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The role of mammalian fetal membranes in early embryogenesis: lessons from marsupials

Running title: Mammalian fetal membranes

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Abstract

Across mammals, early embryonic development is supported by uterine secretions taken up through the yolk sac and other fetal membranes (histotrophic nutrition). The marsupial conceptus is enclosed in a shell coat for the first two-thirds of gestation and nutrients pass to the embryo through the shell and the avascular bilaminar yolk sac. At around the time of shell rupture, part of the yolk sac is trilaminar and supplied with blood vessels. It attaches to the uterus and forms a choriovitelline placenta. Rapid growth of the embryo ensues, still supported by histotrophe as well as exchange of oxygen and nutrients between maternal and fetal blood vessels (haemotrophic nutrition). Few marsupials have a chorioallantoic placenta and the highly altricial newborn is delivered after a short gestation. Eutherian embryos pass through a similar sequence before there is a fully functional chorioallantoic placenta. In most orders, there is transient yolk sac placentation, but even before this, nutrients are transferred through an avascular yolk sac. Yolk sac placentation does not occur in rodents or catarrhine primates. Early embryonic development in the mouse is nonetheless dependent on histotrophic nutrition. In the first trimester of human pregnancy, uterine glands open to the intervillous space and secretion products are taken up by the trophoblast. Transfer of nutrients to the early human embryo also involves the yolk sac, which floats free in the

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exocoelom. Marsupials can therefore inform us about the role of fetal membranes and histotrophic nutrition in early embryogenesis, knowledge that can translate to eutherians.

Keywords:

Histotrophic nutrition

Placentation

Shell coat

Uterine glands

Yolk sac

Research highlights

Early embryogenesis in marsupials is maintained by uterine secretions absorbed initially via an avascular yolk sac.

After loss of the shell coat in marsupials, organogenesis is supported by the yolk sac placenta.

Eutherians similarly depend on histotrophic nutrition for the early phases of embryogenesis.

Graphical abstract image

Yolk sac and chorioallantoic placentas side by side in the hedgehog tenrec

1 INTRODUCTION

Significant progress has been made in researching the initial phases of embryonic development in mammals. The key has been the development of in vitro systems to culture embryos for two weeks in mouse (Bedzhov et al. 2014, Bedzhov and Zernicka-Goetz 2014) and human (Shahbazi et al. 2016, Deglincerti et al. 2016) and for three weeks in crab-eating macaques, *Macaca fascicularis* (Ma et al. 2019, Niu et al. 2019). The genes expressed during differentiation of trophectoderm, epiblast and primitive ectoderm (hypoblast) have been mapped as well as the first steps in formation of amnion and yolk sac.

There remains an interval of days or weeks between these events and the establishment of a chorioallantoic placenta that is poorly understood. In human development there is a “black box” spanning the interval from implantation to 5-6 weeks of pregnancy when access to samples is limited for ethical reasons (James, Carter, and Chamley 2012). Indeed, there is a palpable lack of curiosity about the role played by fetal membranes in support of early human development. Comparative studies can be helpful but also confusing as there are three different patterns of fetal membrane development in eutherian mammals (Carter 2016). Thus, transient yolk sac placentation occurs in most eutherian orders, but not in the mouse, where an inverted yolk sac continues to function through term, nor in the human, where the secondary yolk sac floats free in the exocoelom during the first trimester.

In whatever way the membranes develop, we need to ask how the eutherian embryo is nourished through the earliest stages of its development. Later the chorioallantoic placenta permits exchange of nutrients between maternal and fetal blood, which is known as haemotrophic nutrition. In principle, this can occur at earlier stages with a choriovitelline placenta, since the yolk sac has its own set of blood vessels. However, I shall argue that absorption of uterine gland secretions and other maternal products is more important. This is known as histotrophic nutrition (Enders and Carter 2006). As will be discussed, there is data to show uptake of histotrophe by the inverted yolk sac during early embryonic development in rodents and lagomorphs. In the first trimester of human pregnancy, there is uterine gland secretion to the intervillous space of the placenta and uptake of the secretion products by the trophoblast (Burton et al. 2002, Hempstock et al. 2004).

Marsupials deliver highly altricial neonates after a short gestation. Embryonic development is supported by uterine secretions absorbed, during the first two-thirds of gestation, through the shell coat and an avascular yolk sac and in the remaining third, where most of organogenesis occurs, by both avascular and vascular regions of the yolk sac (Tyndale-Biscoe and Renfree 1987, Guernsey et al. 2017, Renfree 1973). Chorioallantoic placentation is uncommon in marsupials although known from bandicoots, wombats and the koala (Tyndale-Biscoe and Renfree 1987). Thus, studies of fetal membranes in marsupials necessarily deal with the same structures that support early embryonic development in eutherians. Indeed, they have been more extensively studied in marsupials and the present review is predicated on my belief that this body of work can suggest avenues of exploration for early embryonic development in eutherians. Therefore, after a brief digression on monotremes, close consideration will be given to the fetal membranes of marsupials. Attention will then be directed to selected examples of eutherian mammals for which there is data to support the critical role of histotrophic nutrition in early embryogenesis.

2 MONOTREMES

Monotremes are relicts of an ancient radiation. They last shared a common ancestor with therians in the Early Jurassic (Huttenlocker et al. 2018). In addition, there is deep divergence between the extant echidnas (*Tachyglossus aculeatus*; *Zaglossus* spp.) and the platypus (*Ornithorhynchus anatinus*; Rowe et al. 2008). Nonetheless, it is instructive to consider the role of monotreme fetal membranes in embryonic development (Hughes and Hall 1998, Hughes 1993, Flynn and Hill 1947, 1939). Thus, although monotremes are oviparous (Haacke 1885), fully two-thirds of development takes place in the uterus, where the egg grows in size through the absorption of uterine secretions (Hughes 1993). In the platypus at full term (oviposition), there is a neurula-stage embryo with 19-20 somites (Figure 1; Hughes and Hall 1998). Although there is yolk in the ovulated egg and cleavage is meroblastic (Flynn and Hill 1939), histotrophic nutrition from uterine secretions is more important than autotrophic nutrition from the yolk. The secretions are taken up by the yolk sac through the leathery eggshell coat, which is about 70 µm thick (Hughes 1993). The abembryonic pole of the yolk sac is bilaminar whilst the embryonic pole is trilaminar, but lacks blood vessels. The full-term embryo has neither an allantoic sac nor an amnion (Hughes 1993, Hughes and Hall 1998). After oviposition and during incubation, however, the trilaminar omphalopleure and the allantoic sac become richly vascularized with the latter acting as an organ of gas exchange (Semon 1894).

The lessons to be drawn are that an eggshell is no barrier to absorption of fetal secretions and that histotrophic nutrition can occur in the absence of a fetal circulation. This is relevant to the understanding of early development in both marsupials and eutherians.

3 MARSUPIALS

There are 21 families of marsupials (Wilson and Reeder 2005), but most knowledge of their reproductive biology is derived from a handful of species (Table 1).

In marsupials, the length of gestation rarely exceeds that of the oestrous cycle (Tyndale-Biscoe and Renfree 1987) whereas eutherian gestation always exceeds cycle length (Chavan, Bhullar, and Wagner 2016). There is a range of altricialilty in marsupials (Smith and Keyte 2018, Hughes and Hall 1988), but all marsupials are born at a less developed stage than any eutherian (Werneburg et al. 2016).

An egg shell coat is present for two-thirds of gestation and intimate contact between the yolk sac and uterine lining is first achieved around the time of organogenesis (Menkhorst et al. 2009, Tyndale-Biscoe and

Renfree 1987). It was shown in the grey short tailed opossum, that rupture of the egg shell triggers an inflammatory response that may be a limiting factor for prolonged gestation (Griffith, Chavan, et al. 2017). This response has been modified in eutherians with upregulation of genes favouring implantation and downregulation of others (Griffith, Chavan, et al. 2017, Erkenbrack et al. 2018).

A feature of reproduction in several marsupials, including many kangaroos and wallabies, is embryonic diapause (Tyndale-Biscoe and Renfree 1987, Renfree and Fenelon 2017). In these marsupials, the female goes into oestrus shortly after parturition (or even before (Menzies, Hildebrandt, and Renfree 2020)). If this leads to a new pregnancy, embryonic development is arrested at the 100-cell stage and resumed only when the young in the pouch has been weaned (lactational diapause). Researchers can exploit this by removing the pouch young to obtain precisely dated uterine embryos. If conception occurs towards the end of the breeding season, the embryo may remain in diapause for up to eleven months (photoperiod-induced diapause; Renfree and Fenelon 2017).

3.1 Marsupial membranes

The tertiary egg membranes are those added during passage of the egg through the genital tract. They occur in many amniotes and even non-amniotes. The mucoid coat is added by glands in the Fallopian tube. It is clearly seen around uterine eggs in the early stages of cleavage, but rarely persists (Hughes 1977). The shell coat is secreted by the uterine glands (Roberts and Breed 1996, Tyndale-Biscoe and Renfree 1987) and is present throughout the first two-thirds of gestation (Selwood 2000, Renfree 1973). Indeed, marsupial development can be divided into two stages: before and after rupture of the shell coat (Tyndale-Biscoe and Renfree 1987). The first stage, embryogenesis, is relatively slow. In contrast, rapid development and growth (organogenesis) occur after rupture of the shell coat and apposition of the yolk sac to the uterine wall as a choriovitelline placenta.

As in other amniotes, the fetal membranes of marsupials comprise amnion, yolk sac, allantois and chorion. Extraembryonic endoderm and trophoblast constitute the bilaminar omphalopleure, which is the main conduit for maternal-fetal exchange prior to shell rupture. It is converted to the trilaminar omphalopleure by expansion of the extraembryonic mesoderm within which blood vessels appear shortly before shell rupture. Apposition of the vascular trilaminar omphalopleure to the uterine epithelium is the basis for choriovitelline or yolk sac placentation, which supports the final phase of rapid growth. In the following, I shall refer to these membranes as the avascular and vascular yolk sac. The fetal membranes of the tammar wallaby (*Notamacropus eugenii*) in late gestation are shown in Figure 2 (Guernsey et al. 2017).

The trophoblast is moderately invasive in many marsupials including the four-eyed opossum (*Philander opossum*; Enders and Enders 1969) and fat-tailed dunnart (*Sminthopsis crassicaudata*; Roberts and Breed 1994a). Recent studies favour the view that the common ancestor of marsupials had an invasive trophoblast, whereas a completely non-invasive trophoblast, as in the tammar wallaby and common brushtail possum (*Trichosurus vulpecula*), is a derived state (Laird et al. 2018, Mess and Ferner 2010, Laird et al. 2020).

The allantoic sac has its own blood supply but, in most marsupials, does not make contact with the uterine lining. Indeed, it is often hidden in folds of the yolk sac membranes (Tyndale-Biscoe and Renfree 1987). However, true chorioallantoic placentation does occur in bandicoots (*Perameles nasuta*, *P. gunnii*, *Isoodon obesulus*, *I. macrourus*), where fetal and maternal capillaries are separated by a thin layer of syncytium (see below). A chorioallantoic placenta is also established in the koala (*Phascolarctos cinereus*) (Hughes 1974, Semon 1894) and the closely related wombat (*Vombatus ursinus*) (Hughes and Green 1998).

3.2 Uterine responses to pregnancy

Several studies have probed the uterine responses to pregnancy. A redistribution of desmosomes and associated claudins has been noted in the plasma membrane of epithelial cells in the fat-tailed dunnart (Dudley et al. 2015, Buddle et al. 2019) as well as tammar wallaby and brushtail possum (Laird et al. 2018). This response was comparable to that preceding attachment in viviparous squamates and eutherians (Dudley et al. 2015). There was evidence in the three marsupials of a strengthening of the basal plasma membrane (Dudley et al. 2017, Laird et al. 2018, Laird, Turancova, et al. 2017). Thus, comparable changes occurred in a species with invasive trophoblast (the dunnart) and two with non-invasive placentation. Based on the hypothesis that invasive placentation is the ancestral state for marsupials (Mess and Ferner 2010, Freyer, Zeller, and Renfree 2003), it was suggested that strengthening of the basal membrane prevented peeling off of the epithelium during trophoblast invasion; however, it could also have facilitated the transition to a non-invasive state (Laird, Dargan, et al. 2017).

A second approach was to analyse the transcriptomic changes occurring in the uterus of the fat-tailed dunnart prior to rupture of the cell membrane (Whittington et al. 2018). Importantly, many of the top 50 upregulated genes were associated with nutrient transport, underlining the importance of histotrophe secretion in the first two-thirds of embryonic development. A second data set from the grey short-tailed opossum found an upregulation of the same category of genes even after shell rupture (Griffith et al. 2019). Prior to shell rupture in the dunnart, there was some indication of a downregulation of genes with a putative immune function (Whittington et al. 2018). Nevertheless, the overriding impression from the opossum was that exposure to fetal tissue after shell rupture triggered an inflammatory response (Erkenbrack et al. 2018, Griffith et al. 2019).

3.3 Histotrophic nutrition

Uterine gland secretions are the principal source of nutrition prior to shell rupture. The shell coat is permeable to the histotrophe, which is taken up by the bilaminar omphalopleure and later by the trilaminar omphalopleure (Sharman 1961). In macropodids such as the tammar wallaby, which has a relatively long gestation period, uterine gland secretion continues after shell rupture (Freyer, Zeller, and Renfree 2007). A different pattern was found in the grey short-tailed opossum (*Monodelphis domestica*), where a decrease in uterine gland secretion occurred even before shell rupture, suggesting a phase when embryonic growth was supported by nutrients stored in the yolk sac (Freyer, Zeller, and Renfree 2007). Surveying the available evidence, Freyer and colleagues found this pattern was the most widespread and concluded it likely was the ancestral condition (Freyer, Zeller, and Renfree 2002, 2003).

In the four-eyed and grey short-tailed opossums, the yolk sacs of adjacent embryos fuse (although each retains a separate allantois). Formation of a common yolk sac cavity entails a reduction in surface area of the bilaminar yolk sac (Enders and Enders 1969, Freyer, Zeller, and Renfree 2002). This is also the case in the fat-tailed dunnart (Roberts and Breed 1994b). In the tammar wallaby, the trilaminar yolk sac expands from one-third to one-half of the total surface area (Freyer, Zeller, and Renfree 2002). Therefore, it is likely that both parts of the yolk sac are important for uptake of histotrophe, a conclusion strengthened by ultrastructural observations. Thus, the apical surface of the trophoblast is characterized by microvilli, coated pits and vesicles in both, the tammar wallaby and the four-eyed opossum (Figure 3; Enders and Enders 1969, Freyer, Zeller, and Renfree 2002). Nevertheless, the trophoblast associated with the bilaminar yolk sac of the tammar wallaby comprises larger cells than that in the trilaminar area and with a greater number of lysosomes containing degraded histotrophe (Jones et al. 2014).

3.4 Respiratory gas exchange

The oxygen requirements for early embryogenesis are small. Thus a 3-day chick embryo requires just 3.8 µl min^{-1} (Romanoff and Romanoff 1967). The rapid growth of the marsupial embryo following shell rupture necessarily implies an increase in oxygen consumption. However, oxygen consumption is still quite moderate, about 6 µL min^{-1}, in the newborn pouch young of the tammar wallaby (Baudinette et al. 1988, Frappell 2008).

Early descriptions of marsupial fetal membranes assumed a role for the vascular yolk sac in respiratory gas exchange (e.g., Hill 1900). The trophoblast layer is thinner in the trilaminar than in the bilaminar yolk sac of the tammar wallaby (Freyer, Zeller, and Renfree 2002, Jones et al. 2014) and this reduction in diffusion distance would facilitate oxygen transfer (Carter 2015).

The early mammalian embryo needs protection from high oxygen levels because of the threat posed by reactive oxygen species (Burton 2009). Eutherian embryos have embryonic haemoglobins with high oxygen affinities that sequester oxygen and may protect against high oxygen tensions (Kitchen and Brett 1974). Marsupial neonates have several embryonic haemoglobins (Holland and Gooley 1997). However, the oxygen dissociation curve of neonatal blood does not differ from that of adult blood (Henty, Wells, and Brittain 2008). The evolution of alpha and beta globin genes has pursued different paths in marsupials and eutherians (Holland, Gooley, and Hope 1998, Opazo, Hoffmann, and Storz 2008). Nevertheless, the apparent absence of high affinity haemoglobins in marsupial embryos raises the question of whether they mount other defences against reactive oxygen species such as antioxidant enzymes (Burton 2009) or scavenging of reactive oxygen species by tissue globins (Fago et al. 2004).

3.5 Haemotrophic nutrition

The passage of nutrients from maternal to fetal blood vessels, called haemotrophic nutrition, is a principal function of the eutherian placenta. After shell rupture in marsupials, the interhaemal barrier separating fetal capillaries of the vascular yolk sac from uterine capillaries can be rather thin as has been shown for the grey short-tailed opossum (Freyer, Zeller, and Renfree 2007) and long-nosed bandicoot (Figure 4a; Padykula and Taylor 1976). However, the physiological evidence for haemotrophic nutrition is scant. In the tammar wallaby, the composition of yolk sac fluid was found to resemble uterine secretions until rupture of the shell coat (Renfree 1973). The proteins that then appeared, such as transferrin, were not maternal in origin and probably were produced by the fetus (Renfree 1973, Renfree and Tyndale-Biscoe 1973). Recent studies of gene expression give further insight (Guernsey et al. 2017). Genes upregulated in the bilaminar yolk sac fall into ontologies associated with nutrient uptake, whereas those upregulated in the trilaminar yolk sac are associated with respiration (Guernsey et al. 2017).

