Journal of Anatomy

J. Anat. (2017) **231**, pp798–822 doi: 10.1111/joa.12689

Comparative anatomy of neonates of the three major mammalian groups (monotremes, marsupials, placentals) and implications for the ancestral mammalian neonate morphotype

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Abstract

The existing different modes of reproduction in monotremes, marsupials and placentals are the main source for our current understanding of the origin and evolution of the mammalian reproduction. The reproductive strategies and, in particular, the maturity states of the neonates differ remarkably between the three groups. Monotremes, for example, are the only extant mammals that lay eggs and incubate them for the last third of their embryonic development. In contrast, marsupials and placentals are viviparous and rely on intra-uterine development of the neonates via choriovitelline (mainly marsupials) and chorioallantoic (mainly placentals) placentae. The maturity of a newborn is closely linked to the parental care strategy once the neonate is born. The varying developmental degrees of neonates are the main focus of this study. Monotremes and marsupials produce highly altricial and nearly embryonic offspring. Placental mammals always give birth to more developed newborns with the widest range from altricial to precocial. The ability of a newborn to survive and grow in the environment it was born in depends highly on the degree of maturation of vital organs at the time of birth. Here, the anatomy of four neonates of the three major extant mammalian groups is compared. The basis for this study is histological and ultrastructural serial sections of a hatchling of *Ornithorhynchus anatinus* (Monotremata), and neonates of *Monodelphis domestica* (Marsupialia), *Mesocricetus auratus* (altricial Placentalia) and *Macroscelides proboscideus* (precocial Placentalia). Special attention was given to the developmental stages of the organs skin, lung, liver and kidney, which are considered crucial for the maintenance of vital functions. The state of the organs of newborn monotremes and marsupials are found to be able to support a minimum of vital functions outside the uterus. They are sufficient to survive, but without capacities for additional energetic challenges. The organs of the altricial placental neonate are further developed, able to support the maintenance of vital functions and short-term metabolic increase. The precocial placental newborn shows the most advanced state of organ development, to allow the maintenance of vital functions, stable thermoregulation and high energetic performance. The ancestral condition of a mammalian neonate is interpreted to be similar to the state of organ development found in the newborns of marsupials and monotremes. In comparison, the newborns of altricial and precocial placentals are derived from the ancestral state to a more mature developmental degree associated with advanced organ systems.

Key words: altricial; evolution; morphology; neonate; precocial; reproduction.

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Accepted for publication *8 August 2017*

Introduction

Mammals and their close relatives, the extinct mammaliaforms, can be traced back to the Mid-Triassic (Kielan-Jaworowska et al. 2004; Kemp, 2005; Luo, 2007, 2011; Krause et al. 2014; Ekdale, 2016). The three extant mammal groups, that is monotremes (egg-laying mammals), marsupials (pouched mammals) and placentals, have existed since the Jurassic (Luo, 2007; Benton et al. 2009). The separation of Prototheria (monotremes) from therians occurred approximately 220 Mya according to fossil records and molecular data (Bininda-Emonds et al. 2007; Madsen, 2009). Metatheria (including marsupials) and Eutheria (including placentals) separated approximately 140 Mya (Lefevre et al. 2010). In the past 20 years, several new discoveries of fossil mammal skeletons and dentitions have shed light on the anatomy and lifestyle of these early mammals (Luo et al. 2001, 2007; Averianov & Lopatin, 2006; Ji et al. 2006; Krause et al. 2014; Meng et al. 2015). However, discussions about the mode of reproduction in these early mammals remain speculative, because the fossil record consists mainly of the preservation of hard tissues (e.g. teeth and bones), and provides limited to no insight in soft tissues and internal organ structure. For this reason, the main part of the existing discussions about the origin and evolution of mammalian reproduction strategies relies considerably on the interpretation of the different modes of reproduction in living representatives of monotremes, marsupials and placentals (Tyndale-Biscoe & Renfree, 1987; Zeller, 1999).

Mammals have adopted a diversity of reproductive strategies, with clear differences in the three extant major lineages. In contrast to many non-mammalian amniotes, which are lecithotrophic (nutrition comes from embryo's own yolk), all mammals are matrotrophic (nutrition comes from maternal reproductive tract; Blackburn, 2015). Matrotrophic oviparity occurs in monotremes, the closest living representatives of ancestral mammals. Prior to the laying of the egg, the yolk sac functions in uptake of oviductal secretions, and the allantois functions in gas exchange after oviposition (Hughes, 1993). The monotreme eggs are covered by a thin shell, from which the young hatches after the incubation period. The period of lactation is prolonged relative to gestation and incubation time (Griffiths, 1978).

Even though Marsupialia and Placentalia have evolved from a common ancestor, both groups adapted different reproductive strategies. In viviparous mammals, the yolk sac and allantois have been recruited evolutionarily for placentotrophy (Ferner & Mess, 2011). Apposition of the fetal membranes to the uterine lining forms a choriovitelline placenta (in all marsupials and most eutherians) and a chorioallantoic placenta (in all eutherians and a few marsupials; Blackburn, 2015). Marsupials are characterized by a short gestation period (12–38 days) and the birth of a highly immature neonate (Tyndale-Biscoe, 2001; Freyer et al. 2003). Most of their growth and development occurs postnatally, supported by a prolonged lactation cycle (Renfree, 2006; Ferner & Mess, 2011). By contrast, placental females invest more in extended intra-uterine development (16–660 days; Mess & Carter, 2007; Mess & Ferner, 2010; Siniza et al. 2012; Ferner et al. 2014).

With the process of birth the mammalian fetus abandons the physical and physiological protection of the uterus, and the extra-embryonic membranes end their functions as the main gaseous exchange system, waste recycle area and uterine nutrient resource (Hughes & Hall, 1988). The process of birth initiates the most vulnerable time in the life cycle of a neonate (Szdzuy, 2008). Once born, the infant now has to use its own resources to survive risks like hypothermia, desiccation and depletion of energy reserves (Hughes & Hall, 1988). With birth, the 'decision' is made if the organism is capable of surviving, and organ systems (e.g. the cardiorespiratory system, the digestive system, brain) are able to function for extra-uterine life (Mess & Ferner, 2010).

Therefore, the developmental stage of a mammalian neonate offers an exceptional starting point for interpreting structural prerequisites and adaptability in view of survivability of the neonate.

Hatching monotremes and newborn marsupials are characterized by their minute size and high degree of immaturity, much of their development occurs postnatally in a nest or in the pouch attached to one of the maternal teats (Tyndale-Biscoe & Renfree, 1987; Grant, 1995; Zeller & Freyer, 2001; Renfree, 2006; Simpson et al. 2011). In comparison, newborn placentals feature a wide range of developmental stages, from altricial to precocial, but they are always more advanced in development than monotreme hatchlings and marsupial neonates (Eisenberg, 1983; Szdzuy et al. 2008). Altricial placental neonates are born naked with closed eyes in a relatively helpless state, and often stay in nests for parental care for a rather long postnatal period. In contrast, precocial placental neonates are more advanced in development. Typically, they have fully developed fur, eyes are open, and they mature rapidly in locomotor ability, thus achieving independence from their parents relatively early (Hill, 1992; Szdzuy & Zeller, 2009).

Differences in the developing internal organs are expected, considering the different external appearances of altricial and precocial mammalian neonates. The ontogeny of internal organ development has been examined in several mammalian species for a long time (Hill & Hill, 1955; Müller, 1972; Griffiths, 1978; Krause et al. 1979a,b; Walker & Gemmell, 1983; Langston et al. 1984; Gemmell & Selwood, 1994; Buaboocha & Gemmell, 1997; Makanya et al. 2007; Szdzuy et al. 2008; Ferner et al. 2010; Simpson et al. 2011; Modepalli et al. 2015). However, comparative studies on the structural developmental state of mammalian neonates, in particular in view of mammalian evolution, are rare (Müller, 1972; Szdzuy et al. 2008; Ferner et al. 2009).

Much attention has been given in the literature to questions about reproductive evolution in mammals (Freyer et al. 2003; Lillegraven, 2003; Szdzuy & Zeller, 2009; Renfree, 2010; Blackburn, 2015). Nevertheless, the mode of reproduction in ancestral mammals, in particular the developmental state of the mammalian offspring, remains speculative. In particular, the comparison to birds, where precociality seems to be ancestral (Starck & Ricklefs, 1998), raises questions about the mammalian origins. Did the neonate of the mammalian morphotype resemble the precocial neonates, present in many placental orders (similar to their sauropsid ancestors), or was it altricial like today's monotremes and marsupials?

To address these problems, the results and literature data of this study are integrated into a morphotype reconstruction of the mammalian neonate to understand which anatomical features presumably were present in the mammalian ancestor and which traits have been derived in the course of evolution.

Materials and methods

Animals

The taxa selected here represent four critical states in the broad variety of developmental stages that exist in mammalian neonates. The terms neonate and hatchling are defined as the first 24 h at the day of birth. The numbers and specifics of the specimens used for light and transmission electron microscopy in this study are summarized in Table 1. We investigated one hatchling of *Ornithorhynchus anatinus* (platypus; Monotremata) and, respectively, two neonates of the marsupial species *Monodelphis domestica* (the gray short-tailed opossum; Didelphoidea), of *Mesocricetus auratus* (the golden hamster, Rodentia) representing the altricial placental type, and of *Macroscelides proboscideus* (the short-eared elephant shrew; Macroscelidea) representing the precocial placental type.

The *O. anatinus* specimen (collection number M44) was available from the Hill collection (currently located at the Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin). It has been prepared for light microscope studies prior to our investigation (e.g. serially sectioned, transferred to glass slides, stained). The specimens of neonate *M. domestica* were already prepared for light and transmission electron microscopy (coll. U. Zeller, currently located at Humboldt University, Division of Systematic Zoology; collection number 337, 7 μm slice thickness, Azan stained; collection number 137, ultrastructure tissue embedded in Araldite). The neonates of *M. auratus* and *M. proboscideus* (both coll. U. Zeller) used in this study stem from the breeding colonies of the animal facility of the Institute of Systematic Zoology, Museum für Naturkunde (today Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin). All sampling was done under registration (registration number: G 0249/02) of the responsible State Office of Health and Social Affairs Berlin (LaGeSo). The neonates were serially sectioned for histological and ultrastructural investigation. For preparation specifics see below.

Light microscopy

Torsos of the neonates of *M. auratus* and *M. proboscideus* were fixed in Bouin's solution (picric acid, formalin, 100% acetic acid, 15 : 5 : 1; Mulisch & Welsch, 2010) for 1 day, and afterwards rinsed in 70% ethanol. The specimens were decalcified in a stock solution for 4 days [100 mL dimethylsulfoxide, 15 mL nitric acid (65%)], then rinsed in sodium sulfate solution (5%) and distilled water, and transferred to 70% ethanol before automatic paraffin embedding (Shanon Hypercenter XP, Life Science International GmbH, Frankfurt, Germany). The specimens were serially sectioned in a transverse plane. The sections (thickness 7–10 μm) were stained with Azan, trichrome, or hematoxylin and eosin. Selected sections were digitally photographed using a stereomicroscope (Leica MZ 12; Wildt, Switzerland). Morphometric measurements of the lung were the subject of former studies (Table 1; for details, see Szdzuy et al. 2008; Ferner et al. 2009). Measurements of the skin were conducted on the dorsolateral side of the torsos. Assessment of skin thickness and distance between the external surface and most superficial capillaries (in marsupial and monotreme species) were performed by 50 measurements per species with a digital ruler (Image J software), and the mean and SEM were presented.

Transmission electron microscopy

For the ultrastructural investigations, small parts of internal organ tissues from the neonates of *M. auratus* and *M. proboscideus* were dissected, and then fixed in 3.5% glutaraldehyde, buffered in 0.2 M cacodylate (pH 7.4) for 2 h. Afterwards the specimens were treated with 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide for another 2 h. The tissues were embedded in Araldite and thin-sectioned at 70 nm. The thin-sections were transferred to copper grids and stained with uranyl acetate and lead citrate. The thin-sections were examined using transmission electron microscopy (TEM, LEO 912 Omega).

Table 1 Lung morphometry in neonates of a monotreme (*Ornithorhynchus anatinus*), a marsupial (*Monodelphis domestica*), an altricial (*Mesocricetus auratus*) and a precocial (*Macroscelides proboscideus*) placental mammal (data from Szdzuy et al. 2008; Ferner et al. 2009).

Species	CRL (mm)	Specimen no. Light microscopy (LM) Transmission electron microscopy (TEM)	Mean air space diameter (μm)	Mean septum thickness (μm)	Blood–air barrier (nm)
O. anatinus	16.75	M44[a](LM)	320.1 ± 13.1	29.4 ± 0.7	–
M. domestica	10	337[b] (LM), 137[b] (TEM)	453.3 ± 21.9	45.4 ± 1.8	605.2 ± 18.2
M. auratus	30	14 (LM), 41 (TEM)	95.8 ± 3.0	21.7 ± 1.0	368.4 ± 11.3
M. proboscideus	60	9 (LM), 130 (TEM)	26.1 ± 0.6	6.0 ± 0.2	303.1 ± 8.4

The table shows the specimens used in this study.

