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Original Article

Histologic Estimation of Intrauterine Retention Time after Fetal Death

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The intrauterine retention time (IURT) after fetal death can be estimated from the loss of nuclear basophilia. We therefore attempted to derive an autolysis equation to estimate IURT in experimental rat fetuses and human fetal autopsy slides. The degree of loss of nuclear basophilia in various tissues was assessed by hematoxylin and eosin (H & E) staining. Fetal rat tissues showed different rates of autolysis, allowing for the construction of an experimental autolysis curve. We also reviewed the H & E stained slides obtained from 27 human fetal autopsy cases with well-documented death intervals. The degree of autolysis in various tissues was evaluated using percentile scores (PS). Using the findings from H&E staining, we derived the equation Ln (PS/[100-PS]) = 2.62716−0.02377 × IURT. However, this equation or autolysis scores showed some limitations. Owing to the inconsistency of PS, this equation is reliably applicable only within 24 hours of intrauterine fetal death. In the fetal autopsy review, fetal hydrops, local effusion, and sepsis also contributed to accelerated autolysis.

Key Words : Autolysis, Basophilia, Death, Fetus, Histology

Introduction

The fetus undergoes postmortem changes after death in utero due to autolysis. Several reports have suggested that the autolysis may provide a method for determining the intrauterine retention time (IURT).[1-3] Among them, the Genest's study has presented the loss of nuclear basophilia as a most reliable histologic indicator.[3] According to the Genest's criteria, 10 histologic features based on loss of nuclear basophilia were found to have sufficient specificity, sensitivity, and positive predictive values. Specificity and sensitivity were assessed by classifying death to delivery time into 13 time windows, and 86% of the fetuses assayed could be assorted into one of seven time windows.[3]

But applying these criteria, three types of practical limitations are revealed. The first is the internal problem of Genest's methods. Time windows for IURT are arbitrary. For example, a true death-to-delivery time of 23 h 59 min would be classified into the '≥ 4h and ≤ 24h' time window, whereas a death-to-delivery time of 24 h 1 min would be classified into the '≥ 24h' time window. The second is the pathologic conditions that accelerate or decelerate the autolysis rate should be excluded in advance and more detailed. The third is the assessment of 10 histologic features to keep the Genest's criteria is still affected by the kappa value for inter or intra-observer

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affects. In this article, I want to develop the Genest's solution to choose the nuclear basophilia as a histologic indicator for estimation of intrauterine retention time after fetal death. So based on the Genest's criteria, I therefore sought another, more objective method of analyzing these histologic features.

Materials and Methods

1. Animal experiment

Pregnant Sprague-Dawley rats were anesthetized with ether, and their fetuses were harvested by Caesarean section. The fetuses were 20 fetal days (Wight = 4.1 gm). These fetuses were drowned and stored in normal saline solution at 37℃. The fetus of each group was selected at 0, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 196 h after drowned and divided into three pieces. They were immersed in 10% formalin solution for more than 7 days and embedded in paraffin blocks. Each specimen block was sectioned 4 micrometer thickness with microtome (Leica RM2145, rotary type) and stained with hematoxylin and eosin (H & E). At least 5 fields examined under × 400 magnification (Olympus Optical Co. Ltd., Tokyo, Japan). The evaluations were performed twice by one pathologist. When the results were different, another pathologist evaluated the slides and determined the results in consensus. The loss of nuclear basophilia was assessed according to the Genest's criteria: (2) indicating almost total preservation; (1) indicating partial loss of nuclear basophilic (hematoxylin) staining, involving at least 1% of cells, in specific region of organs; (0) indicating maximal loss of nuclear basophilic staining throughout an entire organ (i.e., involving 100% of cells) (Fig. 1). We analyzed these results using percentile scores by setting (2) equal to 100%, (1) equal to 50% and (0) equal to 0%. Using those scores, we could obtain the autolysis curve.

