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REVIEW

Parameters for estimating the time of death at perinatal autopsy of stillborn fetuses: a systematic review

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Abstract

Background Stillbirth is defined by the WHO as birth of a fetus with no vital signs, at or over 28 weeks of pregnancy age. The estimation of time of death in stillbirth appears crucial in forensic pathology. However, there are no validated methods for this purpose.

Objective To perform a systematic review of the available literature regarding the estimation of the time of death in stillborn fetuses, in terms of hours or days.

Methods Electronic databases were searched from their inception to August 2018 for relevant articles. Macroscopic, histologic, and radiologic parameters were evaluated.

Results Nine studies with 664 stillborns were included. The evaluation of extent and location of fetal maceration signs showed good accuracy in estimating the time of death; by contrast, a dichotomous assessment of maceration (present vs absent) was found to be unreliable in a subsequent study. Histologic assessment of the loss of nuclear basophilia in fetal and placental tissues showed excellent accuracy; an "autolysis equation" was proposed to achieve an even higher accuracy in fetuses who had been dead for < 24 h. Magnetic resonance imaging of the lung parenchyma, pleural fluids, and brain parenchyma could estimate the death-to-autopsy time, but the results appeared weak and conflicting.

Conclusion Pathologic examination, based on the assessment of maceration, and even more of the loss of nuclear basophilia, may be a reliable method to estimate the time of death in stillborn fetuses. Further studies should be encouraged to validate these results. Imaging techniques have not yet found application in this field.

Keywords Stillbirth · Intrauterine death · Fetal death · Autopsy · Maceration · Perinatal pathology

Introduction

In 1950, the World Health Organization (WHO) defined fetal death as "death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy; the death is indicated by the fact that after such separation the fetus does not breathe or show any other evidence of life, such as beating of the heart, pulsation of the umbilical cord or definite movement of voluntary muscles" [1]. Miscarriage (spontaneous abortion) and stillbirth are two general terms describing the death of the fetus, but they refer to losses that occur at different times during pregnancy. There is no universally accepted definition when a fetal death is called a stillbirth vs spontaneous abortion; the reporting policies in the different countries and within the states of the same country are not uniformly followed, and

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there are also differences in terms of how the gestational age is assessed and interpreted [2]. In fact, the WHO defines stillbirth as the birth of a fetus with no vital signs, at or over 28 weeks of pregnancy age [3].

In forensic pathology, the diagnosis and estimation of the time of death in stillbirth is crucial. In the case of medical malpractice claims, it is essential to estimate precisely the time of fetal death, in order to assess a potential physicians' answerability more correctly and objectively.

Clinical data and ultrasonographic checking can certainly be helpful for this purpose; however, the time window between the last evidence of fetal life and the first evidence of fetal death may be too wide to allow a reliable estimation. The absence of fetal movements reported by the mother cannot be considered as a reliable parameter, because of the absence of the essential requirements of objectivity and scientific validity.

The forensic and obstetrical literature has reported different criteria used to establish the time of death in stillbirth, according to various parameters involved in the investigation, such as macroscopic, histologic, and radiologic findings.

However, there is still uncertainty in this field, and there are no validated methods to estimate the time of death in stillborn fetuses.

The current study stands out from the necessity to assess the actual validity of the criteria proposed to estimate the time of fetal death, through a systematic review. In particular, we focused on a refined prediction of the time of death in terms of hours or days, rather than the estimation of the gestational age in weeks.

Materials and methods

Methods for search strategy, study selection, risk of bias assessment, and extraction of data were designed a priori. All review stages were performed independently by two authors (AT and MPe). Disagreements were resolved by discussion with a third author (MPa). This study was reported according to the PRISMA statement [4].

Search strategy and study selection

MEDLINE, Scopus, Web of Science, and Google Scholar were searched for relevant articles from the inception of each database to August 2018 by using a combination of the following text words: intrauterine death, fetal death, stillbirth, stillborn, fetal age, macerated, maceration, autopsy, forensic, histology, gestational age, and histologic examination. References of the included studies were also reviewed.

We included all studies assessing macroscopic, histologic, and radiologic parameters to estimate the time of death in stillborn fetuses. Exclusion criteria were studies on animal samples, case reports, and estimation of the gestational age (in weeks) at the time of death.