[a] Hill collection, Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin.

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Phylogenetic reconstruction

To infer evolutionary pathways, it is necessary to link the character states of living organisms to those of the ancestors. One method for phylogenetic reconstruction is to map character states of living organisms on existing phylogenies using the method of 'maximum parsimony' (Cunningham et al. 1998; Zeller & Freyer, 2001). 'Extant phylogenetic bracketing' uses soft tissue evidence of the first two extant outgroups of the fossil taxon of interest to infer character states of the extinct species. This method was described by Witmer (1995), and is based firmly on the relation of biological homology and allows the construction of a hierarchy of inference. For the mammalian morphotype, the outgroup comparison to non-avian sauropsids and Aves is used. A first step was the determination of the character states in extant species (the four investigated species and literature data) of the three mammalian groups and two outgroups (non-avian sauropsids, birds). From this we assumed which characteristics were present in the morphotypes of Prototheria, Theria, Metatheria and Eutheria, respectively. Under consideration of characters present in the outgroups, but also taking into account evolutionary constraints on reproduction, we determined the polarity of the characters and concluded a morphotype of the mammalian neonate.

It is not the intention of this study to draw new phylogenetic trees based on our results, rather to plot our findings on an existing phylogeny and to discuss evolutionary alternatives. This procedure was conducted using the systematics published in Westheide & Rieger (2010).

Results and discussion

The stage of development of newborns of the major mammal groups reflects their different reproductive strategies. The feature that distinguishes monotremes and marsupials from most other mammals is the immaturity of their young at birth. Birth occurring at different times during organogenesis characterizes placental mammals, with a wide spectrum of altricial and precocial neonates, and many intermediate stages (Eisenberg, 1983). Also, marsupials are sorted into three grades of developmental complexity (i.e. G1, G2, G3) based on their organ systems manifested in both the cytological level as well as in the external form of neonates (Hughes & Hall, 1988). However, even the most altricial placental neonate is far more advanced than the most developed marsupial neonate or monotreme hatchling.

General external and internal characteristics

The hatchling of *O. anatinus* and the neonates of *M. domestica, M. auratus* and *M. proboscideus* represent the wide range of different birth stages found in the three major extant mammalian groups (Fig. 1).

Both body dimensions and external morphology of the *O. anatinus* (M44) hatchling are similar to that of newborn marsupials (Fig. 1A). The crown–rump length (CRL) of the monotreme hatchling was 15 mm, and hatching weight was about 0.3–0.4 g (Grant, 1995). The specimen investigated in this study was already sectioned; therefore, the following external description is based on Griffiths (1978) and Zeller (1989). The hatchling of *O. anatinus* shows the following unique characters: (i) altricial appearance; (ii) a prenasal process of the premaxillae, the os caruncle; (iii) the

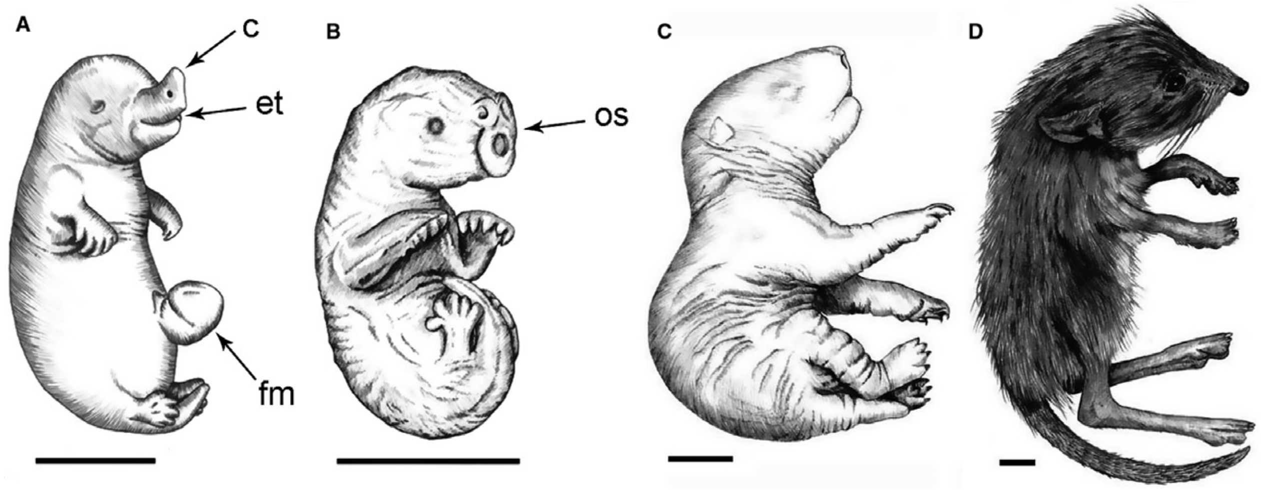

Fig. 1 Drawings of a newly hatched *Ornithorhynchus anatinus* (A; Monotremata; redrawn from Hughes & Hall, 1998) and neonates of *Monodelphis domestica* (B; Didelphoidea, Marsupialia), *Mescocricetus auratus* (C; Rodentia, Glires, Placentalia) and *Macroscelides proboscideus* (D; Macroscelidea, Afrotheria, Placentalia). The appearance of the monotreme hatchling and the marsupial neonate (A, B) is embryonic, with strongly developed forelimbs and undifferentiated paddle-like hindlimbs and an undifferentiated oro-nasal region. An oral shield (os) is prominent in the newborn marsupial, and an egg tooth (et) and a caruncle (C) are visible at the tip of the snout of the newly hatched monotreme. Parts of the fetal membranes (fm) are still attached to the abdomen of *O. anatinus*. The altricial neonate of Placentalia (C) is more advanced with well-developed extremities including claws, but it is naked and eyes and ears are closed. The precocial neonate of Placentalia (D) appears highly developed with open eyes and ears and fully furred. Scale bar: 5 mm.

egg tooth, attached to the premaxillae at their symphysis lying immediately below the os caruncle (used for cutting the egg shell); (iv) eyes and aural apertures covered by a layer of epitrichium, but external nares open and olfactory organs fully functional; (v) cheeks covered with epitrichium, leaving only a small gap but lacking an oral shield; and (vi) well-developed forelimbs with claws, less-developed hindlimbs.

The neonates of the marsupial *M. domestica* are born after a short gestation period (14 days) and have an embryonic appearance (Fig. 1B). The newborn (337) investigated in this study had a CRL of 10 mm and a birth weight of 0.1 g. The newborn *M. domestica* shows the following unique characters: (i) extreme altricial appearance with thin naked skin and apparent erythema; (ii) eye primordium has a prominent ring of retinal pigmentation; (iii) external pinna only recognizable as small bright fields; (iv) oral opening and nose form together the oral shield; and (v) muscular forelimbs with claws well developed, more advanced than paddle-like hindlimbs.

The *O. anatinus* (M44) hatchling and *M. domestica* (337) neonate exhibit several similarities in their internal organization. The skeleton of both species is entirely cartilaginous (Fig. 2A,B). Overall, the musculature is poorly developed in both the hatchling and the neonate, with the exception of the well-developed brachial muscles. The forelimbs show all skeletal parts well developed, including phalanges and metacarpals. The thoracic wall is formed by cartilaginous ribs with thin intercostal muscles. The diaphragm is a thin (thickness: ~ 3 μm) sheet.

When born or hatching, both marsupial and monotreme neonates are extremely small (0.005–0.8 g), and they exhibit a minimum anatomical development possible for a newborn mammal at birth (Tyndale-Biscoe & Renfree, 1987). For example, they lack cranial nerves II–IV and VI, cerebral commissures, eye pigments and eyelids (eyes covered by a protective periderm), skeletal segments are cartilaginous, and the separation of the heart ventricles is incomplete (Lillegraven, 1975; Hughes & Hall, 1988). Although most of their systems are immature, neonates are well developed in certain respects. For instance, in monotreme and most marsupial species, the hatchling and newborn crawl without guidance of the mother from the vagina to the pouch or mammary patch. Neonate marsupials attach itself themselves to a teat and start to suckle immediately. Thus, the locomotor system (pronounced forelimbs) of the

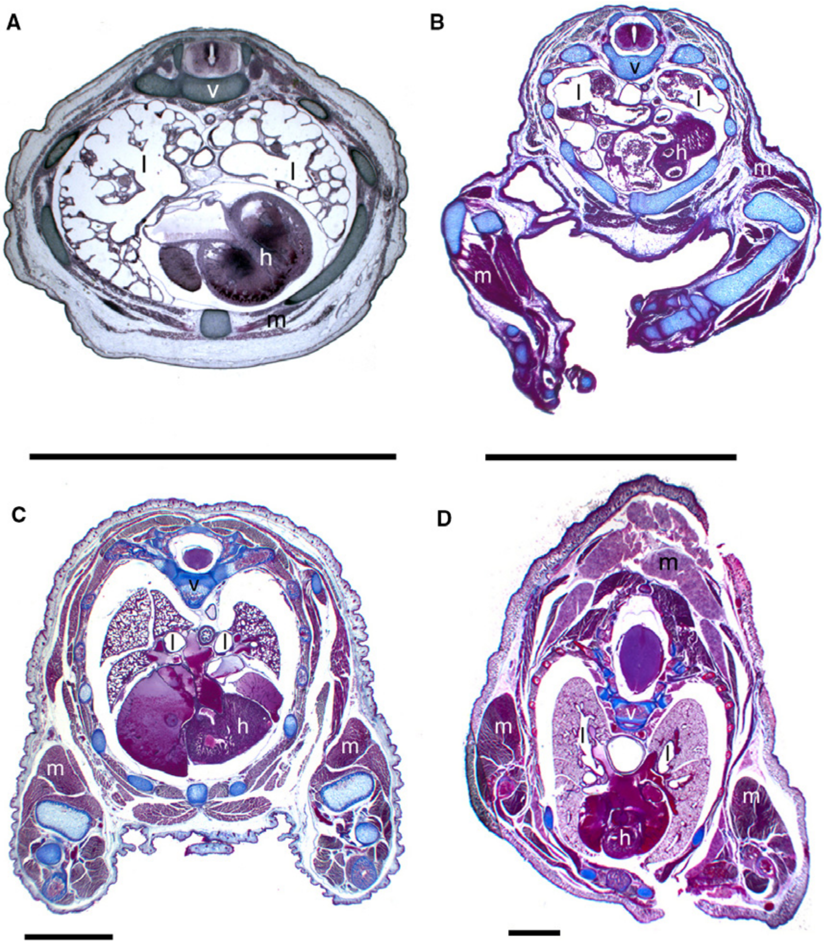

Fig. 2 Light micrographs of histological sections through the anterior abdominal region of a newly hatched *Ornithorhynchus anatinus* (A) and neonates of *Monodelphis domestica* (B), *Mesocricetus auratus* (C) and *Macroscelides proboscideus* (D). The newly hatched monotreme and the newborn marsupial exhibit some similarities in internal organization, although the monotreme hatchling appears slightly more developed than the marsupial neonate. In both, the bronchi end blindly in large saccules, which are slightly smaller and more numerous in *O. anatinus*. The whole skeleton is cartilaginous and only a few muscles are present at birth. *M. domestica* is characterized by a small undifferentiated heart. The newborn *M. auratus*, representing altricial placentals, features a differentiated heart. The lung contains a well-developed bronchial system, which ends in numerous small saccules. The vertebra start to ossify and muscles are distinctive. Precocial placentals, such as *M. proboscideus*, are most advanced in development, characterized by a well-differentiated heart and large alveolarized lungs. The skeleton is completely ossified, and extremities and dorsum are extremely muscular. h, heart; l, lung; m, muscles; v, vertebra. Trichrome (A) and Azan (B–D) staining. Scale bar: 5 mm.

monotreme and marsupial neonates is developed enough to enable active movement. The relatively mature state of the marsupial olfactory system enables finding the pouch and attaching to the teat (Schneider, 2011), and well-developed tongue and mouth muscles enable to suckle (Tyndale-Biscoe & Janssens, 1988). The newborn *M. domestica* (337) investigated here shows prominent brachial musculature and cartilaginous skeletal elements in the forelimbs developed enough to actively climb through the mother's fur to the teat. By contrast, the underdeveloped hindlimbs are paddle-like and project from the body.

Monotreme neonates also show adaptations, associated with their reproductive strategy. To hatch from the egg the monotreme hatchling tears the shell using its egg tooth and caruncle (Dawson, 1983). It has been suggested that the young monotremes do not suck like other mammals but only lick up the milk as it is ejected out of a gland onto the abdominal surface. This is untrue for *Tachyglossus aculeatus*, and presumably also for *O. anatinus,* as Griffiths (1978) has shown that the young *T. aculeatus* sucks vigorously.

