



Performing a Perinatal Autopsy

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Abstract Intrauterine fetal demise or termination for malformation is adverse obstetric outcomes, agonizing for the parents and frustrating for health care providers. These events are often unpredictable and investigation entails not only the evaluation of the fetus/neonate but also the placenta. Though nothing can compensate the bereavement of families who have encountered fetal or a neonatal loss, a meticulous study of the placental, fetal, and neonatal tissues provides succor and help in the healing process. In recent times, with extensive advances in the pediatric specialty, the significance and requirements for perinatal autopsy have also commensurately heightened. There is an inordinate need for an accurate diagnosis in order to render genetic counseling and ascertain the implications of possible recurrence in future pregnancies. A conscientious perinatal autopsy, supplemented by cytogenetic and metabolic evaluation, followed by dissemination of the information to the parents, clinicians, and public health organizations is of paramount importance. This not only assists in the clinical management and bereavement closure but also helps in the reduction of perinatal mortality and morbidity. An autopsy protocol assists in performing an adequate perinatal dissection, facilitates the recognition and documentation of all relevant information and in addition also provides a format for the collection, recording and presenting of data which facilitates collaborative research. A brief account of the benefits and techniques of perinatal autopsy and the protocol are presented.

Keywords Perinatal autopsy · Protocol · Consent form · Autopsy technique · In situ examination · Provisional anatomic diagnosis

Introduction

The Autopsy Committee of the College of American Pathologists describes autopsy as “a medical-surgical procedure by a physician for the welfare of the living through the study of those patients for whom all our current knowledge and technology were inadequate” [1]. An array of brilliant protocols is available, though none of them can be deemed sacrosanct, and one may adopt/adapt any technique to salvage the maximum information for the benefit of the family and the clinician. For an erudite discussion of the plethora of existing protocols, the reader should refer to them [2–5].

The perinatal autopsy compilation here is brief and under the following sections:

1. Benefits of autopsy
2. Autopsy consent and permit
3. Radiographic examination
4. Photography
5. Instruments
6. Clinical and pre-autopsy considerations
7. Cytogenetic study
8. External examination
9. Incision
10. In situ examination and evisceration
11. Provisional anatomic diagnosis (PAD)
12. Placental examination
13. Microscopic examination
14. Final diagnosis
15. Classification of perinatal deaths

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Benefits of Autopsy

An autopsy helps to:

- Ascertain the cause of death
- Corroborate, supplement, or refute clinical diagnosis
- Evaluate diagnosis and therapy including new modalities
- Identify rare diseases
- Detect and discover new pathologic entities
- Document hitherto unknown information on disease manifestations
- Document growth and development
- Provide risk estimates for future pregnancy
- Further the cause of research and education
- Facilitate the investigation of environmental, occupational, and lifestyle causes of diseases
- Improve accuracy and usefulness of biostatistics
- Provide epidemiologic data
- Provide organs for donation and study
- Bereavement closure

Autopsy Consent and Permit

An unsuccessful outcome of pregnancy is a major catastrophe for parents. Seeking consent for performing an autopsy from grieving parents can be difficult. Nevertheless, the peremptory first step with an autopsy is the signed consent form and autopsy permit perusal by the attending pathologist (Fig. 6) in the Appendix. Dissection should be deferred or abandoned without the requisite consent/permit to avoid unnecessary legal entanglements.

Special permissions (dissection and removal of eyes) should be obtained prior to dissection. Autopsy constraints imposed by family or legal restrictions need to be adhered to. The prosector/institute should also familiarize itself with the local laws pertaining to autopsy examination and disposal of the remains.

Radiographic Examination

Every fetus submitted for an autopsy should mandatorily have a roentgenogram. Many conditions, especially suspected skeletal dysplasias, cannot be diagnosed without an X-ray picture. An antero-posterior and lateral view of the entire fetus is essential. Digital radiographs or faxitrons may be used, former being preferred.

