Early postnatal lung development in the eastern quoll (*Dasyurus viverrinus*)

Kirsten Ferner

Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany

Correspondence
Kirsten Ferner, Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Invalidenstraße 43, 10115 Berlin, Germany.
Email: kirsten.ferner@mfn-berlin.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: FE 1878/2-1; Research Foundation

Abstract
Early postnatal lung development (1–25 days) in the eastern quoll (*Dasyurus viverrinus*) was investigated to assess the morphofunctional status of one of the most immature marsupial neonates. Lung volume, surface density, surface area, and parenchymal and nonparenchymal volume proportions were determined using light microscopic morphometry. The lungs of the neonate were at the canalicular stage and consisted of two "balloon-like" airways with few septal ridges. The absolute volume of the lung was only 0.0009 cm³ with an air space surface density of 108.83 cm⁻¹ and a surface area of 0.082 cm². The increase in lung volume in the first three postnatal days was mainly due to air space expansion. The rapid postnatal development of the lung was indicated by an increase in the septal proportion of the parenchyma around day 4, which was reflected by an increase in the airspace surface density and surface area. By day 5, the lung entered the saccular stage of development with a reduction in septal thickness, expansion of the tubules into saccules and development of a double capillary system. The subsequent saccular period was characterized by repetitive septation steps, which increased the number of airway generations. The lungs of the newborn *Dasyurus viverrinus* must be considered as structurally and quantitatively insufficient to meet the respiratory requirements at birth. Hence, cutaneous gas exchange might be crucial for the first three postnatal days. The lung has to mature rapidly in the early postnatal period to support the increased metabolic requirements of the developing young.

Keywords
development, growth, lung, morphology, newborn, pulmonary gas exchange

1 | INTRODUCTION

Mammalian lung development is generally characterized by three major periods: embryonic, fetal, and postnatal. The phases of lung development are based primarily on morphological criteria: the embryonal period encompasses lung organogenesis; the fetal lung development comprises pseudoglandular, canalicular, and saccular stages; and finally, postnatal development comprises alveolarization and microvascular maturation (Schittny, 2017).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Author. The Anatomical Record published by Wiley Periodicals LLC on behalf of American Association for Anatomy.

Anat Rec. 2021;1–18. wileyonlinelibrary.com/journal/ar
The structural development of the mammalian lung is similar in mammals studied to date. However, the time course of mammalian lung development differs between species (Ferner, Zeller, & Renfree, 2009; Szdzuy, Zeller, Renfree, Tzschentke, & Janke, 2008; Tschanz, 2007).

Knowledge about the processes involved in pre- and postnatal remodeling of the lung originates mainly from eutherian species, such as humans (Merkus, Have-Opbroek, & Quanjer, 1996; Mühlfeld et al., 2018; Thurlbeck, 1975; Weibel, 1967, 1970; Zeltner & Burri, 1987), sheep (Davies, Reid, Lister, & Pitt, 1988; Docimo et al., 1991), rats (Burri, 1974, 2006; Burri, Dbaly, & Weibel, 1974), and mice (Bellusci, Henderson, Winner, Oikawa, & Hogan, 1996; Branchfield et al., 2016; Kim et al., 2015; Morrisey & Hogan, 2010; ten Have-Opbroek, 1981).

Recently, an increasing number of studies on lung development have been reported for marsupials. This is because marsupials offer a unique model for understanding mammalian lung development. Marsupials are distinguished from eutherian mammals by their extremely small size and marked immaturity at birth (Figure 1; Ferner et al., 2009; Ferner, Schultz, & Zeller, 2017). Most of their development and growth, including that of the lung, occurs postnatally and are supported by a prolonged lactation period (Ferner & Mess, 2011; Renfree, 2006).

Hughes and Hall (1988) sorted marsupial neonates into three grades of developmental complexity (G1, G2, and G3) based on size variation and the developmental degree of their organ systems. These grades correspond more or less to the altricial-precocial-spectrum of eutherians: G1 (Dasyuridae) is the least developed, G2 (Peramelidae, Phalangeridae, and Didelphidae), and G3 (Macropodidae and Phascolarctidae) represent the most developed marsupial neonates (Figure 1). However, marsupial neonates are always more immature than neonates of altricial eutherians.

The lung structure of marsupial neonates follows the size variation in sequences G1 to G3. Gradation of lung development from the canalicular stage to the saccular stage can be observed in newborn marsupials (Ferner, 2018; Mess & Ferner, 2010).

It seems that the maturity of the respiratory system of newborn marsupials is related to birth weight rather than to the length of gestation (Gemmell & Nelson, 1988). The lungs of the smallest marsupial neonates, the dasyurids (G1), are the most immature among marsupials. Neoneats of the eastern and northern native cat (Dasyurus viverrinus, 12.5 mg, Hill & Hill, 1955; Dasyurus hallucatus, 18 mg, Gemmell & Nelson, 1988), the stripe-faced and fat-tailed dunnart (Sminthopsis macroura, 16 mg, Gemmell & Selwood, 1994; Sminthopsis crassicaudata, 10 mg, Simpson et al., 2011), and the Tasmanian devil (Sarcophilus harrisii; 18 mg, Hughes & Hall, 1988) have lungs consisting of a few well-vascularized air chambers that resemble the early
canalicular stage. Marsupial neonates with intermediate birth weights (G2), such as the gray short-tailed opossum 
(Monodelphis domestica, 100 mg, Szedzuy et al., 2008), North American opossum (Didelphis virginiana, 190 mg, Krause & Leeson, 1975), brushtail possums (Trichosurus vulpecula, 200 mg, Gemmell & Nelson, 1988), and bandicoots (Isoodon macrourus, 180 mg, Gemmell & Little, 1982; Gemmell, 1986) are born with lungs that consist of well-vascularized large terminal air spaces separated by thick septa. The marsupial species with the highest birth weights (G3) have their lungs further subdivided at birth. Neonates of the tammar wallaby (Macropus eugenii, 370 mg, Runciman, Baudinette, & Gannon, 1996; Runciman, Baudinette, Gannon, & Lipsett, 1998; Szedzuy et al., 2008) and koala (500 mg, Phascolarctos cinereus, Ferner, 2018) have lungs at the saccular stage, consisting of a primitive bronchial tree terminating in several terminal saccules, which appear large compared to the altricial neonates of eutherians.

The respiratory system in marsupials was generally considered adequately developed prior to birth, to a level where it was capable of gas exchange commensurate to the metabolic needs of the neonate (Tyndale-Biscoe & Janssens, 1988). However, increasing evidence suggests that the immaturity of the respiratory system at birth in the marsupial necessitates the recruitment of an alternative organ system, such as the skin for gas exchange (Frappell & MacFarlane, 2006; MacFarlane & Frappell, 2001). Given the relative immaturity at birth and the large surface area to volume ratio inherent with small body size, it is not surprising that cutaneous gas exchange occurs to varying extents during the neonatal period of the marsupial (Ferner, 2018; Simpson et al., 2011).

Gas exchange in newborns of two dunnart species was conducted solely via cutaneous respiration (95%–100%) for a number of days; no thoracic movement or pulmonary ventilation was observed, and a rhythmic breathing pattern was not apparent until day 4 (Frappell & MacFarlane, 2006; Frappell & Mortola, 2000; Mortola, Frappell, & Woolley, 1999; Simpson et al., 2011). These physiological studies indicate that lung maturation must proceed very quickly in dasyurids to meet the respiratory requirements of the developing young.

