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The fetal lung 1: developmental aspects

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ABSTRACT

A literature survey is presented on normal fetal development during the embryonic, pseudoglandular, canalicular, saccular and alveolar stages in the human fetus. Normal anatomical and physiological aspects of fetal lung development including the fetal pulmonary circulation are described. Factors which may influence fetal lung growth and consequently may play a role in the development of pulmonary hypoplasia are discussed, such as intrauterine and intrathoracic space, lung fluid, fetal breathing movements, normal balance of volume and pressure in the lung and interference with the blood supply.

INTRODUCTION

A number of papers have recently appeared in this journal on normal and abnormal Doppler velocimetry of the fetal pulmonary circulation[1–8]. To appreciate the significance of this research for clinical monitoring of fetal lung development, insight is needed into growth and development of the fetal lung in general. This review will focus on normal fetal lung development with the emphasis on lung morphology and physiology including various factors influencing fetal lung growth.

NORMAL FETAL LUNG DEVELOPMENT

Human lung development is divided into five stages: embryonic, pseudoglandular, canalicular, saccular and alveolar[9–19] (Figure 1). One should realize that the transition between stages occurs gradually and that there is considerable overlap from one stage to the next, and also between various areas within the lung and between various gestational ages and individuals[9–11]. The anatomic and morphologic characteristics of each stage are as follows:

Embryonic stage (conception – 6th week of gestation)

The human fetal lung arises as a ventral diverticulum from the caudal end of the laryngotracheal groove of the foregut at the end of the fourth week. During the next few weeks this diverticulum grows caudally to form the primitive trachea, and then, the end of the diverticulum divides into two sacs, the lung buds. These two buds develop lobar buds that correspond to the mature lung lobes, i.e. three on the right and two on the left. The lobar buds will have further subdivided and by the end of the sixth week all bronchopulmonary segments will have been formed. The primitive lung bud is lined with endodermally derived epithelium.

Pseudoglandular stage (6th – 16th week of gestation)

Stimulated by the presence of surrounding mesenchyme, conducting airways are formed by repeated dichotomous branching resulting in a tree of narrow, thick epithelial-lined tubules. During this phase the lung has a distinctly glandular appearance, hence the term pseudoglandular. By the 16th week of gestation the tracheobronchial tree has been formed, from the trachea up to and including the terminal bronchioles. These branches, or pre-acinar airways, may increase in size as further growth of the lungs occurs, but new branches cannot be formed after the 16th week. The mesenchyme surrounding the lung bud diverticulum differentiates to form the early rudiments of cartilage, connective tissue, muscle, blood vessels, and lymphatics. The epithelio–mesenchymal interactions play a determining role in regulating growth and branching patterns[16,17]. Recently, it has become clear that both embryonic and pseudoglandular stages are histologically similar and should therefore be referred to as the pseudoglandular period[19].

Canalicular stage (16th – 28th week of gestation)

During this phase, the basic structure of the gas-exchanging portion of the lung is formed and vascularized. Since the cephalad segments of the lungs mature faster than the

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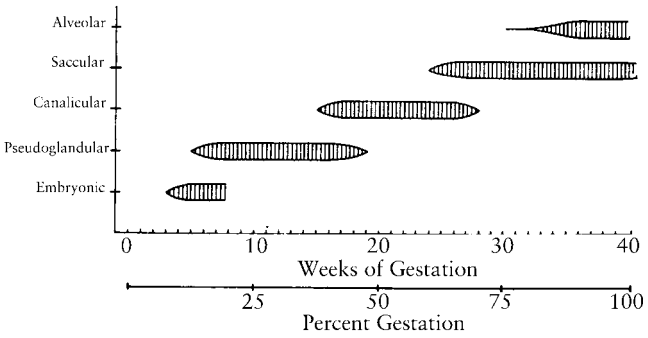

