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Estimating the Time of Death in Stillborn Fetuses: I. Histologic Evaluation of Fetal Organs; an Autopsy Study of 150 Stillborns

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***Objective*: To determine whether the autopsy histology of fetal tissues can determine the time of death of stillborn fetuses.**

***Methods*: Hematoxylin and eosin slides from autopsies of 150 stillborn fetuses with well-timed deaths were evaluated retrospectively. Fetuses were divided into a learning set (100 fetuses) and a test set (50 fetuses).**

***Results*: From assessment of the 100 fetuses in the learning set, 23 histologic features were identified with possible temporal associations with fetal death. When those histologic features were randomly and blindly assessed in the 50 test fetuses, ten features performed well as diagnostic tests (sensitivity, specificity, and positive predictive values at or above 0.875), correctly classifying 43 of 50 fetuses (86%) with respect to the time of death. The ten histologic features and their predicted death-to-delivery intervals were: loss of nuclear basophilia in individual cells in renal cortical tubules (4 hours), liver (24 hours), inner half of the myocardium (24 hours), outer half of the myocardium (48 hours), bronchial epithelium (96 hours), and tracheal cartilage (1 week); and loss of nuclear basophilia of all cells in the liver (96 hours), gastrointestinal tract (1 week), adrenal (1 week), and kidney (4 weeks). The development of these histologic changes appeared to be accelerated by fetal hydrops and a delivery-to-autopsy interval exceeding 24 hours and decelerated by fetal gestational age under 25 weeks.**

Conclusion*: Histologic changes identifiable in hematoxylin and eosin-stained fetal tissue may be useful for estimating the time of death in many stillborn fetuses. *(Obstet Gynecol 1992;80:575–84)

Stillbirth, defined as delivery of a fetus who has died in utero after 20 weeks' gestation, is a relatively common occurrence, accounting for approximately 50% of all perinatal deaths.[1–5] For many stillbirths, the etiology and time of fetal death are unknown. Fetal deaths result from diverse causes; in carefully studied cases, maternal diseases, particularly preeclampsia and diabetes, are present in 40% and placental disorders, especially abruptio placentae and placental underperfusion, in 25%. Other common factors associated with stillbirth include fetal congenital anomalies (10%) and complications of multiple gestation (5%). Several etio-

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logic factors may coexist in individual cases. Approximately 10% of stillbirths are classified as "idiopathic."[6] Studies evaluating the timing of fetal death in stillborns have estimated that 30% occur during labor and 70% before labor.[2,7] In most individual cases, however, the exact timing of fetal death is not known. Because most deaths occur at unknown times under uncertain circumstances, it would be helpful to develop retrospective postmortem techniques to determine accurately the time of fetal death.

During the interval from fetal death until delivery, the retained fetus undergoes maceration, a progressive deterioration of external and internal gross features. Maceration is characterized by softening and peeling of the skin, discoloration and softening of viscera, and fluid accumulation in body cavities.[8,9] The changes of maceration are nonputrefactive and result from fetal immersion in amniotic fluid and digestion of fetal tissues by autolytic enzymes.[10] Investigators have suggested that the extent of maceration in stillborn fetuses (determined from the gross examination) may be a rough indicator of the time interval from fetal death until delivery.[8,9] However, the ability of such gross changes to identify precisely the time of fetal death has not been evaluated systematically. Based on clinical experience, many perinatal pathologists have suggested that such gross changes are not reliable for accurately estimating the time of fetal death.[11,12]

In addition to the gross changes of maceration, the tissues of the retained fetus undergo characteristic histologic alterations (autolysis). In hematoxylin and eosin-stained tissue, these histologic changes consist of gradual loss of nuclear basophilic staining, progressive cytoplasmic eosinophilia, and eventual loss of most cellular detail.[8,13] Two reports[13,14] have suggested that these progressive histologic changes might provide an accurate method for determining the time of fetal death. Although these early studies were promising, the results were inconclusive because precise clinical confirmation of the timing of fetal death was not available for all cases evaluated. Furthermore, these studies relied primarily upon periodic auscultation of the fetal heart for determination of the time of death, a technique with inherent limitations (particularly in evaluating multiple gestations and cases with maternal tachycardia or fetal bradycardia). Over the past few decades, new fetal surveillance and therapeutic techniques have permitted greater accuracy in the timing of fetal deaths: fetal cardiac monitoring in labor, antenatal fetal heart rate testing, fetal ultrasonography, selective termination, and close fetal surveillance after obstetric procedures (ie, fetal intravascular transfusion and external breech version). Because these technical advances now permit more accurate timing of fetal death, we conducted a retrospective study of autopsies performed on stillborn fetuses with accurately timed deaths. Specifically, we sought to determine whether histologic characteristics could be used to predict the timing of fetal death.