Risk of bias assessment

The risk of bias among studies was assessed based on the QUADAS-2 [5]. The risk of bias was evaluated for four domains: (1) sample selection (i.e., if the fetuses were included consecutively without inappropriate exclusions); (2) index test (i.e., if the parameters were assessed blinded to the actual time of death); (3) reference standard (i.e., if the time of death was precisely known); and (4) flow and timing (i.e., if the latency time between intrauterine death and assessment of the parameters did not affect the results). Authors' judgements were categorized as "low," "unclear," or "high" risk of bias.

Data extraction

Data from the included studies were extracted without modifications. The data extracted were country, period of enrollment, study design, criteria for sample selection, sample size, gestational age, intrauterine retention time, delivery-to-autopsy time, criteria for index test, and reference standard. The parameters assessed to estimate the time of death were subdivided into macroscopic, histologic, and radiologic parameters.

Results

Selection and characteristics of the studies

Nine studies assessing 664 stillborn fetuses were included. The whole process of study selection is reported in Fig. 1.

Two studies evaluated macroscopic appearance of the fetuses; two studies evaluated histologic features of fetal tissues and one study evaluated histology of placental tissue; and four studies evaluated magnetic resonance imaging (MRI) findings.

The time of fetal death was retrieved from ultrasonographic reports, based on the last evidence of fetal heartbeat and the first evidence of fetal death.

Characteristics of the included studies are reported in Table 1.

Risk of bias assessment

For the "sample selection" domain, low risk of bias was assigned to all studies, as no particular sources of bias were pointed out.

For the "index test" domain, low risk of bias was assigned to six studies that reported blinding; in two studies, the risk of bias was unclear because they did not report if the parameters were assessed blinded to the actual time of death; in the

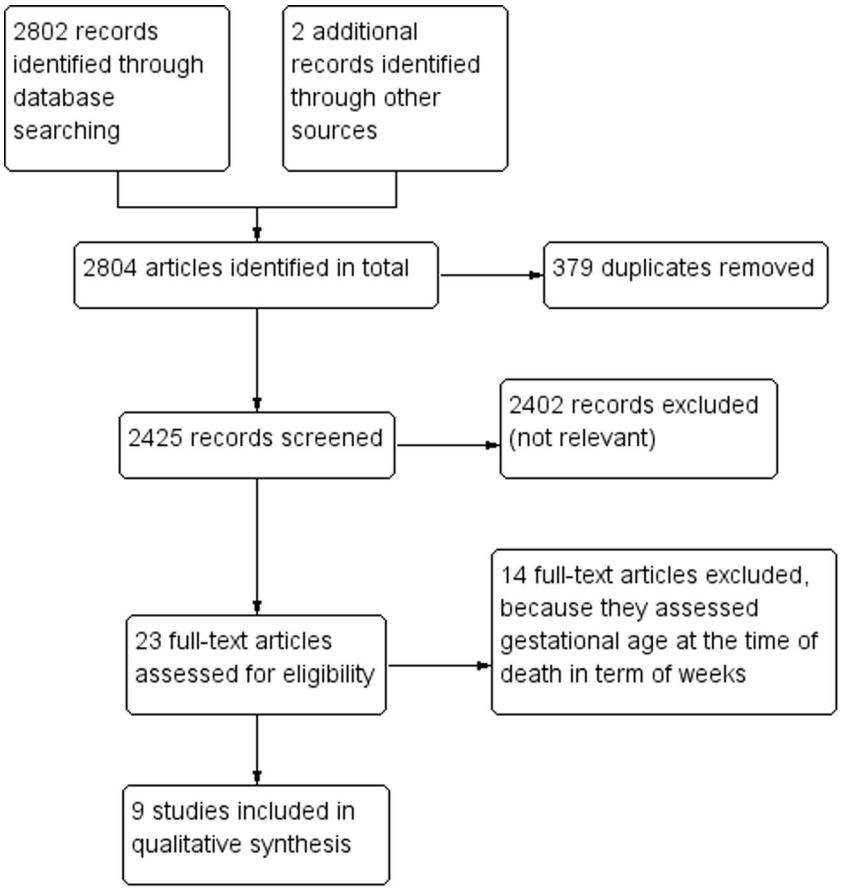

Fig. 1 Flow diagram of studies identified in the systematic review (Prisma template [Preferred Reporting Item for Systematic Reviews and Meta-analyses])

remaining study, the risk of bias was high because no detailed criteria for the index test were provided.

For the "reference standard" domain, all studies were considered at unclear risk of bias, because the interval between the last evidence of fetal life and the first evidence of fetal death was not specified or was too variable among fetuses.

