**DISCUSSION**

In the present study, the histological examination revealed that the fetal lung of the control subgroup at the 18th day of gestation was in the late pseudoglandular and early canalicular stage where there were some air cell spaces rounded and others wavy tubules which were separated by a highly cellular mesenchyme.The primitive bronchioles were lined with columnar epithelium which showed subnuclear vacuolations. They were divided to give tubular air spaces which were lined with a cuboidal epithelium. This was in agreement with **Warburton et al. (2000), Yamada *et al. (2002),*McGowan et al. (2004),and*Maeda* et al. (2007).**

In this study, the presence of highly cellular mesenchyme in between the air spaces might interfere with the process of gas exchange so, in this stage, the lung is non functioning .This was agreed by**Warburton et al. (2010)** who said that at pseudoglandular stage, the epithelial tubes were lined with cubical epithelial cells and resembled an exocrine gland filled with secretions.

In this study, the lung of the diabetic subgroups showed a delay in the lung development and a delay in the differentiation of the type II pneumocytes. At the 18th day of gestation of the diabetic subgroup, the

fetal lungs were in the embryonic stage ,and the bronchial buds were lined by a thick layer of stratified squamous epithelium .The bronchial buds were surrounded by densely packed mesenchymal cells. These bronchial buds branched into primitive bronchioles which were lined with tall columnar epithelium. They were situated within abundant loose mesenchyme. The blood vessels appeared as haloes inside the loose mesenchyme , and were lined with flat endothelial cells and contained erythrocytes. These findings were in agreement with ***Yamada et al. (2002)*** who reported that the process of vasculogenesis was first seen in the rat lung among mesenchymal tissue in the embryonic stage.

In the present study, the fetal lungs at the 20th day of gestation of the control subgroup were in the late canalicular stage. At this stage of development, all airway generations of the fetal lung became greatly widened and elongated resulting in a marked reduction of the pulmonary interstitial connective tissue with increased number of the blood capillaries. The primitive respiratory bronchiole branched into 2 - 3 wide and straight canals termed acinar canals. These findings were in agreement with **Burri(1999),Yamada et al. (2002) and Maeda et al. (2007) .**

The beginning of pneumocyte differentiation in combination with canalization , decreasing the interstitial mesenchyme and increasing number of capillaries had appeared at this stage This was recognized by[**Burri**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Burri%20PH%5BAuthor%5D&cauthor=true&cauthor_uid=16770071)**(2006), Maeda et al. (2007) and Morrisey and Hogan (2013)** who reported that the canalicular stage served as a starting point for the initiation of the cuboidal epithelium that lines the acinar canals to differentiate into type I and type II alveolar cells. **Schittny and Burri (2007)** also mentioned that this step is considered as the birth of the acinus and at the end of this stage, the lung had reached a state of development in which gas exchange was possible.

In the present study, the fetal rat lung of diabetic mother was in the early pseudoglandular stage at 20th day of gestation where the lung showed progressive branching of the bronchial buds to primitive bronchioles in cellular mesenchymal tissue. These airways remained small tubules and were lined with columnar epithelial cells with rounded basal nuclei. Similar findings were recognized by **Yamada et al. (2002)**as pseudo glandular stage that occured at 16th day of gestation and this indicated a delayed development of the lung caused by diabetes mellitus.

In our work, the fetal lungs of the control subgroup at the 18th day of gestation were in the late pseudoglandular. Fine elastic fibers were detected in the developing epithelial tubules .However, at the 20th day of gestation, the fetal lungs were at the late canalicular stage, with fine elastic fibers were detected in the developing acinar canals.The fetal lungs of diabetic groups at 18th and 20th days of gestation, were at embryonic and early pseudoglandular stages, with the elastic fibers absent around the developing air spaces. These findings were in the agreement with **Amy et al. (1977) *and Runciman et al. (1996)*** .

In this study, the ultrastructure of offspring lung of the control subgroup showed that the empty lamellar bodies appeared as vacuolation enclosed by membranes. This finding was in the agreement with **Fehrenbach (2004)** who found that the vacuolated cuboidal cells in the lining of the pseudoglandular stage, and considered it as the precursors of type II pneumocyte of alveolar cells. Also, he suggested that vacuoles represented lamellar bodies' precursors.