Materials and Methods

All perinatal autopsies at the Brigham and Women's Hospital from January 1980 to July 1991 were reviewed to identify stillborn fetuses evaluated pathologically. The mothers' medical records were reviewed to identify fetuses with accurately timed deaths (ie, cases in which clinical confirmation that the fetus was alive was followed shortly by clinical confirmation that the fetus had died). For single gestations, clinical confirmation of fetal status included either fetal heart monitoring by Doppler or ultrasonography. For multiple gestations, only ultrasonographic fetal evaluation was considered acceptable. Maternal recall of fetal movement was not used to assess fetal status. Because a prolonged birth-to-autopsy interval may influence histology, cases with a birth-to-autopsy interval exceeding 7 days were excluded from the study. We identified a total of 150 stillborn fetuses with accurately timed deaths and randomly divided these into a "learning set" (100 cases) and a "test set" (50 cases).

The "time" of each fetal death was the interval, determined clinically, during which death was known to have occurred. This interval was bounded by two points, the last time (hours before birth) that the fetus was confirmed to be alive and the first time (hours before birth) that the fetus was verified to be dead. In certain circumstances (ie, selective termination in early gestation), these two times were identical (11% of all cases); in the majority of cases (89%), they differed. Among the 150 stillborn fetuses, the mean interval from confirmation of cardiac activity until documentation of death was 69.8 hours (range 0–1008); the median interval was 7 hours. Among those who died within 4 hours of birth, the mean interval was 1.4 hours (range 0.1–3). In fetuses who died 4–24 hours before birth, the mean interval was 5.9 hours (range 0.1–16), and for those who died 24–48 hours before birth, the mean interval was 14.4 hours (range 4–24). Among fetuses who died between 48 hours and 7 days before birth, the mean interval was 59 hours (range 12–120), and among those who died more than 7 days before birth, the mean interval was 198.8 hours (range 0–1008).

All fetal autopsies were performed according to a perinatal autopsy protocol.[7,15] Fetuses were kept re-

frigerated at 5C until autopsy, at which time fetal tissues were removed and fixed in 10% buffered formalin solution before processing and staining. Tissue blocks were deparaffinized and stained with hematoxylin and eosin according to a method modified from Preece,[16] using Harris' hematoxylin and eosin-phloxine stains.[17]

The following obstetric data were abstracted from the maternal medical records: expected delivery date (based on last menses and first examination and confirmed by ultrasound in 94% of the cases), gestational age at time of death, intrauterine retention time (death-to-delivery interval), delivery-to-autopsy interval, pregnancy complications, and obstetric procedures (breech version, fetal transfusion, selective termination). From medical records and autopsy reports, we obtained the following fetal information: sex, weight, crown-rump length, congenital anomalies, evidence of fetal infection (positive autopsy blood/lung culture or acute chorioamnionitis), hydrops fetalis, and evidence of fetal hypoxia or severe stress. Fetal hypoxia or severe stress was defined as any of the following clinical or autopsy findings: acute thymic involution,[18] acute adrenal involution,[19] petechial/interstitial hemorrhage,[20] intraventricular hemorrhage, hepatic subcapsular hematoma,[20] retroplacental hematoma, placental infarct, prolapsed cord, or fetal growth retardation.

All hematoxylin and eosin slides from each fetal autopsy were assessed retrospectively by a perinatal pathologist (DRG). To develop a set of proposed histologic criteria for timing fetal death, we evaluated 100 learning cases unblinded, in a temporal sequence beginning with cases with the shortest death-to-delivery intervals. Histologic slides of all organs from each fetus were evaluated collectively to identify sequential autolytic patterns involving multiple tissues. Forty-five histologic features involving 15 organs were assessed (Table 1). These may be summarized in five general patterns: 1) loss of nuclear basophilic (hematoxylin) staining, involving at least 1% of cells, in specific regions of organs; 2) maximal loss of nuclear basophilic staining throughout an entire organ (ie, involving 100% of cells); 3) loss of cartilage matrix basophilic staining in tracheal/bronchial cartilage; 4) nuclear karyorrhexis in thymic cortical lymphocytes; and 5) mucosal epithelial detachment in the bronchi, gastrointestinal tract, or uterus. In normal hematoxylin and eosin-stained tissue, each cell demonstrates nuclear basophilic staining (ie, 100% of nuclei are partially blue). Nuclear basophilic staining is lost when at least 1% of nuclei are entirely devoid of basophilic (blue) staining (ie, at least 1% of nuclei appear entirely pink).