Marsupial offspring prenatally show a wide gaping oral opening, because lips and cheeks are not yet completed. However, a transitory seal develops shortly before the day of birth, and filling soft tissue grows toward the midline from the corners of the mouth. This changes the mouth opening from wide gaping to a round hole, just large enough to accept the mother's teat (Zeller, 1999). This oral shield seals the mouth around the nipple and, in association with a bulbous swelling on the end of the teat, it allows the 'pumpsucking' mode of feeding (Merchant & Sharman, 1966). In addition to the suckling purpose, the oral shield has two other important functions. The fixation of the neonate itself to the nipple, essentially important in pouchless species such as *M. domestica*, and the fixation of the upper and lower jaws to be almost motionless, for the proper development of the temporo-mandibular-jaw joint. It is important to note that newly hatched monotremes also display the transitory closure of the mouth opening to ensure proper jaw development.

The neonate *M. auratus* (14) represents the newborn placental mammal in an altricial condition (Figs 1c and 2C). The specimen had a CRL of 30 mm and a birth weight of 2.2 g. The overall state of the neonate of *M. auratus* is clearly more advanced than the monotreme hatchling or the marsupial neonate. The newborn *M. auratus* (14) shows the following unique characters: (i) naked, skin pinkish and translucent; (ii) oro-nasal region well differentiated, vibrissae visible; (iii) incisors present at birth; (iv) eyes and ears closed, but pinnae clearly visible; (v) fore- and hindlimbs well developed with claws; (vi) most of the skeleton of the limbs and digits consist of cartilage, the ribs and vertebrae show first ossifications; and (vii) musculature well developed, especially in forelimbs for simple crawling movements.

Several authors have described similar characteristics for altricial newborns, mostly rodents and 'insectivores' (Müller, 1972; Jacobsen, 1982; Hertenstein et al. 1987; Bronner, 1992; Gusztak & Campbell, 2004; Szdzuy, 2008). In contrast to newborn marsupials, altricial placentals seem to have no special adaptations or morphological features linked to their behavior directly after birth. Generally, placental mammals give birth to altricial young in a nest-like setting. The juveniles remain in the nest and frequently are nursed by the mother and undergo rapid postnatal development. Blair (1941) describes in some desert rodents (*Neotoma* and *Baiomys*) a marsupial-like method to carry their young around. Like in marsupials the young are firmly attached to the teats, but not for a long period of time. This behavior might be an adaptation to unfavorable habitats with only limited possibilities for nest sites, or simply a mechanism to avoid predation of the young. Müller (1972) assumes that it might be the perpetuation of ancestral reproductive strategies.

The neonate *M. proboscideus* (9) represents the newborn placental mammal in a precocial state (Figs 1d and 2D). After a comparatively long gestation period of 61 days, the neonate of *M. proboscideus* was born with a CRL of 60 mm and a birth weight of 9.5 g. The external appearance of the neonate is already very similar to an adult elephant shrew. The newborn *M. proboscideus* (9) shows the following unique characters: (i) fully covered with fur, vibrissae well developed; (ii) eyes and ears open; (iii) oro-nasal region well differentiated; (iv) teeth present; (v) all four extremities well developed with claws (enable coordinate locomotion shortly after birth); (vi) completely ossified skeleton; and (vii) well-developed musculature in dorsum and extremities allow upright posture and fast cursorial movement.

Precocial placental neonates can be found among ungulates (Cetacea, Sirenia, Proboscidea, Hyracoidea, Perissodactyla and Artiodactyla), elephant shrews (Macroscelidea), hares (Lagomorpha), Pinnipedia (Carnivora), several rodent taxa (Rodentia) and others. A precocial neonate is considered to be well developed, fur-bearing, sensory systems like eyes and ears are fully functional, thermoregulation and locomotion are stable without a nest, and a quick change from milk diet to solid food occurs (Hill, 1992; Vaughan et al. 2000; Hackländer et al. 2002). Precociality associates with different life-history strategies and requires certain structural prerequisites. Survival of newborn elephant shrews in their semiarid habitat, for example, depends on their well-developed sensory abilities (open eyes and ears, mature brain development) and muscle reflexes, necessary for fast movements to avoid predation. In order to maintain a high metabolic rate, precocial young change quickly from mother's milk to solid food. *M. proboscideus* shows a full dentition at the time of birth for effective food breakdown early on.

Lung

The mammalian lung is a highly conservative structure, and the morphogenetic processes of alveolization and septal maturation in monotremes, marsupials and placentals are basically the same, but maturation of this organ differs in timing. The ontogenetic development of air space dimension, thickness of the capillary septum and blood–air barrier were the focus of previous morphometric studies. The results are summarized in Table 1 (for details, see Szdzuy et al. 2008; Ferner et al. 2009).

The lung of the monotreme hatchling *O. anatinus* (M44) shows few large saccules (Fig. 3A,B). Because characteristics of both the canalicular (large primitive airways open into large air spaces) and early saccular (primitive double capillary system, surfactant production) stages are present, the state of lung development is interpreted to be in the transition from one to the other. In general, the bronchial tree is poorly developed at birth with cranially broad, but caudally tapering main bronchi. Short lobar bronchi branch off from the main bronchus. They consist of cuboidal epithelium and a single or double layer of smooth muscle cells. The short bronchi give rise to smooth-walled channels, lined with respiratory epithelium (type I and type II pneumocytes), which open directly in large terminal saccules (~320 µm in diameter). These saccules are lined with respiratory epithelium

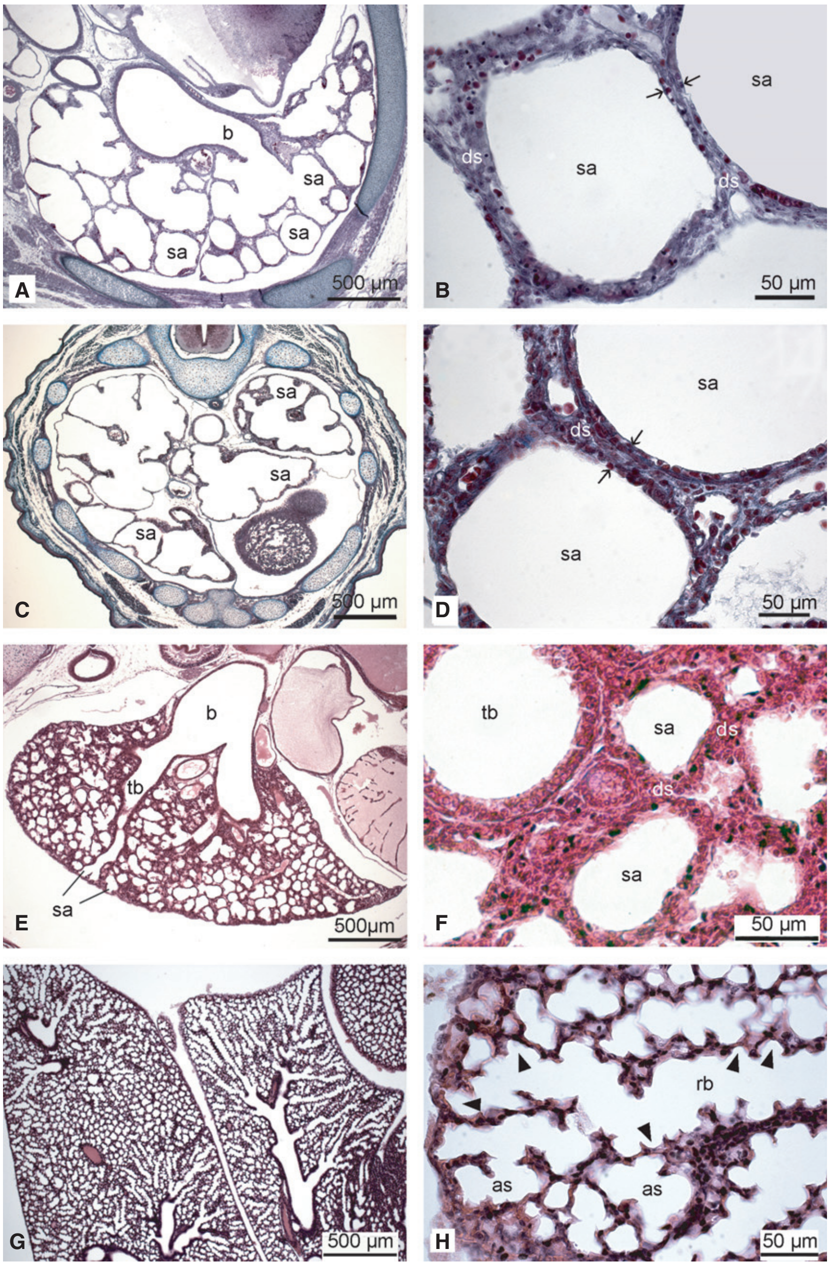

Fig. 3 Light micrographs of the lung in a newly hatched *Ornithorhynchus anatinus* (A, B), and neonate *Monodelphis domestica* (C, D), *Mesocricetus auratus* (E, F) and *Macroscelides proboscideus* (G, H). In the monotreme hatchling and marsupial neonate, the bronchi are very short and end directly in the large terminal saccules, where the gas exchange takes place. The saccules are separated by a double capillary septum (B, D, arrows indicate capillaries). In the altricial placental *M. auratus*, the bronchial tubes lead with many dichotomies deep into the periphery of the lung and terminate in numerous small saccules (E). The small saccules are separated by a double capillary septum. The lung of the precocial placental *M. proboscideus* is characterized by a well-developed system of conducting airways and long respiratory bronchioles (G). The gas exchange takes place in the respiratory bronchioles and in numerous alveolar sacs with small alveoli (arrowheads indicate alveoli). as, alveolar sac; b, bronchus; ds, double capillary septum; rb, respiratory bronchiole; sa, sacculus; tb, terminal bronchiole. Magnification is indicated by the scale bar.

and appear smooth-walled with only a few septal crests protruding from the septum (Fig. 3A). The thick septum separating the saccules consists of a double network of capillaries with abundant interstitial mesenchyme (septum thickness ~30 μm; Fig. 3B).

Like in the monotreme hatchling (M44), the lung development in newborn marsupial *M. domestica* (337) is at the transition from canalicular to early saccular stage (Fig. 3C). The lung consists of a primitive system of short conducting airways that terminate in a number of large terminal saccules (~450 μm in diameter). Single cubodial epithelium lines the main bronchi. Vestigial cartilage is present near the hilum. Bronchial tubes branching off the main bronchi are short and end directly in large saccules. The saccules are lined with a simple squamous epithelium on an extensive capillary bed. Two cell types characterize the respiratory epithelium: cuboidal type II pneumocytes interspersed with simple squamous type I pneumocytes. Each sacculus is supplied by its own capillary bed, separated from the capillary bed of the adjacent sacculus, apparent by the wide double capillary septum (septum thickness ~45 μm; Fig. 3D). The interstitial layer, separating the two capillary beds in the septum, contains delicate reticular fibers of connective tissue, mesenchymal cells and a fine network of deeper lying capillaries (Fig. 4A). Although the lung of *M. domestica* (137) is in an early developmental stage, the pulmonary vasculature is well developed with short blood–air diffusion distances. The blood–air barrier between the capillaries and the saccular lumen is composed of endothelial cells, type I pneumocytes, and a fused basal lamina of both cell types (mean thickness of blood–air barrier ~605 nm). Type II pneumocytes contain lamellar bodies indicating active surfactant production. The erythrocytes found in the capillaries are nucleated at the time of birth.

An immature lung is characteristic for monotreme hatchlings and newborn marsupials, and the lung structures of *O. anatinus* (M44) and *M. domestica* (337) confirm previously published data on lung maturation in marsupial mammals. Because in adult monotremes and marsupials a lung structure typical for adult mammals is present, the immaturity of the neonate and hatchling lung must reflect developmental differences (Szdzuy et al. 2008; Ferner et al. 2009). Among the major marsupial groups, a range of neonate developmental states exists (G1–G3), and each type shows a characteristic state of lung development (Mess & Ferner, 2010). The dasyurids give birth to the smallest newborns in marsupials (G1). Their newborns have the most immature lungs in the canalicular stage, showing tubular-like structures with thick septal walls and the characteristic double-capillary layer (Hill & Hill, 1955; Gemmell & Nelson, 1988; Tyndale-Biscoe & Janssens, 1988; Gemmell & Selwood, 1994; Frappell & Mortola, 2000; Simpson et al. 2011). In comparison, newborns of didelphids, peramelides and phalangerids have an intermediate size (G2) with rudimentary lungs showing large terminal saccules (Krause & Leeson, 1975; Gemmell & Little, 1982; Hughes & Hall, 1984; Gemmell, 1986; Gemmell & Nelson, 1988; Schmidt, 1996; Szdzuy et al. 2008). The Macropodids produce the largest marsupial neonates (G3). Their lungs are greatly enlarged and subdivided by richly vascularized double-capillary septa (Walker & Gemmell, 1983; Gemmell & Rose, 1989; Runciman et al. 1996; MacFarlane & Frappell, 2001; Modepalli et al. 2016).