Figure 1 Schematic outline of the development of the human lung. [Reprinted with permission of Pringle KC. (1986) Human fetal lung development and related animal models. *Clinical Obstetrics and Gynecology* 23: 502–513.][11]

caudal parts, this stage partially overlaps the previous one. Differentiation of the airways starts with widening of the lumina and gradual thinning of the cuboidal epithelium. Proliferation of a rich vascular supply occurs and due to a relative decrease in mesenchyme the capillaries become closer to the airway epithelium. Primitive respiratory bronchioles begin to form, thus delineating the acinus. The acinus is the gas-exchanging portion of the tracheobronchial tree, composed of respiratory bronchioles, alveolar ducts, sacs and alveoli. Flattening of the acinar epithelium at approximately 22–24 weeks marks the initial differentiation of type II pneumocytes, from which type I pneumocytes will be derived later. Type II pneumocytes produce surfactant. Type I pneumocytes are responsible for gas exchange.

Saccular stage (28th – 36th week of gestation)

At the beginning of the saccular stage airways terminate in large smooth-walled cylindrical structures subdivided by ridges called crests. The crests protrude into saccules, pulling a capillary network with them and creating subsaccules, which will eventually become alveoli. The end result of the saccular stage is a rapid increase in the gas-exchange surface of the lung and rapid thinning of the interstitium. During the period of 32–36 weeks, type II (surfactant producing) pneumocytes mature, completing the functional maturity of the lung.

Alveolar stage (36th week – term)

Studies by Langston *et al.*[12] have shown that alveolar structures can be recognized in some fetuses at 30–32 weeks of gestation and are uniformly present at 36 weeks of gestation, challenging previous beliefs that alveoli develop after birth[15,20–22]. The alveolar stage is not completed until 8 years of age, with the greatest increase in the number of alveoli occurring during the first 2 years of life. During this stage further thinning of the blood–gas barrier, increase of surfactant production and progressive branching of the respiratory airways occurs.

Normal lung growth in the human can be summarized according to Reid by three laws[11,20–23]:

Law 1: the bronchial tree is developed by the 16th week of gestation.

Law 2: the majority of alveoli develop after birth, increasing in number until the age of 8 years and in size until growth of the chest wall is finished.

Law 3: The pre-acinar arteries and veins parallel the development of the airways, while the intra-acinar vessels follow the development of the alveoli.

FETAL PULMONARY CIRCULATION

Development

Prenatal growth and development of the pulmonary blood vessels is closely linked to that of the bronchial tree (pre-acinar blood vessels with the conducting airways and intra-acinar blood vessels with alveolar development) (Figure 2). By the third week of gestation (embryonic period), the primitive heart anlage with its arterial and venous communications is present. The sixth branchial arches appear at about 5 weeks and develop into the main pulmonary arterial trunk with right and left branches during the next few weeks of the pseudoglandular period. By 7 weeks of gestation, the adult pattern of vessels connecting heart and lungs is established[9,24]. Just as the branching of the conducting airways is completed by 16 weeks of gestation, all pre-acinar arteries are also present by this time. These arteries grow further by increasing their lengths and diameters, but not in number. By contrast, the intra-acinar arteries develop relatively late in fetal life and continue to form after birth and then

