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Fetal Visceral Maturation: A Useful Contribution to Gestational Age Estimation in Human Fetuses

ABSTRACT: With regard to the law, estimating fetal age is essential to assess viability (after 20 weeks according to the WHO) and the proposed methods generally use long bone measurements. Here, we evaluated the accuracy of soft tissue maturational stage and compared it with long bone measurements. First, eight kinds of tissues or organs from 448 fetuses with known medical history were studied (macroscopically or histologically). We clearly demonstrated that adrenal glands and skin were very good age indicators, because some characteristics appeared only after 20 weeks. We established a linear regression with a 95% confidence interval of +/− 2.9 weeks. Second, we applied our original formula using femur measurement and we combined soft tissues and bones in a multiparametric regression. The confidence interval was reduced to +/− 2.5 weeks. We conclude that the pathologist must use both histological and anthropometric data to determine fetal age as accurately as possible.

KEYWORDS: forensic science, fetus, visceral maturation, age estimation, femur

Determination of age at death is one of the major preoccupations of forensic medicine. With regard to the French law, fetal age determination is extremely important to assess whether a fetus was or was not medically viable. The World Health Organization has set this viability threshold at 20 weeks gestation. This threshold is now considered as an indispensable juristic condition to qualify a homicide in courts.

While age determination methods for adults are somewhat imprecise, those developed for non-matures show interesting determination ranges (1). For fetuses, the proposed age determination methods are quite good but give wide confidence intervals for length of gestation (2). Moreover, some methods suffer from a lack of reliability (3). Methods generally take into account macroscopic or radiographic examinations, including measurements of body weight, crown-heel length, crown-rump length, length of the foot or of the ossified portion of the long bones (4–7). Each of these parameters is generally used to estimate fetal age by comparison with reference tables or regression equations proposed in the literature. Looking at these references, we observed that histological examination of tissues and organs is rarely done, even though the chronology of fetal tissue development is well established (8,9).

This study had a dual purpose: firstly, we wished to determine the maturational level of eight tissues and organs sampled from 448 normal fetuses, on the basis of macroscopic or histological examination. The aim of this part of the study was to assess the usefulness of each tissue or organ in age assessment and especially in situating age around the threshold of 20 weeks. Secondly, we compared the estimated gestational age with that obtained by long bone measurement.

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Material

A light microscopic study was performed on normal human fetuses ranging from 12 to 40 weeks gestation. Fetuses were chosen from anonymous fetopathological records, and we studied those whose cause of death was identified as in utero death or spontaneous abortion. More than 2500 of the above-mentioned reports were studied (this sample corresponded to postmortem examinations performed between 1997 and 2000), and we selected 448 fetuses that were classified as "normal" according to the same criteria applied in our previous studies (10). Gestational age was based on maternal data (last menses: Naegele's rule) and completed with ultrasound data obtained at 10 weeks gestation (this examination is obligatory under French law). We excluded all cases in which these two methods gave discordant age estimations, as well as those that were too poorly preserved.

Methods

We obtained seven different histological samples: skin (three samples, from the scalp, hand, and foot), thymus, lungs, thyroid gland, kidneys, and adrenal glands. The central nervous system was only macroscopically observed to estimate gestational age from Fees-Higgins and Larroche's atlas (11).

All samples were fixed in 10% formalin and embedded in paraffin; 5 μm sections were stained with hematoxylin-phloxine-safran for light microscopy.

The examinations, both microscopic or macroscopic, were done to evaluate the degree of visceral maturation based on knowledge of the developmental chronology of fetal tissue:

- Concerning the skin (example in Fig. 1), thymus, lungs (example in Fig. 3), thyroid and adrenal glands (Fig. 6), we observed

