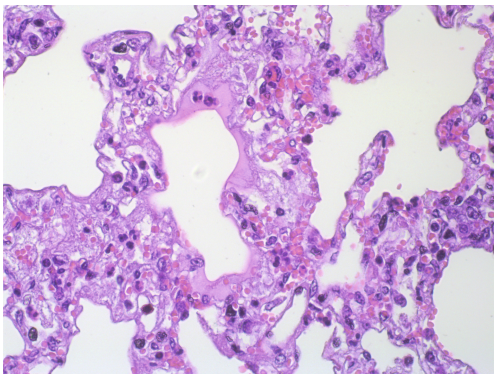
Paula Miriam Martz

Histo-pathologic alterations of lung tissue caused by hypoxia in neonates deceased due to dystocia



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Eingereicht von

Paula Miriam Martz

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For my Family

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Table of Contents

1 Introduction

Perinatal mortality due to dystocia within the first 48 hours of birth is the leading cause of death amongst neonates. In farm animals, this is closely linked to major economic loses (Szenci 2012, Dutra & Banchero 2011, Bleul 2009, Zerbe et al. 2008, Osteras et al. 2007, Bellows 1997, Khan & Khan 1991).

A certain lack of oxygen during birth is physiological, a prolonged birth though causes a pathologic hypoxic state, with ensuing organ damage, leading to unthriftiness and potentially death (Bleul & Götz 2013, Mota-Rojas et al. 2012, Dutra & Banchero 2011, Taverne 2008, Singer & Mühlfeld 2007, Alonso-Spilsbury et al. 2005, Szenci 2012, Ikeda et al. 1998, Tucker & Hauth 1990, Busse et al. 1986). Neonates suffering prolonged episode of hypoxia experience a metabolic shift from an aerobe to an anaerobe metabolism, accompanied by systemic acidosis and finally cellular degeneration. Protracted labour and its deleterious effects on the foetus have been studied in several human and animal trails (Bleul & Götz 2013, Mokra & Calkovska 2013, Castro-Najera et al. 2006, Martinez-Burnes et al. 2002, Ikeda et al. 2000, da Silva et al. 2000, Ikeda et al. 1998, Herpin et al. 1996, Adams et al. 1991, Rurak et al. 1990, Comline & Silver 1972).

One of the consequences of foetal hypoxia is the development of the condition known as Meconium Aspiration Syndrome (MAS) (Swarnam et al. 2012, Castro-Najera et al. 2006, Kirimi et al. 2003, Martines-Burnes et al. 2002, Ikeda et al. 2000). Foetuses experiencing stress during parturition due to a decreased oxygen supply will centralize blood flow, resulting in heightened respiratory movement and an increased intestinal peristalsis and relaxation of the anal sphincter causing premature expulsion of meconium. The early breaths taken intra-partum lead to the aspiration of meconium contaminated amniotic fluid causing airway obstruction, aeration problems and pulmonary inflammation (Swarnam et al. 2012, Mokra & Mokry 2011, Vidyasagar & Zagariya 2008, Castro-Najera et al. 2006, Martines-Burnes et al. 2002, Ikeda et al. 2000).

During foetal life the lungs are a non-vital organ and are therefore excluded from the

blood redistribution which occurs during extended labour, exposing them sooner and longer to an anaerobic metabolism (Szenci 2012, Martines-Burnes et al. 2002, Weinberger et al. 2001, Rurak et al. 1990, Walser & Maurer-Schweizer 1978). The damage of this oxidative stress compounded by the harmful effects of aspired meconium seriously injure the lung tissue.

The aim of this study is to histologically examine the changes to lung tissue of neonates born dead or deceased within 24 hours of birth that experienced dystocia:

- What pathomorphological alterations to lung tissue can be assessed? Can a difference be ascertained between the different examined species?
- Is the distribution of these alterations clustered in specific areas of the lung or are they found disseminated throughout the tissue sample?
- Are there specific inter-species difference regarding the distribution of alterations in the lung tissue?

Furthermore, to use these findings to than create improved resuscitation and first response protocols for newborn.

2 Literature Review

2.1 Foetal/perinatal metabolism and the blood gas and acid-base development during eutotic and dystotic birth

2.1.1 Eutotic Birth

During intrauterine life the foetus is, in most instances, well cared for. Foetal growth and vitality depends on the supply of nutrients and oxygen made possible through the intricate relationship between mother, placenta and foetus (Mota-Rojas et al. 2011). Until birth the foetus is supplied with all the essentials and alleviated from metabolic wastes and carbon dioxide through a transplacental transfer (Tucker & Hauth 1990).

Throughout first stage labour the foetus is still safely sustained through the feto-maternal membranes. With the onset of second stage labour the umbilical cord will be

temporarily occluded during abdominal contractions and the placental membranes begin detaching. This causes a disruption in the blood flow through the placenta depriving the foetus of oxygen as well as nutrients (Taverne & Noakes 2009, Castro-Najera et al. 2006, Alonso-Spilsbury et al. 2005, Rurak et al. 1990, Tucker & Hauth 1990, Walser & Maurer-Schweizer 1978). The circulatory disruption of the uteroplacental unit causes a mixed metabolic-respiratory acidosis, as shown in Figure 1, the foetus experiences periods of hypoxia, hypercapnia and hypoglycemia.

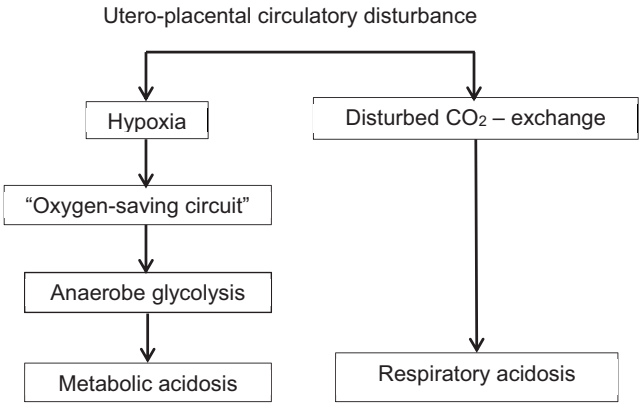


Figure 1: Occurrence of a mixed respiratory-metabolic acidosis during parturition due to disturbances in the uteroplacental circulation. Modified from Walser & Maurer-Schweizer (1978).

Several physiological adjustments must be carried out by the foetus with the onset of birth, such as maintaining adequate glucose levels in the blood as it becomes more and more hypoxic, which causes an increasing respiratory and metabolic acidosis (Bleul & Götze 2013, Mota-Rojas et al. 2011, Szenci et al. 1988, Busse et al. 1986). Walser & Maurer-Schweizer (1978) as well as Rurak et al. (1990) and several others say the physiologically hypoxia and hypercapnia a foetus experiences during birth, due to the

muscular contraction of labour, causes the foetus to switch to an oxygen-saving-circulation favoring the heart, central nervous system (CNS) and adrenal gland. During the perinatal time and shortly after birth the foetus and then neonate depends on its hepatic glycogen reserves, gluconeogenesis and switches to anaerobic metabolic processes to sustain itself, which causes an increase in lactic acid followed by a metabolic acidosis (Bleul & Götz 2013, Szenci 2012, Patterson et al. 1987, Walser & Maurer-Schweizer 1978). This anaerobic state also causes a mild respiratory acidosis and is reflected in a decreased blood pH and an increased base deficit (Kumar & Paterson-Brown 2010, Busse et al. 1986, Comline & Silver 1972).

At birth all vital responsibilities such as blood oxygenation, pH maintenance, thermoregulation and normoglycaemia are transferred over to the neonate. The respiratory acidosis, hypercapnia, is a major incentive to begin breathing a necessity for subsequent independent life. This respiratory acidosis subsides quickly with the onset of breathing, the metabolic acidosis normalises within 24 - 48 hours (Blomhoff Holm 2012, Taverne & Noakes 2009, Schulz 2009, Walser & Bostedt 2009, Busse et al. 1986, Comline & Silver 1972). Table 1 shows an overview of the literature of pH, pCO_2 and Base Excess (BE), in different neonates.

Thrifty neonates have an astonishing ability to self-correct metabolic and respiratory imbalances due to hypoxia, within the first hours of life (Bleul & Götz 2013, Poulsen & McGuirk 2009, Richter 2005, Gorlt 2004, Varga et al. 2001, Herpin et al. 1996, Szenci et al. 1988, Busse et al. 1986, Bostedt & Bellinghausen 1985, Walser & Maurer-Schweizer 1978, Comline & Silver 1972).

2.1.1.1 Acid-Base Status

Blood pH values of neonates are a well-established parameter measured to assess the thriftiness of new borne infants. Comline & Silver (1972) showed in their study with lambs that the pH was relatively steady until approximately 15 minutes ante-natum (a.n.).

A significant drop in pH levels could be observed during birth, with a drop from pH 7.38 one day a.n. to < 7.20 at birth, once breathing was established pH normalized within 30-60 minutes. Busse et al. (1986) presented similar findings in their work with lambs, showing that on average the lambs in the group “Thrifty after birth” (n=22) showed a relatively low venous blood pH of 7.18 at birth, which rapidly increased reaching adult sheep levels within 12 hours post-natum (p.n.). The study done by Bostedt & Bellinghausen (1985) with 12 (6♀, 6♂) thrifty foals showed that venous pH of new born foals had quite a large range, but rising steadily to almost adult levels within the first 4 hours of life, where it then stagnated, which they referred to as late acidosis. This stagnation of the pH levels was also demonstrated by Gorlt (2004) in her study with neonatal foals.

Bovine neonates are no different than other neonates as Richter (2005) showed in her study. Calves started life with a pH lower than the average adult cow (≥ 7.4), and needing approximately 120 minutes to raise their blood pH significantly, within 24 hours adult level were reached. Varga et al. (1999) documented a similar outcome in their examination of bovine neonates. Unlike other authors though, they reported that the metabolic side of the acidosis was alleviated much faster, within one hour, then the respiratory portion, which took up to 48 hours to normalize. Szenci et al. (1988) examined venous blood from 58 calves and was able to show that approximately 25% of the calves were acidotic during second stage labour and approximately 60% directly p.n.. Bleul & Götz (2013) showed in their study with 44 new born calves that the duration of parturition has a great effect on venous pH. Showing that a shorter partus is accompanied by higher pH values, reaching the reference range within four hours of life. This is in accordance with other studies, like Richter (2005), Szenci et al. (1988) and Walser & Maurer-Schweizer (1978). Bleul & Götz (2013) were also able to demonstrate that increased lactic acid levels after birth did not negatively affect the pH, by thrifty neonates. Some authors say the metabolic part of the acidosis subsides faster, most say the respiratory component dose, but all are in agreement that the mixed respiratory-metabolic acidosis due to labour is a physiological aspect of the birthing process and is usually alleviated within 24 hours of birth (Bleul & Götz, 2013, Richter

2005, Gorlt 2004, Varga et al. 2001, Herpin et al. 1996, Szenci et al. 1988, Busse et al. 1986, Bostedt & Bellinghausen 1985, Walser & Maurer-Schweizer 1978, Comline & Silver 1972).

Table 1: Review of pH, $pC0_2$ and BE in thrifty neonates (v = venous blood, a = arterial blood)

Authors	Animal	Mean Blood pH	$pC0_2$ (mmHg)	BE (mmol/L)
Walser & Maurer-Schweizer (1978)	bovine	ca. 7.20(v) 15 min. p.n.	ca. 65	ca. -4
Bostedt & Bellinghausen (1985)	equine	ca. 7.25(v) at birth	67	large individual variance
Szenci et al. (1988)	bovine	7.31(v) at birth	51.0*	0
Herfen (1999)	bovine	7.28(v) at birth	62.58	0.40
Varga et al. (2001)	bovine	7.26(a) 5 min. p.n.	59.8	- 2.3
Gorlt (2004)	equine	7.27(v) at birth	70.38	2.42
Richter (2005)	bovine	7.28 (a) at birth	63.46*	0.97
Bleul & Götz (2013)	bovine	7.24 (v) 10 min p.n.	61.35	- 2.5

*converted from kilopascal into millimeter of mercury (Szenci: 6.8 kPa, Richter: 8.46 kPa); factor 7.5006168

During birth when the blood supply through the foetal-placental unit is intermittent the neonate is not only deprived of oxygen, but also glucose and other nutrients (Mota-Rojas et al. 2011). One of the first challenges a neonate faces is maintaining normoglycaemia intra partum. The foetus and then neonate does this via anaerobic glycolysis owing to its hypoxic state, causing an accumulation of lactic acid (Bleul & Götz 2013, Szenci 2012). Lactic acid is the major metabolite responsible for the decrease in pH resulting in a metabolic acidosis. The BE, a calculated value, allows the determination if an acidosis is metabolic (Bleul & Götz 2013, Busse et al. 1986). Busse

et al. (1986) demonstrated that thrifty lambs had a moderate negative BE which normalized within four hours p.n., most thrifty lambs even showed a positive BE 24 hours p.n.. Bostedt & Bellinghausen (1985) also showed that the BE, which had the highest individual variability, steadily rose far above zero until the fourth day of life. Glucose levels were by all foals in the lower end of the reference range increasing continuously with the commencement of suckling. Noteworthy is a drop in glucose in the first half hour of life. This shows how quickly the glucose reserves are exhausted and how dangerous it can be if a foal does not commence to suckle within the first adaptation phase (Bostedt & Bellinghausen 1985).

Comline & Silver (1972) showed in their study with lambs that lactic acid began rising significantly an hour before parturition, reaching its maximum values 5-10 minutes p.n.. The observations from Comline & Silver (1972) showed a close relationship between the peri-natal fall in pH and rise in lactic acid. They also showed that the glucose levels behaved similar to the lactic acid levels with the greatest rise immediately p.n.. Walser & Maurer-Schweizer (1978) demonstrated in their model with calves that even by thrifty neonates who began breathing immediately after birth that the acidosis worsens in the first 10-15 minutes of life. They attributed this to the abolition of the oxygen-saving-circulation, which had been funnelling oxygenated blood to the CNS, heart, and adrenal gland and the then increased circulating lactic acid. Bleul & Götz (2013) also observed increased L-lactate and partial pressure of carbon dioxide ($pC0_{2)}$ concentrations in thrifty calves, but by a constant pH, disagreeing with Comline & Silver (1972). These values normalized within 48 and 4 hours respectively.

2.1.2 Dystotic Birth

A delayed or difficult parturition, dystocia, is caused by many factors such as foetal-maternal size mismatch, foetal mal-presentation or insufficient widening of the soft birthing canal, all leading to a protracted expulsion und invariable hypoxia (Taverne 2008, Lombard et al. 2007).

As already described all foetuses experience varying degrees of oxygen deprivation during birth, but if this hypoxia persists due to dystocia it will quickly have serious negative long term effects on the survival of the neonate or even be immediately fatal (Barrier et al. 2013, Blomhoff Holm 2012, Bleul et al. 2010, Lombard et al. 2007, Lopez & Bildfell 1992, Adams et al. 1991). The cumulative effects of uterine contractions by pathologically prolonged labour, will lead to cord damage or rupture as well as premature detachment of the placenta (Taverne 2008, Vaala 1999, Herpin et al. 1996). Walser & Maurer-Schweizer (1978) discussed in the late 70's the dangers of neonatal hypoxia, resulting in acidosis. Szenci (1988) and Walser & Maurer-Schweizer (1978) point out that with a pH of approximately 6.7 life expires. Severe acidosis ultimately ends in the death of individual cells. Acidosis which culminates in such disorders as MAS, Hypoxic-Ischemic Encephalopathy (HIE) or enterocolitis and potentially death, is often the consequence of dystocia, depicted in Figure 2 (Armstrong et al. 2012, Jacobson Misbe et al. 2011, Mokra & Mokry 2011, Kumar & Paterson-Brown 2010, Vidyasagar & Zagariya 2008, Castro-Najera 2006, Katz 2006, Martinez-Burnes et al. 2002, Ikeda et al. 2000, Vaala 1999). Mota-Rojas et al. (2012) showed in their study with 120 farrowing sows, through the administration of oxytocin, that heightened myometrium contractions cause severe foetal distress with decreased foetal heart rates and increased meconium staining, leading to higher intra-partum mortality. Cord rupture facilitates placental detachment by causing a decrease in blood pressure and a collapse of the chorion villi (Alonso-Spilsbury et al. 2005). Failure to establish breathing immediately after birth sustains the p.n. vasoconstriction, due to hypoxia, of the lungs, intestines, kidneys, muscles and skin while keeping the blood flow to the CNS, heart and adrenal glands steady (oxygen-saving circulation) (Bleul & Götz 2013, Szenci 2012, Alonso-Spilsbury et. al 2005, Martines-Burnes et al. 2002, Weinberger et al. 2001, Rurak et al. 1990, Walser & Maurer-Schweizer 1978). This redistribution ensures the function of vital organs of the hypoxic foetus.

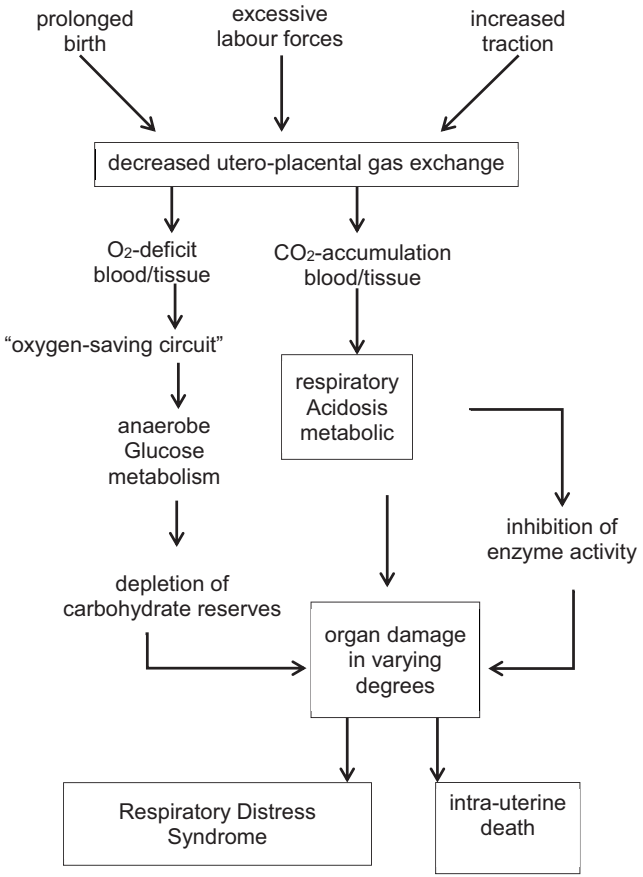


Figure 2: Cause, development and impact of perinatal stress, with focus on the gas exchange. Modified from Schulz 2009, modified from Zaremba et al. 1984.

Any form of dystocia can be the cause of extended labour, facilitating prolonged hypoxia, hypercapnia and acidosis (Barrier et al. 2013, Dutra & Banchero 2011, Bleul et al. 2010, Schulz 2009, Lombard et al. 2007, Adams et al. 1991).

Events causing pathological hypoxia are:

- premature ablation of the placenta (Vaala 1999, Alonso-Spilsbury et al. 2005)
- inadequate placenta perfusion, e.g. uterine torsion, maternal hypotension (Alonso-Spilsbury et al. 2005, Castro-Najera 2006)
- prolonged umbilical occlusion, e.g. sternal presentation (Alonso-Spilsbury et al. 2005, Schulz 2009)
- foetal-maternal disproportion, e.g. heifer, male calve (Berglund 2003, Mee 2008)
- mal-presentation , -position, -posture (Schulz 2009)

2.1.2.1 Acid-Base Status

Busse et al. (1986) showed that unthrifty lambs exhibit only slight variations in the acid/base and blood gas homeostasis, the steep and severe drop in pH in the first 15 minutes of life is owing to the insufficient respiratory response of the lambs. Unthrifty lambs showed on average considerably lower pH levels of 7.08 and a much longer convalescence period then thrifty lambs, reaching adult sheep levels at around 24 hours p.n.. Unthrifty lambs that died within 24 hours of parturition showed diverging severities of acidosis, but all had in common a drastic drop in pH minutes after birth, with venous blood pH remained extremely low until death. Unthrifty lambs exhibited a lower negative BE then thrifty lambs and a drop 15 minutes p.n.. The BE normalized within 12 hours after birth and reaching positive values within 24 hours p.n.. Highly significant differences in the venous pH levels between the thrifty and unthrifty lambs could be shown up until 4 hours p.n..

In Gorlt's (2004) work it was also discernable that foals that were less thrifty had slightly lower pH values than thrifty foals. These depressed foals showed an increase in their

pH values within the first 10 minutes of life, but always remained below the values achieved by thrifty born foals. The stagnation observed in this group of foals was much sooner evident and positive development considerably slower.

Calves experiencing prolonged birth due to dystocia are distinctly less thrifty then calves from eutotic births. Richter (2005) showed that these calves had a noticeably lower blood pH at birth. They also had an obviously reduced respiratory activity, the breathing rate and depth took much longer to regulate. Richter (2005) demonstrated a distinct stagnation of the blood pH in the first 10 minutes p.n.. First in the following 10 minute time interval could a slow increase in blood pH be registered, within the first 60 minutes of life an increase to ≥7.20 was reached. Similar results were presented by Herfen (1999) and Varga et al. (2001).

Differing to other authors Herpin et al. (1996) reported in their study, of 117 new borne piglets, that glucose levels were dramatically increased in neonates with prolonged parturition and showing unthriftiness. They surmised this to be caused by the increased secretion of stress hormones, such as catecholamines. The glucose utilization due to the reduced insulin secretion during hypoxia is still limited, available only to organs whose glucose uptake is insulin independent such as the CNS.

Walser & Maurer-Schweizer (1978) and later Bleul & Götz (2013) showed an increase in the pCO_2 and lactic acid direct p.n. due to reduced respiratory activity and the reperfusion of peripheral organs. This has a deleterious effect on depressed neonates as the lactic acid levels are already high because of the anaerobic glycolysis during partus driving the acidosis so high that it becomes incompatible with life.

Busse et al. (1986) had similar findings where the BE sank steeply (-12 mmol/l) and the pC02 rose drastically (84 mmHg) within 15 minutes p.n., owing to the inadequate respiratory response of the unthrifty lambs. Herfen (1999) confirmed a continued drop in the BE up to 10 minutes p.n. of up to 1.7 units establishing a direct correlation to the pH development. Table 2 shows an over view of the outcome of previous studies.

Table 2: Overview of pH, $pC0_2$ and BE in unthrifty neonates
(v = venous blood, a = arterial blood, n.a. = not available)

Authors	Animal	Mean Blood pH	$pC0_2$ (mmHg)	BE (mmol/L)
Walser & Maurer-Schweizer (1978)	bovine	ca. 7.09 (v)	ca. 74	ca. -10
Szenci et al. (1988)	bovine	7.14 (v)	66.76*	- 6.0
Herfen (1999)	bovine	7.11 (v)	81.14	- 6.9
Varga et al. (2001)	bovine	7.10 (a)	64.5	-10.9
Gorlt (2004)	equine	7.26 (v)	72.76	2.5
Richter (2005)	bovine	7.14 (a)	65.56*	- 7.6
Bleul & Götz (2013)	bovine	7.03(v) 10min p.p.	72.1	n.a.