Small bulges and crests in the larger terminal saccules of the newborn *M. domestica* (337) let us assume that a subdividing process of the terminal saccules is in progress at the time of birth. The decrease of the average size of the saccules during the postnatal lung development in *M. domestica* confirms that such a subdividing process occurs (Szdzuy et al. 2008). In contrast, such crests were not found in the terminal saccules of the altricial newborn *M. auratus* (14), and it is not known from the newborn *Rattus norvegicus* or *Mus musculus* (Burri, 1974; Amy et al. 1977). The double-capillary septa in the lungs of the newborn *M. domestica* (137) are thicker than those in the lungs of *M. auratus* (41) due to the more substantial, loose, interstitial tissue within the septa. The thicker septa suggest that the lungs of the newborn *M. domestica* are in an earlier saccular stage than the lungs of the newborn *M. auratus*. The ultrastructural investigation of the *M. domestica* (137) lung revealed Type II pneumocytes with lamellar bodies, suggesting the production of pulmonary surfactant. Our results confirm similar observations in *Macropus eugenii* (Miller et al. 2001), and assume that marsupials are born with a mature surfactant system despite the short gestation period.

In summary, the minimal required traits for a fully functioning lung at birth are: a large surface area for gas exchange; a thin air–blood barrier; a surfactant system; a conductive airway tree; appropriately developed vasculature; and coordinated neuromuscular effort (Simpson et al. 2011). Many of these structural prerequisites are missing in newborn marsupials, resulting in a neonate with a limited respiratory performance that must rely to varying degrees on transcutaneous gas exchange (MacFarlane & Frappell, 2001). The immature structure of the newborn marsupial lung enables only a low metabolic rate without thermogenic capacity at birth (Loudon et al. 1985; Singer et al. 1995; Rose & Kuswanti, 2004). Marsupial juveniles effectively maintain a fetal metabolism after birth (Singer, 2004), supporting the suggestion of Zeller et al. (2004), who described the marsupial neonate as a 'body appendage' of the mother.

The lungs of the investigated placental neonates are more developed compared with monotreme hatchlings and marsupial neonates. The newborn *M. auratus* (14) (representing the atricial placental mammal neonate) is in the saccular stage of lung development. Saccules are small and more numerous than in the lungs of *O. anatinus* (M44) and *M. domestica* (337) (Fig. 3). The lung parenchyma of *M. auratus* (14) is highly vascularized and extensively subdivided. The conducting airways occupy a large portion of the lung

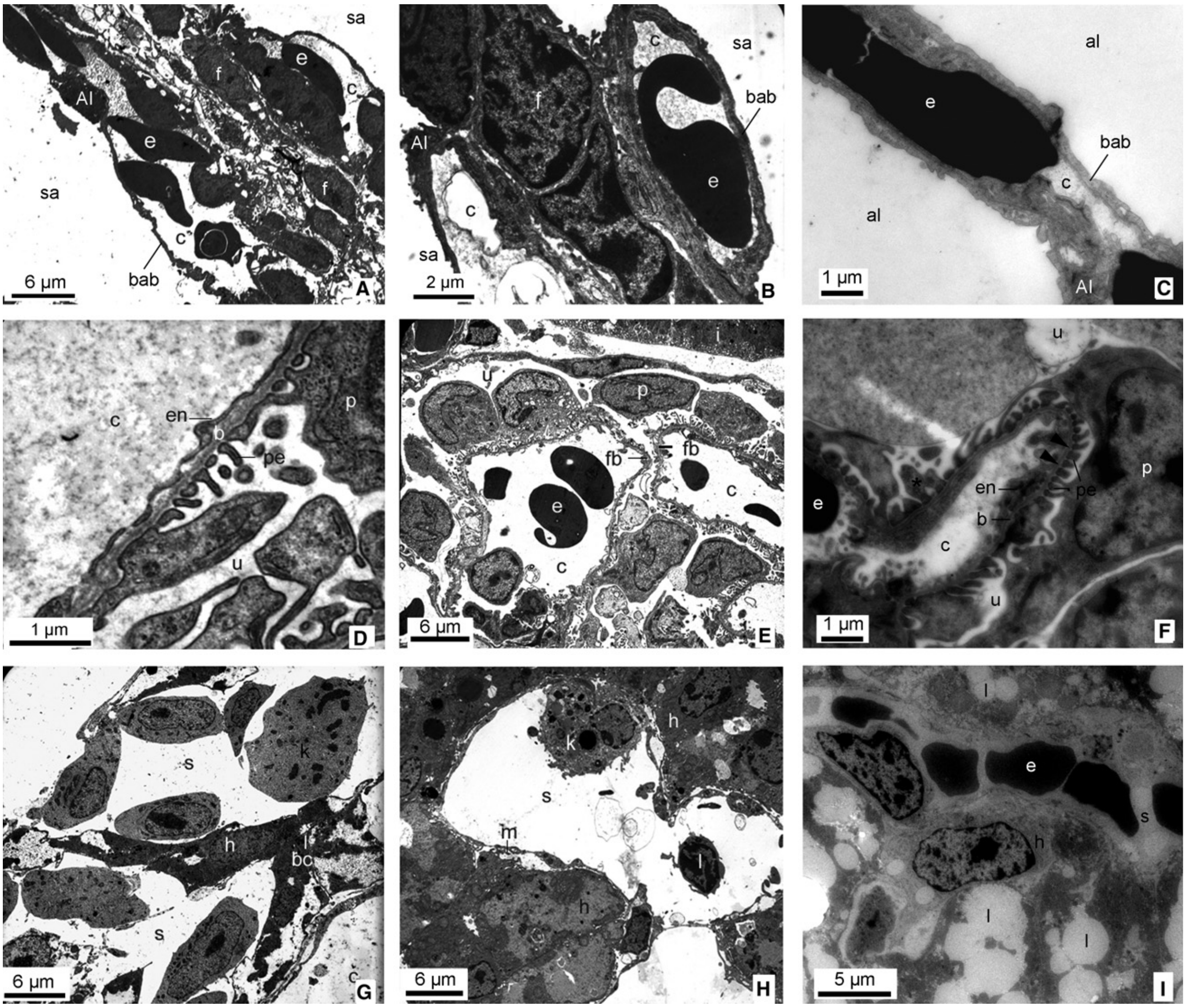

Fig. 4 Ultrastructure of lung, kidney and liver of newborn *Monodelphis domestica* (A, D, G), *Mesocricetus auratus* (B, E, H) and *Macroscelides proboscideus* (C, F, I). In the lung of the neonate marsupial *M. domestica*, an extensive double capillary network with a broad interstitium separates the large saccules (A). The lung of the altricial placental *M. auratus* is at the late saccular stage of lung development and the saccules are separated by a thin double capillary septum (B). The lung of the precocial placental *M. proboscideus* is already at the alveolar stage of lung development and possesses a single capillary septum, dividing adjacent alveoli (C). The kidney of the newborn *M. domestica* is a mesonephros with a simple but functioning filtration membrane in the renal corpuscles (D). In contrast, the filtration barrier of the metanephros of the altricial *M. auratus* is more advanced (E). In the precocial *M. proboscideus*, an advanced filtration system within numerous renal corpuscles is present in the metanephros; the filtration barrier shows structures typical for the adult kidney (F). Adjacent pedicles extending from the podocytes interdigitate with each other (asterisk). Thin filtration slits between pedicles are the final barrier for filtration (arrowheads). The liver parenchyma of the newborn *M. domestica* has a simple sinusoid system (G). In the liver parenchyma of the newborn *M. auratus*, the hepatocytes of the sinusoids are clearly more differentiated (H). The hepatocytes in the liver of *M. proboscideus* show numerous large spaces, sites where lipid was stored prior to its extraction during tissue preparation (I). al, alveole; AI; type I alveolar epithel cells; b, basement membrane; bab, blood–air barrier; bc, bile canalilculi containing microvilli; c, capillary; e, erythrocyte; en, endothelial cell; f, fibroblast; fb, filtration barrier; h, hepatocyte; i, basolateral folds with mitochondria; k, Kupffer cell; l, lipid droplets; m, microvilli; p, podocyte; pe, pedicel; s, sinusoid; sa, sacculus; u, urinary space. Magnification is indicated by the scale bar.

volume. The bronchial tubes reach deep into the periphery of the lung accompanied with many dichotomies. The terminal airways consist of terminal bronchioles only, respiratory bronchioles are missing. The epithelia of the airways flatten towards the periphery of the lung, forming straight and smooth-walled channels. The straight channels terminate in small saccules (~96 µm in diameter), which are separated from each other by a thin septum (thickness ~22 µm; Figs 3F and 4B). This septum in the lung of the *M. auratus* (41) neonate contains a network of capillaries, running on both sides of a central layer of connective tissue or mesenchymal cells. The blood–air barrier, separating incoming gases from the capillary blood in the saccules, is thin (mean thickness of blood–air barrier ~368 nm), and shows the typical trilaminar structure. Squamous type I pneumocytes and cuboidal type II pneumocytes line the respiratory surface of the small saccules. The type II pneumocytes contain lamellar bodies, producing surfactant. Surfactant also lines the surface of the saccules.

Altricial placental mammals have lungs at the late saccular period at birth. This developmental state can be seen in *M. auratus* (14), but also in Soricidae (Foresman, 1994; Szdzuy et al. 2008), *Erinaceus europaeus* (Engel, 1953), *M. musculus* (Ten Have-Opbroek, 1981) and *R. norvegicus* (Burri, 1974). The formation of alveoli induced by the septation process occurs primarily during early postnatal life (Szdzuy et al. 2008). The lungs are characterized by a well-developed bronchial system, which terminates in small saccules. The numerous small sacculi provide a large enough surface area for efficient gas exchange at birth. Thus, the altricial placental neonate can achieve a high metabolic rate (Szdzuy et al. 2008). Generally, the metabolic rate of placental newborns is usually higher than in adults of the same species, and even higher than the expected value for their body weight according to allometric relationships (Hayssen & Lacy, 1985). This pattern is found in numerous mammalian species (for review, see Mortola, 2001). The rise in the metabolic rate is presumably needed for the increased energy costs, required to maintain the growth of various tissues, such as brain, skeletal musculature and gastro-intestinal tract (Adamsons et al. 1969).

The lungs of the newborn *M. proboscideus* (9) (representing the precocial placental mammal neonate) are in the alveolar stage of lung development (Fig. 3G,H). The lung parenchyma is highly differentiated; respiratory bronchioles, alveolar ducts, alveolar sacs and numerous alveoli are present. The multi-branched bronchial tree extends deep into the periphery of the lung. The terminal airways consist of terminal and respiratory bronchioles. Squamous epithelium lines the long respiratory bronchioles. Numerous shallow cavities (alveoli) increase the surface area for gas exchange (Fig. 3H, arrowheads). The remarkable long respiratory bronchioles open via relatively short alveolar ducts into the alveolar sacs. Numerous small alveoli radiate from the center of the terminal alveolar sacs (alveolar size ~26 μm in diameter). Only mature single capillary septa exist in the lung of the newborn *M. proboscideus* (130) (Fig. 4C). Wide capillaries run in the center of each mature septum, or protrude alternating on both sides of the septum surface supported by a thin layer of connective tissue, forming a single-capillary network (septum thickness ~6 μm). The blood–air barrier shows the typical trilaminar structure (mean thickness of blood–air barrier ~303 nm).

The lungs of numerous placental mammal fetuses develop the alveolar stage *in utero* and show alveoli at birth (Table 4). Precocial neonate lungs have a well-developed bronchial system, terminating in respiratory bronchioles, alveolar ducts and alveolar sacs. The numerous small alveoli provide a large gas exchange surface area, needed for the high metabolic rate. In some precocial species the metabolic rate reaches adult levels within the first 24 h of life (Noblet & Le Dividich, 1981; Ousey et al. 1991).

In summary, the highly immature lungs of newly hatched monotremes and newborn marsupials enable a minimal maintenance of their own metabolism (but need support from transcutaneous respiration). No additional metabolic resources (e.g. for autonomous thermoregulation) can be provided. The more advanced lungs of the altricially born placentals enable an increase of the metabolic rate at least for a short time, and the nearly adult lung structure of the precocially born placentals allows an increase of the metabolic rate, if required (e.g. for thermoregulation).

Kidney

Like the lung, also mammalian kidneys follow a similar developmental trajectory; only the time frame with regard to birth varies between species (Zoetis & Hurtt, 2003).

Three different types of renal organ systems develop during the embryonic and fetal development in mammals. Pronephros and mesonephros exist in most cases for a defined period of intra-uterine development. As pronephros and mesonephros regress, the metanephros becomes the permanent adult kidney (Wintour et al. 1998). The metanephros develops sometime after the mesonephros, but for a certain period within gestation time meso- and metanephros co-function.

The *O. anatinus* hatchling (M44) shows both a mesonephros and a similar sized metanephros (Fig. 5A,B). The mesonephros has only a few renal corpuscles and shows signs of atrophy (Fig. 5A). In contrast, the metanephros has definitive glomeruli, collecting tubules and proximal tubules, and appears to be the functionally active excretory organ at hatching (Fig. 5B). Although the metanephros of the *O. anatinus* reveals a simple organization and nephrogenesis is not completed, filtration and urine production is functioning efficient enough for the small hatchling.