After completing clinical and pathologic assessment

Table 1. Histologic Features Assessed in 15 Organs in the 100 Learning Cases

Organ	Feature
Trachea	Cartilage matrix loss of basophilia (any)
	Chondrocyte nuclear loss of basophilia (any cell) [I]*
Liver	Hepatocyte nuclear loss of basophilia (any cell) [C]
	Extramedullary hematopoiesis, nuclear loss of basophilia (any cell)
	Bile ductal nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire organ)
Adrenal	Fetal cortex nuclear loss of basophilia (any cell) [D]
	Adult cortex nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire organ) [H]
Pancreas	Parenchymal nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire organ)
Testis	Seminiferous tubular nuclear loss of basophilia (any cell)
	Interstitial cell nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire organ)
Gastrointestinal tract	Mucosal epithelial intraluminal detachment (any)
	Mucosal epithelial nuclear loss of basophilia (any cell) [B]
	Extent of loss of nuclear basophilia (lamina propria [1+], submucosa [2+], muscularis [3+], transmural [4+])
	Maximal loss of nuclear basophilia (entire tract)
Thymus	Cortical lymphocyte karyorrhexis (nuclear fragmentation)
	Lymphocyte nuclear loss of basophilia (any lymphocyte)
	Maximal loss of nuclear basophilia (entire organ)
Uterus	Mucosal epithelial intraluminal detachment (any)
	Maximal loss of nuclear basophilia (entire organ)
Ovary	Oocyte nuclear loss of basophilia (any oocyte)
	Maximal loss of nuclear basophilia (entire organ)
Spleen	Nuclear loss of basophilia (any splenic cell)
	Maximal loss of nuclear basophilia (entire organ)
Lung	Bronchial cartilage matrix loss of basophilia (any)
	Bronchial cartilage chondrocyte nuclear loss of basophilia (any)
	Bronchial mucosal epithelial intraluminal detachment (any)
	Bronchial epithelial nuclear loss of basophilia (any cell) [G]
	Alveolar wall nuclear loss of basophilia (any interstitial or alveolar epithelial cell)
	Maximal loss of nuclear basophilia (entire organ)
Cerebrum	Cortical neuronal nuclear loss of basophilia (any neuron)
	Maximal loss of nuclear basophilia (entire tissue)
Heart	Loss of nuclear basophilia, inner half of myocardium (any cell) [E]
	Epicardial/outer half of myocardium nuclear loss of basophilia [F]
	Maximal loss of nuclear basophilia (entire organ)
Kidney	Cortical tubule nuclear loss of basophilia (any cell) [A]
	Glomerular nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire organ) [J]
Skin	Epidermal intracytoplasmic vacuolization (any cell)
	Epidermal/dermal separation (any)
	Dermal nuclear loss of basophilia (any cell)
	Maximal loss of nuclear basophilia (entire tissue)

* Letter corresponds to illustration in Figure 1.

of the 100 learning cases, we evaluated the histologic features for possible associations with each of the following intrauterine retention times (death-to-delivery interval): less than 2 hours, at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours, at least 96 hours, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, and at least 8 weeks. Further, we assessed the validity and efficacy of each histologic feature as a possible diagnostic test for these retention times. Measures of diagnostic test validity and performance (sensitivity, specificity, and positive predictive values) were estimated for each histologic feature. Fetuses were excluded from the evaluation of specific retention times when the time interval of death determined clinically included the retention time being assessed. For example, the histologic findings of a fetus who died between 12–20 hours before birth were not assessed for the "at least 18 hours" retention time; this fetus' histologic findings would, however, be evaluated for all other retention times ("at least 12 hours," "at least 24 hours," etc).

Histologic features were also evaluated for possible associations with the following variables: gender, fetal infection, hypoxia/severe stress (defined previously), hydrops, multiple gestation, delivery-to-autopsy interval greater than 24 hours, and gestational age at death (less than 25 weeks, 25–35 weeks, or more than 35 weeks). The median death-to-delivery times for the development of selected histologic features in fetuses affected by each of these conditions were compared with the equivalent times calculated for fetuses unaffected by the conditions.

The test set, consisting of 50 stillborns, was evaluated to determine the accuracy and performance of the proposed histologic criteria developed from the initial 100 stillborns. All slides of the 50 test cases were randomly assessed in a blinded fashion by a perinatal pathologist (DRG). Each organ was evaluated individually, without comparison to other organs from the same fetus. After completing the examination of the test set, the pathologist assessed histologic features individually in terms of sensitivity, specificity, and positive predictive value for predicted death-to-delivery times. Next, histologic features were analyzed collectively to identify which collections of features most accurately classified fetuses according to the time of fetal death.

Finally, to determine the reproducibility of this method, 40 randomly selected cases were blindly re-evaluated by a single observer (DRG). Intra-observer agreement values and kappa values[21] were calculated.

Table 2. Circumstances That Initiated Fetal Surveillance

Premature labor or rupture of membranes		35 (23%)
Multiple gestation		39 (26%)
Spontaneous fetal death	23	
Selective termination*	16	
Fetal hydrops		21 (14%)
Immune†	7	
Nonimmune	14	
Maternal disease		18 (12%)
Preeclampsia	9	
Diabetes	5	
Other‡	4	
Fetal growth retardation		9 (6%)
Multiple fetal anomalies§		10 (7%)
Abruptio placentae		8 (5%)
Placenta previa		1 (1%)
Prolapsed cord		5 (3%)
Maternal motor vehicle accident		2 (1%)
Surveillance following breech version		2 (1%)
Incompetent cervix		2 (1%)
Postdates pregnancy		2 (1%)
Routine obstetric care		13 (9%)

* All cases terminated by intrafetal potassium chloride injection.
† Four cases of Rh erythroblastosis and three cases of Kell erythroblastosis; six cases treated with fetal transfusion.
‡ One case each of asthma, ulcerative colitis, appendicitis, and deep venous thrombosis treated with heparin.
§ Two cases of trisomy 21 and one case each of bladder outlet obstruction, 69,XXX, 45,X, trisomy 18, iniencephaly, holoprosencephaly, hydrocephalus, and osteogenesis imperfecta.