Dasyurid marsupials offer a unique opportunity for a better understanding of mammalian lung development. In contrast to eutherian mammals, the canalicular stage of lung development is encountered postnatally, and the entire process of postnatal lung development occurs in a ventilated functioning state.

The current study used histological studies to examine the structural development and possible functional capacity of the maturing lung during the postnatal period of a very small newborn marsupial, the eastern quoll (Dasyurus viverrinus), whose young are born after 19 days of gestation and with a birth weight of just 12 mg.

2 MATERIALS AND METHODS

2.1 Animal provenance

A total of 45 eastern quoll (Dasyurus viverrinus) pouch youngs between neonate (<24 hr) and 25 days, from which 29 specimens were examined histologically, were enrolled in this study. The specimens were available from the Hubrecht & Hill collection, which is a part of the embryological collection of the Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin.

The Hill collection encompasses a large ontogenetic series of pre- and postnatal stages of eastern quolls, which were collected in Australia between 1898 and 1906 by James Peter Hill (Klima & Bangma, 1987). The collection is dedicated to research and has already been used in numerous scientific studies. These specimens were serially sectioned and whole-mount fixed samples. Details about fixation and processing of the material are unknown, but it is most probable that the material was fixed with Bouin’s solution and preserved in 70% ethanol as state-of-the-art at this time. The fixative Bouin’s solution (picric acid, acetic acid, and formaldehyde in an aqueous solution) and the entire embedding process in paraffin for histology can cause shrinkage of the material (~30%, see Ross, 1953). Considering this shrinkage during the processing of the histological material, the obtained absolute values of lung volume ($V_L$) and surface area ($S_A$) might be underestimated, but comparable to each other because of the same treatment of the material. However, the morphometric measurements of the volume densities of lung components are unaffected by volume changes because these are volume fractions.

The age, head length (HL), and crown-rump length (CRL) of the specimens were documented, and a staging system for the development of D. viverrinus (A–M) was provided by James Peter Hill. To estimate body weight, 16 alcohol-preserved specimens of the corresponding ontogenetic stages of D. viverrinus were investigated. Body weights were matched to the serially sectioned specimens based on the crown-rump length and head length values. The body weights and specifics and numbers of the specimens used in this study are summarized in Table 1. The number of animals investigated per age stage varied depending on the availability in the Hubrecht & Hill collection. Morphological development of the ontogenetic stages of D. viverrinus, investigated here for lung development (0–25 days), is presented in Figure 2.
### Table 1
List of Eastern quoll (*Dasyurus viverrinus*) specimens from the Hubrecht and Hill collection examined in this study

#### (a) Sectioned specimens

| Age (days)/remark | No.      | CRL (mm) | HL (mm) | Stage | Section | Staining      |
|-------------------|----------|----------|---------|-------|---------|----------------|
| 1 (newborn)       | MS 133a  |          |         | A     | 10 μm, longitudinal | H& E          |
| (newborn)         | MS 133b  |          |         | A     | 10 μm, transversal   | H& E          |
| (newborn)         | MS 134b  | 5.50     |         | A     | 10 μm, transversal   | H& E          |
| (4–12 hr old)     | MS 138a  | 5.45     |         | B     | 10 μm, longitudinal  | H & E         |
| (4–12 hr old)     | MS 138b  | 5.45     |         | B     | 10 μm, transversal   | H & E         |
| 2 (26–30 hr old)  | MS 142a  | 6.00     | 3.5     | C     | 10 μm, transversal   | H & E         |
| (26–30 hr old)    | MS 142b  | 6.00     |         | C     | 10 μm, longitudinal  | H & E         |
| (26–30 hr old)    | MS 142c  | 6.00     | 3.5     | C     | 10 μm, transversal   | H & E         |
| 3                 | MS 146   | 6.50–7.25|         | C–D   | 10 μm, transversal   | H & E         |
| (3 days old)      | MS 147   | 7.00     |         | D     | 10 μm, longitudinal  | H & E         |
| (3 days old)      | MS 148a  | 7.00     |         | D     | 10 μm, transversal   | H & E         |
| (3 days old)      | MS 148b  | 7.00     | 4.0     | D     | 10 μm, longitudinal  | H & E         |
| (3 days old)      | MS 148e  | 7.00     |         | D     | 10 μm, transversal   | H & E         |
| 4                 | MS 149a  | 8.00     | 4.5     | E     | 10 μm, transversal   | H & E         |
|                   | MS 150a  | 8.00     | 4.5     | E     | 10 μm, transversal   | H & E         |
|                   | MS 151b  | 8.00     |         | E     | 10 μm, transversal   | H & E         |
| 5                 | MS 152a  | 9.00     | 4.5     | E–F   | 10 μm, transversal   | H & E         |
|                   | MS 152b  | 9.00     | 4.5     | E–F   | 10 μm, transversal   | H & E         |
|                   | MS 154a  | 8.50–9.00| 5.5     | F     | 10 μm, transversal   | H & E         |
|                   | MS 154b  | 8.50     | 5.5     | F     | 10 μm, longitudinal  | H & E         |
| 6                 | MS 157   | 9.50–10.00| 5.5     | F     | 10 μm, transversal   | H & E         |
| 7                 | MS 159   | 10.00–10.50| 6.5     | G     | 10 μm, transversal   | H & E         |
|                   | MS 160   | 11.00    | 7.0     | G     | 10 μm, transversal   | H & E         |
| 14                | MS 164a  | 13.50    | 8.0     | H     | 10 μm, longitudinal  | H & E         |
|                   | MS 164b  | 13.50    | 8.0     | H     | 10 μm, transversal   | H & E         |
|                   | MS 165   | 13.00    | 6.0     | H     | 12 μm, transversal   | Zenker        |
| 19                | MS 167b  | 13.00–15.00| 9.0     | I     | 6 μm, transversal    | H & E         |
| (19 days old)     | MS 172a  | 17.00    | 10.0    | I     | 12 μm, transversal   | H & E         |
| 25 (25 days old)  | MS 176   | 20.00    | 12.5    | J     | 10 μm, transversal   | H & E         |

#### (b) Specimens in alcohol

| Age (days)/remark | No.      | CRL (mm) | HL (mm) | Stage | Body weight (g) |
|-------------------|----------|----------|---------|-------|-----------------|
| 1 (newborn)       | MA 746d  | 5.50     |         | A     | 0.01            |
| 2 (36 hr old)     | MA 724   | 6.00     | 2.75    | C     | 0.02            |
|                   | MA 746a  | 6.00     |         | C     | 0.02            |
| 3 (48 hr old)     | MA 747a  | 6.75     |         | D     | 0.05            |
|                   | MA 744a  | 7.00     | 3.00    | D     | 0.05            |
| 5 (5–6 days old)  | MA 733   | 8.00     | 4.50    | E     | 0.07            |
|                   | MA 744   | 8.00     | 4.50    | E     | 0.07            |
| 7 (7 days old)    | MA 721   | 9.50     | 5.50    | E     | 0.12            |
|                   | MA 754   | 10–10.50 | 6.50    | G     | 0.13            |
| 10                | MA 741   | 11.00    | 7.00    | G     | 0.19            |
| 14 (14 days old)  | MA 738   | 13.00    | 8.00    | H     | 0.32            |
|                   | MA 731   | 13.75    | 8.00    | H     | 0.35            |
2.2 | Lung morphometry

Serial histological sections of whole embedded specimens were investigated by light microscopy using a stereomicroscope (Zeiss Axiokop, Carl Zeiss Microscopy GmbH, Germany) equipped with a digital camera (Leica DFC490, Leica Microsystems, Switzerland Ltd.), connected to a computer (software LAS V4.2, Leica Microsystems, Switzerland Ltd.).

