pathologists, and clinical Geneticist, in order to ensure expertise in assessing the external appearance of the babies, as well examining the placenta and its appendages.
- There is a need to establish computerised database for congenital malformation in this region.

Appendix I

Section I

UCL HOSPITALS
REQUEST FOR POST MORTEM EXAMINATION / OR EXTERNAL ASSESSMENT
(TO BE COMPLETED BY MEDICAL STAFF)

FROM: FETAL MEDICINE UNIT / LABOUR WARD / NEONATAL UNIT
(DELETE AS APPLICABLE)

UCH Booked [Yes] [No]

Booked elsewhere and referred to FMU [Yes] [No]

In Utero / Postnatal transfer from:

Consent included for:
Full Autopsy [Yes] [No]
or External Assessment only [Yes] [No]

Placenta sent for examination: [Yes] [No]

FETUS OF / BABY (Delete as appropriate)

Hospital No.: _____
Name: _____
Sex: _____
Date of Birth: _____
(Computer label if available)

Consultant Obstetrician / Consultant Paediatrician:

SIGNIFICANT MATERNAL OBSTETRIC HISTORY

Parity: Mother's race:

LMP: Father's race:

Problems in Pregnancy:
Fetal
Maternal (PET etc)

Antenatal procedures - CVS / Amnio / FBS etc

Antenatal Chromosomes sent: [Yes] [No]

If yes, Normal [ ] Abnormal [ ] Results awaited [ ]

DELIVERY

Date and time:

Spontaneous [ ] Induced [ ] Augmented [

Method of Induction:

Date and time of ruptured membrane:

Length of Labour:

Vaginal: cephalic [ ] forceps [ ]
breech [ ] ventouse [ ]

Caesarian: [ ]

NEONATAL HISTORY

Gestation by dates: By scan:

Postnatal Chromosomes sent: [Yes] [No]

If yes, Normal [ ] Abnormal [ ] Results Awaited [ ]

Significant microbiology:

Birth Weight: Centile:

BRIEF SUMMARY OF NEONATAL COURSE:

Date and time of death: Age at death:

FURTHER INFORMATION:

Any specific questions for autopsy?

Clinical Diagnosis

Details as entered on the death certificate:

Special instructions re disposal / burial of baby or placenta:

P M REPORT TO BE SENT TO: (Tick as required)

FMU [ ] NNU [ ]

ANC [ ] Other - specify:

U/S [ ]

Signed:

Name (block capitals):

Designation:

Bleep No.:

Section II

UCL Hospitals

Record Of Request And Parental Consent
For A Post Mortem Examination Of A Baby of Any Gestation

(Full autopsy or external examination only)

RECORD OF REQUEST FOR FULL EXAMINATION:

FETUS OF / BABY (Delete as appropriate)

Hospital No.: _____
Name: _____
Sex: _____
Date of Birth: _____
(Computer label if available)

- Date and time of death:
- Date and time of conversation:
- Request made of: Both parents [ ] Mother [ ] Father [ ]*

* **Father's permission alone is not sufficient in case of stillbirth, or where parents are unmarried.**

- Request made by: (Name and designation)
- Have parent(s) seen the explanatory PM leaflet? [Yes] [No]. If no, offer one.
- Reasons for request explained and also how and when the findings will be communicated to the parent(s):

OUTCOME

- Parent(s) request more time to consider - give details:
- Parent(s) refuse full autopsy - record reasons overleaf if parents are willing to give them:

  If parent(s) refuse, ask if they are willing to consent to external examination - PTO for relevant form.
- Parent(s) consent to full autopsy - fill in consent form below:

  Parent(s) consent to external examination - fill in consent form overleaf.
- **Please note that the person administering the form should witness the parent's signature.**
- File this form in the relevant notes. If consent is given, the pathologist will require the original consent form, so detach the section below.

Consent form completed for [full autopsy] [external examination only] Delete as appropriate.

CONSENT FOR A POST MORTEM EXAMINATION

- I agree to a post mortem examination being carried out on:

FETUS OF / BABY (Delete as appropriate)

Hospital No.: _____
Name: _____
Sex: _____
Date of Birth: _____
(Computer label if available)

Name _____

who died on _____

- I understand that this examination is carried out:

a
- to verify the cause of death
- to study the effects of treatment
- to assist in medical education

and that this may involve the retention of tissue for laboratory study.

b . to remove amounts of tissue for medical research. (Section b can be deleted if required.)