2. Human fetal autopsy slide review

Among the 1661 fetal autopsies performed at Department of Pathology in Seoul National University Hospital from 1 January 1991 to 30 June 2001, a total of 30 cases with a specific time of death or a narrow range of estimated death intervals known by history were selected. The medical data of mother and fetus based on the clinical records, and autopsy findings were described on autopsy record. Autopsy data also contained information of abnormal conditions like fetal hydrops, fetal sepsis (confirmed by blood culture), fetal hypoxia, congenital anomalies. As a routine procedures, fetuses had been kept at 5℃ in refrigerator and autopsy was performed within 24 hours after still birth. According to the protocol, the entire organs, from tongue to rectum, were removed en bloc and fixed in 10% buffered formalin solution. The next day, organs were dissected and embedded. The slides were prepared from the paraffin tissue blocks and stained with H&E. The loss of nuclear basophilia was assessed according to the Genest's criteria with the same method of animal experimentation.

The following regions of organs are observed using tissue array slides (Fig. 1). In the kidney, the proximal tubules were evaluated at cortical portion around the fully developed glomeruli and the collecting ducts at medullary portion. In the liver, we were able to discriminate the hepatocytes from hematopoietic cells with H&E. In the heart, the myocardium in the middle of the ventricular wall was evaluated as a whole, excluding the areas just beneath the layer of the endocardium and epicardium. In the lung, the bronchial epithelium and tracheal chondrocytes were evaluated. The fetal adrenal gland is composed of inner fetal and outer permanent zones,[4] each of which was evaluated separately. The central nervous system and gastrointestinal tract could not be evaluated because the exact location and pathologic conditions of each specimen were not identified in the autopsy records and the retina and pancreas was not evaluated because these tissues were not sampled routinely.

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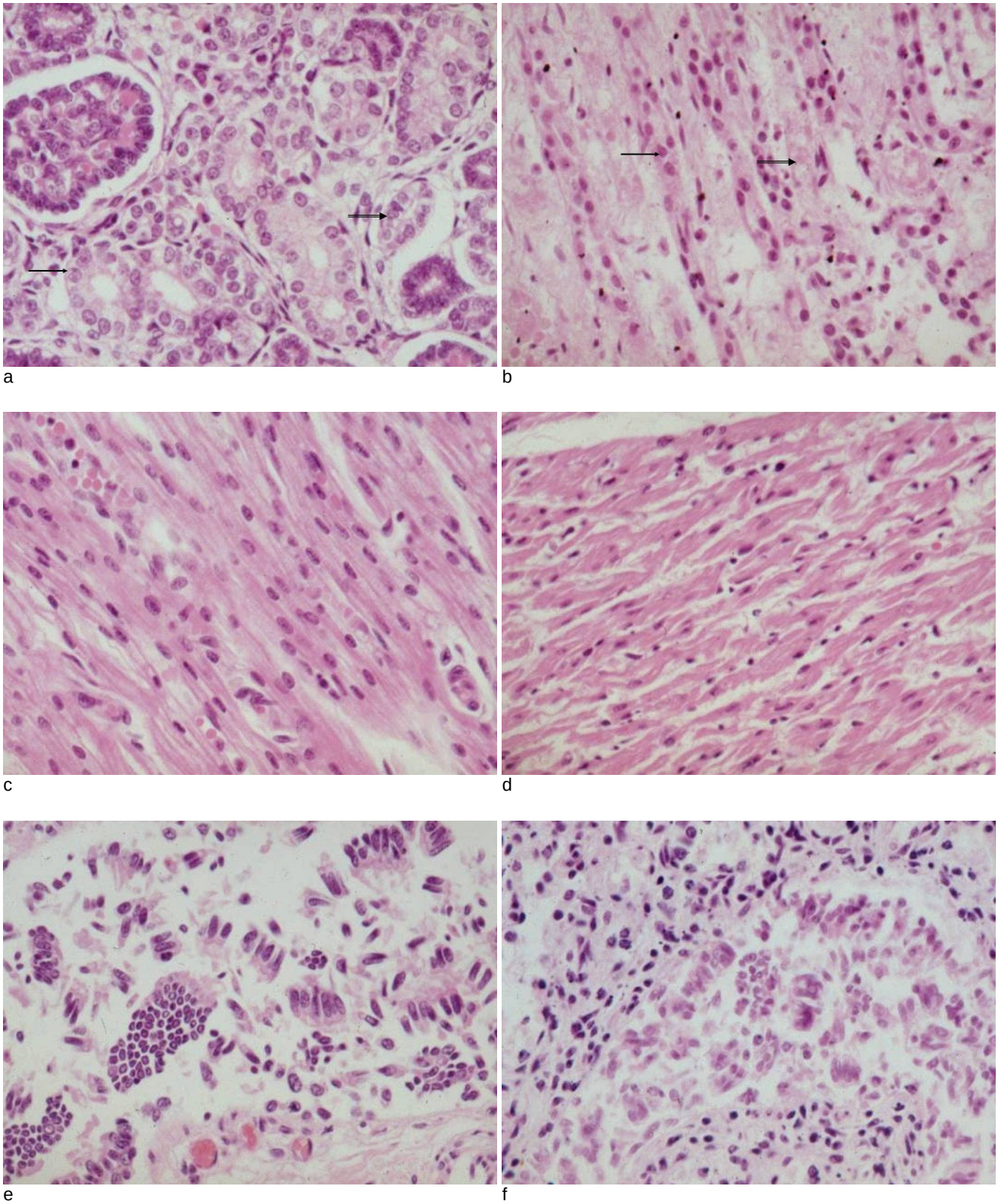