In the newborn marsupial *M. domestica* (337), the mesonephros is the only active organ of the excretory system (Fig. 5C,D). The metanephros is only recognizable as primordium and is non-functional at birth. The mesonephros of *M. domestica* (337) consists of collecting tubules for the transport of urine to the renal sinus. The mesonephric nephrons lack the characteristic loop of Henle in the newborn *M. domestica*. Renal corpuscles located in the peripheral region are surrounded by tubules. Thin layers of connective tissue separate the nephrons from each other. Each renal corpuscle consists of a glomerulus and a Bowman's capsule. Thin squamous epithelium composes the parietal layer of Bowman's capsule.

Two rows of cells with a microvilli brush border line the proximal nephrons (137). Inside a nephron run two capillaries separated by a urinary channel. The capillaries contain nucleated erythrocytes, indicating an immature hematopoietic system in the newborn marsupial. Cells of the distal nephrons show basal folds in the outer rim for ion transportation. The filtration barrier of the mesonephros is composed of endothelial cells of the capillary and podocytes of the corpuscle tissue on the opposite side (Fig. 4D). A very thin basement membrane is located between the endothelial cells and the pedicels of the podocytes. Water and solubles coming from the capillary space enter the urinary space between the pedicels through small slits in the endothelial barrier. The mesonephros has the ability to filter solubles from the blood, to produce urine to transport. Thus, the mesonephros of the marsupial neonate can be considered as an efficient excretion organ with ultrastructural components similar to that of the metanephric kidney.

A functional mesonephros at birth is amongst the most unique features of marsupials (Hughes & Hall, 1988). Investigations on *Didelphis virginiana* (Krause et al. 1979a;), *M. eugenii* (Renfree et al. 2001), *Macropus rufogriseus* (Müller, 1972), *Dasyurus viverrinus* (Hill & Hill, 1955), *Sminthopsis macroura* (Gemmell & Selwood, 1994) and *Trichosurus vulpecula* (Buchanan & Fraser, 1918) support this assumption. In marsupials, the mesonephros persists in a functional state for the first days (10 days in *D. virginiana*, 5–8 days in

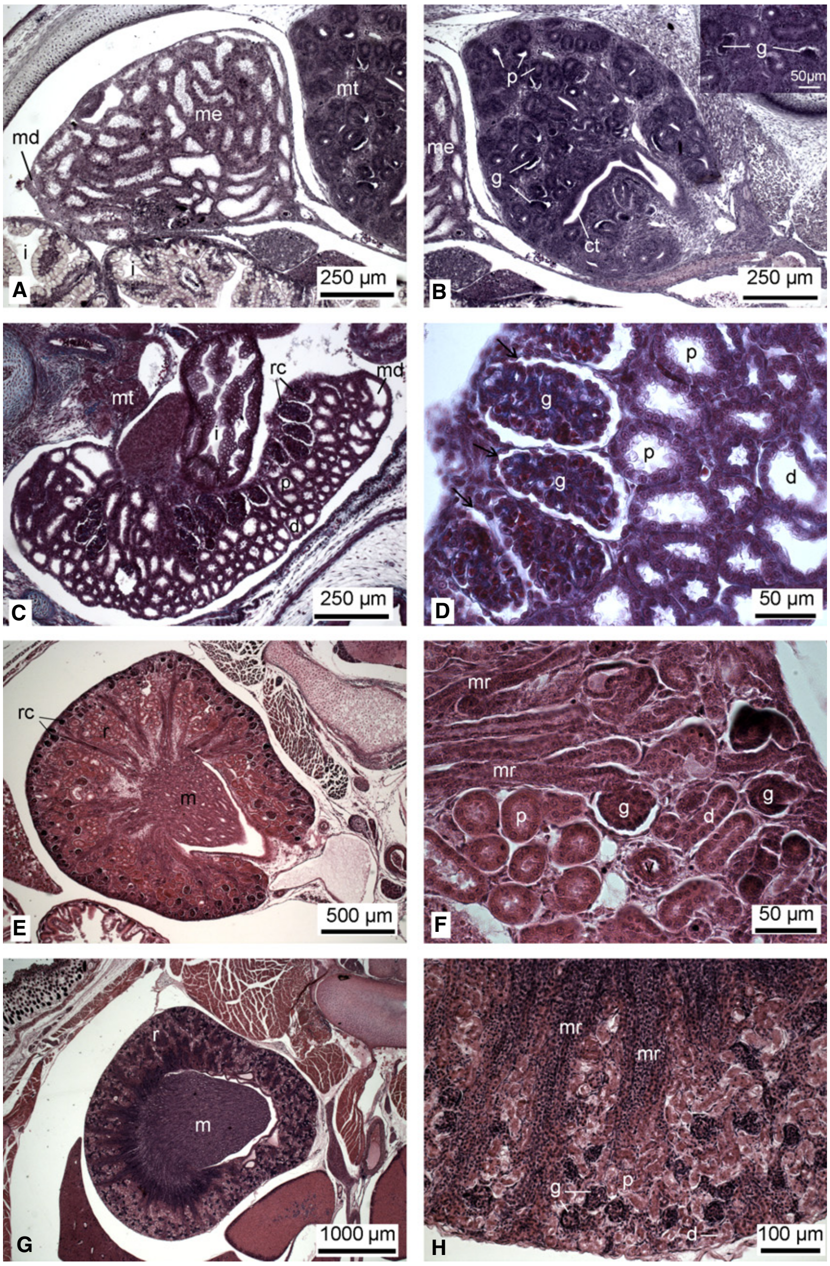

Fig. 5 Light micrographs of the kidney in a newly hatched *Ornithorhynchus anatinus* (A, B), and neonate *Monodelphis domestica* (C, D), *Mesocricetus auratus* (E, F) and *Macroscelides proboscideus* (G, H). In the monotreme hatchling and marsupial neonate a mesonephros is present and still functioning (A, C). The mesonephros of *M. domestica* consists of renal corpuscles (arrows) with glomeruli and numerous proximal and distal tubules (D). In the hatchling of *O. anatinus*, a developing metanephros, consisting of collecting tubules, developing proximal tubules and glomeruli, can be found (B). The well-developed metanephros of the altricial placental *M. auratus* shows already the typical structure of an adult kidney, renal corpuscles and convoluted vessels in the renal cortex and straight nephrons in the medulla (E, F). The precocial placental *M. proboscideus* has a highly differentiated metanephros with a clearly distinguished renal cortex and medulla (G, H). ct, collecting tubulus; d, distal tubulus; g, glomerulus; i, intestine; m, medulla; md, mesonephric duct; me, mesonephros; mr, medullary ray; mt, metanephros; p, proximal tubulus; r, renal cortex; rc, renal corpuscles; v, blood vessel. Magnification is indicated by the scale bar.

M. eugenii) of the postnatal period (Krause et al. 1979a; Wilkes & Janssens, 1988).

In *M. domestica*, the metanephros is primordial and nonfunctional at birth. However, in many marsupial species, the metanephric kidney develops close to the time of birth. Glomeruli have been noted at birth in the metanephros of *D. virginiana* (Krause et al. 1979b;) and *M. eugenii* (Wilkes & Janssens, 1988), on day 2 postpartum in *Isoodon macrourus* (Hall, 1987) and day 8 postpartum in *Dasyurus hallucatus* (Nelson, 1992). Although the mesonephric kidney of the marsupial neonate is functional to filter solubles and produces urine, the structural immaturity of the renal medulla hinders the ability to concentrate urine. Because the renal medulla lacks the countercurrent system of the loop of Henle, a medullary concentration gradient cannot develop and concentration of the urine is impossible (Wilkes & Janssens, 1988).

A mesonephros develops in many placental mammals during their intra-uterine development, but the degree of maturation and the duration of existence vary in different species. The mesonephros of *Ovis aries* (Tiedemann, 1976) exists for a similar period of gestation time to the *Homo sapiens* (Wintour, 1997), whereas the rodent mesonephros is present not only later in gestation but also exists for a shorter time period (Moritz & Wintour, 1999). However, all placental mammals lack a mesonephros at birth (Hughes & Hall, 1988). Excretion is performed in both placental neonates *M. auratus* (14) and *M. proboscideus* (9) by a metanephros.

The metanephros of the altricial placental *M. auratus* (14) is well differentiated in the cortex and medulla (Fig. 5). The renal corpuscles are surrounded by proximal and distal convoluted tubules and several blood capillaries (glomeruli) (Fig. 5F). The convoluted urinary vessels lead to the straight tubules of the collecting ducts. Two kinds of nephrons can be identified, the cortical nephron-system with a short loop of Henle extending to the medulla, and the medullary nephron-system with longer loops of Henle leading deep into the medulla (medullary rays). Short loops of Henle are common in altricial neonates lacking the thin ascending limbs (Schwartz et al. 1999). Because the loops of Henle function to concentrate and dilute urine, the lack of the ascending limb in altricial neonates prevents the excretion of highly concentrated urine, but the excretion of diluted urine is not difficult. The concentrating ability develops postnatally in *H. sapiens*, *R. norvegicus*, *Oryctolagus cuniculus* and *O. aries*, and prenatally in *Canis lupus familiaris* and *Cavia aperea* (Zoetis & Hurtt, 2003).

Basically, the metanephros of the newborn *M. auratus* (14, 41) shows structures similar to those found in adults. The typical filtration barrier with a well-developed basement membrane is present inside the corpuscles (Fig. 4). In comparison to the mesonephridial filtration barrier of *M. domestica* (137), the metanephridial filtration barrier of *M. auratus* (41) is more advanced. One row of podocytes lines the filtration barrier, and their relatively long pedicels are supported by a core of actin filaments. Plasma passes from the vascular into the urinary system through small slits in the diaphragm. Distal nephrons show basolateral folds associated with mitochondria for ion transport, and the proximal nephrons were lined with a brush border of microvilli.

The most advanced metanephros shows the precocial newborn of *M. proboscideus* (9) (Fig. 5G). The cortex contains convoluted nephrons and renal corpuscles with blood capillaries surrounded by the renal capsules. Among the twisted nephrons, long straight tubules of the loop of Henle (medullary rays) extend down into the medulla. They can be differentiated into proximal and distal tubules and collecting ducts with wide lumens (Fig. 5H). Each nephron leads to a collecting duct, which joins with other collecting ducts in the renal pelvis. The length and large volume of the tubules of the loops of Henle allow higher capacities for transport and metabolism (Bernstein, 1991). Large arterial blood vessels supply the organ with oxygen. The newborn *M. proboscideus* kidney (130) is in an advanced developmental stage and shows several adult structures (Fig. 4F). The cortex is scattered with numerous renal corpuscles. This is the place for the initial process of filtration. The glomerulus is in close contact with the initial portion of the nephron (Bowman's capsule) and consists of a tuft of small capillaries. Plasma components are passed from the glomerular capillaries to the urinary space in the process of filtration. The visceral layer of the Bowman's capsule consists of podocytes, which form cytoplasmic extensions that branch into small processes (pedicles) resting on the glomerular basement membrane. The pedicles of adjacent cells interdigitate with one another (asterisk, Fig. 4F). Thin diaphragms traverse the space between adjacent pedicles. Filtration slits of the diaphragms are the final barrier that the plasma encounters after crossing the endothelium and glomerular basement membrane (arrowheads, Fig. 4F).

The precocial neonate produces and concentrates urine effectively supporting higher metabolic rates. Urinary concentration permits the maintenance of body water level under restricted water intake and allows the excretion of waste materials with minimal loss of water. This can be interpreted as an advantage with regard to the semiarid environment in which *M. proboscideus* lives.

In summary, marsupials are born with a functional mesonephros, which produces only dilute urine. The absence of concentrated urine in young marsupials may lead to dehydration. Structural maturity of the metanephric kidney at birth and the rate of nephrogenic maturation vary between placental neonates. Altricial placental neonates are incapable of excreting concentrated urine at birth like newborn marsupials, and are in danger of dehydration. Precocial placental neonates produce concentrated urine at birth to avoid dehydration shortly after.

Liver

The liver is the largest internal organ in neonates responsible for critical functions including: regulation of blood/glucose levels; production of serum proteins (i.e. clotting factors); bile synthesis; biotransformation of xenobiotics and other endogenous byproducts of the metabolism; and metabolism of dietary compounds (for review, see Grijalva & Vakili, 2013). The liver is also the main heat source due to the metabolic processes inside, supporting the thermoregulation of the neonate (Bücker, 1992).

The well-developed liver parenchyma shows the typical sponge-like appearance in the newly hatched *O. anatinus* (M44). A simple sinusoid system penetrates the densely-packed hepatocytes transporting nucleated erythrocytes. Several large capillaries supply the sinusoid system with blood (Fig. 6A). Anastomosing rows and plates of hepatocytes are separated by small hepatic sinusoids, which converge on central veins (Fig. 6B).