Photography

Photographic documentation (external and internal) of the fetus/infant is of paramount importance. The pictures must be focussed to depict the abnormal with adequate reference points within the frame. Photographs may:

- Serve as a record for posterity
- Help ascertain diagnosis of a malformation syndrome
- Depict and preserve anatomic relationships of visceral lesions which otherwise are destroyed after dissection and evisceration
- Assist in identifying tissues submitted for microscopic examination
- Serve as records for family bereavement closure and litigation purposes
- Assist with publication and research

Instruments

The perinatal postmortem requires a fully-equipped laboratory with abundant light and a camera with a good macro-function in order to photograph small fetuses and organs. Ophthalmic instruments are excellent for small dissections. The requirements include (Fig. 1):

- Charts providing normal weights and measurements for newborns
- Sterile syringes and needles
- Sterile forceps and scissors for culture and karyotype
- Mounted magnifying glass
- Dissecting board, absorbent paper towels
- Large scale and electronic digital scale
- Measuring tape, rulers, callipers
- Scalpel handles, blades, large knife
- Small- and medium-sized forceps with and without teeth
- Stout scissors, Metzenbaum scissors, and small scissors
- Fine probes and hemostats of varying sizes

Clinical and Pre-autopsy Considerations

Fetal development is, in part, dependent on maternal health and intrauterine environment. It is imperative that the prosector obtains all relevant clinical and family history prior to the procedure. Genetic syndromes must be identified, if present. Obstetric history, ultrasound reports, including those pertaining to prior pregnancies and/or fetal

Fig. 1 Basic instruments used for perinatal autopsy



demise, hospitalization charts, and complications of delivery should be recorded.

Cytogenetic Study

Babies with malformations and very small fetuses where dysmorphic features may not be apparent should have chromosomal analysis. Samples are usually taken from the skin over the chest at the main incision site or the axilla using a sterile technique, cleansing the skin with sterile saline and not alcohol. Fascia, lungs, Achilles tendon, cartilage, and chorionic villous tissue are best suited for culture. In macerated fetuses, the placenta may be sampled.

External Examination

A complete systematic examination of the fetus/baby is performed using a standardized protocol, regardless of gestational age and proceeding in a cephalo-caudal direction (Fig. 7) in the Appendix. Fetal growth, presence of maceration, edema, dehydration, cyanosis, jaundice, dysmorphic features and injuries related to delivery, meconium staining are some of the features, looked for. External examination is summarized in Fig. 2 (anthropometry) and Fig. 3 (external examination).

Incision

The fetus is placed over a wedge-shaped autopsy block with hyperextended neck with shoulders and chest raised above the dissecting surface. A Y-shaped incision is usually employed such that the arms of the Y commence from the top of the shoulder, descend lateral and inferior to the nipples to meet in the mid-line at the sternal xiphoid process (Fig. 2). The vertical stem extends from the xiphoid, around the umbilicus to terminate at the symphysis pubis. The abdominal cavity is opened by the side of the umbilical vein, finger inserted under the umbilicus and the umbilical arteries, the urachus and the urinary bladder palpated. The course of the umbilical vein and the umbilical arteries is observed. The chest wall is exposed by reflecting the skin upwards and dissecting the subcutaneous and the muscle tissue.

In Situ Examination and Evisceration

Rokitansky method is an in situ examination of viscera, in part combined with en bloc removal. In the Virchow technique, the organs are removed one by one and dissected as removed. Letulle method is an en masse removal of thoracic, cervical, abdominal, and pelvic organs which are subsequently dissected into organ

Fig. 2 **i** Head circumference (HC), **ii** chest circumference (CC), **iii** abdominal circumference, **iv** outer canthus (OC), **v** inner canthus (IC), **vi** interpupillary distance, **vii** philtrum length, **viii** foot length, **ix** wedge-shaped autopsy block, **x** Y-shaped incision