As already noted, a chorioallantoic placenta is found in the koala, wombats and bandicoots. The interhaemal region of the long-nosed bandicoot (*Perameles nasuta*) and southern brown bandicoot (*Isoodon obesulus*) has been examined by transmission electron microscopy (Padykula and Taylor 1976, Padykula and Taylor 1977). The fetal and maternal capillaries are at first separated by the trophoblast and the uterine lining, which is a syncytium formed by fusion of the epithelial cells (Figure 4b). Later this is reduced to a single layer apparently by fusion of trophoblast and syncytium. Although this was seen as evidence for haemotrophic nutrition (Padykula and Taylor 1977), it did not translate into greater maturity of the neonate (Hughes and Hall 1988, Lyne and Hollis 1977). Rather it allowed shortening of the gestation period. At just over 12 days, the northern brown bandicoot (*I. macrourus*) holds the record for the shortest gestation of any mammal (Lyne 1974).

3.6 Hormone secretion

Hormone-related genes are expressed by the fetal membranes of oviparous and viviparous amniotes (Griffith, Brandley, et al. 2017). Notwithstanding, knowledge of the endocrine function of the marsupial yolk sac is based largely on work in the tammar wallaby. Although the yolk sac can convert pregnenolone to progesterone, it likely is not a significant source of steroid hormones (Heap, Renfree, and Burton 1980). It does, however, express several peptide hormones and growth factors, including luteinizing hormone, prolactin and growth hormone as well as insulin-like growth factor-2 and its receptor (Menzies, Pask, and Renfree 2011). Luteinizing hormone could be the signal for pregnancy maintenance perhaps acting in concert with prolactin (Menzies, Pask, and Renfree 2011). Growth hormone, GH receptor and IGF-2 are upregulated in the later phase of organogenesis that follows shell rupture and may act to promote fetal growth (Menzies, Pask, and Renfree 2011).

An interesting form of epigenetic gene regulation, first shown in eutherians, is genomic imprinting, which results in silencing of one of the parental alleles. In marsupials, insulin (*INS*) is paternally expressed in the vascular yolk sac and otherwise only in mammary gland (Ager et al. 2007, Stringer et al. 2012b). Similarly, insulin-like growth factor-2 (*IGF2*) is paternally expressed in the vascular yolk sac and has been ascribed a role in promoting vascularization by increasing expression of vascular endothelial growth factor (Stringer et al. 2012a, Ager et al. 2008).

Finally, the yolk sac of the tammar wallaby produces large amounts of prostaglandin $F_{2\alpha}$ in the last few days of gestation, peaking at term (Shaw, Gehring, and Bell 1999). Although the endometrium also secretes prostaglandins, the fetal signal is thought to initiate parturition (Shaw, Gehring, and Bell 1999, Renfree 2010).

4 EUTHERIANS

Uterine gland secretions have long been recognized as an essential supplement to haemotrophic nutrition via the chorioallantoic placenta of eutherians (Burton 1982, Enders and Carter 2006). Uptake of secretions, cell debris and maternal erythrocytes is by pinocytosis and endocytosis to columnar trophoblast cells often organized in structures such as the chorionic vesicles of lower primates or the areolae of swine, horse and moles (Enders and Carter 2006). Here, we are concerned with the role of histotrophic nutrition in the early stages of embryonic development before there is circulation in the chorioallantoic placenta. An overview of fetal membrane development is given elsewhere (Carter 2016).

4.1 The avascular yolk sac of the horse

Early embryonic development has been well studied in the horse (*Equus caballus*) because the details are important for assisted reproductive technologies (Betteridge 2007). The equine embryo remains spherical in shape and moves about the uterus until about day 17 after ovulation. Between about days 7 and 20 it is enclosed in a capsule (Figure 5a). This is not a tertiary egg membrane, like the marsupial shell coat. It is made up of mucin-like glycoproteins secreted by the trophoblast (Oriol, Sharom, and Betteridge 1993). It is not until dissolution of the capsule around day 21 that there is contact with the endometrium (Betteridge 2007). The conceptus expands during this interval and the capsule with it. The capsule is not a barrier to transfer. Within the capsule is a bilaminar yolk sac comprised of trophoblast and endoderm. The mural trophoblast is endocytotic as indicated by the transfer of tracers (ferritin and peroxidase) from coated pits and tubules to large vacuoles (Enders et al. 1988). Extraembryonic mesoderm appears relatively late and a vascular, trilaminar yolk sac is not present until shortly before the capsule dissolves (Figure 5b). However, the embryo is well formed by the time the capsule is lost and there is then an effective embryonic circulation (Enders et al. 1993). From 22 days embryonic development is supported by a yolk sac placenta

(Enders 2015, Enders et al. 1993). A fully functional chorioallantoic placenta is not established until the end of the first month of gestation (Allen 2001).

Importantly, while the conceptus is enclosed in its capsule yolk sac volume increases over time (Betteridge 2007, Waelchli and Betteridge 1996, Betteridge et al. 1982). Osmolarity, fructose concentration and protein concentration remain constant or increase slightly, but do not fall with increasing volume, showing there must be uptake of solutes from the uterine fluid (Betteridge 2007). The role of the avascular yolk sac in this process was elegantly demonstrated by studies of twinning in horses (Waelchli and Betteridge 2013). If there are two embryos in the capsule and one abuts the membranes of the other, the second twin is unable to survive, presumably because it cannot absorb uterine secretions. With correct orientation of the blastocysts, both can take up nutrients and the twins may survive. This clearly demonstrates that the bilaminar yolk sac has a vital function just as it does in marsupials before egg cell rupture.

4.2 Yolk sac placentas of artiodactyls and tenrecs

A temporary yolk sac or choriovitelline placenta supports embryonic development in most orders of eutherian mammal (Carter 2016). A strict definition requires that this is a vascular yolk sac in intimate apposition to the endometrium (Mossman 1987). As in marsupials, however, the yolk sac may take up histotrophe well before such an attachment is formed. This section will give three examples of how the yolk sac and other membranes support early development. A list of eutherian orders with transient yolk sac placentation is given elsewhere (Carter 2016). Yolk sac placentation is not found in haplorrhine primates, rodents, lagomorphs, armadillos or sloths (Carter 2016).

In the pig (*Sus scrofa*), the blastocyst undergoes a remarkable elongation at 10.5-12 days of gestation, reaching about 1 meter in length and 0.75 mm in diameter (Perry 1981, Perry and Rowlands 1962, Heuser

1927). The elongated part of the blastocyst is a diverticulum of the exocoelom lined by chorion. The chorion participates in maternal-fetal signalling and possibly in absorption of histotrophe secreted by the uterine glands (Bazer and Johnson 2014). At the centre is the embryo and yolk sac, the distal part of which is bilaminar, and the remainder supplied with blood vessels. This likely transfers nutrients to the rapidly developing embryo. Adherence to the uterine wall occurs at 18 days, resulting in transient yolk sac placentation (Figure 6a). At this stage the allantois is just a bud protruding into the exocoelom. However, by 21 days the vascular allantois has displaced the yolk sac from trophoblast and begun to form a chorioallantoic placenta (Figure 6b; Perry 1981, Heuser 1927).

In sheep (*Ovis aries*) and cattle (*Bos taurus*, *B. indicus*), elongation of the chorion is less drastic than in the pig, but its two arms are occupied by the yolk sac (Figure 5c; Rüsse et al. 1992, Assis Neto et al. 2010). As first shown for the sheep, early expansion of the exocoelom hinders contact between yolk sac and endometrium (Assheton 1906). This configuration does not meet the above definition of a yolk sac placenta, but the uterine gland secretions cross the chorion and yolk sac to supply the embryo.

Horses, pigs and cattle belong to the same eutherian clade (Laurasiatheria). My third example, therefore, is the lesser hedgehog tenrec (*Echinops telfairi*), which is from a distant clade (Afrotheria; Murphy et al. 2001). In the tenrec, organogenesis is supported by a yolk sac placenta until it is displaced by expansion of the exocoelom and allantois (Carter et al. 2005). As shown in Figure 7, yolk sac and allantois are richly vascularized and apposed to a thickening of the endometrium called the preplacental pad. Thus, there is the morphological basis for haemotrophic nutrition via a yolk sac placenta.

4.3 The inverted yolk sac of rodents

In rodents, inversion of the germ layers (day E5 in the mouse) and subsequent loss of the outer wall of the yolk sac leaves a visceral yolk sac membrane where extraembryonic endoderm forms an epithelium facing the uterine cavity (Sobotta 1911, Rossant and Cross 2001). There is increasing awareness that yolk sac membranes support embryonic development well before establishment of the chorioallantoic placenta. In the mouse, important events such as closure of the neural folds (Zohn and Sarkar 2010, Terasawa et al. 1999) precede the start of chorioallantoic circulation at embryonic day E10.5 (Cross et al. 2006). The yolk sac endoderm plays a key role in supplying lipids during early embryonic development through synthesis of apolipoprotein B and lipoprotein (Farese et al. 1996, Terasawa et al. 1999). In addition, iron uptake by endocytosis of transferrin has been shown in mouse (Copp et al. 1992) and rat (Young et al. 1997).

The rodent yolk sac continues to serve as an accessory to the chorioallantoic placenta through to term. Its transport functions have been studied extensively in mid to late gestation in the guinea pig (e.g., King and Enders 1970). The yolk sac is also an important conduit for maternal immunoglobulins (Brambell, Hemmings, and Rowlands 1948, King and Enders 1970).

4.4 The yolk sac in haplorrhine primates

Early in human development, the extraembryonic mesoderm separates the endoderm from the trophoblast resulting in a “secondary” yolk sac comprised of endoderm and mesenchyme that comes to lie within the exocoelom (Enders and King 1993, Hertig, Rock, and Adams 1956, Jauniaux et al. 2004, Jones 1997). Indeed, precocious development of the mesoderm and exocoelom with formation of a secondary yolk sac is a characteristic feature of haplorrhine primates (tarsiers, Old and New World monkeys and apes; Luckett 1974). In the human, a vitelline circulation is established starting around day 16 and yolk sac function persists through 9-11 weeks of gestation after which there is a gradual decline. In the first trimester chorioallantoic placenta, the orifices of the maternal arteries are blocked by plugs of trophoblast and there is no flow before 6-7 weeks and restricted flow until 12 weeks (Roberts et al. 2017, James et al. 2018). However, uterine glands open to the intervillous space of the placenta and their secretion products are taken up by the trophoblast (Burton et al. 2002, Hempstock et al. 2004, Moser et al. 2015). Thus, the yolk sac functions through a period when perfusion of the intervillous space is minimal and uterine gland secretions are an important source of nutrients for the developing embryo. Indeed, the uterine glands may be stimulated by hormones released from the trophoblast, such as human placental lactogen and human chorionic gonadotrophin, while trophoblast invasion could be promoted by “uterine milk proteins” and growth factors (Burton, Cindrova-Davies, and Turco 2020).

Exocoelomic fluid from the first trimester of human pregnancy has been characterized as an ultrafiltrate of maternal plasma with additional proteins from the placenta and secondary yolk sac. It is therefore regarded as an important interface for maternal-fetal nutrient transfer and as a nutrient reservoir for the embryo (Jauniaux and Gulbis 2000). Yolk sac fluid is not easily obtained, but its protein content is consistent with exchange between exocoelomic and yolk sac compartments including transfer of hCG derived from the placental trophoblast (Gulbis et al. 1998). The yolk sac mesothelium faces the exocoelomic cavity and has the ultrastructural features of pinocytotic activity (Jones 1997, Jones and Jauniaux 1995). Finally, the inward-facing endodermal layer expresses the endocytic receptors megalin and cubilin (Burke et al. 2013). Transcriptome analysis has confirmed the expression of a broad range of transporters in first trimester human yolk sac (Cindrova-Davies et al. 2017). Together these observations support the argument that uterine gland secretions and other nutrients are transported to the exocoelom and absorbed by the secondary yolk sac whence they are transferred to the vitelline circulation (Jauniaux, Gulbis, and Burton

2003). Additional functions of human yolk sac are synthesis of α-fetoprotein and other proteins (Gulbis et al. 1998), haematopoiesis (Tavian et al. 2010) and as a repository for germ cells (De Felici 2013).

5 CONCLUSIONS

The first lesson to be drawn from this review is that uptake of uterine secretions does not require a placenta. The monotreme embryo has reached 18-20 somites by oviposition, its growth supported both by yolk and by histotrophe taken up through the avascular yolk sac. Similarly, the first two-thirds of development in marsupials is dependent on histotrophe absorbed by the avascular bilaminar yolk sac and by a trilaminar yolk sac that has no blood vessels until shortly before shell rupture. An essential role for the bilaminar yolk sac in eutherian development is confirmed by studies of twinning in the horse.

Secondly, a yolk sac placenta supports the growth of the marsupial embryo after shell rupture. It may allow for haemotrophic nutrition although the physiological evidence is scant. The vascular yolk sac might be of greater importance for the oxygen supply to the embryo. The ancestral condition in eutherians and in most extant clades is for a yolk sac placenta to provide nutrition in the early stages of fetal growth. Rodents and higher primates do not conform. However, in rodents the inverted yolk sac is functional well before establishment of a circulation in the chorioallantoic placenta. In the mouse, that transition occurs at day E10.5. Human development is unusual in that the yolk sac never approaches the uterus. However, there is ample evidence that early embryonic development is supported by histotrophe transferred via the exocoelom and yolk sac. The human embryo is at least partly dependent upon uptake of uterine secretions by the trophoblast facing the intervillous space, where blood flow is negligible in the first few weeks of pregnancy.

Marsupials provide important information about histotrophic nutrition via the avascular yolk sac and later the yolk sac placenta. To a large extant these translate to early embryonic development in eutherians. Thus there is every reason to incorporate this knowledge when considering the role of fetal membranes in eutherians and to encourage further studies of marsupials as essential to a fuller understanding of mammalian development and its evolution.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Figure legends

FIGURE 1 Intrauterine development of monotremes is supported by yolk and absorption of uterine secretions. 18-20-somite embryo of the platypus (*Ornithorhynchus anatinus*). H, Hensen's node; S, somites; TGP, trigeminal ganglionic primordia. Reprinted with permission of The Royal Society from (Hughes and Hall 1998) © The Royal Society (U.K.).

FIGURE 2 Fetal membranes of the tammar wallaby (*Notamacropus eugenii*) at 24 days gestation. Right: schematic representation of the fetal membranes. Left and middle: cellular components of the avascular yolk sac or bilaminar omphalopleure (BOM) and vascular yolk sac or trilaminar omphalopleure (TOM). Cell layers are: TL, trophoblast; EL, endoderm; ML, mesoderm (with blood vessels). Reprinted from (Guernsey et al. 2017) © the authors.

FIGURE 3 Electron micrographs of trophoblast from the vascular yolk sac. (a) Tammar wallaby (*Notamacropus eugenii*) at 20 days gestation. Structures shown include coated pits (long arrowheads), vesicles (v), and nucleus (N). Reprinted with permission from (Freyer, Zeller, and Renfree 2002) © Anatomical Society of Great Britain and Ireland 2002. (b) Grey four-eyed opossum (*Philander opossum*). Note that the apical microvilli are long but do not interdigitate with those of the uterine epithelial cell. Arrows indicate coated pits. At the opposite surface, microvilli project into basal infoldings situated beneath the basal lamina. Image courtesy of Dr. Allen C. Enders.

FIGURE 4 The interhaemal barrier in the placentae of the long-nosed bandicoot (*Perameles nasuta*); crown-rump length of embryo 10-11 mm. (a) Yolk sac placenta. Fetal blood vessels (fv) appear in the attenuated mesoderm between endoderm (E) and trophoblast (T). Maternal capillaries are superficial and above the syncytial masses of uterine epithelium (S). CT, endometrial stroma; m, mitochondrion; n, nucleus; x, artefactual space. (b) Chorioallantoic placenta. There is a short diffusion distance between fetal capillaries (fv) and maternal ones (mv) separated only by trophoblast (T) and the syncytial masses (S) derived from uterine epithelial cells. Reprinted with permission from (Padykula and Taylor 1976) Copyright © 1976 Wiley-Liss, Inc.

FIGURE 5 The capsule and yolk sac of the horse and the bovine yolk sac. (a) In the horse at 14.5 days the resilient capsule is so tightly applied to the yolk sac that it is invisible. (b) At 17.5 days the flaccid capsule hangs intact from the yolk sac. Reprinted from (Betteridge 2007) © (2007) with permission from Elsevier. (c) The bovine yolk sac at 20 days comprises a central portion (YSC) and two extended arms (YSE); allantoic vessels (arrows) are seen extending from the embryo. Courtesy of Dr. Antonio Assis Neto.

