Fetal and Perinatal Autopsy in Prenatally Diagnosed Fetal Abnormalities With Normal Karyotype

This technical update has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecology Canada (SOGC), reviewed by the Family Physicians Advisory Committee and the Medico–Legal Committee of the SOGC and approved by the Executive of the SOGC.

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Abstract

Objective: To review the information on fetal and perinatal autopsies, the process of obtaining consent, and the alternative information-gathering options following a prenatal diagnosis of non-chromosomal malformations, and to assist clinicians in providing postnatal counselling regarding fetal diagnosis and recurrence risks.

Outcomes: To provide better counselling about fetal and perinatal autopsies for women and families who are dealing with a prenatally diagnosed non-chromosomal fetal anomaly.

Evidence: Published literature was retrieved through searches of PubMed or Medline, CINAHL, and The Cochrane Library in 2009 and 2010, using appropriate key words (fetal autopsy, perinatal autopsy, postnatal autopsy).
The major objectives of the fetal or perinatal autopsy are to determine gestational age, document growth and development, detect congenital abnormalities, analyze clinical diagnosis and treatment, and determine the cause of death and possible recurrence risk.1

The approach to fetal or perinatal autopsy is very different from the approach to adult autopsy. Fetal development is, in part, dependent on maternal health and intrauterine environment. In addition, the diseases and conditions considered in the fetus are not the same as in adults. Genetic syndromes must be identified if present.

The answers provided by the fetal or perinatal autopsy benefit the parents and extended family as well as allowing the treating physician to understand and counsel about the etiology identified for the loss. Most parents after losing a pregnancy or newborn have many questions that can be answered only after a high quality autopsy. The autopsy can provide valuable explanations, and it allows care providers to offer more accurate genetic counselling to the family and helps in planning for the management of future pregnancies.

Autopsy findings are more likely to be useful when no clear clinical diagnosis is available or when there is a fetal malformation.2 The need for a fetal or perinatal autopsy must be evaluated by the clinician and adapted to the results of the investigation performed before fetal or neonatal demise.3 For example, the need for a fetal autopsy after pregnancy termination for a confirmed diagnosis of fetal trisomy 18 is different from the need for autopsy of a fetus with unexplained multiple malformations. The presence of prenatally diagnosed fetal malformation(s) with no chromosomal diagnosis is a clear indication for a fetal autopsy.

The main objectives of this technical update are to review

1. The benefits of a fetal or perinatal autopsy
2. The consent process
3. The alternatives when the family declines the full autopsy.

A standardized approach to the fetal and perinatal autopsy is provided in the Appendix.

DEMONSTRATED BENEFITS OF A FETAL OR PERINATAL AUTOPSY

Gordijn et al.4 reviewed the performance of perinatal autopsies by comparing the clinical and autopsy diagnoses in stillbirths, neonatal deaths, and therapeutic terminations.
The autopsy revealed a change in diagnosis or additional findings in 22% to 76% of cases. If confirmation of clinical findings is also considered, then perinatal autopsy had value in up to 100% of cases. The factors that may influence the value of perinatal autopsies include the type and definitions of perinatal loss, autopsy rates and protocol use, expertise of pathologists and level of hospital care, and antenatal diagnosis.4

More recently, Phadke and Gupta5 obtained similar indicators of performance in 91 autopsies performed after antenatal identification of fetal malformations: fetal autopsy provided a definite diagnosis in 79.1% of the cases and confirmed the sonographic findings in 97.8%. Additional findings helped in redefining the diagnosis in 33% of the cases.5

Dickinson et al.6 published a series of 1012 consecutive terminations for fetal abnormality. Autopsy was performed in 809 cases (79.9%). In euploid cases, autopsy confirmed the prenatal diagnosis with no additional information in 63.5% (357 of 562). In 1.1% (6 cases), autopsy added major diagnostic information, and in 15.1% (85 cases), significant information was provided. Autopsy provided diagnosis or clarification of some prenatal findings in 16% of cases.6

Previous studies reported an overall refinement of the recurrence risk in 27% of the cases.7