Results

Most of the 150 stillborn fetuses were under close surveillance at the time of intrauterine death because of high-risk maternal or fetal conditions (Table 2). Among the 150 fetuses, the mean (± standard deviation [SD]) birth weight was 1073 ± 1028 g (range 10–4200), and the mean gestational age at the time of death was 26.5 ± 7.7 weeks (range 10–43). The median time from death until birth was 30 hours (range 0.25 hours to 203 days) for all cases, 38 hours for the 100 learning cases, and 22 hours for the 50 test cases. The intrauterine retention time was less than 6 hours in 29% of the learning set and 31% of the test set; other intervals were 6–48 hours in 26 and 25%, 48 hours to 2 weeks in 23 and 26%, and more than 2 weeks in 22 and 18%, respectively. Table 3 compares clinical and pathologic data for the learning cases and the test cases. Virtually all characteristics were similar for the learning and test sets.

Among the 45 histologic features assessed, 23 features correlated reasonably well with the time of fetal death in terms of sensitivity, specificity, and positive predictive value (Table 4). The earliest histologic change noted was loss of nuclear basophilic staining in renal cortical tubules; this finding was rarely seen in fetuses who had died within 4 hours of birth (one of

Table 3. Clinical and Pathologic Features of 150 Stillborn Fetuses

	Learning set (N = 100)	Test set (N = 50)
Birth weight (g)		
Mean ± SD	1046 ± 974	1129 ± 1158
<1000	64 (64%)	31 (62%)
1000–2500	23 (23%)	13 (26%)
>2500	13 (13%)	6 (12%)
Gestational age at death (wk)		
Mean	26.5 ± 7.7	26.4 ± 7.7
<25	44 (44%)	24 (48%)
25–36	43 (43%)	21 (42%)
>36	13 (13%)	5 (10%)
Male gender*	46 (51%)	21 (48%)
Hypoxia/severe stress†	51 (51%)	24 (48%)
Hydrops	11 (11%)	10 (20%)
Multiple gestation		
Total	27 (27%)	12 (24%)
Spontaneous death	17 (17%)	6 (12%)
Selective termination	10 (10%)	6 (12%)
Infection	40 (40%)	19 (38%)
Mean birth-to-autopsy interval (h)	36.1 ± 37.6	23.9 ± 19.9

* Fetal gender unknown for nine cases in the learning set and six cases in the test set.

† Acute thymic involution, acute adrenal involution, interstitial hemorrhage, intraventricular hemorrhage, hepatic subcapsular hematoma, abruptio placentae, prolapsed cord, fetal growth retardation.

17, 6%) but was present almost universally in fetuses with an intrauterine retention interval of 4 or more hours (60 of 61, 98%) ($P < .001$). Other early histologic changes that accurately classified fetuses by time of death included loss of nuclear basophilia in the gastrointestinal tract mucosa (8 or more hours) and loss of nuclear basophilia of hepatocytes and myocardial cells of the inner one-half of the heart (24 or more hours). Of note, autolysis proceeded most slowly in the lungs and the cerebral cortex; the lungs of one fetus who died at least 14 weeks before birth had persistent nuclear basophilia in interstitial/alveolar regions. Residual nuclear basophilia in cerebral cortical neurons was present at least focally in all fetuses evaluated, including a fetus papyraceous whose death was documented 25 weeks before birth. Figure 1 presents selected histologic features.

To determine how factors other than the death-to-delivery interval might influence fetal histology, we evaluated selected histologic features for possible associations with fetal gender, infection, multiple gestation, hydrops fetalis, hypoxia/severe stress, gestational age at death, and birth-to-autopsy interval. Fetal sex, infection, multiple gestation, and hypoxia did not appear to influence consistently the median times at

Table 4. Histologic Features in the 100 Learning Cases That Correlated Best With Timing of Fetal Death