Systematic sampling for estimation of the volume fractions of the lung components was combined with the Cavalieri volume estimation to obtain the absolute volume. The entire right and left lungs were examined for lung morphometry, and the stereological analysis followed the procedure of systematic uniform random sampling (SURS) as proposed by Hsia, Hyde, Ochs, and Weibel (2010). To ensure that the selected lung sections represent the whole and all parts of the lung have an equal probability of being sampled, the fractionator method was applied. The lungs were serially sliced to a constant thickness (mostly 10 μm). The total length of the lung was calculated from the number of all lung sections, and a fractionator sequence with a sampling fraction of 1/8 was applied. The first section selected for morphometry was randomly chosen using a random number table within the sampling fraction. Thus, for each specimen, eight micrographs were obtained at magnifications of ×50 or ×100 depending on the size of the lung. The morphometric analysis program STEPanizer (Tschanz, Burri, & Weibel, 2011) was used to determine the lung volume and volume densities of parenchymal and nonparenchymal lung components.

Lung volume was determined as a reference to translate relative stereological parameters (e.g., volume and surface density) into absolute values by using the Cavalieri principle and point counting methods (Hsia et al., 2010; Tschanz, Schneider, & Knudsen, 2014). The total lung volume was calculated as follows:

---

TABLE 1 (Continued)

| Age (days)/remark | No. | CRL (mm) | HL (mm) | Stage | Section | Staining |
|-------------------|-----|----------|---------|-------|---------|----------|
| 19 (19 days old)  | MA 745 | 16.50     | 10.00   | I     | 0.60    |
|                   | MA 753 | 15.50     | 10.00   | I     | 0.58    |
| 25 (25 days old)  | MA 739 | 20.00     | 12.50   | J     | 1.05    |
|                   | MA 750 | 24.00     | 13.50   | J     | 1.45    |

Abbreviations: CRL, crown-rump length; HL, head length.

---

FIGURE 2 Postnatal development of *Dasyurus viverrinus*. The macrographs reflect the morphological transformation from neonate to day 25: neonate (a) 0–12-hr-old, (b) 7-day-old, (c) 14-day-old, (d) 19-day-old, and (e) 25-day-old. The highly immature neonate (a) had a massive oral shield and large prominent nostrils; eye and ear primordia were barely visible and a definitive neck was missing. The characteristics of marsupial neonates are strongly developed forelimbs and rudimentary hindlimbs. On day 7 (b), a definitive neck was visible; the nasal swelling was moderate and a simple oral shield was present. The morphological transformation of the fore- and hindlimbs started at this time. A clear developmental progress occurred between days 14 (c) and 19 (d), where ear and eye primordia and the area of the later mystacial vibrissae became visible. On day 25 (e), the head appeared even more differentiated, with a lateral lip line, eyelids, and auricle, which were still fused. Scale bar = 0.5 cm
where $P$ is the number of points falling on the lung tissue, $d$ is the distance between adjacent grid points, and $t$ is the distance between the selected lung sections.

To examine the structural changes in the developing lung, the volume densities of the lung parenchyma ($V_{vp}$) and nonparenchyma ($V_{vnp}$) were obtained by point counting methods as proposed by Makanya, Haenni, and Burri (2003). Furthermore, the volume densities of the coarse constituent components of the parenchyma, tubular, or saccular air spaces ($V_{va}$), and septa ($V_{vs}$), were estimated.

The volume densities of the nonparenchymal components were also estimated: conducting airways ($V_{vb}$), blood vessels with diameters >25 μm ($V_{vv}$), and connective tissue ($V_{vt}$). The magnification for the morphometric analysis was performed. All values were set at a significance level of $p < .05$.

### 3.1 | Lung structure

The structural changes in the lung during the first 25 postnatal days are summarized in Figures 3, 4, 5, and 6. The whole lung of *D. viverrinus* comprised a single left lung and a right lung with an accessory lobe (Figure 3b). The left lung was smaller than the right lobe. Fissures subdividing the lung were not evident in the lungs of the neonate but developed by days 4–5 (Figure 4e,g). The left and right lobes were each served by the main bronchus or emerged directly from the trachea for the first postnatal day (Figures 3e,f, and 4a). The accessory lobe was intimately joined to the right lobe through the parenchymatous lung tissue (Figure 3b and 4b).

The lungs of the neonate *D. viverrinus* were at the canalicular stage of lung development, characterized by primitive “balloon-like” airways that consisted of large tubules. Only a few septal ridges protruded into the lumen and poorly subdivided the lung (Figure 3a–c). The air space surface area $S_v(a)$ of the lungs was obtained using the intersection counting method (see Howard & Reed, 2005 for details). Multiplying $S_v(a)$ by the volume density of the lung parenchyma and the absolute lung volume yielded the absolute surface area $S(a)$.

### 2.3 | Statistical analysis

All the age group data are presented as mean ± 1 SD. Differences in the weight-specific lung volumes and volume proportions of the various components of the lung were statistically tested using one-way analysis of variance (ANOVA). Age groups (days 0, 2, 3, 4, 5, and 14) that had statistically viable numbers of animals were tested. Tukey’s test was used to identify the specific values with differences. The data in the form of ratios were arcsine-transformed before the statistical analysis was performed. All values were set at a significance level of $p < .05$.
FIGURE 3  Histological sections of the lung in the (a–d) neonate and (e–h) 2-day-old Dasyurus viverrinus. The lung of the newborn D. viverrinus consisted of poorly vascularized airspaces (tubules). The respiratory epithelium is located on the opposite side of the wall of the lung from the visceral epithelium. The epithelial lining of the airways consisted of mostly cuboidal cells, interspersed with a few respiratory capillaries (d, inset). No double capillary septa were present. This points to the canalicular stage of lung development. The lung appeared mostly undivided, and only a few septal ridges were protruding from the walls. (g) The lung of the 2-day-old joey resembled that of the neonate; however, more septal ridges subdivided the lung. A bronchial tree was not present yet, and the airspaces opened directly from the trachea (e, f). The epithelium lining the tubules were mainly cuboidal with some respiratory capillaries in between (h, inset). a, accessory lobe; aa, ascending aorta; ce, cuboidal epithelium; dea; descending aorta; h, heart; i, intestine; k, kidney (mesonephros); l, liver; ll, left lung; o, oesophagus; r, rib; rc, respiratory capillary; re, respiratory epithelium; revc, right vena cava; rl, right lung; sc, spinal cord; sr, septal ridge; t, trachea; tu, tubule; ve, visceral epithelium; vb, vertebral body. The respiratory capillaries are indicated by arrowheads. The magnification is indicated by the scale bar.
FIGURE 4  Histological sections of the lung in the (a–c) 3-day-old, (d–f) 4-day-old, and (g–h) 5-day-old Dasyurus viverrinus. By day 3, the lung became more compartmentalized by thick septal ridges (a). Thick intertubular septa were lined by parenchymal epithelium alternating with respiratory capillaries (c, inset). The bronchial system consisted of short main bronchi that opened immediately to the tubular airways. By day 4, the sub septation of the lung continued and numerous smaller tubules resulted, a double capillary system was developing, indicating the transition from the canalicular to the saccular stage of lung development (f, inset). In the 5-day-old young, the sub septation of the lung parenchyma progressed resulting in numerous large saccules. (a), accessory lobe; (b), bronchus; dea, descending aorta; h, heart; ll, left lung; lmb, left main bronchus; mb, main bronchus; o, oesophagus; pa, pulmonary artery; pv, pulmonary vein; r, rib; rc, respiratory capillary; revc, right vena cava; rl, right lung; rmb, right main bronchus; sa, saccule; sr, septal ridge; t, trachea; tu, tubule; vb, vertebral body. The respiratory capillaries are indicated by arrowheads. The magnification is indicated by the scale bar.
had entered the saccular stage of development with a reduction in septal thickness, expansion of the tubules into sacculae, and development of a consistent double capillary system (Figure 4g,h).