*converted from Kilopascal into Millimeter of mercury (Szenci: 8.9 kPa, Richter: 8.74 kPa); factor 7.5006168

When the organism finds itself in a hypoxic state lactic acid is accumulated which is the cause of metabolic acidosis (Bleul & Götz 2013, Poulsen & McGuirk 2009). Reduced myocardial contractility, hypotension and eventual coagulopathies are the sequela of the inevitable systemic metabolic acidosis (Seri & Evans 2001).

The decline in pH will cause cell injury in multiple organs and the oxygen insufficiency will force a shift to an anaerobic metabolism (Bleul & Götz 2013, Alonso-Spilsbury et al. 2005). Meconium staining of the neonate or the amniotic fluids is a sign of peri-partal distress (Mota-Rojas et al. 2012, Castro-Najera 2006, Martinez-Burnes et al. 2002, Vaala 1999).

2.2 Effects of pathological hypoxia on specific organs

Oxygen deprivation triggers a cascade of cellular biochemical events bringing about an alteration in cell function as far as cell death (Alonso-Alconada 2012, Alonso-Spilsbury et. al 2005, Galvin & Collins 2004). Hypoxia affects most cells similarly through the change from aerobe to anaerobe metabolism. The cell loses its ability of efficient oxidative phosphorylation and initiates anaerobic glycolysis. The adenosine triphosphate (ATP) dependent sodium pump fails, exchange of ions across the cell membrane is disrupted and metabolites accumulate causing damage to the structural integrity of the cell (Myer & McGavin 2007). Further insults can be attributed to an over-production of free radicals followed by oxidative stress and increased stimulation of pro-inflammatory cytokines (Alonso-Alconada 2012). This multi-factorial process acts synergistically leading eventually to cellular necrosis (Alonso-Spilsbury et al. 2005).

Peri-partal hypoxia, depending on the severity, has in varying degrees multisystemic effects (Vaala, 1999). Meconium staining of amniotic fluid is a macroscopic clinical sign for intrauterine hypoxia, foetal stress, in animals as well as humans. Hypoxia experienced in utero causes an increased peristalsis of the intestines and a relaxation of the anal sphincter as well as increased respiratory movement. This results in the passage of meconium into the amniotic fluid, consequentially causing a staining of the neonate and potential aspiration (Mota-Rojas et al. 2012, Swarnam et al. 2012, Castro-Najera 2006, Martinez-Burnes et al. 2002, Vaala 1999).

2.2.1 Lungs

The lungs develop in six stages embryonic, pseudoglandular, canalicular, saccular, alveolar and vascular maturation, beginning as a ventral diverticulum in the foregut endoderm and completing their development after birth (Caswell & Williams 2007, Pringle 1986). The lungs progress through phases of development, going through a series of epithelial-mesenchymal changes. A healthy lung exhibits histologically approximately the same diameter of an airway and its accompanying artery (Caswell & Williams 2007). Disproportion of this dyad is indicative of a pathological condition. The

airway basement membrane region consist of three layers which are the lamina lucida, lamina densa, lamina reticulairis, and can be stained using the digested Periodic Acid-Schiff (PAS) reaction. Interstitial widening and increased cellularity are normal histological findings in children. Therefore it can be assumed that this would also pertain to young and even neonatal animals (Colby et al. 2007). In infants large elastic pulmonary arteries are structurally very similar to the makeup of the aorta. The bronchus-associated lymphoid tissue (BALT) is not present at birth, establishing with increasing age. Lymphocytes are generally only present and grouped together in a pathological state in neonatal lungs (Colby et al. 2007). The mucosal epithelium of the conducting airways shows great cell diversity, depending upon species and section of airway under consideration.

The lungs of most neonates continue to mature after partus, not being fully developed at birth (Haworth & Hislop 2003, Pringle 1986). The stage of microvascular maturation of the lung is the period of maturation of the air-blood interface, a single capillary layer, which occurs after birth by nidicolous animals and peri-partal by nidifugous animals (Caswell & Williams 2007, Roth-Kleiner & Post 2003).

Normal term foetuses may exhibit a minor sloughing of epithelial cells, but large quantities, especially if meconium is also present, indicate aspiration of amniotic fluid. This is a sign for increased intrauterine respiratory movement due to hypoxia in utero. Hypoxia can also be responsible for weak respiratory movements p.n. causing patchy congenital atelectasis due to incomplete expansion of the lung. Acute hypoxia due to prolonged parturition may be the cause of MAS, where histological sections of affected lungs show amorphous yellow-orange meconium, keratin, and/or squamous epithelial cells (SEC). This is associated with atelectasis, hemorrhage, and a mild but diffuse alveolar infiltrate of neutrophils and occasionally multinucleate cells (Mokra & Calkovska 2013, Poulsen & McGuirk 2009, Caswell & Williams 2007, Satas et al 2003, Martinez-Burnes et al. 2002). Satas et al. (2003) showed that global hypoxic-ischemia produce multiple small haemorrhages as well as the deposition of fibrin debris in the alveoli.

Lungs are affected by prolonged uterine hypoxia two fold, firstly mechanically through

the aspiration of amniotic fluid which may be contaminated with meconium and secondly on a cellular level due to the anaerobic metabolism (Bleul & Götz 2013, Alonso-Spilsbury et al. 2005).

Hypoxia exacerbates cellular injury in lung tissue. Lung macrophages are activated by prolonged episode of hypoxia increasing the production and release of inflammatory mediators. This initiates the inflammatory cascades ending in the release of reactive oxygen and nitrogen species that can be damaging to pulmonary cells (Mokra & Calkovska 2013, Weinberger 2001).

With the onset of breathing, after birth, the neonatal lungs experiences increased alveolar oxygen tension and ensuing oxidative stress. The organism counters this by increasing antioxidant defenses. The expression of anti-oxidative enzymes is negatively influenced by pre-/perinatal hypoxic stress (Giles et al. 2002). This increases the probability of pulmonary oxidative damage in neonates experiencing prolonged labour.

As mentioned above premature passage of meconium in utero is a sign of a hypoxic stress. Figure 3 depicts the pathway of how meconium causes damage to lung tissue. The aspiration of meconium contaminated amniotic fluid results in MAS and is a terminal event associated with prolonged intrauterine hypoxia. Neonates borne with MAS experience respiratory distress, clinical signs are caused by airway obstruction, impaired gas exchange, atelectasis or dysdelectasis, pneumonia and/or surfactant dysfunction (Zagariya et al. 2000, Vaala 1999). These structural and functional alterations have in turn major effect on the acid-base regulation resulting in acidosis (Lopez & Bildfell 1992). Clinical effects are not limited to the respiratory tract, since the acidosis and hypoxia affects all tissues in the body. Histological examinations of lungs from neonates that experienced respiratory distress due to prolonged labour show in a hematoxylin and eosin (H&E) stain, SEC, meconium and inflammation (Lopez & Bildfell 1992). Zagariya et al. (2000) were able to show in a model with rabbit pups that meconium triggers an increased polymorph-nuclear leukocytes response of the lung tissue. Intrapulmonary neutrophil accumulation gravely increasing the expression of pro-inflammatory chemotactic cytokines (most prominent: tumor-necrosis factor-α,

interleukin-6, interleukin-1ß), phospholipase A_2 and levels of prostaglandin E_2, exacerbating inflammatory reactions of lung tissue and having a deleterious effect on alveolar cells (Mokra & Mokry 2011, Vidyasagar & Zagariya 2008, Zagariya et al. 2000). These inflammatory reactions cause increased vascular permeability leading to pulmonary edema and proteinaceous exsudative liquid to accumulate in the alveoli and an increased release of oxidative species potentially initiating increased apoptosis (Vidyasagar & Zagariya 2008, Kirimi et al. 2003).

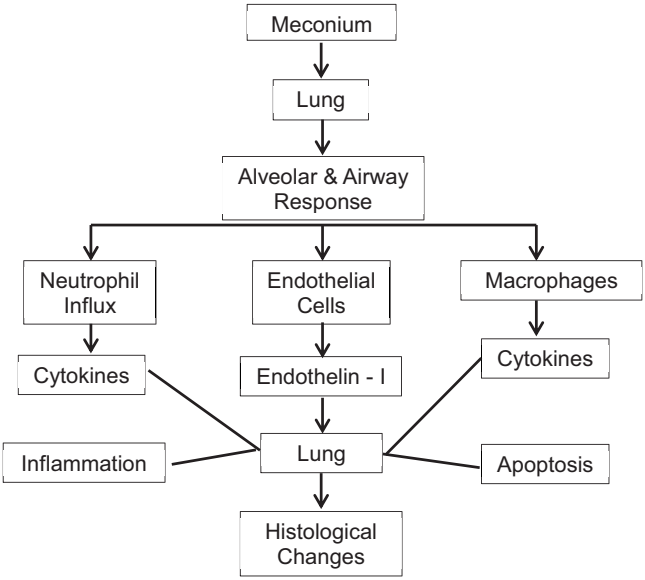


Figure 3: The pathways in which meconium induces lung injuries, modified from Vidyasagar and Zagariza 2008.

2.2.2 Central nervous system

A prolonged partus with ensuing oxygen deprivation induces a specific oxygen-saving circulation to ensure adequate oxygenation of the brain, initially this is essential for the survival of the brain cells. However, the increased blood flow also compromises the vascular self-regulation of the brain, inhibiting the ability of the arterioles to respond to pressure fluctuations. If the hypoxic state persists for too long an imbalance in the metabolic demand and cellular energy supply will result in a disruption of critical cellular processes, finally resulting in HIE (Kasdorf & Perlman. 2013, Alonso-Spilsbury et al. 2005). HIE is histologically associated with hemorrhage, edema and neuronal necrosis (Vaala 1999). Neurons succumb to the shift from aerobic to anaerobic metabolism yielding to the collective assault of acidosis, neurotoxic activities of glutamate, nitric oxide and free radicals, lipid peroxidation, accumulation of intercellular calcium and destructive over-activity of intercellular enzymes (Galvin 2004, Vaala 1999). The capillary endothelium becomes hyper-permeable leaking fluids and osmotic agents into the interstitium resulting in edema (Vaala 1999). Ikeda et al (2000) showed that damage to brain tissue is quite variable, but if umbilical cord occlusion occurs even just partially and persists for any length of time (60 min.) alterations ranging from vacuolization, loss of myelin sheath, increased cellularity, up to extensive necrosis are very likely. In an earlier paper Ikeda et al. (1998) are able to demonstration that especially prolonged lactate concentrations and cerebral hypotension seems to influence the severity of damage to neurons. Alonso-Alconada et al. (2012) showed a correlation between pro-inflammatory cytokines and reactive oxygen species and the number of neuronal apoptotic cells after perinatal hypoxia.

2.2.3 Cardiovascular System

Human neonates respond quickly and efficiently to hypoxia by redirecting an increased blood flow to vital organs such as the brain, heart and adrenal gland. This is achieved through a deceleration of the heart rate and increased systemic blood pressure which redirects the cardiac output (Alonso-Spilsbury et al. 2005). Walser & Maurer-Schweizer

(1978) as well as Rurak et al. (1990) have also described this oxygen-saving-circulation by calves and lambs favoring the heart, brain and adrenal gland. Further studies with porcine neonate have shown that they respond in a similar manner (Leffer et al. 1986). The lung, being a non-vital organ to the foetus, also responds with vasoconstriction to hypoxia to help redirect the blood flow. If the pulmonary hypertension continues p.n. the foramen ovale will not close and/or a ductus arteriosus persistence will manifest itself (Alonso-Spilsbury et al. 2005). Persistent pulmonary hypertension, due to vasoconstriction, a reaction to continued hypoxemia will cause a failure of the transition from foetal to neonatal cardiopulmonary circulation. This maintains a right-to-left shunting and the persistence of the ductus arteriosus and foramen ovale (Poulsen & McGuirk 2009). The increased resistance from both the small and the large circulatory system puts a magnificent mechanical load on the heart, if this continues p.n. eventually the heart, especially the right ventricle and atrium, will capitulate with ensuing right heart dilatation, tricuspid insufficiency and eventual heart failure (Alonso-Spilsbury et al. 2005). Del Duca et al. (2012) have differing findings in their work with rats, describing the exact opposite, saying that the heart seems to experience most of its permanent structural and functional changes in the left ventricle. Donnelly et al. 1980 show that the myocardial dysfunctions appear to be based on ischemia found mainly in sub-endocardial and papillary muscles. Ikeda et al. (2000) and Donnelly et al. (1980) also describe that the papillary muscles are the sight of most necrotic damage.

Alterations which can be identified using an H&E stain include hydropic cell swelling, wavy arrangement of cardiac architecture, cytoplasmic eosinophilia, nuclear pyknosis, necrosis, phagocytosis, contraction band, and edema. Some changes are not detectable until up to 12 hours post hypoxic insult (Faa et al. 2012, Ikeda et al. 2000, Donnelly et al. 1980). Satas et al. (2003) similar to Ikeda et al. (2000) and Donnelly et al. (1980) showed that small necrotic lesions are the main changes found in the heart after a hypoxic episode. Oxidative stress as well as high lactic acid levels are indicators for an anaerobic metabolism. Both parameters can be identified in the tissue of the heart during umbilical cord occlusion (Ikeda et al. 2000). Myocardial dysfunctions display themselves as tricuspid regurgitation and decreased contractility. Clinically the

damage presents itself inconsistently with tachypnea, congestive heart failure, cardiogenic shock (Borke 2004).

2.2.4 Liver

The increase of hepatic glycogen stores in the last trimester of gestation is one of the measures taken by the foetus to prepare for the transition to extra-uterine life. The liver is the major supplier of energy for the new born by orchestrating the increase of glycogenolysis and gluconeogenesis and the decrease of glycogenesis and glycolysis in order to increase blood glucose levels (Mota-Rojas et al. 2011). In neonates experiencing hypoxia an oxygen-saving blood circulation is initiated, the liver is one of the few organs whose blood supply is initially unaffected (Leffler et al. 1986). This is probably due to its crucial role as energy supplier. The liver does this by converting its glycogen stores into glucose, glycogenolysis is stimulated by hypoxia (Grongnet 1984). In the macroscopic necropsy the liver sometimes appears slightly enlarged, its colouration red-black, crumbly and blood filled (Alonoso-Spilsbury et al. 2005). One study from Ikeda et al. (2000), working with porcine neonates, showed that morphological deviations in the liver concentrated around hepatocytes and were due to inflammatory reactions. Following alterations were found: cytoplasmic eosinophilia, canalicular cholestasis, fatty changes, eosinophilic hyaline globules, centrilobular necrosis, inflammatory cell infiltration, congestion. Hepatic damage was only noted in neonates with severe oxygen deprivation, which indicates that the liver may enjoy a form of cellular protection (Ikeda et al. 2000). Satas et al. (2003) identified two types of lesions, subcapsular haemorrhagic and ischaemic, in their histological examination of liver tissue after hypoxic episodes. Impaired hepatic function due to parenchymal liver demise renders the neonate subjected to fluctuations in the glucose homeostasis and more vulnerable to sepsis (Vaala 1999).

2.2.5 Intestinal tract

The gastrointestinal tract is a tube system divided into specific anatomical sections. The foetal gastrointestinal tract is sterile. The intestine establishes its own unique microflora by being colonized with bacteria mainly from its mother and the surrounding environment during parturition and shortly thereafter (Mackie et al. 1999). The soundness of the intestinal tract relays on a fine tuned balance between the epithelium, immune cells and the establishing resident microflora (Smith et al. 2012). The commensal bacteria not only help maintain the integrity of the mucosal barrier and stimulate the intestinal immune defences, but also control pathogenic bacterial colonization (Moore et al. 2011). When a foetus experiences oxygen shortage due to dystocia decreased blood flows to the small intestine, this oxygen saving circulation as mentioned earlier is seen as a protective mechanism to ensure continued oxygenation of vital organs (Rurak et al. 1990, Leffler et at. 1986, Maurer-Schweizer 1978). Touloukain et al. (1972) showed in their study with twenty-three neonatal piglets, that if this shunting is sustained for too long, due to dystocia, ischemic changes could lead to necrosis. Further they showed that vascular injury accompanied by intramural hemorrhage is one of the earliest lesions evident. Hypoxic episodes affect the intestinal epithelium, the monolayer of cells which form the critical barrier between external (luminal) and internal (vascular) compartments (Taylor & Colgan 2007). Lacerations can be observed spanning all layers of the intestinal wall, necrotic lesions as well as congestion are found in the mucosa, submucosa and muscular propria after hypoxic incidents as caused by prolonged labour (Satas et al. 2003). Taylor & Colgan (2007) showed that inflammatory cells are mostly recruited to the areas experiencing hypoxia, leading to intestinal injury due to common inflammatory processes and cytokine activity. These injuries show themselves as destruction of villi and crypts and loss of submucosal architecture, as well as edema, lymphatic dilatation, hemorrhage and inflammatory cell infiltration (Canpolat et al. 2006, Clark et al. 1988). The compromised intestinal wall cannot withstand colonization and proliferation of invasive bacteria. Neonatal ischemic enterocolitis is a devastating disease affecting many neonates who suffer hypoxia during birth. This is undoubtedly caused by profound and/or prolonged

ischemia of the gut (Lu et al. 2012, Canpolat et al. 2006, Clark et al. 1988, Touloukain et al. 1972).

2.3 Clinical consequences due to the pathological hypoxia

Consequences of prolonged hypoxia intra-partum are unthriftiness, a longer time needed to make first contact with the udder and begin suckling, weak suckling, which has a negative effect on the intake of colostrum and failure of passive transfer (Barrie et al. 2013, Lombard 2007, Alonso-Spilsbury et al. 2005, Lopez & Bildfell 1992, Grongent 1984). This reduced intake of colostrum is partially responsible for an inadequate transfer of passive immunity which speaks for a probable hypogamma-globulinemia and an increased risk of infection. As well as high chance of a hypoglycemia as well as a reduced absorption of other vital nutrients and minerals (Edwards 2012, Lopez & Bildfell 1992). Low glucose levels and high lactate levels due to an anaerobic metabolism and the depleted hepatic glycogen can often be associated with depression of the neonate (Mota-Rojas et al. 2011, Schulz 2009). Linke et al. (2013) showed in his work with bovine neonates that at the end of the first hour p.n. the lungs of thrifty born calves are still not fully ventilated, showing a ventilation of up to 80% cranially and 55% ventrally. So it can be expect that the lungs of depressed neonates, which show lower respiratory activity, prolonged laying periods and incomplete left-right shunting of the cardio-vascular system, are even more poorly ventilated (Linke et al. 2013). Further diagnostic examinations with ultra sound, as established by Jung & Bostedt (2004), clearly showed increased atelectasis of the lungs by neonates that are unthrifty and show clinical signs of amniotic fluids aspiration at birth. More concrete disease complexes such as pneumonia, HIE, neonatal Respiratory Distress Syndrome (nRDS), MAS, heart disease and necrotizing enterocolitis are also commonly seen in neonates that experienced prolonged hypoxic episode during birth (Faa et al. 2012, Lu et al. 2012, Taylor & Colgan 2007, Canpolat et al. 2006, Satas et al. 2003, Ikeda et al. 2000, Zagariya 2000, Vaala 1999, Lopez & Bildfell 1992, Clark et al. 1988, Donnelly 1980, Touloukain et al. 1972).

3 Materials and Methods

3.1 Objective

The aim of this study was to examine neonatal lungs for tissue damage and/or corpuscular deposits in the bronchiolar and alveolar lumen due to hypoxia caused by dystocia in cows, horses and dogs. Data and samples were collected from animals presented at the clinic for Obstetrics, Gynaecology and Andrology for large and small animals, with Ambulatory service (the clinic) of the Justus-Liebig University in Giessen, experiencing dystocia.

3.2 Anamnesis and first measurements

Information about case history of the mother animals was obtained from the owners and first attending veterinarian as well as the medical records, information was also gathered regarding resuscitation measures and applied treatments of the neonates p.n..

3.2.1 Mother animals

If possible the time labour began was documented, parity, type of dystocia presented, and what measures had been taken by the owner and/or first attending veterinarian, were recorded. At the clinic all animals were given a general and obstetric examination.

Where possible conservative measures were implemented to deliver the neonate, when necessary, a caesarean operation or fetotomy was performed.

3.2.2 Neonates

When unthrifty mature young were delivered specific resuscitation measures were taken. Included, clearing of airways, oxygenation via an incubator, tracheal tube or face mask, prevention of hypothermia through drying and/or placing the neonates in incubators and/ or under a heat lamp. Further, an indwelling vein catheter was implanted, as well as the antagonization of anaesthesia administered for caesarean

section (by dogs) with Narcanti-vet® (Naloxonhydrochlorid 0.04 mg/kg body weight). If necessary, emergency medical treatment was administered (Table 3). After fist measures were successful, glucose-solution 5 present ad us. vet. B. Braun® (10-25ml/kg body weight/hour) for energy was infused, vitamins and minerals (vitamin B complex 5ml/animal, Ursovit AD$_3$EC® 2-5ml/animal, Belfer® - iron 5ml/animal, vitamin e/selenium 5ml/animal) were supplemented, para-immunity inducers (Zylexis®, one dose per animal) and a decongestant (bromhexin, 0.5mg/kg body weight, Bisolvon®) were given. Furthermore, colostrum or a milk substitute was fed or drenched via a stomach tube to ensure antibody and nourishment uptake.

Table 3: Drugs and their dosages, administered as part of resuscitation and stabilization measures

Drug (active ingredient)	Dosage
Doxapram-V® ad us. vet. (Doxapram)	Dog: 1-5mg analogous to 0.05-0.25ml Foal: 2mg/kg body weight Calf: 40-100mg analogous to 2.0-5.0ml iv, im, sc or po
Dexasel 2%® (Dexamethason)	Dog: 0.02-0.04 mg /kg body weight analogous to 0.01-0.02 ml/kg body weight Foal: 0.01-0.02mg/kg body weight analogous to 0.005-0.01ml/kg body weight Calf: 0.03-0.06mg/kg body weight analogous to 0.015-0.03ml/kg body weight iv, im

Suprarenin® 1mg/ml injectable solution (Epinephrinhydrochlorid)	Neonate: initial dose 0,01mg/kg body weight iv, intra ossar or 0,1 mg/kg body weight diluted in salin solution instilled endotracheal
Bisolvon® (Bromhexinhydrochlorid)	Neonate: 0,5mg/kg/body weight analogous to 0,17ml injectable solution/kg/body weight im, sc

3.3 Organ tissue samples

Organ tissue samples were taken from neonates which died intra partum or up to 24 hours p.n. due to dystocia. In order to ensure the cells had not succumbed to the natural process of post mortem autolysis, a necropsy was done immediately after the foetus was delivered. If this was not possible the neonate was secured in the cooling chamber (<10°C) of the clinic to slow the degradation of the material. The lung samples were always taken from approximately the same location as depicted in Figure 4 and of approximately the same size to support consistency, as shown in Table 4. Neonates with apparent macroscopic malformations as described in Table 5, as well as animals that showed signs of advanced autolysis due to prolonged inter uterine death were excluded.