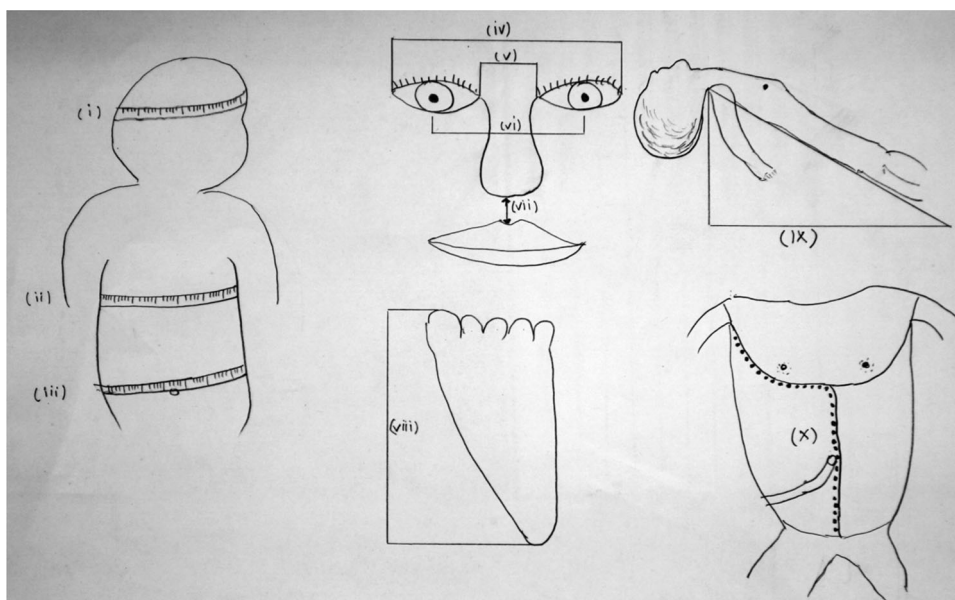


Fig. 3 **i** Amniotic band syndrome, **ii** nonimmune hydrops fetalis (Turner syndrome), **iii** blue sclera, **iv** microphthalmia, **v** Duane anomaly, **vi** occipital cephalocele, **vii** scoliosis, **viii** omphalocele, **ix**

hypercoiled umbilical cord, **x** per-auricular skin tag, **xi** amputated fingers due to amniotic bands, **xii** post-axial polysyndactyly, **xiii** pre-axial polydactyly, **xiv** rocker bottom foot, **xv** radial aplasia

blocks. An en bloc removal of viscera into functionally-related blocks (Gohn) is a compromise between the Virchow and en masse techniques. The choice of

technique is made at the time of the autopsy by the prosector with the intent to salvage the maximum information possible (Figs. 4 and 5).