During the following 2 days, the lung development progressed and led to further subdivision of the lung parenchyma (Figure 5a,e). By day 6, the bronchial trees began to form. Several short smooth-walled conducting airways (later lobar bronchi) branched off the main bronchi and communicated directly with the large terminal sacculae (Figure 5a–c). The large sacculae were characterized by numerous septal ridges, leading to the formation of new sacculae (Figure 5c,f). The thick septa separating the smaller sacculae consisted of a double capillary network and an abundant cellular and acellular connective tissue layer in between (Figure 5d,g–j). Centrally located capillaries or small blood vessels were often encountered in the septa (Figure 5d,h,j). Large blood vessels were commonly found at the thick septal junctions (Figure 5h). By day 7, the bronchial tree consisted of the main and lobar bronchi. The smooth-walled conducting airways extended to the lung periphery, where they opened into the terminal sacculae (Figure 5e). The larger sacculae in the lung of the 7-day-old D. viverrinus were due to numerous septal ridges more irregular in shape than those seen in earlier stages (Figure 5f), whereas the smaller new-formed sacculae were smooth-walled (Figure 5h). Septa with a double capillary network separated the numerous small sacculae.

Between days 14 and 25, the parenchymal structure increased in complexity (Figure 6). The development of the bronchial tree progressed, and lobar and segmental bronchi were present (Figure 6a,b,d,e,g,h). The lung parenchyma was further subdivided into numerous smaller sacculae separated by thick septa. At 25 days, the air compartments of the lung parenchyma had markedly increased in number and decreased in size (compare Figures 3bf; 4b,e,g; 5b,f; and 6a,d,h). By day 14, 19, and 25, many of the respiratory capillaries were positioned close to the airspaces on both sides of a thickened interstitium, indicating the presence of a double capillary network (Figure 6c,f,i). By day 25, the double capillary septum appeared slightly thinner due to a reduction in the interstitial layer.

3.2 | Lung morphometry

3.2.1 | Lung volume, surface densities, surface area, and body mass

Body mass and lung volumes ($V_L$) for the various developmental stages of D. viverrinus are presented in Table 2 and Figure 7.

At birth, the neonate weighed approximately 0.01 g and the lung volume was only 0.000894 ± 0.000348 cm$^3$. By day 2, both bodyweight and lung volume nearly doubled. From day 3 to day 25, a steady increase in lung volume was observed (Figure 7). Specific $V_L$ was significantly higher in neonates and 2-day-old young than in all other developmental stages. Overall the developmental stages, $V_L$ was closely correlated with body mass ($r = .986$).

The surface densities $Sv(a)$ and absolute surface areas $S(a)$ for the airspaces are listed in Table 2. The airspace surface density was lowest in the neonate D. viverrinus (108.83 ± 19.93 cm$^{-1}$). The airspace surface density increased steadily with progressive structural lung development via subseptation of the lung parenchyma. $Sv(a)$ more than doubled from day 1 to day 25 (260.53 cm$^{-1}$). The absolute surface area of the neonatal lung (0.082 ± 0.020 cm$^2$) increased 26-fold by day 14 (2.122 ± 0.569 cm$^2$). The airspace surface area was positively correlated with the body mass (Figure 7). When scaled against $V_L$, $Sv(a)$ increased at a rate close to that of the lung volume (Figure 8).

3.2.2 | Lung composition

The volume densities of the various coarse parameters of the lung are provided in Table 3, and the absolute volumes are scaled against the body mass in Figure 9.

The densities of the parenchyma ($V_{vp}$) ranged from 0.779 ± 0.014 (day 7) to 0.900 ± 0.019 (day 1); the highest value was observed in the neonatal group. Within the parenchymal components, the volume density of the airspaces $V_{va}$ was highest in the neonatal group (0.627 ± 0.081) and remained significantly higher than the proportion of the septal tissue ($V_{vs}$) for the first three postnatal days (> 0.5). From days 4 to 25, the volume density of the airspaces decreased and remained low. The volume density of the parenchymal septa was reciprocal to that of the alveolar space. During the first 3 days, the volume densities of the septa were low and increased to higher values from days 4 to 25. A shift around day 4 was clearly noticeable when comparing the absolute volumes of parenchymal components (Figure 9). The volumes of both parenchymal components increased with body mass; however, the volume of the septal tissue was higher than that of the air spaces.

The values for the nonparenchymal volume density were reciprocal to the volume density of the parenchyma, with a low of 0.100 ± 0.019 by day 0 and a high of 0.221 ± 0.014 by day 7. The volume density of the non-parenchymal showed a slight (not significant) increase from days 5 to 6 on. The primary airways and the
FIGURE 5  Histological sections of the lung in the (a–d) 6-day-old and (e–j) 7-day-old Dasyurus viverrinus. By day 6, the development of the bronchial tree had progressed. Several short bronchi (lobar bronchi), branching off the main bronchi, communicated directly with large terminal saccules. The large saccules were further subdivided by septal ridges. The thick septa separating the smaller saccules consisted of a double capillary network (d, inset). By day 7, three generations of bronchi were present. The bronchial tree consisted of the main, lobar, and segmental bronchi, which communicated with the terminal saccules. A septum with a double capillary network separated the numerous small saccules (j, inset). b, bronchus; bv, blood vessel; dea; descending aorta; la, left atrium; ll, left lung; lmb, left main bronchus; mb, main bronchus; o, oesophagus; pa, pulmonary artery; pv, pulmonary vein; r, rib; ra, right atrium; rc, respiratory capillary; rl, right lung; rmb, right main bronchus; s, septum; sa, saccule; sr, septal ridge. The respiratory capillaries are indicated by arrowheads. The magnification is indicated by the scale bar.
pulmonary vascular system began to develop around this time. The values of the volume densities of the non-parenchymal components (airways, blood vessels, connective tissue) are summarized in Table 3, and the absolute values are presented in Figure 9. The differences were not statistically significant. The volume densities of the airways (Vvb) and blood vessels (Vvv) were lowest in the neonatal group and highest by day 7. Generally, the volume densities and absolute volumes of the airways, blood vessels, and connective tissue showed a tendency to increase during the saccular period, reflecting the formation of the bronchial tree and pulmonary vasculature.