- I understand that my clinical team will explain and discuss the findings with me at a later date if I wish.

CONSENT GIVEN BY:	WITNESSED BY:
Signature:	Signature:
Name (block capitals):	Name (block capitals):
Relationship to baby:	Designation:
	Date:

PARENT(S) REASONS FOR REFUSING REQUEST:

CONSENT FOR A POST MORTEM EXAMINATION (EXTERNAL ONLY)

. I agree to a post mortem examination (external only) being carried out on: FETUS OF / BABY (Delete as appropriate)

Name _____

who died on _____

Hospital No.: _____
Name: _____
Sex: _____
Date of Birth: _____
(Computer label if available)

. I understand that this will not give such detail as a full post mortem examination, but that it may:

- help to verify the cause of death
- assist in medical education
- provide information that could be helpful in the future.

. The examination consists of careful external inspection, weighing and measuring the baby and possible use of X-ray and/or ultrasound examination, medical photography and collection of specimens.

. I understand that my clinical team will explain and discuss the findings with me at a later date if I wish.

CONSENT GIVEN BY:	WITNESSED BY:
Signature:	Signature:
Name (block capitals):	Name (block capitals):
Relationship to baby:	Designation:

Appendix II

Growth Charts

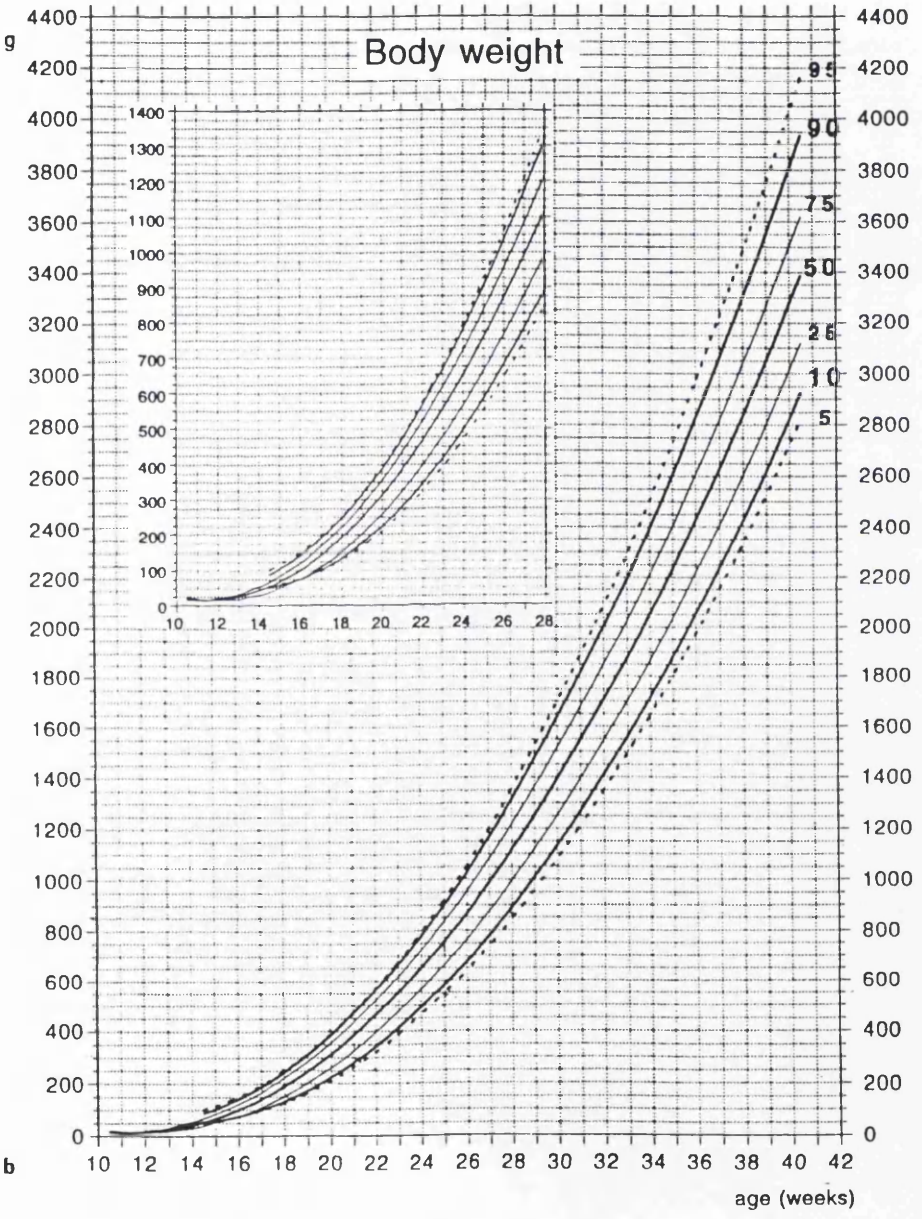


Fig. 2b. Body weight of 'normal' fetuses. Smoothed curves of the 5th, 10th, 25th, 50th, 75th, 90th, 95th pecentiles of the distribution. Inset: detail of body weights for ages below 28 weeks.

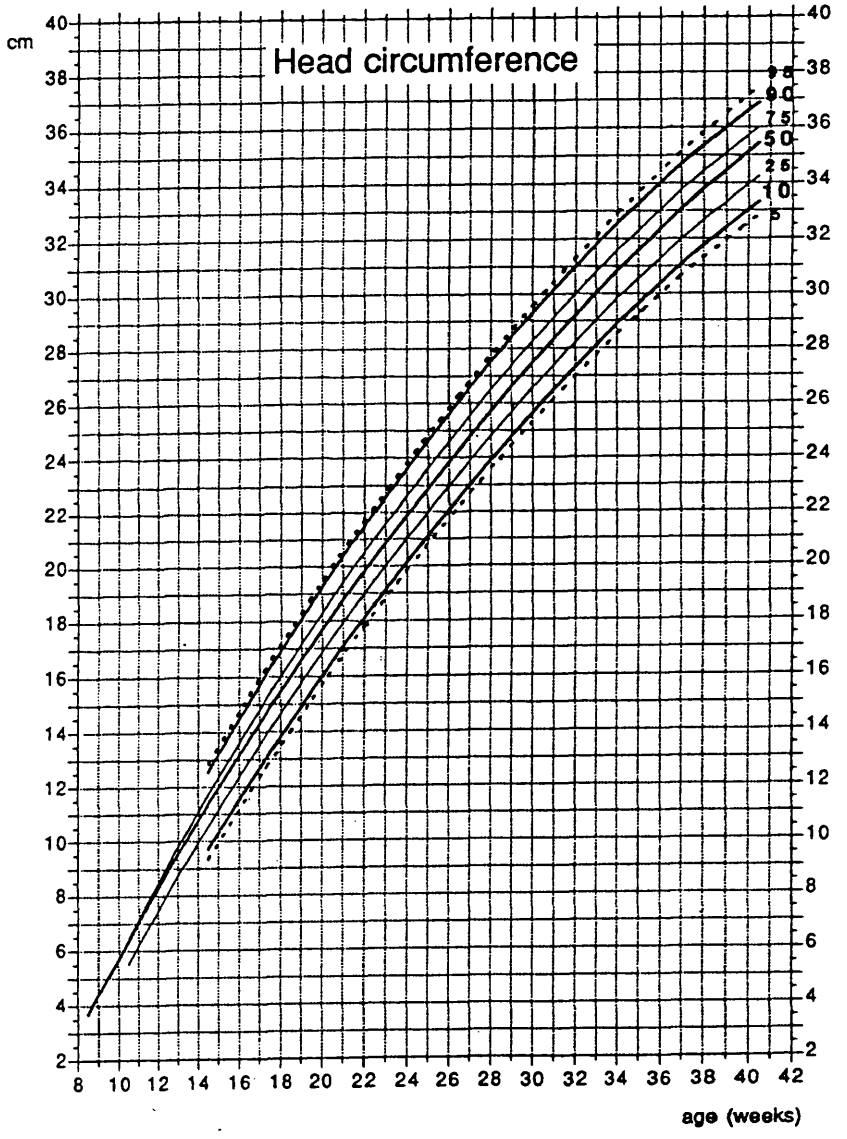


Fig. 6. Head circumference. Smoothed curves of the 5th, 10th, 25th, 50th, 75th, 90th, 95th percentiles of the distribution.