Tissue feature	N	(NA)	Death-to-delivery time	Sensitivity	Specificity	Positive predictive value
Kidney: cortical tubular nuclear loss of basophilia [A]*	78	(13)	≥4 h	0.983	0.800	0.934
GI tract: mucosal epithelial nuclear loss of basophilia [B]	85	(7)	≥8 h	0.966	0.875	0.952
Lung: bronchial mucosal epithelial detachment	84	(8)	≥18 h	1.000	0.903	0.946
Lung: bronchial cartilage matrix loss of basophilia	87	(5)	≥24 h	0.820	0.919	0.932
Liver: hepatocyte nuclear loss of basophilia [C]	88	(4)	≥24 h	0.957	0.902	0.957
Thymus: cortical lymphocyte karyorrhexis	76	(5)	≥24 h	1.000	0.850	0.857
Heart: inner half of myocardium nuclear loss of basophilia [E]	81	(4)	≥24 h	0.909	0.919	0.930
Adrenal: fetal adrenal cortex nuclear loss of basophilia [D]	81	(5)	≥24 h	0.762	0.897	0.889
Pancreas: maximal loss of nuclear basophilia	52	(7)	≥36 h	0.917	0.900	0.753
Thymus: lymphocyte nuclear loss of basophilia	73	(8)	≥48 h	0.958	0.837	0.742
Kidney: glomerular nuclear loss of basophilia	83	(8)	≥48 h	0.806	0.942	0.893
Heart: outer half of myocardium nuclear loss of basophilia [F]	78	(7)	≥48 h	0.839	0.915	0.867
GI tract: transmural [4+] bowel wall loss of nuclear basophilia	79	(12)	≥72 h	0.966	0.960	0.933
Adrenal: adult adrenal cortex nuclear loss of basophilia	75	(11)	≥72 h	1.000	0.946	0.864
Lung: bronchial epitheliál nuclear loss of basophilia [G]	80	(12)	≥96 h	0.920	0.945	0.885
Liver: maximal loss of nuclear basophilia	80	(12)	≥96 h	0.917	0.929	0.846
GI tract: maximal loss of nuclear basophilia	78	(13)	≥1 wk	0.950	0.983	0.950
Adrenal: maximal loss of nuclear basophilia [H]	74	(12)	≥1 wk	0.929	0.917	0.722
Trachea: chondrocyte nuclear loss of basophilia [I]	62	(1)	≥1 wk	0.750	0.963	0.750
Lung: alveolar wall nuclear loss of basophilia	88	(4)	≥2 wk	0.938	0.958	0.833
Kidney: maximal loss of nuclear basophilia [J]	89	(2)	≥4 wk	1.000	0.976	0.750
Lung: maximal loss of nuclear basophilia	92	(0)	≥8 wk	0.900	0.988	0.900
Cerebrum: cortical neuronal nuclear loss of basophilia	70	(2)	≥8 wk	0.714	1.000	1.000

NA = not available for evaluation because the clinically timed interval during which death occurred included the death-to-delivery time being assessed; GI = gastrointestinal.

* Letter corresponds to illustration in Figure 1.

