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No. 365-Fetal and Perinatal Autopsy in Prenatally Diagnosed Fetal Abnormalities with Normal Chromosome Analysis

This revised Technical Update has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC), reviewed by the Clinical Practice Committee of the Canadian College of Medical Geneticists (CCMG) and approved by the Board of the SOGC.

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Key Words: Fetal autopsy, perinatal autopsy, perinatal postmortem examination, fetal anomalies, autopsy protocol, postmortem magnetic resonance imaging, postmortem MRI, autopsy consent, tissue retention, autopsy evaluation

CHANGES IN PRACTICE

1. Autopsy should be an essential part of investigating fetal loss, stillbirths, and neonatal deaths associated with non-chromosomal fetal anomalies.
2. Options for a full, limited, or step-wise postmortem examination should be discussed with parents.
3. 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.

KEY MESSAGES

1. The synthesis of fetal and perinatal autopsy data should be performed by trained perinatal or pediatric pathologists.
2. Timely pre and post autopsy communication between the most responsible health provider, the fetopathologist and ultimately the parents, is essential.
3. The most responsible health 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.

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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 anomalies in order to assist health care providers in providing postnatal counselling regarding diagnosis and potential 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 2010, 2011, and 2017, using appropriate key words (fetal autopsy postmortem, autopsy, perinatal postmortem examination, autopsy protocol, postmortem magnetic resonance imaging, autopsy consent, tissue retention, autopsy evaluation). Results were restricted to systematic reviews, randomized controlled trials/controlled clinical trials, and observational studies. Additional publications were identified from the bibliographies of these articles. There were no date or language restrictions. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Benefits, harms, and costs: This update educates readers about (1) the benefits of a fetal perinatal autopsy, (2) the consent process, and (3) the alternatives when the family declines autopsy. It also highlights the need for a standardized approach to fetal and perinatal autopsies, emphasizing pertinent additional sampling when indicated. The authors recognize that there is variability across Canada in access to the cited services and resources. As such, these recommendations were developed in an attempt to promote access and to provide a minimum standard for all provinces and territories across the country.

Values: The quality of evidence was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table).

Recommendations:

1. Standard autopsy should ideally be an essential part of investigating second trimester fetal loss, stillbirths, and neonatal deaths associated with non-chromosomal fetal anomalies, as it has been demonstrated to add clinical information on potential etiology and recurrence risks in about 25% of cases (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 decline 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. At this time, postmortem magnetic resonance imaging cannot function as a substitute for standard full autopsy (III-A).
5. External physical examination, and when clinically indicated medical photographs, standard radiographic or computed tomography, should be offered in all cases of fetal anomaly(ies) of non-chromosomal etiology (II-2A).
6. The synthesis of the fetal and perinatal autopsy data 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. If a biochemical disorder is suspected, fibroblast culture may allow future metabolic studies as well as DNA analysis, when clinically indicated (II-3A).
8. In cases requiring special evaluation, the most responsible health provider should have direct communication with the feto-pathologist to ensure that all necessary sampling is performed in a timely manner (II-3A).
9. The most responsible health provider must see the families in follow-up to share autopsy findings and plan for the management of future pregnancies. Consent for additional testing and genetic counselling to the couple and other family members should be performed through referral to a Medical Genetics service when clinically indicated and depending on local resources (III-A).

Table. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

Quality of Evidence Assessment[a]	Classification of Recommendations[b]
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group	C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in the category	D. There is fair evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	I. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

[a]The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.

[b]Recommendations included in these guidelines have been adapted from the Classification of recommendations criteria described in The Canadian Task Force on Preventive Health Care.

Taken from: Canadian Task Force on Preventive Health Care. New grades for recommendations from the Canadian Task Force on Preventive Health Care. CMAJ 2003;169:207–8.

INTRODUCTION

The major objectives of the fetal or perinatal autopsy are to determine gestational age, document growth and development, confirm the prenatal US and/or MR findings, identify congenital abnormalities not detectable by US, clarify the cause of lesions, analyze clinical diagnosis and treatment, and determine the cause of death with the aim of providing parents with accurate information. Fetal and perinatal autopsies are also essential in a Public Health perspective, a useful audit tool for the quality control of prenatal diagnosis and for epidemiological studies.[1,2]

The approach to fetal or perinatal autopsy is very different from the approach to adult autopsy. The fetus is not isolated but a part of the feto-maternal unit including placenta. 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. All these reasons make it essential for the perinatal autopsy to be performed by personnel trained in this field, as the yield of this examination is typically very low when carried out by someone without this specific expertise.

The answers provided by the fetal or perinatal autopsy benefit the parents and extended family as well as allowing the most responsible health provider 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 answers, and it allows care providers to offer more accurate genetic counselling to the family concerning the risk of recurrence or the possibility of prevention, 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 and/or in the presence of a fetal malformation.[3] The need for a fetal or perinatal autopsy must be evaluated by the most responsible health provider and adapted to the results of the investigation performed before fetal or neonatal demise.[4] 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 anomaly(ies) with no chromosomal diagnosis is a clear indication for a fetal autopsy.