FIGURE 6 Fetal membranes of the domestic pig (*Sus scrofa*). Ectoderm is in blue, mesoderm in red and endoderm in green. (a) Longitudinal section of the elongated blastocyst at 18 days post coitum. The yolk sac is attached to the chorion partly as the bilaminar omphalopleure and partly as a choriovitelline placenta. The allantois is expanding into the exocoelom but has not yet reached the chorion. (b) By 21 days post coitum, the allantois and chorion have fused to form the allanto-chorion. In the process the yolk sac has been displaced. Reprinted with permission from (Perry 1981). Copyright © 1981, Society for Reproduction and Fertility.

FIGURE 7. Fetal membranes of the lesser hedgehog tenrec (*Echinops telfairi*) at 22 days of gestation. (a) Overview showing amniotic cavity (Amn), yolk sac (YS), allantoic cavity (All) and exocoelom (Exo). (b) Yolk sac placenta (YS Pl) and allantoic placenta (All Pl). At this stage neither vessels from the yolk sac nor vessels from the allantois have entered the preplacental pad (PP). (c) Vessels of yolk sac (above) and allantois (below) abut the preplacental pad. Note the more cuboidal endoderm lining the yolk sac vessels. MBS, maternal blood space. (d) The allantois displacing the yolk sac. The allantoic cavity (All) is encroaching from

either side on the remnants of the exocoelom (Exo) and the yolk sac (YS). Haemotoxylin and eosin. Scale bars: 1 mm (a), 100 mm (b), 20 mm (c-d). Reprinted from (Carter et al. 2005) © (2005) with permission from Elsevier.

TABLE 1 Marsupial orders and model species. Orders and suborders from (Wilson and Reeder 2005). The species shown are those featured in the recent literature.

Order and suborder	Species	Common name
Didelphimorphia	*Monodelphis domestica*	Grey Short-Tailed Opossum
	Philander opossum	Grey Four-Eyed Opossum
Paucituberculata	None	
Microbiotheria	None	
Notoryctemorphia	None	
Dasyuromorphia	*Sminthopsis crassicaudata*	Fat-Tailed Dunnart
Peramelemorphia	*Isoodon macrourus*	Northern Brown Bandicoot
	Isoodon obesulus	Southern Brown Bandicoot
	Perameles nasuta	Long-nosed Bandicoot
Diprotodontia: Vombatiformes	*Phascolarctos cinereus*	Koala
	Vombatus ursinus	Common wombat
Diprotodontia: Phalangeriformes	*Trichosurus vulpecula*	Common Brushtail Possum
	Pseudocheirus peregrinus	Common Ringtail Possum
Diprotodontia: Macropodiformes	*Potorous tridactylus*	Long-Nosed Potoroo
	Notamacropus eugenii	Tammar Wallaby

REFERENCES

Ager, E. I., A. J. Pask, G. Shaw, and M. B. Renfree. 2008. "Expression and protein localisation of IGF2 in the marsupial placenta." *BMC Dev Biol* 8:17. doi: 10.1186/1471-213X-8-17.

Ager, E., S. Suzuki, A. Pask, G. Shaw, F. Ishino, and M. B. Renfree. 2007. "Insulin is imprinted in the placenta of the marsupial, Macropus eugenii." *Dev Biol* 309 (2):317-28. doi: 10.1016/j.ydbio.2007.07.025.

Allen, W. R. 2001. "Fetomaternal interactions and influences during equine pregnancy." *Reproduction* 121 (4):513-27.

Assheton, R. 1906. "The morphology of the ungulate placenta, particularly the development of that organ in the sheep, and notes upon the placenta of the elephant and hyrax." *Phil Trans R Soc Lond B* 198:239-250.

Assis Neto, A. C., F. T. Pereira, T. C. Santos, C. E. Ambrosio, R. Leiser, and M. A. Miglino. 2010. "Morpho-physical recording of bovine conceptus (Bos indicus) and placenta from days 20 to 70 of pregnancy." *Reprod Domest Anim* 45 (5):760-72. doi: 10.1111/j.1439-0531.2009.01345.x.

Baudinette, R. V., B. J. Gannon, R. G. Ryall, and P. B. Frappell. 1988. "Changes in metabolic rates and blood respiratory characteristics during pouch development of a marsupial, Macropus eugenii." *Respir Physiol* 72 (2):219-28. doi: 10.1016/0034-5687(88)90008-4.

Bazer, F. W., and G. A. Johnson. 2014. "Pig blastocyst-uterine interactions." *Differentiation* 87 (1-2):52-65. doi: 10.1016/j.diff.2013.11.005.

Bedzhov, I., C. Y. Leung, M. Bialecka, and M. Zernicka-Goetz. 2014. "In vitro culture of mouse blastocysts beyond the implantation stages." *Nat Protoc* 9 (12):2732-9. doi: 10.1038/nprot.2014.186.

Bedzhov, I., and M. Zernicka-Goetz. 2014. "Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation." *Cell* 156 (5):1032-44. doi: 10.1016/j.cell.2014.01.023.

Betteridge, K. J. 2007. "Equine embryology: an inventory of unanswered questions." *Theriogenology* 68 Suppl 1:S9-21. doi: 10.1016/j.theriogenology.2007.04.037.

Betteridge, K. J., M. D. Eaglesome, D. Mitchell, P. F. Flood, and R. Beriault. 1982. "Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens." *J Anat* 135 (1):191-209.

Brambell, F. W. R., W. A. Hemmings, and W. T. Rowlands. 1948. "The passage of antibodies from the maternal circulation into the embryo in rabbits." *Proc R Soc Lond B* 135 (880):390-403.

Buddle, A. L., M. B. Thompson, L. A. Lindsay, C. R. Murphy, C. M. Whittington, and B. M. McAllan. 2019. "Dynamic changes to claudins in the uterine epithelial cells of the marsupial Sminthopsis crassicaudata (Dasyuridae) during pregnancy." *Mol Reprod Dev* 86 (6):639-649. doi: 10.1002/mrd.23140.

Burke, K. A., E. Jauniaux, G. J. Burton, and T. Cindrova-Davies. 2013. "Expression and immunolocalisation of the endocytic receptors megalin and cubilin in the human yolk sac and placenta across gestation." *Placenta* 34 (11):1105-9. doi: 10.1016/j.placenta.2013.08.003.

Burton, G. J. 1982. "Review article. Placental uptake of maternal erythrocytes: a comparative study." *Placenta* 3 (4):407-34.

Burton, G. J. 2009. "Oxygen, the Janus gas; its effects on human placental development and function." *J Anat* 215 (1):27-35. doi: 10.1111/j.1469-7580.2008.00978.x.

Burton, G. J., T. Cindrova-Davies, and M.Y. Turco. 2020. "Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy." *Placenta*. doi: https://doi-org.proxy1-bib.sdu.dk/10.1016/j.placenta.2020.02.008.

Burton, G. J., A. L. Watson, J. Hempstock, J. N. Skepper, and E. Jauniaux. 2002. "Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy." *J Clin Endocrinol Metab* 87 (6):2954-9. doi: 10.1210/jcem.87.6.8563.

Carter, A. M. 2015. "Placental Gas Exchange and the Oxygen Supply to the Fetus." *Compr Physiol* 5 (3):1381-403. doi: 10.1002/cphy.c140073.

Carter, A. M. 2016. "IFPA Senior Award Lecture: Mammalian fetal membranes." *Placenta* 48 Suppl 1:S21-S30. doi: 10.1016/j.placenta.2015.10.012.

Carter, A. M., T. N. Blankenship, H. Kunzle, and A. C. Enders. 2005. "Development of the haemophagous region and labyrinth of the placenta of the tenrec, Echinops telfairi." *Placenta* 26 (2-3):251-61. doi: 10.1016/j.placenta.2004.06.001.

Chavan, A. R., B. A. Bhullar, and G. P. Wagner. 2016. "What was the ancestral function of decidual stromal cells? A model for the evolution of eutherian pregnancy." *Placenta* 40:40-51. doi: 10.1016/j.placenta.2016.02.012.

Cindrova-Davies, T., E. Jauniaux, M. G. Elliot, S. Gong, G. J. Burton, and D. S. Charnock-Jones. 2017. "RNA-seq reveals conservation of function among the yolk sacs of human, mouse, and chicken." *Proc Natl Acad Sci U S A* 114 (24):E4753-E4761. doi: 10.1073/pnas.1702560114.

Copp, A. J., J. P. Estibeiro, F. A. Brook, and K. M. Downs. 1992. "Exogenous transferrin is taken up and localized by the neurulation-stage mouse embryo in vitro." *Dev Biol* 153 (2):312-23. doi: 10.1016/0012-1606(92)90116-x.

Cross, J. C., H. Nakano, D. R. Natale, D. G. Simmons, and E. D. Watson. 2006. "Branching morphogenesis during development of placental villi." *Differentiation* 74 (7):393-401. doi: 10.1111/j.1432-0436.2006.00103.x.

De Felici, M. 2013. "Origin, migration, and proliferation of human primordial germ cells." In *Oogenesis*, edited by G. Coticchio, D.F. Albertini and L. De Santis, 19-37. London: Springer-Verlag.

Deglincerti, A., G. F. Croft, L. N. Pietila, M. Zernicka-Goetz, E. D. Siggia, and A. H. Brivanlou. 2016. "Self-organization of the in vitro attached human embryo." *Nature* 533 (7602):251-4. doi: 10.1038/nature17948.

Dudley, J. S., C. R. Murphy, M. B. Thompson, and B. M. McAllan. 2015. "Desmoglein-2 during pregnancy and its role in the evolution of viviparity in a marsupial (Sminthopsis crassicaudata; Dasyuridae)." *J Morphol* 276 (3):261-72. doi: 10.1002/jmor.20333.

Dudley, J. S., C. R. Murphy, M. B. Thompson, and B. M. McAllan. 2017. "Epithelial cadherin disassociates from the lateral plasma membrane of uterine epithelial cells throughout pregnancy in a marsupial." *J Anat* 231 (3):359-365. doi: 10.1111/joa.12648.

Enders, A. C. 2015. "Equine development - blastocyst to endometrial cup." Centre for Trophoblast Research, accessed 13 November 2019. https://www.trophoblast.cam.ac.uk/Resources/enders.

Enders, A. C., and A. M. Carter. 2006. "Comparative placentation: some interesting modifications for histotrophic nutrition -- a review." *Placenta* 27 Suppl A:S11-S16. doi: 10.1016/j.placenta.2005.10.013.

Enders, A. C., and R. K. Enders. 1969. "The placenta of the four-eye opossum (Philander opossum)." *Anat Rec* 165 (3):431-49. doi: 10.1002/ar.1091650311.

Enders, A. C., and B. F. King. 1993. "Development of the human yolk sac." In *The human yolk sac and yolk sac tumors*, edited by F. F. Nogales, 33-47. Berlin; London: Springer-Verlag.

Enders, A. C., K. C. Lantz, I. K. Liu, and S. Schlafke. 1988. "Loss of polar trophoblast during differentiation of the blastocyst of the horse." *J Reprod Fertil* 83 (1):447-60. doi: 10.1530/jrf.0.0830447.

Enders, A. C., S. Schlafke, K. C. Lantz, and I. K. Liu. 1993. "Endoderm cells of the equine yolk sac from day 7 until formation of the definitive yolk sac placenta." *Equine Vet J Suppl 15*:3-9.

Erkenbrack, E. M., J. D. Maziarz, O. W. Griffith, C. Liang, A. R. Chavan, M. C. Nnamani, and G. P. Wagner. 2018. "The mammalian decidual cell evolved from a cellular stress response." *PLoS Biol* 16 (8):e2005594. doi: 10.1371/journal.pbio.2005594.

Fago, A., C. Hundahl, H. Malte, and R. E. Weber. 2004. "Functional properties of neuroglobin and cytoglobin. Insights into the ancestral physiological roles of globins." *IUBMB Life* 56 (11-12):689-96. doi: 10.1080/15216540500037299.

Farese, R. V., Jr., S. Cases, S. L. Ruland, H. J. Kayden, J. S. Wong, S. G. Young, and R. L. Hamilton. 1996. "A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice." *J Lipid Res* 37 (2):347-60.

Flynn, T. T., and J. P. Hill. 1939. "The development of the Monotremata. Part IV. Growth of the ovarian ovum, maturation, fertilization and early cleavage." *Trans. Zool. Soc. Lond.* 26:445-622.

Flynn, T. T., and J. P. Hill. 1947. "The development of the Monotremata- Part VI. The later stages of cleavage and the formation of the primary germ-layers." *Trans. Zool. Soc. Lond.* 26:1-151.

Frappell, P. B. 2008. "Ontogeny and allometry of metabolic rate and ventilation in the marsupial: matching supply and demand from ectothermy to endothermy." *Comp Biochem Physiol A Mol Integr Physiol* 150 (2):181-8. doi: 10.1016/j.cbpa.2008.02.017.

Freyer, C., U. Zeller, and M. B. Renfree. 2002. "Ultrastructure of the placenta of the tammar wallaby, Macropus eugenii: comparison with the grey short-tailed opossum, Monodelphis domestica." *J Anat* 201 (2):101-19. doi: 10.1046/j.1469-7580.2002.00084.x.

Freyer, C., U. Zeller, and M. B. Renfree. 2003. "The marsupial placenta: a phylogenetic analysis." *J Exp Zool A Comp Exp Biol* 299 (1):59-77. doi: 10.1002/jez.a.10291.

Freyer, C., U. Zeller, and M. B. Renfree. 2007. "Placental function in two distantly related marsupials." *Placenta* 28 (2-3):249-57. doi: 10.1016/j.placenta.2006.03.007.

Griffith, O. W., M. C. Brandley, C. M. Whittington, K. Belov, and M. B. Thompson. 2017. "Comparative genomics of hormonal signaling in the chorioallantoic membrane of oviparous and viviparous amniotes." *Gen Comp Endocrinol* 244:19-29. doi: 10.1016/j.ygcen.2016.04.017.

Griffith, O. W., A. R. Chavan, M. Pavlicev, S. Protopapas, R. Callahan, J. Maziarz, and G. P. Wagner. 2019. "Endometrial recognition of pregnancy occurs in the grey short-tailed opossum (Monodelphis domestica)." *Proc Biol Sci* 286 (1905):20190691. doi: 10.1098/rspb.2019.0691.

Griffith, O. W., A. R. Chavan, S. Protopapas, J. Maziarz, R. Romero, and G. P. Wagner. 2017. "Embryo implantation evolved from an ancestral inflammatory attachment reaction." *Proc Natl Acad Sci U S A* 114 (32):E6566-E6575. doi: 10.1073/pnas.1701129114.

Guernsey, M. W., E. B. Chuong, G. Cornelis, M. B. Renfree, and J. C. Baker. 2017. "Molecular conservation of marsupial and eutherian placentation and lactation." *Elife* 6:e27450. doi: 10.7554/eLife.27450.

Gulbis, B., E. Jauniaux, F. Cotton, and P. Stordeur. 1998. "Protein and enzyme patterns in the fluid cavities of the first trimester gestational sac: relevance to the absorptive role of secondary yolk sac." *Mol Hum Reprod* 4 (9):857-62. doi: 10.1093/molehr/4.9.857.

Heap, R. B., M. B. Renfree, and R. D. Burton. 1980. "Steroid metabolism in the yolk sac placenta and endometrium of the tammar wallaby, Macropus eugenii." *J Endocrinol* 87 (3):339-49. doi: 10.1677/joe.0.0870339.

Hempstock, J., T. Cindrova-Davies, E. Jauniaux, and G. J. Burton. 2004. "Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: a morphological and immunohistochemical study." *Reprod Biol Endocrinol* 2:58. doi: 10.1186/1477-7827-2-58.

Henty, K., R. M. Wells, and T. Brittain. 2008. "Characterization of the hemoglobins of the neonatal brushtailed possum Trichosurus vulpecula (Kerr): evidence for a highly cooperative, aggregated isoform of hemoglobin." *Comp Biochem Physiol A Mol Integr Physiol* 150 (1):52-7. doi: 10.1016/j.cbpa.2008.03.001.

Hertig, A. T., J. Rock, and E. C. Adams. 1956. "A description of 34 human ova within the first 17 days of development." *Am J Anat* 98 (3):435-93. doi: 10.1002/aja.1000980306.

Heuser, C.H. 1927. "A study of the implantation of the ovum of the pig from the stage of the bilaminar blastocyst to the completion of the fetal membranes." *Contrib Embryol Carnegie Inst* 19:229-243.

Hill, J.P. 1900. "On the foetal membranes, placentation and parturition of the native cat (Dasyurus viverrinus)." *Anat Anz* 18:364-373.

Holland, R. A., and A. A. Gooley, 2nd. 1997. "Characterization of the embryonic globin chains of the marsupial Tammar wallaby, Macropus eugenii." *Eur J Biochem* 248 (3):864-71. doi: 10.1111/j.1432-1033.1997.00864.x.

Holland, R. A., A. A. Gooley, and R. M. Hope. 1998. "Embryonic globins of the marsupial the Tammar Wallaby (Macropus eugenii): bird like and mammal like." *Clin Exp Pharmacol Physiol* 25 (9):740-4. doi: 10.1111/j.1440-1681.1998.tb02288.x.