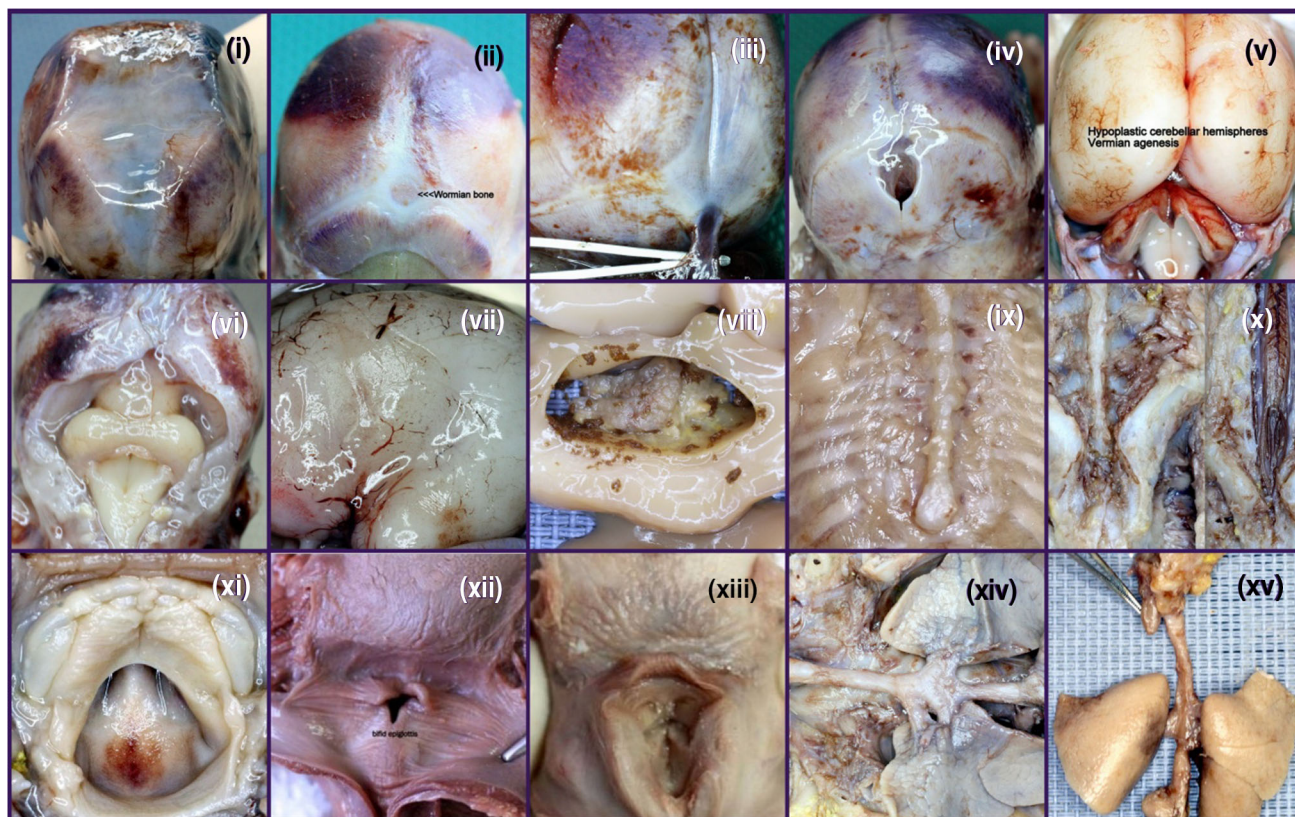


Fig. 4 **i** Wide anterior fontanel, **ii** wormian bone, **iii** and **iv** occipital cephalocle, **v** vermian agenesis, **vi** question mark dissection to display the brain stem, **vii** lissencephaly, **viii** ventriculomegaly with nodular

protrusions, **ix** caudal dysplasia sequence, **x** sacral agenesis (diabetes associated), **xi** cleft palate, **xii** bifid epiglottis, **xiii** congenital tracheal stenosis, **xiv** and **xv** tracheoesophageal fistula

The abdominal organs are usually examined first commencing with recording of the situs. The position of appendix (which denotes the completion of mid-gut rotation), and the mesenteric attachment along with the position of ascending colon, transverse colon, descending colon, sigmoid colon, rectum, and anal canal inspected. The transverse colon can now be separated from the stomach allowing visual inspection of the spleen, splenic artery, and the pancreas. The descending colon can be retracted medially along with the peritoneum to visualize left kidney, left supra renal gland, and the left ureter. The presence of anomalies is documented. Uterus, ovaries, and fallopian tubes in the female and testes in the male are identified. The domes of the diaphragm are inspected. The liver, its visceral surface along with gall bladder, porta-hepatis, and the lesser omentum are examined.

The thoracic cavity is entered by first incising the sternoclavicular joints and then cutting the ribs 4 mm from the costochondral junction in an inverted V pattern. After examining the xiphoid process, the ribs are lifted off by grasping the xiphoid with toothed forceps. The external surface of the lungs and the lobations recorded. The heart and the great vessels are inspected next by removing the thymus and the pericardium. Absence of left brachiocephalic vein suggests persistent left superior vena cava. Cardiothoracic ratio is recorded and the thoracic situs determined. The position of the heart and the apex documented. The aorta, the great vessels, ductus arteriosus, and the pulmonary trunk and the pulmonary veins examined.