4 | DISCUSSION

4.1 | Lung structure

It can be assumed that mammalian lung development is highly conserved and follows similar developmental pathways in all mammalian species, including marsupials and monotremes (Ferner et al., 2009; Schittny, 2017; Szdzuy et al., 2008). However, difficulties in the classification of marsupial lung development may arise from the fact that mammalian lung development has been studied mainly in eutherian species and definitions for the different stages of lung development (e.g., canalicular, saccular, and alveolar) focused on the situation found in altricial eutherian species, mostly during the prenatal period in the nonventilated state. In marsupials, the characteristics of lung developmental stages might be slightly different, because lung maturation takes place postnatally in a ventilated and functioning state. Compared to eutherians, marsupials and monotremes are born earlier during the developmental trajectory of lung development. Generally, the lungs of newborn marsupials are reported to be in the terminal sac (saccular) stage (Gemmell & Nelson, 1988; Krause & Leeson, 1975; Mess & Ferner, 2010; Runciman et al., 1996, 1998) or even the canalicular stage of lung development (Burri, 1999; Runciman et al., 2003; Ferner, 2018; Makanya et al., 2003; Makanya, Sparrow, Warui, Mwangi, & Burri, 2001; Makanya, Tschanz, Haenni, & Burri, 2007; Modepalli et al., 2018; Simpson et al., 2011). However, all marsupial newborns show qualitative characteristics of a mature gas-exchanging organ, such as the full complement of surfactant proteins (Makanya et al., 2007; Miller, Orgeig, Daniels, & Baudinette, 2001; Ribbons, Baudinette, & McMurchie, 1989) and a thin blood-gas barrier (Runciman et al., 1996; Szdzuy et al., 2008). This seems to be valid even for the most immature marsupial neonates, as reported for the newborn dasyurids Dasyurus hallucatus (Gemmell & Nelson, 1988) and Sminthopsis crassicaudata (Simpson et al., 2011). In contrast, branching morphogenesis, which is completed during the canalicular stage in fetal eutherians, is postponed in marsupials and takes place postnatally during the saccular period. This indicates that lung structures indispensable for respiratory function (e.g., stabilization of air spaces by surfactant and thin blood-gas barrier for gas exchange) have to be mature in newborn marsupials, whereas the formation of distal airways is delayed and follows the structural development of the lung parenchyma.

The first description of the lung of the newborn eastern native cat, D. viverrinus, originated from Hill and Hill (1955). They stated that the lungs are remarkably simple organs, being structurally at the lowest level, compatible with some degree of functional efficiency. A later ultrastructural study of the lungs of the newborn northern native cat D. hallucatus described the lung as similar in structure to two balloons, which were lined internally with a respiratory epithelium formed by both squamous cells and surfactant-secreting cells (Gemmell & Nelson, 1988). This describes the lung structure of the newborn D. viverrinus quite well and is confirmed by the present study. However, it appears difficult to fit this “balloon-like” lung structure into the developmental trajectory of mammalian lung development. The parenchymal phenotypes during pre- and postnatal lung development have been well documented and used, among other characteristics, to define the different stages of lung development (Burri, 1999; Schittny, 2017). Previous descriptions of the canalicular stage were based on species that were not exposed to air. However, the presence of stretches of cuboidal epithelium and a few portions of the thin blood-gas barrier in the inner lining of the lung, as observed in the newborn D. viverrinus, are some of the essential characteristics of the canalicular stage. The inner lining of the air spaces resembles that described in the lungs of the neonates of the northern native cat (Gemmell & Nelson, 1988) and the quokka (Makanya et al., 2007), two marsupial species that also have lungs at the canalicular stage at birth. Gemmell and Nelson (1988) stated that the respiratory lining of the lung is composed of surfactant-secreting cells (cuboidal) and squamous cells that were separated from the endothelial cells of the underlying capillaries by a composite of two basal laminae; thus, a true blood-gas barrier was present. However, Makanya et al. (2007) suggested that the squamous epithelium differentiates earlier than the capillary system. The delay in the formation of capillaries and their opposition to the tubular epithelium indicates increased diffusion distance in several parenchymal regions (Makanya et al., 2007).
FIGURE 6  Histological sections of the lung in the (a–c) 14-day-old, (d–f) 19-day-old, and (g–i) 25-day-old *Dasyurus viverrinus*. By day 14, the lung parenchyma appeared further subdivided with numerous saccules separated by thick double capillary septa (c, inset). Until 25 days, a reduction in the size of the saccules and an increase in the surface area available for gas exchange were achieved by septation. By day 25, the double capillary septum appeared thinner due to a reduction in the interstitium (i, inset). br, bronchiolus; h, heart; lb, lobar bronchus; mb, main bronchus; o, oesophagus; pa, pulmonary artery; r, rib; rc, respiratory capillary; s, septum; sa, saccule; sb, segmental bronchus; sr, septal ridge. The respiratory capillaries are indicated by arrowheads. The magnification is indicated by the scale bar.
In the most mature marsupial neonates (G3), a functional lung develops prenatally in a very short time. The formation of a functional lung occurs in the bandicoot in the last 3–4 days of gestation (Gemmell & Little, 1982), in which the canalicular stage is converted into the saccular stage in the last 3 days of gestation (Runciman et al., 1996). Marsupial species, born with lungs at the canalicular stage, attain the saccular stage rapidly after birth. In the quokka wallaby, for example, the canalicular stage is converted to the saccular stage within the first four postnatal days (Makanya et al., 2001). In addition, the gray short-tailed opossum and the fat-tailed dunnart reached the saccular stage in the postnatal period by days 8 and 10, respectively (Modepalli et al., 2018; Simpson et al., 2011). In the lungs of the D. viverrinus examined in this study, the formation of the primary septa started by day 6, indicating that the transition from canalicular to saccular stage occurred around this time. The following saccular period was rather long, and the lung was still at the saccular stage on day 25, when this study ended. The saccular stage was characterized by the formation of transitory saccules, which were progressively subdivided by septation into more generations of saccules. The process of saccule multiplication is very similar to that of alveolar formation (Burri, 1974) and is accompanied by tissue proliferation (Burri et al., 2003). In contrast to alveolization, microvascular maturation, a process that leads to the

| Age (days) | n | Mass (g) | Total lung volume, $V_L$ (cm³) | Specific lung volume, $V_L$ (cm³ kg⁻¹) | Air space surface density, $Sv(a)$ (cm⁻¹) | Surface area $S(a)$, (cm²) |
|-----------|---|----------|-----------------|-----------------|-----------------|-----------------|
| 1         | 5 | 0.01     | 0.000894 ± 0.000348 | 89.37* ± 34.76 | 108.83 ± 19.93 | 0.082 ± 0.020   |
| 2         | 3 | 0.02     | 0.001459 ± 0.000358 | 72.96* ± 17.91 | 127.33 ± 28.61 | 0.156 ± 0.017   |
| 3         | 5 | 0.05     | 0.001714 ± 0.000439 | 34.27 ± 8.78   | 136.75 ± 18.03 | 0.197 ± 0.041   |
| 4         | 3 | 0.07     | 0.002184 ± 0.000987 | 31.20 ± 14.10  | 176.86 ± 29.94 | 0.307 ± 0.071   |
| 5         | 4 | 0.10     | 0.003376 ± 0.000647 | 33.76 ± 6.47   | 169.27 ± 12.24 | 0.478 ± 0.053   |
| 6         | 1 | 0.12     | 0.004366           | 36.39           | 169.55          | 0.606           |
| 7         | 2 | 0.13     | 0.006339 ± 0.000985 | 49.18 ± 7.57   | 208.77 ± 34.68 | 1.013 ± 0.031   |
| 14        | 3 | 0.34     | 0.010062 ± 0.001338 | 29.50 ± 2.94   | 245.30 ± 46.57 | 2.122 ± 0.569   |
| 19        | 2 | 0.59     | 0.021025 ± 0.00327  | 35.55 ± 4.94   | 217.98 ± 27.56 | 3.876 ± 1.298   |
| 25        | 1 | 1.05     | 0.034354           | 32.72           | 260.53          | 7.267           |

Note: The body mass used for calculation of the specific lung volume was measured in alcohol preserved eastern quoll specimens with corresponding age and body size (see Table 1). * indicates a value significantly different $(p \leq .05, \text{ANOVA, Tukey})$. Values are given as means ± SD for those age groups where more than one animal was measured, n is the number of individuals in the group.