Hughes, R. L. 1974. "Morphological studies on implantation in marsupials." *J Reprod Fertil* 39 (1):173-86. doi: 10.1530/jrf.0.0390173.

Hughes, R. L. 1993. "Monotreme development with particular reference to the extraembryonic membranes." *J Exp Zool* 266 (6):480-94. doi: 10.1002/jez.1402660602.

Hughes, R. L., and L. S. Hall. 1998. "Early development and embryology of the platypus." *Philos Trans R Soc Lond B Biol Sci* 353 (1372):1101-14. doi: 10.1098/rstb.1998.0269.

Hughes, R.L. 1977. "Egg membranes and ovarian function during pregnancy in monotremes and marsupials." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J.H. Calaby and C. H. Tyndale-Biscoe, 281-291. Canberra: Australian Academy of Science.

Hughes, R.L., and R.H. Green. 1998. "Placentation and adaptations for survival at birth in the common wombat." In *Wombats*, edited by R.T. Wells and P. A. Pridmore, 86-108. Chipping Norton, Surrey: Beatty & Sons.

Hughes, R.L., and L.S. Hall. 1988. "Structural adaptations of the newborn marsupial." In *The developing marsupial: models for biomedical research*, edited by C.H. Tyndale-Biscoe and P. A. Janssens, 8-27. Berlin: Springer-Verlag.

Huttenlocker, A. K., D. M. Grossnickle, J. I. Kirkland, J. A. Schultz, and Z. X. Luo. 2018. "Late-surviving stem mammal links the lowermost Cretaceous of North America and Gondwana." *Nature* 558 (7708):108-112. doi: 10.1038/s41586-018-0126-y.

Haacke, W. 1885. "On the marsupial ovum, the mammary pouch, and the male milk glands of Echidna hystris." *Proc R Soc Lond B* 38:72-74.

James, J. L., A. M. Carter, and L. W. Chamley. 2012. "Human placentation from nidation to 5 weeks of gestation. Part I: What do we know about formative placental development following implantation?" *Placenta* 33 (5):327-34. doi: 10.1016/j.placenta.2012.01.020.

James, J. L., R. Saghian, R. Perwick, and A. R. Clark. 2018. "Trophoblast plugs: impact on utero-placental haemodynamics and spiral artery remodelling." *Hum Reprod* 33 (8):1430-1441. doi: 10.1093/humrep/dey225.

Jauniaux, E., T. Cindrova-Davies, J. Johns, C. Dunster, J. Hempstock, F. J. Kelly, and G. J. Burton. 2004. "Distribution and transfer pathways of antioxidant molecules inside the first trimester human gestational sac." *J Clin Endocrinol Metab* 89 (3):1452-8. doi: 10.1210/jc.2003-031332.

Jauniaux, E., and B. Gulbis. 2000. "Fluid compartments of the embryonic environment." *Hum Reprod Update* 6 (3):268-78. doi: 10.1093/humupd/6.3.268.

Jauniaux, E., B. Gulbis, and G. J. Burton. 2003. "The human first trimester gestational sac limits rather than facilitates oxygen transfer to the foetus--a review." *Placenta* 24 Suppl A:S86-93. doi: 10.1053/plac.2002.0932.

Jones, C. J. 1997. "The life and death of the embryonic yolk sac." In *Embryonic medicine and therapy*, edited by E. Jauniaux, E. R. Barnea and R. G. Edwards, 180-196. Oxford: Oxford University Press.

Jones, C. J., and E. Jauniaux. 1995. "Ultrastructure of the materno-embryonic interface in the first trimester of pregnancy." *Micron* 26 (2):145-73. doi: 10.1016/0968-4328(95)00002-l.

Jones, C. J., J. N. Skepper, M. B. Renfree, and J. D. Aplin. 2014. "Trophoblast specialisations during pregnancy in the tammar wallaby, Macropus eugenii: a morphological and lectin histochemical study." *Placenta* 35 (7):467-75. doi: 10.1016/j.placenta.2014.03.018.

King, B. F., and A. C. Enders. 1970. "Protein absorption and transport by the guinea pig visceral yolk sac placenta." *Am J Anat* 129 (3):261-87. doi: 10.1002/aja.1001290303.

Kitchen, H., and I. Brett. 1974. "Embryonic and fetal hemoglobin in animals." *Ann N Y Acad Sci* 241:653-71. doi: 10.1111/j.1749-6632.1974.tb21921.x.

Laird, M. K., J. R. Dargan, L. Paterson, C. R. Murphy, B. M. McAllan, G. Shaw, M. B. Renfree, and M. B. Thompson. 2017. "Uterine molecular changes for non-invasive embryonic attachment in the marsupials Macropus eugenii (Macropodidae) and Trichosurus vulpecula (Phalangeridae)." *Mol Reprod Dev* 84 (10):1076-1085. doi: 10.1002/mrd.22861.

Laird, M. K., V. L. Hansen, B. M. McAllan, C. R. Murphy, and M. B. Thompson. 2020. "Uterine epithelial remodelling during pregnancy in the marsupial Monodelphis domestica (Didelphidae): Implications for mammalian placental evolution." *J Anat*. doi: 10.1111/joa.13162.

Laird, M. K., H. McShea, C. R. Murphy, B. M. McAllan, G. Shaw, M. B. Renfree, and M. B. Thompson. 2018. "Non-invasive placentation in the marsupials Macropus eugenii (Macropodidae) and Trichosurus vulpecula (Phalangeridae) involves redistribution of uterine Desmoglein-2." *Mol Reprod Dev* 85 (1):72-82. doi: 10.1002/mrd.22940.

Laird, M. K., M. Turancova, B. M. McAllan, C. R. Murphy, and M. B. Thompson. 2017. "Uterine focal adhesion dynamics during pregnancy in a marsupial (Sminthopsis crassicaudata; Dasyuridae)." *Anat Rec (Hoboken)* 300 (6):1150-1159. doi: 10.1002/ar.23535.

Luckett, W. P. 1974. "Comparative development and evolution of the placenta in primates " In *Reproductive biology of the primates*, edited by W. P. Luckett, 142-234. Basel: Karger.

Lyne, A. G. 1974. "Gestation period and birth in the marsupial Isoodon macrourus." *Aust J Zool* 22 (3):303-309.

Lyne, A.G., and D.E. Hollis. 1977. "The early development of marsupials, with special reference to bandicoots." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J. H. Calaby and C. H. Tyndale-Biscoe, 293-302. Canberra: Australian Academy of Science.

Ma, H., J. Zhai, H. Wan, X. Jiang, X. Wang, L. Wang, Y. Xiang, X. He, Z. A. Zhao, B. Zhao, P. Zheng, L. Li, and H. Wang. 2019. "In vitro culture of cynomolgus monkey embryos beyond early gastrulation." *Science* 366 (6467):eaax7890. doi: 10.1126/science.aax7890.

Menkhorst, E., A. Nation, S. Cui, and L. Selwood. 2009. "Evolution of the shell coat and yolk in amniotes: a marsupial perspective." *J Exp Zool B Mol Dev Evol* 312 (6):625-38. doi: 10.1002/jez.b.21235.

Menzies, B. R., T. B. Hildebrandt, and M. B. Renfree. 2020. "Unique reproduction strategy in the swamp wallaby." *Proc Natl Acad Sci U S A*. doi: 10.1073/pnas.1922678117.

Menzies, B. R., A. J. Pask, and M. B. Renfree. 2011. "Placental expression of pituitary hormones is an ancestral feature of therian mammals." *Evodevo* 2:16. doi: 10.1186/2041-9139-2-16.

Mess, A. M., and K. J. Ferner. 2010. "Evolution and development of gas exchange structures in Mammalia: the placenta and the lung." *Respir Physiol Neurobiol* 173 Suppl:S74-82. doi: 10.1016/j.resp.2010.01.005.

Moser, G., G. Weiss, M. Gauster, M. Sundl, and B. Huppertz. 2015. "Evidence from the very beginning: endoglandular trophoblasts penetrate and replace uterine glands in situ and in vitro." *Hum Reprod* 30 (12):2747-57. doi: 10.1093/humrep/dev266.

Mossman, H. W. 1987. *Vertebrate fetal membranes : comparative ontogeny and morphology; evolution; phylogenetic significance; basic functions; research opportunities*. New Brunswick: Rutgers University Press.

Murphy, W. J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W. de Jong, and M. S. Springer. 2001. "Resolution of the early placental mammal radiation using Bayesian phylogenetics." *Science* 294 (5550):2348-51. doi: 10.1126/science.1067179.

Niu, Y., N. Sun, C. Li, Y. Lei, Z. Huang, J. Wu, C. Si, X. Dai, C. Liu, J. Wei, L. Liu, S. Feng, Y. Kang, W. Si, H. Wang, E. Zhang, L. Zhao, Z. Li, X. Luo, G. Cui, G. Peng, J. C. Izpisua Belmonte, W. Ji, and T. Tan. 2019. "Dissecting primate early post-implantation development using long-term in vitro embryo culture." *Science* 366 (6467):eaaw5754. doi: 10.1126/science.aaw5754.

Opazo, J. C., F. G. Hoffmann, and J. F. Storz. 2008. "Genomic evidence for independent origins of beta-like globin genes in monotremes and therian mammals." *Proc Natl Acad Sci U S A* 105 (5):1590-5. doi: 10.1073/pnas.0710531105.

Oriol, J. G., F. J. Sharom, and K. J. Betteridge. 1993. "Developmentally regulated changes in the glycoproteins of the equine embryonic capsule." *J Reprod Fertil* 99 (2):653-64. doi: 10.1530/jrf.0.0990653.

Padykula, H. A., and J. M. Taylor. 1976. "Ultrastructural evidence for loss of the trophoblastic layer in the chorioallantoic placenta of Australian bandicoots (Marsupialia: Peramelidae)." *Anat Rec* 186 (3):357-85. doi: 10.1002/ar.1091860303.

Padykula, H.A., and J.M. Taylor. 1977. "Uniqueness of the bandicoot chorioallantoic placenta (Marsupialia: Peramelidae) cytological and evolutionary interpretations." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J. H. Calaby and C. H. Tyndale-Biscoe, 303-311. Canberra: Australian Academy of Science.

Perry, J. S. 1981. "The mammalian fetal membranes." *J Reprod Fertil* 62 (2):321-35. doi: 10.1530/jrf.0.0620321.

Perry, J. S., and I. W. Rowlands. 1962. "Early pregnancy in the pig." *J Reprod Fert* 4 (2):175-188.

Renfree, M. B. 1973. "The composition of fetal fluids of the marsupial Macropus eugenii." *Dev Biol* 33 (1):62-79. doi: 10.1016/0012-1606(73)90165-6.

Renfree, M. B. 2010. "Review: Marsupials: placental mammals with a difference." *Placenta* 31 Suppl:S21-6. doi: 10.1016/j.placenta.2009.12.023.

Renfree, M. B., and J. C. Fenelon. 2017. "The enigma of embryonic diapause." *Development* 144 (18):3199-3210. doi: 10.1242/dev.148213.

Renfree, M. B., and C. H. Tyndale-Biscoe. 1973. "Transferrin variation between mother and fetus in the marsupial, Macropus eugenii." *J Reprod Fertil* 32 (1):113-5. doi: 10.1530/jrf.0.0320113.

Roberts, C. T., and W. G. Breed. 1994a. "Embryonic-maternal cell interactions at implantation in the fat-tailed dunnart, a dasyurid marsupial." *Anat Rec* 240 (1):59-76. doi: 10.1002/ar.1092400107.

Roberts, C. T., and W. G. Breed. 1994b. "Placentation in the dasyurid marsupial, Sminthopsis crassicaudata, the fat-tailed dunnart, and notes on placentation of the didelphid, Monodelphis domestica." *J Reprod Fertil* 100 (1):105-13. doi: 10.1530/jrf.0.1000105.

Roberts, C. T., and W. G. Breed. 1996. "The marsupial shell membrane: an ultrastructural and immunogold localization study." *Cell Tissue Res* 284 (1):99-110. doi: 10.1007/s004410050570.

Roberts, V. H. J., T. K. Morgan, P. Bednarek, M. Morita, G. J. Burton, J. O. Lo, and A. E. Frias. 2017. "Early first trimester uteroplacental flow and the progressive disintegration of spiral artery plugs: new insights from contrast-enhanced ultrasound and tissue histopathology." *Hum Reprod* 32 (12):2382-2393. doi: 10.1093/humrep/dex301.

Romanoff, A.L., and A.J.S. Romanoff. 1967. *Biochemistry of the avian embryo : a quantitative analysis of prenatal development*. New York: Interscience.

Rossant, J., and J. C. Cross. 2001. "Placental development: lessons from mouse mutants." *Nat Rev Genet* 2 (7):538-48. doi: 10.1038/35080570.

Rowe, T., T. H. Rich, P. Vickers-Rich, M. Springer, and M. O. Woodburne. 2008. "The oldest platypus and its bearing on divergence timing of the platypus and echidna clades." *Proc Natl Acad Sci U S A* 105 (4):1238-42. doi: 10.1073/pnas.0706385105.

Rüsse, I., F. Sinowatz, L. Richter, M. Lehmann, and E. Schallenberger. 1992. "Die Entwicklung des Dottersackes beim Wiederkäuer (Schaf und Rind)." *Anat Histol Embryol* 21 (4):324-47. doi: 10.1111/j.1439-0264.1992.tb00464.x.

Selwood, L. 2000. "Marsupial egg and embryo coats." *Cells Tissues Organs* 166 (2):208-19. doi: 10.1159/000016733.

Semon, R. 1894. "Die Embryonalhüllen der Monotremen und Marsupialer " In *Zoologische Forschungsreisen in Australien und dem Malayischen Archipel Band 2*, edited by R. Semon, 19-58. Jena: Gustav Fischer.

Shahbazi, M. N., A. Jedrusik, S. Vuoristo, G. Recher, A. Hupalowska, V. Bolton, N. N. M. Fogarty, A. Campbell, L. Devito, D. Ilic, Y. Khalaf, K. K. Niakan, S. Fishel, and M. Zernicka-Goetz. 2016. "Self-organization of the human embryo in the absence of maternal tissues." *Nat Cell Biol* 18 (6):700-708. doi: 10.1038/ncb3347.

Sharman, G. B. 1961. "The embryonic membranes and placentation in five genera of diprotodont marsupials." *Proc Zool Soc Lond* 137:197-220.

Shaw, G., H. M. Gehring, and E. C. Bell. 1999. "Production of prostaglandin f2alpha and its metabolite by endometrium and yolk sac placenta in late gestation in the tammar wallaby, Macropus Eugenii." *Biol Reprod* 60 (3):611-4. doi: 10.1095/biolreprod60.3.611.

Smith, K. K., and A. L. Keyte. 2018. "Adaptations of the marsupial newborn: birth as an extreme environment." *Anat Rec (Hoboken)*. doi: 10.1002/ar.24049.

Sobotta, J. 1911. "Die Entwicklung des Eies der Maus vom ersten Auftreten des Mesoderms an bis zur Ausbildung der Embryonalanlage und dem Auftreten der Allantois. I. Teil: Die Keimblase." *Arch Mikrosk Anat* 78:271-352.

Stringer, J. M., S. Suzuki, A. J. Pask, G. Shaw, and M. B. Renfree. 2012a. "Promoter-specific expression and imprint status of marsupial IGF2." *PLoS One* 7 (7):e41690. doi: 10.1371/journal.pone.0041690.

Stringer, J. M., S. Suzuki, A. J. Pask, G. Shaw, and M. B. Renfree. 2012b. "Selected imprinting of INS in the marsupial." *Epigenetics Chromatin* 5 (1):14. doi: 10.1186/1756-8935-5-14.

Tavian, M., K. Biasch, L. Sinka, J. Vallet, and B. Peault. 2010. "Embryonic origin of human hematopoiesis." *Int J Dev Biol* 54 (6-7):1061-5. doi: 10.1387/ijdb.103097mt.

Terasawa, Y., S. J. Cases, J. S. Wong, H. Jamil, S. Jothi, M. G. Traber, L. Packer, D. A. Gordon, R. L. Hamilton, and R. V. Farese, Jr. 1999. "Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development." *J Lipid Res* 40 (11):1967-77.

Tyndale-Biscoe, C. H., and M. Renfree. 1987. *Reproductive Physiology of Marsupials*. Cambridge: Cambridge University Press.

Waelchli, R. O., and K. J. Betteridge. 1996. "Osmolality of equine blastocyst fluid from day 11 to day 25 of pregnancy." *Reprod Fertil Dev* 8 (6):981-8. doi: 10.1071/rd9960981.

Waelchli, R. O., and K. J. Betteridge. 2013. "Morphology of twin and triplet equine conceptuses during weeks 3 and 4 of pregnancy." *Reprod Fertil Dev* 25 (5):762-74. doi: 10.1071/RD12067.

Werneburg, I., M. Laurin, D. Koyabu, and M. R. Sanchez-Villagra. 2016. "Evolution of organogenesis and the origin of altriciality in mammals." *Evol Dev* 18 (4):229-44. doi: 10.1111/ede.12194.