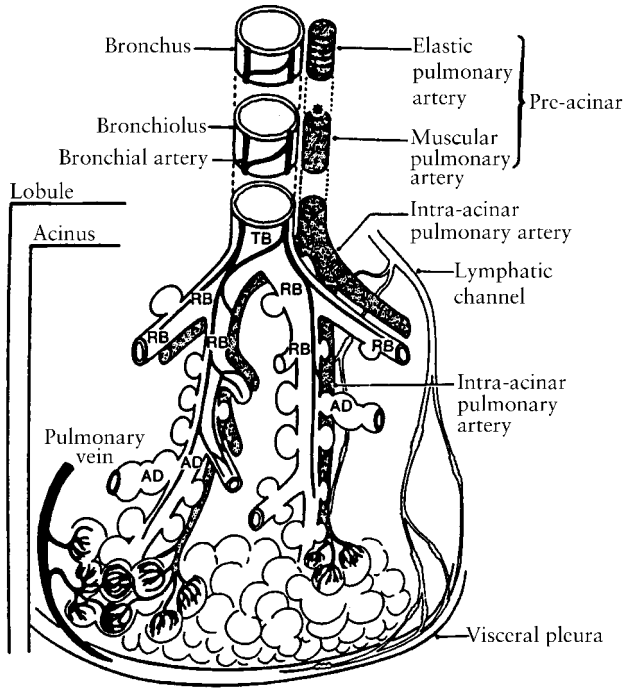

Figure 2 Diagram of the branching pattern of airways and pulmonary artery in the mature lung. TB, terminal bronchiolus; RB, respiratory bronchiolus; AD, alveolar duct. [Reprinted by permission of Anderson RH, Macartney FJ, Shineboune EA, Tynan M. (1987) *Paediatric Cardiology.* Edinburgh: Churchill Livingstone].

multiply rapidly to keep pace with alveolar multiplication[9,10,24,25]. There are two types of pulmonary arterial branches in the lung: conventional arteries, which course with the conducting airway branches, and ultimately provide terminal branches to supply the acini. From these conventional arteries numerous and smaller branches arise, called supernumerary arteries, and pass into the adjacent respiratory tissue to supply the alveoli directly. The supernumerary arteries may serve for collateral circulation if blood flow through the conventional arteries is impeded. The pre-acinar conventional and supernumerary arteries appear simultaneously and the adult ratio of 2.5–3.5 supernumaries to one conventional artery is present by 12 weeks of gestation[9,10,24–26]. Development of the pulmonary veins occurs after that of the arteries, but by 20 weeks of gestation all pre-acinar veins are present. The development of the venous system parallels that of the arteries and airways in the fetus, with the exception that the venous system of the lungs is more extensive than the arterial system: the conventional veins correspond in number and branches to conventional arteries and conducting airways but the supernumerary veins outnumber the supernumerary arteries. Both types of veins appear together and their number maintains the same ratio (3.5–4.3 supernumerary to one conventional vein) to each other throughout pre- and postnatal life. The veins do not accompany the arteries and airways but lie in the intersegmental plane[9,24,25,27].

Structure

Whereas the total adult complement of branches of blood vessels forming the pre-acinar region is completed half-way through fetal life, the intra-acinar blood vessels develop as alveoli form and further changes of the pulmonary circulation are characterized by remodeling of the arteries, with changes in wall-thickness and in extension and distribution of muscle within their walls[9]. In the adult lung the proximal half of the pre-acinar arterial pathway down to the seventh generation has an elastic wall structure, and beyond this it is muscular. In fetal life the adult distribution of elastic structure is achieved by the 19th week of gestation[25]. The elastic arteries may play a role during fetal life as their high tensile strength may counteract the distending tendency of the blood pressure and also maintain the vessels patent[26]. The fetal pulmonary arteries are completely muscularized to the level of the terminal bronchioles, and only after birth does muscle extend to the level of alveolar ducts and beyond[10,26]. In the fetus the arteries are more muscular than in the adult, with the wall thickness being higher relative to external diameter. The wall thickness of a given size of artery in the fetus is double that in the adult[24]. This increases the resistance in the pulmonary arteries to maintain the reduced flow through the lungs seen during fetal life[26]. As gestation advances, the number of muscularized arteries rises, increasing the total amount of smooth muscle per unit area of lung tissue. In contrast to those investigators who found a rise in the amount of pulmonary arterial smooth muscle with increasing gestational age, Hislop and Reid concluded that thickness of the muscle coat of each individual artery does not change, regardless of age[25,26,28–31].

Unlike the arteries, the intrapulmonary veins have thinner walls and muscle cells are rarely found in the wall before 28 weeks of gestation[27].