The heart is dissected in situ following a six step technique. *Step 1:* The right atrium is nicked at the lateral aspect, the incision extended cranially through the superior

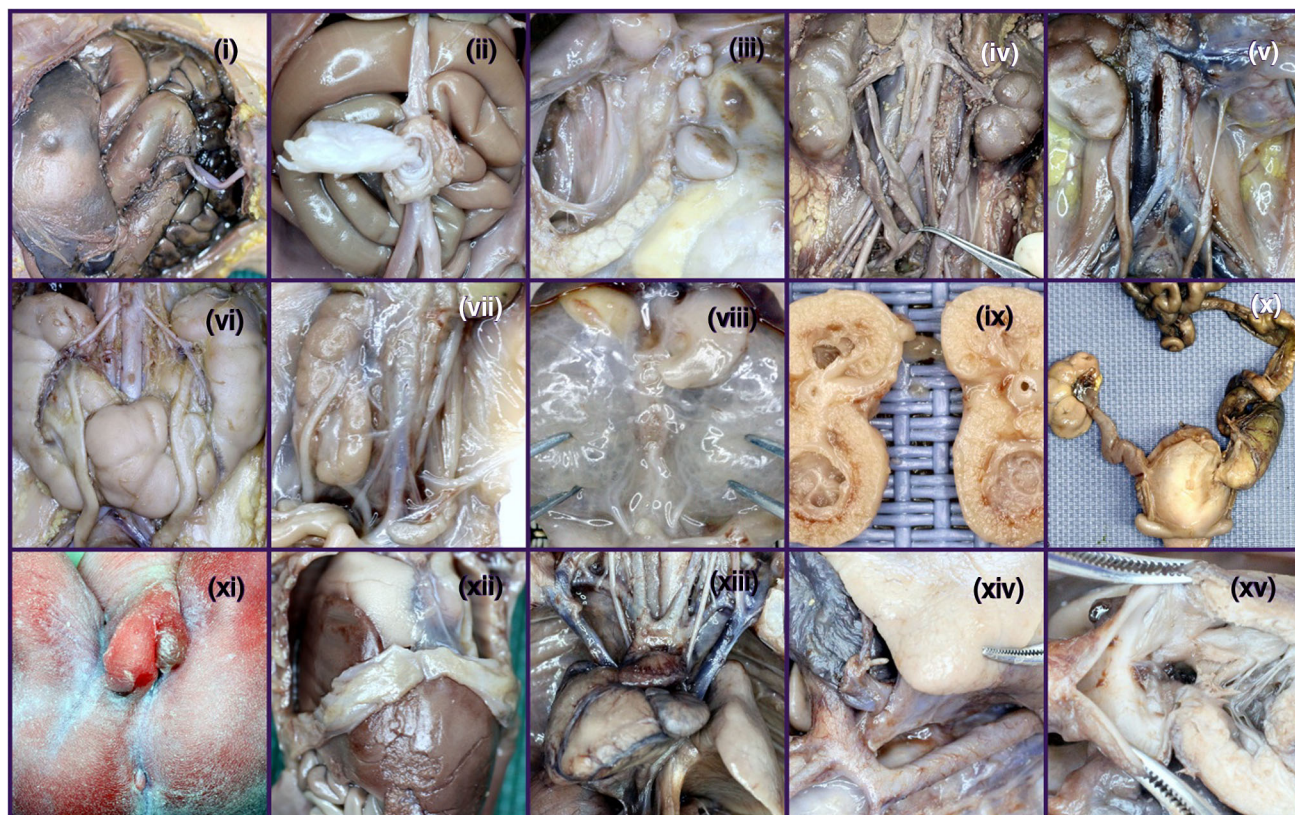


Fig. 5 **i** Aberrant umbilical vein, **ii** single umbilical artery, **iii** splenicules, **iv** double ureters, **v** ureteral dysplasia, **vi** horse shoe kidney, **vii** unilateral renal agenesis, **viii** polycystic kidney disease (autosomal recessive) with duplex ureters (*right*), **ix** renal cystic

dysplasia, **x** Uro-Rectal-Septal-Malformation Sequence (URSMS) with vesico-uretero-rectal fistula, **xi** ambiguous genitalia with phallic like structure (URSMS), **xii** diaphragmatic hernia, **xiii** pulmonary agenesis (*right*), **xiv** right sided aorta, **xv** ventricular septal defect