Fig. 1. The loss of nuclear basophilia was assessed according to the Genest's criteria: a) Kidney proximal tubules (arrow, PS = 100) and distal tubules (double line arrow, PS = 100) (H & E, ×400). b) Kidney collecting tubule (arrow, PS = 50, double line arrow, PS = 0) (H & E, ×400). c) Heart cardiac myocytes show (PS = 100), (H & E, ×400). d) Cardiac myocytes (PS = 50), (H & E, ×200). e) Bronchial epithelium shows (PS = 100), (H & E, × 400). f) Bronchial epithelium shows (PS = 50), (H & E, ×400).

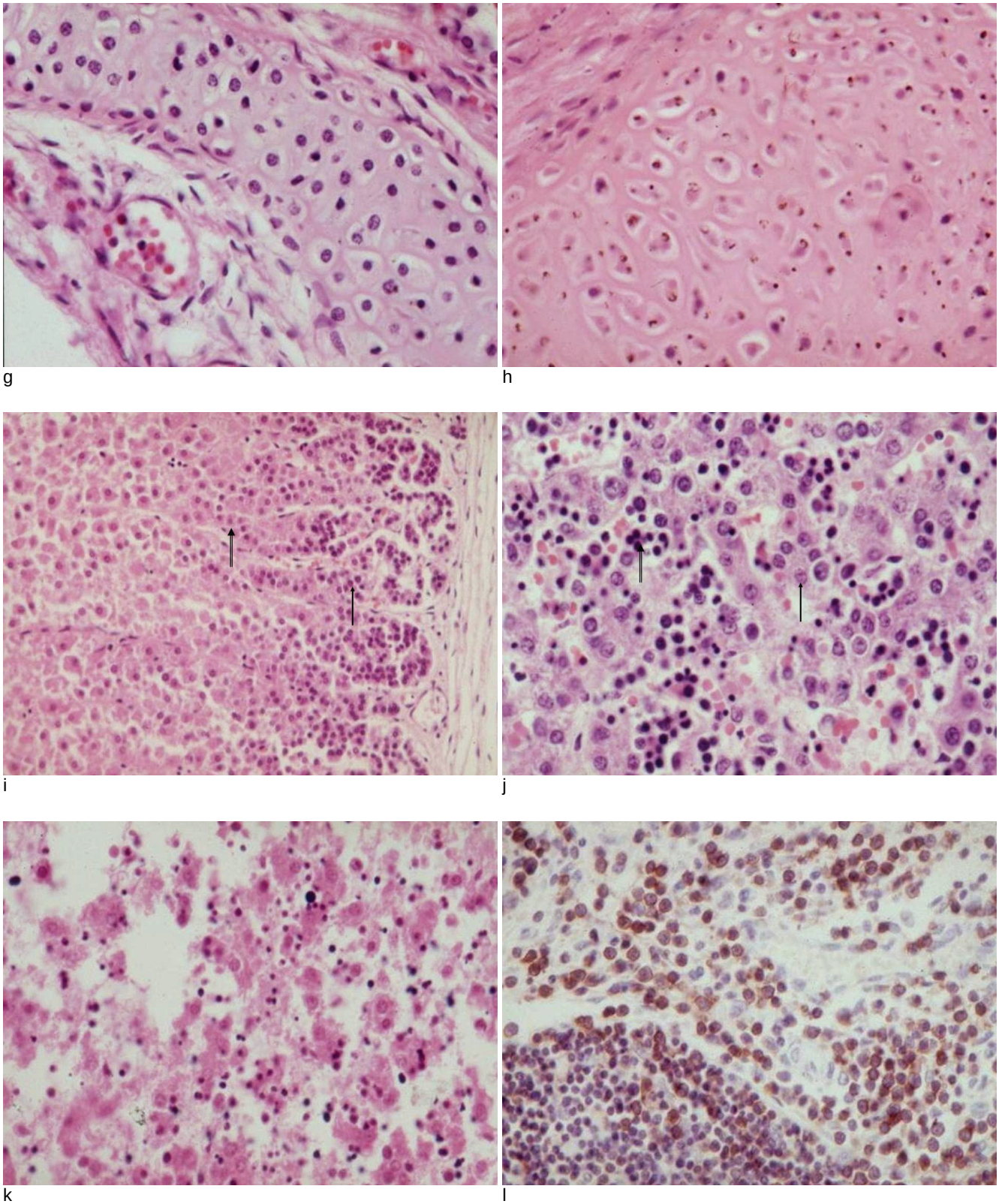

Fig. 1. g) Bronchial cartilage (PS = 100), (H & E, ×400). h) Bronchial chondrocytes (PS = 50), (H & E, ×400). i) Outer fetal zone of adrenal gland shows (arrow, PS = 100) and inner permanent zone (double line arrow) shows (arrow, PS = 50), (H & E, ×200). j) Hepatocytes (arrow, PS = 100) (arrow) and hematopoietic cells (double line arrow), (H & E, ×400). k) Hepatocytes (PS = 50), (H & E, ×400). l) Leukocyte common antigen staining of thymocytes shows (PS = 100), (H & E, ×400) (PS, percentile score).

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Results

1. Animal experiment

We observed a loss of nuclear basophilia (Table 1). The autolysis curve derived from these results is shown (Fig. 2).

2. Human fetal autopsy slide review