Physiology

The *in utero* pulmonary vascular circuit is a high-resistance, high-pressure, low-flow system[32]. In the fetus, normal gas exchange is placental in origin and pulmonary blood flow is low, merely supplying nutritional requirements for lung growth and some metabolic functions[33]. In the fetal lamb 3–4% of the total cardiac output supplies the lungs in early gestation, rising to about 7% near term[33–35]. In human fetuses this value is 13% at 20 weeks increasing to 25% at 30 weeks, but it remains constant during the last trimester[36]. This implies that pulmonary blood flow increases and pulmonary vascular resistance decreases with advancing of gestation. Fetal pulmonary blood flow is low despite the dominance of the right ventricle, which ejects about two-thirds of total cardiac output. Most of the right ventricular output is diverted away from the lungs through the widely patent ductus arteriosus to the thoracic aorta, then reaching the placenta through the umbilical circulation for oxygenation[34,35]. Since the ductus arteriosus is patent during intrauterine life, pulmonary arterial pressure is considered to be maintained at a level at least equal to systemic arterial pressure[37]. Maintenance of a low-flow pulmonary vascular circuit depends on the high-resistance of the pulmonary vasculature. In the fetal lamb, lung development is characterized by a marked increase in the number of pulmonary resistance arteries[28]. Both the total vascular cross-sectional area and the total amount of vascular wall muscular tissue increase significantly during fetal development The increased cross-sectional area of the pulmonary vascular bed produces a lower total resistance, while the greater number of small vessels with muscular walls provides a widespread mechanism for controlling resistance[28,32]. Nevertheless, while absolute pulmonary flow increases with advancing gestation, Morin and Egan determined in fetal sheep that when corrected for wet weight of the lungs, pulmonary flow actually falls somewhat and total pulmonary resistance rises. When corrected for dry weight of the lungs neither pulmonary blood flow nor total pulmonary resistance change with advancing gestation[38].

Regulation of fetal pulmonary vascular resistance

During fetal life and the transition to extrauterine air breathing, pulmonary vascular tone is regulated by a complex, interactive group of mechanisms, including mechanical influences and the release of a variety of vaso-active substances, which have been the subject of several reviews[33,35,39–43] (Table 1). Much of our knowledge

Table 1 Regulators of pulmonary vascular resistance

	Fluid in alveolar space	*Pulmonary PO_2 ↑/↓*	*Acidosis/Alkalosis*	*Leucotrienes*	*PGI_2*	*NO*	*ET-1*
Pulmonary vascular resistance	↑	↑/↓	↑/↓	↑	↓	↓	↑

of fetal blood flow and its distribution is based on animal studies. Data on human fetuses and newborn are sparse because of the invasive techniques that would be necessary to obtain them[33].

In unventilated fetal lungs, fluid filling the alveolar space compresses the small pulmonary arteries, thereby increasing pulmonary resistance. A high pulmonary vascular resistance is associated with a normally low oxygen tension in pulmonary and systemic arterial blood. However, fetal pulmonary vascular resistance decreases with increasing oxygen tension[38,44]. This leads to the suggestion that vasoconstriction to the normal, relatively hypoxic status of the fetus is a mechanism that restricts blood flow to the expanding vascular bed in the fetal lungs, thereby protecting the fetal heart from unnecessary increases in output [38,45]. O_2-related changes in pulmonary vascular resistance are also affected by pH. As acidosis is a pulmonary vasoconstrictor, alkalosis is a potent pulmonary vasodilator at all stages of development[39,46]. The exact mechanism of hypoxic pulmonary vasoconstriction in the fetal pulmonary circulation is unclear. Although a change in O_2 environment could directly relax or vasoconstrict pulmonary vascular smooth muscle, it could also modify the local production and release of vaso-active substances, which then could act directly or indirectly on the pulmonary circulation [33,41,47,48]. Arachidonic acid metabolites have been suggested to play an important role in the regulation of the pulmonary vascular tone. Leukotrienes represent one such group of metabolites and are potent smooth muscle constrictors[33,43,49]. Prostaglandins form another group of potent vaso-active substances, although none of the prostaglandins is truly specific for the pulmonary circulation. PGI_2 seems to be the major prostaglandin with an effect on the pulmonary vascular resistance and is produced in vascular endothelial cells including those in the lungs. Throughout gestation, a maturational increase in PGI_2 production parallels the decrease in pulmonary vascular resistance in the fetal third trimester[33,41,43,46,47,49]. In addition to PGI_2, vascular endothelial cells produce other vaso-active factors, clearly involved in the regulation of the vascular tone of the fetal pulmonary circulation. These include potent vasodilators, such as endothelium-derived relaxing factor, also known as nitric oxide (NO), and potent vasoconstrictors, such as endothelin type 1 (ET-1)[33,42,43,48].