Medical termination may be preferred for fetal anomalies for which a diagnosis has not been reached prenatally, since surgical termination via dilation and evacuation is likely to decrease the quality of the examination, despite the lack of evidence-based data. However, patient choice and available resources should be taken into consideration. Surgical management has been suggested to reduce maternal morbidity compared with labor induction[5-7] and may be the preferred method of termination for maternal reasons.

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 online Appendix.

DEMONSTRATED BENEFITS OF A FETAL OR PERINATAL AUTOPSY

The benefits of fetal and perinatal autopsy have been highlighted in a retrospective study of 300 fetuses examined after termination performed following a prenatal diagnosis of fetal malformation, with the objective of comparing *postmortem* findings with those of prenatal diagnosis. In this study, autopsy confirmed the diagnosis in 38.7% of cases, and provided additional information in 41% of cases. *Postmortem* findings modified the prenatal diagnosis in 20.3% cases, with a potential impact on subsequent genetic counselling.[8] In a recent study of 100 perinatal autopsies, *postmortem* examination added major findings in 42.5% cases, changing the prenatal diagnosis in 25% of fetuses.[9]

Gordijn et al.[10] reviewed the performance of perinatal autopsies by comparing the clinical and autopsy diagnoses in stillbirths, neonatal deaths, and terminations. The autopsy revealed a change in diagnosis or additional findings in 22% to 76% of cases. Phadke and Gupta[11] 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.[11]

ABBREVIATIONS

CGH	comparative genomic hybridization
MR	magnetic resonance
PMMR	Postmortem magnetic resonance
US	ultrasound

Dickinson et al.[12] 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.[12] Previous studies reported an overall refinement of the recurrence risk in 27% of the cases.[13]

In the study of Nayak et al.[14] where 230 fetal autopsies were conducted for spontaneous abortions, termination of pregnancy for fetal anomalies or intrauterine fetal deaths and neonatal deaths, a definitive diagnosis or documentation of anomalies was possible in 87% (200 cases). In 13% (30 cases) no anomaly was documented and the cause of death could not be established. Analysis of the impact on genetic counselling demonstrated that autopsy was valuable in 77.5% of cases by confirming the prenatal findings in 35.2% and by providing additional information in 42.2%.[14]

A recent meta-analysis covering *postmortem* examination of 3534 fetuses with congenital anomalies concluded that autopsy provided additional information in 22.5% cases and changed the final diagnosis in 3.8% cases. Postmortem findings refuted prenatal diagnosis findings in 9.2%.[15] A prospective study of over 500 stillbirths by Page et al. reported that the two most useful diagnostic investigations in establishing cause of death were placental pathology and fetal autopsy.[16]

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, as well as antenatal diagnosis.[10]

Autopsy findings are more likely to contribute additional information when the examination is completed as soon as possible after fetal demise.[3]

Even in cases where a full autopsy is not completed, external post-mortem clinical examination alone can provide additional information in about 20% of cases.[17]

Health care providers can confidently advise parents of the usefulness of autopsy in ascertaining the cause of death and for counselling them in their future pregnancies.[10] 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.[18]

Recommendation

1. Standard autopsy should ideally be an essential part of investigating second trimester fetal loss, stillbirths, and neonatal deaths associated with non-chromosomal fetal anomalies, as it has been demonstrated to add clinical information on potential etiology and recurrence risks in about 25% of cases (II-3A).

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.[19] The topic of a postmortem examination can be brought up when withdrawal of treatment and/or pregnancy termination is first considered. The most responsible health provider approaching parents for autopsy consent should discuss the options for a full, limited, or step-wise postmortem examination. The issue of retaining fetal samples, the value of autopsy, and the possibility that information gained may not benefit them directly but may benefit others should also be discussed.[20] Written information should be made 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 its alternatives to parents is as crucial as the ability to be skillful in the collection of specimens and performance of autopsy.[18] The most responsible health provider 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, with published studies available to care providers for guidance on this specific aspect.[21] 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.

European Parliament and Council guidelines advise that postmortem consent forms should include a section explicitly addressing the issue of organ retention.[22] Parental agreement to organ retention has been reported to be as high as 60%.[23] Laws specific to fetal autopsy may vary by jurisdiction.

The consent for autopsy should be recorded on an institution-approved consent form relevant to the jurisdiction.[24] Consent for molecular diagnostic or microarray (CGH) analyses from a fetal sample should also be recorded, ideally on specific consent forms. If parents decline a full autopsy, alternatives to autopsy must be presented in a manner that includes disclosure of limitations.[25]

Recommendation

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).

ALTERNATIVES WHEN A FAMILY DECLINES AN AUTOPSY

Standard autopsy provides the only process for fully investigating second trimester 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.[26] The controversies surrounding the issue of organ retention are likely to have had an impact.[25] 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.[3] A similar decrease has been reported in other countries.[25,27,28] Khong and Tanner reported a 58% acceptance for fetal autopsy in a group of 305 women following pregnancy terminations.[19]

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 of postmortem examination.[29] 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 health care provider's ability to discuss in a sensitive manner the options acceptable to the family.[28] Cultural and religious considerations pertaining to fetal and perinatal autopsies are reviewed in the literature.[21,28] Parents' decision should be respected.