Therefore, in around 30% of perinatal postmortem examinations, some additional information is obtained from autopsy that changes either the underlying diagnosis or information given to parents during counselling.8 Autopsy findings are more likely to contribute additional information when the examination is completed as soon as possible after fetal demise.2 Clinicians can confidently advise parents of the usefulness of autopsy in ascertaining the cause of death and for counselling them in their future pregnancies.4 Other benefits of perinatal autopsy include auditing of perinatal program outcomes, ensuring that families receive emotional support and bereavement care, and enhancing teaching and medical knowledge.9

OBTAINING CONSENT FOR A FETAL OR PERINATAL AUTOPSY

It is imperative that clear information be provided to the parents so their consent for full or limited autopsy is truly informed.10 The topic of a postmortem examination can be brought up when withdrawal of treatment and/or pregnancy termination is first considered. Clinicians approaching parents for autopsy consent should discuss the options for a full, limited, or step-wise postmortem examination. They should also discuss the issues of retained fetal tissues, the value of autopsy, and the possibility that information gained may not benefit them directly but may benefit others.11 There must also be written information available to parents, describing the perinatal autopsy, as an adjunct to the explanations given by the clinical team.

Health care professionals need to tailor the information they provide to each specific situation, as some people may insist...
on in-depth detail, whereas others would prefer to have only the basics of the procedure explained to them. The ability to be sensitive in communicating the rationale for postmortem examination and alternatives to parents is as crucial as the ability to be skilful in the collection of specimens and performance of autopsy. The treating team should discuss the autopsy with the family in a timely and accurate manner in a quiet environment, allowing sufficient time to answer questions. Addressing specific cultural and/or religious values is essential, and there are published studies that will help care providers in doing this. It is important to inform the parents that their baby will be treated with respect and dignity at all times. Agreeing to autopsy does not prevent a family from spending time with their baby or choosing to have a funeral or memorial service.

The guidelines from the European Parliament and Council advised that postmortem consent forms should include a section explicitly addressing the issue of organ retention. Parental agreement to organ retention has been reported to be as high as 60%. Laws specific to fetal autopsy may vary by jurisdiction.

The consent for autopsy should be recorded on an approved consent form relevant to the jurisdiction. Molecular diagnostic analyses from a fetal sample may be discussed and recorded on a specific consent form. If parents are unwilling to give consent for a full autopsy, alternatives to autopsy must be presented in a manner that includes disclosure of limitations.

**APPROACH TO THE FETAL OR PERINATAL AUTOPSY**

These autopsies should be performed by trained perinatal, pediatric, or fetal pathologists and should follow accepted protocols.

Information for the pathologist should be provided in a timely and accurate manner and should include the details of the complete obstetrical and medical history, invasive testing, imaging, and family history. In cases requiring special evaluation (e.g., eye examination, metabolic autopsy, and suspected myopathies), direct communication with the pathologist is preferable to ensure that all necessary sampling is performed. Details of the approach to the perinatal autopsy are included in the Appendix.

**ALTERNATIVES WHEN A FAMILY DECLINES AN AUTOPSY**

Standard autopsy provides the only process for fully investigating fetal loss, stillbirths, and neonatal deaths associated with non-chromosomal fetal malformations. The rates of fetal and perinatal autopsy are higher than the rates of autopsy for any other age group but have experienced a decline in recent decades. The controversies surrounding the issue of organ retention are likely to have had an impact. The year after the implementation of a guideline for investigating stillbirths in Alberta there was an increase from 54% to 74.5% in fetal autopsies and a decrease to 48% 3 years later. A similar decrease has been reported in other countries.

Khong and Tanner reported a 58% acceptance for fetal autopsy in a group of 305 women following pregnancy terminations.

Autopsy may be declined because (a) the parents feel the baby has already suffered enough, (b) the parents assume that prenatal investigations were sufficient, (c) health care professionals failed to provide adequate explanation of autopsy, and (d) the parents were not offered options to postmortem examination. Declining autopsy rates may also be linked to personal values and cultural or religious prohibitions. Knowledge of the circumstances in which a postmortem examination is permitted may improve the clinician’s ability to discuss in a sensitive manner the options acceptable to the family. Cultural and religious considerations pertaining to fetal and perinatal autopsies are reviewed in the literature. The treating team should respect the parents’ decision.