In this study, there was glycogen which was detected in the cytoplasm type II pneumocytes of the fetal lung of the diabetic subgroup at 18th day of gestation.This was in the agreement with **Trevin˜o-Alanı´s et al.(2009)** who observed abundant amount of glycogen in the apical part of cytoplasm in offspring of diabetic group and said that this was sign of immaturity. **Ridsdale and Post,(2004)** have suggested that glycogen is used as a carbon source to generate the surfactant lipids but a direct precursor-prouduct relationship has not been demonstrated**. Rannel et al .,(1991)** suggested that glycogen acts as an energy source for rapidly maturating type II pneumocytes.

In the present work and at the day of birth, the offspring lungs of the control subgroup were in the saccular stage of development. During this stage, the terminal portions of the acinar canals were formed of typical clusters of widened air spaces termed air saccules. The surrounding interstitial tissue was condensed to form thick primary septa that contained a double blood capillary layer. These findings were in agreement with **Joshi and Kotecha (2007), Schittny and Burri (2007), and Smith et al. (2010) . Schittny et al. (1998)** said that mammals were born with a functioning lung but still immature.

At the7th day after birth in the present work, the offspring lung of the control subgroup was in the alveolar stage of development. There were large numbers of secondary septa which were recognized along the primary septa. The secondary septa subdivided the air saccules into smaller units (primitive alveoli). These findings were in agreement with **Prodhan and Kinane (2002), Roth-Kleiner and Post (2005) and Joshi and Kotecha (2007).** These septa still contained double blood capillary layer and this allowed a small surface area of the capillaries to be exposed to gas exchange. These findings were in agreement with **Bolle et al. (2007).**

**Cardoso, (2010)** said that, in the developing respiratory system, the airway branching was a prenatal event, and the formation of the alveoli spanned pre- and postnatal life, and in many species such as the rat and mouse, it occured only postnatally. The secondary septa subdivided the air saccules into smaller units (primitive alveoli).

In this study, at 14thday after birth, the offspring lungs of the control subgroup were in the mature alveolar stage.The lung was formed of alveoli with thin interalveolar septa. The epithelial lining of the alveoli became differentiated into flat cell with flat nucleus; type I alveolar cells and the other epithelial cells remained cuboidal with rounded nucleus,type II alveolar cells. These findings were in agreement with **Schittny et al. (1998), Joshi and Kotecha (2007), Schittny and Burri (2007) and Rutter (2008).** The double capillary layers inside the primary and secondary septa were transformed into a single central capillary layer. This allowed an increase of the surface area of the capillary exposed to gas exchange and allowed more than one pneumocyte facing one capillary. These findings were in agreement with **Bolle et al. (2007)** who said that during alveolarization, the septa with double capillary networks were restructured to the mature form with a single capillary network interwoven with connective tissue strands, which stabilized the interalveolar wall. This process resulted in a reduction of tissue mass and alveolar sepal thickness**.**

**Harding et al. (2004) and McGrath et al., (2005)** reported that the number of type II cells decrease. This decrease in type II cells postnatally occured by means of programmed cell death or apoptosis. This explained a decrease in the total number of type II epithelial cells and fibroblasts in offspring lung during the postnatal weeks. So, apoptosis, therefore, played a key role in thinning of the alveolar septa that occured after alveolarization. **Kresch et al. (1998*)*** said that an apoptosis of mesenchymal cells epithelium was present in the fetal lung at 18 days of gestation and there was a 14-fold increase in apoptosis at birth and in the first postnatal day of life (9-12% of cells) compared with fetal lung (0.6-1% of cells).

In the present work, the lungs of offspring of diabetic rats were in the canalicular stage at the day of birth and even at the 7th day after birth. The lungs were also still in the saccular phase, with thick inter-saccular septa and narrow saccular spaces at the 14th day after birth .Such findings support the well-known effect of diabetes mellitus(DM) on fetal lung development and they were in the agreement with **Pinter et al.,(1991).**This effect was also described by **Sosenko and Frank (1986)and Trevin˜o-Alanı´s M et al.(2009)** as a delay in the alveolarization of the lungs in the offspring of the diabetic rats. Although, **Harding *et al. (2004)*** reported that the postnatal apoptosis continued in the immature lung and this might cause thinning of the inter-saccular septum.