When parents decline a full autopsy, they may agree to a limited or minimally invasive 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.[29]

Prior to autopsy, consent specifying the extent of the examination must be obtained and recorded, then communicated to the pathologist. 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. Examination of targeted internal organs or specific tissue sampling, as dictated by the phenotype of the fetus, can be suggested to the family.

Recommendation

3. If parents decline 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).

MRI may be offered to parents who decline an autopsy investigation,[30] although the limited availability of MRI and the need for prioritization are concerns in most countries.

Health care providers 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.[28,31] MRI also provides suboptimal resolution in assessing certain malformations such as skeletal dysplasia.[32]

A 2008 overview underlined the non-invasive nature of the MRI examination and the detection of pathologies and malformations of the central nervous system.[33] 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 missed 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.[34] A meta-analysis comparing the 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.[35] Other large studies have reported utility for abdominal anomalies[36] but more mitigated results for thoracic anomalies.[37] At this time and based on current literature, 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.[25] However, there is now public awareness of this procedure, and a new specialty in radiology seems to be emerging.[38]

A 2016 study by Vullo et al. correlated morphological data from PMMR with conventional autopsy and histological data in a small cohort of 15 antenatal demises.[39] In 8/15 fetuses, no structural anatomical anomalies were found through either PMMR or autopsy. In the remaining 7 fetuses, anomalies detected by PMMR were confirmed by autopsy. The authors demonstrate that PMMR could provide useful information before proceeding to autopsy for planning of a more suitable technique and focusing on particular organ pathology otherwise not detectable, with the aim of improving the identification of the cause of fetal death. The authors suggest that possibly, *postmortem* MR could allow a selective sampling of abnormal organs when parents do not consent to a full autopsy. This study has obvious limitations (small sample size, having been performed in a reference center) and does not take into account the high cost of MR compared with that of routine autopsy.

Recommendation

4. At this time, postmortem magnetic resonance imaging cannot function as a substitute for standard full autopsy (III-A).

Other options such as postmortem needle biopsy, laparoscopic autopsy, and small incision access are alternatives to a full postmortem examination for focused investigation of suspected anomalies, and 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.[25] When a fetal infection is suspected, it is often possible to specify the infectious agent, particularly when cultures of the placenta or of fetal tissues are initiated promptly after delivery, or through molecular techniques when available. Finally, readers are reminder that fetuses with congenital malformations known to be associated with a high underlying risk of chromosomal anomalies should have cytogenetic analyses performed. If this was not done antenatally, it can be performed on cord blood, fetal tissues, or placenta through rapid aneuploidy detection (QF-PCR) followed by chromosome microarray (CGH) if clinically indicated and where available. This last technique has the benefit, over standard karyotyping, of not requiring dividing or live cells. Fetal tissues and placenta are a good source of fetal DNA that can be banked for further studies as clinically indicated.

APPROACH TO THE FETAL OR PERINATAL AUTOPSY

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

The approach to fetal or perinatal autopsy is different from that to adult autopsy as it evaluates a developing being part of a feto-maternal unit. Fetal well-being is dependent on its environment in a broad sense, including its intra and extra uterine components.

The fetal autopsy should therefore incorporate and be based on clinical history and results of prenatal investigations (prenatal imaging, ancillaries tests), as well as external and internal macroscopic examination, microscopic description, neuropathological findings and clinico-pathological correlations.

For these reasons, the fetal 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 clinicopathologic discussion form the completed autopsy report. The autopsy protocol must document both normal and abnormal findings. As a basic rule, everything that can be observed must be described and documented with clinical photography, 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 face sheet (demographics and list of anatomic diagnosis and findings), a clinical summary, an objective description of the gross autopsy observations including those

of the placenta, 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 most responsible health provider in a timely manner. Published guidelines regarding these items are available.[27] The written report complements but cannot replace verbal communication between the pathologist and the most responsible health provider.

Recommendations

5. External physical examination, and when clinically indicated medical photographs, standard radiographic or computed tomography, should be offered in all cases of fetal anomaly(ies) of non-chromosomal etiology (II-2A).
6. The synthesis of the fetal and perinatal autopsy data should be performed by trained perinatal or pediatric pathologists (II-2A).

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 the absence of a clear diagnosis, tissue samples (liver, kidney and/or thymus, organs which are rich in nucleated cells), 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 amnion, chorionic villi or psoas muscle.

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.

Recommendations

7. In cases requiring special evaluation, the most responsible health provider should have direct communication with the feto-pathologist to ensure that all necessary sampling is performed in a timely manner (II-3A).
8. 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. If a biochemical disorder is suspected, fibroblast culture may allow future metabolic studies as well as DNA analysis, when clinically indicated (II-3A).

Details of the approach to the perinatal autopsy are included in the online Appendix.

As the fetal autopsy is performed to provide couples with information regarding their reproductive health, it is essential that the results of this clinical evaluation, when completed, be communicated in a timely manner.

Recommendation

9. The most responsible health provider must see the families in follow-up to share autopsy findings and plan for the management of future pregnancies. Consent for additional testing and genetic counselling to the couple and other family members should be performed through referral to a Medical Genetics service when clinically indicated and depending on local resources (III-A).