In the most mature marsupial neonates (G3), a functional lung develops prenatally in a very short time. The formation of a functional lung occurs in the bandicoot in the last 3–4 days of gestation (Gemmell & Little, 1982), in which the canalicular stage is converted into the saccular stage in the last 3 days of gestation (Runciman et al., 1996). Marsupial species, born with lungs at the canalicular stage, attain the saccular stage rapidly after birth. In the quokka wallaby, for example, the canalicular stage is converted to the saccular stage within the first four postnatal days (Makanya et al., 2001). In addition, the gray short-tailed opossum and the fat-tailed dunnart reached the saccular stage in the postnatal period by days 8 and 10, respectively (Modepalli et al., 2018; Simpson et al., 2011). In the lungs of the D. viverrinus examined in this study, the formation of the primary septa started by day 6, indicating that the transition from canalicular to saccular stage occurred around this time. The following saccular period was rather long, and the lung was still at the saccular stage on day 25, when this study ended. The saccular stage was characterized by the formation of transitory saccules, which were progressively subdivided by septation into more generations of saccules. The process of saccule multiplication is very similar to that of alveolar formation (Burri, 1974) and is accompanied by tissue proliferation (Burri et al., 2003). In contrast to alveolization, microvascular maturation, a process that leads to the
formation of secondary septa with a single capillary layer, does not occur during sacculation.

Since the subsequent course of lung development could not be followed in *D. viverrinus*, the time of alveolization remains unknown. However, the structural lung development of *D. viverrinus* may follow a time course similar to that of the fat-tailed dunnart, a dasyurid species with similar birth weight (13 mg) and developmental degree at birth (G1). Simpson et al. (2011) reported that in the fat-tailed dunnart, the first secondary septa did not occur until day 45 when alveolization started. The period between days 70 and 100 was the most important time for bulk alveolization (Simpson et al., 2011).

In marsupial species that have neonates of developmental stage G2, similar times of alveolization have been reported (Buaboocha & Gemmell, 1997; Gemmell, 1986; Krause & Leeson, 1975; Modepalli et al., 2018; Szdzuy et al., 2008). In contrast, marsupial species that have neonates of developmental stage G3 usually have longer sac
cular periods and enter the alveolar stage later (Runciman et al., 1996).

### 4.1.1 Lung volume and body mass

At birth, *D. viverrinus* weighs approximately 12 mg and has a lung volume ($V_L$) of only 0.000894 ml. Bodyweights of 750 g in females and 1.1 kg in males have been reported for adult eastern quolls (Cooper & Withers, 2010). This means that from birth to adulthood, the bodyweight increase lies in the range of 63,000–92,000 times. If the $V_L$ increase is proportional to body weight, the $V_L$ of adult *D. viverrinus* may be approximately 56–82 ml. However, the actual lung volume might be lower, since comparison to marsupials with similar body mass revealed lower $V_L$ values (e.g., quokka wallaby (210-day-old, mass: 711 g, $V_L$: 21.3 mL; Burri et al., 2003) and tammar wallaby (250-day-old, mass: 775 g, $V_L$: 30.5 ml; Runciman et al., 1998).

Since the present study examined lung development only in the early postnatal period (until day 25), the course of complete lung development cannot be described for *D. viverrinus*. Given the fact that $V_L$ doubled in 2 days, it can be assumed that in *D. viverrinus*, the

---

**TABLE 3** Proportions of the volumes of the various coarse components of the lung for the various ages of *Dasyurus viverrinus* determined with light microscopy at $\times 100$ magnification

| Age (days) | n | Vvp | Vva | Vvs | Vvnp | Vvb | Vvv | Vvt |
|-----------|---|-----|-----|-----|-------|-----|-----|-----|
| 1         | 5 | 0.900 ± 0.019 | 0.627* ± 0.081 | 0.273* ± 0.090 | 0.100 ± 0.019 | 0.064 ± 0.025 | 0.022 ± 0.006 | 0.014 ± 0.008 |
| 2         | 3 | 0.869 ± 0.031 | 0.613* ± 0.038 | 0.257* ± 0.032 | 0.131 ± 0.031 | 0.068 ± 0.035 | 0.045 ± 0.014 | 0.018 ± 0.007 |
| 3         | 5 | 0.856 ± 0.020 | 0.564* ± 0.076 | 0.291* ± 0.057 | 0.144 ± 0.020 | 0.097 ± 0.012 | 0.030 ± 0.012 | 0.017 ± 0.007 |
| 4         | 3 | 0.865 ± 0.033 | 0.362 ± 0.153 | 0.503 ± 0.179 | 0.135 ± 0.033 | 0.065 ± 0.018 | 0.052 ± 0.016 | 0.017 ± 0.010 |
| 5         | 4 | 0.850 ± 0.036 | 0.359 ± 0.121 | 0.491 ± 0.136 | 0.150 ± 0.036 | 0.094 ± 0.025 | 0.037 ± 0.011 | 0.019 ± 0.009 |
| 6         | 1 | 0.818 | 0.301 | 0.517 | 0.182 | 0.095 | 0.068 | 0.018 |
| 7         | 2 | 0.779 ± 0.014 | 0.323 ± 0.027 | 0.456 ± 0.041 | 0.221 ± 0.014 | 0.132 ± 0.020 | 0.062 ± 0.007 | 0.027 ± 0.002 |
| 14        | 3 | 0.855 ± 0.021 | 0.289 ± 0.035 | 0.566 ± 0.048 | 0.145 ± 0.021 | 0.085 ± 0.016 | 0.037 ± 0.010 | 0.022 ± 0.002 |
| 19        | 2 | 0.815 ± 0.052 | 0.332 ± 0.036 | 0.483 ± 0.089 | 0.185 ± 0.052 | 0.090 ± 0.032 | 0.052 ± 0.011 | 0.043 ± 0.010 |
| 25        | 1 | 0.812 | 0.299 | 0.513 | 0.188 | 0.105 | 0.056 | 0.027 |

Note: The parenchymal proportion (Vvp) is the summation of the airspace (Vva) and septal tissue (Vvs) proportions. The nonparenchymal proportion (Vvnp) comprises the airway (Vvb), the blood vessel (Vvv and the connective tissue (Vvt) components. * value significantly different ($p \leq .05$, ANOVA, Tukey). Values are given as means $\pm SD$ for those age groups where more than one animal was measured, n is the number of individuals in the group.
most dramatic increase in $V_L$ occurs within the first postnatal stage of development (~3–5 postnatal days) when the lung changes from the canalicular stage to the saccular stage. The increase in $V_L$ may be mainly due to airspace expansion, as indicated by the significantly high volume densities of the airspaces during the first three postnatal days. It has been reported that $V_L$ increase from birth to adulthood is approximately 23 times in humans and rats (Zeltner & Burri, 1987; Zeltner, Caduff, Gehr, Pfenninger, & Burri, 1987), 3,800 times in the tammar wallaby (Runciman et al., 1998), and 8,000 times in the quokka wallaby (Makanya et al., 2003). Considering the enormous gain in body mass from newborns (12 mg) to adults (780–1,100 g) in *D. viverrinus*, the increase in $V_L$ should be even more dramatic, possibly approximately 30,000 times. However, the low absolute $V_L$ in the neonate *D. viverrinus* may be related not only to the small body size, but also to the stage of lung development. Makanya et al. (2001) proposed that the earlier the developmental stage of lung development, the lower the $V_L$ at birth.