vena cava into the left brachiocephalic vein and caudally into the inferior vena cava to the diaphragm. *Step 2:* The right atrial wall is incised to the right ventricular apex 1 cm above the posterior descending coronary artery. *Step 3:* The right ventricular outflow tract is incised next from the apex, across the pulmonary valve and into the left pulmonary artery. *Step 4:* The left atrial appendage is nicked next with extension of the cut into the pulmonary veins. *Step 5:* The postero-lateral wall of the left ventricle is incised across the mitral valve to the apex. *Step 6:* Commencing from the apex, the anterior wall of the left ventricle, the aortic valve, ascending aorta and the aortic arch are cut next. At this juncture, the thoracic and the abdominal cavity are completely exposed and the evisceration of the organs can be done utilizing any of the aforementioned techniques.

The brain removal is facilitated by an incision over the cranium extending from behind one ear to the other. The

conversion of this incision into a question mark posteriorly is useful for delineating the brainstem and herniations through the foramen magnum. After reflecting the skin, the length and the breadth of the fontanel are measured and the degree of tension noted. The skull is next opened by cutting along the suture lines commencing at the lateral corner of the anterior fontanel, thus producing frontal and parietal bone flaps. The frontal, parietal, and the occipital bones are further cut in an oval fashion such that a flap of bone is left at the lateral aspect. The brain is inspected in situ and so are falx and the tentorium. The falx is now cut along the sagittal sinus antero-posteriorly. The brain can be removed by cradling the skull and the brain in the palm of the left hand at the occiput and tilting the head backwards gently allowing the brain to dislodge from the calvarium. The cranial nerves are inspected and gently cut close to the bone and the stalk of the pituitary severed close

to the brain. The tentorium is dissected around the periphery exposing the cerebellum. The cervical spinal column is transected as far into the foramen magnum as possible. The completely-disengaged brain is removed, weighed, inspected, and placed in formalin tank for 10–20 days for fixation. The brain can also be removed by using the same method under the water, a technique which eliminates parenchymal tear due to brain weight and gravity. Post removal of the brain, the base and the foramen magnum are inspected.

Provisional Autopsy Diagnosis (PAD)

The PAD usually is an extrapolation of gross external, internal, radiograph, frozen section, and Gram stain findings compiled within 24–48 h of the postmortem examination.

Placental Examination

The post mortem examination is incomplete without the placenta. Gross examination of the placenta is discussed in a separate write-up in this issue of the journal and is thus not included here.

Microscopic Examination

With the exception of malformations, the macroscopic appearance of fetal organs does not give specific information. Apart from identifying a cause or mechanism of death, microscopic examination can also help in assessment of gestational age.

Final Diagnosis

The final diagnosis is furnished only after the completion of microscopy. A summary of the case, comments, and pertinent references should be included in the final report.

Classification of Perinatal Deaths

The aim of classifying perinatal deaths is to derive strategies towards understanding the cause and ultimately prevent perinatal mortality. A good classification system integrates clinical factors, autopsy, and placental findings, takes into account the causative and contributory factors; thereby yielding a high percentage of classifiable causes. The ReCoDe system (Fig. 8) is a hierarchical classification that identifies the relevant condition at the time of death. It identifies what went wrong, not necessarily why [6]. The Tulip classification (Fig. 9) allows classification of underlying causes and mechanisms of perinatal mortality [7]. Both these systems have a low percentage of unexplained deaths (10–15 %). Classifying perinatal death is a complex process that requires a high level of expertise and is best accomplished at a well-organized, multidisciplinary, perinatal mortality meeting, where a conclusion is reached after discussion of all available clinical, pathological, and ancillary information.

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Compliance with Ethical Standards

Conflict of interest None.

Appendix

See Figs. 6, 7, 8, and 9.

FETAL / PERINATAL / NEONATAL DEATH POST MORTEM EXAMINATION CONSENT FORM

I, _____ (insert full name) give my consent for a complete / partial
(limited to _____) autopsy examination of _____ (my
fetus/baby/ward/relative) and I am the mother/father/competently authorized maternal or paternal relative of the deceased.