Control of the perinatal pulmonary circulation probably reflects a balance between factors producing pulmonary vasoconstriction (low O_2, leukotrienes, ET-1, and other vasoconstricting substances) and those producing pulmonary vasodilatation (high O_2, PGI_2, NO, shear stress and other vasodilatating substances)[33].

FACTORS INFLUENCING FETAL LUNG GROWTH

Fetal lung development incorporates a combination of two processes: lung growth and lung maturation, which are related, but appear to be separately controlled. Normal lung growth refers to increase in cell number and seems to be influenced primarily by physical factors such as intrauterine (i.e. amniotic fluid volume) and intrathoracic space, lung liquid volume and pressure and breathing movements. Lung maturation refers to the distensibility or compliance of the lung and is divided into two components, structural and biochemical (surfactant). Structural maturation is regulated by physical factors, whereas biochemical maturation appears to be controlled by several endocrine organs (pituitary, adrenal, thyroid) and a host of endocrine factors including corticotrophin, cortisol, thyroid hormones and others[10,11,13,50,51]. Detailed discussion of surfactant production and regulation is beyond the scope of this overview.

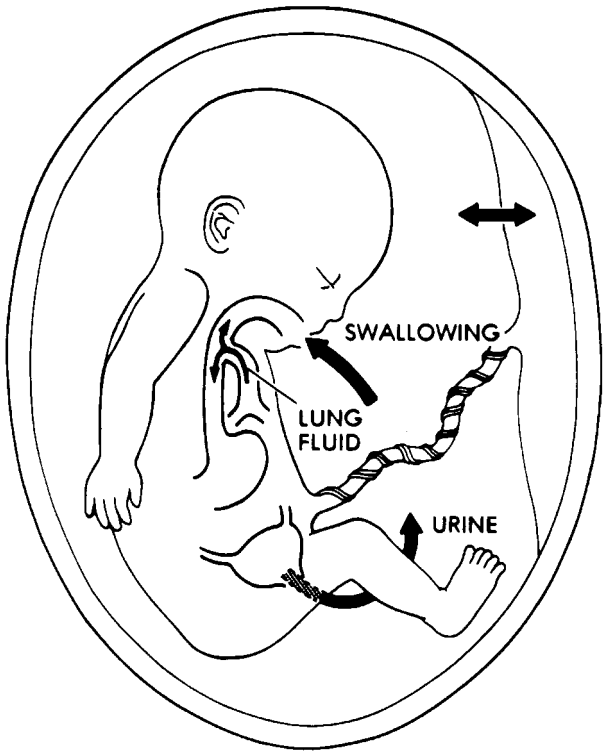

Figure 3 A schematic illustration of the dynamic process of amniotic fluid formation and resorption. [Reprinted with permission of Adzick NS, *et al*. (1984) Experimental pulmonary hypoplasia and oligohydramnios: relative contributions of lung fluid and fetal breathing movements. *J Pediatr Surg* 19: 658–665.][52]