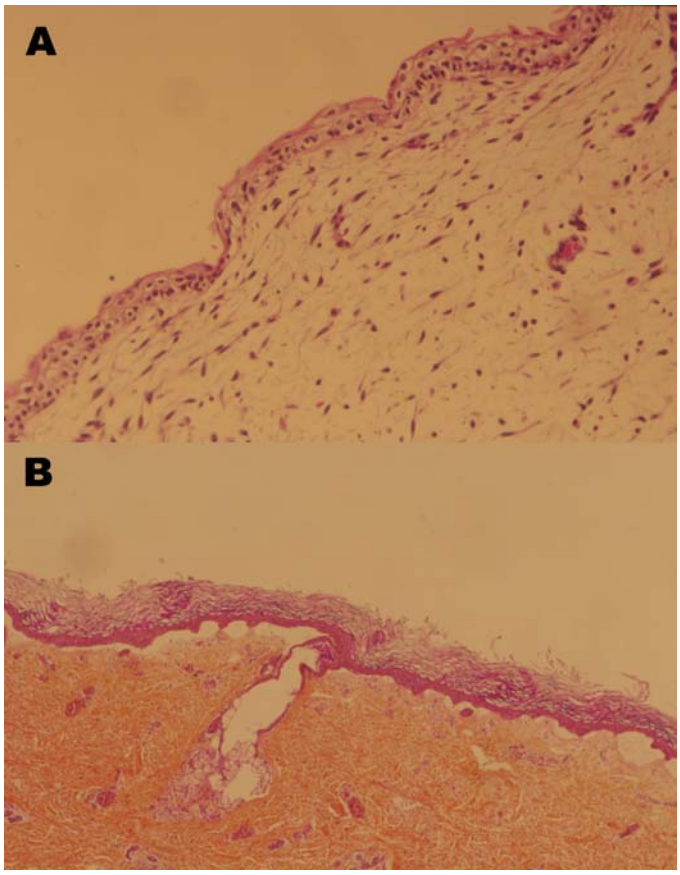

FIG. 1—*Skin of the abdomen × 100. A: 13 weeks fetus. B: 38 weeks fetus.*

the developmental stage of specific cellular elements. The selected elements, description of the developmental stages and chosen coding method are summarized in Table 1.

- We also carried out quantitative analysis of the kidneys and lungs. For the kidneys, gestational age was estimated by counting the rows of glomeruli between two well-oriented columns of Bertin running from the arcuate artery to the nephrogenic zone. For the lungs, we added the radial alveolar count described by Emery and Mithal: we counted the number of alveoli crossed by an imaginary straight line drawn from the center of a terminal bronchiole to the nearest pleural surface (12). In both cases, we coded "0" when putrefaction was observed (Figs. 4, 5).
- For the central nervous system, we chose macroscopic examination because the brain gyration pattern is well known