For the "flow and timing" domain, low risk of bias was assigned to eight studies: three studies by Genest et al., as they included only fetuses with a delivery-to-autopsy time < 7 days and assessed whether a time > 24 h affected results; the study by Kim, as delivery-to-autopsy time was < 24 h for all fetuses; and the studies by Arthur et al., Barber et al., Papadopoulou et al., and Shelmerdine et al., as delivery-to-autopsy time was > 24 h for all fetuses. Unclear risk was assigned to one study, because the delivery-to-autopsy time was not specified (Fig. 2).

Table 1 Characteristics of the included studies

Study	Country	Period of enrollment	Study design	Sample size	Gestational age (weeks)	Intrauterine retention time	Delivery-to-autopsy time
Genest I [6]	USA	1980–1991	Retrospective	150	10–43	0.25 h–203 days	≤ 7 days
Genest II [7]	USA	1980–1991	Retrospective	71	11–43	Unclear	≤ 7 days
Genest III [8]	USA	1980–1991	Retrospective	86	18–41	0.3–3528 h	≤ 7 days
Kim [9]	Korea	1991–2001	Retrospective	30	22–40	0.3–240 h	≤ 24 h
Gold [10]	Ghana	2011–2012	Prospective	337	> 28	0–275 h	Unclear
Arthurs [11]	UK	2013	Prospective	15	23–50	0.5–2 days	2–21 days
Barber [12]	UK	2012–2014	Retrospective	23	24–48	0 h	5–23 days
Papadopoulou [13]	UK	2012–2014	Prospective	43	21–41	0–14 days	4–23 days
Shelmerdine [14]	UK	2013–2016	Prospective	66	18–41	Unclear	1–18 days
Total	–	1980–2016	–	664	10–50	0 h–203 days	1–23 days

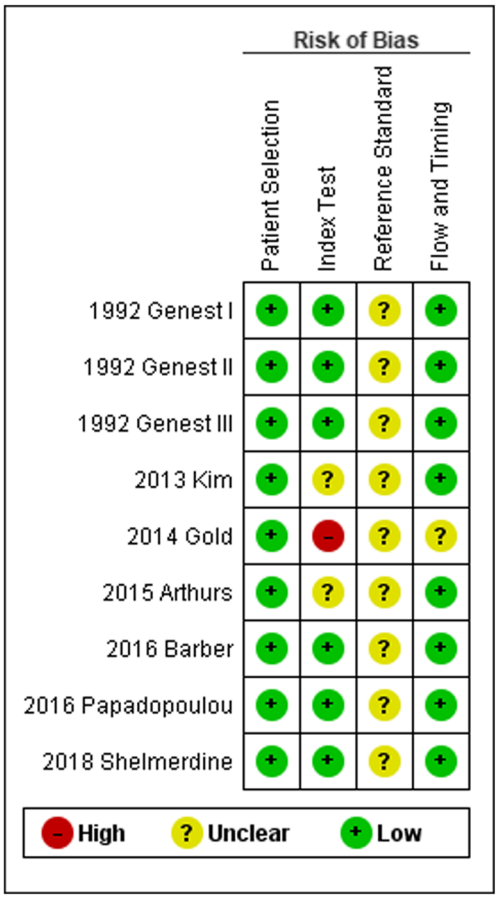

Fig. 2 Assessment of risk of bias. Summary of the risk of bias for each study. Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias

Macroscopic factors

In 1992, Genest et al. [15] assessed several external macroscopic characteristics (Table 2) of 60 stillborns > 18 weeks of gestation. Then, they tested the accuracy of such parameters on further 26 stillborns. They were able to correctly estimate the time of death in 85% of cases. Gross features with high accuracy are reported in Table 3.

In 2014, Gold et al. [8] used maceration as a parameter to estimate the time of death in 141 stillborns with a gestational age > 28 weeks. They considered that maceration—regardless of extent and location—indicated a death-to-delivery interval > 8 h. They correctly estimated the time of death in 22% of cases, concluding that maceration is not a reliable parameter.

Histologic factors

Genest et al. [10] evaluated five histologic patterns (Table 2) in organs of 100 stillborns of any gestational age. Each feature was assessed for association with each of the intrauterine retention time intervals defined a priori (Table 2). Based on the associations found, they tested the accuracy of these patterns on further 50 stillbirths. They showed that, in several organs, the loss of nuclear basophilia was a good predictor of the time of death. In the evaluation of confounding factors, they found that histologic changes were decelerated in fetuses < 25 weeks, ad accelerated in case of hydrops fetalis, gestational age > 35 weeks, birth-to-autopsy time > 24 h. Histologic features with high accuracy are reported in Table 3.