### 4.1.2 Surface densities and surface area

The absolute surface area of the airspace increased ~89-fold from day 1 to day 25, reflecting a remarkable increase in the surface area for gas exchange. The mass-normalized surface area of the newborn *D. viverrinus* (0.82 m$^2$kg$^{-1}$) was low compared to the mass-normalized values of airspace surface area reported for other newborn marsupials (quokka wallaby: 1.34 m$^2$ kg$^{-1}$, tammar wallaby: 1.22 m$^2$ kg$^{-1}$), and for altricial eutherians (newborn rat: 5.31 m$^2$ kg$^{-1}$; Makanya et al., 2007).

The low surface density of the air spaces in the newborn lung reflected the simple “balloon-like” structure at birth. Marsupial newborns with developmental degrees 2 and 3 have more subdivided lungs than newborn dasyurids, which is reflected by the higher surface densities of the air spaces (for review see Fermer, 2018).

Continuous reorganization of the septal components during the early postnatal period (days 1–25) of *Dasyurus viverrinus* resulted in a doubling of the surface density of the air spaces.

### 4.1.3 Lung composition

The proportion of the lung parenchyma Vvp (90%) in the newborn *D. viverrinus* is comparable to that reported for the quokka wallaby (93%; Makanya et al., 2007) and the tammar wallaby (88%; Runciman et al., 1998). The low proportion of nonparenchymal (10%; conducting airways, blood vessels, and connective tissue) in the lungs of the newborn *D. viverrinus* reflects the virtual nonexistence of a bronchial tree and poor vascularization at birth.

The high proportion of the airspaces (63%) in the newborn lung of *D. viverrinus* result from the “balloon-like” lung structure with only a few septal ridges protruding in the airspace. The proportions of airspaces reported for the newborn quokka wallaby (70%; Makanya et al., 2007) and tammar wallaby (63%, Runciman et al., 1998) are quite similar.

Starting from day 4, a reversal in the parenchymal proportions (air spaces, 36%; septa, 50%), reflected the extensive septal formation and increasing subdivision of the lung parenchyma, which continued in the saccular period. A similar situation was observed in the 3-day-old quokka wallaby, where the proportions of the airways and septa were 30% and 58%, respectively (Makanya et al., 2003).

Since the present study examined the lungs of *D. viverrinus* only in the early postnatal period, when the lung was still at the saccular stage, no statements can be made about the timing of alveolization and possible changes in lung composition during this period. However, this process might be similar to that reported for other marsupial species. In the quokka wallaby, after a relatively brief canalicular stage, a prolonged saccular stage markedly increased the lung parenchyma. The terminal wave of saccule septation, in the quokka around day 125, led to the formation of the first generation of alveoli, causing a dramatic increase in the gas exchange area (Makanya et al., 2003). It can be hypothesized that the process of alveolization might occur earlier in *D. viverrinus* than in the quokka wallaby. However, the structural changes in the lungs associated with alveolization might be comparable.

### 4.1.4 Gas exchange through the skin

In the neonate of *D. viverrinus*, the small lung volume and the highly immature lung structure, resulting in low airspace surface density and total surface area, might affect pulmonary function. Although the lungs of the newborn *D. viverrinus* show qualitative characteristics (surfactant system and blood-gas-barrier) of a gas-exchanging organ, quantitatively, this organ is poorly developed to meet the metabolic demands at birth. It is most probable that the neonate of *D. viverrinus* is almost totally dependent on the gas exchange via the skin, as has been reported for other newborn dasyurids (Mortola et al., 1999; Frappell & MacFarlane, 2006).

Factors that likely contribute to cutaneous exchange in the newborn marsupial include the properties of the
skin (Ferner, 2018, 2020; Makanya et al., 2007; Randall, Gannon, Runciman, & Baudinette, 1984), the low oxygen demands of the pouch young (Frappell & MacFarlane, 2006; Szdzuy et al., 2008), neural, mechanical, or chemical constraints on ventilation (Frappell & MacFarlane, 2006), and the presence of cardiac shunts (Runciman, Gannon, & Baudinette, 1995). A thin epidermis (diffusion barrier), no hair follicles, and the presence of numerous capillaries in the dermal layer of the skin of many marsupial neonates support the notion that cutaneous exchange in newborn marsupials might be commonplace (Ferner, 2018; MacFarlane, Frappell, & Mortola, 2002).

Recent studies on the skin structure of *D. viverrinus* revealed that the neonate possesses an extensive subepidermal capillary network, characterized by low diffusion distances and high capillary volume density, a well-developed vascular system for communication between cutaneous capillaries and the cardiac system, and an undivided ventricle (Ferner, 2018, 2020). These structural prerequisites allow for extensive transcutaneous gas exchange. The duration of cutaneous gas exchange during the postnatal period seems to be determined primarily by the maturation of the cardiorespiratory system. In particular, the closing of the shunts, resulting in the separation of the left and right ventricles, necessitates a transition from cutaneous to pulmonary gas exchange around 3 days after birth (Ferner, 2020; Runciman et al., 1995). The rapid postnatal development of the lung in *D. viverrinus* is indicated by a marked increase in the septal proportion of the parenchyma around day 4, which is reflected by an increase in the air space surface density and surface area at this time. These findings support the assumption that the time of transition from cutaneous to pulmonary respiration was around day 4. The pulmonary system has to mature quickly to be functional to meet the metabolic needs of the developing young.

**ACKNOWLEDGMENTS**

I thank Peter Giere for providing access to the Hubrecht & Hill collection and for his help in finding the appropriate specimens. In addition, I appreciate the support and confidence in my research by the Museum für Naturkunde Berlin. Furthermore, I would like to thank the reviewers for their inspiring comments and helpful suggestions, which improved the clarity of the paper. I acknowledge the financial support from the German Research Foundation (DFG) with the module “temporary position for the principal investigator” (Grant No. FE 1878/2-1).

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available under https://doi.org/10.7479/wcst-nn56.

**ORCID**

Kirsten Ferner https://orcid.org/0000-0001-9646-9759

**REFERENCES**

Bellusci, S., Henderson, R., Winnier, G., Oikawa, T., & Hogan, B. L. (1996). Evidence of normal expression and targeted misexpression that bone morphogenetic protein (Bmp-4) plays a role in mouse embryonic lung morphogenesis. *Development*, 122, 1693–1702.