By giving my consent, I understand,

1. That, it may include removal, retention, macroscopic, microscopic examination or use of any of the body part/organ as deemed proper by the physicians.
2. That the autopsy is being done for academic reasons and not for medico legal purpose.
3. That any derived information may be utilized for diagnostic, therapeutic, scientific, research or publication purposes.
4. That the information would not be used for commercial or advertising purpose.
5. That my consent is only partially applicable for and inclusive of

S. No	PROCEDURE	CONSENT	
		YES	NO
I	Photography		
II	Radiological examination		
III	External examination		
IV	Internal examination		
V	Removal of organs for examination purpose		
VI	Retention of organs		
VII	Removal and examination of the brain and the spinal cord		
VIII	Mounting for academic purpose		
IX	Publication		

6. That I can revoke my consent any time prior to the autopsy. However once the autopsy has been performed by the physicians, it will not be possible to revoke the consent.
7. That if the information is utilized for publication purpose, every attempt would be made to ensure anonymity.
8. That I would have no objection to the text of the article and its publication and distribution.
9. That the consent form and information contained in the consent form as well as procedures pertaining to the autopsy have been explained to me in a language that I can understand.
10. That once the autopsy is completed I wish,

The baby to be returned to the family		
The baby to be sent to the burial ground for the performance of the final rites		
The baby be retained by the institute for academic purpose		

Consenter's Signature: _____ Physician's signature: _____

Date: _____

Fig. 6 Perinatal autopsy consent form template used at the institute

Date:		Death: Neonatal / Non-macerated stillbirth (SB) / Macerated SB	
Autopsy number:		Consent authorized by: Restrictions:	
Birth date and time:		Birth sequence: Singleton / Twin A / Twin B / Others	
Gestational age:		Birth weight and gender:	
Infant's name:		Postnatal age at the time of death:	
Mother's name and age:		Obstetric index:	
Blood group and Rh type:		Consanguinity:	
Medical/surgical history, substance abuse:		Ethnicity:	
Prior pregnancy loss and cause if known:		Parental karyotype:	
Antepartum/intrapartum complications:			
Prenatal ultrasound findings:			
Fetal karyotype:			

Anthropometry	Measurements (mm)	
Crown rump		
Crown heel		
Head circumference		
Biparietal diameter		
Occipito frontal diameter		
Inner canthus		
Outer canthus		
Palpebral fissure	Right (R)	Left (L)
Nose		
Philtrum		
Oral fissure		
Ears	R	L
Chest circumference		
Inter-nipple distance		
Breast bud	R	L
Abdominal circumference		
Arm length (acromion to elbow)	R	L
Forearm length	R	L
Hand length	R	L
Thigh length (trochanter to knee)	R	L
Leg length (knee to lateral malleolus)	R	L
Foot length	R	L
Radiology	Right	Left
Humerus		
Ulna		
Radius		
Femur		
Tibia		
Fibula		

External examination
Skin colour
Skin peeling
Maceration
Cyanosis
Dehydration
Oedema
Jaundice
Petechial haemorrhage
Meconium staining
Bruising
Vernix
Ischaemia (gangrene)
Skin lesions suggestive of infection
Trauma due to mechanical devices
Excessive tissue folds
Lanugo
Limb changes
Extent of plantar crease
Eyes
Ears
Nose
Hard and soft palate
Descent of testis
Scrotal rugae
Female genitalia
Vaginal patency
Anal patency

Internal examination
Abdomen and pelvis
Peritoneal cavity and surface
Diaphragm
Umbilical vein
Umbilical arteries
Liver
Gall bladder
Spleen
Pancreas
Stomach
Small intestine
Caecum and appendix
Large intestine
Adrenal glands
Kidneys
Ureters and urinary bladder
Gonads
Thorax
Thymus
Lungs
Azygous and hemiazygous vein
Pericardial cavity
Position of the heart
Atria
Ventricles
Aorta
Pulmonary arteries
Superior vena cava
Inferior vena cava
Pulmonary veins
Foramen ovale
Ductus arteriosus
Myocardium
Coronary ostia and coronary sinus
Neck
Thyroid
Larynx
Brain