Typical adult liver structures (e.g. periportal fields with vena interlobularis, arteria interlobularis, bile duct) are lacking in the liver parenchyma of the newborn marsupial *M. domestica* (337) (Fig. 6C,D). Cell rows of hepatocytes, characteristic for the adult sinusoid system, are not very distinctive in the neonate liver parenchyma of *M. domestica* (137) (Fig. 4G). Blood vessels and capillaries are lined with

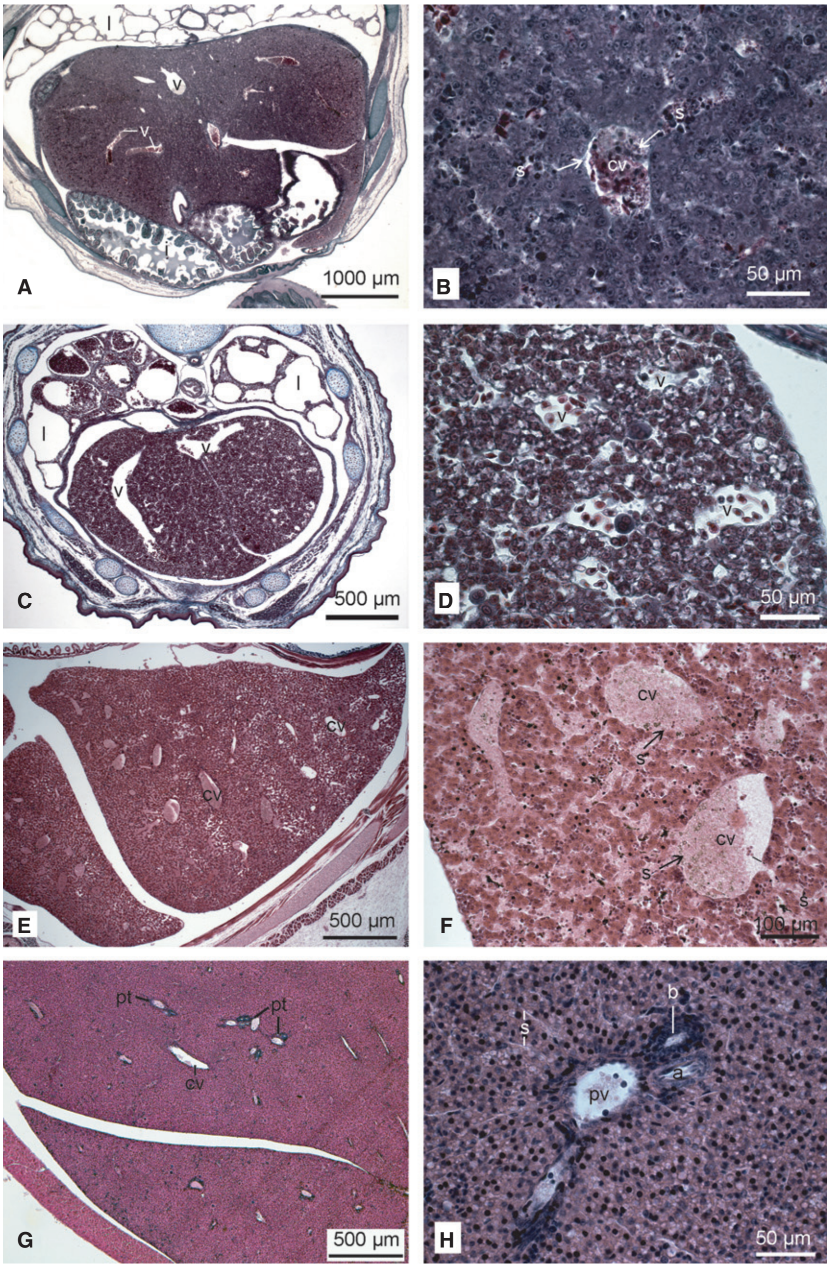

Fig. 6 Light micrographs of the liver in a newly hatched *Ornithorhynchus anatinus* (A, B), and neonate *Monodelphis domestica* (C, D), *Mesocricetus auratus* (E, F) and *Macroscelides proboscideus* (G, H). The poor-developed liver parenchyma of the marsupial *M. domestica* appears undifferentiated and is characterized by large blood vessels (C, D). In the monotreme *O. anatinus*, the liver parenchyma looks more differentiated, and typical liver structures, such as central veins and scattered sinusoids, can be found (A, B; arrows indicate sinusoid flow). The liver of the altricial placental *M. auratus* shows an advanced sinusoid system converging on central veins (E, F). The liver of the precocial *M. proboscideus* is characterized by typical structures of the adult liver. Besides a highly differentiated sinusoid system and central veins, periportal fields (portal triad) that consist of a portal venule, hepatic arteriole and a bile duct can be found (G, H). a, hepatic arteriole; b, bile duct; cv, central vein; i, intestine; pt, portal triad; pv, portal venule; s, sinusoid; v, blood vessel. Magnification is indicated by the scale bar.

endothelial cells. Beside erythrocytes, the capillaries transport Kupffer cells, specialized macrophages that are abundant in the liver. Small bile canaliculi are found between adjacent hepatocytes. The hepatocytes contain only a few stored glycogen droplets. The endoplasmic reticulum of the hepatocytes synthesize enzymes required for lipid breakdown, which are stored inside the hepatocytes.

The liver structures of the hatchling of *O. anatinus* (M44) and the neonate *M. domestica* (337, 137) exhibit histological similarities to findings in the newborn *D. virginiana* (Block, 1964), *Marmosa robinsoni* (Bryant & Shifrine, 1974), *I. macrourus* (Cisternas & Armati, 1999), *S. macroura* (Old et al. 2004), *Setonix brachyurus* (Ashman & Papadimitriou, 1975) and *M. eugenii* (Basden et al. 1996). In contrast to placentals, where the liver ends the hematopoietic function prior to birth, the marsupial liver is the main site of hematopoiesis at birth and during the early postnatal life (for review, see Borthwick et al. 2014). Generally, in all marsupial species hematopoietic tissue is present at birth. It increases during the first days and then gradually decreases during the second week postpartum (Cisternas & Armati, 1999). The neonate and early pouch young marsupial liver shows relatively high enzyme activity. This catalyzes the formation of glucose-1-phosphate from galactose, essential for the conversion to glucose or glycogen, enabling the neonate to digest the milk with high galactose content (Janssens & Messer, 1988).

The production of bile is important for the digestion of lipids. The newborn *M. domestica* (137) shows small bile canaliculi between the rows of hepatocytes in the parenchyma, even in this immature state bile is produced for digestion of milk.

Compared with the marsupial neonate, a more differentiated liver parenchyma is present in both placental neonates at birth. However, differences in the structural maturity of the liver were observed between altricial and placental mammals.

Primitive periportal fields are present in the liver parenchyma of the newborn *M. auratus* (14, 41), consisting of two blood vessels and a small bile duct. A well-developed sinusoid system passes through the rows of hepatocytes and converges on central veins (Fig. 6,F). Several large capillaries supply the sinusoid system. Hepatocyte tissue surrounds the sinusoids interspersed with bile canaliculi that contain small microvilli (Fig. 4H). The apical parts of the hepatocytes, lining the sinusoids, also show microvilli. Glycogen is stored in droplets inside the cell arranged in large rosettes. Kupffer cells and lymphocytes are frequently present in the sinusoid system. Occasionally, the Kupffer cells coalesce with hepatocytes for anchoring inside the sinusoid.

Structural changes of the liver parenchyma (metabolic zonation), associated with the alteration of the nutritional state, were observed by Katz et al. (1976) in the newborn *M. auratus*. Prior birth glucose utilized from the maternal blood is stored as the main energy substrate, visible in the *M. auratus* (41) neonate as large rosettes of glycogen droplets. The glucose supply from the maternal blood is suddenly interrupted with birth. Maintenance of the glucose supply depends now on glycogenolysis of the limited hepatic glycogen stores in the liver until other energy substrates are offered by fat- and protein-rich but carbohydrate-poor maternal milk (Grijalva & Vakili, 2013).

Fatty acid metabolism provides another significant energy source for the neonate during the early postnatal period. Elevated lipolysis shortly after birth provides non-esterified fatty acids for oxidation and ketone body synthesis, which may provide up to 25% of the neonate's basal energy during the first few days of life (Hawdon et al. 1992).

Fully developed periportal fields are present in the liver of the newborn *M. proboscideus* (9, 130), clearly showing a bile duct in the center (Fig. 6G,H). The hepatocytes sit radially around the vena centralis. A well-defined sinusoid system branches through the liver parenchyma and transports blood from the larger blood vessels to the periportal fields. Numerous large spaces within the hepatocytes can be seen, sites of lipid storage, which got dissolved during tissue preparation (Fig. 4I).

Higher structural maturity and advanced lipid storage capacity in the liver parenchyma of the precocial neonate *M. proboscideus* suggest that precocial species have additional resources available for autonomous energy supply directly after birth. In another precocial species, *C. aperea*, a marked glycogenolysis occurs at birth, resulting in the complete loss of liver glycogen within a period of the first 10 h. It is followed by a marked reduction of liver fat concentrations until adult levels are reached within the first 3–4 days (Hard et al. 1944).

Although the basic liver development is similar in marsupials and placentals, the timing of structural changes differs. As pointed out, the liver is an essential hematopoietic component in the marsupial neonate. In comparison, in the placental neonate, the liver has stopped its hematopoietic role prior to birth. The liver undergoes dynamic changes during the neonatal period. Immediately after birth, the liver has a vital key role in glucose and fatty acid metabolism, to ensure the continuous supply of energy as the neonate adapts to autonomous feeding.

Skin structure and thermoregulatory abilities

Generally, the basic skin structure is very similar in the four investigated neonates; however, differences in thickness and development could be observed. The integument basically consists of two main layers, epidermis and dermis. The thin skin of the newly hatched *O. anatinus* (M44) and the newborn *M. domestica* (337) is structurally similar and differs only in thickness (75.3 ± 4.6 μm in *O. anatinus* and 63.2 ± 2.2 μm in *M. domestica*; Fig. 7A,B). The epidermis is a thin layer of stratum corneum composed of squamous keratinized cells and the stratum basale with 1–2 rows of columnar and rounded cells. The underlying layer is the dermis consisting of loose connective tissue. Superficial subcutaneous capillaries are running close to the epidermis. The diffusion distance (distance between the external surface and most superficial capillaries) measures 38.5 ± 1.4 μm in the neonate *M. domestica* and 46.9 ± 2.2 μm in the newly hatched *O. anatinus* (M44). Dermal papillae, sweat glands or hair follicles are absent. In the adult *O. anatinus*, sweat glands are present and generously scattered over the snout of the *O. anatinus.* In addition, one apocrine gland opens into the follicle of each of the large hairs (Robinson, 1954). Adult marsupials have sweat glands as well (Green, 1963). Hence, the lack of sweat glands in the *O. anatinus* hatchling and *M. domestica* neonate can be explained by developmental differences related to the young age.

The extremely small body sizes of monotreme hatchlings and marsupial neonates expose them to hypothermia and potential desiccation. The very thin translucent skin provides almost no protection from water and heat loss. However, adult monotremes and marsupials evolved strategies to establish a humid and warm environment for their neonates. For example, the monotreme *T. aculeatus* and most marsupial species raise their offspring in a pouch, which offers a thermally stable and humid environment. The monotreme *O. anatinus* does not have a pouch; eggs and hatchlings are held between the broad flat tail and the abdominal surface to provide shelter and warmth. The muzzle of the curled-up mother is overlapped by the tail and warm humid air is expired into the belly area, providing a warm and humid environment for incubation of the small young (Dawson, 1983). A similar curled-up position is applied from time to time by pouch-less marsupials where the young are firmly attached to the teat, like *M. domestica* or *M. robinsoni*. Marsupial and monotreme young are ectothermic when born. Hence, it is necessary that they develop in a relatively stable environment with constant temperature close to the body temperature of the mother. Hair growth starts with gradual attainment of thermogenic capacity, and by the time full thermogenic capacity is achieved a complete fur is present (Hulbert, 1988).