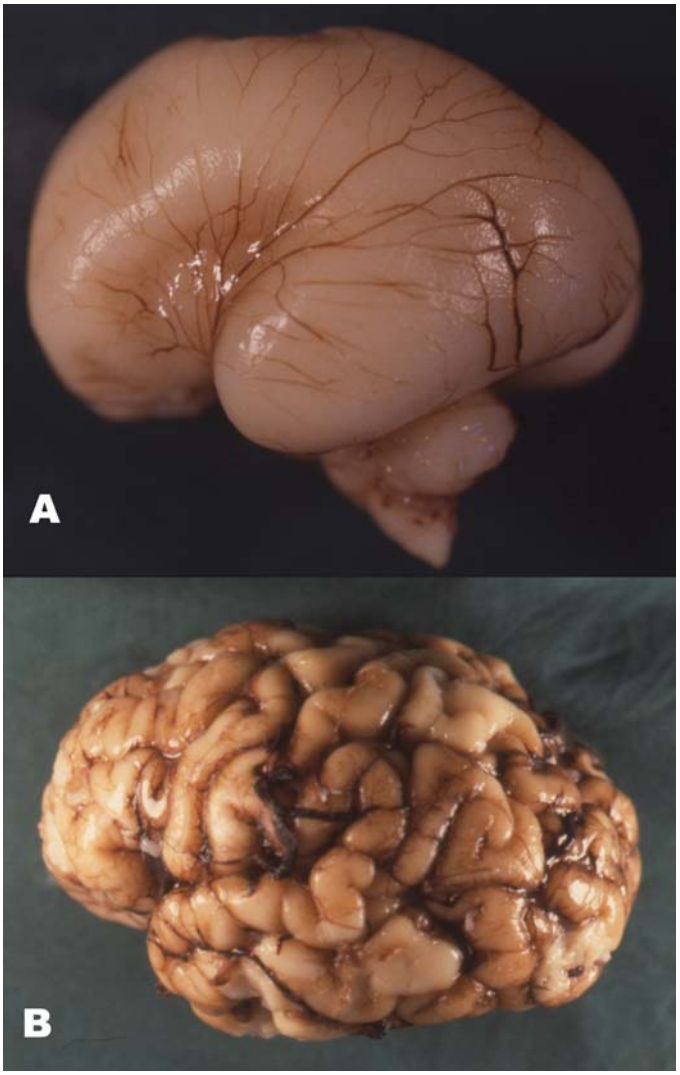

FIG. 2—*Macroscopic view of fetal brain. A: 17 weeks fetus. B: 37 weeks fetus.*

to be particularly valuable for assessing gestational age. It has been clearly established that the surface of the brain is smooth at 20 weeks gestation and that it progressively takes on its mature appearance with the development of secondary gyri. Firstly, we compared maturational level with maturational stages and we estimated gestational age from the anatomical atlas (11) (Fig. 2). Secondly, we measured the anteroposterior

TABLE 1—*Coding method to describe the developmental stages.*

	Skin					
Code	Stratum Corneum	Pacini Bodies (or Annexial Glands)	Thymus	Thyroid Gland	Adrenal Glands	Lungs
0			Putrefactive Sample			
1	Absent	Absent	No Hassal corpuscle	Small vesicles without colloids	Presence of neuroblastic nests	Glandular stage
2	Present	Present in the hand	One Hassal corpuscle per field (×400)	Few vesicles without colloids	No neuroblastic nests	Canalicular stage
3	Present	Present in the hand and foot	Two Hassal corpuscles per field (×400)	Adult pattern	. . .	Saccular stage
4	. . .	. . .	Three Hassal corpuscles per field (×400)	. . .	. . .	Alveolar stage