The histologic changes that occurred in human fetal tissues are shown in tables 2, 3 and figure 1. We analyzed these changes using PS (Table 2). The glomeruli of the kidney and the thymus showed no nuclear changes over 7.5 days, excluding them from logistic regression analysis. Using the S obtained from H & E, we obtained the autolysis equation, Ln(PS/(100-PS)) = 2.62716 − 0.02377 × IURT. For this equation, standard error of estimated intercept (2.62716) is 0.280969 and p-value is < 0.0001. Standard error of estimated beta (0.02377) is 0.002972 and p-value is < 0.0001. Determinant coefficient R2 = 0.719 and pearson correlation of R is 0.516961 (Fig. 3).

In H & E, the weighted kappa value for intra-observer agreement (the first two evaluations by one pathologist calculated by SAA version 8.02) of our

Table 1. Loss of Nuclear Basophilia from Rat Fetal Tissues

IT* (hour)	Kidney p‖	d¶	c**	g††	Heart	Lung	Liver	GI†	GI‡	PS§ %
0	2	2	2	2	2	2	2	2		100.00
2	1	2	2	2	2	2	2	2		93.45
4	1	2	2	2	2	2	2	2		93.45
8	1	2	2	2	2	2	2	2		93.45
12	1	2	2	2	2	2	2	2		93.45
24	1	2	2	2	2	2	2	2		93.45
48	1	2	2	2	1	2	1	2		81.25
72	1	1	2	2	1	2	1	1		68.75
96	0	1	1	2	1	1	0	1	0	43.75
120	0	1	1	1	1	1	0	1	0	37.50
144	0	0	1	1	1	1	0	1		31.25
168	n††	n	n	n	n	n	n	n		

*IT: Immersion time ; †GI: Gastrointestinal tract; ‡GI: Gastrointestinal tract after exposure to saline ; §PS: Percentile score ; ‖p: Proximal tubule; ¶d: Distal tubule ; **c: Collecting tubule ; ††g: Glomerulus ; ††n: Not evaluable