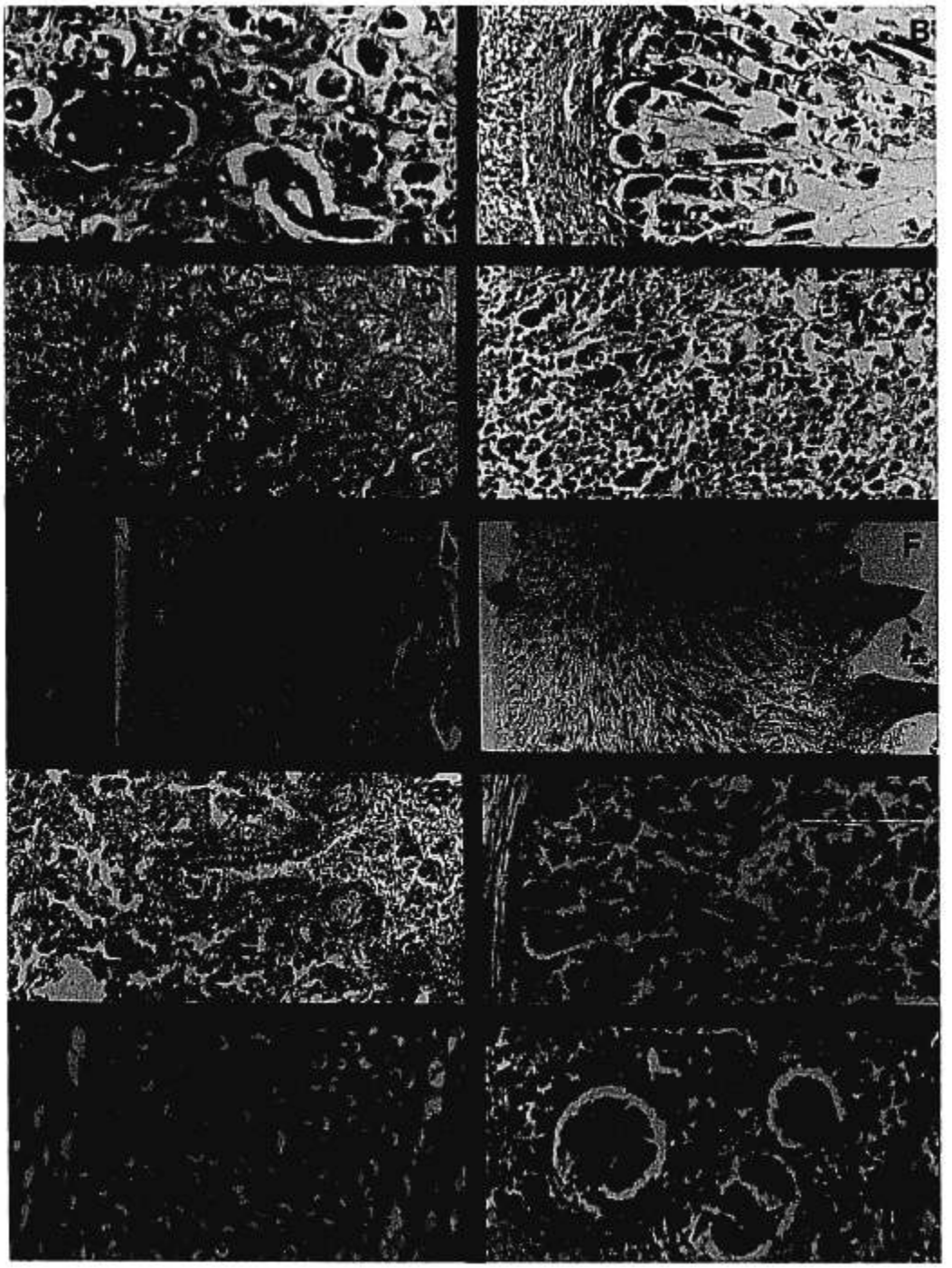

Figure 1. Hematoxylin and eosin-stained fetal tissues. A) Loss of nuclear basophilia in some renal cortical tubules (*arrow*) 4 hours or more after fetal death (× 120). B) Loss of mucosal epithelial nuclear basophilia in intestine 8 hours or more after fetal death (× 100). C) Loss of hepatocyte nuclear basophilia (*small arrows*); normal nuclear staining in some hepatocytes (*large arrow*) and in extramedullary hematopoietic elements (*curved arrow*) 24 hours or more after fetal death (× 100). D) Loss of nuclear basophilia in fetal adrenal cortical cells (*arrow*) 24 hours or more after fetal death (× 100). E) Loss of nuclear basophilia involving the inner half of the myocardium, extending from the endocardial surface (*small arrow*) to the middle of the myocardium (*curved arrow*); insets demonstrate higher magnification of myocytes of the inner (*top*) and outer (*bottom*) myocardium 24 hours or more after fetal death (× 50; insets × 200). F) Loss of nuclear basophilia involving outer half of the myocardium, extending from the endocardial surface (*small arrow*) to the epicardium (*curved arrow*) 48 hours or more after fetal death (× 50). G) Loss of nuclear basophilia in detached bronchial epithelial cells (*arrow*) and persistent nuclear basophilic staining in chondrocytes and alveolar septal cells 96 hours or more after fetal death (× 100). H) Adrenal demonstrating "maximal" loss of nuclear basophilia; in contrast to illustration D, there is no basophilic staining in any cell 1 week or more after fetal death (× 100). I) Tracheal cartilage demonstrating loss of nuclear basophilia in chondrocytes 1 week or more after fetal death (× 160). J) Kidney with "maximal" loss of nuclear basophilia; in contrast to illustration A, there is no nuclear basophilic staining throughout the entire organ 4 weeks or more after fetal death (× 120).

Table 5. Histologic Features in the Test Set: Analysis as Diagnostic Tests for Specific Death-to-Delivery Interval

Tissue feature	*N*	Death-to-delivery time	Sensitivity	Specificity	Positive predictive value
Good predictors					
Kidney: loss of tubular nuclear basophilia [A]*	44	≥4 h	0.971	0.889	0.971
Liver: loss of hepatocyte nuclear basophilia [C]	41	≥24 h	1.000	0.920	0.889
Myocardium: inner half loss of nuclear basophilia [E]	39	≥24 h	0.938	1.000	1.000
Myocardium: outer half loss of nuclear basophilia [F]	38	≥48 h	1.000	0.964	0.909
Bronchus: loss of epithelial nuclear basophilia [G]	47	≥96 h	1.000	0.973	0.909
Liver: maximal loss of nuclear basophilia	46	≥96 h	0.909	1.000	1.000
GI tract: maximal loss of nuclear basophilia	47	≥1 wk	0.900	1.000	1.000
Adrenal: maximal loss of nuclear basophilia [H]	40	≥1 wk	1.000	1.000	1.000
Trachea: chondrocyte loss of nuclear basophilia [I]	37	≥1 wk	0.889	1.000	1.000
Kidney: maximal loss of nuclear basophilia [J]	49	>4 wk	1.000	0.976	0.875
Intermediate predictors					
GI epithelium: loss of nuclear basophilia [B]	44	>8 h	0.930	0.800	0.900
Adrenal: fetal cortical loss of nuclear basophilia [D]	39	≥24 h	0.813	0.957	0.929
Pancreas: maximal loss of nuclear basophilia	32	≥36 h	0.714	1.000	1.000
GI tract: transmural [4+] nuclear loss of basophilia	43	≥72 h	1.000	0.909	0.769
Lung: alveolar wall loss of nuclear basophilia	48	≥2 wk	1.000	0.949	0.800
Poor predictors					
Bronchus: mucosal epithelial detachment	35	≥18 h	1.000	0.588	0.720
Bronchus: cartilage matrix loss of basophilia	40	≥24 h	0.941	0.625	0.640
Thymus: cortical lymphocyte karyorrhexis	30	≥24 h	1.000	0.429	0.429
Thymus: lymphocyte nuclear loss of basophilia	29	≥48 h	1.000	0.880	0.571
Kidney: glomerular nuclear loss of basophilia	39	≥48 h	1.000	0.821	0.688
Adrenal: adult cortical nuclear loss of basophilia	42	≥72 h	1.000	0.875	0.714
Lung: maximal nuclear loss of basophilia	49	>8 wk	1.000	0.976	0.875
Cerebral cortex: neuronal nuclear loss of basophilia	37	>8 wk	0.750	0.970	0.750