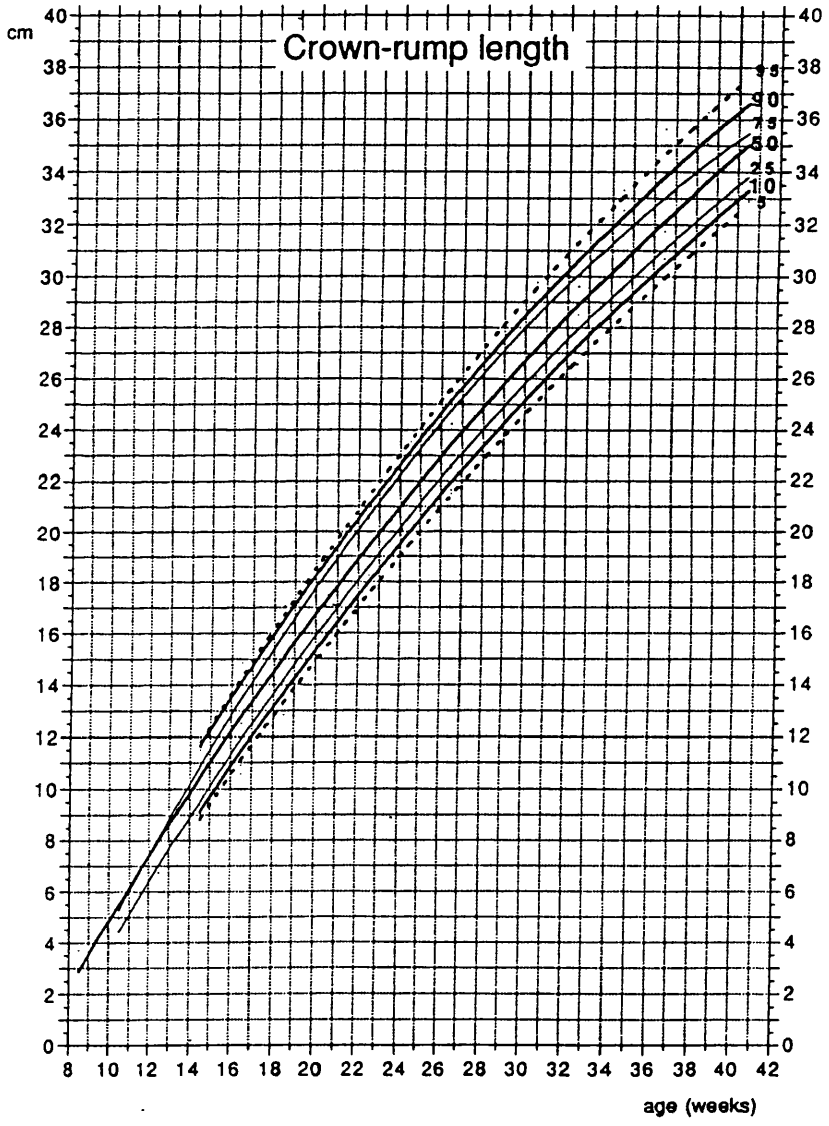


Fig. 12. Crown-rump length. Smoothed curves of the 5th, 10th, 25th, 50th, 75th, 95th percentiles of the distribution.