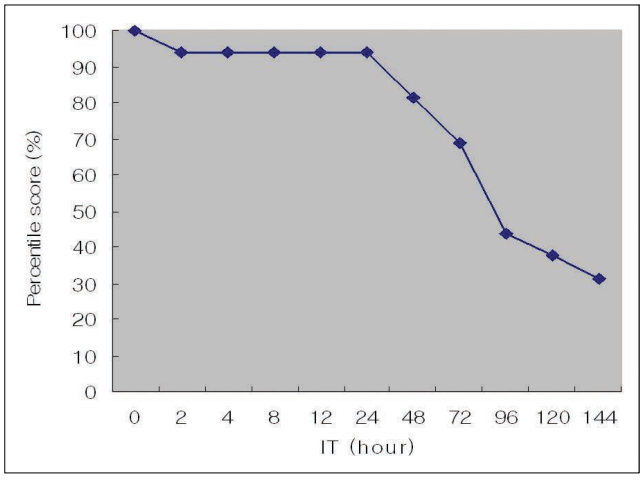

Fig. 2. Autolysis curve derived from fetal rats. Percentile Score (PS) shows a steep downward slope after 24 hours immersion time (IT).

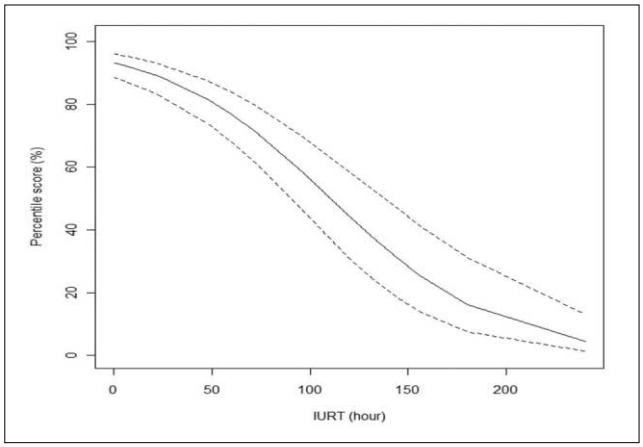

Fig. 3. IURT derived from the percentile score (PS) of H & E. Ln(PS/(100−PS)) = 2.62716 − 0.02377 × IURT, which is shown in the middle line. The other lines represent the upper and lower limits of Intrauterine Retention Time (IURT).

study ranged 0.65 (chondrocyte in trachea) ~ 1.0 (collecting duct in kidney) which were similar with the Genest's kappa value ranged 0.711 ~ 1.0.

We observe 3 cases with abnormal pathologic findings, each of which was the cause of accelerated autolysis (Table 3). These included fetal sepsis, fetal hydrops and local serous effusion in the subcapsular lesion of the liver. In local effusion case, even we exclude the percentile scores of hepatocytes from the calculation, the estimated IURTs of 80.84 h by H & E and 84.22 h by both H & E and Immunohistochemical stain, both indicate generalized accelerated autolysis.

Discussion

IURT after fetal death is another form of estimating the hours post-mortem. IURT can be used for the resolution of disputes over the legal problems especially fetal death related. For example, the disputes over obstetric problems or traffic accident associated with fetal death would be helped by the estimation IURT.

The ideal animal model of experimental autolysis involves sacrificing the fetus within the mother's uterus by ligation of the umbilical vessels, followed by harvesting of the fetuses by caesarean section after

Table 3. Abnormal Cases Showing Accelerated Autolysis

Case	GA* (day)	Wt† (gm)	PS‡ (%) H & E	IURT§: Chart H & E‖ (hour)	
Local effusion	287	3100	55.60	8~48	80.84
Fetal sepsis	255	3420	38.90	≤120	113.74
Fetal hydrops	214	1820	31.30	24~120	128.71

*GA: Gestational age ; †Wt: Weight ; ‡PS: Percentile score ; §IURT: Intrauterine retention time ; ‖H & E: Hematoxylin and eosin.