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. Such a protocol can facilitate the use of newer technologies to attain a diagnosis.[3,40] Frozen samples should be collected, and cells with the capacity for future culture and growth (such as fibroblasts) kept when clinically indicated. This will allow the most responsible health provider to offer, later if not at the time of fetal or neonatal death, precise diagnosis, genetic counselling, and prenatal diagnosis for future pregnancies.

Fetuses with congenital malformations known to be associated with a high risk of underlying chromosome anomaly should have chromosomal analysis performed if this was not done antenatally.[41] The key is to learn to recognize situations in which other types of tissue handling is required.[42]

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.

In remote settings where a pathologist may not be available, should anomalies only be noted at delivery in a stillborn (or in a perinatal death), it is recommended that gestational age and biometry be documented, photographs and X-rays be taken as described, and tissue sampling (either from placenta, umbilical cord or skin) be performed

(see following online Appendix). Communication with a medical genetics services (on-call service available in tertiary care centers) can facilitate these investigations and help coordinate further evaluations when clinically indicated.

CONCLUSION

The fetal or perinatal autopsy has the potential to provide information about the cause of the fetal or perinatal loss and to assist in reaching a final diagnosis, while contributing to the determination of potential recurrence risks. It allows health care providers to offer more accurate genetic counselling to the family regarding this risk or the possibility of prevention and planning of future pregnancies.

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SUPPLEMENTARY DATA

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REFERENCES

1. Sauvegrain P, Carayol M, Ego A, et al. Comment comprendre le risque élevé de mortalité infantile et périnatale dans une zone géographique ? L'exemple de la situation en Seine-Saint-Denis. Bull Epidemiol Hebd (Paris) 2015;6–7:116–22.
2. Pauli RM, Reiser CA, Lebovitz RM, et al. Wisconsin Stillbirth Service Program: I. Establishment and assessment of a community-based program for etiologic investigation of intrauterine deaths. Am J Med Genet 1994;50:116–34.
3. Corabian P, Scott NA, Lane C, et al. Guidelines for investigating stillbirths: an update of a systematic review. J Obstet Gynaecol Can 2007;29:560–7.
4. Gagnon A, Wilson RD, Allen VM, et al. Evaluation of prenatally diagnosed structural congenital anomalies. J Obstet Gynaecol Can 2009;31:875–81, 82-9.
5. Edlow AG, Hou MY, Maurer R, et al. Uterine evacuation for second-trimester fetal death and maternal morbidity. Obstet Gynecol 2011;117:307–16.
6. Lohr PA. Surgical abortion in the second trimester. Reprod Health Matters 2008;16:151–61.
7. Grimes DA, Smith MS, Witham AD. Mifepristone and misoprostol versus dilation and evacuation for midtrimester abortion: a pilot randomised controlled trial. BJOG 2004;111:148–53.
8. Laussel-Riera A, Devisme L, Manouvrier-Hanu S, et al. [Value of fetopathological examination in medical abortions: comparison of prenatal diagnosis and autopsy results of 300 fetuses]. Ann Pathol 2000;20:549–57.
9. Grover S, Garg B, Sood N, et al. Lethal congenital malformations in fetuses-antenatal ultrasound or perinatal autopsy. Fetal Pediatr Pathol 2017;36:220–31.
10. Gordijn SJ, Erwich JJ, Khong TY. Value of the perinatal autopsy: critique. Pediatr Dev Pathol 2002;5:480–8.
11. Phadke SR, Gupta A. Comparison of prenatal ultrasound findings and autopsy findings in fetuses terminated after prenatal diagnosis of malformations: an experience of a clinical genetics center. J Clin Ultrasound 2010;38:244–9.
12. Dickinson JE, Prime DK, Charles AK. The role of autopsy following pregnancy termination for fetal abnormality. Aust N Z J Obstet Gynaecol 2007;47:445–9.
13. Boyd PA, Tondi F, Hicks NR, et al. Autopsy after termination of pregnancy for fetal anomaly: retrospective cohort study. BMJ 2004;328:137.
14. Nayak SS, Shukla A, Lewis L, et al. Clinical utility of fetal autopsy and its impact on genetic counseling. Prenat Diagn 2015;35:685–91.
15. Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: a systematic review. Eur J Obstet Gynecol Reprod Biol 2017;210:201–6.
16. Page JM, Christiansen-Lindquist L, Thorsten V, et al. Diagnostic tests for evaluation of stillbirth: results from the stillbirth collaborative research network. Obstet Gynecol 2017;129:699–706.
17. Vander Wielen B, Zaleski C, Cold C, et al. Wisconsin stillbirth services program: a multifocal approach to stillbirth analysis. Am J Med Genet A 2011;155A:1073–80.
18. Putman MA. Perinatal perimortem and postmortem examination: obligations and considerations for perinatal, neonatal, and pediatric clinicians. Adv Neonatal Care 2007;7:281–8.
19. Khong TY, Tanner AR. Foetal and neonatal autopsy rates and use of tissue for research: the influence of "organ retention" controversy and new consent process. J Paediatr Child Health 2006;42:366–9.
20. Hutchins GM, Berman JJ, Moore GW, et al. Practice guidelines for autopsy pathology: autopsy reporting. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med 1999;123:1085–92.
21. Chichester M. Requesting perinatal autopsy: multicultural considerations. MCN Am J Matern Child Nurs 2007;32:81–6.
22. Samuels A. Human Tissue Act 2004: the removal and retention of human organs and tissue. Med Leg J 2004;72:148–50.
23. Wallace SV, Semeraro D, Darne FJ. Consent for fetal necropsy. Lancet 2001;357:1884.
24. Alberta Medical Association Ad Hoc Working Group on Investigation of Stillbirths. Investigation of stillborn protocol; February 10, 2009. Alberta Medical Association. Available at: http://aphp.dapasoft.com/Lists/HTMLPages/resources/stillbornprotocol/investigationProtocol.aspx. Accessed on May 2018.
25. Wright C, Lee RE. Investigating perinatal death: a review of the options when autopsy consent is refused. Arch Dis Child Fetal Neonatal Ed 2004;89:F285–8.
26. Battaglia A. Paying our last respects: the neonatal autopsy as continuing care and ethical obligation. Neoreviews 2003;4:E207.
27. Adappa R, Paranjothy S, Roberts Z, et al. Perinatal and infant autopsy. Arch Dis Child Fetal Neonatal Ed 2007;92:F49–50.
28. Burton JL, Underwood J. Clinical, educational, and epidemiological value of autopsy. Lancet 2007;369:1471–80.