Intrauterine space

Amniotic fluid formation and resorption is a dynamic process. Fetal urine is the primary component of amniotic fluid, but actively secreted fetal lung fluid also constitutes a significant portion, which is either swallowed or flows into the amniotic space. Fetal swallowing is the principal means of amniotic fluid resorption[52] (Figure 3). A normal amount of amniotic fluid seems to be important for fetal lung growth[10,50,53]. Bilateral renal agenesis, obstructive lesions of the urinary tract and early preterm rupture of membranes are the most common conditions that cause oligohydramnios[54]. Although the association between oligohydramnios and pulmonary hypoplasia is well-documented, the mechanisms of this phenomenon remain to be fully elucidated. Several possibilities have been adopted such as: (1) decreased space for lung growth due to compression of the uterine wall upon the fetal chest and abdomen; (2) restriction of fetal breathing movements by prolonged thoracic compression; and (3) increased efflux of lung liquid from the intrapulmonary space to the amniotic space, resulting in a decrease of intrapulmonary pressure[10,50,53].

Intrathoracic space

All abnormalities which result in a smaller than normal intrathoracic cavity can interfere with fetal lung growth and be responsible for the development of fetal pulmonary hypoplasia both in animals and in man[10,50,51]. Among the conditions known in this respect are congenital diaphragmatic hernia, fetal hydrops, tumors of the thorax including adenomatoid malformations and skeletal anomalies deforming the thoracic cage[23,50,53,55–58]. Paralysis of the diaphragm may, apart from limiting intrathoracic space, interfere with fetal breathing movements and this may have an additional negative effect on fetal lung growth[50,51,59].

Lung fluid

Fetal intrapulmonary fluid is formed by active transport across the pulmonary epithelium into the tracheo-bronchial lumen, where it establishes positive pressure within the developing lung. Lung-liquid volume and intratracheal pressure are maintained within very precise ranges by the larynx, which through unknown mechanisms regulates the efflux of lung liquid volume from the trachea to the amniotic space[51,60]. Lung liquid contributes up to one-third of amniotic fluid volume. Oligohydramnios may increase lung fluid loss with consequent decreased lung volume within the potential airways[38]. In 1977, Alcorn *et al.*[61] demonstrated in fetal sheep that chronic drainage of lung liquid through a tracheal cannula led to pulmonary hypoplasia, while tracheal ligation resulted in increased lung growth, although with a negative influence on lung maturation as assessed by the enhanced ratio of type 2 to type 1 pneumocytes. Others reported that tracheal ligation resulting in increased intratracheal pressure, is responsible for lung growth through cell proliferation. In these studies, however, lung maturation was normal, as confirmed by measurement of the surfactant phospholipids, or by quantitative morphometrics[60,62]. Several lung hypoplasia animal experiments (tracheal ligation in combination with amniotic fluid shunting in fetal rabbits, with lung fluid drainage or bilateral nephrectomy in fetal lambs or in combination with a model of congenital diaphragmatic hernia also in fetal lambs) showed that tracheal ligation did not only reverse the pulmonary hypoplasia, but could actually accelerate lung growth even beyond normal limits [10,52,60,63].

Fetal breathing movements

Fetal breathing movement in humans can be recorded by ultrasonography from about 10 weeks' gestation. These respiratory movements produce significant changes in intrathoracic pressure, which may influence lung development[50,64]. A fetal breathing movement is usually defined as a downward movement of the diaphragm with concomitant inward movement of the chest wall and outward movement of the abdominal wall[65]. However, different types of respiratory movements during gestation have been described[65–67]. Early in gestation fetal breathing is usually irregular and sporadic; from about 28 weeks of gestation onward it becomes more regular and episodic[67,68]. During the last 10 weeks of pregnancy, fetuses make spontaneous breathing movements approximately 30% of the time over a 24-h period[69]. Several factors have been identified that may influence the presence and maybe also the pattern of fetal breathing movements. Among these are gestational age, time of the day, maternal food intake and maternal plasma glucose level, maternal end-tidal carbon dioxide level, fetal behavioral state, fetal distress, maternal use of nicotine, drugs or alcohol, and labor. Some of these factors may be interrelated[66,67].