Table 4: Location and size of tissue samples taken for histological examination

Organ	Abbreviation	Location	Size (approximate)*
Lung Tissue Sample 1	Lu1	left cranial lob	10 mm x10 mm x 5mm
Lung Tissue Sample 2	Lu2	left caudal lob	10 mm x10 mm x 5mm
Lung Tissue Sample 3	Lu3	right cranial lob	10 mm x10 mm x 5mm
Lung Tissue Sample 4	Lu4	right caudal lob	10 mm x10 mm x 5mm

* sample size may vary slightly due to the overall organ size

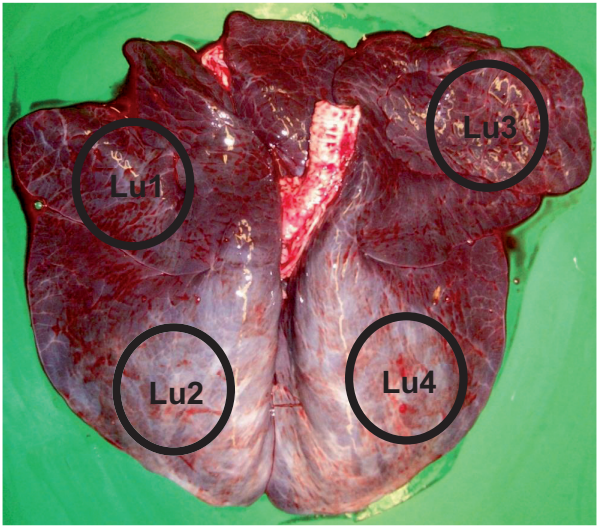


Figure 4: Neonatal calf lung depicting the approximate locations from where tissue samples were taken.

Table 5: Exclusion criteria for neonates for the study

Apparent macroscopic malformations	Signs of prolonged uterine death
Palatoschisis	aberrance of smell, colour, viscosity
Schistosoma reflexum	impurities of amniotic fluid
Hydrocephalus externus	looseness of fur
Atresia ani and/or recti	edema

3.4 Histology

3.4.1 Tissue preservation

The tissue samples were placed in cassettes (Tissue-tek R tissue-processing containers, Sakura Finetek Europe B.V., Zoeterwoude, Netherlands) and fixed for a

minimum of 7 days in buffered 10% formalin, protocol described in Table 6 and stored therein until further processing.

Table 6: Protocol for neutral buffered 10% formalin (Formol as per Lillie) for 1L solution

Formaldehyde solution (~37%) (Merck, Darmstadt)	100 ml
Natriumdihydrogenphosphat-Monohydrat (Merck)	4 g
Di-Natriumhydrogenphosphat (Merck)	6.5 g
Aqua destillata	900 ml

3.4.2 Dehydration of tissue samples

After fixation, the tissue samples were washed with a buffer solution (Table 7) changing the solution every 24 hours for 48 hours, and then placed in 70% alcohol, protocol described in Table 8, until the embedding process could start approximately 24 hours later.

Table 7: Protocol for neutral buffer, for 1L solution (used to wash the tissue samples)

Natriumdihydrogenphosphat-Monohydrat (Merk, Darmstadt)	13.8 g
Di-Natriumhydrogenphosphat (Merk, Darmstadt)	17.8 g
Aqua destillata	1000 ml

Table 8: Protocol for 70% isopropanol solution (used to wash the tissue samples)

98% Isopropanol	700 ml
Aqua destillata	300 ml

3.4.3 Embedding

The tissue samples were further dehydrated (Table 9) and then embedded in paraffin blocks. The process was carried out with the embedding machine *Leica TO 1050* (Leica Mikrosystem Vertrieb GmbH, Wetzlar) at the Institute for Veterinary-Anatomy, Histology, and Embryology of the Justus-Liebig University, Giessen, Germany.

Table 9: Tissue embedding in paraffin; blocking up for cutting and further processing

Step	Reagent	Time (in minutes)
1. Dehydration	Isopropanol 70%	15, at room temperature
Incremental alcohol wash (Carl Roth, Karlsruhe)	Isopropanol 80%	15, at room temperature
	Isopropanol 96%	15, at room temperature
	Isopropanol 100%	15, at room temperature
	Isopropanol 100%	15, at room temperature
2. Clearing	Xylol	15, at room temperature
(Merck, Darmstadt)	Xylol	15, at room temperature
3. Infiltration	Paraffin	15, at 60 °C
(Merck, Darmstadt)	Paraffin	

The tissue samples were taken out of the liquid paraffin after complete infiltration, and then placed in adequate moulds in order to produce paraffin embedded tissue samples (paraffin blocks) which could then be cut.

3.4.4 Creating tissue section

The paraffin blocks were cooled for 24 hour, to ensure accurate slicing. Four micrometer thick slices were cut at the clinic using the Microtome Leica RM 2125 (Leica Mikrosysteme Nussloch GmbH, Nussloch), and disposable blades Leica DB 80 L (Leica Mikrosysteme Nussloch GmbH, Nussloch). The slices were placed in a 35-38 °C water bath (G FL 1052, Gesellschaft für Labortechnik mgH, Burgwedel) of Aqua destillata for levelling. The levelled slices were then mounted on with 3-aminopropyltriethoxysilan (APES) coated slides, (Table 10). Subsequently the tissue section were dried in a heating cabinet (Memmert SL 40, Memmert, Schwabach) for 24 hours at 38 °C and then stored at room temperature until stained.

Table 10: APES coating of the slides, to functionalize the surface for better adhesion

APES (Merck, Darmstadt)	20 seconds in 2% solution
Aceton, pure (Merck, Darmstadt)	2 x rinses
Aqua destillata	2 x rinses

3.4.5 Tissue section staining

The tissue sections were dyed using an H&E, protocol described in Table 11, allowing a histological examination of the lung sections to identify pathological changes. A second batch of tissue sections was stained using a PAS reaction, protocol described in Table 12, to better accentuate aspirated meconium, amniotic fluid and the basement layer of the airways. After staining the tissue sections were immediately covered with Etallen® (Merck, Darmstadt) and a cover glass.

Table 11: H&E tissue staining for visualization of tissue and cell structures

Step	Reagent	Time (in minutes)
1. Clearing	Xylol	20
(Merck, Darmstadt)		
2. Rehydrated	Ethanol absolute	5
Incremental alcohol wash	Ethanol 96%	5
	Ethanol 80%	5
(Carl Roth, Karlsruhe)	Ethanol 70%	5
	Ethanol 60%	5
	Ethanol 50%	5
	Ethanol 50%	5
3. Rinse	Aqua destillata	5
4. Stain (Merck, Darmstadt)	Haematoxylin	8
5. Rinse	Tap water (running)	10-15
6. Stain (Merck, Darmstadt)	Eosin 0.1%	6
7. Rinse	Tap water	Flush
8. Dehydrated	Ethanol 80%	flush
Alcohol	Ethanol 96%	flush
(Carl Roth, Karlsruhe)	Ethanol absolute	2.5
	Ethanol absolute	2.5
9. Clearing	Xylol	10
(Merck, Darmstadt)	Xylol	10

Table 12: PAS staining for visualization glycoconjugates in tissue samples; neutral mucopolysaccharides, mucoproteins, glucoproteins, glycogen and the basement layer of the bronchial epithelium

Step	Reagent	Time (in minutes)
1. Clearing (Merck, Darmstadt)	Xylol	20
2. Rehydrated	Ethanol absolute	5
Incremental alcohol wash (Carl Roth, Karlsruhe)	Ethanol 96%	5
	Ethanol 80%	5
	Ethanol 70%	5
	Ethanol 60%	5
	Ethanol 50%	5
3. Rinse	Aqua destillata	Flush
4. Hydrolyse (Carl Roth, Karlsruhe)	Periodic acid 1%	10
5. Rinse	Tap water	10
6. Rinse	Aqua destillata	2 x 2
7. Stain (Carl Roth, Karlsruhe)	Schiff's reagent	10-20
8. Rinse	Tap water (min 35°C)	5
9. Rinse	Aqua destillata	Flush
10. Stain (Carl Roth, Karlsruhe)	Hemalum sol. acc. to Mayer	5
11. Rinse	Tap water (running)	10-15

12. Dehydrated	Ethanol 80%	flush
Alcohol	Ethanol 96%	flush
(Carl Roth, Karlsruhe)	Ethanol absolute	2.5
	Ethanol absolute	2.5
13. Clearing	Xylol	10
(Merck, Darmstadt)		

3.4.6 Light microscopic examination

All slides were analyzed using the light microscope Leica DMR (Leica Mikrosystem GmbH, Wetzlar) at the clinic. The tissue slides were stained with H&E and examined for signs of foetal dystelectasis, interstitial edema, lymphangiectasis and neutrophils in blood vessels, congestion of blood vessels, and the presence of corpuscular and amorphous elements and cellular infiltration in the interstitium and lumen of the bronchioles and alveoli. Further, the alveolar maturity was assessed using the grading developed and used by Schoon (1989). Pictures of histological alterations can be found in the appendix.

To better visualize meconium and amniotic fluid as well as evaluate the basement layer of the respiratory epithelium a second batch of tissue sections were stained with PAS reaction and counterstained with hematoxylin.

The slides were first examined in terms of the stain quality and the depiction of the typical tissue structures, their integrity and cut accuracy. Only slides of good quality were evaluated. Images of the evaluated elements were captured with a digital camera Leica DC 300 (Leica Mikrosystem AG, Heerbrugg, Switzerland) attached to the light microscope Leica DMR and stored on the computer GX 240 Dell using the corresponding picture archiving software (Leica Mikrosystem AG, Heerbrugg, Switzerland). The extent of the histopathological alterations were examined, first at a 25 times magnification to ascertain the extent of the dystelectasis and measure the examined area using the above mentioned software. Then the cuts were evaluated

closer with a 100, 200 and 400 times magnification to better visualize cellular alterations. This was done using a standardized protocol for the H&E staining and a second time with a modified protocol for the PAS stains (Waldner et al. 2010, Zagariya et al. 2010, Castro-Najera et al. 2006, Ikeda et al. 1998, Staribratova & Belovejdov 2011).

In the H&E stain the frequency and severity of the following variables were scored either on a scale from 0 to 3; 0 representing no alterations and 3 severe alterations or with a binary score; 0 meaning not present and 1 meaning present (Castro-Najera et al. 2006, Martinez-Burnes et al. 2002 Eigenmann et al. 1984) :

- Foetal dystelectasis: poorly aerated lung tissue section → a ruffled/wrinkled ("Kraeuselstruckture") to a hepatic like appearance
- Interstitial edema: separation of the connective tissue elements
- Lymphangiectasis: dilatation of lymphatic vessels
- Blood vessel:
    - Neutrophils: light eosinophilic cytoplasm with a 3-5 lobed nucleus
    - Blood vessel congestion: hyperaemia/haemorrhage

Presence of corpuscular and amorphous elements

- Meconium: amorphous usually yellow to red-orange material aggregates
- Keratin: eosinophilic filamentous material, without a nucleus
- Squamous epithelial cells (SEC): weak eosinophilic polygonal cell with a nuclear "shadow"
- Cell debris: basophilic nuclear detritus
- Hair: segmented eosinophilic filamentous material (PAS positive stain)
- Amniotic fluid: basophilic cloudy substance

- Edema (around the bronchioles and blood vessels): separation of the connective tissue elements
- Hyaline membranes: eosinophilic homogeneous material resting on the epithelium and protruding into the lumen

Cellular infiltration

- Erythrocytes (Hemorrhage: congestion with erythrocytes
- Neutrophils: light eosinophilic with a 3-5 lobed nucleus

Alveolar tissue maturity (Histological characteristics modified from Schoon 1989)

- Alveolar epithelium continual cubical; scarcely capillarized; substantial mesenchyme
- Alveolar epithelium continual cubical; intensive capillarisation; substantial mesenchyme
- Alveolar epithelium in individual alveoli still cubical; intensive capillarization (two rows); substantial mesenchyme
- Alveolar epithelium generally discontinues cubical; intensive capillarization (one and two rows); reduced mesenchyme
- Differentiated alveolar epithelium; capillarization one row; mesenchyme reduced to a minimum

For the PAS reaction the frequency and severity of the following variables were scored the same way the H&E variables were scored.

- Meconium: amorphous pink to orange material aggregates
- Amniotic fluid: pink cloudy substance

3.5 Analytical goal

The histological examination of neonatal lungs in four different locations allowed us to gain a perspective of the alterations found in the lungs due to dystocia and their potential hazards. Three different species were examined, 37 calves, 10 foals and 16 puppies in four different locations. This enabled us to compare variations between different locations within one species and compare how the different species vary.

Using the same protocol for all examined lung sections, 34 different parameters were assessed. The occurrence of each parameter was counted, the frequency was then calculated and the distribution within the four examined locations analyzed.

Also examined, for the bovine neonates, was the occurrence of neutrophils in the bronchioles and alveoli in relation with the occurrence of corpuscular and amorphous elements with respect to their distribution within the lungs. This was done only for calves due to the low case numbers by the other two species.

3.6 Statistical analysis

The statistical analysis of the data was carried out with the help of the unit for Biomathematics and Data processing of the Faculty of Veterinary Medicine of the Justus-Liebig-University, Giessen using the statistic program packages BMDP/Dynamic®, Release 8.1 (Dixon 1993) and StatXact (Cytel 2010). The graphics were generated on a personal computer using the program Microsoft® Office Excel 2010.

Qualitative parameters were displayed in two dimensional frequency tables according to location and separated for species. The statistical significance of differences between locations was verified with the exact-Friedman-test using the program StatXact (Cytel 2010). Additionally, a two dimensional analysis of dependency between the appearance of neutrophils and the occurrence of corpuscular and amorphous elements was carried out for the bovine neonates. Two dimensional frequency tables were generated and their relationship was tested with the exact-Wilcoxon-Mann-Whitney-test also using the

StatXact (Cytel 2010) program. In the assessment of the statistical significances a level of significance of $\alpha = 0.05$ related to each target variable was used, all p-value ≤ 0.05 were interpreted as statistically significant.

4 Results

4.1 Hematoxylin and eosin tissue staining

4.1.1 Histopathological alterations of lung tissue in neonatal calves

4.1.1.1 Foetal dystelectasis

It could be shown that 37 (100%) of the animals had some signs of foetal dystelectasis (Figure 5) in at least two locations. Both caudal lung lobes showed the most severe (100%) signs of dystelectasis (Table 13 and Graph 1). A significant association between the occurrence of dystelectasis and its distribution in the four locations could be shown ($p = 0.008$).

Table 13: Dystelectasis in the four lung locations in calves (n=37)

dystelectasis	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	8	21.6	5	13.5	7	18.9	2	5.4
< 50%	8	21.6	4	10.8	6	16.2	4	10.8
> 50%	6	16.2	6	16.2	6	16.2	8	21.6
100%	15	40.5	22	59.5	18	48.6	23	62.2

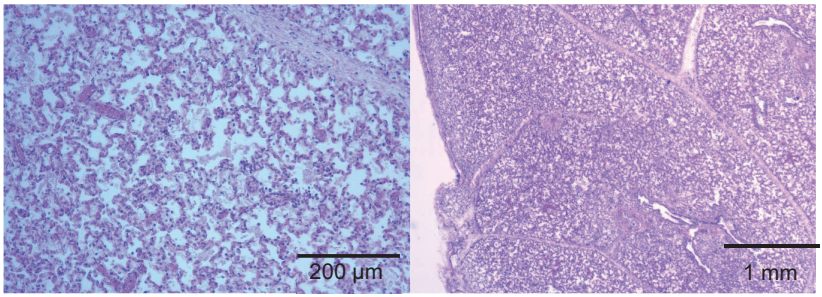
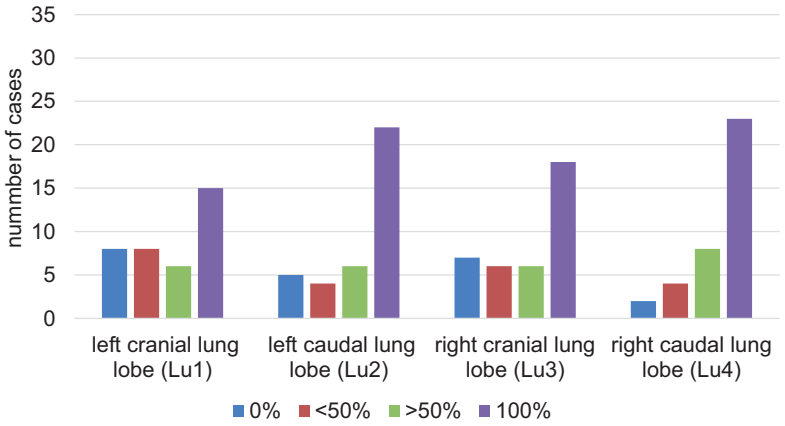


Figure 5: Dystelectasis in bovine neonatal lungs, H&E stain



Graph 1: Occurrence of dystelectasis in the four lung locations in calves (n = 37)

4.1.1.2 Interstitial edema

31 (83.8%) of the calves exhibited interstitial edema in three of the four localizations. The cranial lobes had a higher occurrence of edema then the caudal lobes (Table 14). The occurrence of interstitial edema and the distribution showed no association (p = 0.47).

Table 14: Interstitial edema in the four lung locations in calves (n=37)

location	interstitial edema			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	32	86.5	5	13.5
left caudal lung lobe (Lu2)	31	83.8	6	16.2
right cranial lung lobe (Lu3)	33	89.2	4	10.8
right caudal lung lobe (Lu4)	28	75.7	9	24.3

4.1.1.3 Lymphangiectasis

Of the examined calves 24 (64.9%) showed lymphangiectasis in three of the four locations. The presence of lymphangiectasis was greater in the cranial lobes then in the caudal lobes (Table 15). The occurrence of the defect and its distribution were not related (p = 0.98).

Table 15: Lymphangiectasis in the four lung locations in calves (n=37)

location	lymphangiectasis			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	28	75.7	9	24.3
left caudal lung lobe (Lu2)	26	70.3	11	29.7
right cranial lung lobe (Lu3)	28	75.7	9	24.3
right caudal lung lobe (Lu4)	26	70.3	11	29.7

4.1.1.4 Neutrophils in blood vessels

Of the calves examined 31 (83.8%) showed neutrophils in at least 3 locations. In the cranial lobes more cells could be detected then in the caudal lobes, the left cranial lobe had the most neutrophils (Tables 16). The findings are not statistically significant (p = 0.14).

Table 16: Neutrophils in the four lung locations in calves (n=37)

location	neutrophils in the blood vessels			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	32	86.5	5	13.5
left caudal lung lobe (Lu2)	30	81.1	7	18.9
right cranial lung lobe (Lu3)	32	86.5	5	13.5
right caudal lung lobe (Lu4)	26	70.3	11	29.7

4.1.1.5 Blood vessel congestion

32 (86.5%) of the animals showed in all four examined locations congestion of the blood vessels. The left cranial lobe showed congestion in 36 of the cuts (Table 17). There was no relationship between the distribution and the occurrence of congestion in blood vessel ($p = 0.07$).

Table 17: Congestion of the blood vessels in the four lung locations in calves (n=37)

blood vessel congestion	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	1	2.7	4	10.8	2	5.4	2	5.4
< 50%	1	2.7	4	10.8	0	0.0	4	10.8
> 50%	7	18.9	7	18.9	11	29.7	8	21.6
100%	28	75.7	22	59.5	24	64.9	23	62.2

4.1.1.6 Corpuscular and amorphous elements in the bronchioles

4.1.1.6.1 Meconium

Meconium could be displayed in 24 (64.9%) of the examined animals in at least one of

the four locations. In the cranial lobes meconium was detected, though only mildly (< 3), most often (Table 18). The appearance of meconium was not dependent on its distribution in the bronchioles (p = 0.96).

Table 18: Meconium in the bronchiolar lumen in the four lung locations in calves (n=37)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	25	67.6	26	70.3	23	62.2	27	73.0
< 3	8	21.6	6	16.2	13	35.1	7	18.9
< 5	3	8.1	2	5.4	1	2.7	1	2.7
> 5	1	2.7	3	8.1	0	0.0	2	5.4

4.1.1.6.2 Keratin

Of the calves examined 28 (75.7%) showed keratin in at least three of the four locations. The distribution is fairly even, as shown in Table 19. A relationship between the distribution and the occurrence of keratin could not be made (p = 0.52).

Table 19: Keratin in the bronchiolar lumen in the four lung locations in calves (n=37)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	N	%
none	11	29.7	12	32.4	13	35.1	14	37.8
< 3	6	16.2	6	16.2	6	16.2	7	18.9
< 5	7	18.9	7	18.9	5	13.5	6	16.2
> 5	13	35.1	12	32.4	13	35.1	10	27.0

4.1.1.6.3 Squamous epithelial cells

16 (43.2%) of the examined calves showed SEC in at least one of the analysed areas in

the bronchiolar lumen. SEC were most often detected only mildly (< 3). In the right cranial lobe, in 2 cases, were more than 5 fragments detected (Table 20). The occurrence of SEC has no relationship to its distribution (p = 0.36).

Table 20: SEC in the bronchiolar lumen in the four lung locations in calves (n=37)

fragments of squamous epithelial cells	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	N	%	n	%
none	29	78.4	31	83.8	31	83.8	29	78.4
< 3	5	13.5	6	16.2	4	10.8	4	10.8
< 5	3	8.1	0	0.0	0	0.0	4	10.8
> 5	0	0.0	0	0.0	2	5.4	0	0.0

4.1.1.6.4 Cell debris

Cell debris could be detected in all four locations in 31 (83.8%) of the calves. The right caudal lung lobe showed the most severe signs of cell debris (Table 21). An association between cell debris and its distribution could not be made (p = 0.68)

Table 21: Cell debris in the bronchiolar lumen in the four lung locations in calves (n=37)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	N	%	n	%
none	3	8.1	2	5.4	3	2.7	1	2.7
< 3	4	10.8	3	8.1	1	10.8	3	8.1
< 5	3	8.1	5	13.5	4	21.6	4	10.8
> 5	27	73.0	27	73.0	24	64.9	29	78.4

4.1.1.6.5 Hair

In only one of the samples a mild amount of hair was found, 36 (97.2%) of the calves displayed no hair. A dependency between occurrence and location could not be calculated.

4.1.1.6.6 Amniotic fluid

25 (67.6%) of the examined animals showed amniotic fluid in at least three of the four lung lobes. The left lung lobes seem to be slightly more affected then the right (Table 22). No association could be made between findings of amniotic fluid and its distribution ($p = 0.47$).

Table 22: Amniotic fluid in the bronchiolar lumen in the four lung locations in calves (n=37)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	8	21.6	9	24.3	11	29.7	12	32.4
< 50%	19	51.4	18	48.6	20	54.1	17	45.9
> 50%	8	21.6	8	18.9	3	8.1	6	16.2
100%	2	5.4	3	8.1	3	8.1	2	5.4

4.1.1.6.7 Edema

22 (59.5%) of the calves showed signs of edema around the bronchioles and vessels, fairly even distributed throughout the four lobes (Table 23), therefore no relationship could be found between the occurrence of edema and its location ($p = 0.89$).

Table 23: Edema around the bronchioles in the four lung locations in calves (n=37)

location	edema			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	22	59.5	15	40.5
left caudal lung lobe (Lu2)	23	62.2	14	37.8
right cranial lung lobe (Lu3)	22	59.5	15	40.5
right caudal lung lobe (Lu4)	25	67.6	12	32.4

4.1.1.6.8 Hyaline membrane

Hyaline membranes were detected in 11 (30%) of the examined calves in at least two of the four locations. The left lung appears to be more affected than the right lung (Table 24). A relationship between distribution and appearance could not be found (p = 0.51).