29. Lyon A. Perinatal autopsy remains the "gold standard". Arch Dis Child Fetal Neonatal Ed 2004;89:F284.

30. Alderliesten ME, Peringa J, van der Hulst VP, et al. Perinatal mortality: clinical value of postmortem magnetic resonance imagning compared with autopsy in routine obstetric practice. BJOG 2003;110:378–82.

31. Huisman TA. Magnetic resonance imaging: an alternative to autopsy in neonatal death? Semin Neonatol 2004;9:347–53.

32. Thayyil S, Chitty LS, Robertson NJ, et al. Minimally invasive fetal postmortem examination using magnetic resonance imaging and computerised tomography: current evidence and practical issues. Prenat Diagn 2010;30:713–8.

33. Cohen MC, Paley MN, Griffiths PD, et al. Less invasive autopsy: benefits and limitations of the use of magnetic resonance imaging in the perinatal postmortem. Pediatr Dev Pathol 2008;11:1–9.

34. Woodward PJ, Sohaey R, Harris DP, et al. Postmortem fetal MR imaging: comparison with findings at autopsy. AJR Am J Roentgenol 1997;168:41–6.

35. Thayyil S, Chandrasekaran M, Chitty LS, et al. Diagnostic accuracy of post-mortem magnetic resonance imaging in fetuses, children and adults: a systematic review. Eur J Radiol 2010;75:e142–8.

36. Arthurs OJ, Thayyil S, Owens CM, et al. Diagnostic accuracy of post mortem MRI for abdominal abnormalities in foetuses and children. Eur J Radiol 2015;84:474–81.

37. Arthurs OJ, Thayyil S, Olsen OE, et al. Diagnostic accuracy of post-mortem MRI for thoracic abnormalities in fetuses and children. Eur Radiol 2014;24:2876–84.

38. Whitby EH, Paley MN, Cohen M, et al. Postmortem MR imaging of the fetus: an adjunct or a replacement for conventional autopsy? Semin Fetal Neonatal Med 2005;10:475–83.

39. Vullo A, Panebianco V, Cannavale G, et al. Post-mortem magnetic resonance foetal imaging: a study of morphological correlation with conventional autopsy and histopathological findings. Radiol Med 2016;121:847–56.

40. Newton D, Coffin CM, Clark EB, Lowichik A. How the pediatric autopsy yields valuable information in a vertically integrated health care system. Arch Pathol Lab Med 2004;128:1239–46.

41. Armour CM, Dougan SD, Brock JA, et al. Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. J Med Genet 2018;55:215–21.

42. Kapur R. Use of ancillary tests in perinatal autopsy. In: Gilbert-Barness E, Kapur RP, Oligny LL, Siebert JR, editors. Potter's pathology of the fetus, infant, and child. 2nd ed. Philadelphia: Mosby Elsevier; 2007.

APPENDIX. STANDARDIZED APPROACH TO THE PERINATAL AUTOPSY

Photographs

High-quality photographs are an important part of the fetal autopsy procedure. Frontal, lateral, and dorsal pictures of the whole body of the fetus next to a ruler, with a close up of the cleaned face, front- view and both side-view and 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 labeled and filed in the medical record or in a computerized archival system.

At present, the use of digital imaging for this purpose is optimal; however, issues regarding patient consent and confidentiality should be considered. Photographs can also 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.

x-Rays

Standard radiographic images of the whole body provide additional significant information and should be used in cases of fetal anomaly(ies) of non-chromosomal etiology when growth restriction and/or skeletal anomalies are suspected. Some advocate the use of radiography in every fetal or perinatal death.[1] The so-called fetogram is useful in all cases for the assessment of ossification centers and evaluation of bone age, when clinically indicated. For most cases, a single antero-posterior and lateral projections of the entire fetus are sufficient. When skeletal malformations or dysplasia are detected or suspected, a whole body radiograph is a minimum standard, as in those circumstances more detailed views of the skull, spine, extremities (full length, with separate images of hands and feet) and pelvic bones are generally needed. This should be discussed with the clinical radiologist prior to acquisition of images.

