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Radial alveolar count as a tool for the estimation of fetal age

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Abstract A total of 53 normal fetuses with a gestational age ranging from 15 up to 39 weeks was investigated and the radial alveolar count (RAC) was estimated as a parameter for lung maturation. Values lower than 2.0 could only be found in lungs of fetuses aged less than 18 weeks. Between 18 and 25 weeks of gestation, relatively constant levels of RAC were observed but with considerable interindividual variation. In fetuses with a gestational age of more than 25 and especially 30 weeks, a slight or rapid increase in RAC occurred respectively. Values lower than 3.0 were found up to a fetal age of less than 30 weeks and a RAC of more than 4.0 was only found in lungs of fetuses aged more than 30 weeks. Values exceeding 6.0 occurred only in fetuses at near full-term birth. Since the estimation of RAC overcomes the effects of varying degrees of alveolar collapse, such an analysis also seems to be useful for the determination of fetal age in cases of advanced putrefaction.

Key words Fetal age · Radial alveolar count · Lung maturation · Morphometry

Introduction

The estimation of the individual age of an unknown corpse is a relatively frequent problem in forensic medicine [11] while determination of fetal age can be of significance in illegal abortions. The most reliable methods are measurements of fetal body length and body weight but these parameters are only useful in the examination of intact fetuses. Furthermore, body length and body weight are considerably influenced by postmortem changes in particular putrefaction or drying artefacts which limits their use for the determination of gestational age in cases with lengthy postmortem intervals. The maturation of internal organs also depends on fetal age and an analysis of organ development should provide further information on this topic. In this context, the fetal lung is of particular interest [4, 8] since the evaluation of lung maturation is useful not only as a parameter of fetal age but also as a means of estimating a theoretical survival chance of a fetus.

Material and methods

A total of 53 stillborn fetuses with a fetal age ranging from 15 to 39 weeks autopsied at the Department of Pathology of the University of Munich, Germany, was investigated. The fetuses were delivered spontaneously or by induced labour after intrauterine death from different causes (malfunction of the placenta, chorioamnionitis etc.). Cases with macroscopical or microscopical signs of malformation or pulmonary conditions which could have influenced

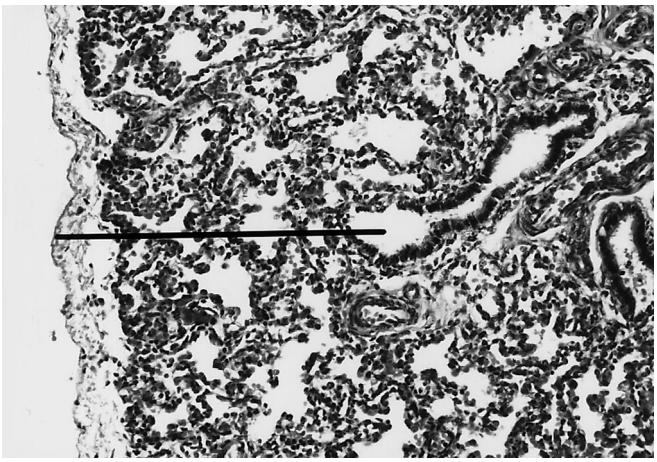

Fig. 1 Lung of a fetus aged 28 weeks of gestation, mean radial alveolar count (RAC): 3.3 (paraffin, H&E, magnification 95 ×)

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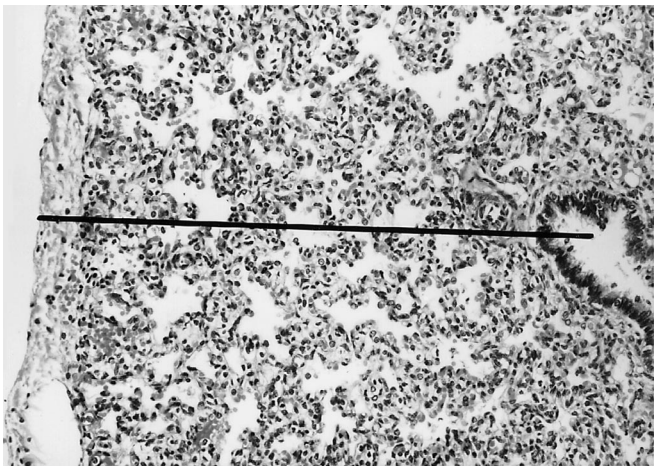

Fig. 2 Lung section of a fetus with a gestational age of 38 weeks, mean radial alveolar count (RAC): 6.2 (paraffin, H&E, magnification 95 ×)

the radial alveolar count such as pulmonary hypoplasia, diaphragmatic hernia, anencephaly etc. [1, 5, 10, 12] were not included. The gestational age had been determined according to detailed clinical (date of last menstrual cycle of the mother) and ultrasonographical data and fetal body length and weight. Following fixation in 4% formaldehyde solution the lung tissue was embedded in paraffin and 3–5 μm sections were cut. After staining with H&E, a morphometrical analysis was performed estimating the radial alveolar count as described by Emery and Mithal [3]. According to this method, bronchioles partly lined by epithelium were selected. From the centre of such a respiratory tube, a line was drawn to the nearest and definite connective tissue septum at right angles to the epithelium (Figs. 1 and 2). The number of alveoli cut by this line was then counted and at least ten counts were made for each fetal lung, mainly from two or three histological sections. The mean value for the radial alveolar count was taken as a measure of lung maturation.