Whittington, C. M., D. O'Meally, M. K. Laird, K. Belov, M. B. Thompson, and B. M. McAllan. 2018. "Transcriptomic changes in the pre-implantation uterus highlight histotrophic nutrition of the developing marsupial embryo." *Sci Rep* 8 (1):2412. doi: 10.1038/s41598-018-20744-z.

Wilson, D. E., and D. M. Reeder. 2005. *Mammal species of the world : a taxonomic and geographic reference*. 3rd ed. Baltimore, Md.: Johns Hopkins University Press.

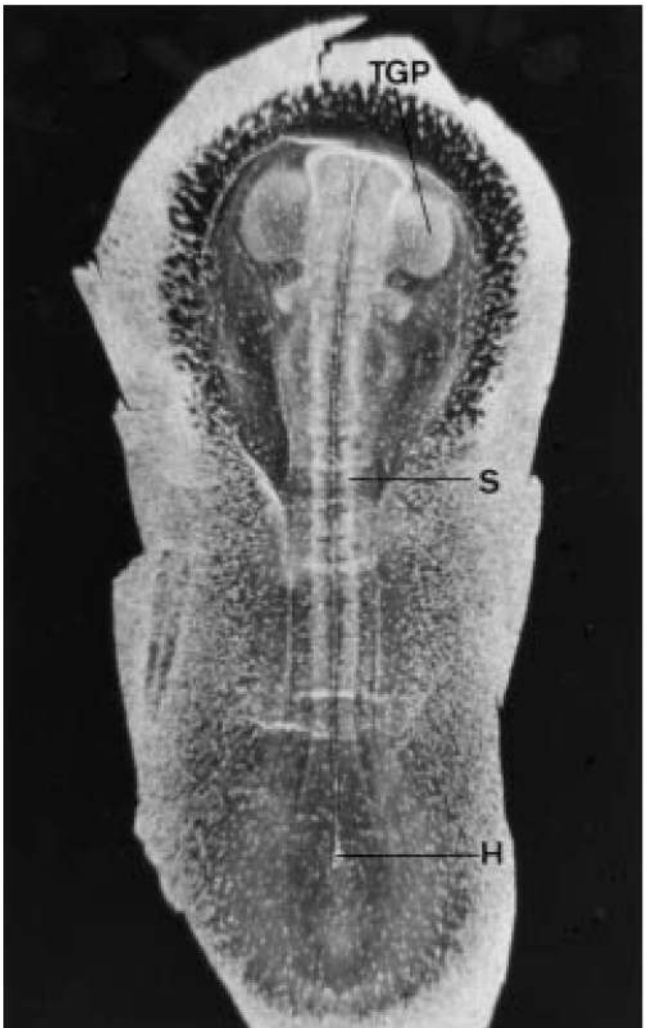
Young, D., A. R. Klemm, D. A. Beckman, R. L. Brent, and J. B. Lloyd. 1997. "Uptake and processing of 59Fe-labelled and 125I-labelled rat transferrin by early organogenesis rat conceptuses in vitro." *Placenta* 18 (7):553-62. doi: 10.1016/0143-4004(77)90010-8.

Zohn, I. E., and A. A. Sarkar. 2010. "The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation." *Birth Defects Res A Clin Mol Teratol* 88 (8):593-600. doi: 10.1002/bdra.20705.

TABLE 1 Marsupial orders and model species. Orders and suborders from (Wilson and Reeder 2005). The species shown are those featured in the recent literature.

Order and suborder	Species	Common name
Didelphimorphia	*Monodelphis domestica*	Grey Short-Tailed Opossum
	Philander opossum	Grey Four-Eyed Opossum
Paucituberculata	None	
Microbiotheria	None	
Notoryctemorphia	None	
Dasyuromorphia	*Sminthopsis crassicaudata*	Fat-Tailed Dunnart
Peramelemorphia	*Isoodon macrourus*	Northern Brown Bandicoot
	Isoodon obesulus	Southern Brown Bandicoot
	Perameles nasuta	Long-nosed Bandicoot
Diprotodontia: Vombatiformes	*Phascolarctos cinereus*	Koala
	Vombatus ursinus	Common wombat
Diprotodontia: Phalangeriformes	*Trichosurus vulpecula*	Common Brushtail Possum
	Pseudocheirus peregrinus	Common Ringtail Possum
Diprotodontia: Macropodiformes	*Potorous tridactylus*	Long-Nosed Potoroo
	Notamacropus eugenii	Tammar Wallaby

Yolk sac and chorioallantoic placentas exist side by side in the hedgehog tenrec



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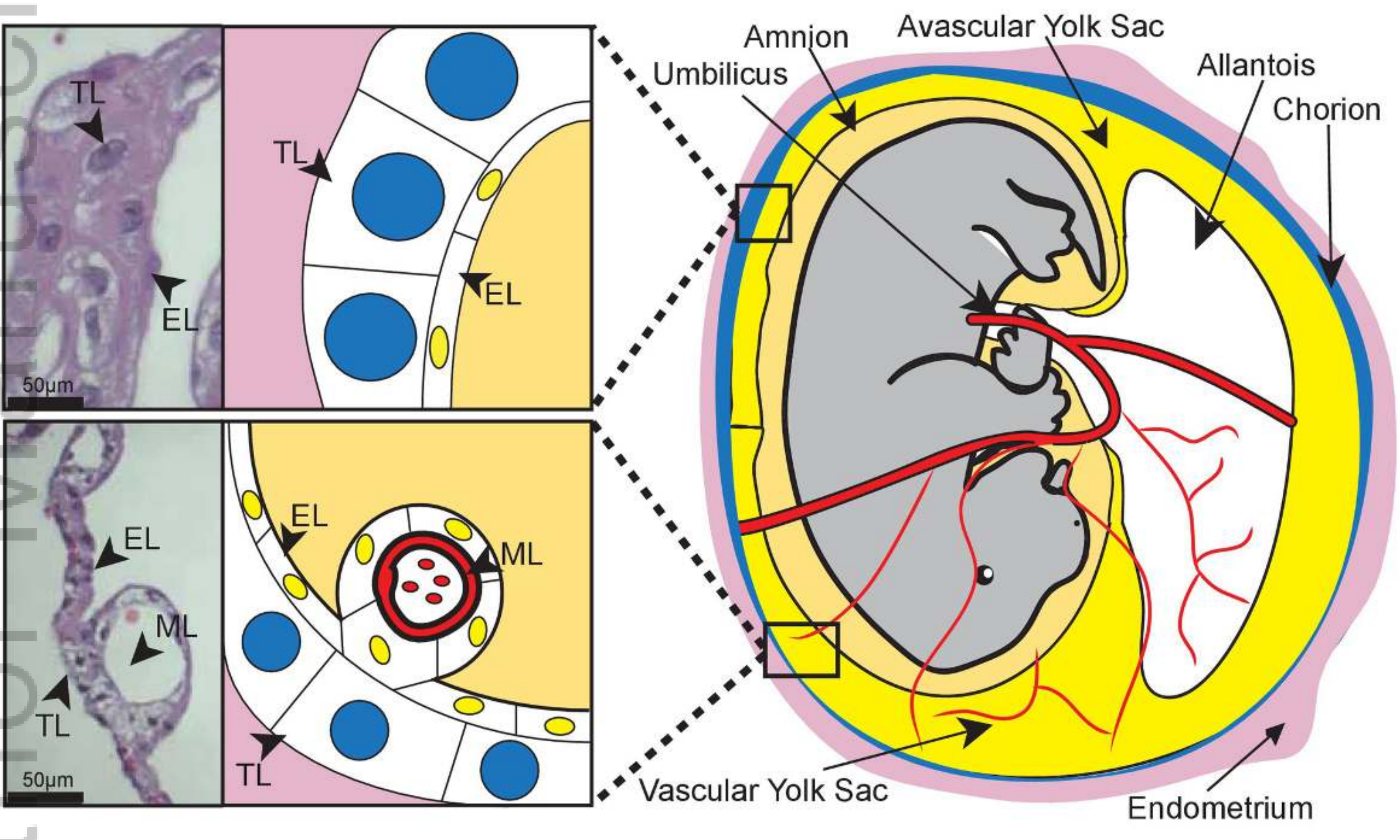

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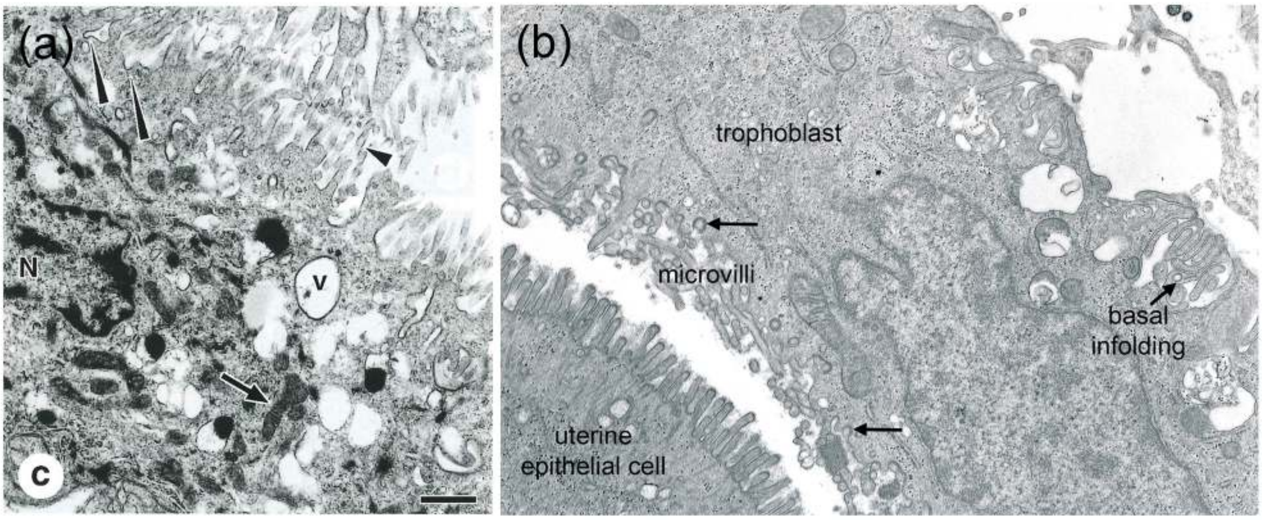

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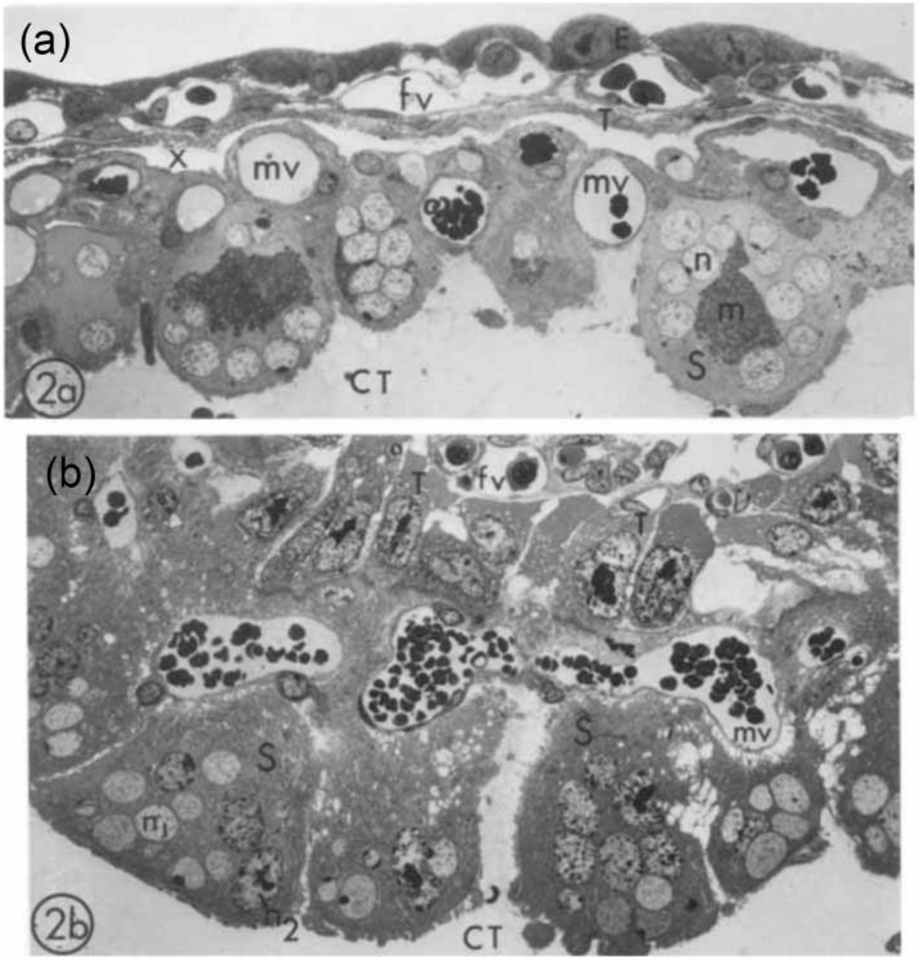

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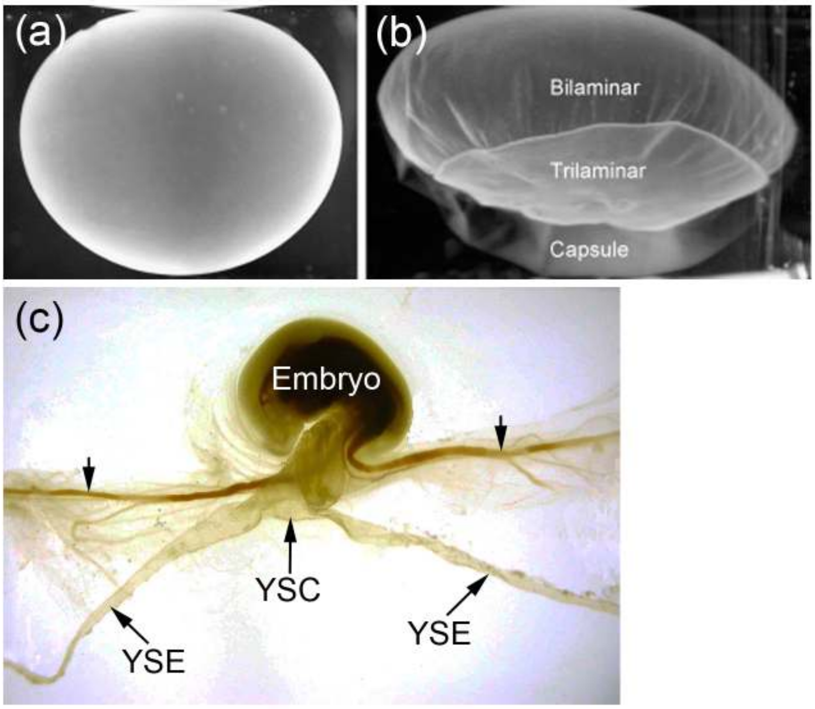

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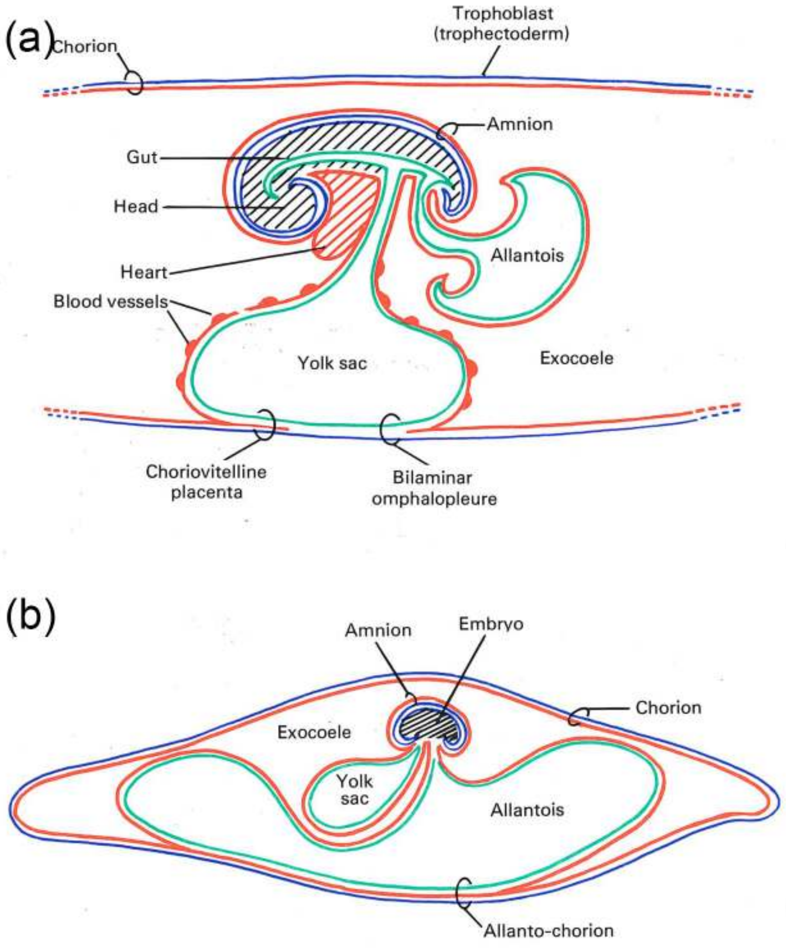