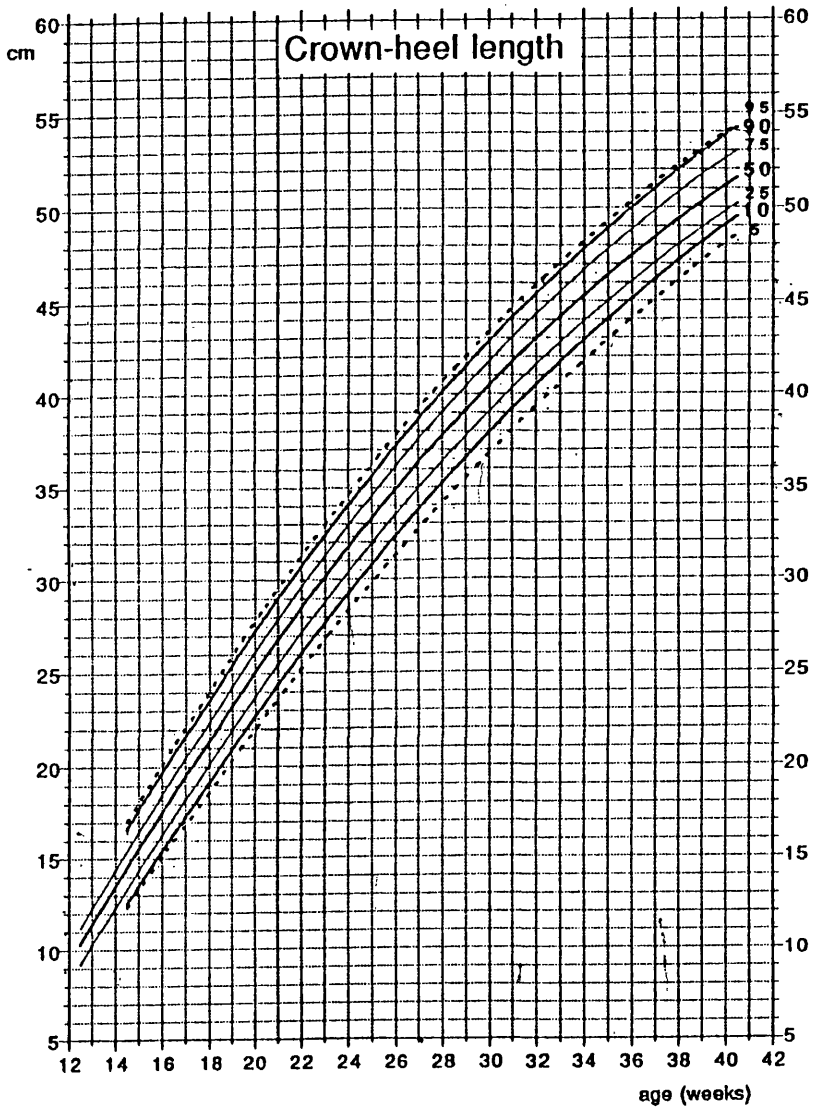


Fig. 9. Crown-heel length. Smoothed curves of the 5th, 10th, 25th, 50th, 75th, 90th, 95th percentiles of the distribution.