Table 24: Hyaline membranes in the bronchiolar lumen in the four lung locations in calves (n=37)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	12	32.4	25	67.6
left caudal lung lobe (Lu2)	11	29.7	26	70.3
right cranial lung lobe (Lu3)	7	18.9	30	81.1
right caudal lung lobe (Lu4)	8	21.6	29	78.4

4.1.1.7 Cellular infiltration

4.1.1.7.1 Bronchiolar interstitium

Erythrocytes (hemorrhage) in the bronchiolar interstitium could be detected in 25 (67.6%) of the calves in at least one of the four examined lobes. The right cranial lobe showed the highest occurrence of erythrocytes. 13 (35.1%) of the calves displayed neutrophils, which seem to appear slightly more frequently in the right side of the lung. Table 25 and 26 show the distribution of the hemorrhage and neutrophils respectively. The occurrence of the cellular infiltration and its location showed no relationship (p = 0.12 & p = 0.64 respectively).

Table 25: Hemorrhage in the bronchiolar interstitium in the four lung locations in calves (n=37)

location	hemorrhage in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	14	37.8	23	62.2
left caudal lung lobe (Lu2)	10	27.0	27	73.0
right cranial lung lobe (Lu3)	18	48.6	19	51.4
right caudal lung lobe (Lu4)	14	37.8	23	62.2

Table 26: Neutrophils in the bronchiolar interstitium in the four lung locations in calves (n=37)

location	neutrophils in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	4	10.8	33	89.2
left caudal lung lobe (Lu2)	4	10.8	33	89.2
right cranial lung lobe (Lu3)	7	18.9	30	81.1
right caudal lung lobe (Lu4)	6	16.2	31	83.8

4.1.1.7.2 Bronchiolar lumen

In the bronchiolar lumen by 24 (64.9%) calves erythrocytes could be detected in at least one of the four examined lobes (Table 27). These results are not statistically significant (p = 0.21).

Table 27: Hemorrhage in the bronchiolar lumen in the four lung locations in calves (n=37)

location	hemorrhage in the lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	12	32.4	25	67.6
left caudal lung lobe (Lu2)	17	45.9	20	54.1
right cranial lung lobe (Lu3)	10	27.0	27	73.0
right caudal lung lobe (Lu4)	13	35.1	24	64.9

Further in 26 (70.3%) of the examined calves neutrophils could be detected in the bronchiolar lumen. The left caudal and right cranial lobe appear to have a slightly higher occurrence of neutrophils (Table 28). This is simply a numerical increase, not statistically significant (p = 0.22).

Table 28: Neutrophils in the bronchiolar lumen in the four lung locations in calves (n=37)

location	neutrophils in the lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	9	24.3	28	75.7
left caudal lung lobe (Lu2)	16	43.2	21	56.8
right cranial lung lobe (Lu3)	15	40.5	22	59.5
right caudal lung lobe (Lu4)	14	37.8	23	62.2

4.1.1.8 Corpuscular and amorphous elements in the alveolar lumen

4.1.1.8.1 Meconium

In the alveoli of 33 (89.2%) calves meconium could be detected in at least one of the four examined locations. Table 29 shows the distribution of meconium in the four lung lobes, the right cranial lobe appears to have a lower occurrence of meconium. These results are not statistically significant (p = 0.49).

Table 29: Meconium in the alveoli in the four lung locations in calves (n=37)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	14	37.8	12	32.4	16	43.2	14	37.8
< 3	5	13.5	10	27.0	10	27.0	10	27.0
< 5	10	27.0	5	13.5	3	8.1	3	8.1
> 5	8	21.6	10	27.0	8	21.6	10	27.0

4.1.1.8.2 Keratin

29 (78.4%) of the animals showed keratin in their alveoli in at least two of the analyzed locations. The distribution of keratin appeared to be relatively equal (Table 30), there was no relationship to the location where it was found (p = 0.60).

Table 30: Keratin in the alveoli in the four lung locations in calves (n=37)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	N	%	n	%
none	11	29.7	15	40.5	10	27.0	11	29.7
< 3	4	10.8	4	10.8	7	18.9	5	13.5
< 5	6	16.2	5	13.5	3	8.1	5	13.5
> 5	16	43.2	13	35.1	17	45.9	16	43.2

4.1.1.8.3 Squamous epithelial cells

In the alveoli of 23 (62.2%) of the examined calves SEC could be detected in at least one of the lobs. The left cranial and right caudal lobs appear to have a higher occurrence of SEC (Table 31). An association to its locations could not be established (p = 0.76).

Table 31: SEC in the alveoli in the four lung locations in calves (n=37)

fragments of SEC	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	24	64.9	29	78.4	28	75.7	26	70.3
< 3	12	32.4	5	13.5	5	13.5	10	27.0
< 5	0	0.0	2	5.4	3	8.1	1	2.7
> 5	1	2.7	1	2.7	1	2.7	0	0.0

4.1.1.8.4 Cell debris

37 (100%) of the examined calves had at least moderate signs of cell debris in the alveoli in all four locations. The distribution of the debris is fairly uniform (Table 32), a relationship to the location was not found (p = 1).

Table 32: Cell debris in the alveoli in the four lung locations in calves (n=37)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	0	0.0	0	0.0	0	0.0	0	0.0
< 3	0	0.0	0	0.0	0	0.0	0	0.0
< 5	1	2.7	1	2.7	2	5.4	1	2.7
> 5	36	97.3	36	97.3	35	94.6	36	97.3

4.1.1.8.5 Hair

Hair could not be found in the alveoli of any of the examined animals.

4.1.1.8.6 Amniotic fluid

All of the calves (100%) showed amniotic fluid in at least one of the examined locations and 32 (86.5%) in at least two locations. Table 33 shows a relatively uniform distribution, therefore no relationship to its distribution (p = 0.76).

Table 33: Amniotic fluid in the alveoli in the four lung locations in calves (n=37)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	4	10.8	4	10.8	3	8.1	5	13.5
< 50%	18	48.6	17	45.9	18	48.6	18	48.6
> 50%	14	37.8	14	37.8	14	37.8	13	35.1
100%	1	2.7	2	5.4	2	5.4	1	2.7

4.1.1.8.7 Hyaline membrane

Of the calves examined 35 (94.6%) showed hyaline membranes in at least one of the four locations. The detection of hyaline membranes, as seen in Figure 6, in the alveoli showed an association with their location (p = 0.04). Table 34 and Graph 2 show the distribution throughout the four examined areas.

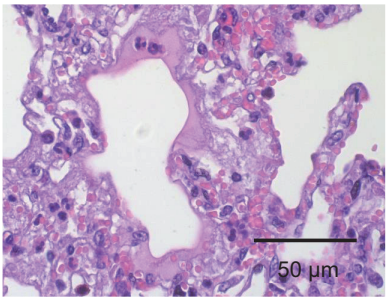
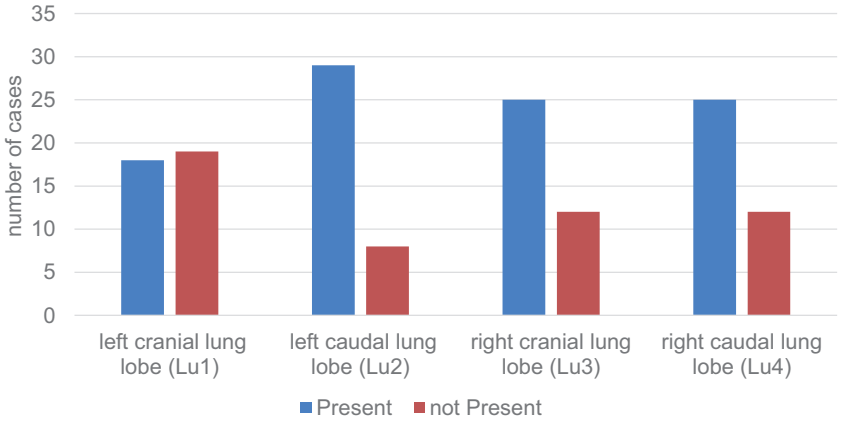


Figure 6: Hyaline membrane in the alveoli of bovine neonates, H&E stain

Table 34: Presence of hyaline membranes in the alveoli in the four lung locations in calves (n=37)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	18	48.6	19	51.4
left caudal lung lobe (Lu2)	29	78.4	8	21.6
right cranial lung lobe (Lu3)	25	67.6	12	32.6
right caudal lung lobe (Lu4)	25	67.6	12	32.6



Graph 2: Presence of hyaline membranes in the alveoli in the four lung locations in calves (n=37)

4.1.1.9 Cellular infiltration

4.1.1.9.1 Alveolar septs

In the tissue examined at least 32 (86.5%) calves showed hemorrhage in the alveolar septs in at least two of the four analyzed locations. The distribution in the four different locations was relatively uniform (Table 35). These findings were not statistically significant (p = 0.11).

Table 35: Hemorrhage in the alveolar septs in the four lung locations in calves (n=37)

location	hemorrhage in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	33	89.2	4	10.8
left caudal lung lobe (Lu2)	29	78.4	8	21.6
right cranial lung lobe (Lu3)	32	86.5	5	13.5
right caudal lung lobe (Lu4)	31	83.8	6	16.2

30 (81.1%) of the calves exhibited neutrophils in at least two of the four examined locations. The cells are distributed evenly throughout the lung tissue (Tables 36). The occurrence of the cellular infiltration and its appearance show no association (p = 0.82).

Table 36: Neutrophils in the alveolar septs in the four lung locations in calves (n=37)

location	neutrophils in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	26	70.3	11	29.7
left caudal lung lobe (Lu2)	29	78.4	8	21.6
right cranial lung lobe (Lu3)	28	75.7	9	24.3
right caudal lung lobe (Lu4)	28	75.7	9	24.3

4.1.1.9.2 Alveolar lumen

Of the calves examined 29 (78.4%) showed in at least two of the four locations hemorrhage in the alveoli. The left caudal lobe has a slightly higher occurrence of erythrocytes then the other three locations (Table 37). The occurrence of the alteration and its dissemination were not related (p =0.44). The analyzed tissue showed that 34 (91.9%) of these calves displayed neutrophils in at least three of four locations. The distribution of the neutrophils was relatively uniform (Tables 38). An association between neutrophils and their distribution could not be made (p = 0.98)

Table 37: Hemorrhage in the alveoli in the four lung locations in calves (n=37)

location	hemorrhage in the lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	23	62.2	14	37.8
left caudal lung lobe (Lu2)	28	75.7	9	24.3
right cranial lung lobe (Lu3)	23	62.2	14	37.8
right caudal lung lobe (Lu4)	24	64.9	13	35.1

Table 38: Neutrophils in the alveoli in the four lung locations in claves (n=37)

location	neutrophils in the alveoli			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	29	75.7	9	24.3
left caudal lung lobe (Lu2)	30	81.1	7	18.9
right cranial lung lobe (Lu3)	29	78.4	8	21.6
right caudal lung lobe (Lu4)	28	75.7	9	24.3

4.1.1.10 Alveolar tissue maturity

The lungs examined from all calves (37, 100%) showed an alveolar tissue maturity of at least grade 4 from 5. Eleven (29.7%) of the calves exhibited different maturity grads within the examined locations. Table 39 shows the distribution of alveolar maturity, a significant relationship to its location could not be established (p =0.98).

Table 39: Maturity of alveolar tissue in calves (n=37)

grade	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
1	0	0.0	0	0.0	0	0.0	0	0.0
2	0	0.0	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0	0	0.0
4	9	24.3	7	18.9	8	21.6	8	21.6
5	28	75.7	30	81.1	29	78.4	29	78.4

4.1.1.11 Analysis of Dependency

Dependency analyses were carried out between the occurrence of neutrophils and the appearance of corpuscular and amorphous elements with respect to the location.

4.1.1.11.1 Bronchioles

In the analysis of the occurrence of neutrophils and the detections of corpuscular and amorphous elements in the bronchioles with respect to the location, a relationship could be shown for the detection of meconium in the left cranial lung lobe ($p = 0.038$).
In the same location an association could also be made with the appearance of cell debris ($p = 0.047$). Table 40 shows all the p-values of the compared elements and the presence of neutrophils with respect to the location, significant ($p \leq 0.05$) findings are bold.

Table 40: p-values of the relationship between the detection of different elements and neutrophils with respect to the location in the bronchioles in claves (n=37)

examined lung lobe	location of neutrophils	meconium	keratin	debris	mucus
left cranial lung lobe (Lu1)	interstitium	**0.0378**	0.5466	**0.0469**	0.9518
	lumen	0.5101	0.3735	0.7977	0.3639
left caudal lung lobe (Lu2)	interstitium	0.3406	0.3015	0.7786	0.0608
	lumen	0.6675	0.6111	0.1201	0.6552
right cranial lung lobe (Lu3)	interstitium	1.0000	0.4308	0.6202	0.7538
	lumen	0.8502	0.6953	0.3417	0.9633
right caudal lung lobe (Lu4)	interstitium	0.5788	0.1214	0.0947	0.4458
	lumen	0.4107	0.9013	0.8300	0.2939

4.1.1.11.2 Alveoli

In the alveoli a relationship could be shown for the occurrence of neutrophils and keratin in the right caudal lung lobe ($p = 0.039$). Table 41 shows all the p-values of the

compared elements and the presence of neutrophils with respect to the location, significant ($p \leq 0.05$) findings are bold.

Table 41: p-values of the correlation between the detection of different elements and neutrophils with respect to the location in the alveoli in calves (n=37)

examined lung lobe	location of neutrophils	meconium	keratin	debris	mucus
left cranial lung lobe (Lu1)	interstitium	0.9277	0.1566	1.0000	0.3353
	lumen	0.4238	0.0805	1.0000	0.1659
left caudal lung lobe (Lu2)	interstitium	0.9386	0.3488	1.0000	0.4233
	lumen	0.6732	0.8811	1.0000	0.6085
right cranial lung lobe (Lu3)	interstitium	0.9941	0.1743	1.0000	0.9905
	lumen	0.5514	0.5505	1.0000	0.4687
right caudal lung lobe (Lu4)	interstitium	0.1043	**0.0392**	1.0000	0.9262
	lumen	0.8052	0.3660	1.0000	0.1242

4.1.2 Histopathological alterations of lung tissue in neonatal foals

4.1.2.1 Foetal dystelectasis

All of the animals (10) showed signs of foetal dystelectasis in at least two of the locations examined. The caudal lobes showed a more severe occurrence of improper inflation (Table 42). The results are not statistically significant ($p = 0.51$).

Table 42: Dystelectasis in the four lung locations in foals (n=10)

dystelectasis	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	1	10	0	0	1	10	0	0
< 50%	1	10	0	0	1	10	2	20
> 50%	2	20	3	30	3	30	0	0
100%	6	60	7	70	5	50	8	80

4.1.2.2 Interstitial edema

In six (60%) of the foals interstitial edema could be identified in 3 localizations[1]. The left caudal and right cranial lung lobes were affected most often (Table 43). The occurrence of interstitial edema in the distribution showed no association (p = 0.42).

Table 43: Interstitial edema in the four lung locations in foals (n=10)

location	interstitial edema			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	50	5	50
left caudal lung lobe (Lu2)	6	85.7	1	14.3
right cranial lung lobe (Lu3)	7	70	3	30
right caudal lung lobe (Lu4)	5	55.6	4	44.4

4.1.2.3 Lymphangiectasis

Five (50%) of the examined foals showed lymphangiectasis in two of the four locations. The right cranial lobe had the least occurrence of lymphangiectasis (Table 44). The appearance of the defect and its dispersion were not related (p =0.27).

[1] Four of the cuts were not counted because no interstitial connective tissue was present

Table 44: Lymphangiectasis in the four lung locations in foals (n=10)

location	lymphangiectasis			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	50	5	50
left caudal lung lobe (Lu2)	6	60	4	40
right cranial lung lobe (Lu3)	2	20	8	80
right caudal lung lobe (Lu4)	6	60	4	40

4.1.2.4 Neutrophils in blood vessels

Eight (80%) foals showed neutrophils in all four locations. The distribution was relatively even and so a relationship to their location could not be established (p = 1) (Table 45).

Table 45: Neutrophils in the four lung locations in foals (n=10)

location	neutrophils			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	8	80	2	20
left caudal lung lobe (Lu2)	9	90	1	10
right cranial lung lobe (Lu3)	8	80	2	20
right caudal lung lobe (Lu4)	8	80	2	20

4.1.2.5 Blood vessel congestion

All 10 examined foals (100%) showed congestion in all four locations. Only one animal showed mild congestion in the left caudal lobe. All the other foals (90%) showed at least a moderate congestion of the blood vessels in all lung lobes (Table 46). There was no

relationship between the distribution and the occurrence of blood vessel congestion (p = 0.27).

Table 46: Congestion of the blood vessels in the four lung locations in foals (n=10)

blood vessel congestion	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	0	0	0	0	0	0	0	0
< 50%	0	0	1	10	0	0	0	0
> 50%	4	40	4	40	2	20	3	30
100%	6	60	5	50	8	80	7	70

4.1.2.6 Corpuscular and amorphous elements in the bronchioles

4.1.2.6.1 Meconium

Five (50%) of the foals showed a mild (< 3) incidence of meconium in the bronchioles, of these 80% in only 1 lung lobe. In the right cranial lung lobe meconium could not be detected in any of the examined animals (Table 47). Due to the reasonably even distribution, no association to the location could be found (p = 0.69).

Table 47: Meconium in the bronchiolar lumen in the four lung locations in foals (n=10)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	8	80	8	80	10	100	8	80
< 3	2	20	2	20	0	0	2	20
< 5	0	0	0	0	0	0	0	0
> 5	0	0	0	0	0	0	0	0

4.1.2.6.2 Keratin

In seven (70%) of the foals keratin could be detected and in five (50%) of these in all four locations, with a fairly even distribution (Table 48). The results were not statistically significant (p = 0.58).

Table 48: Keratin in the bronchiolar lumen in the four lung locations in foals (n=10)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	4	40	5	50	5	50	4	40
< 3	2	20	2	20	0	0	2	20
< 5	0	0	0	0	0	0	0	0
> 5	4	40	3	30	5	50	4	40

4.1.2.6.3 Squamous epithelial cells

SEC could be detected in four (40%) of the examined foals in at least two of the four locations. The left caudal lung lobe showed no signs of SEC and in the sections were they could be observed SEC occurred mildly (<3) to moderately (3-5) (Table 49). The occurrence of SEC and their distribution had no association (p = 0.16).

Table 49: SEC in the bronchiolar lumen in the four lung locations in foals (n=10)

fragments of SEC	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	7	70	10	100	7	70	6	60
< 3	0	0	0	0	2	20	4	40
< 5	1	10	0	0	1	10	0	0
> 5	0	0	0	0	0	0	0	0

4.1.2.6.4 Cell debris

All but one animal (90%) showed cell debris in all four locations. Of these animals 50% display a severe (> 5) amount of debris in all four locations. Both caudal lobes were slightly more affected than the cranial lung lobes (Table 50). A significant difference could not be calculated (p =0.12).

Table 50: Cell debris in the bronchiolar lumen in the four lung locations in foals (n=10)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
None	1	10	0	0	1	10	0	0
< 3	1	10	0	0	1	10	0	0
< 5	1	10	1	10	2	20	3	30
> 5	7	70	9	90	6	60	7	70

4.1.2.6.5 Hair

One of the samples presented a mild (<3) amount of hair. 97.5% of the examined slides showed no hair. A statistically significant was not calculable.

4.1.2.6.6 Amniotic fluid

Six (60%) of the examined foals presented amniotic fluid in at least two of the four analyzed areas. Most of these animals showed mild (< 3) amounts of amniotic fluid (Table 51). A statistical significance could not be found (p = 0.60).

Table 51: Amniotic fluid in the bronchiolar lumen in the four lung locations in foals (n=10)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	7	70	4	40	6	60	5	50
< 50%	2	20	5	50	4	40	5	50
> 50%	1	10	1	10	0	0.0	0	0.0
100%	0	0.0	0	0.0	0	0.0	0	0.0

4.1.2.6.7 Edema

All (100%) foals displayed signs of edema around the bronchioles and seven (70%) in three of the four examined locations. The edema was fairly evenly distributed throughout the four lobes (Table 52), therefore not statistically significant (p = 0.96).

Table 52: Edema around the bronchioles in the four lung locations in foals (n=10)

location	edema			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	7	70	3	30
left caudal lung lobe (Lu2)	8	80	2	20
right cranial lung lobe (Lu3)	7	70	3	30
right caudal lung lobe (Lu4)	6	60	4	40

4.1.2.6.8 Hyaline membranes

Hyaline membranes could be detected in five (50%) of the foal, 60% of these showed signs in three of four lobes. Most of the hyaline membranes were found in the right cranial lung lobe (Table 53). An association between hyaline membranes and its distribution could not be made (p = 0.11).

Table 53: Hyaline membranes in the bronchiolar lumen in the four lung locations in foals (n=10)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	1	10	9	90
left caudal lung lobe (Lu2),	3	30	7	70
right cranial lung lobe (Lu3),	5	50	5	50
right caudal lung lobe (Lu4)	2	20	8	80

4.1.2.7 Cellular infiltration

4.1.2.7.1 Bronchiolar interstitium

Hemorrhage could be detected in eight (80%) of the neonates in at least one of the four examined locations, of these 37.5% showed erythrocytes in more than one lung lobe. The distribution is quite even (Table 54). Neutrophils were found in four (40%) of the foals in at least one lobe (Table 55). The occurrence of the cellular infiltration had no relationship to its dissemination ($p = 0.79$ & $p = 1$, respectively).

Table 54: Hemorrhage in the bronchiolar interstitium in the four lung locations in foals (n=10)

location	hemorrhage in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	4	40	6	60
left caudal lung lobe (Lu2)	4	40	6	60
right cranial lung lobe (Lu3)	4	40	6	60
right caudal lung lobe (Lu4)	2	20	8	80

Table 55: Neutrophils in the bronchiolar interstitium in the four lung locations in foals (n=10)

location	neutrophil in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	1	10	9	90
left caudal lung lobe (Lu2)	2	20	8	80
right cranial lung lobe (Lu3)	2	20	8	80
right caudal lung lobe (Lu4)	1	10	9	90

4.1.2.7.2 Bronchiolar lumen

In the bronchiolar lumen of nine (90%) foals erythrocytes could be detected in at least one of the four examined lobe. The erythrocytes are almost completely evenly distributed, except in the right caudal lobe were 80% of the animals displayed hemorrhage (Table 56). In seven (70%) of the foals neutrophils could be detected in the bronchiolar lumen in at least one of the four examined locations. The neutrophils that were found were spread out fairly evenly (Table 57), thus an association to their distribution could not be made ($p = 0.37$ & $p = 0.80$, respectively).

Table 56: Hemorrhage in the bronchiolar lumen in the four lung locations in foals (n=10)

location	hemorrhage in the bronchiolar lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	50	5	50
left caudal lung lobe (Lu2)	5	50	5	50
right cranial lung lobe (Lu3)	5	50	5	50
right caudal lung lobe (Lu4)	8	80	2	20

Table 57: Neutrophils in the bronchiolar lumen in the four lung locations in foals (n=10)

location	neutrophils in the bronchiolar lumen			
	present		not present	
	N	%	n	%
left cranial lung lobe (Lu1)	4	40	6	60
left caudal lung lobe (Lu2)	3	30	7	70
right cranial lung lobe (Lu3)	3	30	7	70
right caudal lung lobe (Lu4)	5	50	5	50

4.1.2.8 Corpuscular and amorphous elements in the alveolar lumen

4.1.2.8.1 Meconium

In ten (100%) of the foals meconium could be detected in at least two of the four examined locations. The right caudal lung lobe shows the least amount of meconium (Table 58). These results are not statistically significant (p = 0.75).