When parents are reluctant to consent to a full autopsy, they may agree to a limited autopsy, including examination of specific body cavities, or full body imaging techniques, which will allow specific questions or concerns to be addressed and which may be more acceptable to some families. Alternatives to autopsy must be presented in a manner that includes the disclosure of limitations and how those limitations may affect the management of future pregnancy.

The pathologist should be informed if parents have consented to a limited autopsy. Consent specific to the extent of the examination should be obtained and recorded. Biometric measurements, clinical photographs, external examination, and radiographs are generally acceptable to most parents. The findings should be recorded in the medical chart. Obtaining samples of blood, body fluids, skin, and placenta is important to allow specific ancillary testing (Tables 2 and 3). Examination of targeted internal organs or specific tissue sampling, as dictated by the phenotype of the fetus, can be suggested to the family.

MRI may be offered to parents who decline an autopsy investigation, although the limited availability of MRI and the need for prioritization are concerns in most countries.
Clinicians should explain to the parents that a full autopsy remains the gold standard because the MRI does not supply tissue samples, and important information may therefore be missed. Many limitations of using perinatal postmortem MRI are cited in the literature: high cost, limited availability, lack of experience, need for specialist equipment, lower resolution, lack of detection of changes at the histological level, and uncertain value when there is an advanced degree of maceration or autolysis.\textsuperscript{19,22} MRI also provides suboptimal resolution in assessing certain malformations such as skeletal dysplasia.\textsuperscript{8}

A 2008 overview underlined the non-invasive nature of the MRI examination and the detection of pathologies and malformations of the central nervous system.\textsuperscript{23} There was complete agreement in 60% of cases between MRI and autopsy findings. The autopsy was essential to finding the cause of death in 37% of the cases. If MRI had been the only investigation, essential information would have been lost in 17 of 24 cases (71%). Another smaller study (n = 26) comparing postmortem MRI and autopsy for all malformations demonstrated a 79% detection rate for major malformations and a 9% detection rate for minor malformations.\textsuperscript{24} A recent meta-analysis comparing the

### Table 2. Additional sampling in fetal anomalies

<table>
<thead>
<tr>
<th>Collection of placenta tissue for cytogenetic studies (if karyotype not known)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. After delivery of the placenta and before the placenta is sent to pathology, a sample of placenta tissue should be collected from all stillborns and stored until the clinician or pathologist decides whether or not the specimen requires cytogenetic studies.</td>
</tr>
<tr>
<td>2. Collect the placenta tissue sample from the fetal side by the site of cord insertion beneath the amnion. Remove 1 cm(^3) of placenta tissue with a sterile surgical knife and dissecting forceps. The sample should be placed in sterile saline or other appropriate tissue culture media, sealed, and labelled. Ensure that the media container is completely filled, as the sample may stick to the lid of the container in transport.</td>
</tr>
<tr>
<td>3. If necessary, the sample can be stored in the refrigerator at 4°C. DO NOT FREEZE. (The length of time the specimen can be stored should be determined locally in conjunction with the genetics laboratory.) The sample should be transported to the cytogenetics laboratory promptly.</td>
</tr>
</tbody>
</table>

### Table 3. Algorithm for use of ancillary tests in perinatal pathology

<table>
<thead>
<tr>
<th>For every fetal case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detailed gross description and detailed records (written, radiographic, photographic)</td>
</tr>
<tr>
<td>Frozen samples of liver and placenta (–70 °C)</td>
</tr>
<tr>
<td>And if the phenotype</td>
</tr>
<tr>
<td>Includes ≥ 1 major malformation</td>
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<tr>
<td>Suggests aneuploidy</td>
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<tr>
<td>Suggests DiGeorge/velo-cardio-facial syndrome</td>
</tr>
<tr>
<td>Suggests hypokinesia</td>
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<tr>
<td>Suggests Fanconi anemia</td>
</tr>
<tr>
<td>Suggests skeletal dysplasia</td>
</tr>
<tr>
<td>Is unexplained fetal hydrops</td>
</tr>
<tr>
<td>Suspected metabolic</td>
</tr>
<tr>
<td>Suggests Fanconi anemia</td>
</tr>
<tr>
<td>Suggests skeletal dysplasia</td>
</tr>
</tbody>
</table>

**Microbiology (when needed)**

**Placenta tissue**

1. Using sterile technique, collect and send placenta tissue culture for Group B Steptococcus, Listeria, Enterovirus, and Cytomegalovirus (check with laboratory for appropriate culture media).
2. To collect: swab surface of placenta membranes with alcohol prior to cutting, incise amnion and peel away from incision site, and then resect a 1 cm\(^3\) segment of subamniotic chorionic plate along with underlying villi. Place in culture medium.