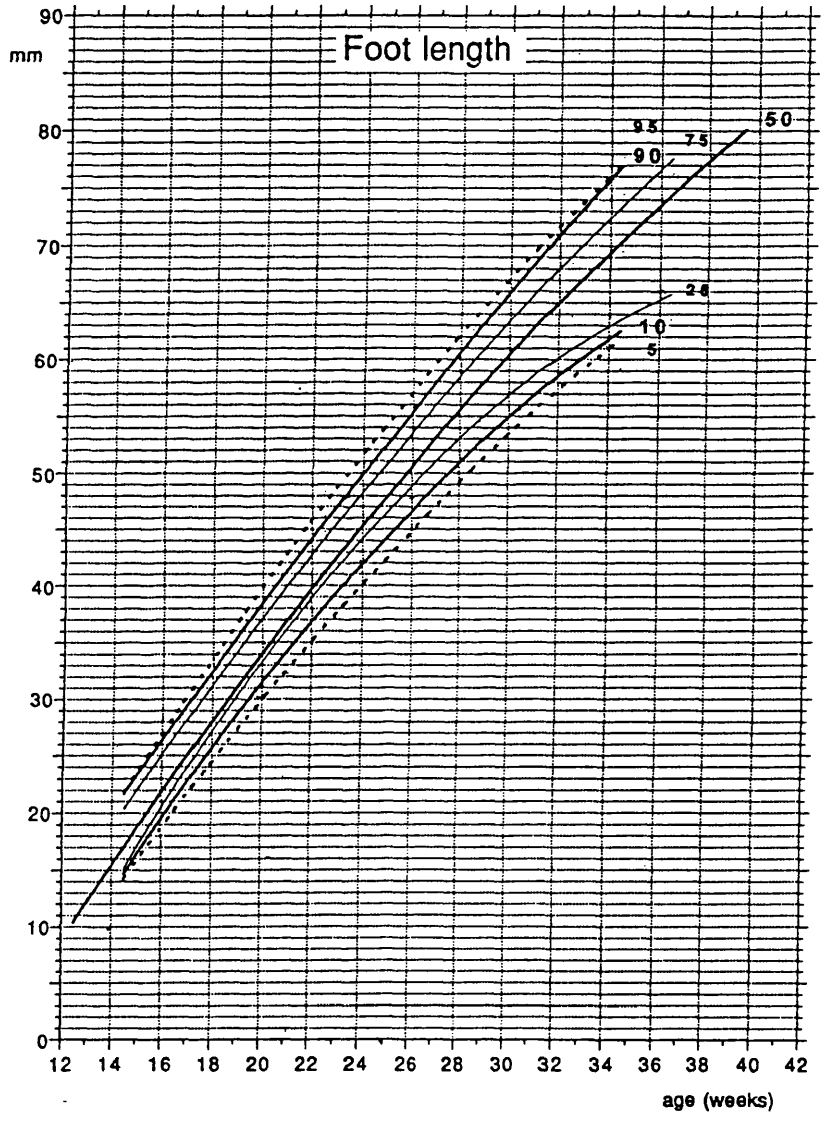


Fig. 15. Foot length. Smoothed curves of the 5th, 10th, 25th, 50th, 75th, 90th, 95th percentiles of the distribution.

Appendix III

Non-invasive PM Protocol

Section I

Obstetric History[1]

Maternal illness:

Diabetes yes / no / unknown If yes:

Insulin dependent / Non-Insulin dependent / Gestational

Epilepsy Yes / No / unknown If yes: was there drug treatment in pregnancy? Yes / No / unknown

If yes, specify.....

Other illnesses; Yes / No / unknown

If yes - specify.....

Maternal infections Yes / No / unknown

Maternal therapy Yes / No / unknown

Bleeding needing transfusion Yes / No

Family history of genetic or handicapping disorder Yes / No

if yes - specify.....

Antenatal care in this pregnancy Yes / No

Mode of delivery (normal, vaginal breech, forceps or suction, caesarean)

Obstructed labour Yes / No

Prolonged labour (> how many hours?)

Best estimate of gestational age of baby in weeks.....

Previous pregnancy out come: Please fill the following Table

[1] Modified From unpublished report from WHO, Regional Office for Eastern Mediterranean, 1995

Pregnancy outcome Present state

Pregnancy	Abortion / SB		LB	if dead		if alive		Any abnormality	if unwell
No	wks	Cause	wks/ mo	Died	Cause of death	age years	general condition	(specify)	Diagnosis
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		
				<1mo, 1mo-1yr, > 1yr			Well/ Unwell		

Abbreviations; SB= stillbirth, LB=live born, mo= months, Yr= year

Section II

Clinical Investigations:

The following tests should be preferably performed for each case of pregnancy loss:

- Parents' blood groups and maternal antibody screen
- Maternal and paternal haemoglobinopathy status
- TORCH screen and virology antibody* to teratogenic maternal infections such as rubella, toxoplasma and syphilis, and to other infections relevant to maternal health, such as chlamydia, trachoma, herpes virus, CMV, and other sexually transmitted diseases.
- Check for maternal urine culture and cervical and vaginal swabs results
- Liver function test
- Random blood sugar ± glucose tolerance test
- Thyroid function test
- Lupus anticoagulant
- Anticardiolipin antibodies*
- Kleihauer test

* some of theses tests are considerable very advanced to be carried out in most Middle East countries

Section III

Checklist for the external examination:[2]

Skin	Meconium staining Pallor/ plethora/mottling Blisters and rashes Nail development
Skull	Fontanelle size and tension Hydrocephaly Microcephaly Encephalocele
Eyes	Lids fused/open Size Proportion Pupillary defects
Nose	Choanal atresia
Ears	Position and size of pinna External auditory meatus Pits/tags Meconium
Mouth	Width Micrognathia Clefts Natal teeth Tongue size Palatal clefts
Neck	Webs Goitre Cysts Branchial remnants
Trunk	Body wall defects Neural tube defects Asymmetry Nipple position Abdominal distension Prune belly Patent urachus

[2] Reference No 159

Checklist for the external examination (continue):

Limbs

Absent
Hypertrophied
Shortened: Proximal
Distally
Combined
Curved or bowed
Contractures