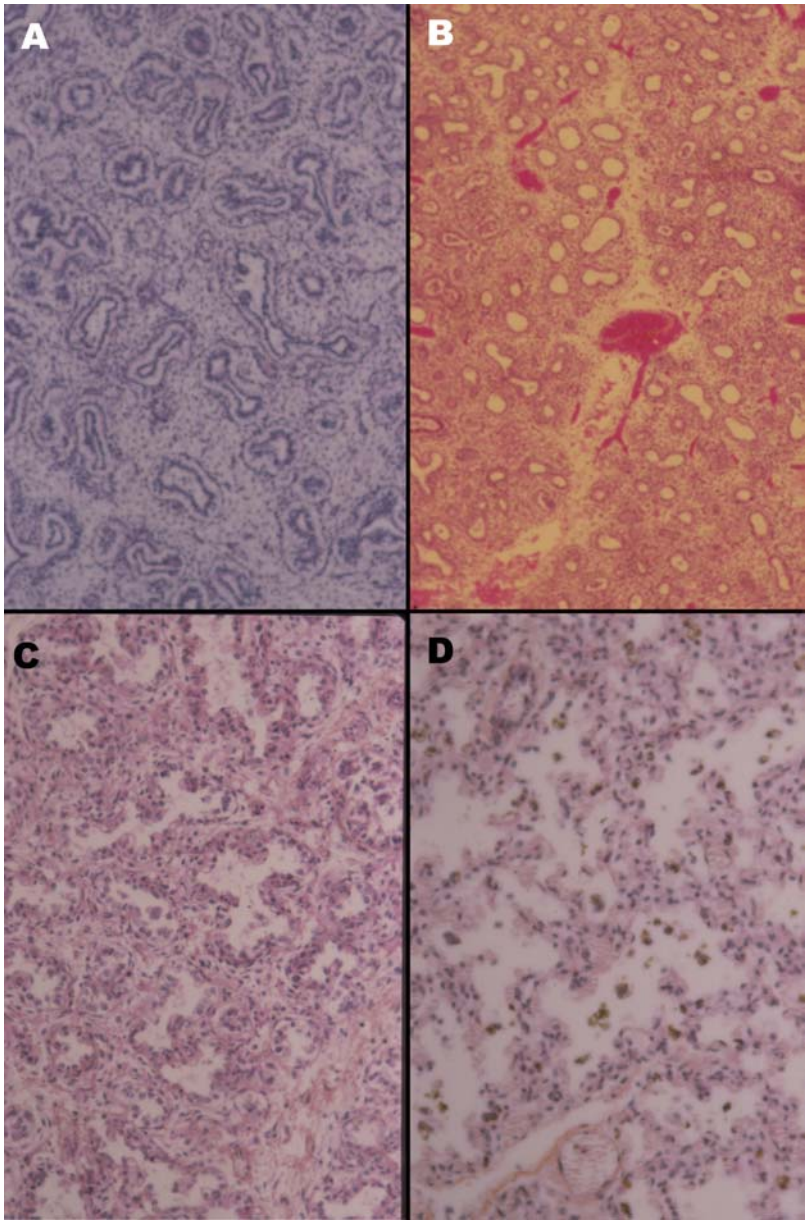

FIG. 3—*Histologic pattern of lungs and illustration of developmental levels. A: Pseudo glandular (×200). B: Canalicular (×100). C: Saccular (×200). D: Alveolar (×200).*

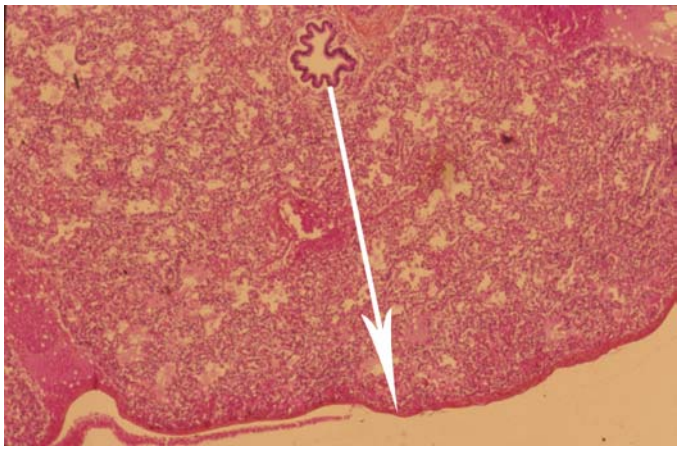

FIG. 4—*Count of the number of alveoles from the last bronchiole (white arrow) (×50).*

hemispheric length of the left lobe, since it has been demonstrated that hemispheres increase by about 10 mm every two weeks. Samples were coded "0" when cerebral tissue was putrefied.

- For measurement of femur length, we used the age determination method which was developed in our previous papers from radiographic studies (10). Following this experimental protocol, we measured 344 femurs and proposed the following regression equation:

$$\text{Predicted age (weeks)} = 0.434 \times \text{femur length (mm)} + 6.93$$

Statistics

The complete sample of normal fetuses was divided into *learning* and *validation* samples. In this way, the established formula can be tested on a sample that was not used for its computation, avoiding