Large surface area to volume ratios coupled with small body sizes favor cutaneous gas exchange for at least a short period of time in marsupial neonates (Simpson et al. 2011). In the newborn *M. eugenii*, cutaneous exchange accounts for approximately one-third of total oxygen uptake (MacFarlane & Frappell, 2001), while the smaller *Sminthopsis douglasi* (birth weight 17–20 mg) relies entirely on cutaneous exchange at birth, with the onset of pulmonary ventilation being delayed for a number of days (Frappell & Mortola, 2000). The epidermis of the

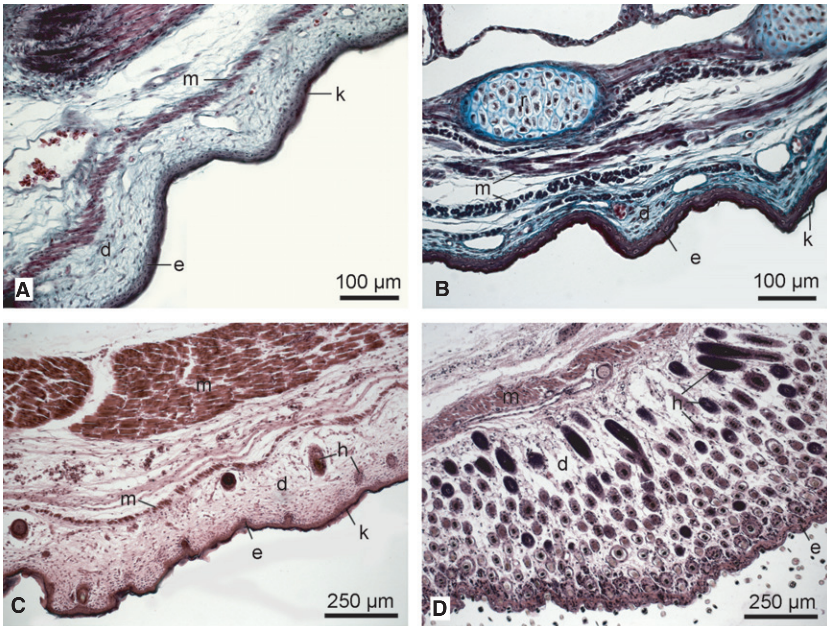

Fig. 7 Light micrographs of the skin in a newly hatched *Ornithorhynchus anatinus* (A), and neonate *Monodelphis domestica* (B), *Mesocricetus auratus* (C) and *Macroscelides proboscideus* (D). The skin of all four investigated species is similar in structure and differs mainly in thickness. The skin contains two major components, the epidermis with a thin superficial layer of keratin, and the thicker dermis, an underlying layer of connective tissue. In the monotreme hatchling and the newborn marsupial, both components are comparatively thin and no hair follicles ca be found (A, B). In the altricial placental *M. auratus*, first hair follicles and glands can be recognized in the dermis, but the epidermis is not penetrated by hair (C). The skin of the precocial placental neonate *M. proboscideus* is densely packed with hair follicles that penetrate the epidermis (D). Melanocytes can be observed in the cell layer that caps the follicles. d, dermis; e, epidermis; h, hair follicle; k, keratin; m, muscle. Magnification is indicated by the scale bar.

O. anatinus (M44) hatchling and *M. domestica* (337) neonate is poorly developed with a thin layer of keratin and no hair follicles, making transcutaneous gas exchange possible. Cutaneous diffusion of oxygen can reach as deep as 250–400 μm (Makanya et al. 2007). Therefore, the average diffusion distances (distance between dermal capillaries and external surface) of ~40 μm in *M. domestica* (337) and ~47 μm in *O. anatinus* (M44) are suitable for gaseous exchange.

The skin structure of the neonate *M. domestica* (337) is very similar to that found in neonates of other marsupial species (Gibbs, 1938; Krause et al. 1978; Pralomkarn et al. 1990; Makanya et al. 2007). The striking skin erythema of newborn marsupials and monotremes is very likely the result of the transparency of the thin epidermis; fewer collagen fibers in the dermis result in poorer light scattering (Baudinette et al. 1988).

The skin of the altricial newborn *M. auratus* (14) measures 242.6 ± 6.3 μm in thickness. Few hair follicles sweat and sebaceous glands are present in the dermal tissue (Fig. 7C). The epidermis consists of a top layer of squamous keratinized cells (stratum corneum) and a well-defined basal layer with 2–3 rows of columnar and rounded cells (stratum basale).

The small size of altricial placental neonates endangers them to hypothermia and desiccation like marsupial neonates. Because the internal organs are further developed, they support the maintenance of vital functions and a short metabolic increase if required (e.g. for the time the juvenile is left in the nest). However, this is always at the expense of growth (Hill, 1992).

Most altricial neonates completely depend on their mother for body temperature and nourishment. They have a poor thermoregulation, poor insulation and limited heat production ability (Harjunpää & Rouvinen-Watt, 2004). For example, *M. auratus* shows no endogenous thermoregulatory response to cold temperatures until 7–10 days old (Hissa, 1968). The main mechanisms for producing heat in very small altricial newborns are non-shivering thermogenesis (via brown adipose tissue) and behavioral mechanisms (huddling with nest-mates, seeking a warmer place or calling for their mother; Bryant & Hails, 1975; Sundin & Cannon, 1980; Withers & Jarvis, 1980; Asakura, 2004). Achievement of full thermogenic capacity correlates with the development of insulating fur in altricial species (Szdzuy, 2008).

The integument of the newborn *M. proboscideus* (9) appears to be the most advanced in development (thickness 605.0 ± 22.1 μm) of all four investigated neonates. Numerous hair follicles associated with sweat and sebaceous glands (Fig. 7D) are present in the massive dermal layer. Melanocytes can be identified within the dermis. Hair follicles of epidermal origin deeply penetrate into the dermis. Hair pierces through the epidermal surface and covers the animal in dense fur.

Because of their fur and rapid growth as well as their thermoregulatory abilities, precocial neonates respond to cold by increasing heat production from very early on in life (Hart et al. 1961; Le Dividich & Noblet, 1981; Waldschmidt & Müller, 1988; Hackländer et al. 2002). A useful strategy is lowering thermal conductance by means of effective insulation. Neonates of cold-adapted species developed

Table 2 Summary of histological and ultrastructural findings for selected organ systems of the four species examined.

Structure	*Ornithorhynchus anatinus*	*Monodelphis domestica*	*Mesocricetus auratus*	*Macroscelides proboscideus*
Respiratory system	Medium to large terminal saccules, short bronchi end in saccules, wide double capillary septum	Large terminal saccules, saccules branch off directly from the main bronchus, wide double capillary septum	Numerous small saccules, differentiation in dichotomies and fine bronchial tubes, small double capillary septum	Alveoli, differentiation in many dichotomies and long respiratory bronchioles, single capillary septum
Excretory system	Transition period from meso- to metanephros, metanephros exhibits a few definitive glomeruli	Functioning mesonephros with few small glomeruli, similar ultrastructural components as a metanephros	Metanephros in a low developmental stage, glomeruli with the typical filtration system	Large metanephros with an advanced filtration and transportation system, many glomeruli in the periphery
Liver parenchyma	Simple sinusoid system with central veins and thin sinusoid channels	Simple cell rows as a sinusoid system, few large blood vessels, small bile canaliculi between the hepatocytes	Sinusoid system with simple periportal fields and many larger blood vessels, stored glycogen and lipids	Advanced parenchyma with periportal fields and differentiated sinusoid system, substantial lipid storage in hepatocytes
Skin development	Thin, without hair follicles, dermal capillaries		Hair follicles and glands present in dermis	Well-developed hair follicles penetrating the epidermis, fur
Locomotory system	Distinctive muscles in the forelimbs and at the back, cartilaginous skeleton		Muscular extremities, first ossifications in the ribs and the vertebrae	Fully developed muscular system and ossifications in the whole skeleton

additional strategies to prevent heat loss. For example, a thick layer of subcutaneous blubber provides insulation in most aquatic mammals (Vaughan et al. 2000). Newborn *Lepus europaeus* use peripheral vasoconstriction to reduce heat loss in the cold (Hackländer et al. 2002). In larger precocial newborns, both non-shivering and shivering thermogenesis can be found (Brück & Wünneberg, 1966).

However, precocial development is characterized by high maintenance costs because of rapid growth and thermoregulation, which may lead to negative energy balances when food supply is restricted and ambient temperatures are low (all results are summarized in Table 2).

The mammalian neonate morphotype

Outgroup comparison to non-mammalian amniotes, in which oviparity is plesiomorphic (Ferner & Mess, 2011), supports the theory that for the mammalian morphotype oviparity is plesiomorphic as well. Monotremes and marsupials share the ‘early hatching’ of the embryo from the egg, extra-uterine in monotremes or intra-uterine in marsupials (Tyndale-Biscoe & Renfree, 1987). Furthermore, the yolk elimination and resorption of yolk from the blastocyst cavity during early development in marsupials corresponds to the situation found in monotremes (Zeller, 1999). This supports the view that newborns of the common ancestor of mammals also hatched from an egg, similar to extant monotremes. The question about the developmental degree of the ancestral mammalian hatchling remains.

The morphotype of the non-avian sauropsid hatchling was probably precocial, as today lizards, snakes, crocodiles and turtles are. They hatch from the egg as miniature replications of the adults, with open eyes, ossified skeleton, able to coordinated locomotion, the chambered lungs are fully developed, they have a well-developed metanephros and they are scaled, but ectothermic. There is still a controversy about the evolution of the altricial–precocial spectrum in birds. However, it is most likely that the morphotype of Aves was precocial, and altriciality is a derived character in the higher taxa of birds (Starck & Ricklefs, 1998). Within the mostly altricial Neoaves, precocity arose secondarily and independently in several taxa (e.g. Ciconiiformes, Gruiformes). The comparison to these outgroups suggests that also the morphotype of mammals was precocial, and altriciality evolved in monotremes, marsupials and some eutherian taxa independently. Although this theory is possible, our knowledge of the anatomy and lifestyle of Mesozoic mammals argues for an altricial mammalian morphotype.

Mesozoic mammals were remarkably similar in structure and level of organization. They were very small, 10–15 cm long and 20–30 g in body weight. Features of the axial skeleton and limbs are still typical of small marsupials and placentals alive today, which are arboreal or forage in forest litter (Tyndale-Biscoe & Renfree, 1987). The small size may have imposed constraints on reproduction. A consequence of homeothermy and small adult size would have been the production of altricial young (to avoid high energetic costs for an endothermic infant; Hopson, 1973). Alternatively, with the production of an independent homeothermic (precocial) offspring, the egg would have been inordinately large, with a large amount of non-thermogenic yolk, which would have imposed a serious energetic burden on the homeothermic female (Case, 1978). Also, there might have been anatomical constraints of the pelvic canal, favoring the production of small eggs and resultant altricial offspring (Tyndale-Biscoe & Renfree, 1987). It is likely that a certain degree of parental care predated the development of both lactation and altriciality. The close similarity of mammary glands and milk secretion in all three groups of living mammals suggests that these are shared derived (synapomorphic) characters (Griffiths, 1978). There are many similarities in several aspects of reproduction between small marsupials (e.g. opossums) and small placental mammals (e.g. shrews) reflecting constraints imposed by adult size. Therefore, it is reasonable to suppose that Mesozoic mammals, which were likewise small, displayed the same reproductive characteristics of small mammals today: polyoestry, polyovulation, short gestation and extremely altricial offspring (Tyndale-Biscoe & Renfree, 1987).

To reconstruct the morphotype of the mammalian neonate, it is necessary first to reconstruct the morphotypes of the monotreme hatchling, and the marsupial and placental neonates, respectively. The morphotypes of monotremes and marsupials seem to be non-controversial, as both groups have only embryonic, highly altricial offspring. A more difficult situation arises in placental mammals, as the developmental degrees of neonates are more variable, ranging from altricial (semi-precocial) to highly precocial. There is consensus that marsupial and placental mammals share a common ancestor that had most probably altricial offspring (Lillegraven, 1975; Tyndale-Biscoe & Renfree,

Table 3 Characters and character states used for reconstruction of the stem species pattern of the mammalian neonate.

No.	Character at birth	Character state 0 (plesiomorphy)	Character state 1 (apomorphy)
1	Neonate weight	Low (< 1 g)	High (> 1 g)
2	Eyes	Closed	Open
3	Ears	Closed	Open
4	Skeletal composition	Cartilaginous	Ossified
5	Locomotor activity	Limited	Present
6	Lung development	Canalicular/ saccular	Alveolar
7	Kidney development	Mesonephros	Metanephros
8	Liver development	Periportal fields absent	Periportal fields present
9	Hairs, pelage	Absent	Present
10	Thermoregulation	Absent	Present

Table 4 Distribution of character states among mammals used for morphotype reconstruction of the mammalian neonate (based on literature data; own results are highlighted).