**Table 2. Additional sampling in fetal anomalies**

**Table 3. Algorithm for use of ancillary tests in perinatal pathology**

performance of MRI with that of conventional autopsies demonstrated a 69% sensitivity (95% CI 56% to 80%) and 95% specificity (95% CI 88% to 98%) in determining the final cause of death or most clinically significant abnormality in 146 fetuses. Well-designed large prospective studies are needed to evaluate the accuracy of postmortem MRI. Therefore, the integrated result obtained from the traditional autopsy remains crucial in determining the cause of the malformation or of the fetal or perinatal death.

MRI can detect some malformations and other macroscopic lesions, but it cannot function as a substitute for standard autopsy. However, there is now public awareness of this procedure, and a new specialty in radiology seems to be emerging.

New options such as postmortem needle biopsy, laparoscopic autopsy, and small incision access are other alternatives to a full postmortem examination for focused investigation of suspected anomalies, and these should be discussed with the pathologist. Aspiration of body fluids (cord blood, cerebrospinal fluid, urine, cyst, edema) for biochemical, hematologic, microbiologic, or metabolic investigations may be considered. These methods have not been fully assessed in the specific context of perinatal death. Biopsy of individual organs clearly has a role in selected cases. When a fetal infection is suspected, it is often possible to specify the infectious agent, particularly when cultures of the placenta or infant are initiated promptly after delivery. Finally, all fetuses with known congenital malformations should have at least cytogenetic analyses performed if this was not done antenatally. This analysis can be done on cord blood, fetal tissues, or the placenta. Fetal tissues and placenta are a good source of fetal DNA that can be banked for further studies as indicated.

**SUMMARY**

Fetal and perinatal autopsies are an essential part of the clinical management of families experiencing the loss of a fetus or newborn with prenatally identified non-chromosomal anomalies. The current literature emphasizes the importance of autopsy in providing accurate etiologic diagnosis necessary for genetic counselling.

A standardized approach to fetal and perinatal autopsy is crucial. It increases uptake after counselling from better-trained care providers and allows consultation with experienced colleagues by sharing samples. Such a protocol can facilitate the introduction and use of newer technology. By banking frozen samples and dividing cells, patients and their families can access testing for genetic disorders, currently and in the future. This will allow the clinician to offer, later if not at the time of fetal or neonatal death, precise diagnosis, genetic counselling, and prenatal diagnosis for future pregnancies.

The option of minimally invasive autopsy is available to parents, but it has limitations that should be presented to them. In this case, consent should be obtained for biometry, clinical photographs, X-rays, placental pathologic examination, and, when indicated, fluid aspiration/targeted organ biopsies. The performance of postmortem MRI remains to be established. Conventional autopsies remain the gold standard.

**Recommendations**

1. Standard autopsy should ideally be an essential part of fully investigating fetal loss, stillbirths, and neonatal deaths associated with non-chromosomal fetal malformations. (II-3A)

2. Clinicians and health care providers approaching parents for autopsy consent should discuss the options for a full, limited, or step-wise postmortem examination; the issue of retained fetal tissues; and the value of autopsy and the possibility that the information gained may not benefit them but may be of benefit to others. This information should be provided while respecting the personal and cultural values of the families. (III-A)

3. If parents are unwilling to give consent for a full autopsy, alternatives to full autopsy that provide additional clinical information must be presented in a manner that includes disclosure of limitations. (III-A)

4. External physical examination, medical photographs, and standard radiographic or computed tomography should be offered in all cases of fetal anomaly(ies) of non-chromosomal etiology. (II-2A)