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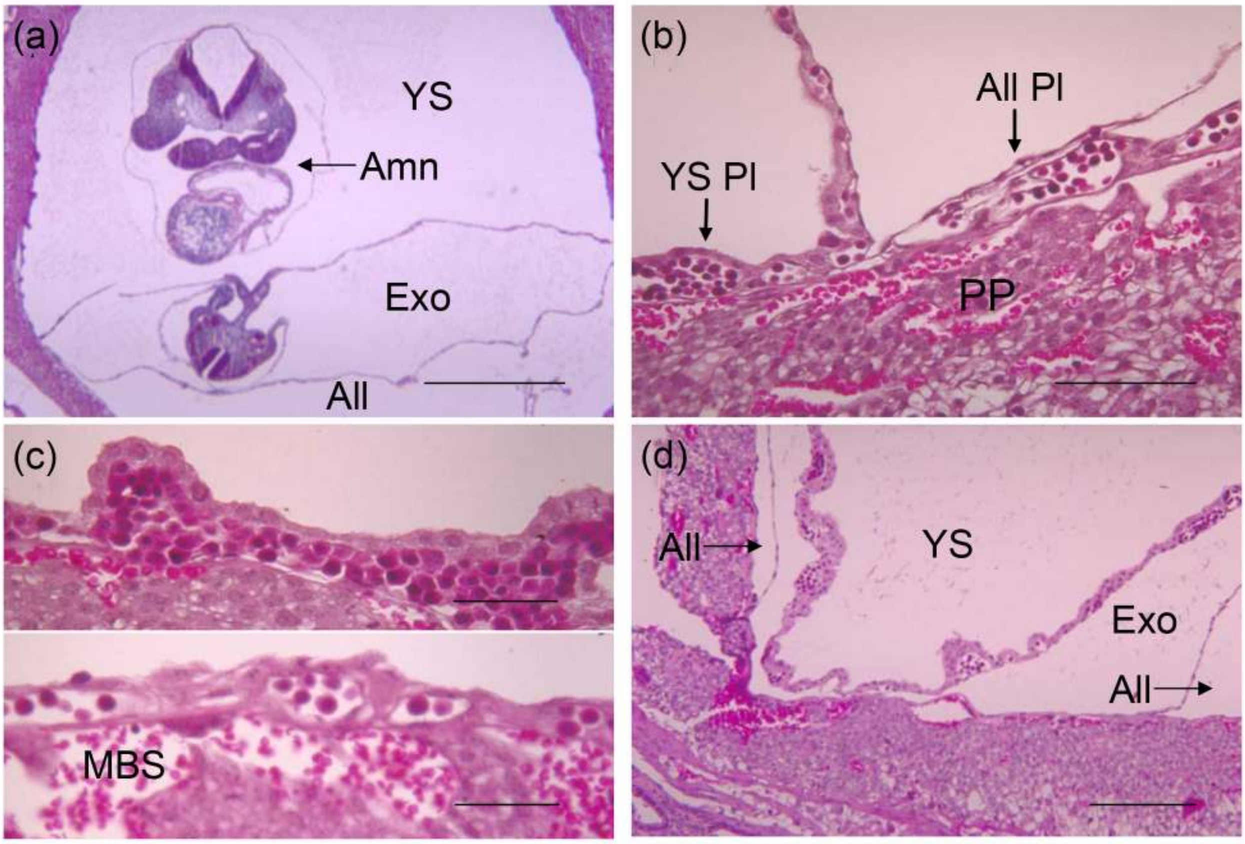

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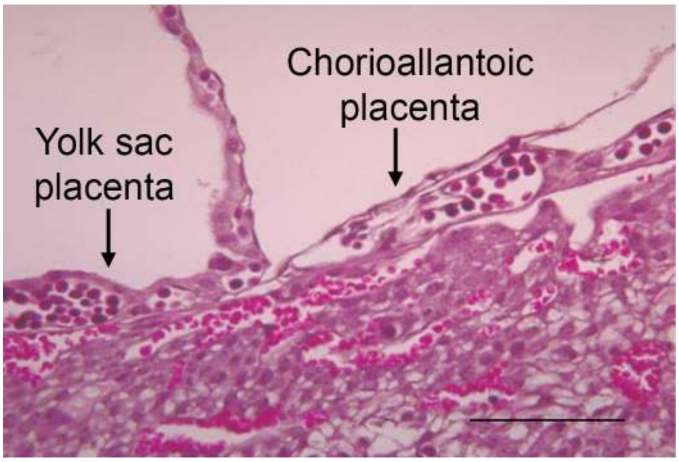

JMOR_21140_Carter Graphic Abstract.tif

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	Notamacropus eugenii	Tammar Wallaby

The role of mammalian fetal membranes in early embryogenesis: lessons from marsupials

Running title: Mammalian fetal membranes

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Abstract

Across mammals early embryonic development is supported by uterine secretions taken up through the yolk sac and other fetal membranes (histotrophic nutrition). The marsupial conceptus is enclosed in a shell coat for the first two-thirds of gestation and nutrients pass to the embryo through the shell and the avascular bilaminar yolk sac. At around the time of shell rupture, part of the yolk sac is trilaminar and supplied with blood vessels. It attaches to the uterus and forms a choriovitelline placenta. Rapid growth of the embryo ensues, still supported by histotrophe as well as exchange of oxygen and nutrients between maternal and fetal blood vessels (haemotrophic nutrition). Few marsupials have a chorioallantoic placenta and the highly altricial newborn is delivered after a short gestation. Eutherian embryos pass through a similar sequence before there is a fully functional chorioallantoic placenta. In most orders there is transient yolk sac placentation, but even before this, nutrients are transferred through an avascular yolk sac. Yolk sac placentation does not occur in rodents or catarrhine primates. Early embryonic development in the mouse is nonetheless dependent on histotrophic nutrition. In the first trimester of human pregnancy, uterine glands open to the intervillous space and secretion products are taken up by the trophoblast. Transfer of nutrients to the early human embryo also involves the yolk sac, which floats free in the exocoelom. Marsupials can therefore inform us about the role of fetal membranes and histotrophic nutrition in early embryogenesis, knowledge that can translate to eutherians.

Keywords:

Histotrophic nutrition

Placentation

Shell coat

Uterine glands

Yolk sac

Research highlights

Early embryogenesis in marsupials is maintained by uterine secretions absorbed initially via an avascular yolk sac.

After loss of the shell coat in marsupials, organogenesis is supported by the yolk sac placenta.

Eutherians similarly depend on histotrophic nutrition for the early phases of embryogenesis.

Graphical abstract image

Yolk sac and chorioallantoic placentas side by side in the hedgehog tenrec

1 INTRODUCTION

Significant progress has been made in researching the initial phases of embryonic development in mammals. The key has been the development of in vitro systems to culture embryos for two weeks in mouse (Bedzhov et al. 2014, Bedzhov and Zernicka-Goetz 2014) and human (Shahbazi et al. 2016, Deglincerti et al. 2016) and for three weeks in crab-eating macaques (*Macaca fascicularis*) (Ma et al. 2019, Niu et al. 2019). The genes expressed during differentiation of trophectoderm, epiblast and primitive ectoderm (hypoblast) have been mapped as well as the first steps in formation of amnion and yolk sac.

There remains an interval of days or weeks between these events and the establishment of a chorioallantoic placenta that is poorly understood. In human development there is a “black box” spanning the interval from implantation to 5-6 weeks of pregnancy when access to samples is limited for ethical reasons (James, Carter, and Chamley 2012). Indeed, there is a palpable lack of curiosity about the role played by fetal membranes in support of early human development. Comparative studies can be helpful but also confusing as there are three different patterns of fetal membrane development in eutherian mammals (Carter 2016). Thus, transient yolk sac placentation occurs in most eutherian orders, but not in the mouse, where an inverted yolk sac continues to function through term, nor in the human, where the secondary yolk sac floats free in the exocoelom during the first trimester.

In whatever way the membranes develop, we need to ask how the eutherian embryo is nourished through the earliest stages of its development. Later the chorioallantoic placenta permits exchange of nutrients between maternal and fetal blood, which is known as haemotrophic nutrition. In principle this can occur at earlier stages with a choriovitelline placenta, since the yolk sac has its own set of blood vessels. However, I shall argue that absorption of uterine gland secretions and other maternal products is more important. This is known as histotrophic nutrition (Enders and Carter 2006). As will be discussed, there is data to show uptake of histotrophe by the inverted yolk sac during early embryonic development in rodents and lagomorphs. In the first trimester of human pregnancy, there is uterine gland secretion to the intervillous space of the placenta and uptake of the secretion products by the trophoblast (Burton et al. 2002, Hempstock et al. 2004).

Marsupials deliver highly altricial neonates after a short gestation. Embryonic development is supported by uterine secretions absorbed, during the first two-thirds of gestation, through the shell coat and an avascular yolk sac and in the remaining third, where most of organogenesis occurs, by both avascular and vascular regions of the yolk sac (Tyndale-Biscoe and Renfree 1987, Guernsey et al. 2017, Renfree 1973). Chorioallantoic placentation is uncommon in marsupials although known from bandicoots, wombats and the koala (Tyndale-Biscoe and Renfree 1987). Thus, studies of fetal membranes in marsupials necessarily deal with the same structures that support early embryonic development in eutherians. Indeed, they have been more extensively studied in marsupials and the present review is predicated on my belief that this body of work can suggest avenues of exploration for early embryonic development in eutherians. Therefore, after a brief digression on monotremes, close consideration will be given to the fetal membranes of marsupials. Attention will then be directed to selected examples of eutherian mammals for which there is data to support the critical role of histotrophic nutrition in early embryogenesis.

2 MONOTREMES

Monotremes are relicts of an ancient radiation. They last shared a common ancestor with therians in the Early Jurassic (Huttenlocker et al. 2018). In addition, there is deep divergence between the extant echidnas (*Tachyglossus aculeatus*; *Zaglossus* spp.) and the platypus (*Ornithorhynchus anatinus*) (Rowe et al. 2008). Nonetheless, it is instructive to consider the role of monotreme fetal membranes in embryonic development (Hughes and Hall 1998, Hughes 1993, Flynn and Hill 1947, 1939). Thus, although monotremes are oviparous (Haacke 1885), fully two-thirds of development takes place in the uterus, where the egg grows in size through the absorption of uterine secretions (Hughes 1993). In the platypus at full term (oviposition), there is a neurula-stage embryo with 19-20 somites (Figure 1) (Hughes and Hall 1998). Although there is yolk in the ovulated egg and cleavage is meroblastic (Flynn and Hill 1939), histotrophic nutrition from uterine secretions is more important than autotrophic nutrition from the yolk. The secretions are taken up by the yolk sac through the leathery eggshell coat, which is about 70 µm thick (Hughes 1993). The abembryonic pole of the yolk sac is bilaminar whilst the embryonic pole is trilaminar but lacks blood vessels. The full-term embryo has neither an allantoic sac nor an amnion (Hughes 1993, Hughes and Hall 1998). After oviposition and during incubation, however, the trilaminar omphalopleure and the allantoic sac become richly vascularized with the latter acting as an organ of gas exchange (Semon 1894).

The lessons to be drawn are that an eggshell is no barrier to absorption of fetal secretions and that histotrophic nutrition can occur in the absence of a fetal circulation. This is relevant to the understanding of early development in both marsupials and eutherians.

3 MARSUPIALS

There are 21 families of marsupials (Wilson and Reeder 2005), but most knowledge of their reproductive biology is derived from a handful of species (Table 1).

In marsupials, the length of gestation rarely exceeds that of the oestrous cycle (Tyndale-Biscoe and Renfree 1987) whereas eutherian gestation always exceeds cycle length (Chavan, Bhullar, and Wagner 2016). There is a range of altriciality in marsupials (Smith and Keyte 2018, Hughes and Hall 1988), but all marsupials are born at a less developed stage than any eutherian (Werneburg et al. 2016).

An egg shell coat is present for two-thirds of gestation and intimate contact between the yolk sac and uterine lining is first achieved around the time of organogenesis (Menkhorst et al. 2009, Tyndale-Biscoe and Renfree 1987). It was shown in the grey short tailed opossum, that rupture of the egg shell triggers an inflammatory response that may be a limiting factor for prolonged gestation (Griffith, Chavan, et al. 2017). This response has been modified in eutherians with upregulation of genes favouring implantation and downregulation of others (Griffith, Chavan, et al. 2017, Erkenbrack et al. 2018).

A feature of reproduction in several marsupials, including many kangaroos and wallabies, is embryonic diapause (Tyndale-Biscoe and Renfree 1987, Renfree and Fenelon 2017). In these marsupials, the female goes into oestrus shortly parturition (or even before (Menzies, Hildebrandt, and Renfree 2020)). If this leads to a new pregnancy, embryonic development is arrested at the 100-cell stage and resumed only when the young in the pouch has been weaned (lactational diapause). Researchers can exploit this by removing the pouch young to obtain precisely dated uterine embryos. If conception occurs towards the end of the breeding season, the embryo may remain in diapause for up to eleven months (photoperiod-induced diapause) (Renfree and Fenelon 2017).

3.1 Marsupial membranes

The tertiary egg membranes are those added during passage of the egg through the genital tract. They occur in many amniotes and even non-amniotes. The mucoid coat is added by glands in the Fallopian tube. It is clearly seen around uterine eggs in the early stages of cleavage, but rarely persists (Hughes 1977). The shell coat is secreted by the uterine glands (Roberts and Breed 1996, Tyndale-Biscoe and Renfree 1987) and is present throughout the first two-thirds of gestation (Selwood 2000, Renfree 1973). Indeed, marsupial development can be divided into two stages: before and after rupture of the shell coat (Tyndale-Biscoe and Renfree 1987). The first stage, embryogenesis, is relatively slow. In contrast, rapid development and growth (organogenesis) occur after rupture of the shell coat and apposition of the yolk sac to the uterine wall as a choriovitelline placenta.

As in other amniotes, the fetal membranes of marsupials comprise amnion, yolk sac, allantois and chorion. Extraembryonic endoderm and trophoblast constitute the bilaminar omphalopleure, which is the main conduit for maternal-fetal exchange prior to shell rupture. It is converted to the trilaminar omphalopleure by expansion of the extraembryonic mesoderm within which blood vessels appear shortly before shell rupture. Apposition of the vascular trilaminar omphalopleure to the uterine epithelium is the basis for choriovitelline or yolk sac placentation, which supports the final phase of rapid growth. In the following I shall refer to these membranes as the avascular and vascular yolk sac. The fetal membranes of the tammar wallaby (*Notamacropus eugenii*) in late gestation are shown in Figure 2 (Guernsey et al. 2017).

The trophoblast is moderately invasive in many marsupials including the four-eyed opossum (*Philander opossum*) (Enders and Enders 1969) and fat-tailed dunnart (*Sminthopsis crassicaudata*) (Roberts and Breed 1994a). Recent studies favour the view that the common ancestor of marsupials had invasive trophoblast, whereas completely non-invasive trophoblast, as in the tammar wallaby and common brushtail possum (*Trichosurus vulpecula*), is a derived state (Laird et al. 2018, Mess and Ferner 2010, Laird et al. 2020).

The allantoic sac has its own blood supply but, in most marsupials, does not make contact with the uterine lining. Indeed, it is often hidden in folds of the yolk sac membranes (Tyndale-Biscoe and Renfree 1987). However, true chorioallantoic placentation does occur in bandicoots (*Perameles nasuta*, *P. gunnii*, *Isoodon obesulus*, *I. macrourus*), where fetal and maternal capillaries are separated by a thin layer of syncytium (see below). A chorioallantoic placenta is also established in the koala (*Phascolarctos cinereus*) (Hughes 1974, Semon 1894) and the closely related wombat (*Vombatus ursinus*) (Hughes and Green 1998).

3.2 Uterine responses to pregnancy

Several studies have probed the uterine responses to pregnancy. A redistribution of desmosomes and associated claudins has been noted in the plasma membrane of epithelial cells in the fat-tailed dunnart (Dudley et al. 2015, Buddle et al. 2019) as well as tammar wallaby and brushtail possum (Laird et al. 2018). This response was comparable to that preceding attachment in viviparous squamates and eutherians (Dudley et al. 2015). There was evidence in the three marsupials of a strengthening of the basal plasma membrane (Dudley et al. 2017, Laird et al. 2018, Laird, Turancova, et al. 2017). Thus, comparable changes occurred in a species with invasive trophoblast (the dunnart) and two with non-invasive placentation. Based on the hypothesis that invasive placentation is the ancestral state for marsupials (Mess and Ferner 2010, Freyer, Zeller, and Renfree 2003), it was suggested that strengthening of the basal membrane prevented peeling off of the epithelium during trophoblast invasion; however, it could also have facilitated the transition to a non-invasive state (Laird, Dargan, et al. 2017).