Table 58: Meconium in the alveoli in the four lung locations in foals (n=10)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	3	30	2	20	2	20	4	40
< 3	3	30	5	50	6	60	4	40
< 5	4	40	1	10	1	10	2	20
> 5	0	0.0	2	20	1	10	0	0.0

4.1.2.8.2 Keratin

Seven (70%) of the animals showed keratin in their alveoli in all four analyzed locations. The dissemination of keratin appears to be relatively even (Table 59), therefore a relationship to its location cannot be found (p = 0.53).

Table 59: Keratin in the alveoli in the four lung locations in foals (n=10)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	1	10	3	30	2	20	3	30
< 3	1	10	2	20	2	20	0	0.0
< 5	1	10	0	0.0	1	10	0	0.0
> 5	7	70	5	50	5	50	7	70

4.1.2.8.3 Squamous epithelial cells

Of the examined animals SEC could be found in three of four locations in three (30%) foals. The appearance of SEC is equally distributed among all the lobes (Table 60). The occurrence of the alteration and its distribution showed no association (p = 0.89).

Table 60: SEC in the alveoli in the four locations in foals (n=10)

fragments of SEC	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	7	70	7	70	7	70	7	70
< 3	2	20	1	10	2	20	3	30
< 5	0	0.0	2	20	1	10	0	0.0
> 5	1	10	0	0.0	0	0.0	0	0.0

4.1.2.8.4 Cell debris

All of the examined foals showed at least moderate (< 5) amounts of cell debris, of these seven (70%) showed severe (> 5) sings in their alveoli in all four locations. The right cranial lung lobe showed the least amount of debris (Table 61 and Graph 2). The association between the amount of debris and its location was significant (p = 0.047). The distribution of cell debris is shown in Figure 7.

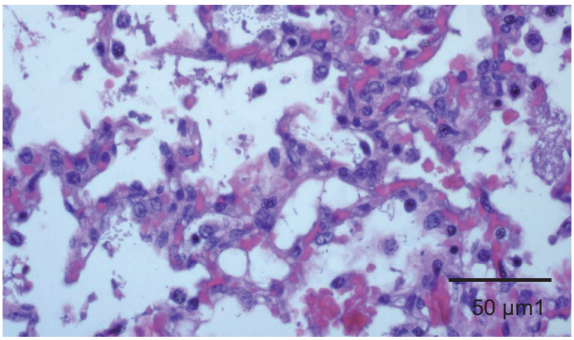
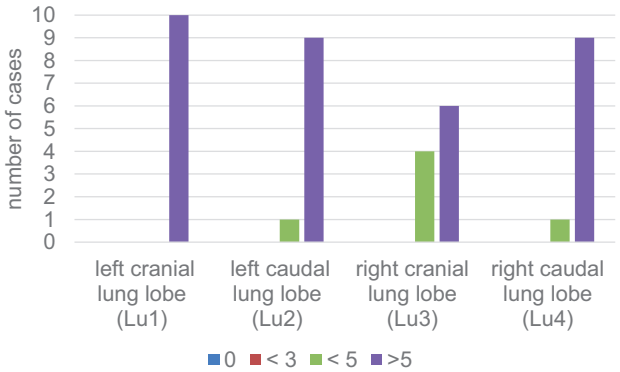


Figure 7: Cell debris in the alveoli of a foal, H&E stain

Table 61: Cell debris in the alveoli in the four lung locations in foals (n=10)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	N	%
none	0	0.0	0	0.0	0	0.0	0	0.0
< 3	0	0.0	0	0.0	0	0.0	0	0.0
< 5	0	0.0	1	10	4	40	1	10
> 5	10	100	9	90	6	60	9	90



Graph 3: Presence of cell debris in the alveoli in foals (n=10)

4.1.2.8.5 Hair

Hair could not be found in the alveoli of any of the animals examined.

4.1.2.8.6 Amniotic fluid

All of the analyzed foals (100%) showed amniotic fluid in at least two of the examined locations. The left caudal lobe showed the least amount of amniotic fluid and the right caudal lung lobe the most (Table 62). No statistically significant could be calculated (p = 0.65).

Table 62: Amniotic fluid in the alveoli in the four locations in foals (n=10)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	1	10	3	30	2	20	0	0.0
< 50%	6	60	4	40	7	70	7	70
> 50%	2	20	3	30	3	30	3	30
100%	1	10	0	0.0	0	0.0	0	0.0

4.1.2.8.7 Hyaline membrane

Six (60 %) of the examined foals showed hyaline membranes in three of the four locations. Hyaline membranes were most often detected in the right caudal lobe (Table 63). There was no relationship between the distribution and the occurrence of hyaline membranes (p = 0.75).

Table 63: Presence of hyaline membranes in the alveoli in the four lung locations in foals (n=10)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	50	5	50
left caudal lung lobe (Lu2)	5	50	5	50
right cranial lung lobe (Lu3)	6	60	4	40
right caudal lung lobe (Lu4)	7	70	3	30

4.1.2.9 Cellular infiltration

4.1.2.9.1 Alveolar septs

Of the examined tissue sections nine (90%) of the foals showed hemorrhage in the alveolar septs in all analysed locations. The distribution in the four different locations was relatively uniform (Table 64) and so an association could not be shown (p = not calculable). Seven (70%) of the foals displayed neutrophils in at least three of the examined locations. The cells were distributed quite evenly throughout the lung tissue (Tables 65). The occurrence of cellular infiltration had no association to its distribution (p = 0.89).

Table 64: Hemorrhage in alveolar septs in the four lung locations in foals (n=10)

location	hemorrhage in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	10	100	0	0
left caudal lung lobe (Lu2)	10	100	0	0
right cranial lung lobe (Lu3)	10	100	0	0
right caudal lung lobe (Lu4)	9	90	1	10

Table 65: Neutrophils in the alveolar septs in the four lung locations in foals (n=10)

location	neutrophils in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	7	70	3	30
left caudal lung lobe (Lu2)	6	60	4	40
right cranial lung lobe (Lu3)	7	70	3	30
right caudal lung lobe (Lu4)	8	80	2	20

4.1.2.9.2 Alveolar lumen

All foals showed in at three of the four examined locations hemorrhage in the alveoli. The right cranial lobe has a slightly higher occurrence of erythrocytes then the other three locations (Table 66). There was no relationship between the distribution and the occurrence of hemorrhage ($p = 0.91$). The analyzed tissue shows that eight (80%) of the foals display neutrophils in at least three of the four locations. The distribution of cells is relatively uniform (Tables 67) and has no association ($p = 0.93$).

Table 66: Hemorrhage in the alveolar lumen in the four lung locations in foals (n=10)

location	hemorrhage in the alveoli			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	9	90	1	10
left caudal lung lobe (Lu2)	8	80	2	20
right cranial lung lobe (Lu3)	10	100	0	0
right caudal lung lobe (Lu4)	9	90	1	10

Table 67: Neutrophils in the alveolar lumen in the four lung locations in foals (n=10)

location	neutrophils in the alveoli			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	8	80	2	20
left caudal lung lobe (Lu2)	8	80	2	20
right cranial lung lobe (Lu3)	7	70	3	30
right caudal lung lobe (Lu4)	9	90	1	10

4.1.2.10 Alveolar tissue maturity

Nine (90%) of the foal lungs examined showed an alveolar tissue maturity of grade 5. Of these 20% displayed a different maturity grad in the left cranial lobe (Table 68). The tissue maturity showed no dependence to location (p = 0.25).

Table 68: Maturity of alveolar tissue in foals (n=10)

grade	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	3	30	1	10	1	10	1	10
5	7	70	9	90	9	90	9	90

4.1.3 Histopathological alterations of lung tissue in neonatal puppies

4.1.3.1 Foetal dystelectasis

14 (87.5%) of the animal showed signs of foetal dystelectasis in at least one of the locations examined of these 63% had signs in all four areas. Most of the puppies (12, 75%) displayed severe signs of inadequate ventilation in at least three of the four locations. The alterations were quite evenly distributed throughout the lung tissue (Table 69) and so a relationship could not be established (p = 0.86).

Table 69: Dystelectasis in the four lung locations in puppies (n=16)

dystelectasis	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	4	25.0	3	18.8	3	18.8	4	25.0
< 50%	0	0.0	2	6.2	1	6.2	1	6.2
> 50%	0	0.0	0	0.0	1	6.2	1	6.2
100%	12	75.0	12	75.0	11	68.7	10	62.5

4.1.3.2 Interstitial edema

8 (50%) of the neonates showed connective tissue in the histological sections, in at least one lung lobe. Of these 4 animals had signs of interstitial edema, one of these puppies showed edema in two of the examined locations. An association could not be calculated (p = not calculable).

4.1.3.3 Lymphangiectasis

Of the examined puppies 10 (63%) showed, in two of the four locations lymphangiectasis. The left cranial lobe had the least signs of lymphangiectasis (Table 70). The occurrence of the defect and its dissemination were not related (p = 0.40).

Table 70: Lymphangiectasis in the four lung locations in puppies (n=16)

location	lymphangiectasis			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	31.3	11	68.8
left caudal lung lobe (Lu2)	9	56.3	7	43.8
right cranial lung lobe (Lu3)	8	50	8	50
right caudal lung lobe (Lu4)	9	56.3	7	43.8

4.1.3.4 Neutrophils in blood vessels

10 (62.5%) of the puppies showed neutrophils in at least two of the four locations. The distribution was relatively even, a relationship could not be made. (Table 71) (p = 0.90).

Table 71: Neutrophils in the four lung locations in puppies (n=16)

location	neutrophils in the blood vessels			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	9	56.2	7	43.7
left caudal lung lobe (Lu2)	9	56.2	7	43.7
right cranial lung lobe (Lu3)	8	50	8	50
right caudal lung lobe (Lu4)	7	43.7	9	56.2

4.1.3.5 Blood vessel congestion

All examined neonatal lung sections (16, 100%) showed congestion, 9 (56.3 %) showed severe congestion in all four locations (Table 72). The distribution of the occurrence of blood vessel congestion was not related (p = 0.40).

Table 72: Congestion of the blood vessels in the four locations in puppies (n=16)

blood vessel congestion	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	0	0.0	0	0.0	0	0.0	0	0.0
<50%	0	0.0	2	12.5	1	6.2	1	6.2
>50%	4	25	4	25	3	18.8	3	18.8
100%	12	75	10	62.5	12	75	12	75

4.1.3.6 Corpuscular and amorphous elements in the bronchioles

4.1.3.6.1 Meconium

Of the examined puppies 12 (75%) showed a mild to moderate (< 3 - < 5) incidence of meconium in the bronchioles. The right cranial lung lobe had the least signs of meconium (Table 73). An association between the occurrence of meconium and its location could be found (p = 0.98).

Table 73: Meconium in the bronchiolar lumen in the four lung locations in puppies (n=16)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	11	68.7	11	68.7	12	75	10	62.5
< 3	5	31.3	5	31.3	2	12.5	6	37.5
< 5	0	0.0	0	0.0	2	12.5	0	0.0
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.1.3.6.2 Keratin

16 (100%) of the puppies displayed keratin, 7 (43.8%) of them in at least three of the four locations (Table 74). There was no relationship between the dissemination and the occurrence of Keratin (p =0.78).

Table 74: Keratin in the bronchiolar lumen in the four lung locations in puppies (n=16)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	2	12.5	3	18.8	1	6.2	1	6.2
< 3	5	31.3	1	6.2	7	43.7	6	37.5
< 5	4	25	6	37.5	5	31.3	1	6.2
> 5	5	31.3	6	37.5	3	18.8	8	50

4.1.3.6.3 Squamous epithelial cells

SEC could be detected in 7 (43.8%) of the examined puppies in at least one of the four locations. The caudal lobe showed the least signs of SEC. One animal showed more than a mild occurrence of SEC (Table 75). An association between SEC and its distribution could not be made (p = 0.41).

Table 75: SEC in the bronchiolar lumen in the four lung locations in puppies (n=16)

fragments of squamous epithelial cells	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	13	81.2	15	93.7	12	75	15	93.7
< 3	2	15.2	1	6.2	4	25	1	6.2
< 5	1	6.2	0	0.0	0	0.0	0	0.0
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.1.3.6.4 Cell debris

All but one animal (15, 93.7%) showed cell debris in all four locations. Of these animals 11 (68.8%) display severe (> 5) amounts of debris in at least three of the four locations. The distribution of cell debris was quite even throughout the lung (Table 76) and so not related (p = 0.73).

Table 76: Cell debris in the bronchiolar lumen in the four lung locations in puppies (n=16)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	0	0.0	0	0.0	1	6.2	0	0.0
< 3	1	6.2	0	0.0	2	12.5	1	6.2
< 5	3	18.8	2	12.5	2	12.5	3	18.8
> 5	12	75	14	87.5	11	68.7	12	75

4.1.3.6.5 Hair

None of the examined slides (16, 100%) showed any hair.

4.1.3.6.6 Amniotic fluid

14 (87.5%) of the analyzed puppies displayed amniotic fluid in at least one of the four examined areas. Most of the animals (13, 81.3%) showed mild to moderate (< 3 - < 5) amounts of amniotic fluid in the bronchiolar lumen (Table 77). An association could not be made (p = 0.59).

Table 77: Amniotic fluid in the bronchiolar lumen in all four locations in puppies (n=16)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	4	25	4	25	1	6.2	3	18.8
< 50%	8	50	6	37.5	10	62.5	10	62.5
> 50%	2	12.5	5	31.3	4	25	2	12.5
100%	2	12.5	1	6.2	1	6.2	1	6.2

4.1.3.6.7 Edema

Edema could be detected in 15 (93.7%) of the puppies in at least one of the four examined locations. The edema was fairly evenly distributed throughout the four lobes (Table 78). The location and appearance of edema are not related (p = 1).

Table 78: Edema around the bronchioles in all four locations in puppies (n=16)

location	edema			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	11	68.7	5	31.3
left caudal lung lobe (Lu2)	10	62.5	6	37.5
right cranial lung lobe (Lu3)	11	68.7	5	31.3
right caudal lung lobe (Lu4)	11	68.7	5	31.3

5.1.3.6.8 Hyaline membranes

4 (25%) of the puppies showed signs of hyaline membranes and always only in one examined section. The hyaline membranes that were found presented themselves only in the right lung (Table 79). The occurrence of the alteration and its distribution show no association (p = 0.34).

Table 79: Hyaline membranes in the bronchiolar lumen in the four lung locations in puppies (n=16)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	0	0.0	16	100
left caudal lung lobe (Lu2)	0	0.0	16	100
right cranial lung lobe (Lu3)	2	12.5	14	87.5
right caudal lung lobe (Lu4)	2	12.5	14	87.5

4.1.3.7 Cellular infiltration

4.1.3.7.1 Bronchiolar interstitium

Hemorrhage could be detected in 10 (62.5%) of the neonates in at least one of the four examined lobes. The distribution is quite even (Table 77). Neutrophils were found in 7 (43.8%) of the puppies in at least one lobe (Table 80). The occurrence of the cellular infiltration and its dissemination were not related ($p = 1$ & $p = 0.36$ respectively).

Table 80: Hemorrhage in the bronchiolar interstitium in the four lung locations in puppies (n=16)

location	hemorrhage in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	31.3	11	68.7
left caudal lung lobe (Lu2)	6	37.5	10	62.5
right cranial lung lobe (Lu3)	6	37.5	10	62.5
right caudal lung lobe (Lu4)	5	31.3	11	68.7

Table 81: Neutrophils in the bronchiolar interstitium in the four lung locations in puppies (n=16)

location	neutrophils in the interstitium			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	4	25	12	75
left caudal lung lobe (Lu2)	1	6.2	15	93.7
right cranial lung lobe (Lu3)	3	18.8	13	81.2
right caudal lung lobe (Lu4)	1	6.2	15	93.7

4.1.3.7.2 Bronchiolar lumen

In the bronchiolar lumen of 14 (87.5%) of the puppies hemorrhage could be detected in at least one of the four examined lobes. In the left caudal lung lobe, erythrocytes could be found most frequently (Table 82). These findings were not related ($p = 0.20$). In 10 (62.5%) of the puppies neutrophils could be detected in the bronchiolar lumen of all four examined locations. The neutrophils that were found were spread out fairly evenly (Table 83), correspondingly an association to their location could not be made ($p = 0.24$).

Table 82: Hemorrhage in the bronchiolar lumen in the four lung locations in puppies (n=16)

location	hemorrhage in the lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	31.3	11	68.7
left caudal lung lobe (Lu2)	11	68.7	5	31.3
right cranial lung lobe (Lu3)	8	50	8	50
right caudal lung lobe (Lu4)	7	43.7	9	56.2

Table 83: Neutrophils in the bronchiolar lumen in the four lung locations in puppies (n=16)

location	neutrophils in the lumen			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	11	68.7	5	31.3
left caudal lung lobe (Lu2)	14	87.5	2	12.5
right cranial lung lobe (Lu3)	14	87.5	2	12.5
right caudal lung lobe (Lu4)	15	93.7	1	6.2

4.1.3.8 Presence of corpuscular and amorphous elements in the alveolar lumen

4.1.3.8.1 Meconium

Meconium could be detected in the alveoli of 13 (81.3%) of the examined puppies in at least two of the four analyzed locations. The right cranial lobe appeared to have a lower occurrence of meconium than the other locations (Table 84). An association could not be found (p = 0.68).

Table 84: Meconium in the alveoli in the four lung locations in puppies (n=16)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	5	31.3	5	31.3	8	50	4	25
< 3	9	56.2	7	43.7	5	31.3	9	56.2
< 5	2	12.5	3	18.8	3	18.8	2	12.5
> 5	0	0.0	1	6.2	0	0.0	1	6.2

4.1.3.8.2 Keratin

15 (93.7%) of the animals showed keratin in their alveoli in three of the analyzed

locations. The distribution appease to be relatively equal (Table 85) and therefore shows no association to the location (p = 0.98).

Table 85: Keratin in the alveoli in the four lung locations in puppies (n=16)

fragments of keratin	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	2	12.5	2	12.5	1	6.2	1	6.2
< 3	2	12.5	2	12.5	3	18.8	3	18.8
< 5	3	18.8	3	18.8	4	25	3	18.8
> 5	9	56.2	9	56.2	8	50	9	56.2

4.1.3.8.3 Squamous epithelial cells

In the alveoli of 11 (68.7%) of the examined puppies SEC could be detected in at least one of the lobes. The left caudal lobs appeared to have the lowest occurrence of SEC (Table 86). A relationship between the distribution and the SEC could not be made (p = 0.55).

Table 86: SEC of in the alveoli in the four lung locations in puppies (n=16)

fragments of squamous epithelial cells	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	11	68.7	13	81.2	10	62.5	11	68.7
< 3	4	25	3	18.8	5	31.3	2	12.5
< 5	1	6.2	0	0.0	1	6.2	3	18.8
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.1.3.8.4 Cell debris

By all (16, 100%) puppies cell debris was found in the alveoli, 75% of them had sever signs of cell debris in all four locations. The distribution of the debris is fairly uniform

(Table 87). An association could not be made (p =0.66).

Table 87: Cell debris in the alveoli in all four lung locations in puppies (n=16)

fragments of cell debris	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	0	0.0	0	0.0	0	0.0	0	0.0
< 3	0	0.0	1	6.2	0	0.0	0	0.0
< 5	3	18.8	1	6.2	1	6.2	1	6.2
> 5	13	81.2	14	87.2	15	93.7	15	93.7

4.1.3.8.5 Hair

Hair could not be found in the alveoli of any of the animals examined.

4.1.3.8.6 Amniotic fluid

13 (81.3%) of the puppies showed amniotic fluid in at least one of the examined locations and 62.5% in at least three of the four locations. Most neonates displayed mild signs of amniotic fluid with a fairly even distribution through the lung (Table 88). The occurrence of the alteration and its appearance were not related (p = 0.60).

Table 88: Amniotic fluid in the alveoli in the four lung locations in puppies (n=16)

mucus	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	5	31.3	6	37.5	4	24	6	37.5
< 50%	8	50	8	50	10	62.5	9	56.2
> 50%	3	18.8	2	12.5	2	12.5	1	6.2
100%	0	0.0	0	0.0	0	0.0	0	0.0

4.1.3.8.7 Hyaline membrane

Of the puppies examined 11 (68.8%) showed hyaline membranes in at least one of the

four locations. The left cranial lung lobe showed the least signs of hyaline membranes (Table 89). An association between hyaline membranes and its distribution could not be found (p = 0.31).

Table 89: Hyaline membranes in the alveoli in the four lung locations in puppies (n=16)

location	hyaline membranes			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	5	31.3	11	68.7
left caudal lung lobe (Lu2)	7	43.7	9	56.2
right cranial lung lobe (Lu3)	9	56.2	7	43.7
right caudal lung lobe (Lu4)	8	50	8	50

4.1.3.9 Cellular infiltration

4.1.3.9.1 Alveolar septs

In the tissue examined 13 (81.3%) of the neonates showed hemorrhage in the alveolar septs in at least three of the four analysed locations. The distribution in the four different locations was relatively uniform (Table 90). The occurrence of hemorrhage and the distribution showed no association (p = 0.61).

Table 90: Hemorrhage in the alveolar septs in the four lung locations in puppies (n=16)

location	hemorrhage in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	11	68.7	5	31.3
left caudal lung lobe (Lu2)	13	81.2	3	18.8
right cranial lung lobe (Lu3)	13	81.2	3	18.8
right caudal lung lobe (Lu4)	11	68.7	5	31.3

10 (62.5%) of the puppies exhibited neutrophils in at least one of the four examined locations. The right caudal lobe was slightly more affected than the others (Tables 91). The appearance of cellular infiltration was not dependent on its distribution (p = 0.32).

Table 91: Neutrophils in the alveolar septs in the four locations in puppies (n=16)

location	neutrophils in the septs			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	6	37.5	10	62.5
left caudal lung lobe (Lu2)	4	25	12	75
right cranial lung lobe (Lu3)	3	18.8	13	81.2
right caudal lung lobe (Lu4)	7	43.7	9	56.2

4.1.3.9.2 Alveolar lumen

In 13 (81.3%) of the puppies hemorrhage could be detected in the alveoli in at least three of the four examined locations. The left caudal and right cranial lobe had a slightly higher occurrence of erythrocytes than the other two locations (Table 92). The distribution and appearance of hemorrhage was not related (p = 0.086).

Table 92: Hemorrhage in the alveoli in the four lung locations in puppies (n=16)

location	hemorrhage in the alveoli			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	10	62.5	6	37.5
left caudal lung lobe (Lu2)	14	87.5	2	12.5
right cranial lung lobe (Lu3)	14	87.5	2	12.5
right caudal lung lobe (Lu4)	10	62.5	6	37.5

Neutrophils could be found in the lungs of all neonates (16, 100%). Only one cut displayed no signs of neutrophils. The distribution of neutrophils was relatively uniform (Tables 93), an association to their location could not made (p = not calculable).

Table 93: Neutrophils in the alveoli in puppies (n=16)

location	neutrophils in the alveoli			
	present		not present	
	n	%	n	%
left cranial lung lobe (Lu1)	15	93.7	1	6.2
left caudal lung lobe (Lu2)	15	93.7	1	6.2
right cranial lung lobe (Lu3)	16	100	0	0.0
right caudal lung lobe (Lu4)	16	100	0	0.0

4.1.3.10 Alveolar tissue maturity

The examined lungs of all puppies (16, 100%) showed an alveolar tissue maturity of grade 3 (3, 18.8%) or 4 (8, 50%). 5 (31.2%) of the puppies exhibited different maturity grads between the examined locations (Table 94). An association could not be found (p = 0.94).