Table 2. Loss of Nuclear Basophila from Human Fetal Tissues

IURT* (hour)	GA† (day)	Wt‡ (gram)	Kidney			Heart	Lung		Liver	Adrenal		Thymus	PS§ (%)
			P‖	D¶	C**		B††	T‡‡		Pe§§	Fe‖‖		
0.3	153	440	2	2	2	2	2	2	2	2	2	n	100.00
0.5	155	293	1	2	2	2	2	2	2	2	2	2	95.00
1	164	670	2	2	2	2	2	2	2	2	2	2	100.00
1	189	920	2	2	2	2	2	2	2	1	1	2	90.00
1	243	1550	1	2	2	2	2	2	2	2	2	n	94.44
1	169	500	1	2	2	n¶¶	n	n	2	2	2	2	92.85
1.5	154	560	1	2	2	2	2	2	2	2	2	2	95.00
1.5	154	555	1	2	2	2	2	2	2	2	2	2	95.00
1.63	171	684	1	2	2	2	2	2	2	2	2	n	94.44
1.63	159	439	2	2	2	2	2	2	2	2	1	n	94.44
1.75	159	459	1	2	2	2	2	2	1	2	1	n	83.33
6	281	3050	1	2	2	2	2	2	2	2	1	2	90.00
7	158	690	1	2	2	2	2	2	2	2	1	2	90.00
17.5	203	1900	1	1	2	1	2	2	2	2	1	1	75.00
24	272	3500	1	1	2	1	2	2	2	2	1	n	77.78
48	261	2000	0	1	2	0	2	2	1	0	0	1	45.00
60	184	1000	0	1	2	n	2	2	1	1	1	1	61.11
72	200	920	0	1	1	n	0	2	0	0	0	1	27.78
96	198	330	0	1	2	1	2	2	n	n	n	n	66.67
120	282	2950	0	1	1	0	1	2	1	2	1	2	55.56
132	237	1070	0	1	2	n	0	0	0	n	n	1	28.57
144	221	880	0	1	1	0	0	0	0	0	0	n	11.11
144	166	360	0	1	2	1	1	1	1	n	n	1	50.00
156	190	650	0	1	2	1	2	2	0	1	1	2	60.00
180	262	2790	0	0	0	0	1	2	0	0	0	1	20.00
180	277	2890	n	n	n	n	2	2	0	n	n	n	66.67
240	269	3860	0	0	0	0	0	0	0	0	0	0	0.00

*IURT: Intrauterine retention time ; †GA: Gestational age ; ‡Wt: Weight ; §PS: Percentile score; ‖P: Proximal tubule; ¶D: Distal tubule ; **C: Collecting tubule ; ††B: Bronchial epithelium ; ‡‡T: Tracheal cartilage; §§Pe: Permanent zone; ‖‖Fe: Fetal zone; ¶¶n: Not evaluable.

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various time intervals.[5] This type of experiment is performed to evaluate the real autolysis rates of specific tissues. Our model using Sprague-Dawley rat fetuses is designed to determine the relative rates of autolysis of tissues. The autolysis curve obtained from this model shows a relatively flat slope over the first 24 hours.

In our work, we classify each tissue type specifically as possible. For example, we evaluate proximal and distal renal cortical tubules separately. The previous work for fetal death evaluation used the adrenal gland as a single tissue type,[3] but the fetal adrenal gland can be divided into inner fetal and outer permanent zone, and the only fetal zone showed early autolytic changes. Therefore, we evaluate each zone of the adrenal gland separately. In addition, while it has been reported that the thymus is a poor predictor of autolysis,[3] we use antibodies to LCA and CK to discriminate between leukocytes and epithelial cells before evaluating them separately (Fig. 1).

While we are able to generate an autolysis equation from the scores obtained from H&E staining alone, some limitations were also revealed. The autolysis score were dropped from 77.78 (24 hours after intrauterine death) to 44.44 (48 hours after intrauterine death), and elevated to 62.50 (60 hours after intrauterine death). The fluctuation of autolysis scores after 24 hours after intrauterine death was a main problem of this autolysis equation. We propose this autolysis equation or using the autolysis score to estimate the intrauterine fetal death should be confined within 24 hours after intrauterine fetal death. The more data would be needed to define more elaborate autolysis equation.

In evaluating the tissue features of autolysis, any pathologic conditions affecting autolysis rate should be excluded too. In the fetal autopsy review, fetal hydrops, local effusion, and sepsis would cause the accelerated autolysis. Previous study has suggested that extremely premature human fetuses (before 25 weeks) undergo decelerated autolysis.[3] But in our study, the gestational age of human fetus is not concerned with the autolysis rates, especially which confined the cases within 24 hours after intrauterine fetal death. The validity of estimating IURT by an autolysis equation depends on the number of histologic predicators but we could not determined how many tissue samples are required to generate a valid autolysis equation. Even that IURT could estimate prior to 180 hours, we recommend these data should be used for 24 hours after fetal death *in utero*.

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