GI = gastrointestinal.
* Letter corresponds to illustration in Figure 1.

which the histologic changes first appeared (data not shown; available upon request). Median times for histologic changes appeared to be decelerated in extremely premature fetuses (before 25 weeks) and accelerated in fetuses with hydrops fetalis, advanced gestational age (after 35 weeks), and birth-to-autopsy interval exceeding 24 hours (data not shown; available upon request). Because of the small numbers of fetuses with hydrops, advanced gestational age, extreme prematurity, and prolonged birth-to-autopsy intervals, the precise influence of these factors upon the progression of histologic changes is unclear and remains to be determined in larger data sets.

The 23 histologic criteria developed using the 100 learning cases were evaluated using the 50 test cases. Hematoxylin and eosin slides of the test cases were reviewed randomly and blindly by one author (DRG). Initially, each feature was evaluated as a single diagnostic test (independent of the other histologic findings in the same fetus) in terms of its sensitivity, specificity, and positive predictive value. Ten of the 23 histologic variables (Table 5) performed well as diagnostic tests for the predicted time of fetal death (sensitivity, specificity, and positive predictive values at least 0.875); among the 13 remaining histologic features, five variables performed moderately well and eight performed poorly (poor predictors). The earliest histologic change consistently noted among the 50 test cases was absence of renal cortical tubular nuclear basophilia; this change was infrequently found in fetuses dead less than 4 hours (one of 13, 8%), but was seen in 34 of 35 (97%) of cases dead 4 or more hours ($P < .001$). These results are comparable to the results obtained in the learning set.

After each histologic feature was analyzed as an independent diagnostic test, we analyzed collectively the complete set of histologic features observed in each fetus to determine whether a composite could correctly predict the timing of fetal death. In this analysis, the single most advanced histologic feature was used to predict the time of fetal death. When all 23 histologic features were considered, 27 of the 50 cases (54%) were correctly classified into one of 13 time "windows" regarding fetal death (less than 4 hours; 4–8, 8–18, 18–24, 24–36, 36–48, 48–72, and 72–96 hours; 96 hours to 1 week; 1–2, 2–4, and 4–8 weeks; and more than 8 weeks). When the nine poor predictors were excluded from this analysis, 32 of the 50 cases (64%) were

Table 6. Categorization of 50 Test Cases into Seven Time "Windows" Based on the Ten Best Histologic Predictive Features

Predicted time (histology)	Actual time (from chart review)							Total
	<4 h	≥4 h to <24 h	≥24 h to <48 h	≥48 h to <96 h	≥96 h to <1 w	≥1 w to <4 w	≥4 w	
<4 h	**13**	1*						14
>4 h to <24 h	1†	**8**						9
≥24 h to <48 h		2‡§	**2**					4
≥48 h to <96 h			1‖	**9**	1¶			11
≥96 h to <1 wk					**2**			2
≥1 wk to <4 wk						**2**		2
≥4 wk						1#	**7**	8
Total	14	11	3	9	3	3	7	50

Bold type = correct prediction.
* Premature labor, 25 wk; birth-to-autopsy 4 h.
† Prolapsed cord, 24 wk; birth-to-autopsy 25 h.
‡ Rh hydrops fetalis, 36 wk; birth-to-autopsy 23 h.
§ Rh hydrops fetalis, 24 wk; birth-to-autopsy 8 h.
‖ Trisomy 21 and hydrops, 34 wk; birth-to-autopsy 13 h.
¶ Death of one twin, 36 wk; birth-to-autopsy 88 h.
\# Selective termination of one triplet at 11 wk; birth-to-autopsy 12 h.

correctly classified into one of 11 time windows (less than 4 hours; 4–8, 8–24, 24–36, 36–48, 48–72, and 72–96 hours; 96 hours to 1 week; 1–2 and 2–4 weeks; and more than 4 weeks). Further, when the analysis was restricted to the ten best histologic features (good predictors), 43 of the 50 cases (86%) were correctly classified into one of seven time windows (Table 6); in this assessment, all incorrect predictions were misclassified by one window. Table 6 lists the clinical features of the seven misclassified cases. Of note, fetal hydrops was present in three of the five cases in which the death-to-delivery interval was overestimated.

To determine intra-observer variability in assessment of the ten good predictors, one author (DRG) blindly reviewed the slides of 40 randomly selected cases. The percentage of agreement between the two observations ranged from 89% (maximal loss of hepatocyte nuclear basophilia) to 100% (maximal loss of kidney nuclear basophilia). Kappa values[21] for the ten histologic features ranged from 0.711–1.0.

Discussion

Following fetal death, parents and obstetricians pose three major questions to the obstetric pathologist: 1) When did the fetal death occur? 2) Why did the fetus die? and 3) What are the implications for future pregnancies? Because stillbirths are relatively common, the answers to these questions are also of interest to the medical community at large.[2] This retrospective histologic study of 150 stillborns demonstrates that autopsy histology is useful for answering the first of these questions. Using hematoxylin and eosin histology for selected features, we have observed that in most instances, the approximate time of fetal death can be accurately estimated.