Results

In fetuses with a gestational age of less than 18 weeks, the values of the radial alveolar count (RAC) did not exceed 2.0. In the period between 18 and 25 weeks, relatively constant levels with considerable interindividual variation were observed. The values ranged between 2.2 in a fetus aged 18 weeks and 3.3 in a fetus with a gestational age of 20 weeks. In fetuses aged between 25 and 30 weeks of gestation a continuous slight increase was found and after 30 weeks a rapid increase in the values of RAC occurred dependent on gestational age. In this interval of fetal life, values between 2.5 (25 weeks of gestation) and 4.0 (29 weeks of gestation) could be found. The maximum value was about 6.3 and was detected in fetuses aged 37 or 39 weeks (Fig. 3).

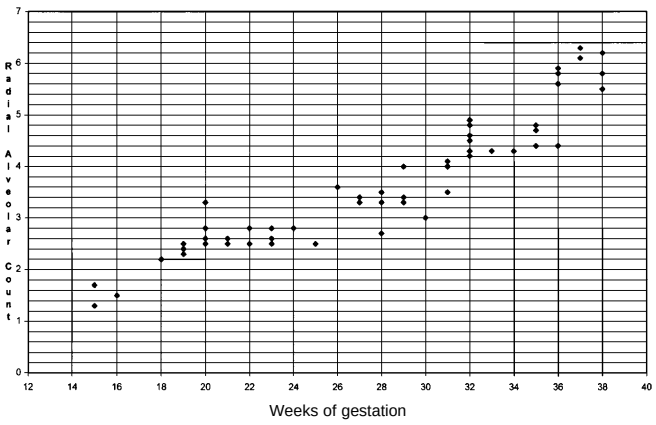

Fig. 3 Increase of the mean radial alveolar count (RAC) in relation to gestational age (n = 53)

Discussion

Emery and Mithal [3] estimated the number of alveoli cut by a line which was dropped at right angles to the epithelium from the centre of terminal respiratory bronchioles to the nearest connective tissue septum as a criterion of lung maturation. This parameter was termed the radial alveolar count (RAC) and the authors found it to increase continuously during fetal and postnatal development. In that study, a great variability was reported. The authors, however, did not perform an analysis of the RAC in relation to different gestational ages. In particular, the considerable interindividual variability described by Emery and Mithal as well as the small number of cases evaluated could explain the somewhat reduced values reported. A further study dealing with RAC values in normal fetuses was performed by Askenazi and Perlman [1] but because only two groups of fetal age less than or greater than 36 weeks of gestation were evaluated, no detailed information on a fetal age-dependent increase in the RAC-values was obtained. In contrast, no clusters of fetal age groups were analysed in our study but in the current series of fetuses of various ages between 15 and 39 weeks of gestation more detailed information was provided on an age-dependent increase in RAC. Our results indicate that values of RAC lower than 2.0 can be found exclusively in fetuses aged less than 18 weeks of gestation. In the period between 19 and 25 weeks, the levels of RAC are relatively constant and start to increase slightly up to a gestational age of 30 weeks with a rapid increase being observed after 30 weeks of gestational age. These observations which correspond to the results of Emery and Mithal [3] can be interpreted as an indicator of significant changes in lung maturation as also confirmed by recent findings obtained by the analysis of surfactant-producing alveolar type II cells in fetal lungs. Production of surfactant can be expected earliest after approximately 25 weeks up to 30 weeks of gestation [2, 6, 7, 13]. These observations support the assumption of Murphy [9] that this stage of fetal life can be regarded as a „watershed of survival“ since up to this period no significant stages of lung maturation occur. In contrast to the morphometrical analysis of surfactant-producing alveolar type II cells detected by immunohistochemistry [2], the radial alveolar count has the advantage of showing an almost continuous increase with advancing gestational age

while the number of surfactant-producing alveolar type II cells per microscopic field is influenced by the maturation-induced increase in volume of alveolar spaces leading to a relative decrease of these cells per unit area. This limits the use of such an analysis for an estimation of the gestational age in older fetuses. Furthermore, measuring the RAC should overcome the effects of varying degrees of alveolar collapse and could therefore possibly provide useful information on lung maturation even in cases of putrefaction although we did not test this situation. Since RAC estimates depend exclusively on such structures as alveolar septa, bronchioles and connective tissue septa, the estimation of this parameter can also be performed even in fetal lungs showing loss of nuclear staining due to severe putrefactive processes.

As reported by Askenazi and Perlman [1] and Reale and Esterly [10], the RAC is influenced by disturbances in pulmonary development such as lung hypoplasia, so that in these cases the RAC provides no reliable estimation of fetal age.

It must be emphasized that RAC measurements cannot specify the exact age in weeks because of the considerable interindividual variation. This method can, however, provide the following information on the gestational age of an unknown (even fragmented and/or putrefied) fetus:

1. Values of RAC lower than 2.0 indicate a gestational age of less than approximately 18 weeks and can exclude a potential for extrauterine survival due to the stage of lung maturation.

2. RACs of less than 3.0 can be observed exclusively up to a fetal age of approximately 30weeks indicating a corresponding maximum gestational age.

3. Values of RAC exceeding 4.0 or 6.0 indicate a gestational age of more than (approximately) 30 weeks or a fetal age near to full-term birth, respectively.

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