A second approach was to analyse the transcriptomic changes occurring in the uterus of the fat-tailed dunnart prior to rupture of the cell membrane (Whittington et al. 2018). Importantly, many of the top 50 upregulated genes were associated with nutrient transport, underlining the importance of histotrophe secretion in the first two-thirds of embryonic development. A second data set from the grey short-tailed opossum found an upregulation of the same category of genes even after shell rupture (Griffith et al. 2019). Prior to shell rupture in the dunnart, there was some indication of a downregulation of genes with a putative immune function (Whittington et al. 2018). Nevertheless, the overriding impression from the opossum was that exposure to fetal tissue after shell rupture triggered an inflammatory response (Erkenbrack et al. 2018, Griffith et al. 2019).

3.3 Histotrophic nutrition

Uterine gland secretions are the principal source of nutrition prior to shell rupture. The shell coat is permeable to the histotrophe, which is taken up by the bilaminar omphalopleure and later by the trilaminar omphalopleure (Sharman 1961). In macropodids such as the tammar wallaby, which has a relatively long gestation period, uterine gland secretion continues after shell rupture (Freyer, Zeller, and Renfree 2007). A different pattern was found in the grey short-tailed opossum (*Monodelphis domestica*), where a decrease in uterine gland secretion occurred even before shell rupture, suggesting a phase when embryonic growth was supported by nutrients stored in the yolk sac (Freyer, Zeller, and Renfree 2007). Surveying the available evidence, Freyer and colleagues found this pattern was the most widespread and concluded it likely was the ancestral condition (Freyer, Zeller, and Renfree 2002, 2003).

In the four-eyed and grey short-tailed opossums, the yolk sacs of adjacent embryos fuse (although each retains a separate allantois). Formation of a common yolk sac cavity entails a reduction in surface area of the bilaminar yolk sac (Enders and Enders 1969, Freyer, Zeller, and Renfree 2002). This is also the case in the fat-tailed dunnart (Roberts and Breed 1994b). In the tammar wallaby, the trilaminar yolk sac expands from one-third to one-half of the total surface area (Freyer, Zeller, and Renfree 2002). Therefore, it is likely that both parts of the yolk sac are important for uptake of histotrophe, a conclusion strengthened by ultrastructural observations. Thus, the apical surface of the trophoblast is characterized by microvilli, coated pits and vesicles in both the tammar wallaby and the four-eyed opossum (Figure 3) (Enders and Enders 1969, Freyer, Zeller, and Renfree 2002). Nevertheless, the trophoblast associated with the bilaminar yolk sac of the tammar wallaby comprises larger cells than that in the trilaminar area and with a greater number of lysosomes containing degraded histotrophe (Jones et al. 2014).

3.4 Respiratory gas exchange

The oxygen requirements for early embryogenesis are small. Thus a 3-day chick embryo requires just 3.8 µl min^{-1} (Romanoff and Romanoff 1967). The rapid growth of the marsupial embryo following shell rupture necessarily implies an increase in oxygen consumption. However, oxygen consumption is still quite moderate, about 6 µL min^{-1}, in the newborn pouch young of the tammar wallaby (Baudinette et al. 1988, Frappell 2008).

Early descriptions of marsupial fetal membranes assumed a role for the vascular yolk sac in respiratory gas exchange (e.g. (Hill 1900)). The trophoblast layer is thinner in the trilaminar than in the bilaminar yolk sac of the tammar wallaby (Freyer, Zeller, and Renfree 2002, Jones et al. 2014) and this reduction in diffusion distance would facilitate oxygen transfer (Carter 2015).

The early mammalian embryo needs protection from high oxygen levels because of the threat posed by reactive oxygen species (Burton 2009). Eutherian embryos have embryonic haemoglobins with high oxygen affinities that sequester oxygen and may protect against high oxygen tensions (Kitchen and Brett 1974). Marsupial neonates have several embryonic haemoglobins (Holland and Gooley 1997). However, the oxygen dissociation curve of neonatal blood does not differ from that of adult blood (Henty, Wells, and Brittain 2008). The evolution of alpha and beta globin genes has pursued different paths in marsupials and eutherians (Holland, Gooley, and Hope 1998, Opazo, Hoffmann, and Storz 2008). Nevertheless, the apparent absence of high affinity haemoglobins in marsupial embryos raises the question of whether they mount other defences against reactive oxygen species such as antioxidant enzymes (Burton 2009) or scavenging of reactive oxygen species by tissue globins (Fago et al. 2004).

3.5 Haemotrophic nutrition

The passage of nutrients from maternal to fetal blood vessels, called haemotrophic nutrition, is a principal function of the eutherian placenta. After shell rupture in marsupials, the interhaemal barrier separating fetal capillaries of the vascular yolk sac from uterine capillaries can be rather thin as has been shown for the grey short-tailed opossum (Freyer, Zeller, and Renfree 2007) and long-nosed bandicoot (Figure 4 (a)) (Padykula and Taylor 1976). However, the physiological evidence for haemotrophic nutrition is scant. In the tammar wallaby, the composition of yolk sac fluid was found to resemble uterine secretions until rupture of the shell coat (Renfree 1973). The proteins that then appeared, such as transferrin, were not maternal in origin and probably were made by the fetus (Renfree 1973, Renfree and Tyndale-Biscoe 1973). Recent studies of gene expression give further insight (Guernsey et al. 2017). Genes upregulated in the bilaminar yolk sac fall into ontologies associated with nutrient uptake, whereas those upregulated in the trilaminar yolk sac are associated with respiration (Guernsey et al. 2017).

As already noted, a chorioallantoic placenta is found in the koala, wombats and bandicoots. The interhaemal region of the long-nosed bandicoot (*Perameles nasuta*) and southern brown bandicoot (*Isoodon obesulus*) has been examined by transmission electron microscopy (Padykula and Taylor 1976, Padykula and Taylor 1977). The fetal and maternal capillaries are at first separated by trophoblast and the uterine lining, which is a syncytium formed by fusion of the epithelial cells (Figure 4 (b)). Later this is reduced to a single layer apparently by fusion of trophoblast and syncytium. Although this was seen as evidence for haemotrophic nutrition (Padykula and Taylor 1977), it did not translate into greater maturity of the neonate (Hughes and Hall 1988, Lyne and Hollis 1977). Rather it allowed shortening of the gestation period. At just over 12 days, the northern brown bandicoot (*I. macrourus*) holds the record for the shortest gestation of any mammal (Lyne 1974).

3.6 Hormone secretion

Hormone-related genes are expressed by the fetal membranes of oviparous and viviparous amniotes (Griffith, Brandley, et al. 2017). Notwithstanding, knowledge of the endocrine function of the marsupial yolk sac is based largely on work in the tammar wallaby. Although the yolk sac can convert pregnenolone to progesterone, it likely is not a significant source of steroid hormones (Heap, Renfree, and Burton 1980). It does, however, express several peptide hormones and growth factors, including luteinizing hormone, prolactin and growth hormone as well as insulin-like growth factor-2 and its receptor (Menzies, Pask, and Renfree 2011). Luteinizing hormone could be the signal for pregnancy maintenance perhaps acting in concert with prolactin (Menzies, Pask, and Renfree 2011). Growth hormone, GH receptor and IGF-2 are upregulated in the later phase of organogenesis that follows shell rupture and may act to promote fetal growth (Menzies, Pask, and Renfree 2011).

An interesting form of epigenetic gene regulation, first shown in eutherians, is genomic imprinting, which results in silencing of one of the parental alleles. In marsupials, insulin (*INS*) is paternally expressed in the vascular yolk sac and otherwise only in mammary gland (Ager et al. 2007, Stringer et al. 2012b). Similarly, insulin-like growth factor-2 (*IGF2*) is paternally expressed in the vascular yolk sac and has been ascribed a role in promoting vascularization by increasing expression of vascular endothelial growth factor (Stringer et al. 2012a, Ager et al. 2008).

Finally, the yolk sac of the tammar wallaby produces large amounts of prostaglandin $F_{2\alpha}$ in the last few days of gestation, peaking at term (Shaw, Gehring, and Bell 1999). Although the endometrium also secretes prostaglandins, the fetal signal is thought to initiate parturition (Shaw, Gehring, and Bell 1999, Renfree 2010).

4 EUTHERIANS

Uterine gland secretions have long been recognized as an essential supplement to haemotrophic nutrition via the chorioallantoic placenta of eutherians (Burton 1982, Enders and Carter 2006). Uptake of secretions, cell debris and maternal erythrocytes is by pinocytosis and endocytosis to columnar trophoblast cells often organized in structures such as the chorionic vesicles of lower primates or the areolae of swine, horse and moles (Enders and Carter 2006). Here we are concerned with the role of histotrophic nutrition in the early stages of embryonic development before there is circulation in the chorioallantoic placenta. An overview of fetal membrane development is given elsewhere (Carter 2016).

4.1 The avascular yolk sac of the horse

Early embryonic development has been well studied in the horse (*Equus caballus*) since the details are important for assisted reproductive technologies (Betteridge 2007). The equine embryo remains spherical in shape and moves about the uterus until about day 17 after ovulation. Between about days 7 and 20 it is enclosed in a capsule (Figure 5 (a)). This is not a tertiary egg membrane, like the marsupial shell coat. It is made up of mucin-like glycoproteins secreted by the trophoblast (Oriol, Sharom, and Betteridge 1993). It is not until dissolution of the capsule around day 21 that there is contact with the endometrium (Betteridge 2007). The conceptus expands during this interval and the capsule with it. The capsule is not a barrier to transfer. Within the capsule is a bilaminar yolk sac comprised of trophoblast and endoderm. The mural trophoblast is endocytotic as indicated by the transfer of tracers (ferritin and peroxidase) from coated pits and tubules to large vacuoles (Enders et al. 1988). Extraembryonic mesoderm appears relatively late and a vascular, trilaminar yolk sac is not present until shortly before the capsule dissolves (Figure 5 (b)). However, the embryo is well formed by the time the capsule is lost and there is then an effective embryonic circulation (Enders et al. 1993). From 22 days embryonic development is supported by a yolk sac placenta (Enders 2015, Enders et al. 1993). A fully functional chorioallantoic placenta is not established until the end of the first month of gestation (Allen 2001).

Importantly, while the conceptus is enclosed in its capsule yolk sac volume increases over time (Betteridge 2007, Waelchli and Betteridge 1996, Betteridge et al. 1982). Osmolarity, fructose concentration and protein concentration remain constant or increase slightly, but do not fall with increasing volume, showing there must be uptake of solutes from the uterine fluid (Betteridge 2007). The role of the avascular yolk sac in this process was elegantly demonstrated by studies of twinning in horses (Waelchli and Betteridge 2013). If there are two embryos in the capsule and one abuts the membranes of the other, the second twin is unable to survive, presumably because it cannot absorb uterine secretions. With correct orientation of the blastocysts, both can take up nutrients and the twins may survive. This clearly demonstrates that the bilaminar yolk sac has a vital function just as it does in marsupials before egg cell rupture.

4.2 Yolk sac placentas of artiodactyls and tenrecs

A temporary yolk sac or choriovitelline placenta supports embryonic development in most orders of eutherian mammal (Carter 2016). A strict definition requires that this is a vascular yolk sac in intimate apposition to the endometrium (Mossman 1987). As in marsupials, however, the yolk sac may take up histotrophe well before such an attachment is formed. This section will give three examples of how the yolk sac and other membranes support early development. A list of eutherian orders with transient yolk sac placentation is given elsewhere (Carter 2016). Yolk sac placentation is not found in haplorrhine primates, rodents, lagomorphs, armadillos or sloths (Carter 2016).

In the pig (*Sus scrofa*), the blastocyst undergoes a remarkable elongation at 10.5-12 days of gestation, reaching about 1 meter in length and 0.75 mm in diameter (Perry 1981, Perry and Rowlands 1962, Heuser 1927). The elongated part of the blastocyst is a diverticulum of the exocoelom lined by chorion. The chorion participates in maternal-fetal signalling and possibly in absorption of histotrophe secreted by the uterine glands (Bazer and Johnson 2014). At the centre is the embryo and yolk sac, the distal part of which is bilaminar, and the remainder supplied with blood vessels. This likely transfers nutrients to the rapidly developing embryo. Adherence to the uterine wall occurs at 18 days, resulting in transient yolk sac placentation (Figure 6 (a)). At this stage the allantois is just a bud protruding into the exocoelom. However, by 21 days the vascular allantois has displaced the yolk sac from trophoblast and begun to form a chorioallantoic placenta (Figure 6 (b)) (Perry 1981, Heuser 1927).

In sheep (*Ovis aries*) and cattle (*Bos taurus*, *B. indicus*), elongation of the chorion is less drastic than in the pig, but its two arms are occupied by the yolk sac (Figure 5 (c)) (Rüsse et al. 1992, Assis Neto et al. 2010). As first shown for the sheep, early expansion of the exocoelom hinders contact between yolk sac and endometrium (Assheton 1906). This configuration does not meet the above definition of a yolk sac placenta, but the uterine gland secretions cross the chorion and yolk sac to supply the embryo.

Horses, pigs and cattle belong to the same eutherian clade (Laurasiatheria). My third example, therefore, is the lesser hedgehog tenrec (*Echinops telfairi*), which is from a distant clade (Afrotheria) (Murphy et al. 2001). In the tenrec, organogenesis is supported by a yolk sac placenta until it is displaced by expansion of the exocoelom and allantois (Carter et al. 2005). As shown in Figure 7, yolk sac and allantois are richly vascularized and apposed to a thickening of the endometrium called the preplacental pad. Thus, there is the morphological basis for haemotrophic nutrition via a yolk sac placenta.

4.3 The inverted yolk sac of rodents

In rodents, inversion of the germ layers (day E5 in the mouse) and subsequent loss of the outer wall of the yolk sac leaves a visceral yolk sac membrane where extraembryonic endoderm forms an epithelium facing the uterine cavity (Sobotta 1911, Rossant and Cross 2001). There is increasing awareness that yolk sac membranes support embryonic development well before establishment of the chorioallantoic placenta. In the mouse, important events such as closure of the neural folds (Zohn and Sarkar 2010, Terasawa et al. 1999) precede the start of chorioallantoic circulation at embryonic day E10.5 (Cross et al. 2006). The yolk sac endoderm plays a key role in supplying lipids during early embryonic development through synthesis of apolipoprotein B and lipoprotein (Farese et al. 1996, Terasawa et al. 1999). In addition, iron uptake by endocytosis of transferrin has been shown in mouse (Copp et al. 1992) and rat (Young et al. 1997).

The rodent yolk sac continues to serve as an accessory to the chorioallantoic placenta through to term. Its transport functions have been studied extensively in mid to late gestation in the guinea pig (e.g. (King and Enders 1970)). The yolk sac is also an important conduit for maternal immunoglobulins (Brambell, Hemmings, and Rowlands 1948, King and Enders 1970).

4.4 The yolk sac in haplorrhine primates

Early in human development, the extraembryonic mesoderm separates the endoderm from the trophoblast resulting in a “secondary” yolk sac comprised of endoderm and mesenchyme that comes to lie within the exocoelom (Enders and King 1993, Hertig, Rock, and Adams 1956, Jauniaux et al. 2004, Jones 1997). Indeed, precocious development of the mesoderm and exocoelom with formation of a secondary yolk sac is a characteristic feature of haplorrhine primates (tarsiers, Old and New World monkeys and apes) (Luckett 1974). In the human, a vitelline circulation is established starting around day 16 and yolk sac function persists through 9-11 weeks of gestation after which there is a gradual decline. In the first trimester chorioallantoic placenta, the orifices of the maternal arteries are blocked by plugs of trophoblast and there is no flow before 6-7 weeks and restricted flow until 12 weeks (Roberts et al. 2017, James et al. 2018). However, uterine glands open to the intervillous space of the placenta and their secretion products are taken up by the trophoblast (Burton et al. 2002, Hempstock et al. 2004, Moser et al. 2015). Thus, the yolk sac functions through a period when perfusion of the intervillous space is minimal and uterine gland secretions are an important source of nutrients for the developing embryo. Indeed, the uterine glands may be stimulated by hormones released from the trophoblast, such as human placental lactogen and human chorionic gonadotrophin, while trophoblast invasion could be promoted by “uterine milk proteins” and growth factors (Burton, Cindrova-Davies, and Turco 2020).

Exocoelomic fluid from the first trimester of human pregnancy has been characterized as an ultrafiltrate of maternal plasma with additional proteins from the placenta and secondary yolk sac. It is therefore regarded as an important interface for maternal-fetal nutrient transfer and as a nutrient reservoir for the embryo (Jauniaux and Gulbis 2000). Yolk sac fluid is not easily obtained but its protein content is consistent with exchange between exocoelomic and yolk sac compartments including transfer of hCG derived from the placental trophoblast (Gulbis et al. 1998). The yolk sac mesothelium faces the exocoelomic cavity and has the ultrastructural features of pinocytotic activity (Jones 1997, Jones and Jauniaux 1995). Finally, the inward-facing endodermal layer expresses the endocytic receptors megalin and cubilin (Burke et al. 2013). Transcriptome analysis has confirmed the expression of a broad range of transporters in first trimester human yolk sac (Cindrova-Davies et al. 2017). Together these observations support the argument that uterine gland secretions and other nutrients are transported to the exocoelom and absorbed by the secondary yolk sac whence they are transferred to the vitelline circulation (Jauniaux, Gulbis, and Burton 2003). Additional functions of human yolk sac are synthesis of α-fetoprotein and other proteins (Gulbis et al. 1998), haematopoiesis (Tavian et al. 2010) and as a repository for germ cells (De Felici 2013).