Traditionally, obstetric pathologists have suggested that standardized protocols be used to optimize the performance of the perinatal autopsy. Most proposed protocols include routine use of photography, radiography, and bacteriology; karyotyping of selected cases; gross and histologic examination of the placenta; external examination and gross dissection of the fetus; and histologic examination of multiple fetal organs.[7,9,15] More recently, it has been suggested that routine histologic examinations of fetal tissues are not warranted in autopsies of stillborns because in many cases they provide little useful information[22]; this conclusion has been challenged.[23,24] The findings of the current study support the viewpoint that fetal histology is an important part of the autopsy of a stillborn fetus.

Two previous studies evaluated the use of fetal histology for timing intrauterine death.[13,14] In 1964, Shanklin[13] applied histologic criteria developed from a rabbit model to 53 stillborn humans. Forty-six histologic features in four organs (skin, liver, kidney, and lung) were graded as 0, trace, 1+, 2+, 3+, or 4+; the precise method used for the grading of histologic variables was not defined. In the majority of the cases (47 of 53, 89%), the author reported a "poor" level of confidence regarding the clinical documentation of the time of fetal death. Nevertheless, it was concluded that fetal histology was potentially valuable for estimating the time of fetal death. The second study, published in 1970 by Babala,[14] evaluated 110 stillborns. The investigators studied 15 organs, identifying six "stages" of histologic change; these were not described in detail.

They concluded that microscopic changes were capable of identifying the time of fetal death; furthermore, they noted that progression of microscopic changes was accelerated by postmaturity and hydrops, and decelerated by prematurity and anencephaly. Although the methods and definitions of these two investigators were not described in detail, both studies of fetal death and histology reached conclusions similar to those of our study.

In the current study, the principal histologic feature associated with the time of fetal death is the absence of nuclear basophilia (hematoxylin staining) in hematoxylin and eosin-stained tissues (Figure 1). The use of this histologic variable has several rationales and advantages. It is a fairly objective microscopic criterion that is easy to apply: "Presence of nuclear basophilia" means all nuclei are partially blue, and "loss of nuclear basophilia" means at least 1% of nuclei are totally pink. For decades, pathologists have recognized this histologic finding as characteristic of fetal death.[8] Hematoxylin and eosin staining is used universally in histopathology laboratories. This methodology readily lends itself to retrospective assessment of archival pathologic material. Although this study assessed several histologic criteria other than nuclear basophilia (karyorrhexis, mucosal epithelial detachment, and eosinophilia of cartilage matrix), the ten microscopic features that performed best among the unknown cases all involved assessment of nuclear basophilic staining. Nuclear basophilia may be superior to the other features assessed either because it can be more objectively measured or because it is more strongly associated with the time of fetal death than the other microscopic features. Hematoxylin dyes bind to the proteins in the cell nucleus. During autolysis, these proteins initially are separated from nucleoproteins by activated proteinases, then they are degraded by polynucleotidases.[10] According to Janssen,[10] these biochemical processes might suggest the nucleus for measuring autolysis because there may be an "autolytic regularity" in these nuclear biochemical reactions in various tissues.

Several limitations of our study deserve further comment and additional evaluation. First, pathologists recognize that tissue histology varies considerably between institutions. It is not clear whether variations in the methods of tissue fixation, tissue preparation, or hematoxylin and eosin staining could alter the histologic appearance of fetal tissues sufficiently to cause miscalculations of the time of fetal death. Second, although certain factors (gestational age, hydrops, and birth-to-autopsy interval) might either accelerate or decelerate the development of histologic changes following fetal death, the extent to which predictions about the time of fetal death should be modified because of these factors is currently not known.

Third, it is not clear how often a pathologic process unrelated to the time of fetal death might mimic a histologic change linked to the time of death. For example, if acute renal tubular necrosis were to develop in a fetus who died within 4 hours of delivery, this change might mimic absence of renal tubular nuclear basophilia, the change that in this study is associated with fetal death 4 or more hours before birth. Alternatively, the almost universal finding of absent renal tubular nuclear basophilia in fetuses who died more than 4 hours before birth in this study raises the following question: Can acute tubular necrosis be diagnosed in a fetus who died more than 4 hours before birth? We believe that acute tubular necrosis should be diagnosed in a stillborn fetus only when ancillary microscopic findings (such as hemoglobin and cellular casts) support such a diagnosis.[25–27]

Fourth, most stillborns evaluated in this study died within a few days of delivery; only 20% of cases were retained in utero for more than 7 days. This suggests that the predictive strength of "early" histologic changes may be better than that of "late" changes.

Finally, the retrospective design of our study warrants that the results be interpreted with caution. Although several small prospective animal studies have appeared in the veterinary and medical literature, the applicability of the results to humans is uncertain.[28–31] To minimize observation bias in this study, we developed histologic criteria in 100 cases and then blindly evaluated those criteria in a test set of 50 stillborns. These criteria need further prospective testing.

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