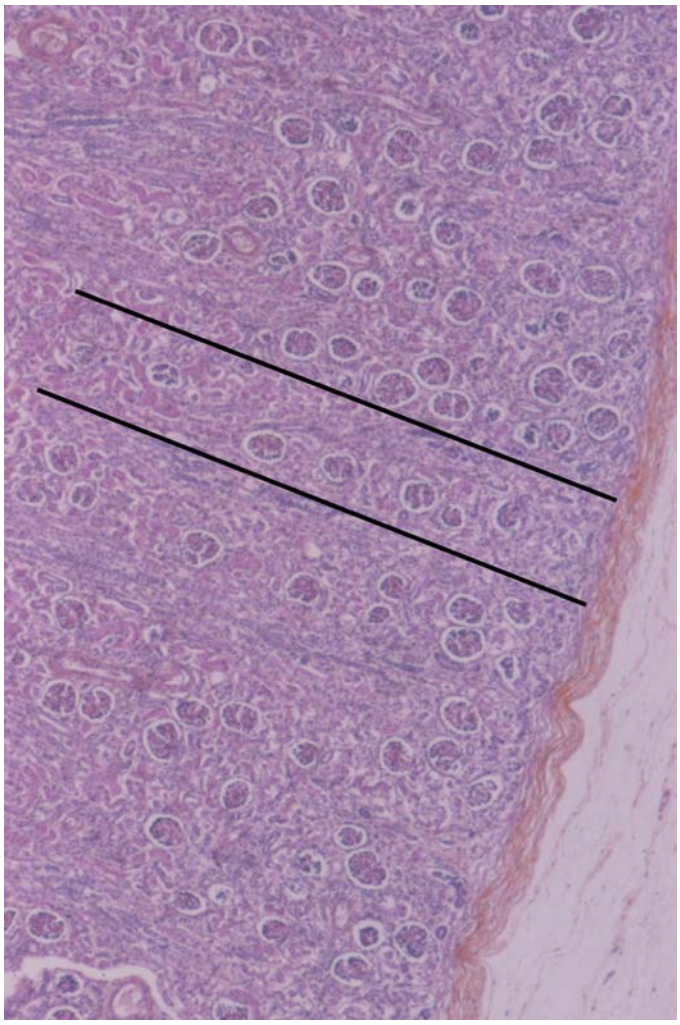

FIG. 5—*Histologic view of a kidney from a 22 weeks fetus (×100). The black lines delimitate the column used for glomerular count.*

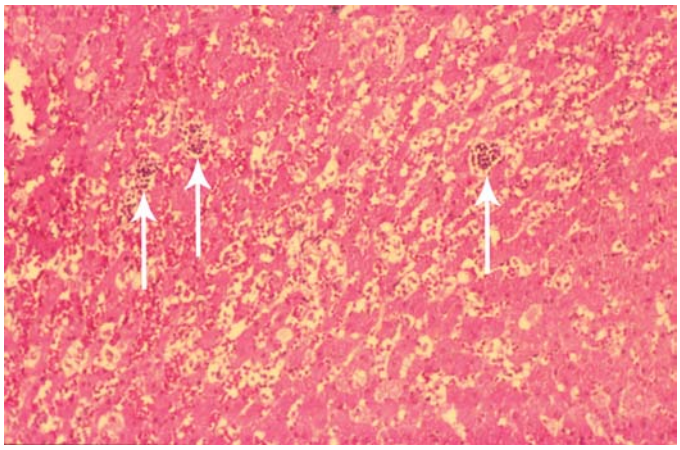

FIG. 6—*Surrenal gland: neuroblastic nests of a 12 weeks fetus (×100). White arrows show the nests.*

the statistical bias of auto-validation. The validation sample was completed by 30 randomly selected fetuses.

Both qualitative and quantitative parameters were used to evaluate maturational stages. Because qualitative parameters can be subjective, we focused on the reliability of the observations. So as in our earlier studies, we tested "repeatability," which is the difference between two measurements taken at two different times by the same observer and "reproducibility," which is the error attributable to the change of observers (10). In order to test this phenomenon, we again observed the validation sample after a three-month interval and asked a second observer (a trained fetopathologist) to re-evaluate the same cases.

The distribution of maturational stages around the threshold of 20 weeks gestation (i.e., 22 weeks amenorrhea) was expressed as a percentage and age was determined using uniparametric and multiparametric linear regression models.

Results

Reliability Testing

Observations concerning brain, skin and adrenal gland samples gave exactly the same results in both repeatability and reproducibility tests. Lung sample examinations showed no statistical differences, with a mean difference of 0.2 for both repeatability and reproducibility tests. Thyroid and thymus samples showed marked differences between the two observers. For kidney samples, there were some differences in 25% of the observations made by two different observers and in 10% of the observations made by the same observer at two different times. Nevertheless, these differences in the count never exceeded 1 glomerulus.

Value of Maturational Stages in Age Assessment

Distributions of maturational levels in relation to gestational age are presented in Table 2. We observed that some maturational levels are seen only after 20 weeks, and are therefore very good indicators for age assessment. An example is the absence of neuroblastic nests in the adrenal glands (stage coded "2"), as these disappear only after 20 weeks gestation. Some other maturational levels are also good indicators, such as the presence of the stratum corneum in the skin, which corresponds to an age of over 20 weeks in 94% of cases. The presence of Pacini bodies corresponds to an age of over 20 weeks in 98% of cases when they are located in the hand and in 100% of cases when they are also located in the foot.