In the same year, Genest et al. [6] also evaluated placental histology in 51 stillbirths. Fifteen parameters (Table 2) were

Table 2 Macroscopic and histologic factors evaluated in the studies by Genest et al., with the intrauterine retention time intervals defined a priori [6, 10, 15]

Macroscopic factors
Skin color: normal/pink, partially red, totally red, at least partially brown, at least partially tan
Cord color: normal, brown, brown red
Mouth: closed, partially open, widely open
Lip color: normal, abnormal (red/brown)
Eyelid color: normal, abnormal (dark red)
Cranium: not collapsed, partially collapsed, severely collapsed
Desquamation
- Extent: none, slight degree; moderate degree; severe degree
- Surface: < 5%, 5–10%, 10–25%, 25–50%, 50–75%, > 75%
- Diameters of largest area of exposed dermis (cm)
- Location: scalp, face, neck, chest, abdomen, back, arm, hand, leg, foot, scrotum
Mummification: none, regional, diffuse
Histologic factors—fetal tissues
Loss of nuclear basophilic staining in at least 1% of the cells in specific regions of organs
Loss of nuclear basophilia in an entire organ
Loss of basophilic staining in the tracheal/bronchial cartilage matrix
Nuclear karyorrhexis in thymic cortical lymphocytes
Mucosal epithelial detachment in the bronchi, gastrointestinal tract, or uterus
Histologic factors—placental tissues
Cord: vasculitis, loss of nuclear basophilia of stromal cells, loss of nuclear basophilia of vascular smooth-muscle cells
Chorionic plate: acute chorioamnionitis, meconium-laden macrophages in chorion
Stem villi: vascular luminal abnormalities
Terminal villi
- Stroma: infarction, edema, extensive fibrosis, microcalcification
- Vessels: nucleated red blood cells, intravascular karyorrhexis
- Trophoblast: increased syncytial knots, basement membrane thickening, increased cytotrophoblastic cells
Retention time intervals
Less than 24 h: < 2 h, ≥ 2 h, ≥ 4 h, ≥ 6 h, ≥ 8 h, ≥ 12 h, ≥ 18 h
More than 24 h: ≥ 24 h, ≥ 36 h, ≥ 48 h, ≥ 72 h, ≥ 96 h
Weeks: ≥ 1 week, ≥ 2 weeks, ≥ 3 weeks, ≥ 4 weeks, ≥ 8 weeks

Table 3 Macroscopic and histologic features that showed high accuracy in predicting the time of death in stillbirths in the studies by Genest et al. [6, 10, 15]

Macroscopic factor	Retention time
Desquamation ≥ 1 cm	≥ 6 h
Desquamation of the face, back, or abdomen	≥ 12 h
Desquamation ≥ 5% of the body	≥ 18 h
Desquamation 2 or more of 11 zones	≥ 18 h
Mummification	≥ 2 weeks
Histologic factor—fetal tissues	**Retention time**
Kidney: loss of tubular nuclear basophilia	≥ 4 h
Liver: loss of hepatocyte nuclear basophilia	≥ 24 h
Myocardium: inner half loss of nuclear basophilia	≥ 24 h
Myocardium: outer half loss of nuclear basophilia	≥ 48 h
Bronchus: loss of epithelial nuclear basophilia	≥ 96 h
Liver: maximal loss of nuclear basophilia	≥ 96 h
Gastrointestinal tract: maximal loss of nuclear basophilia	≥ 1 week
Adrenal: maximal loss of nuclear basophilia	≥ 1 week
Trachea: chondrocyte loss of nuclear basophilia	≥ 1 week
Kidney: maximal loss of nuclear basophilia	≥ 4 weeks
Histologic factor—placental tissues	**Retention time**
Intravascular karyorrhexis	≥ 6 h
Stem vessel luminal abnormalities: multifocal	≥ 48 h
Stem vessel luminal abnormalities: extensive	≥ 14 days
Extensive villous fibrosis	≥ 14 days

assessed for association with the abovementioned retention times and were subsequently tested on further 20 stillborns. Good predictors are shown in Table 3.