There is experimental evidence that fetal respiration is critical to lung growth and development. Wigglesworth *et al.*[59] abolished fetal breathing movements in an animal model by selective destruction of the upper cervical cord, which arrested lung development. Bilateral phrenic nerve section in sheep produces pulmonary hypoplasia, but also results in diaphragmatic atrophy and raises the issue that there may be compression of the lung by abdominal contents[13,70]. Adzick and coworkers[52] showed in another animal study that when oligohydramnios and cervical cord transection were combined, a further significant decrease in lung growth was observed. Thus, even when fetal breathing was eliminated by cord transection, oligohydramnios caused even more hypoplasia. They concluded that although fetal breathing is clearly important for fetal lung growth, it seems unlikely that inhibition of fetal breathing is the predominant etiology of oligohydramnios-related pulmonary hypoplasia. Fetal breathing movements may stimulate lung growth by intermittently distending the lungs with fluid aspirated into the trachea. It has been suggested that integration of fetal breathing movements

and fluid secretion by the lungs is necessary for lung growth [50,59].

Normal balance of volume and pressure in the lung

Fetal lung fluid establishes a positive pressure within the lung of the fetal lamb[51,54]. The presence of a positive pressure in the trachea relative to amniotic pressure shows that there is resistance to outflow[51]. When breathing movements are absent, the pressure in the fetal trachea is even higher, which may be important in regulating the volume of fluid within the lungs. So, there is evidence that lung fluid acts as an internal stent for the lung, distending potential airways and stimulating growth and differentiation[54,59]. Oligohydramnios may increase lung fluid loss by compression of the lungs, thus leading to pulmonary hypoplasia, which was demonstrated by Harding and coworkers in the fetal lamb[64]. Lung fluid production was not affected by oligohydramnios according to this study. During breathing movements in the fetal lamb a considerable negative pressure is created on inspiration and the volume of fluid within the potential airways and air spaces increases when fetal breathing occurs[50]. Based on several experimental animal studies it may be concluded that positive intratracheal pressure, amplitude of pressure changes as well as tidal volume changes during fetal breathing movements may influence fetal lung growth[50,51].

Interference with the blood supply

Ligation of the left pulmonary artery during the late canalicular stage of lung development in the fetal sheep creates pulmonary hypoplasia with significantly reduced lung weight and lung volume of the future gas-exchange portion of the lung (future air spaces, parenchymal tissue and capillary content). These results suggest that normal pulmonary arterial flow during the canalicular and alveolar stages of fetal lung development is essential for normal lung growth[11,71].

Other factors involving fetal lung development

Several transcription factors may play an important role in lung development, regulating cell growth and differentiation by modulating expression of target genes. Two functional domains are important in these proteins, one responsible for transcription activation and the other for DNA recognition and binding. Although the lung is the site of expression of several transcription factors, such as *Hox*-genes, retinoid receptors, hepatocyte nuclear factors and *myc*, sparse information is available on how they influence lung pattern[72]. Cardoso[72] has recently published an extensive review regarding these factors.

Important growth factors involving lung development are insulin-like growth factor I and II (IGF-I and IGF-II), platelet-derived growth factor (PDGF), fibroblast pneumocyte factor (FPF), transforming growth factor-β (TGF-β) and epidermal growth factor (EGF). These growth factors may regulate the extracellular matrix formation, which regulates cellular growth, migration and differentiation[73].

Pulmonary neuro-endocrine cells are amine- and peptide producing cells distributed throughout the airway mucosa. Several observations indicate that these cells are involved in lung development[74].

In case of controlling vascular growth the following growth factors have been suggested to be involved: fibroblast growth factors (FGFs), TGF-β, PDGFs, IGF-I and II and vascular endothelial growth factor[43].

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