Organs	Weights (g) / Measurements (cm)	
Body weight		
Brain		
Thyroid		
Thymus		
Lungs	R	L
Heart		
Liver		
Spleen		
Pancreas		
Adrenal	R	L
Kidneys	R	L
Small intestine		
Large intestine		
Appendix		
Gonads		
Pituitary		
Brain and liver weight ratio		
Lung weight/body weight ratio		

Fig. 7 Abridged version of the perinatal autopsy protocol template in use at the institute



A. Fetus	1. Lethal congenital anomaly 2. Infection 2.1 Chronic – e.g. TORCH 2.2 Acute 3. Non-immune hydrops 4. Iso-immunisation 5. Fetomaternal haemorrhage 6. Twin-twin transfusion 7. Fetal growth restriction ¹ 8. Other
B. Umbilical Cord	1. Prolapse 2. Constricting loop or knot ² 3. Velamentous insertion 4. Other
C. Placenta	1. Abruptio 2. Praevia 3. Vasa Praevia 4. Placental insufficiency /infarction ³ 5. Other
D. Amniotic fluid	1. Chorioamnionitis 2. Oligohydramnios ² 3. Polyhydramnios ² 4. Other
E. Uterus	1. Rupture 2. Other
F. Mother	1. Diabetes 2. Thyroid diseases 3. Essential Hypertension 4. Hypertensive diseases in pregnancy 5. Lupus/Antiphospholipid Syndrome 6. Cholestasis 7. Drug abuse 8. Other
G. Intrapartum	1. Asphyxia 2. Birth Trauma
H. Trauma	1. External 2. Iatrogenic
I. Unclassified	1. No relevant condition identified 2. No information available

ReCoDe

Classification of stillbirth by Relevant Condition at Death

This system seeks to identify the condition(s) which existed at the time of death in-utero. The classification is based on the following principles:

1. Stillbirths are distinct from neonatal deaths and warrant their own classification.
2. There is hence no need for a sub-classification according to gestation, as 'prematurity' is not a relevant cause or condition for stillbirths.
3. There is no subclassification according to weight, but one related to fetal growth status, based on weight-for-gestation.
4. The classification emphasises what went wrong, not necessarily 'why'. Hence, more than one category can be coded.
5. The hierarchy starts from conditions affecting the fetus and moves outwards, in simple anatomical categories (A-F) which are subdivided into pathophysiological conditions.
6. The primary condition should be the highest on the list that is applicable to a case.

Footnotes in table:

1. Defined as < 10th customised weight-for-gestation percentile (centile calculator is available at www.gestation.net/centile)
2. If severe enough to be considered relevant
3. Histological diagnosis

www.perinatal.nhs.uk/recode

Fig. 8 ReCoDe classification

Cause of death	Subclassification	
1 Congenital anomaly	1 Chromosomal defect	1 Numerical 2 Structural 3 Microdeletion/uniparental disomy
	2 Syndrome	1 Monogenic 2 Other
	3 Central nervous system	
	4 Heart and circulatory system	
	5 Respiratory system	
	6 Digestive system	
	7 Urogenital system	
	8 Musculoskeletal system	
	9 Endocrine/metabolic system	
	10 Neoplasm	
	11 Other	1 Single organ 2 Multiple organ
2 Placenta	1 Placental bed pathology	
	2 Placental pathology	1 Development 2 Parenchyma 3 Localisation
	3 Umbilical cord complication	
3 Prematurity/immaturity	4 NOS	
	1 PPRM	
	2 Preterm labour	
	3 Cervical dysfunction	
	4 Iatrogenous	
4 Infection	5 NOS	
	1 Transplacental	
	2 Ascending	
	3 Neonatal	
	4 NOS	
5 Other	1 Fetal hydrops of unknown origin	
	2 Maternal disease	
	3 Trauma	1 Maternal 2 Fetal
	4 Out of the ordinary	
6 Unknown	1 Despite thorough investigation	
	2 Important information missing	

Fig. 9 Tulip classification

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