In 2013, Kim [7] assessed the changes in nuclear basophilia in rat's fetuses immersed in saline solution for a defined time. Then, he evaluated such parameters in 30 stillborns > 22 weeks. Compared to Genest's study, Kim made a more elaborate evaluation of fetal tissue by assessing separately different regions of organs. He created an "autolysis equation" to reduce subjectivity. Such equation appeared to be reliable for an intrauterine retention time < 24. The author found that fetal hydrops, local effusion, and sepsis accelerated autolysis, creating a bias in the estimation of the time of death; he reported that gestational age was not a confounding factor instead.

Radiologic factors

Arthurs et al. [9] performed MRI on 15 stillborns. They analyzed the apparent diffusion coefficient (ADC) related to several organs, comparing it with ADC values from 44 live infants < 6 months. They found a significant, non-linear correlation between mean ADC values in the lung parenchyma and post-mortem interval, but not for gestational age, intrauterine retention interval, or maceration grade. There were no correlations between ADC values of any abdominal organ and any of these parameters.

Barber et al. [11] performed thoracic MRI on 12 stillbirths and 11 childhood deaths (1 day–4 years), assessing the correlation between thoracic fluids and post-mortem interval. They subjectively assessed pleural fluid volume, pericardial volume, or ascites volume, finding no correlation with post-mortem interval. When the fluid accumulation was assessed objectively, a significant correlation was found with post-mortem interval in pediatric cases, but not in perinatal cases.

Papadopoulou et al. [14] carried out post-mortem brain MRI on 43 stillborns. They evaluated perinatal brain ADC values at post-mortem, assessing its correlation with maceration, gestational age, and post-mortem interval. They showed that maceration was the strongest predictor of ADC values in all anatomical areas of the brain. Gestational age affected the ADC of the thalamus, and post-mortem interval only affected the basal ganglia.

Shelmerdine et al. [12] performed MRI on 14 neonatal deaths and 66 stillbirths. They evaluated ADC values related to several organs, confirming the significant association with the degree of maceration, but not with post-mortem interval.

Discussion

We found that macroscopic evaluation of maceration was useful in the estimation of the time of death in stillbirths if its extent and location were thoroughly quantified. Histologic assessment of nuclear basophilia appeared as the most reliable

parameter. On the other hand, imaging techniques such as MRI have not yet a definite utility in this field.

The estimation of the time of death in stillbirths may be crucial in forensic field. Stillbirth is a devastating event for the parents and the wider family, and medical malpractice claims are common in this regard. If the physician and/or medical staff performed an action that led to a stillbirth, or failed to perform medical intervention that could have prevented a fetal death, they may be liable for damage. The American College of Obstetricians and Gynecologists (ACOG) found in their 2006 survey that the primary clinical issue in an obstetric claim was a neurologically impaired baby (34.3%) followed by stillbirth or neonatal death (15.3%). Hospital-based treatments such as fetal monitoring (47.7% for a neurologically impaired baby; 21.9% for a stillbirth or neonatal death) and oxytocin-impaired administration (43.3% for a neurologically impaired baby; 14.7% for a stillbirth or neonatal death) were significant factors in both types of claims [13].

In medico-legal settings, when the time of death is unknown, or even when the time of intrauterine demise in stillbirth is unclear, several problems may arise in legal process. In particular, it is necessary to evaluate whether the time of death matches correctly with the time of the alleged malpractice case. In the case of stillbirth, it may be important to know if fetal death occurred before, during, or after admission, to assess a potential physicians' answerability more correctly and objectively. Unfortunately, there are no validated criteria to estimate the time of death in stillbirth fetuses.

Genest et al. were the first authors to systematically address the problem of the estimation of the time of death in stillbirths. They assessed a quite large sample (150 fetuses) in relation to both macroscopic and histologic factors. They performed a thorough analysis of the fetus appearance in 86 stillbirth cases assessing separately quality, extent, and location of the macroscopic alterations, finding an accuracy of 85% in estimating the time of death. Histologic examination of fetal (on 150 stillborns) and placental (on 71) tissues was thorough as well. Each histologic feature was categorized as "good," "moderate," or "poor" predictor based on sensitivity, specificity, and predictive values. Good predictors showed all values > 75%. The accuracy of all parameters was blindly assessed on a "test set" of stillborns, demonstrating good intraobserver reproducibility. They also considered and evaluated possible confounding factors. Based on a good methodology, Genest et al. provided a complete and accurate overview of macroscopic and microscopic parameters that may help to estimate the time of death in stillbirth cases.