The fetogram should be performed before the internal examination is done with equipment that depends on the local resources. For most fetuses, optimal radiographic imaging could be obtained using a digitized specimen radiography system as used for assessment of surgical or biopsied breast specimens, such as Faxitron cabinet X-rays (System-Faxitron series; Faxitron Bioptics, Lincolnshire, IL).[2,3] Olsen et al.[4] report abnormal radiographs in 30% of fetograms performed in a population-based set of 542 perinatal deaths. New information about the pathological process was obtained in 8.6% of cases. Radiographs were of vital importance for establishing cause of death in 3.1% of cases. Contrast studies can also be useful for evaluation of certain malformations, particularly vascular malformations, and may help to direct subsequent dissections.

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.[5-7]

The crown–heel and crown–rump lengths should be determined to the nearest 5 mm. Normally in fetuses and young infants, occipito-frontal circumference and crown–rump lengths should not differ by more than 10 mm. Distances between inner canthi, outer canthi and eyelid slit should be obtained. Chest and abdominal circumference are measured at the level of the nipples and umbilicus respectively, as well as inter-nipple distance. Foot lengths should be obtained, as this measurement correlates well with gestational age. Other specialized measurements can be obtained such as lengths of limbs when asymmetrical.

External Examination

This examination is of particular importance when autopsy is declined. Ideally, it should be performed by experienced clinicians in the field of perinatal/pediatric pathology, clinical genetics or a pediatric pathologist**.** 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.

Inspection of the external features of the body is similar to the physical examination on newborns performed in the clinical setting (online Table). Developing and following such a routine ensures that no pertinent feature is overlooked.[8]

The extent of maceration must be documented, as it correlates somewhat with the duration of postmortem retention.[9] Photographs are indicated.

The fetal morphological examination should aim towards attaining an accurate diagnosis. Dysmorphism, deformations, disproportions, and malformations should be described. The shape of the skull and facial hair should be noted. The face must be described accurately, with attention to dysmorphism, deformations, and iatrogenic lesions. The following must be described: height and shape of forehead, size, shape and orientation of the eyes eyelids and eyebrows; nasal shape/insertion and angulation; nostrils orientation, choanal permeability; length and shape of the philtrum; size of the mouth and size/orientation of lips; palate integrity, size and position of tongue, size and shape

Online Table. Data collection sheet for postmortem medical examination when autopsy is declined (adapted from the Alberta Medical Association, 2009[13])

Structure	Normal	Abnormal	Comments
General appearance			
Skin			
Head			
Scalp			
Eyes			
Nose			
Nostrils			
Ears			
Mouth			
Mandible			
Neck			
Chest			
Abdomen			
Cord			
Genitalia			
Anus			
Spine			
Arms			
Hands			
Legs			
Feet			
Other			

Gestational age (wks)	*Birth weight (g)*	*Circumference of head (cm)*	*Crown-heel length (cm)*	*Crown-rump length (cm)*	*Foot length (cm)*
Completed by		Signature/Status		Date/time	

Adapted from: Alberta Medical Association Ad Hoc Working Group on Investigation of Stillbirths .Investigation of stillborn protocol; February 10, 2009. Alberta Medical Association.[13]

of chin; ear orientation/implantation, auditory canal permeability and presence of pre-auricular pits or branchial sinuses. Features of the neck, thorax, abdomen, umbilical cord including abdominal insertion and vessel count, anus position and permeability; genital area must be described. Back and spine should be carefully examined. 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.

Internal Examination and Routine Microscopic Sections

Several publications provide technical considerations.[8,10,11] All major organs must be weighed after careful dissection guided by the published methodology. The weight of each organ is compared with weight standards in relation with gestational age and body weight.[7,12] Organ maturity and structure can later be assessed by macroscopic 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 (such as Parvovirus, Zika virus, HIV and syphilis) rubella, cytomegalovirus, herpes simplex virus).

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. When clinically indicated, obtaining skin or fasciae biopsy for fibroblast cultures may allow future studies, particularly 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).

In the absence of a clear diagnosis, samples of fetal liver, kidney and/or thymus, organs which are rich in nucleated cells, 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 amnion, chorionic villi, or psoas muscle.

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.

Neuro-pathological examination

Central nervous system malformations or lesions represent the most frequent indication for termination of pregnancy.[14,15] Whether isolated or part of various syndromes, few of these lesions are amenable to treatment and most are associated with a poor prognosis. Brain examination is an integral part of any fetal, stillbirth or perinatal necropsy, as it is needed to confirm and detail prenatal imaging findings, as well as record potential consequences of antenatal or perinatal injuries. Brain is also the most reliable organ for assessment of fetal maturity, as cerebral development is usually preserved in most pathologies affecting fetal growth.

Removal and examination of the fetal and neonatal brain requires a different approach from that used for older children and adults.[10,11,16] In certain circumstances, techniques should be adapted.[16]

An *in situ* fixation performed shortly after the delivery may be used to facilitate the removal of the brain while preserving the integrity of its structures. Fetal brains often present with autolysis at the time of autopsy either because fetal death occurred some time before delivery, or because of delays between the time of delivery and time of autopsy. This procedure, which can be performed shortly after birth without altering the external appearance of the fetus, can allow the mother to keep her infant for longer periods with her without compromising neuropathology examination.