Table 94 Maturity of alveolar tissue in puppies (n=16)

grade	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
1	0	0.0	0	0.0	0	0.0	0	0.0
2	0	0.0	0	0.0	0	0.0	0	0.0
3	10	62.5	10	62.5	11	68.7	9	56.2
4	6	37.5	6	37.5	5	31.3	7	43.7
5	0	0.0	0	0.0	0	0.0	0	0.0

4.2 Periodic Acid Schiff's staining

4.2.1 Histopathological alterations of lung tissue in neonatal calves

4.4.1.2 Corpuscular and amorphous elements in the bronchioles

4.2.1.2.1 Meconium

Meconium was found in 10 (27.8%) of the examined animals in at least one of the four locations. Most of the animals showed only a mild (< 3) occurrence of meconium, the right cranial lung in only one cut (Table 95). The occurrence of meconium and its distribution showed no association (p = 0.43).

Table 95: Meconium in the bronchiolar lumen in the four lung locations in calves (n=37)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	31	86.1	33	89.2	36	97.3	33	89.2
< 3	4	11.1	4	10.8	1	2.7	4	10.8
< 5	1	2.8	0	0.0	0	0.0	0	0.0
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.2.1.2.2 Amniotic fluid

Of the examined calves 15 (41.7%) showed amniotic fluid in at least one of the four locations. The occurrence of amniotic fluid was quite evenly distributed throughout the lung (Table 96) and showed no association (p = 0.77).

Table 96: Amniotic fluid in the bronchiolar lumen in the four lung locations in calves (n=37)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	12	33.3	13	35.1	13	35.1	14	37.8
< 50%	17	47.2	17	45.9	17	45.9	16	43.2
> 50%	4	11.1	5	13.5	6	16.2	6	16.2
100%	3	8.3	2	5.4	1	2.7	1	2.7

4.2.1.3 Presence of corpuscular and amorphous elements in the alveolar lumen

4.2.1.3.1 Meconium

In the alveoli of 31 (83.8%) calves meconium could be detected in at least one of the four examined locations. The right caudal lung lobe appeared to have a higher occurrence of meconium than the other lobes (Table 97). A relationship could not be made ($p = 0.37$).

Table 97: Meconium in the alveoli in the four lung locations in calves (n=37)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	20	54.1	22	59.5	22	59.5	18	48.6
< 3	12	32.4	9	24.3	12	32.4	13	35.1
< 5	2	5.4	4	10.8	1	2.7	1	2.7
> 5	3	8.1	2	5.4	2	5.4	5	13.5

4.2.1.3.2 Amniotic fluid

All of the calves (37, 100%) showed amniotic fluid in at least one of the examined locations and 33 (89.2%) in all four locations. The neonates showed mild to moderate (>

5) incidences with a fairly uniform distribution (Table 98), so an association to its location could not be made (p = 0.89).

Table 98: Amniotic fluid in the alveoli in the four lung locations in calves (n=37)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	1	2.7	2	5.4	0	0.0	1	2.7
< 50%	29	78.4	28	75.7	33	89.2	31	83.8
> 50%	7	18.9	7	18.9	4	10.8	5	13.5
100%	0	0.0	0	0.0	0	0.0	0	0.0

4.2.2 Histopathological alterations of lung tissue in neonatal foals

4.2.2.2 Corpuscular and amorphous elements in the bronchioles

4.2.2.2.1 Meconium

In only two cases could signs of meconium be found (20%), both in the right caudal lung lobe (Table 99). The appearance of meconium and its distribution in the bronchioles were not related (p = 0.25).

Table 99: Meconium in the bronchiolar lumen in the four lung locations in foals (n=10)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	10	100	10	100	10	100	8	80
< 3	0	0.0	0	0.0	0	0.0	2	20
< 5	0	0.0	0	0.0	0	0.0	0	0.0
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.2.2.2.2 Amniotic fluid

All of the foals (10, 100%) showed amniotic fluid in at least one of the four examined locations. Most of the neonates show only mild (< 3) signs, the right lung lobes seem to be slightly more affected then the left (Table 100). The occurrence of the alteration and its dissemination were not related (p = 0.79).

Table 100: Amniotic fluid in the bronchiolar lumen in the four lung locations in foals (n=10)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	5	50	4	40	6	60	6	60
< 50%	3	30	6	60	4	40	4	40
> 50%	2	20	0	0.0	0	0.0	0	0.0
100%	0	0.0	0	0.0	0	0.0	0	0.0

4.2.2.3 Presence of corpuscular and amorphous elements in the alveolar lumen

4.2.2.3.1 Meconium

In the alveoli of eight (80%) of the foals meconium could be detected in at least one of the four examined locations. All of the foals showed a mild (> 3) occurrence and fairly even distribution of meconium in the four lung lobes (Table 101). An association could not be made (p = 0.86).

Table 101: Meconium in the alveoli in the four lung locations in foals (n=10)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	6	60	8	80	6	60	7	70
< 3	4	40	2	20	4	40	3	30
< 5	0	0.0	0	0.0	0	0.0	0	0.0
> 5	0	0.0	0	0.0	0	0.0	0	0.0

4.2.2.3.2 Amniotic fluid

10 (100%) of the foals showed amniotic fluid in at least one of the examined locations and seven (70%) in all locations. Most animals showed a mild (< 3) amount of amniotic fluid, the right cranial lung lobe appeared to have the least incidents (Table 102). A relationship between the distribution and amniotic fluid could not be established (p = 0.42).

Table 102: Amniotic fluid in the alveoli in the four lung locations in foals (n=10)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	1	10	0	0.0	2	20	1	10
< 50%	8	80	9	90	8	80	9	90
> 50%	1	10	1	10	0	0.0	0	0.0
100%	0	0.0	0	0.0	0	0.0	0	0.0

4.2.3 Histopathological alterations of lung tissue in neonatal puppies

4.2.3.2 Corpuscular and amorphous elements in the bronchioles

4.2.3.2.1 Meconium

Meconium could be shown in 11 (68.8%) of the examined animals in at least one of the four locations. In the right lung lobes meconium could be detected more often than in the left (Table 103). The appearance of meconium in the bronchioles was not dependent on its distribution (p = 0.77).

Table 103: Meconium in the bronchiolar lumen in the four lung locations in puppies (n=16)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	12	75	12	75	10	62.5	9	56.2
< 3	4	25	3	18.8	6	37.5	6	37.5
< 5	0	0.0	1	2.8	0	0.0	0	0.0
> 5	0	0.0	0	0.0	0	0.0	1	6.2

4.2.3.2.2 Amniotic fluid

16 (100%) of the examined animals showed amniotic fluid in at least two of the four locations and 13 (81.3%) in all four. The right cranial lung lobe seemed to be slightly more affected than the others (Table 104). An association could not be established (p = 0.86).

Table 104: Amniotic fluid in the bronchiolar lumen in the four lung locations in puppies (n=16)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	2	12.5	3	18.8	0	0.0	2	12.5
< 50%	10	62.5	8	50	12	75	10	62.5
> 50%	3	18.8	4	25	4	26	4	25
100%	1	6.2	1	6.2	0	0.0	0	0.0

4.2.3.3 Presence of corpuscular and amorphous elements in the alveolar lumen

4.2.3.3.1 Meconium

Of the examined puppies 12 (75%) showed meconium in at least one of the four locations. The cranial lung lobes appeared to be slightly more affected then the caudal

lobes (Table 105). The occurrence of meconium in the alveoli was not related to its distribution (p = 0.28).

Table 105: Meconium in the alveoli in the four lung locations in puppies (n=16)

fragments of meconium	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
none	8	50	11	68.7	8	50	10	62.5
< 3	6	37.5	5	31.3	5	31.5	5	31.3
< 5	1	6.2	0	0.0	3	18.8	1	6.2
> 5	1	6.2	0	0.0	0	0.0	0	0.0

4.2.3.3.2 Amniotic fluid

All of the puppies (16, 100%) showed amniotic fluid in at least one of the examined locations and 8 (50%) in all the locations. Table 106 shows a relatively uniform distribution between the four locations. The appearance of amniotic fluid and its distribution showed not association (p = 0.81).

Table 106: Amniotic fluid in the alveoli in the four lung locations in puppies (n=16)

amniotic fluid	left cranial lung lobe (Lu1)		left caudal lung lobe (Lu2)		right cranial lung lobe (Lu3)		right caudal lung lobe (Lu4)	
	n	%	n	%	n	%	n	%
0%	3	18.8	3	18.8	2	12.5	3	18.8
< 50%	11	68.7	9	56.2	12	75	12	12.5
> 50%	2	12.5	4	25	2	12.5	1	6.2
100%	0	0.0	0	0.0	0	0.0	0	0.0

5 Discussion

5.1 Discussion of the motivation

Peri-natum mortality continues to cause high losses in farm as well as companion animals, causing not only great economic losses but also emotional distress. In cattle the peri-natum mortality varies between 2-10% internationally (Mee et al. 2008), approximately 17% of foals die in the perinatal time (Haas et al. 1996) and in dogs the p.n. mortality is around 24% (Tønnessen 2012). The World Health Organization (2015) states that the neonatal mortality rate in humans is by 0.02% internationally.

Compared to humans the peri-natal mortality of animals is staggeringly high. This situation has continued to persist over decades being mainly due to housing and management failures and inefficiencies. Short comings in medical care, though, should also be considered. The aim of this study was to improve the understanding of peri-natum respiratory distress suffered by neonates. Therefore the focus was on lung tissue alterations which cause neonatal Respiratory Distress Syndrome (nRDS) and Meconium Aspiration Syndrome (MAS), some of the main causes for neonatal fatalities.

nRDS is a disorder found in neonates experiencing dystocia. The neonates are presented with reduced reflexes, lateral recumbency, gasping/grunting noises and tachypnea, resulting from an insufficient oxygen uptake and an increase carbon dioxide retention, leading to a respiratory acidosis (Sweet et al. 2010, Bleul 2009). Most commonly this disorder is seen in premature neonates who exhibit immature type 2 pneumocytes with insufficient surfactant production, causing the fluid-air interface to be inadequate for proper oxygen transfer (Speer 2014, Vannucchi et al. 2012, Sweet et al. 2013, Danlois et al. 2000). The presence of meconium also inactivates the efficiency of surfactant production, making nRDS also a disorder of mature neonates (Swarnam et al. 2012, Zimmermann et al. 2005, Vaala 1999). MAS is another disorder seen in neonates caused by the aspiration of meconium contaminated amniotic fluid (Mokra & Calkovska 2013, Swarnam et al. 2012). Due to these disorders, gas exchange is impaired and extra uterine life threatened. Edwards et al. (2013) states that 7% of newborn humans suffer from respiratory distress. In human neonatology, 1% of all

newborn suffer from nRDS usually due to dysfunctional surfactant production (Speer 2014). According to Swarnam et al. (2012) 10% of all respiratory failure in human neonates is due to MAS with a mortality of 39%. After the birth specific protocols should be followed, especially by neonates born after dystocia were nRDS and/or MAS could be a potential complication. Guidelines for the resuscitation of foals are available from Palmer (2007) and Vaala (1999). For the neonatal calf, guidelines are provided by Nagy (2009) and Minor & Riese (1984). Lee & Cohn (2015) and Wehrend & Wehrend (2013) present guidelines on pediatric care for puppies. It is essential that guidelines and protocols continue to be revised, adapted and improved, especially pertaining to respiratory disorders. This is a very slow process in veterinary neonatology.

A major hurdle is the fact that there are no approved medicines for newborn animals and so treatment strategies are usually based on experience and extrapolation (Collins 2012). There is little to no research concerning neonatal pharmacodynamics and/or pharmacokinetics making accurate and precise recommendations for the use of specific medicines is very difficult. Nevertheless the goal to strive for improved treatment procedures for neonates should always be worked towards. In order to facilitate this it was aim of this study to identify and display lung tissue alterations that occurred peri-natum during a dystotic birth and use the insight gained from these examinations to create an improved practical therapy protocol to increase neonatal survival. As well as enabling diagnostics, such as ultrasound, to be more effective.

5.2 Discussion of the literature

Many studies have been done on respiratory distress in the peri-natal phase due to dystocia, causing nRDS and MAS.

Research on lung pathology in veterinary neonatology, though, is limited. Especially when looking specifically at morphological alterations, which could lead to improved treatment strategies. In Table 107 authors that have published work focusing on neonatal lung alterations are summarized.

Table 107: Publications on neonatal lung examinations using varying methods in veterinary medicine

Author & year of publication	Species examined	Method of lung examination
Linke et al., 2013	Calves	Computer tomography
Waldner et al., 2010	Calves	Histopathological examination
Junge & Bostedt, 2004	Calves	Ultrasonography
Lopez & Bildfell, 1992	Calves	Histopathological examination
Schoon, 1989	Calves	Histopathological examination
Eigenmann et al., 1984	Calves	Histopathological examination
Schoon & Kikovic , 1987	Calves & Foals	Histopathological examination
Bedenice et al., 2003	Foals	Radiographic examination
Kirim et al., 2003	Puppies	Histopathological examination
Zagariya et al., 2000	Rabbit pups	i.a. Histopathological examination
Tyler et al., 1978	Rabbit pups	i.a. Histopathological examination
Castro-Najera et al., 2006	Piglets	Histopathological examination
Korhonen et al., 2003	Piglets	i.a. Histopathological examination

Linke et al. (2013) used computer tomography to comparatively examine and visualize the ventilation of bovine lungs. They were able to show a discrepancy of aeration between the dorsal and ventral lung locations. Linke et al. (2013) also showed that at birth bovine lungs were not fully ventilated, in fact calves did not reach their full gas exchanging capacity until fourteen days p.n.. With this study they were able to give insight into the potential ventilation pattern and duration to full aeration of the bovine neonatal lungs. Jung & Bostedt (2004) used ultra-sonographic examinations as a diagnostic tool in calves that experienced a dystotic birth to better describe ensuing

respiratory disorders. They were able to show that such alterations as amniotic fluid and/or meconium aspiration, lung edema and atelectasis could be visualized using ultra-sonography if the alterations were near the pleura. What Jung & Bostedt (2004) did not do was to comparatively examine different regions of the lung, which could have given insight into the potential distribution of different pneumopathies.

Lopez & Bildfell (1992) showed in the histological examination of neonatal calf lungs that meconium, keratin and SEC were commonly depicted and could be associated with mild alveolitis. A comparison of different locations was not done, never the less the lung alterations found in this study support the importance of critical care for neonates that suffered dystocia at birth. Calves delivered via caesarean section near term (prematurely) were analyzed histopathologically by Eigenmann et al. (1984). These calves showed signs of disturbed vascular permeability (edema, lymphatic ectatic dilatation, blood vessel hyperaemia) and degenerative epithelia lesions. Lesions which are in accordance with surfactant deficiency due to an immature surfactant system, with little or no evidence of inflammation. Even though the lung tissue examined in Eigenmann et al. (1984) study came from premature neonates similarities in alterations were seen, such as lymphangiectasis, hyaline membranes and cell debris. Schoon & Kikovic (1987) comparatively examined several locations of the calf and foal lungs. They were able to display cellular amniotic fluid components in all examined animals. They were further able to show a species specific distribution pattern. The presence of meconium was always accompanied by neutrophils and alveolar macrophages, indicative of an inflammatory reaction.

In human medicine studies are based on animal models or in-vitro experiments. Castro-Nájera et al. (2006), Korhonen et al. (2003), Zagariya et al. (2010, 2000) and Tyler et al. (1978) using animal models to induce a MAS showed such histological alterations as hyaline material (membranes), edema, epidermal cells, keratin, cell debris, cell death as well as an increased infiltration of alveolar macrophages and neutrophils. Zagariya et al. (2000) detected in their examination of lung lavages increased levels of inflammatory cytokines. Kääpä & Soukka (2008) induced MAS in piglets and found a high

phospholipase A_2 activity, a pro-inflammatory agent. The activation of the complement system could also be observed after the induction of MAS in piglets by Lindenskov et al. (2004). Further, Zagariya et al. (2011) showed that meconium induced lung cell apoptosis. Morka & Calkovsak (2013), Swarnam et al. (2012), van Ierland & de Beaufort (2009), Vidyasagar & Zagariya (2008) and Alonso-Spilsbury et al. (2005) showed in their reviews and discussion of the literature, that the aspiration of meconium is harmful to the lungs in many ways. It causes mechanical obstruction, contributes to persistent pulmonary hypertension, increased vascular permeability, an inflammatory response through the heightened activity of phospholipase A_2, cytokine and chemokine activation and complement activation as well as surfactant inactivation. Aspired meconium leads to severe respiratory distress in the newborn. As neutrophilic infiltration can also be demonstrated in calves, foals and puppies similar complement and cytokine reactions can be expected in these animals.

Work done with foals focuses mainly on the deleterious effects hypoxia has on the brain rather than the lungs under the syndrome name perinatal asphyxia syndrome. In perinatal asphyxia syndrome all organs can be effected but according to the literature most detrimental is HIE (Giguère 2008, Katz 2006, Galvin & Collins 2004, Vaala 1999). Craig et al. (2003) exposed rat alveolar macrophage cell lines to foal meconium which caused an increased stimulation of respiratory burst. nRDS can be seen on radiographs as an alveolar-interstitial radiographic pattern, which is a commonly observed thoracic radiographic alteration in foals with respiratory distress (Bedencie et al. 2003).

Kirimi et al. (2003) showed in their histopathological examination of neonatal canine lung tissue, that 20 hours after administration of meconium 26-50% of the tissue samples were infiltrated with leukocytes and markers indicating high activation of free oxygen radicals. Substantiating the increase of inflammatory process due to meconium aspiration due to a prolonged birth. Further supporting the need for medical intervention after a dystotic birth.

As has been shown studies pertaining to peri-partum hypoxia, meconium aspiration and the resulting syndromes such as nRDS and MAS have been extensively studies

especially in human medicine. But actual pathomorphological examinations of lung tissue and a comparison of locations is limited. An inter-species comparison is even rarer. Making this one of a very few study, to our knowledge, to not only compare different lung areas but also different species using one standardised protocol.

5.3 Discussion of the material and methods

Material

The tissue used in this study was from individuals delivered from mothers brought to the clinic experiencing dystocia. The neonates who did not survive the delivery (or died within 24 hours of birth) were included in the study, causing the examined group to be quite heterogeneous, which mirrors the diversity of the population. Through the strict inclusion criteria (all foetuses were term and showed no signs of malformations) a comparative study of the neonates was possible. The animals chosen for this study were the most common presented in the clinic and are also the most common farm and companion animals.

Four different lung locations were examined, this enabled us to compare different regions of the lungs to see if one area was more prone to pathological alterations. This could allow us to focus diagnostic examinations such as ultra-sonographic imaging, as done by Junge & Bostedt (2004), of surviving neonates to specific regions. Schoon (1989) compared different locations within the neonatal bovine lungs, finding differences in the cranial and caudal lobes. Eigenmann et al. (1984) examined multiple locations, but did not compare the different areas. As discussed earlier, with exception of Schoon & Kikovic (1987) who included foals in their study, the comparative analysis of multiple regions of the lungs has up until now, to our knowledge, been done only in calves. In this study we did not only examine multiple locations within the lungs, but also compared the different species (calf, foal and puppy).

Method

The frequency and severity of the variables were scored either on a scale from 0 to 3 (absent to severe) or with a binary score. A similar quantification of findings as used by Castro-Nájera et al. (2006) in their examination of meconium stained piglets, Kirimi et al. (2003) in their work with puppies and Eigenmann et al. (1984) who examined bovine neonates that died due to nRDS. Lopez & Bildfell (1992) used a binary (present and not present) scoring system when examining their calf lungs for inflammation. The H&E stain is a well-established technique to stain and evaluate histological slides, done in almost every study were histological sections are examined (Zagariya et al. 2011 & 2000, Waldner et al. 2010, Castro-Nájera et al. 2006, Kirimi et al. 2003, Korhnen et al. 2003, Lopez & Bildfell 1992, Schoon 1989, Eigenmann et al. 1984, Tyler et al. 1978).

The PAS reaction can be used to visualize fibrinous precipitation (plasma protein leakage), examine the congruity of the basement membrane, depict meconium, and demonstrate the presence of carbohydrate and glycoproteins (Waldner et al. 2010, Castro-Nájera et al. 2006, Lopez & Bildfell 1992, Eigenmann et al. 1984).

In this study both descriptive (absent to severe) and binary (present and not present) grading scales were used to quantify the examined variables, as these are well established methods of gathering data. When collecting qualitative data, a binomial scale can be misleading as the severity of the insult cannot be established, which could be essential for determining potential impact of a parameter. On the other hand grading an event from mild to severe is very observer biased, which could be avoided by a blinded examination carried out by at least two observers.

The qualitative parameters were displayed in two-dimensional frequency tables arranged according to location and separated by species. This was done to determine if one region of the lung is statistically more affected by the negative effects caused by dystocia. And to then allow us to compare these findings between the different species. Furthermore a two-dimensional analysis of dependency between appearance of neutrophils and the occurrence of corpuscular and amorphous elements was calculated. This was done for the bovine neonates as they presented the largest cohort (n = 37).

This enabled us to determine if a significant relationship can be found between the presence of neutrophils and occurrence of corpuscular and amorphous elements and their location.

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5.4 Discussion of the results

Over forty variables were examined, here a focus will be placed on the significant findings.

Dystelectasis was found in all species. In puppies an improper aeration of the tissue was found throughout the lungs. For the foals and the calves the caudal lungs appeared to be less ventilated. In the bovine neonates the insufficient inflation of both caudal sections was significant ($p = 0.008$). Schoon (1989) also found dystelectasis in his examination of term-calves mainly in the caudal lobes. Linke et al. (2013) described that thrifty calves showed a better aeration of the dorsal than the ventral lung segments. Linke et al. (2013), Lopez & Bildfell (1992) and Eigenmann (1984) all reported finding atelectasis in their examination of bovine lungs, but none of made any reference to specific locations.

Hyaline membranes in the alveoli were detected less frequently in the cranial lobes by both puppies and claves. Calves displayed hyaline membranes in the alveoli significantly less often in the left cranial lobe ($p = 0.04$). In foals hyaline membranes could be shown most often in the alveoli of the right caudal lobe. According to Vaala (1999) hyaline membrane disease is associated with intrapartum hypoxia. Neonatal hyaline membrane disease is a well-recognized disease in foals, with such histological findings as edematous alveoli, cellular debris, hyaline membranes lining the alveoli as well as thickened and hyper-cellular alveolar septs (Caswell & Williams 2007). For both puppies and foals a significance could not be calculated. This pattern of distribution, in the calves, could be caused by more severe hypoxia in the caudal lobes causing more tissue damage and endothelial leakage due to the greater occurrence of dystelectasis (Locci et al. 2014). Schoon (1989), Schoon & Kikovic (1987) and Eigenmann et al. (1984) all reported finding hyaline membranes in their pathohistological examination of

bovine lungs. They also found that the longer the neonate survived p.n. the more severe the alteration were. Schoon & Kikovic (1987) found in their work that calf lungs were more severely affected in the caudal lobes, which are similar findings to ours.