In the thyroid and thymus, we found no significant pattern changes that could reliably determine age.

Study of the gyral pattern and age determination using the atlas accurately predicted age in 36% of cases, and the difference between true gestational age and estimated age was less than two weeks in 92% of cases.

Value of Quantitative Parameters in Age Assessment

The linear regression equations are presented in Table 3. For uniparametric regressions, we observed that the better 95% confidence interval was obtained with brain length measurement and glomerular count since they both showed a determination range of 8.8 weeks (centered prediction plus or minus 4.4 weeks). Multiparametric stepwise regression showed a better determination range since its 95% confidence interval was $+/-$ 2.9 weeks.

Comparison of these formulas with that using femur length measurement showed that the femur was the best parameter with simple regression: its 95% confidence interval was $+/-3.3$ weeks. Nevertheless, when entering histological parameters and femur length in stepwise regression, we observed that the glomerular count and the femur improved the regressions previously obtained. This multiparametric regression showed an excellent determination coefficient ($R^2 = 95\%$) and a good prediction interval since its 95% confidence interval was defined by the centered prediction $+/-2.5$

TABLE 2—*Statistical analysis*.

Stratum Corneum			Pacini Bodies or Annexial Glands			Adrenal Glands		
Number of Observations		329	Number of Observations		329	Number of Observations		329
0	Number	22	0	Number	32	0	Number	39
	Frequency	6.68%		Frequency	9.72%		Frequency	11.85%
	Min Age	11		Min Age	11		Min Age	10
	Max Age	33		Max Age	37		Max Age	38
1	Number	137	1	Number	176	1	Number	196
	Frequency	41.64%		Frequency	53.49%		Frequency	59.57%
	Min Age	10		Min Age	10		Min Age	12
	Max Age	31		Max Age	29		Max Age	26
	Percentage < 20 weeks	63.50%		Percentage < 20 weeks	51.13%		Percentage < 20 weeks	44.38%
	Percentage > = 20 weeks	36.49%		Percentage > = 20 weeks	48.86%		Percentage > = 20 weeks	55.61%
2	Number	170	2	Number	51	2	Number	94
	Frequency	51.67%		Frequency	15.50%		Frequency	28.57%
	Min Age	11		Min Age	18		Min Age	21
	Max Age	40		Max Age	31		Max Age	39
	Percentage < 20 weeks	5.88%		Percentage < 20 weeks	1.96%		Percentage < 20 weeks	0%
	Percentage > = 20 weeks	94.11%		Percentage > = 20 weeks	98.03%		Percentage > = 20 weeks	100%
			3	Number	70			
				Frequency	21.27%			
				Min Age	22			
				Max Age	39			
				Percentage < 20 weeks	0%			
				Percentage > = 20 weeks	100%			

TABLE 3—*Linear regression to predicted age with each tissue or organ studied*.

Parameter	Formula	95% CI
Central nervous system	Age = 2.25 × hemisphere length + 8.45	+/−4.4 weeks
Lung maturational stage	Age = 1.24 × lung maturational stage − 5.56	+/−5.9 weeks
Radial alveolar count	Age = 6.06 × radial alveolar count + 8.86	+/−5.5 weeks
Kidneys	Age = 1.51 × glomerular count + 14.37	+/−4.4 weeks
Multiparametric analysis	Age = (1.025 × glomerular count) + (0.499 × hemisphere length) + 14.786	+/−2.9 weeks

weeks. This equation is:

$$\text{Predicted age (weeks)} = 0.715 \times \text{glomerular count} + 0.201 \times \text{femur length (mm)} + 10.59$$

When we applied this age prediction to the validation sample, we observed that all residues (differences between true age and predicted age) were within the confidence interval.

Discussion

Over three consecutive years, we selected 448 fetuses in order to study the value of various parameters for the determination of fetal age, including histological parameters. For several decades, gestational age has been assessed only by fetal biometry using ultrasound techniques (4,13–17). Even if it appears that the biometric expression of growth changes through the ages (secular tendencies) and varies under different conditions (environmental, economic . . .), the variability of organ maturation is very weak. Therefore, the association of metric expression of growth with organ maturation could provide further information, increase the applicability of the results and improve the accuracy of age assessment (8).