The procedure consists in injecting a fixative (such as formalin) into the subarachnoïd spaces to perform *in situ* fixation. The fixative is injected in the anterior and posterior fontanels, on each side of the midline, as well as in the mastoid fontanel for the posterior fossa. The needle penetrates perpendicularly to the fontanel and is next directed parallel and in close apposition to the bone to avoid injury to the cerebral tissues.[17,18]

Macroscopic examination

Biometric parameters should be recorded including brain weight, occipito-frontal length of each hemisphere, biparietal diameter, transverse cerebellar diameter and weight of brainstem and cerebellum. They are evaluated in relation to gestational age using biometry charts for fetuses.[12] The pattern of gyri and sulci should be carefully recorded as they are the most reliable indices for the evaluation of the gestational age. The brain is usually studied on sections performed on the hemispheres, in a coronal plane and, on brainstem, in an axial plane. However, sagittal sections are required for the study of midline anomalies, corpus callosum or vermian anomalies. All anomalies should be documented with clinical pictures.

Histological examination

Histological examination should always be performed on specimens from both hemispheres, brain stem and cerebellum. Standard blocks are systematically sampled, even in the absence of macroscopic lesions. Additional blocks should be obtained when such lesions are recorded.

Muscle examination

Muscle examination is mandatory in fetuses presenting with fetal akinesia sequence, multiple contractures and/or pterygia, when an disorder of fetal movement is suspected. For such cases, a comprehensive examination of skeletal muscle includes routine histology, frozen-section histochemistry, histoenzymology, and electron microscopy. In addition, muscle is rapidly frozen and stored for additional biochemical studies.[19] Quadriceps and deltoid should be sampled in all cases, but other muscles could be also sampled depending on the topography of observed muscular lesions.

Placenta

The placental examination is essential to the investigation of stillbirth, the diagnosis of ante or perinatal infection, and in cases where maternal disease plays a large role in pregnancy outcome.[19] 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.[20] 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). The placenta should be weighted trimmed of its membranes and cord.

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.[21]

Macroscopic examination is divided into portions of the placenta: umbilical cord, extra-placental 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).

Multiple Gestation, Stillborn Fetuses and Fragmented Fetuses

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.[10]

Stillborn Fetuses

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. Examination is often hampered by maceration of the body that may vary from mild to extreme. Maceration is more rapid in the presence of chorioamnionitis.[9] 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. In macerated fetuses, sampling of the amnion, of the subchorionic plate or of the cord's attachment to the placental disk generally yields good quality DNA to perform microarray (CGH) analysis if clinically indicated.[22]

Fragmented Fetuses

Few publications have assessed the value of examination of fragmented fetuses. Ernst et al. (2013)[11] reported that careful examination of surgical specimens can identify significant fetal and placental anomalies when performed within a standardized protocol. They suggested guidelines for a complete and detailed examination of surgical specimens similar to those described by Klatt (1995),[23] using a standardized checklist including radiography, pictures of fetal tissues oriented in anatomic position, and a systematic macroscopic and histological examination of fetal and placental tissues. In their experience, pathologic examination of surgical specimens with a rigorous protocol can confirm clinical diagnosis, provide a definitive diagnosis when the clinical diagnosis includes several diagnostic hypotheses, as well as identify unexpected fetal anomalies that may be useful to establish a definitive diagnosis and/or for genetic counseling.[11] The same group, in a retrospective review of 118 surgical fetal specimens, assessed the results of pathologic examination and noted that when performed by a perinatal pathologist using a standardized examination checklist, this examination identified more anomalies (77.3%) than when performed by general surgical pathologists (9.5%).[24] These results confirmed that pathologic examination of fragmented fetuses can provide valuable information when performed within a standardized protocol developed by a pediatric or perinatal pathologist, similar to the autopsy protocol used for intact fetuses.

Metabolic autopsy

When there is a strong suspicion of an underlying metabolic condition, either based on clinical findings (for example cardiomyopathy or hydrops) or on family history, prior consultation with a physician specializing in metabolic disorders is recommended. In those circumstances, autopsy should be performed ideally within 4 to 6 hours of death to promptly store tissues for subsequent diagnostic enzymatic studies, as in some instances molecular studies may not be sufficient as a stand-alone test to reach a definite diagnosis. In the context of a neonatal death, the collection of blood and urine for biochemical testing prior to death, when feasible, may be sufficient.

Samples of kidney, liver, muscle, brain and spinal cord are frozen and kept at −80°C, and others fixed in glutaraldehyde for electron microscopy.

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.[8,10] When indicated, blood, urine, bile, and liver can be submitted to the appropriate toxicology laboratory.[8]

Ancillary Tests

A frozen tissue sample (liver or 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, storage of fetal thymus, liver or placenta is optimal because of high DNA concentration. Hepatic
extracts contain a large amount of DNA, and some enzyme deficiencies may be specific to the liver. 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 approaches, currently not universally available clinically across Canada, include whole exome or genome analysis, which rely on detailed and specific description of phenotype.[25,26] Consultation with a medical geneticist is strongly recommended to assist in the selection of the most appropriate molecular investigations.