Meconium was found in the alveoli of 89.2% of the examined claves (n=33), all of the foals (n=10) and 81.3% of the puppies (n=13). The right lungs seemed to be effected less often in all species. For calves and puppies it was the right cranial lobe and in the foals it was the right caudal lobe which was least effected. For the foals and claves our findings are similar to those from Schoon & Kikovic (1987). The calves being more frequently affected in the caudal lung lobes and foals more often in the cranial lung lobes. This was a regular finding by Schoon & Kikovic (1987), allowing them to come to the conclusion that lesions occurred in an opposite pattern of distribution by foals and calves. Schoon & Kikovic (1987) attributed this distribution to the different maturation processes of the foal and calf lungs. A significance could not be established for the distribution of meconium.

The appearance of neutrophils and meconium in specific locations, was evaluated for the neonatal calves. In the bronchioles a significant association between the appearance of inflammation (neutrophils) and meconium could be calculated. In the bronchioles the right caudal ($p = 0.46$) lung lobes showed a significant association between inflammation and meconium. In 34% of the calves examined by Lopez & Bildfell (1992) meconium was present. They were further able to show that 22 of the analyzed calves who displayed meconium and/or SEC were positive for signs of inflammation. With the presence of meconium there was a 1.5 higher chance of finding sings of inflammation. Tyler et al. (1978) showed that in rabbit pups meconium aspiration increased the alveolar sept infiltration with neutrophils as well as the influx of hyaline material. The association between meconium and the presence of neutrophils speaks for cytokine and chemokine activation. This is substantiated by Yamada et al. (2000) who showed that amniotic fluid contaminated with meconium had a chemotactic activity for neutriphils. Swarnam et al. (2012) and Mokra & Calkovska (2013) both state in their work that meconium not only exhibits pro-inflammatory mediators but also

proteolytic enzymes. Many other authors have similar findings (Kopincova & Calkovska 2016, Zagariya et al. 2011, Poulsen & McGuirk 2009, van Ierland & de Beaufort 2009, Kääpä & Soukka 2008, Vidyasagar & Zagariya 2008, Lopez & Bildfell 1992). Homberg (2015) showed that new borne calves that were meconium stained had a prolonged time to sternal recumbence (T-SE) and a reduced milk uptake. Meconium stained neonates should be seen as critical patients.

Cell debris was an alteration found in all samples taken. The calves and puppies showed a fairly uniform distribution of cell debris. In the foals cell debris was found significantly less often in the alveoli of the right cranial lung lobe. Bedenice et al. (2003) in his study with, under four week old, foals found abnormalities in their radiographs most frequently in the caudal lung regions.

The alveolar tissue maturity of the neonates was also examined in this study. This was done using a modified protocol as described by Schoon (1989) in his work with calves. The same protocol was used for all species, identifying specific histological characteristics at the alveolar level to ascertain the maturity. 11 calves, two foals and five of the puppies exhibited different maturity levels in the four examined locations, this was also found by Schoon (1989). 23 calves and seven foals showed mature alveolar tissue (grade = 5), none of the puppies did. That the lungs of the puppies are less developed then those of the foals or calves (nidifugous species) is due to their different foetal development. Sipriani et al. (2009) gave a detailed summarization of the development of the canine lung. They showed that the pseudoglandular stage begins between the 35th and 46th day of gestation. The canalicular stage, which is the phase of maturation when pneumocytes I & II begin developing, was observed between the 48th and 57th day of gestation. From day 60 onwards the saccular stage starts occurring, followed by the alveolar phase most likely p.n.

Due to ethical reason this study was carried out without a control group, the information acquired from the literature allowed us to create protocols with likely alterations to expect within the lung tissue samples.

5.5 Conclusion

A significant difference in alterations between the species could not be shown. The increased migration of neutrophils into the lung which could be shown in all animals leads to the conclusion that neonates that are born following dystocia should not only be closely monitored concerning their acid-base status but an anti-inflammatory treatment should be considered. The influx of neutrophils into the lung tissue and lumen is a consistent feature with MAS (Korhonen et al. 2003). Meconium plays a major role in interstitial pneumonia in the peri-natal phase (Tyler et al. 1978). The effects triggered and the pathophysiology of aspired meconium are multi-factorial, van Ierland & de Beaufort (2009) highlight some of these, such as airway obstruction, toxic damage of lung tissue, inactivation of surfactant, pulmonary inflammation and hypertension. Mechanical obstruction causes insufficient ventilation as well as respiratory epithelial shedding (Zagariya et al 2000). Meconium also causes the inactivation of surfactant a major aspect of nRDS and MAS (Swarnam et al. 2012, Mokra & Calkovska 2013). The inflammatory response due to cytokine and chemokine and complement activation and the presents and activation of phospholipase A_2, caused by meconium, lead to interstitial pneumonia (Vidyasagar et al. 2005, Lindenskov et al. 2004, Zagariya et al 2000). Another problem that arises in the reanimation of unthrifty neonates is the pathologic metabolic/respiratory acidosis. The most common used buffer in veterinary neonatology is sodium bicarbonate, an inappropriate choice for neonates after dystocia (Bleul 2009). When treating neonates born after dystocia an alternative buffer to sodium bicarbonate, such as carbicarb should be used to balance acidosis, because of the neonates already compromised lung function (Bleul 2009).

Since it can be concluded that the migration of neutrophils is increased in neonates experiencing dystocia a non-steroidal anti-inflammatory drugs (NSAID) could be considered as part of future treatment regimens. There is need for further work in this area, as there are no studies addressing how newborns react and/or metabolize NSAID's. Until this is done, NSAID's should be implemented with caution.

6 Summary

It was the aim of this study to depict lung alterations in neonates, that died due to dystocia, histologically and to examine species specific distribution differences. Emphasis was placed on bovine neonate.

The lungs of 37 calves, 10 foals and 16 puppies were examined. The tissue samples were taken from the same locations and worked up with an H&E stain and a PAS reagent for histological examination.

The following relevant results were found:

- 37 calves, 10 foals and 14 puppies showed at least mild signs of foetal dystelectasis. In the calves the caudal lung lobes were affected significantly more severely than the cranial lobes ($p = 0.008$)
- Keratin and cell debris showed themselves in the lung tissue with a relative equal distribution in all neonates. In foals cell debris was found significantly less often in the right cranial lung lobe compared with the other lobes ($p = 0.05$).
- Hyaline membranes were found in all neonates (35 calves, 5 foals, 11 puppies). In the calves it could be shown that the left cranial lung lobes was significantly less affected ($p = 0.04$).
- In the analysis of dependency between the appearance of neutrophils and the occurrence of corpuscular and amorphous elements in relation to the location in calve lungs, it could be shown that in the bronchioles a relationship between the occurrence of neutrophils and meconium ($p = 0.04$) and neutrophils and cell debris ($p = 0.05$) exists. In the alveoli a dependency exists between the incidence of neutrophils and keratin ($p = 0.04$).

In summary it could be shown, that in deceased neonates lung alterations play an important role. Therefore a treatment with NSAID drugs in neonates form dystocia could be considered.

7 Zusammenfassung

Ziel der Studie war es, Lungenveränderungen von Neonaten, die wegen einer Dystokie verstarben, histologisch darzustellen und speziesspezifische Verteilungsunterschiede der Veränderungen zu untersuchen.

Lungengewebe von 37 Kälbern, 10 Fohlen und 16 Welpen, die verstorben waren, wurde untersucht. Die Gewebsproben wurden aus den gleichen Lokalisationen entnommen und zur histologischen Analyse mit einer H&E Färbung und einer PAS Reaktion aufgearbeitet.

Folgende relevanten Ergebnisse wurden erzielt:

- 37 Kälber, 10 Fohlen und 14 Welpen zeigten zumindest geringgradige. Zeichen von fetaler Dystelektase. Bei den Kälbern waren die kaudalen Lungenlappen signifikant hochgradiger betroffen als die kranialen Lappen ($p = 0,008$).
- Keratin und Zellreste im Lungengewebe zeigten sich mit einer relativ gleichmäßigen Verteilung bei allen Neonaten. Bei Fohlen wurden Zellreste signifikant seltener im rechten kranialen Lungenlappen dargestellt ($p = 0,05$).
- Hyaline Membranen konnten bei einigen Neonaten (35 Kalb, 5 Fohlen, 11 Welpen) dargestellt werden. Bei den Kälber konnte gezeigt werden, dass der linke kraniale Lungenlappen signifikant seltener betroffen war ($p = 0,04$).
- In der Zusammenhangsanalyse des Auftretens von Entzündungszellen und korpuskularen und amorphen Elementen in Relation zur Lokalisation in Kälberlungen wurde gezeigt, dass in den Bronchiolen eine Beziehung zwischen dem Auftreten von Neutrophilen und Mekonium ($p = 0,04$) und Neutrophilen und Zellresten ($p = 0,05$) herrscht. In den Alveolen existiert ein Zusammenhang zwischen dem Auftreten von Neutrophilen und Keratin ($p=0,04$).

Zusammenfassend konnte gezeigt werden, dass entzündlichen Lungenaffektionen eine große Bedeutung bei verstorbenen Neonaten zukommt. Eine Therapie mit NSAID Medikamenten erscheint bei Neonaten aus Dystokien indiziert.

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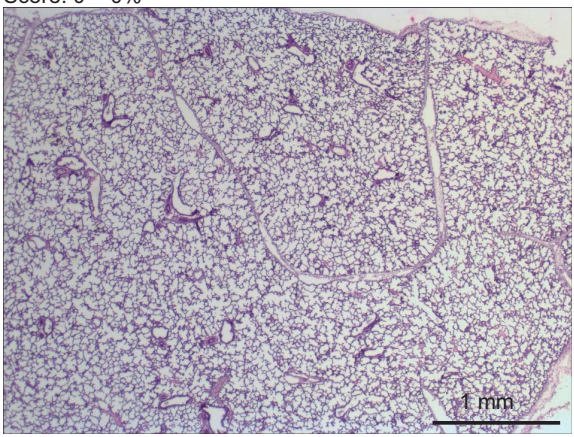
9 Appendix

Analysis examples for the descriptive and binomial scoring

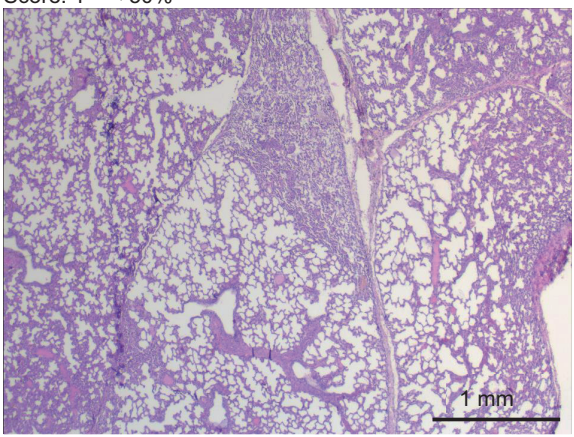
Hematoxylin and eosin stain

Foetal dystelectasis (magnification 2,5 x)*:*

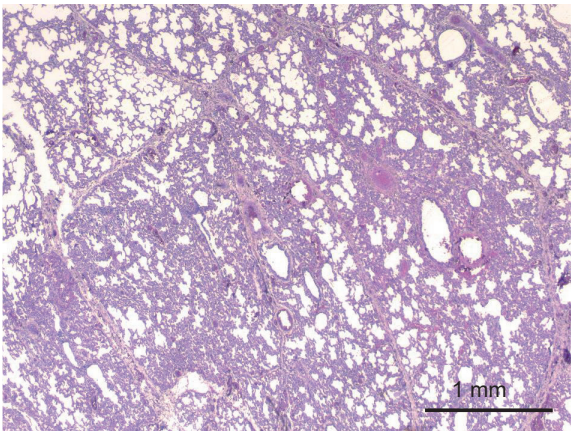
Score: 0 = 0%



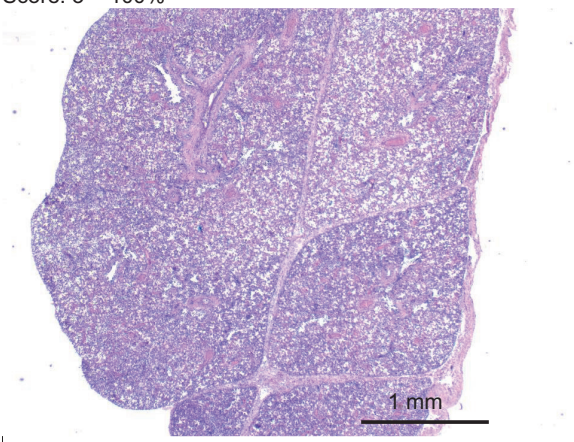
Score: 1 = < 50%



Score: 2 = > 50%

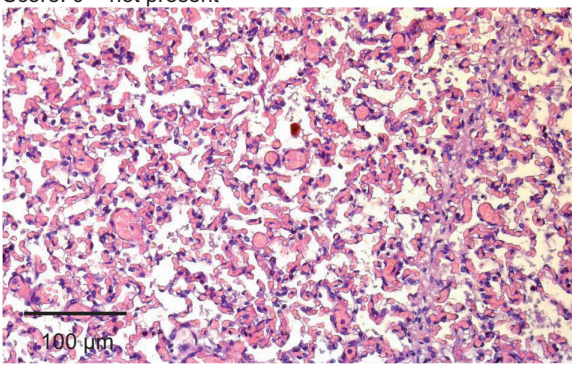


Score: 3 = 100%

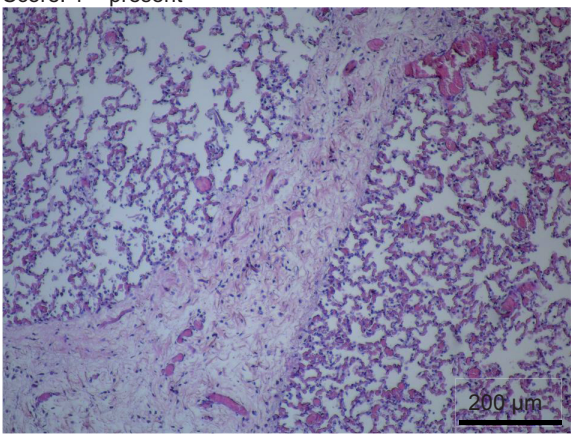


Interstitial edema (magnification 10x, 20x & 40x) :

Score: 0 = not present

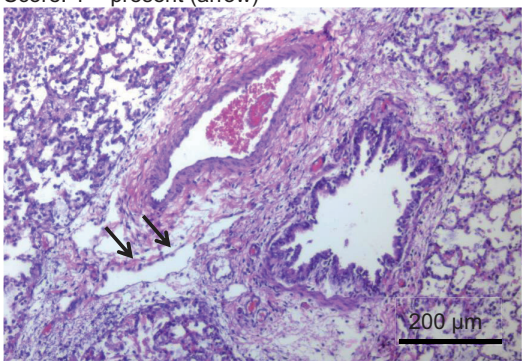


Score: 1 = present



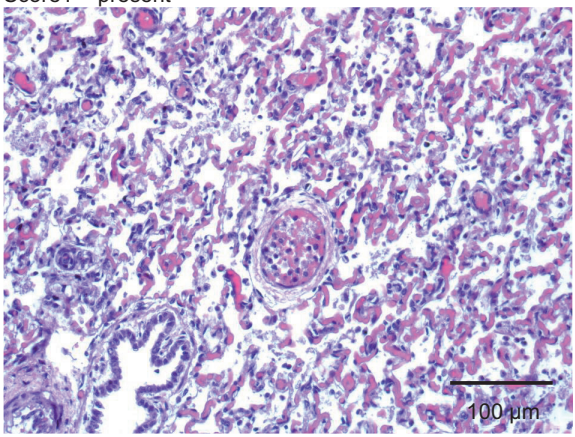
Lymphangiectasis (magnification 10x, 20x & 40x):

Score: 1 = present (arrow)



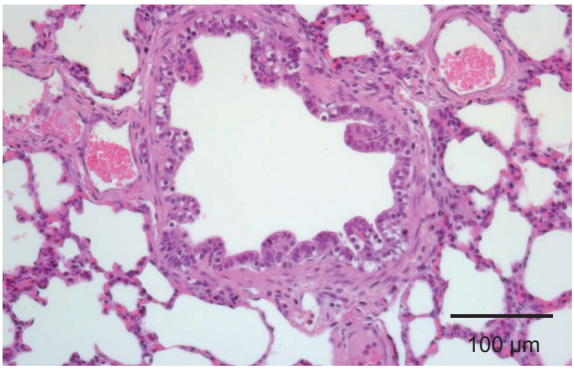
Neutrophils in blood vessels (magnification 10x, 20x & 40x):

Score1 = present

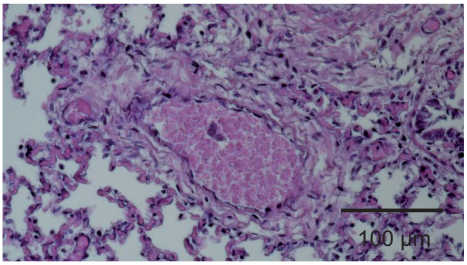


Blood vessel congestion (magnification 10x, 20x & 40x):

Score: 2 = > 50%

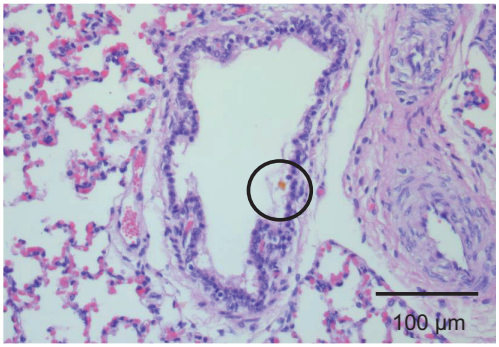


Score: 3 = 100%

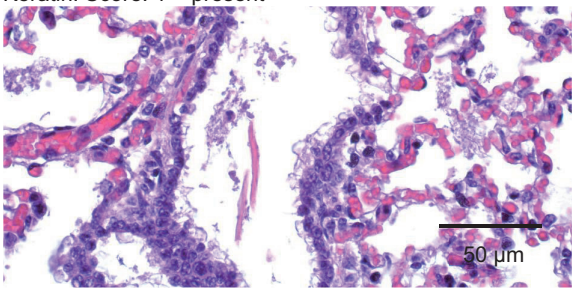


Presence of corpuscular and amorphous elements in the bronchiolar lumen (magnification 10x, 20x & 40x)

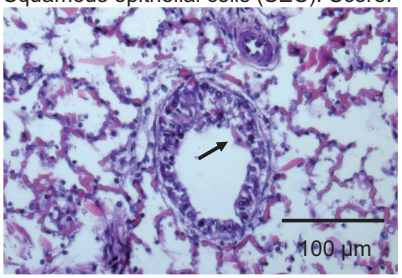
Meconium: Score: 1 = < 3



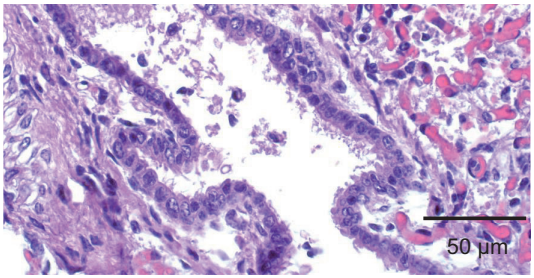
Keratin: Score: 1 = present



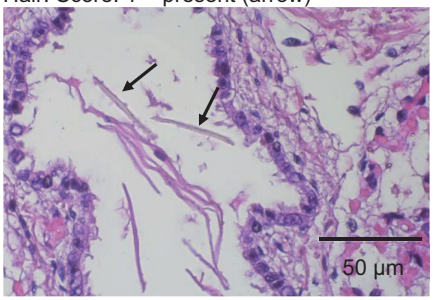
Squamous epithelial cells (SEC): Score: 1 = present (arrow)



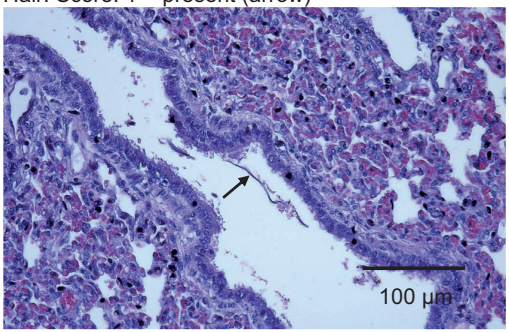
Cell debris: Score: 3 = > 5 elements



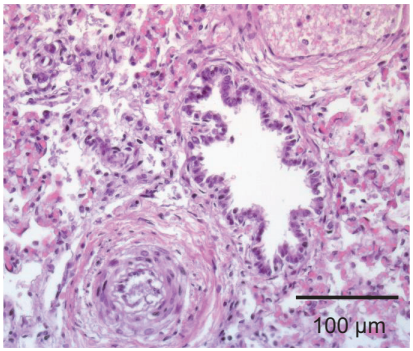
Hair: Score: 1 = present (arrow)



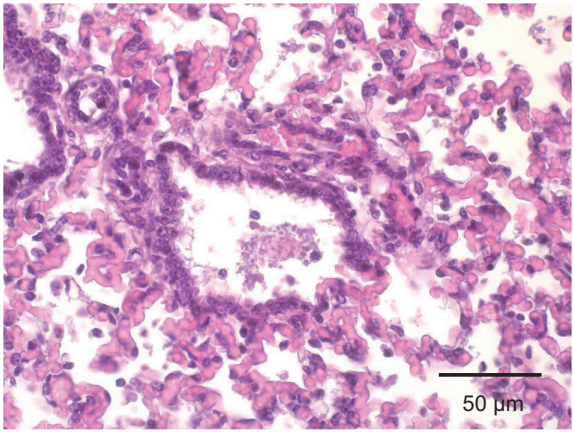
Hair: Score: 1 = present (arrow)



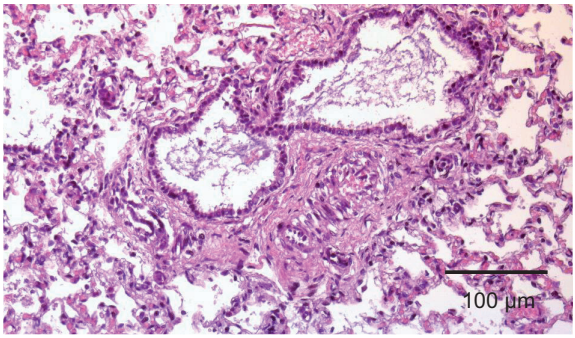
Amniotic fluid: Score: 0 = 0%



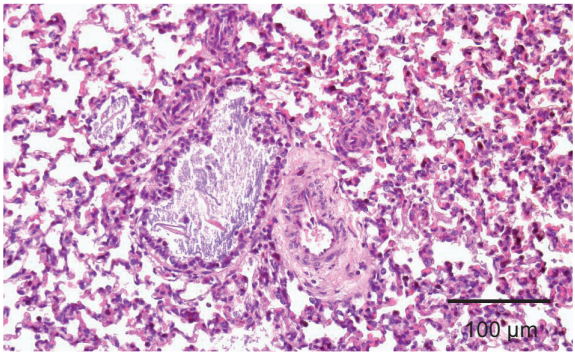
Amniotic fluid: Score: 1 = < 50%



Amniotic fluid: Score: 2 = > 50%

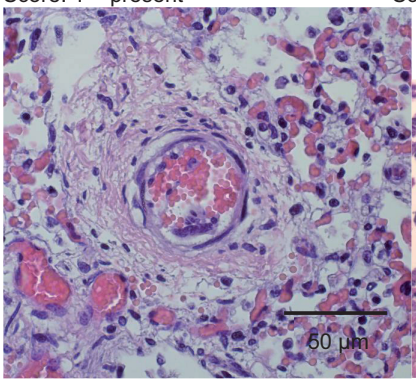


Amniotic fluid: Score: 3 = 100%

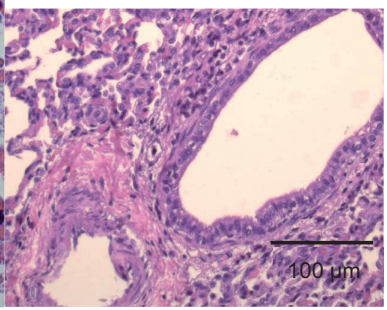


Edema (around bronchioles and vessels):