	1	2	3	4	5	6	7	8	9	10	Source
Monotremata											
Ornithorhynchidae	**0**	**0**	**0**	**0**	**0**	**0**	**0/1**	**0**	**0**	**0**	Griffiths (1978), Grant (1995), Ferner et al. (2009)
Metatheria											
Didelphidae	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	Krause & Leeson (1975), Krause et al. (1979a), Szdzuy (2008), Szdzuy & Zeller (2009)
Peramelidae	0	0	0	0	0	0	0	0	0	0	Gemmell & Little (1982), Gemmell & Johnston (1985), Gemmell (1986), Hughes & Hall (1988)
Dasyuridae	0	0	0	0	0	0	0	0	0	0	Hill & Hill (1955), Geiser et al. (1986), Gemmell & Nelson (1988), Gemmell & Selwood (1994), Frappell & Mortola (2000)
Phalangeridae	0	0	0	0	0	0	0	0	0	0	Hughes & Hall (1984), Gemmell et al. (1987), Buaboocha & Gemmell (1997)
Macropodidae	0	0	0	0	0	0	0	0	0	0	Setchell (1974), Walker & Gemmell (1983), Loudon et al. (1985), Wilkes & Janssens (1988), Runciman et al. (1996), Rose & Kuswanti (2004), Szdzuy et al. (2008)
Eutheria											
Afrotheria											
Elephantidae	1	1	1	1	1	1	1	?	1	1	McKay (1973), Gaeth et al. (1999), West et al. (2003)
Macroscelidae	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	Perrin (1995), Szdzuy (2008)
Laurasiatheria											
Soricidae	1	0	0	1	0	0	1	1	0	0	Nagel (1989), Foresman (1994), Szdzuy et al. (2008)
Erinaceidae	1	0	0	1	0	0	?	?	0	0	Engel (1953), Müller (1972)
Megachiroptera[a]	1	1	1	1	1	1	1	?	1	1	Patil & Janbandhu (2011), Patil (2013)
Microchiroptera[b]	1	0	0	1	0	?	1	?	0	0	Kleiman (1969), Patil (2013)
Felidae	1	0	0	1	0	1	1	?	1	0	Hull (1965), Hemmer (1976), Olmstead et al. (1979), Mercurio & Rhodin (1984), Knospe (2002)
Canidae	1	0	0	1	0	1	1	1	1	0	Boyden & Tompsett (1961), Hay & Evan (1979)
Pinnipedia[c]	1	1	1	1	1	1	?	?	1	1	Engel (1953), Bryden (1972), Denison & Kooyman (1973), Blix et al. (1979), Whittow (1987), Miller et al. (2005)
Mustelidae	1	0	1	1	0	1	?	?	0	0	Denison & Kooyman (1973), Harjunpää & Rouvinen-Watt (2004),
Equidae	1	1	1	1	1	1	1	?	1	1	Engel (1953), Alexander (1975), Maloney (1984)
Suidae	1	1	1	1	1	1	1	?	1	1	Eisenberg (1983), Moritz & Wintour (1999), Alexander, (1975), Winkler & Cheville (1985), Mortola (2001)
Bovidae	1	1	1	1	1	1	1	?	1	1	Alexander (1975), Blix et al. (1984), Castleman & Lay (1990), Moritz & Wintour (1999)
Cetacea[d]	1	1	1	1	1	1	1	?	1	1	Bryden (1972), Drabeck & Kooyman (1983), Whittow (1987)
Euarchontoglires											
Tupaidae	1	0	0	1	0	1	1	1	0	?	Hertenstein et al. (1987), Szdzuy et al. (2008), Ferner et al. (2010)
Cercopithecidae	1	1	1	1	0	1	1	1	1	1	Quattropani et al. (1974), Hislop et al. (1984), Dettmer et al. (2007), Batchelder et al. (2010)
Hominidae	1	1	1	1	0	1	1	1	1	1	Weibel (1970), Zeltner & Burri (1987), Eisenberg (1983), Moritz & Wintour (1999)
Leporidae	1	0/1	0/1	1	0/1	1	1	1	0/1	0/1	Lewis (1958), Leeson & Cutts (1972), Snyder & Magliato (1991), Hull (1965), Hackländer et al. (2002)
Muridae	**1**	**0**	**0**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	Burri (1974), Weibel (1970), Katz et al. (1976), Ten Have-Opbroek (1981), Eisenberg (1983), Moritz & Wintour (1999), Mortola (2001)
Cavidae	1	1	1	1	1	1	1	1	1	1	Weir (1974), Adamsons et al. (1969), Szdzuy et al. (2008), Mortola (2001)

[a] Data from Pteropodidae, Megadermatidae.

[b] Data from Vespertilionidae, Hipposideridae.

[c] Data from Phocidae, Otariidae, Odobenidae.

[d] Data from Delphinidae, Phocoenidae, Physeteridae, Eschrichtiidae.

Character state 0: plesiomorpy; character state 1: apomorphy. For character coding, see Table 3.

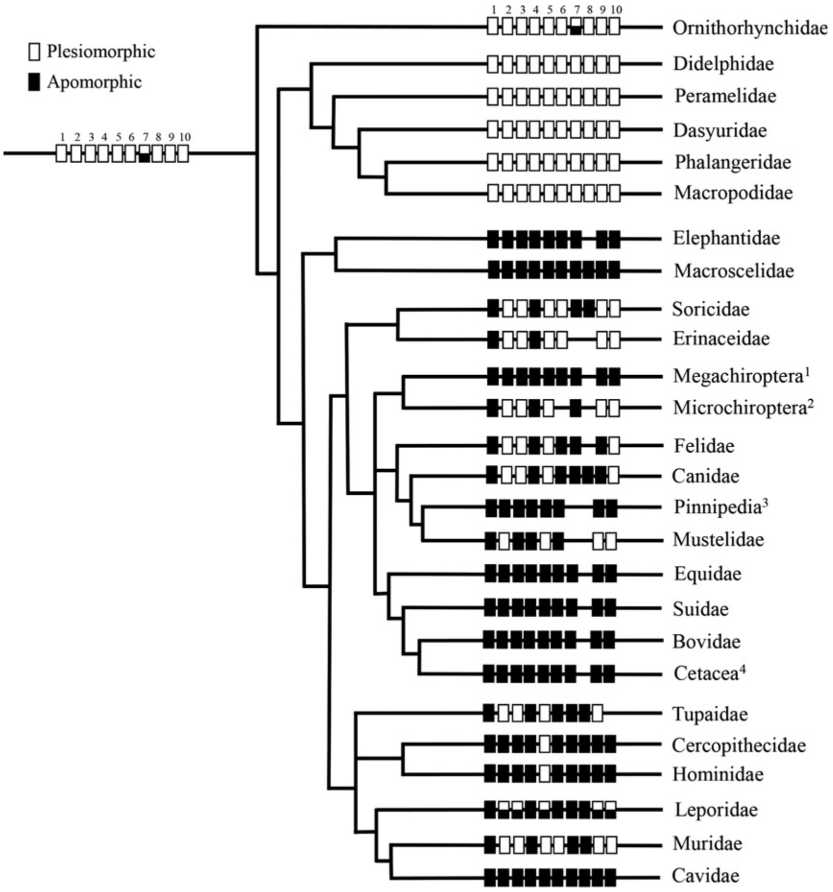

Fig. 8 Reconstruction of the neonate in the mammalian morphotype. For character coding, see Table 3. The numbers above the rectangle are character numbers. Plesiomorphic characters appear white, while apomorphic characters are marked black. [1]Megachiroptera data from Pteropodidae, Megadermatidae; [2]Microchiroptera data from Vespertilionidae, Hipposideridae; [3]Pinnipedia data from Phocidae, Otariidae, Odobenidae; [4]Cetacea data from Delphinidae, Phocoenidae, Physeteridae, Eschrichtiidae.

1987; Blackburn, 2015). Based on this therian morphotype, there are two alternative scenarios: (1) placental mammals transformed to a precocial neonate and from this altriciality re-evolved in nearly all mammalian major lineages independently (two transformations); or (2) the neonate of the placental morphotype was altricial, similar to the therian morphotype, and evolution to precociality occurred in multiple clades (one transformation). Since scenario (1) involves more transformations than scenario (2), it is less parsimonious. Further evidence for an altricial placental morphotype comes from the mammalian phylogeny. Some phylogenetic old mammalian orders like Afrosoricida (e.g. Tenrecidae), Scandentia (Tupaidae) and Eulipotyphla (Soricidae, Erinaceidae) produce neonates in an altricial state, what argues for a placental morphotype with an altricial neonate (Szdzuy & Zeller, 2009). Placental orders with highly precocial offspring (e.g. Perrissodactyla, Cetartiodactyla) diversified much later (Bininda-Emonds et al. 2007). With the changing climates at the beginning of the Tertiary, major adaptive radiations of mammals occurred. These radiations were accompanied by an increase in body size of species in many placental orders (Tyndale-Biscoe & Renfree, 1987). We already referred to the constraints that small body size imposes on reproduction. Conversely, large size correlates with increased gestation length, reduced litter size, proportionally reduced maternal investment and with the delivery of precocial young. Thus, it is reasonable to assume that precociality spread during the adaptive radiations of placental mammals and was not present in the placental morphotype.

Considering the morphotypes of Marsupialia (altricial) and Placentalia (altricial), with an outgroup comparison to Monotremata (altricial), the therian morphotype would have been altricial. There is still the question if viviparity evolved before the split of marsupials and placentals or in each group independently. Today, there is consensus that therians are derived from a single origin of viviparity and placentation that postdates their divergence from their common ancestors with extant monotremes (Blackburn, 2015).

Extensive studies on embryos and neonates of a wide range of mammals showed that marsupials pass through a series of temporary closures for the mouth, eyes and ears that develop shortly before birth and are functionally significant after birth (Müller, 1972). Also, placental mammals form transitory mouth, eye and ear closures at early stages of development that may be lost well before birth in precocial neonates. These closures are today without function in precocial neonates and seem only to be conservative features retained from placental ancestors that were born, as are marsupials today, in an extreme altricial state following a short period of internal gestation. Thus, the findings of Müller (1972) strengthen our hypothesis of an altricial mammalian morphotype.

Emanating from a primary precocial hatchling (resulting from lecithotrophic oviparity), present in extant non-mammalian amniotes and the hypothetical extinct pre-mammalian ancestor, a primary altricial hatchling (resulting from matrotrophic oviparity) must have been derived. This condition would have been present in the mammalian morphotype. From this the monotremes split, which retain until today matrotrophic oviparity and exhibit the closest resemblance to the mammalian morphotype. In therian mammals viviparity evolved, characterized by a primary altricial neonate, the therian morphotype. Whereas in marsupials this type of neonate is still present (marsupial morphotype), placentals transformed to further developed altricial (semi-precocial) neonates (placental morphotype), from which secondary highly precocial neonates evolved.

The character states used for the reconstruction of the stem species pattern of the mammalian neonate are listed in Table 3; the plesiomorphic characters present in the mammalian morphotype and those that are considered apomorphic are summarized in Table 4 and Fig. 8. The characters are based on the distribution within the four mammalian species examined here and literature available for the broad variety of marsupial and placental species. As concluded above, the neonate in the mammalian morphotype was probably similar to the monotreme hatchling, represented here by *O. anatinus* (M44): born naked, with limited mobility and a low birth weight of $<$ 1 g. Other characteristics were very likely closed eyes and ears, a cartilaginous skeleton, lungs in the saccular state with large terminal saccules, kidneys in the transition of meso- to metanephros, a liver with a simple sinusoid system without defined periportal fields and no thermoregulation. The marsupial neonate fits into this morphotype, with the exception that no functional metanephros is present at birth. The further developed altricial (semi-precocial) placental neonates (e.g. Soricidae, Erinaceidae, Muridae, Microchiroptera) also share some characters of the mammalian morphotype, born hairless, limited mobility, closed eyes and ears, the lungs in the saccular state, and very poor thermoregulation. However, they are derived in characters like higher birth weights, metanephric kidneys and a liver with periportal fields. More apomorphies (lungs at alveolar stage, pelage present) are present in intermediate developmental stages (e.g. Carnivora). Finally, transformations in all characters (high birth weight, open eyes and ears, ossified skeleton, mobility, alveolar lungs, metanephric kidneys, liver with periportal fields, pelage and thermoregulation present) can be seen in precocial placental neonates (e.g. Cetacea, Artiodactyla, Perissodactyla, Megachiroptera, Macroselidea, Proboscidea).

Conclusion

The results presented here illustrate the strong correlation of the state of organ development and the capacity of a newborn organism to cope with the challenges of the environment it is born in. Monotreme and marsupial hatchlings/newborns are viable at a very early developmental stage, but are unable to live separated from their mother for a long time. On one hand, structures for maintaining high metabolic rates and thermoregulation are missing; on the other hand, the newborns save energy by getting the energy they need from the mother, just as a placental fetus but outside the uterus. The overall immaturity is a challenge to survive the hazards of the extra-uterine environment. From the evolutionary perspective, the survival of such immature mammalian offspring is at great risk during an extinction event (e.g. K/Pg-event), but marsupials adapted a unique lactation system to support postnatal development of their offspring. In contrast to monotremes and marsupial neonates, both altricial and precocial placental mammals are born in a more mature state reflected in external and internal characters. They develop fast into an independent individual able to survive self-sufficiently. Their more advanced developmental state at birth very likely is an advantage for resisting unfavorable conditions.

The differences in structural development of external characters, lungs, kidneys, liver and skin between monotremes, marsupials and placentals help to develop an evolutionary holistic scenario that sheds light on some aspects of mammalian reproductive strategies in Mesozoic times. Monotreme hatchlings and marsupial neonates very likely represent the degree of development at birth that was present in the common mammalian ancestor. Placental neonates transformed to neonates ranging from slightly further developed (altricial/semi-precocial) to very far-developed with adult features (precocial).

Acknowledgements

This study was carried out within the graduate research program GRK 503, founded by the Deutsche Forschungsgemeinschaft (DFG). The authors thank Mrs Jutta Zeller, Mrs Gabriele Drescher and Mrs Anke Sänger for their technical support with the histological and ultrastructural processing of the material. The authors are grateful to Peter Bartsch for comments on an earlier version of the paper.

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