Digits: Proportions
Number
Syndactyly
Position of thumb
Plantar and palmar creases

Nails: Meconium
Hypoplastic

Perineum

Genitalia: Male
Female
Ambiguous
Position of testes
Urethra patent
Anus perforate

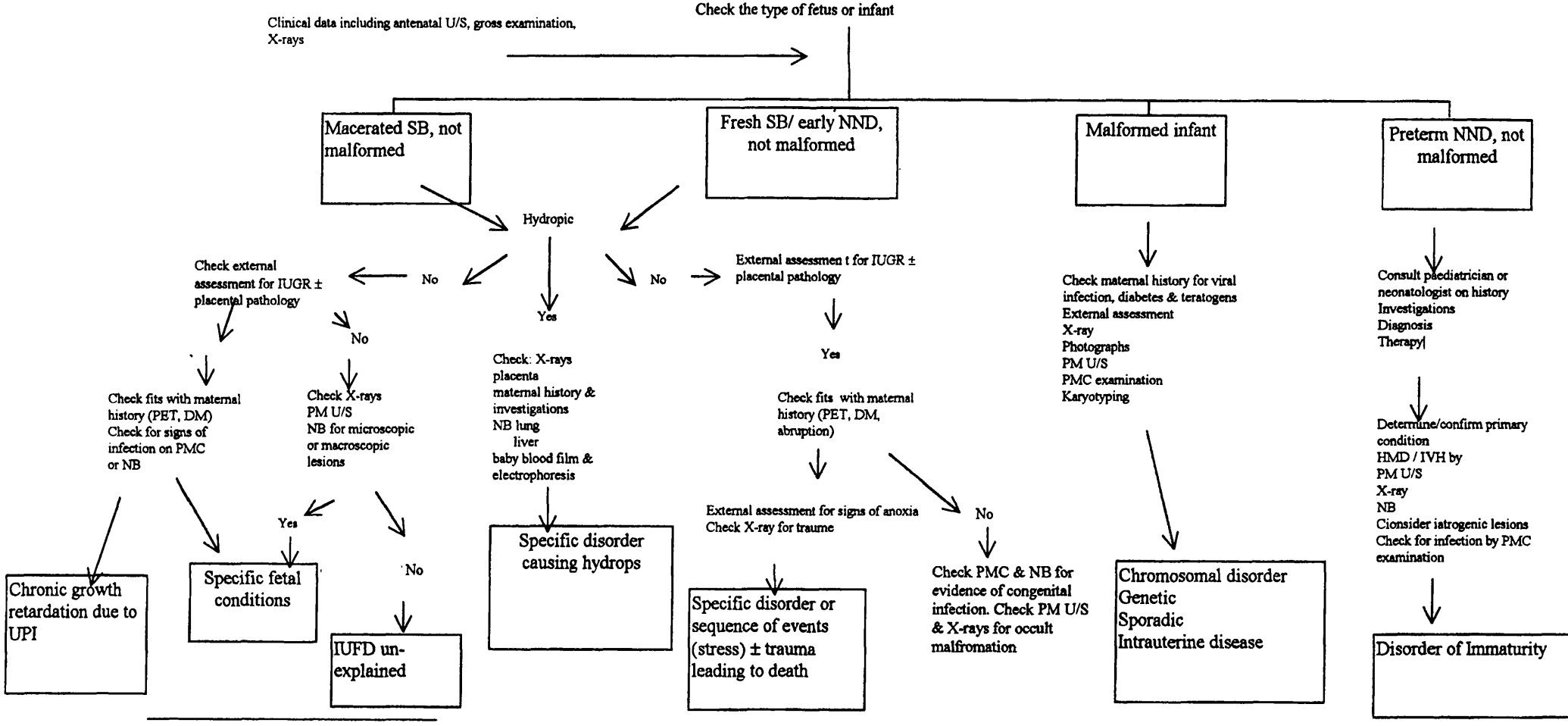
Iatrogenic

Cannula
Airways
Drain
Trauma

Section IV

Flow Chart[3]

Check the type of fetus or infant



[3] Modified from Reference No 54, SB= stillbirth, NND= neonatal death, PET= pre-eclampsia, DM= diabetes melitus, PMC= placenta, membrane, cord HMD= hyaline membrane disease, IVH= intraventricular haemorrhage

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