Although there are cogent scientific reasons for relating growth to the time elapsed since conception, the practical problems this implies mean that few studies have been done, especially in postmortem conditions. Biometric studies based on necropsy series of a large number of fetuses are less numerous than those using ultrasound measurements of surviving infants (18). In our study, we selected a large population of normal fetuses and we also took maternal health into consideration. The relationship between fetal growth and maternal health has been widely studied and maternal diabetes and hypertension have been found to play an important role in fetal growth; they must therefore be excluded from this type of study (19,20). Concerning the age distribution of our study sample, it corresponds to the usual distribution of in utero deaths and spontaneous abortions and involves an imbalance in the number of fetuses for each age (9). This difference was probably responsible for the size of the confidence intervals. In a further study, we will equalize the cases of normal fetuses per age.

The second difficulty frequently encountered in fetal growth studies concerns true fetal age. Many postmortem studies were based on forensic cases and the "reference" age was based on a single parameter, generally crown-foot length or femur length (21–24). It was therefore an approximate age and not true age. In our study, fetal age was known because it was determined by the first day of the woman's last menstrual period (Naegele's rule). Temptation to correct this gestational age is prevalent now that obstetric ultrasound is routinely used to supplement menstrual data, and we excluded all cases in which menstrual age did not agree with sonographic age.

Histological Examination

Histological examination of fetal viscera can be very helpful for forensic pathologists since most organs show marked qualitative or quantitative changes during development. Of course, individual variations exist but visceral development is less variable than some anthropometric parameters such as weight (9).

The gyral pattern was particularly valuable for assessing gestational age, in the middle of fetal development, between the 20th and the 36th week. Gyral and sulcal development is not usually affected by the conditions that can result in intrauterine growth retardation (25,26). Nevertheless, tissue is poorly preserved and brain maceration often makes study impossible.

The count of the glomerular zone extends from the top of the superficial definitive glomerulus to the bottom of the deepest glomerulus, at the junction with the medulla. The nephrogenic zone is quiescent until about 23 weeks (two rows of mature glomeruli are present in the deeper cortex), when glomeruli start to form at the rate of one row per week and continue for the next 12 weeks. Individual variation exists and fetuses of the same age may differ by one row of mature glomeruli (9). We also observed this difference in the reproducibility test, perhaps because the number of rows may vary in different zones of the same kidney. Histological examination of the kidney has the advantage of revealing clearly visible structural changes that are still recognizable in cases of advanced necrosis, which is frequently encountered in forensic practice.

The adrenal gland was an interesting parameter since the fetuses did not present any neuroblastic nests after 20 weeks. This result was also similar to those reported in the literature (8,26).

Maturation of the skin is easy to observe and was very useful in situating gestational age around week 20 (27). Unfortunately, after this date, the annexial gland did not show enough significant changes to determine fetal age with any great accuracy.

The lung was also a reliable parameter in cases of putrefactive change, but hypertensive maternal disease could hasten its maturation (28) and radial alveolar count required a pleural section parallel to the bronchiolar tree.

Other organs have been described as important for estimating gestational age, but our experience of fetal autopsies and some reports in the literature have shown that many pathological conditions can modify the histological examination (9,26,29). This is true of the pancreas, spleen, genital organs, and liver. For example, liver erythropoiesis decreases progressively through the last half of gestation and is quite sparse at term, but hypoxia or anemia, pathological conditions that are frequently present before fetal death (30), can be responsible for persistent erythropoiesis.

Comparison with Femur Length

In a previous study, we showed that femur length was an important parameter for estimating gestational age and we proposed a regression equation allowing age determination with a 95% confidence interval of 3.3 weeks (10). This interval was better than those obtained in the present work when using histological parameters alone. But the association of femur length and some pertinent histological parameters resulted in a notable decrease of the prediction interval.

In practice, it appears that the pathologist must correlate histological with anthropometric data in order to estimate gestational age as accurately as possible. While most organs, such as the central nervous system or thyroid gland, are very liable to autolysis, others (skin, lungs, kidneys) are still recognizable even in cases of advanced maceration and are very useful in forensic determination.

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