5. Well-designed, large prospective studies are needed to evaluate the accuracy of postmortem magnetic resonance imaging. It cannot function as a substitute for standard full autopsy. (III-A)

6. The fetal and perinatal autopsies should be performed by trained perinatal or pediatric pathologists. (II-2A)

7. The need for additional sampling is guided by the results of previous prenatal and/or genetic investigations, as well as the type of anomalies identified in the fetus. Fibroblast cultures may allow future laboratory studies, particularly in the absence of previous karyotyping or if a biochemical disorder is suspected, and DNA analysis. (II-3A)
8. In cases requiring special evaluation, the most responsible health care provider should have direct communication with the fetopathologist to ensure that all necessary sampling is performed in a timely manner. (II-3A)

9. The most responsible health care provider must see the families in follow-up to share autopsy findings, plan for the management of future pregnancies, obtain consent for additional testing, and offer genetic counselling to other family members when appropriate. (III-A)

REFERENCES


The approach to autopsy must be systematic. Use of an autopsy protocol to record information ensures that all required data are obtained and recorded. Measurements and their normal values for gestational age are recorded onto these forms, and organ-directed checklists are useful time-saving adjuncts. The autopsy protocol and a list of possible diagnoses, a clinical summary, and a clinico-pathologic discussion form the completed autopsy report. In the context of a fetal autopsy, as opposed to those performed in adults, normality cannot be assumed; therefore, the autopsy protocol must document both normal and abnormal findings. As a basic rule, everything that can be observed must be described and possibly photographed, and everything that can be measured or weighed must be, with value recorded and compared with normality charts. If the autopsy report fails to state that a particular structure is normal, the genetic syndromic differential diagnosis may be hampered.

The autopsy results should be reported in a standard format: autopsy facesheet (demographics and list of anatomic diagnosis and findings), a clinical summary, an objective description of the gross autopsy observations, a slide and block catalogue, reports of ancillary studies, and a clinico-pathologic clinical summary. Communication of provisional autopsy findings should be relayed to the clinician in a timely manner. Published guidelines regarding these items are available. The written report complements but cannot replace verbal communication between the pathologist and the clinical team.

1. Biometry
The size and weight of the body should correlate with age and are affected by disorders of growth and development. These weights and measurements must be accurate and compared with normal charts. The crown–heel and crown–rump lengths should be determined to the nearest 5 mm. Normally in fetuses and young infants, occipitofrontal circumference and crown–rump lengths should not differ by more than 10 mm. Distances between inner canthi and outer canthi should be obtained. Chest and abdominal circumference are measured at the level of the nipples and umbilicus respectively. Foot lengths should be obtained, as this measurement correlates well with gestational age. Other specialized measurements can be obtained and compared with published norms: width and length of fontanelles, nasal height, philtrum length, mouth width, ear length, inter-nipple distance, lengths of limbs.

2. Photographs
High-quality photographs are an important part of the fetal autopsy procedure. Frontal, lateral, and dorsal pictures of the fetus, with a close up of the cleaned face, of any unusual findings, and of both maternal and fetal placental surfaces, are a strict minimum. A list of photographs should be mentioned in the report. The photographs should be labelled and filed in the medical record or in a computerized archival system. The use of digital imaging for this purpose is optimal; however, issues regarding patient consent and confidentiality should be considered. The photographs can be used to facilitate parental grief, for consultation with colleagues, and even for resolving medico-legal issues. Photographs are useful for teaching and publication and can be used for external consultation when needed.

3. External Examination
This examination is of particular importance when the autopsy is declined.

It should be performed by experienced clinicians in the field of perinatal/pediatric pathology or clinical genetics or by a pediatrician. In the absence of such expertise, detailed photographs must be taken for future evaluation (see prior section). The clinician should document in the mother’s chart the presence of major fetal external malformations.
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Inspection of the external features of the body is similar to the physical examination on newborns performed in the clinical setting (Table 4). Developing and following such a routine ensures that no pertinent feature is overlooked. The extent of maceration must be documented, as it correlates somewhat with the duration of postmortem retention. Photographs are indicated.