Score: 1 = present

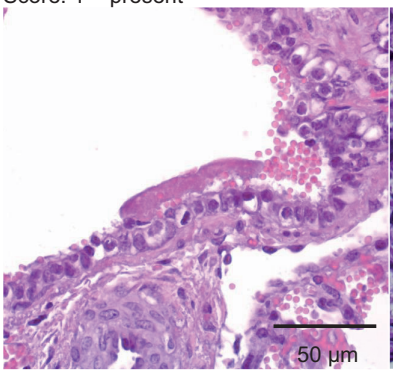


Score: 0 = not present

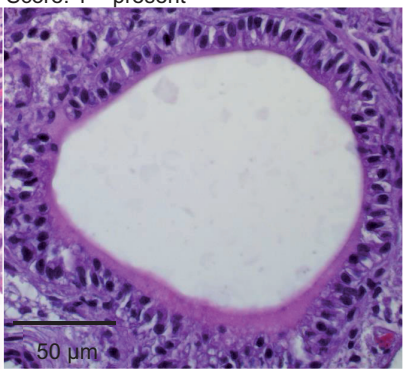


Hyaline membranes:

Score: 1 = present

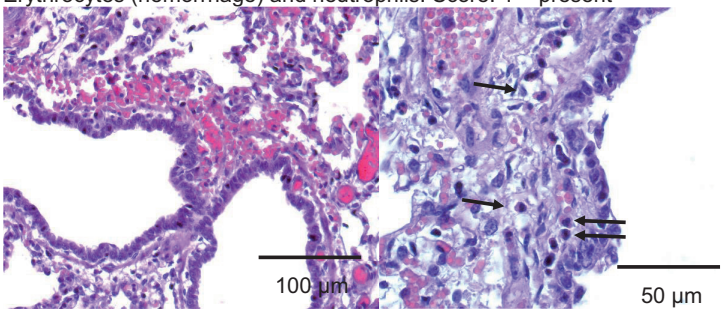


Score: 1 = present

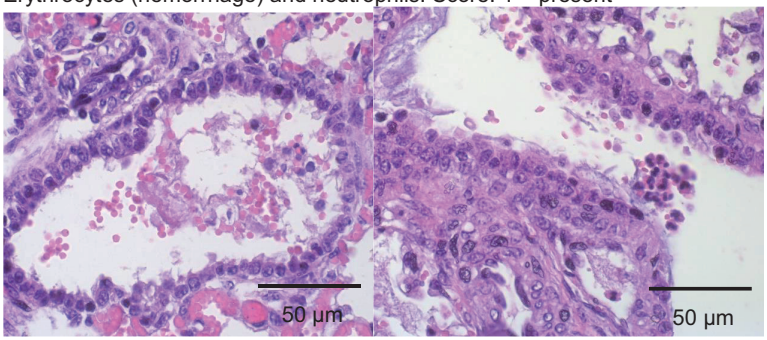


Cellular infiltration (bronchioles):

Interstitium:
Erythrocytes (hemorrhage) and neutrophils: Score: 1 = present

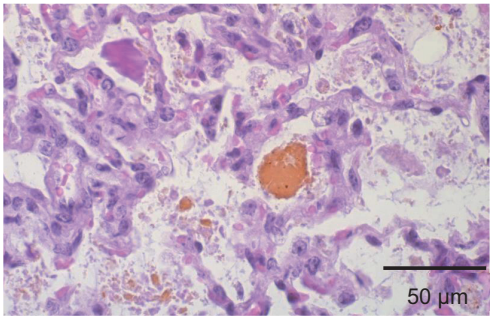


Lumen:
Erythrocytes (hemorrhage) and neutrophils: Score: 1 = present

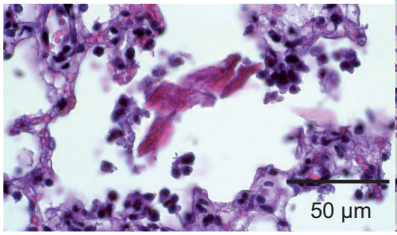


Presence of corpuscular and amorphous elements in the bronchiolar lumen (magnification 10x, 20x & 40x)

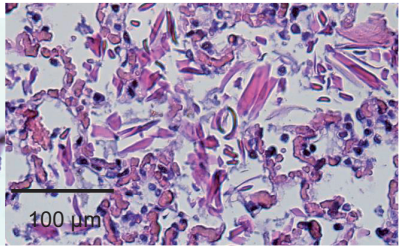
Meconium: Score: 3 = > 5 elements



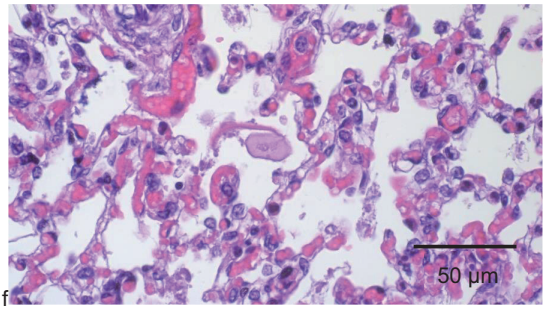
Keratin: Score: 2 = < 5 elements



Score: 3 = > 5 elements



SEC: Score: 1 = < 3 elements

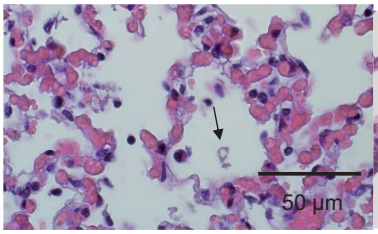


f

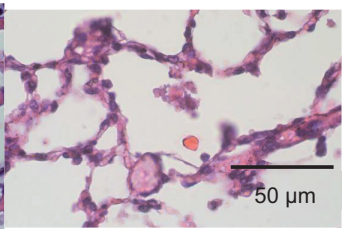
(Keratin: 1)

Cell debris:

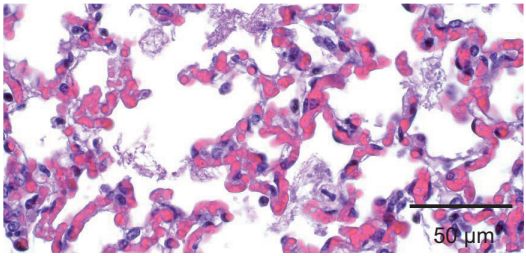
Score: 1 = < 3 elements



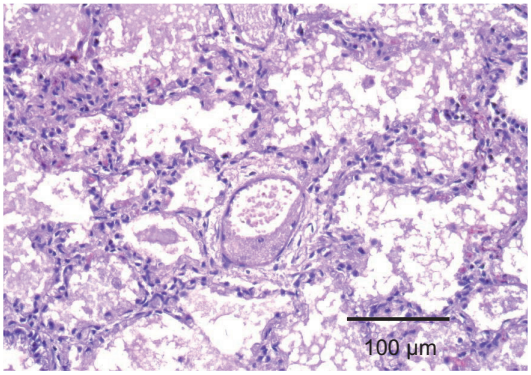
Score: 2 = < 5 elements



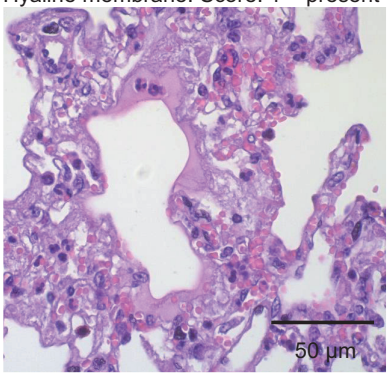
Amniotic fluid: Score: 1 = < 50%



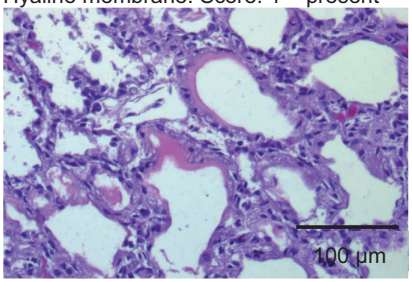
Amniotic fluid: Score. 2 = > 50%



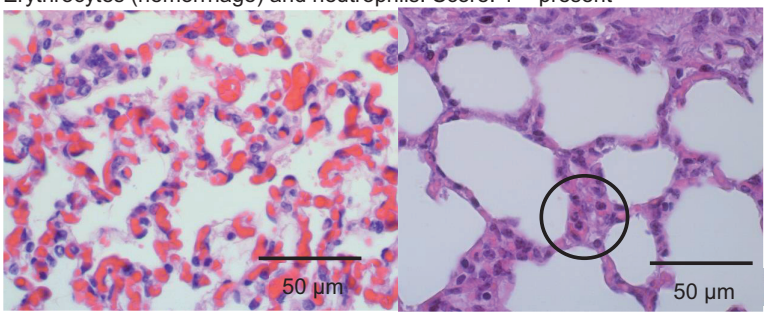
Hyaline membrane: Score: 1 = present



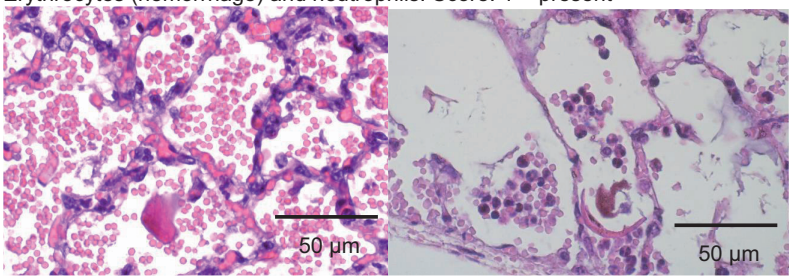
Hyaline membrane: Score: 1 = present



Cellular infiltration (alveoli):
Interstitium:
Erythrocytes (hemorrhage) and neutrophils: Score: 1 = present

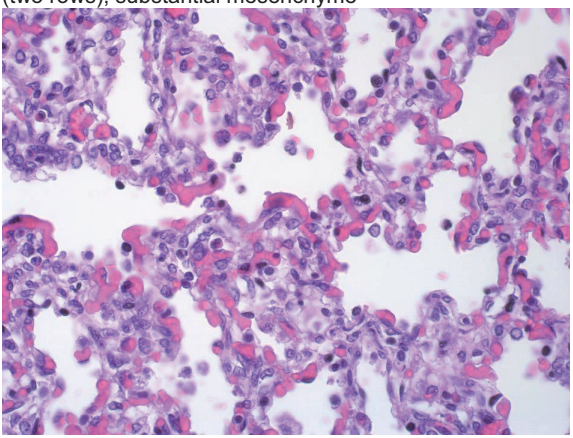


Lumen:
Erythrocytes (hemorrhage) and neutrophils: Score: 1 = present

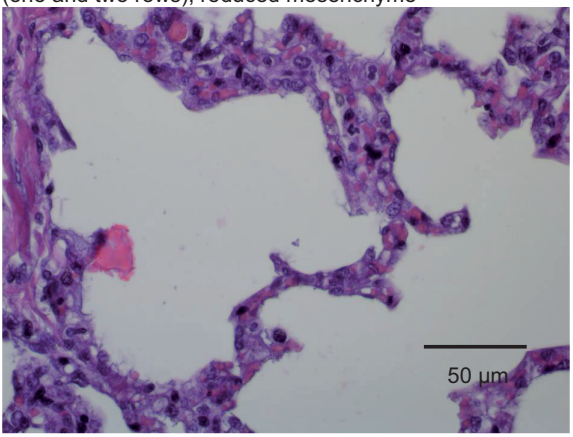


Alveolar tissue maturity (Histological characteristics modified from Schoon 1989)

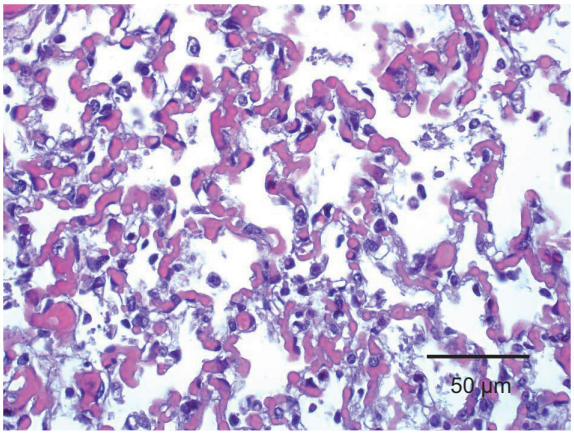
- Alveolar epithelium continual cubical; scarcely capillarized; substantial mesenchyme
- Alveolar epithelium continual cubical; intensive capillarisation; substantial mesenchyme
- Alveolar epithelium in individual alveoli still cubical; intensive capillarization (two rows); substantial mesenchyme



- Alveolar epithelium generally discontinues cubical; intensive capillarization (one and two rows); reduced mesenchyme



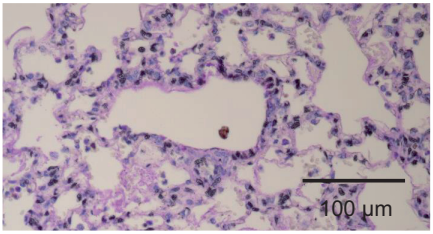
- Differentiated alveolar epithelium; capillarization one row; mesenchyme reduced to a minimum



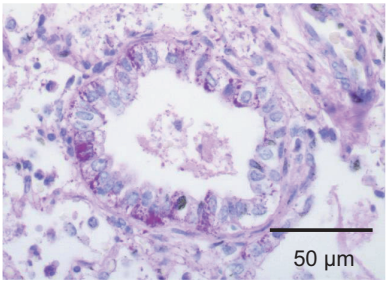
Periodic Acid Schiff reaction

Airway basement membrane region

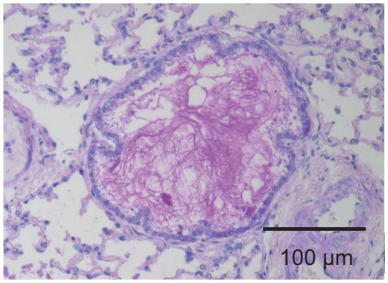
Bronchioles:
Meconium: Score: 1



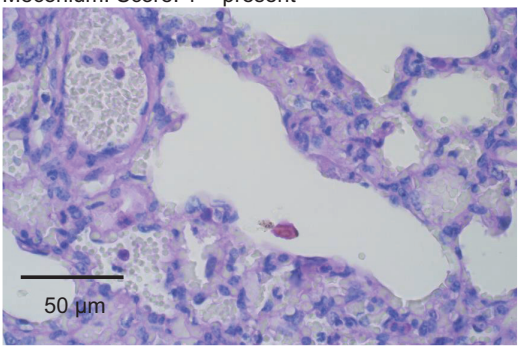
Amniotic fluid:
Score: 2 = > 50%



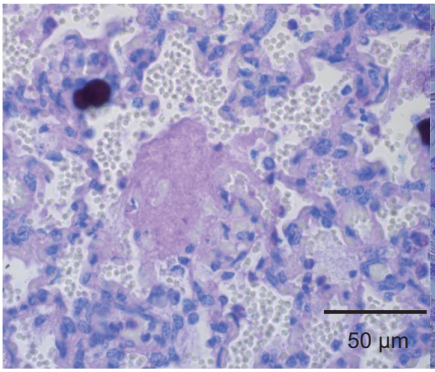
Score: 3 = 100%



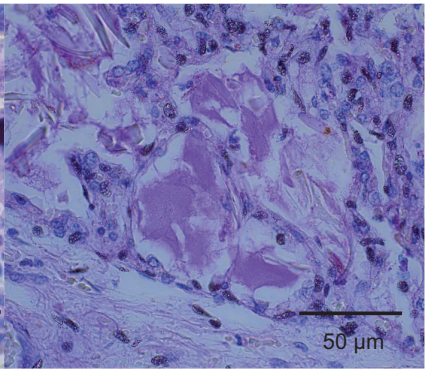
Alveoli
Meconium: Score: 1 = present



Amniotic fluid
Score: 2 = > 50%



Score: 3 = 100%



Index of Abbreviations

MAS	Meconium Aspiration Syndrome
HIE	hypoxic ischemic encephalopathy
CNS	central nervous system
BE	Base Excess
pCO_2	partial pressure of carbon dioxide
CO_2	carbon dioxide
a.n.	ante natum
p.n.	post-natum
ATP	adenosine triphosphate
PAS	Periodic Acid-Schiff
BALT	bronchus-associated lymphoid tissue
H&E	hematoxylin and eosin
SEC	squamous epithelial cells
nRDS	neonatal Respiratory Distress Syndrome
i.a.	among others
iv	intravenous
im	intramuscular
sc	subcutaneous
po	orally
NSAID	anti-inflammatory drugs

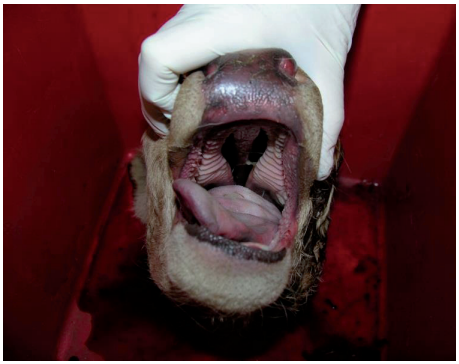
Index of Figures

Index of Graphs

Index of Tables

Figure 8: Criteria for exclusion: Depiction of apparent macroscopic malformations

- Palatoschisis

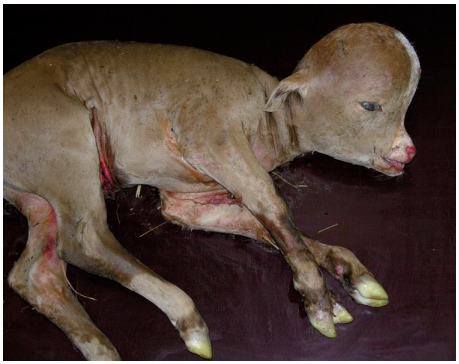


Courtesy of the clinic

- Schistosoma reflexus

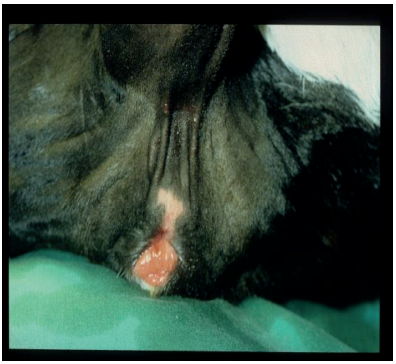
Courtesy of the clinic

- Hydrocephalus externus



Courtesy of the clinic

- Atresa ani and/or recti



Courtesy of the clinic

Protocol 1: Neonatal Lung Analysis – Protocol: H&E stain (with DLF Filter on)

Foetal dystelectasis: 0 1 2 3
(magnification 2,5 x) *Scoring: 0 = 0%, 1 = < 50%, 2 = > 50%, 3 = 100%*

(magnification 10, 20 & 40x) *Scoring: 0 = not present, 1 = present*
Interstitial edema: 0 1
(Multifocal irregular peripheral/ Pulmonary edema)
Lymphangiectasis: 0 1
Neutrophils in blood vessels: 0 1

Scoring: 0 = 0%, 1 = < 50%, 2 = >50%, 3 = 100% of the vessel filled
Blood vessel congestion: 0 1 2 3

Presence of corpuscular and amorphous elements in the bronchioles lumen
(magnification 10, 20 & 40x) *Scoring: 0 = none, 1 = < 3, 2 = < 5, 3 = > 5 elements*

Meconium (amorphous yellow-orange): 0 1 2 3
Keratin: 0 1 2 3
Squamous epithelial cells: 0 1 2 3
Cells debris: 0 1 2 3
Hair: 0 1 2 3
Scoring: 0 = 0%, 1 = < 50%, 2 = >50%, 3 = 100% of the lumen filled
Amniotic fluid: (PAS-stain) 0 1 2 3
Scoring: 0 = not present, 1 = present
Edema: 0 1
Hyaline membranes: 0 1

Cellular infiltration:
Bronchioles interstitium *Scoring: 0 = not present, 1 = present*
Erythrocytes (Hemorrhaging): 0 1
Neutrophils: 0 1
Bronchioles lumen *Scoring: 0 = not present, 1 = present*
Erythrocytes (Hemorrhaging): 0 1
Neutrophils: 0 1

Presence of corpuscular and amorphous elements in the alveolar lumen
(magnification 10, 20 & 40x) *Scoring: 0 = none, 1 = < 3, 2 = < 5, 3 = > 5 elements*

Meconium (amorphous yellow-orange):	0	1	2	3
Keratin:	0	1	2	3
Squamous epithelial cells:	0	1	2	3
Cells debris:	0	1	2	3
Hair:	0	1	2	3
Scoring: 0 = 0%, 1 = < 50%, 2 = >50%, 3 = 100% of the lumen filled				
Amniotic fluid: (PAS-stain)	0	1	2	3
Scoring: 0 = not present, 1 = present				
Hyaline membranes:	0	1		

Cellular infiltration:

Alveolar septum *Scoring: 0 = not present, 1 = present*		
Erythrocytes (Hemorrhaging):	0	1
Neutrophils:	0	1
Alveolar lumen *Scoring: 0 = not present, 1 = present*		
Erythrocytes (Hemorrhaging):	0	1
Neutrophils:	0	1

Alveolar tissue maturity:
modified from Schoon (1989).

Grade:	1	2	3	4	5

Description:

Grade	Histological characteristics at the alveolar level
1	Alveolar epithelium continual cubical; scarcely capillarised; substantial mesenchyme
2	Alveolar epithelium continual cubical; intensive capillarisation; substantial mesenchyme
3	Alveolar epithelium in individual alveols still cubical; intensive capillarisation (two rows); substantial mesenchyme
4	Alveolar epithelium generally discontinues cubical; intensive capillarisation (one and two rows); reduced mesenchyme
5	Differentiated alveolar epithelium; capillarisation one row; mesenchyme reduced to a minimum

Comments:

Protocol 2: Neonatal Lung Analysis – Protocol: PAS stain

(magnification 10 - 40X)

Presence of corpuscular and amorphous elements in the bronchioles lumen

Scoring: 0 = none, 1 = < 3, 2 = < 5, 3 = > 5 elements

Meconium (amorphous yellow-orange): 0 1 2 3

Scoring: 0 = 0%, 1 = < 50%, 2 = >50%, 3 = 100% of the lumen filled

Mucus: 0 1 2 3

Presence of corpuscular and amorphous elements in the alveolar lumen

Scoring: 0 = none, 1 = < 3, 2 = < 5, 3 = > 5 elements

Meconium (amorphous yellow-orange): 0 1 2 3

Scoring: 0 = 0%, 1 = < 50%, 2 = >50%, 3 = 100% of the lumen filled

Mucus: 0 1 2 3

Acknowledgements

I would like to thank the following people for enabling me to write this dissertation:

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