Gold et al. criticized the use of maceration status to estimate the time of death. They reported an accuracy of 22% for such method, compared to the 85% reported by Genest. However, the study by Gold appears to be affected by important limitations. Although the sample was larger than Genest's (141 vs 86), the evaluation of maceration was definitely rougher. In fact, Gold et al. only assessed whether or not any sign of maceration was present, regardless of the extent and location. Moreover, blinding to the reference standard was not reported, and the cut-off considered (8 h) was arbitrary.

Kim pointed out some limitations in Genest's method to evaluate fetal histology. He pointed out three possible concerns with Genest's criteria: the arbitrariness of the time windows considered, the presence of pathologic conditions that influence autolysis process, and inter/intraobserver variability in the assessment of histologic parameters. The author tried to overcome them by using an even more elaborate and objective methodology. Kim evaluated histologic changes in rat's fetuses after immersion in saline solution for a defined time, subdividing histologic alterations based on their position across the organ. He also elaborated an "autolysis equation" to reduce subjectivity. This equation appeared to be reliable only when the fetus had been dead for < 24 h. However, the accuracy of this equation was not blindly evaluated on a "test set," and the reference standard used to define the time of death was not reported. Moreover, the reproducibility did not appear to be improved if compared to Genest's method, as the pathologist should subjectively assess the values to be included in the analysis.

Regarding imaging techniques, four studies from a British group involved performing MRI on stillborn fetuses. One of these studies (Arthurs et al.) showed a correlation between ADC values in the lung parenchyma and post-mortem interval. Despite appearing interesting, this result was limited by the high interindividual variability for organ ADC values. This would invalidate the significance of ADC in the medico-legal practice.

The second study (Barber et al.) found a significant correlation between the pleural fluid volume and post-mortem interval in pediatric death cases, but not in perinatal cases. Authors hypothesized that such a difference might be due to meconium inhalation in fetal cases, which would have reduced fluid accumulation by osmotic action.

The third study (Papadopoulou et al.) showed a strong association between maceration and ADC values in all anatomical areas of the brain. On the other hand, ADC values of the thalamus were associated with gestational age, while values in the basal ganglia were only weakly associated with post-mortem interval.

The other study (Shelmerdine et al.) re-assessed ADC values of several organs on a larger sample, not confirming the previously found correlation with post-mortem interval. Instead, they showed that ADC values were affected by maceration, in accordance with Papadopoulou's study.

Contrary to macroscopic and histologic features, studies assessing MRI found significant correlation for post-mortem interval, but not for intrauterine retention time. This same conclusion was found by Keller et al. [16], who assessed ADC values of the hepatic parenchyma at multiple time points after death in adult population sample.

Furthermore, authors were not able to evaluate the accuracy of MRI parameters in terms of hours (all fetuses had been dead for at least 1 day). To date, MRI and other imaging techniques have still not a role in this field. Further studies are necessary to assess the possible relevance of imaging techniques in this field.

Given these observations, the studies by Genest et al. appear as the most reliable to help the pathologist in the estimation of the time of death in stillborn fetuses. However, these studies still have some limitations. In fact, the reliability of the reference standard was not the same in all cases, as the interval between the last confirmation of fetal cardiac activity and the confirmation of death ranged from 0 to 1008 h. Although all fetuses were stored at 5 °C, the delivery-to-autopsy time was not taken into account; this might affect pathologic examination, as the autolysis process is not completely stopped by the low temperature. Anyway, all autopsies were performed within 1 week from the delivery, and the authors limited the risk of bias by assessing whether a post-mortem interval > 24 h affected the results. Furthermore, the assessment of maceration was performed retrospectively, based on photographs. Finally, since only one pathologist performed all evaluations, the interobserver reproducibility should not be assessed. Further studies would be necessary to validate Genest's approach in the estimation of the time of death in stillbirth cases.

Conclusion

Following an accurate methodology as the one proposed by Genest et al., macroscopic evaluation of the extent and location of fetal maceration may allow a reliable estimation of the time of death in stillbirths. An even more accurate estimation may be achieved by quantifying the loss of nuclear basophilia on histologic examination of fetal and placental tissues. Subsequent data contrasting with Genest's results appeared to be based on low-quality evidence. In the absence of further evidence, the use of Genest's criteria would be advisable in this field. Further studies should be encouraged to confirm the accuracy of these parameters and to validate their use.

Until now, there has been no application for imaging techniques in this field.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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