Branchfield, K., Li, R., Lungova, V., Verheyden, J. M., McCulley, D., & Sun, X. (2016). A three-dimensional study of alveologenesis in mouse lung. *Developmental Biology*, 409, 429–441. https://doi.org/10.1016/j.ydbio.2015.11.017

Buaboocha, W., & Gemmell, R. T. (1977). Development of lung, kidney and skin in the brushtail possum, *Trichosurus vulpecula*. *Acta Anatomica*, 159, 15–24. https://doi.org/10.1159/000147960

Burri, P. H. (1974). The postnatal growth of the rat lung. III. Morphology. *The Anatomical Record*, 180, 77–98. https://doi.org/10.1002/ar.1091800109

Burri, P. H. (1999). Lung development and pulmonary angiogenesis. In C. Gaultier, J. R. Bourbon, & M. Post (Eds.), *Lung development. Clinical physiology series*. New York, NY: Springer. https://doi.org/10.1007/978-1-4614-7537-8_5
Burri, P. H. (2006). Structural aspects of postnatal lung development: Alveolar formation and growth. *Biologia Neonatorum*, 89, 313–322. https://doi.org/10.1159/000092868

Burri, P. H., Dbaly, J., & Weibel, E. R. (1974). The postnatal growth of the rat lung. I. Morphometry. *The Anatomical Record*, 178, 711–730. https://doi.org/10.1002/ar.1091780405

Burri, P. H., Haenni, B., Tschanz, S. A., & Makanya, A. N. (2003). Morphometry and allometry of the postnatal marsupial lung development: An ultrastructural study. *Respiratory Physiology & Neurobiology*, 138, 309–324. https://doi.org/10.1016/S1569-9048(03)00197-6

Cooper, C. E., & Withers, P. C. (2010). Comparative physiology of Australian quolls (Dasyurus; Marsupialia). *Journal of Comparative Physiology B*, 180, 857–868. https://doi.org/10.1007/s00360-010-0452-3

Davies, P., Reid, L., Lister, G., & Pitt, B. (1988). Postnatal growth of the sheep lung: A morphometric study. *The Anatomical Record*, 220, 281–286. https://doi.org/10.1002/ar.1092200308

Docimo, S. G., Crone, R. K., Davies, P., Reid, L., Retik, A. B., & Mandell, J. (1991). Pulmonary development in the fetal lamb: A morphometric study of the alveolar phase. *The Anatomical Record*, 229, 495–498.

Ferner, K. (2018). Skin structure in newborn marsupials with focus on cutaneous gas exchange. *Journal of Anatomy*, 233, 311–327. https://doi.org/10.1111/joa.12843

Ferner, K. (2020). Development of the skin in the eastern quoll (*Dasyurus viverrinus*) with focus on cutaneous gas exchange in the early postnatal period. *Journal of Anatomy*, 00, 1–20. https://doi.org/10.1111/joa.13316

Ferner, K., & Mess, A. (2011). Evolution and development of fetal membranes and placentaion in amniote vertebrates. *Respiratory Physiology and Neurobiology*, 178, 39–50. https://doi.org/10.1016/j.resp.2011.03.029

Ferner, K., Zeller, U., & Renfree, M. B. (2009). Lung development of monotremes: Evidence for the mammalian morphotype. *The Anatomical Record*, 292, 190–201. https://doi.org/10.1002/ar.20825

Ferner, K., Schultz, J. A., & Zeller, U. (2017). Comparative anatomy of neonates of the three major mammalian groups (monotremes, marsupials, placentals) and implications for the ancestral mammalian neonate morphotype. *Journal of Anatomy*, 231, 798–822. https://doi.org/10.1111/joa.12689

Frappell, P. B., & MacFarlane, P. M. (2006). Development of the respiratory system in marsupials. *Respiratory Physiology and Neurobiology*, 154, 252–267. https://doi.org/10.1016/j.resp.2006.05.001

Frappell, P. B., & Mortola, J. P. (2000). Respiratory function in a newborn marsupial with skin gas exchange. *Respiratory Physiology*, 120, 35–45. https://doi.org/10.1016/S0034-5687(99)00103-6

Gemmell, R. T. (1986). Lung development in the newborn marsupial bandicoot, *Isoodon macrourus*. *Journal of Anatomy*, 148, 193–204.

Gemmell, R. T., & Little, G. J. (1982). The structure of the lung of the newborn marsupial bandicoot, *Isoodon macrourus*. *Cell and Tissue Research*, 223, 445–453. https://doi.org/10.1007/BF01258501

Gemmell, R. T., & Nelson, J. (1988). The ultrastructure of the lung of two newborn marsupial species, the northern native cat, *Dasyurus hallucatus*, and the brush-tail possum, *Trichosurus vulpecula*. *Cell and Tissue Research*, 252, 683–685. https://doi.org/10.1007/BF00216657

Gemmell, R. T., & Selwood, L. (1994). Structural development in the newborn marsupial, the stripe-faced dunnart, *Sminthopsis macroura*. *Acta Anatomica*, 149, 1–12. https://doi.org/10.1159/000147549

Hill, J. P., & Hill, W. C. O. (1955). The growth stages of the pouch young of the native cat (*Dasyurus viverrinus*) together with observations on the anatomy of the newborn young. *Transactions of the Zoological Society of London*, 28, 349–352.

Howard, C. V., & Reed, M. G. (2005). *Unbiased stereology*. New York: Garland Science/BIOS Scientific.

Hisia, C. C., Hyde, D. M., Ochs, M., & Weibel, E. R. (2010). An official research policy statement of the American Thoracic Society/ European Respiratory Society: Standards for quantitative assessment of lung structure. *American Journal of Respiratory and Critical Care Medicine*, 181, 394–418. https://doi.org/10.1164/rccm.200809-1522ST

Hughes, R. L., & Hall, L. S. (1988). Structural adaptations of the newborn marsupial. In C. H. Tyndale-Biscoe & P. A. Janssens (Eds.), *The developing marsupial. Models for biomedical research* (pp. 8–27). Berlin, Heidelberg: Springer.

Kim, H. Y., Pang, M. F., Varner, V. D., Kojima, L., Miller, E., Radisky, D. C., & Nelson, C. M. (2015). Localized smooth muscle differentiation is essential for epithelial bifurcation during branching morphogenesis of the mammalian lung. *Developmental Cell*, 34, 719–726. https://doi.org/10.1016/j.devcel.2015.08.012

Klima, M., & Bangma, G. C. (1987). Unpublished drawings of marsupial embryos from the Hill collection and some problems of marsupial ontogeny. *Zeitschrift für Säugetierkunde*, 52, 201–211.

Krause, W. J., & Leeson, C. R. (1975). Postnatal development of the respiratory system of the opossum. II. Electron microscopy of the epithelium and pleura. *Acta Anatomica*, 92, 28–44. https://doi.org/10.1159/000144429

MacFarlane, P. M., & Frappell, P. B. (2001). Convection requirement is established by total metabolic rate in the newborn tammar wallaby. *Respiratory Physiology*, 126, 221–231. https://doi.org/10.1016/S0034-5687(01)00227-4

MacFarlane, P. M., Frappell, P. B., & Mortola, J. P. (2002). Mechanics of the respiratory system in the newborn tammar wallaby. *The Journal of Experimental Biology*, 205, 533–538.

Makanya, A. N., Sparrow, M. P., Warui, C. N., Mwangi, D. K., & Burri, P. H. (2001). Morphological analysis of the postnattally developing marsupial lung: The quokka wallaby. *The Anatomical Record*, 262, 253–265. https://doi.org/10.1002/1097-0185(20010301)262:3<253::AID-AR1025>3.0.CO;2-B

Makanya, A. N., Haenni, B., & Burri, P. H. (2003). Morphometry and allometry of the postnatal lung development in the quokka wallaby (*Setonix brachyrurus*): A light microscopic study. *Respiratory Physiology and Neurobiology*, 134, 43–55. https://doi.org/10.1016/S1569-9048(02)00204-5

Makanya, A. N., Tschanz, S. A., Haenni, B., & Burri, P. H. (2007). Functional respiratory morphology in the newborn quokka wallaby (*Setonix brachyrurus*). *Journal of Anatomy*, 211, 26–36. https://doi.org/10.1111/j.1469-7580.2007.00744.x

Merkus, P. J. F. M., Have-Opbroek, A. T., & Quanjer, P. H. (1996). Human lung growth: A review. *Pediatric Pulmonology*, 21, 383–397. https://doi.org/10.1002/sipul.1099-0496(199606)21:6<383::AID-PPUL6>3.0.CO;2-M