When clinically indicated, tissue culture can be obtained from fibroblast (skin, Achilles tendon, epicardium, subamniotic chorion). These dividing cells can be used for cytogenetic testing (metaphase fluorescence in situ hybridization, chromosome breakage studies) or biochemical testing.

REFERENCES

1. Wright C, Lee RE. Investigating perinatal death: a review of the options when autopsy consent is refused. Arch Dis Child Fetal Neonatal Ed 2004;89:F285–8.
2. Calder AD, Offiah AC. Foetal radiography for suspected skeletal dysplasia: technique, normal appearances, diagnostic approach. Pediatr Radiol 2015;45:536–48.
3. Gitz L, Khung-Savatovsky S, Viot G, et al. Fetopathologic examination for early termination of pregnancy: dogma or necessity? Am J Obstet Gynecol 2011;205:467, e1-9.
4. Olsen EO, Espeland A, Maartmann-Moe H, et al. Diagnostic value of radiography in cases of perinatal death: a population based study. Arch Dis Child Fetal Neonatal Ed 2003;88:F521–4.
5. Gilbert-Barness ED-SD. Embryo and fetal pathology: color atlas with ultrasound correlation. Cambridge: Cambridge University Press; 2004.
6. Guihard-Costa AM, Larroche JC, Droulle P, Narcy F. Fetal Biometry. Growth charts for practical use in fetopathology and antenatal ultrasonography. Introduction. Fetal Diagn Ther 1995;10:211–78.
7. Maroun LL, Graem N. Autopsy standards of body parameters and fresh organ weights in nonmacerated and macerated human fetuses. Pediatr Dev Pathol 2005;8:204–17.
8. Siebert J. Perinatal, fetal and embryonic autopsy. Perinatal, fetal and embryonic autopsy. In: Gilbert-Barness E, Kapur RP, Oligny LL, Siebert JR, editors. Potter's pathology of the fetus, infant, and child. 2nd ed. Philadelphia: Mosby Elsevier; 2007.
9. Genest DR, Singer DB. Estimating the time of death in stillborn fetuses: III. External fetal examination; a study of 86 stillborns. Obstet Gynecol 1992;80:593–600.
10. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med 1997;121:368–76.
11. Ernst LM. A pathologists perspective on the perinatal autopsy. Semin Perinatol 2015;39:55–63.
12. Guihard-Costa AM, Menez F, Delezoide AL. Organ weights in human fetuses after formalin fixation: standards by gestational age and body weight. Pediatr Dev Pathol 2002;5:559–78.
13. Alberta Medical Association Ad Hoc Working Group on Investigation of Stillbirths. Investigation of stillborn protocol; February 10, 2009. Alberta Medical Association. Available at: http://aphp.dapasoft.com/Lists/HTMLPages/resources/stillbornprotocol/investigationProtocol.aspx. Accessed on May 2018.
14. Grover S, Garg B, Sood N, et al. Lethal congenital malformations in fetuses-antenatal ultrasound or perinatal autopsy. Fetal Pediatr Pathol 2017;36:220–31.
15. Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: a systematic review. Eur J Obstet Gynecol Reprod Biol 2017;210:201–6.
16. Keeling JW, Khong TY. Neonatal autopsy. Semin Neonatol 2004;9:245.
17. Pomara C, Karch SB, Fineschi V. Forensic autopsy: a handbook and atlas. CRC Press; 2010. ISBN 9781439800645.
18. Rorke LB, Riggs HE. Myelination of the brain in the newborn. J.B. Lippincott Company; 1969.
19. Cox PM, Brueton LA, Bjelogrlic P, et al. Diversity of neuromuscular pathology in lethal multiple pterygium syndrome. Pediatr Dev Pathol 2003;6:59–68.
20. Boyd TR. Pathology of the placenta. In: Gilbert-Barness E, Kapur RP, Oligny LL, Siebert JR, editors. Potter's pathology of the fetus, infant, and child. 2nd ed. Philadelphia: Mosby Elsevier; 2007.
21. Kapur R. Use of ancillary tests in perinatal autopsy. In: Gilbert-Barness E, Kapur RP, Oligny LL, Siebert JR, editors. Potter's pathology of the fetus, infant, and child. 2nd ed. Philadelphia: Mosby Elsevier; 2007.
22. Armour CM, Dougan SD, Brock JA, et al. Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. J Med Genet 2018;55:215–21.
23. Klatt EC. Pathologic examination of fetal specimens from dilation and evacuation procedures. Am J Clin Pathol 1995;103:415–8.
24. Gawron LM, Hammond C, Ernst LM. Perinatal pathologic examination of nonintact, second-trimester fetal demise specimens: the value of standardization. Arch Pathol Lab Med 2013;137:1083–7.
25. Filges I, Friedman JM. Exome sequencing for gene discovery in lethal fetal disorders–harnessing the value of extreme phenotypes. Prenat Diagn 2015;35:1005–9.
26. Fletcher E, Porteous M, McKenzie KJ, et al. Fetal dysmorphology-still an essential art. Analysis of the limitations of microarray in a fetal population and a look toward the genome sequencing era. Pediatr Dev Pathol 2017;20:288–97.