Dysmorphism, deformations, disproportions, and malformations should be described. The shape of the skull and facial hair should be noted. The face and palate must be described accurately, with attention to dysmorphism, deformations, and iatrogenic lesions. The following must be described: size, shape and orientation of the eyes and eyelids; eyebrows; nasal shape/insertion and angulation; choanal permeability; palate integrity; shape of the philtrum; size of the mouth; ear orientation/implantation; chin; and presence of preauricular pits or branchial sinuses. Features of the neck, thorax, abdomen, umbilical cord including abdominal insertion and vessel count, anus position and patency, spine, and genital area must be noted. Limbs including digits, palmar creases, muscle mass, disproportion or abnormal positioning must be described in detail. Skin is examined for meconium staining, edema, constriction, pallor/anemia, petechia, purpura, pustules, size of nails, sites of aplasia and, when present, position of shunts (see Table 4 for a summary sheet).

### Table 4. Data collection sheet for postmortem medical examination when autopsy is declined (adapted from Alberta Medical Association, 2009)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>General appearance</td>
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<tr>
<td>Skin</td>
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<tr>
<td>Head</td>
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<td>Scalp</td>
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<td>Eyes</td>
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<td>Nose</td>
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<td>Nostrils</td>
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<td>Ears</td>
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<td>Mouth</td>
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<td>Mandible</td>
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<td>Neck</td>
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<td>Chest</td>
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<td>Abdomen</td>
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<tr>
<td>Cord</td>
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<td>Genitalia</td>
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<td>Anus</td>
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<td>Spine</td>
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<td>Arms</td>
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<td>Hands</td>
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<td>Legs</td>
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<td>Feet</td>
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<tr>
<td>Other</td>
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<tr>
<td>Gestational age (wks)</td>
<td>Birth weight (g)</td>
<td>Circumference of head (cm)</td>
<td>Crown-heel length (cm)</td>
</tr>
<tr>
<td>Completed by</td>
<td></td>
<td>Signature/Status</td>
<td>Date/time</td>
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</tbody>
</table>

4. Internal Examination and Routine Microscopic Sections
Several publications provide technical considerations. All major organs must be weighed after careful dissection guided by the published methodology, thereby allowing comparison with expected values. Organ maturity and structure can later be assessed by macroscopic (e.g., cerebral gyration) and/or histologic (e.g., lungs and kidneys) evaluation. Histological examination must assess the presence of changes that could indicate a storage disease or an intrauterine infection (TORCH: toxoplasmosis, other infections, rubella, cytomegalovirus, herpes simplex virus).

5. Additional Sampling
The need for additional sampling is guided by the results of previous investigations, along with the type of anomalies identified in the fetus. Obtaining skin or fasciae biopsy for fibroblast cultures may allow future studies, particularly in the absence of previous karyotyping or if a biochemical disorder is suspected. Many units have protocols for handling the tissue samples (e.g., refrigerated if not handled immediately by the laboratory). When there is a strong suspicion of a metabolic disorder, prior consultation with a physician specializing in metabolic disorders is recommended.

In the absence of a clear diagnosis, a sample of fetal liver should be obtained and frozen at −70°C for future studies if tissues are not too macerated. Otherwise, less macerated tissues should be sampled, including chorionic villi.

A variety of muscles must be examined when central nervous or muscular system diseases are being considered. If sampling of muscle and of central nervous system is performed adequately, a neuropathologist will subsequently be able to assist with the diagnosis. Myopathies should be suspected if a fetus shows stigmata of in utero dyskinesia such as multiple contractures and pterygia in association with polyhydramnios. For such cases, a comprehensive examination of skeletal muscle includes routine histology, frozen-section histochemistry, and electron microscopy. In addition, muscle is rapidly frozen and stored for additional biochemical studies.

Bony tissue should be studied grossly and microscopically. A section of the growth plate should be frozen and kept long term for additional studies as required. In some forms of skeletal dysplasia, the examination of long bones or of the entire rib cage with the vertebral column may be necessary. Specific tissue sampling is presented in Table 3.

6. Placenta
The placental examination is useful in verifying infection, investigating stillbirth, and in cases where maternal disease plays a large role in pregnancy outcome. Gross examination of the placenta should follow a routine methodology and should ideally be performed on a fresh sample following brief drainage and removal of non-adherent blood. Fresh examination allows a better assessment of discoloration and weight (formalin fixation increases weight by 6% to 10%), better photography of placental lesions, and special procedures requiring fresh tissues (culture, cytogenetics, RNA).