However, **Bourbon et al., (1985); Gewolb et al., (1985); Gewolb, (1993); Rotenberg and Gewolb, (1993); Gewolb and O\_Brien, (1997)** focused their studies on the biochemical development of lung surfactant factors. They found, in fetuses of diabetic mother rats, a significant decrease in the total amount of lipids of the pulmonary surfactant factor at 20th day of intrauterine development, which was related to the pathogenesis of the delay of fetal lung development in gestational DM **Nold and Georgieff,( 2004).** This evidence thus suggested that there was a delay in the development of the offspring lungs in rats obtained from STZ-induced diabetic mothers.

In this study, the offspring lungs at the day of birth in the control subgroup were found at the saccular stage and the elastic fibers could be detected in the walls of the developing saccules. At 7th day after birth,

the lungs became in the alveolar stage and the elastic fibers were detected around tips of the secondary alveolar septa and in the wall of primitive alveoli. So, the present findings suggested that the elastic fibers play a critical role in septation and alveolar formation in which elastin deposition in the thickness of primary septa is one of the factors that participate in the control of budding of secondary septa. Similar findings were recognized by **Amy et al. (1977)**in mice, by ***Maritz and Woolward (1992) ,Runciman et al. (1996)***,in human and by ***Yamada et al. (2002)*** in rat. Also, ***Nogachi and Samaha (1991)*** said that lung elastic fibers are involved in septation by providing structural support for newly emerging secondary septa. Inhibition of elastic fibers has been linked to impaired septation.

In this study, the ultrastructure of the type II pneumocyte of offspring

of the control subgroup showed that the empty lamellar bodies appeared as vacuolation enclosed by membranes. By increasing of the offspring age, the numbers of empty lamellar bodies decreased and became all full lamellar bodies inside the cell. This indicated secretion of surfactant and maturation of type II pneumocyte with increasing number of cytoplasmic organelles. This was in the agreement of **Ridsdale and Post (2004).**

In our work, the ultrastructure of the type II pneumocyte of offspring of the diabetic mother showed increase number of the empty lamellar bodies. This was in the agreement of **Trevin˜o-Alanı´s et al.(2009)**that have described the ultra structure of the type II pneumocytes of the offspring of diabetes-induced mothers at the 21st gestational day with, empty lamellar bodies appeared as empty spaces enclosed by membranes and scarce cytoplasmatic organelles ,while **(Gewolb, 1993 and Benachi et al. 1999)**reported that the scare lamellar bodies and the absence of microvilli are typical features in the delay of the differentiation of the type II pneumocytes in the lung.

In this study, the presence of multivesicular bodies in the cytoplasm of type II pneumocytes of the diabetic group indicated that the cell was immature and still in process of surfactant synthesis. This was in the agreement with**(Singh and Katyal 1992).**

In this study, the offspring of diabetic mothers which were given hydrocortisone showed an encouraged lung maturity and enhanced excessive branching and thinning of the intersaccular septa. Also, there was an increase in the number of lamellar bodies. This finding indicated increased maturity of pneumocyte type II and increased surfactant secretions .These findings were in the agreement with **Bolt et al.( 2001)** who said that in humans, the late gestational increase in cortisol in the fetal circulation coincides with important pulmonary maturational events, such as surfactant synthesis, alveolar septal thinning, and reduction of the double capillary system to a single layer. If the birth occurs before full term, this late gestational age increase in cortisol has not yet occurred, and this results in failure of glucocorticoid-induced pulmonary maturation, causing low levels of surfactant with the subsequent development of RDS in these infants. **Hay(2012)** said that introduction of corticosteroids for fetal lung maturity at risk of preterm labor was the major milestonein reducing neonatal morbidity and mortality from respiratory distress syndrome RDS.

However, **Anttila et al.,(2005) ,DeCastro et al.,(2009), and Halliday et al.,( 2011)**added that the main role of corticosteriod in preterm infants is its anti- inflammatory effect and inhibition of the progression of acute lung injury to chronic lung disease.