In specific situations, tissue sampling should occur prior to gross examination, to avoid contamination. A fresh placental sample, from the fetal surface, should be obtained and frozen at −70°C. The excision of placenta should be shallow to minimize the risk of maternal cell contamination.

Macroscopic examination is divided into portions of the placenta: umbilical cord, extraplacental membranes, and the disc proper (fetal surface, maternal surface, and the cut surface). Careful examination may help distinguish between amniotic fluid infection (progression including both fetal and maternal inflammation) and hematogenous placental infection (placental disc).

7. Multiple Gestation and Stillborn Fetus

Multiple gestation
In a case of multiple gestations, additional studies should be considered. Examining the dividing membranes to establish chorionicity is required. When a twin-to-twin transfusion is suspected, injection studies of the fetal vessels may help demonstrate abnormal vascular communications on both amniotic surface and placenta body.

Stillborn fetus
It is important to establish the cause of death, and, if possible, to exclude the presence or role of congenital anomalies, infection, or other diseases. Establishing the time of death may be difficult. Tables are available for estimation but can be of limited accuracy (maceration is more rapid with chorioamnionitis). Examination is hampered by maceration of the body which may vary from mild to extreme. The pathologist may still derive meaningful findings, such as congenital
anomalies, even when tissues are in poor condition. Histologically, viral inclusions are generally still apparent in fetuses with advanced maceration. Skin or viscera are inappropriate for karyotyping in cases of advanced maceration. The placenta can be useful in such cases, but genomic array-based techniques may well replace standard karyotyping in the near future.

8. X-Rays
Standard radiographic or computed tomography should be used in all cases of fetal anomaly(ies) of non-chromosomal etiology. Some advocate the use of radiography in every fetal or perinatal death. The fetogram should be performed before the internal examination. Olsen et al. report abnormal radiographs in 30% of fetograms performed in a population-based set of 542 perinatal deaths. New information about the pathological process was found in 8.6%. Radiographs were of vital importance for establishing the cause of death in 3.1% of the cases. In the presence of fetal anomalies, it is essential to obtain a fetogram to evaluate skeletal anomalies that could lead to the identification of a fetal genetic syndrome.

9. Cultures and Toxicology
Specifying the infectious agent is often possible, particularly when cultures of the placenta or infant are initiated promptly after delivery. Fetal fluid (blood, cerebrospinal fluid) and fetal tissues (spleen or lung) can be used for bacterial or viral cultures. Increasingly, morphologic studies aided by molecular techniques may assist in the identification of pathogenic organisms. When indicated, blood, urine, bile, and liver can be submitted to the appropriate toxicology laboratory.

10. Ancillary Tests
All fetuses with known congenital malformations should have at least cytogenetic analyses performed if these were not done antenatally. The key is to learn to recognize situations in which other types of tissue handling are required. A frozen tissue sample (liver, placenta) is an important component of the perinatal pathologic examination. Frozen tissue provides a source of DNA, RNA, proteins, and molecules that can be used in a variety of ways. When maceration is not advanced, we recommend storage of fetal liver and placenta because of high concentration of DNA. Hepatic extracts contain a large amount of DNA, and enzyme deficiencies may be specific to the liver. In addition, maternal perfusion of the intervillous space maintains viability of fetal cells in the placenta long after fetal death. Targeted molecular testing can later be performed after careful analysis of the autopsy findings that may suggest a specific genetic syndrome. Other, newer approaches include microarrays and whole genome sequencing array. The contribution of these new molecular tools in the etiologic elucidation of congenital anomalies is currently under evaluation. Consultation with a medical geneticist is recommended to assist in the selection of the most appropriate molecular investigation.

Tissue culture can be obtained from fibroblast (skin, Achilles tendon, epicardium, subamnion chorion). These dividing cells can be used for cytogenetic testing (karyotyping, metaphase fluorescence in situ hybridization breakage studies) or metabolic testing. Table 2 shows additional sampling in fetal anomalies, and Table 3 present a series of tests indicated in specific clinical situations.