5 CONCLUSIONS

The first lesson to be drawn from this review is that uptake of uterine secretions does not require a placenta. The monotreme embryo has reached 18-20 somites by oviposition, its growth supported both by yolk and by histotrophe taken up through the avascular yolk sac. Similarly, the first two-thirds of development in marsupials is dependent on histotrophe absorbed by the avascular bilaminar yolk sac and by a trilaminar yolk sac that has no blood vessels until shortly before shell rupture. An essential role for the bilaminar yolk sac in eutherian development is confirmed by studies of twinning in the horse.

Secondly, a yolk sac placenta supports the growth of the marsupial embryo after shell rupture. It may allow for haemotrophic nutrition although the physiological evidence is scant. The vascular yolk sac might be of greater importance for the oxygen supply to the embryo. The ancestral condition in eutherians and in most extant clades is for a yolk sac placenta to provide nutrition in the early stages of fetal growth. Rodents and higher primates do not conform. However, in rodents the inverted yolk sac is functional well before establishment of a circulation in the chorioallantoic placenta. In the mouse, that transition occurs at day E10.5. Human development is unusual in that the yolk sac never approaches the uterus. However, there is ample evidence that early embryonic development is supported by histotrophe transferred via the exocoelom and yolk sac. The human embryo is at least partly dependent upon uptake of uterine secretions by the trophoblast facing the intervillous space, where blood flow is negligible in the first few weeks of pregnancy.

Marsupials provide important information about histotrophic nutrition via the avascular yolk sac and later the yolk sac placenta. To a large extant these translate to early embryonic development in eutherians. Thus there is every reason to incorporate this knowledge when considering the role of fetal membranes in eutherians and to encourage further studies of marsupials as essential to a fuller understanding of mammalian development and its evolution.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Figure legends

FIGURE 1 Intrauterine development of monotremes is supported by yolk and absorption of uterine secretions. 18-20-somite embryo of the platypus (*Ornithorhynchus anatinus*). H, Hensen's node; S, somites; TGP, trigeminal ganglionic primordia. Reprinted with permission of The Royal Society from (Hughes and Hall 1998) © The Royal Society (U.K.).

FIGURE 2 Fetal membranes of the tammar wallaby (*Notamacropus eugenii*) at 24 days gestation. Right: schematic representation of the fetal membranes. Left and middle: cellular components of the avascular yolk sac or bilaminar omphalopleure (BOM) and vascular yolk sac or trilaminar omphalopleure (TOM). Cell layers are: TL, trophoblast; EL, endoderm; ML, mesoderm (with blood vessels). Reprinted from (Guernsey et al. 2017) © the authors.

FIGURE 3 Electron micrographs of trophoblast from the vascular yolk sac. (a) Tammar wallaby (*Notamacropus eugenii*) at 20 days gestation. Structures shown include coated pits (long arrowheads), vesicles (v), and nucleus (N). Reprinted with permission from (Freyer, Zeller, and Renfree 2002) © Anatomical Society of Great Britain and Ireland 2002. (b) Grey four-eyed opossum (*Philander opossum*). Note that the apical microvilli are long but do not interdigitate with those of the uterine epithelial cell. Arrows indicate coated pits. At the opposite surface, microvilli project into basal infoldings situated beneath the basal lamina. Image courtesy of Dr. Allen C. Enders.

FIGURE 4 The interhaemal barrier in the placentae of the long-nosed bandicoot (*Perameles nasuta*); crown-rump length of embryo 10-11 mm. (a) Yolk sac placenta. Fetal blood vessels (fv) appear in the attenuated mesoderm between endoderm (E) and trophoblast (T). Maternal capillaries are superficial and above the syncytial masses of uterine epithelium (S). CT, endometrial stroma; m, mitochondrion; n, nucleus; x, artefactual space. (b) Chorioallantoic placenta. There is a short diffusion distance between fetal capillaries (fv) and maternal ones (mv) separated only by trophoblast (T) and the syncytial masses (S) derived from uterine epithelial cells. Reprinted with permission from (Padykula and Taylor 1976) Copyright © 1976 Wiley-Liss, Inc.

FIGURE 5 The capsule and yolk sac of the horse and the bovine yolk sac. (a) In the horse at 14.5 days the resilient capsule is so tightly applied to the yolk sac that it is invisible. (b) At 17.5 days the flaccid capsule hangs intact from the yolk sac. Reprinted from (Betteridge 2007) © (2007) with permission from Elsevier. (c) The bovine yolk sac at 20 days comprises a central portion (YSC) and two extended arms (YSE); allantoic vessels (arrows) are seen extending from the embryo. Courtesy of Dr. Antonio Assis Neto.

FIGURE 6 Fetal membranes of the domestic pig (*Sus scrofa*). Ectoderm is in blue, mesoderm in red and endoderm in green. (a) Longitudinal section of the elongated blastocyst at 18 days post coitum. The yolk sac is attached to the chorion partly as the bilaminar omphalopleure and partly as a choriovitelline placenta. The allantois is expanding into the exocoelom but has not yet reached the chorion. (b) By 21 days post coitum, the allantois and chorion have fused to form the allanto-chorion. In the process the yolk sac has been displaced. Reprinted with permission from (Perry 1981). Copyright © 1981, Society for Reproduction and Fertility.

FIGURE 7. Fetal membranes of the lesser hedgehog tenrec (*Echinops telfairi*) at 22 days of gestation. (a) Overview showing amniotic cavity (Amn), yolk sac (YS), allantoic cavity (All) and exocoelom (Exo). (b) Yolk sac placenta (YS Pl) and allantoic placenta (All Pl). At this stage neither vessels from the yolk sac nor vessels

from the allantois have entered the preplacental pad (PP). (c) Vessels of yolk sac (above) and allantois (below) abut the preplacental pad. Note the more cuboidal endoderm lining the yolk sac vessels. MBS, maternal blood space. (d) The allantois displacing the yolk sac. The allantoic cavity (All) is encroaching from either side on the remnants of the exocoelom (Exo) and the yolk sac (YS). Haemotoxylin and eosin. Scale bars: 1 mm (a), 100 mm (b), 20 mm (c-d). Reprinted from (Carter et al. 2005) © (2005) with permission from Elsevier.

TABLE 1 Marsupial orders and model species. Orders and suborders from (Wilson and Reeder 2005). The species shown are those featured in the recent literature.

Order and suborder	Species	Common name
Didelphimorphia	*Monodelphis domestica*	Grey Short-Tailed Opossum
	Philander opossum	Grey Four-Eyed Opossum
Paucituberculata	None	
Microbiotheria	None	
Notoryctemorphia	None	
Dasyuromorphia	*Sminthopsis crassicaudata*	Fat-Tailed Dunnart
Peramelemorphia	*Isoodon macrourus*	Northern Brown Bandicoot
	Isoodon obesulus	Southern Brown Bandicoot
	Perameles nasuta	Long-nosed Bandicoot
Diprotodontia: Vombatiformes	*Phascolarctos cinereus*	Koala
	Vombatus ursinus	Common wombat
Diprotodontia: Phalangeriformes	*Trichosurus vulpecula*	Common Brushtail Possum
	Pseudocheirus peregrinus	Common Ringtail Possum
Diprotodontia: Macropodiformes	*Potorous tridactylus*	Long-Nosed Potoroo
	Notamacropus eugenii	Tammar Wallaby

REFERENCES

Ager, E. I., A. J. Pask, G. Shaw, and M. B. Renfree. 2008. "Expression and protein localisation of IGF2 in the marsupial placenta." *BMC Dev Biol* 8:17. doi: 10.1186/1471-213X-8-17.

Ager, E., S. Suzuki, A. Pask, G. Shaw, F. Ishino, and M. B. Renfree. 2007. "Insulin is imprinted in the placenta of the marsupial, Macropus eugenii." *Dev Biol* 309 (2):317-28. doi: 10.1016/j.ydbio.2007.07.025.

Allen, W. R. 2001. "Fetomaternal interactions and influences during equine pregnancy." *Reproduction* 121 (4):513-27.

Assheton, R. 1906. "The morphology of the ungulate placenta, particularly the development of that organ in the sheep, and notes upon the placenta of the elephant and hyrax." *Phil Trans R Soc Lond B* 198:239-250.

Assis Neto, A. C., F. T. Pereira, T. C. Santos, C. E. Ambrosio, R. Leiser, and M. A. Miglino. 2010. "Morpho-physical recording of bovine conceptus (Bos indicus) and placenta from days 20 to 70 of pregnancy." *Reprod Domest Anim* 45 (5):760-72. doi: 10.1111/j.1439-0531.2009.01345.x.

Baudinette, R. V., B. J. Gannon, R. G. Ryall, and P. B. Frappell. 1988. "Changes in metabolic rates and blood respiratory characteristics during pouch development of a marsupial, Macropus eugenii." *Respir Physiol* 72 (2):219-28. doi: 10.1016/0034-5687(88)90008-4.

Bazer, F. W., and G. A. Johnson. 2014. "Pig blastocyst-uterine interactions." *Differentiation* 87 (1-2):52-65. doi: 10.1016/j.diff.2013.11.005.

Bedzhov, I., C. Y. Leung, M. Bialecka, and M. Zernicka-Goetz. 2014. "In vitro culture of mouse blastocysts beyond the implantation stages." *Nat Protoc* 9 (12):2732-9. doi: 10.1038/nprot.2014.186.

Bedzhov, I., and M. Zernicka-Goetz. 2014. "Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation." *Cell* 156 (5):1032-44. doi: 10.1016/j.cell.2014.01.023.

Betteridge, K. J. 2007. "Equine embryology: an inventory of unanswered questions." *Theriogenology* 68 Suppl 1:S9-21. doi: 10.1016/j.theriogenology.2007.04.037.

Betteridge, K. J., M. D. Eaglesome, D. Mitchell, P. F. Flood, and R. Beriault. 1982. "Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens." *J Anat* 135 (1):191-209.

Brambell, F. W. R., W. A. Hemmings, and W. T. Rowlands. 1948. "The passage of antibodies from the maternal circulation into the embryo in rabbits." *Proc R Soc Lond B* 135 (880):390-403.

Buddle, A. L., M. B. Thompson, L. A. Lindsay, C. R. Murphy, C. M. Whittington, and B. M. McAllan. 2019. "Dynamic changes to claudins in the uterine epithelial cells of the marsupial Sminthopsis crassicaudata (Dasyuridae) during pregnancy." *Mol Reprod Dev* 86 (6):639-649. doi: 10.1002/mrd.23140.

Burke, K. A., E. Jauniaux, G. J. Burton, and T. Cindrova-Davies. 2013. "Expression and immunolocalisation of the endocytic receptors megalin and cubilin in the human yolk sac and placenta across gestation." *Placenta* 34 (11):1105-9. doi: 10.1016/j.placenta.2013.08.003.

Burton, G. J. 1982. "Review article. Placental uptake of maternal erythrocytes: a comparative study." *Placenta* 3 (4):407-34.

Burton, G. J. 2009. "Oxygen, the Janus gas; its effects on human placental development and function." *J Anat* 215 (1):27-35. doi: 10.1111/j.1469-7580.2008.00978.x.

Burton, G. J., T. Cindrova-Davies, and M.Y. Turco. 2020. "Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy." *Placenta*. doi: https://doi-org.proxy1-bib.sdu.dk/10.1016/j.placenta.2020.02.008.

Burton, G. J., A. L. Watson, J. Hempstock, J. N. Skepper, and E. Jauniaux. 2002. "Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy." *J Clin Endocrinol Metab* 87 (6):2954-9. doi: 10.1210/jcem.87.6.8563.

Carter, A. M. 2015. "Placental Gas Exchange and the Oxygen Supply to the Fetus." *Compr Physiol* 5 (3):1381-403. doi: 10.1002/cphy.c140073.

Carter, A. M. 2016. "IFPA Senior Award Lecture: Mammalian fetal membranes." *Placenta* 48 Suppl 1:S21-S30. doi: 10.1016/j.placenta.2015.10.012.

Carter, A. M., T. N. Blankenship, H. Kunzle, and A. C. Enders. 2005. "Development of the haemophagous region and labyrinth of the placenta of the tenrec, Echinops telfairi." *Placenta* 26 (2-3):251-61. doi: 10.1016/j.placenta.2004.06.001.

Chavan, A. R., B. A. Bhullar, and G. P. Wagner. 2016. "What was the ancestral function of decidual stromal cells? A model for the evolution of eutherian pregnancy." *Placenta* 40:40-51. doi: 10.1016/j.placenta.2016.02.012.

Cindrova-Davies, T., E. Jauniaux, M. G. Elliot, S. Gong, G. J. Burton, and D. S. Charnock-Jones. 2017. "RNA-seq reveals conservation of function among the yolk sacs of human, mouse, and chicken." *Proc Natl Acad Sci U S A* 114 (24):E4753-E4761. doi: 10.1073/pnas.1702560114.

Copp, A. J., J. P. Estibeiro, F. A. Brook, and K. M. Downs. 1992. "Exogenous transferrin is taken up and localized by the neurulation-stage mouse embryo in vitro." *Dev Biol* 153 (2):312-23. doi: 10.1016/0012-1606(92)90116-x.

Cross, J. C., H. Nakano, D. R. Natale, D. G. Simmons, and E. D. Watson. 2006. "Branching morphogenesis during development of placental villi." *Differentiation* 74 (7):393-401. doi: 10.1111/j.1432-0436.2006.00103.x.

De Felici, M. 2013. "Origin, migration, and proliferation of human primordial germ cells." In *Oogenesis*, edited by G. Coticchio, D.F. Albertini and L. De Santis, 19-37. London: Springer-Verlag.

Deglincerti, A., G. F. Croft, L. N. Pietila, M. Zernicka-Goetz, E. D. Siggia, and A. H. Brivanlou. 2016. "Self-organization of the in vitro attached human embryo." *Nature* 533 (7602):251-4. doi: 10.1038/nature17948.

Dudley, J. S., C. R. Murphy, M. B. Thompson, and B. M. McAllan. 2015. "Desmoglein-2 during pregnancy and its role in the evolution of viviparity in a marsupial (Sminthopsis crassicaudata; Dasyuridae)." *J Morphol* 276 (3):261-72. doi: 10.1002/jmor.20333.

Dudley, J. S., C. R. Murphy, M. B. Thompson, and B. M. McAllan. 2017. "Epithelial cadherin disassociates from the lateral plasma membrane of uterine epithelial cells throughout pregnancy in a marsupial." *J Anat* 231 (3):359-365. doi: 10.1111/joa.12648.

Enders, A. C. 2015. "Equine development - blastocyst to endometrial cup." Centre for Trophoblast Research, accessed 13 November 2019. https://www.trophoblast.cam.ac.uk/Resources/enders.

Enders, A. C., and A. M. Carter. 2006. "Comparative placentation: some interesting modifications for histotrophic nutrition -- a review." *Placenta* 27 Suppl A:S11-S16. doi: 10.1016/j.placenta.2005.10.013.

Enders, A. C., and R. K. Enders. 1969. "The placenta of the four-eye opossum (Philander opossum)." *Anat Rec* 165 (3):431-49. doi: 10.1002/ar.1091650311.

Enders, A. C., and B. F. King. 1993. "Development of the human yolk sac." In *The human yolk sac and yolk sac tumors*, edited by F. F. Nogales, 33-47. Berlin; London: Springer-Verlag.

Enders, A. C., K. C. Lantz, I. K. Liu, and S. Schlafke. 1988. "Loss of polar trophoblast during differentiation of the blastocyst of the horse." *J Reprod Fertil* 83 (1):447-60. doi: 10.1530/jrf.0.0830447.

Enders, A. C., S. Schlafke, K. C. Lantz, and I. K. Liu. 1993. "Endoderm cells of the equine yolk sac from day 7 until formation of the definitive yolk sac placenta." *Equine Vet J Suppl 15*:3-9.

Erkenbrack, E. M., J. D. Maziarz, O. W. Griffith, C. Liang, A. R. Chavan, M. C. Nnamani, and G. P. Wagner. 2018. "The mammalian decidual cell evolved from a cellular stress response." *PLoS Biol* 16 (8):e2005594. doi: 10.1371/journal.pbio.2005594.

Fago, A., C. Hundahl, H. Malte, and R. E. Weber. 2004. "Functional properties of neuroglobin and cytoglobin. Insights into the ancestral physiological roles of globins." *IUBMB Life* 56 (11-12):689-96. doi: 10.1080/15216540500037299.

Farese, R. V., Jr., S. Cases, S. L. Ruland, H. J. Kayden, J. S. Wong, S. G. Young, and R. L. Hamilton. 1996. "A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice." *J Lipid Res* 37 (2):347-60.

Flynn, T. T., and J. P. Hill. 1939. "The development of the Monotremata. Part IV. Growth of the ovarian ovum, maturation, fertilization and early cleavage." *Trans. Zool. Soc. Lond.* 26:445-622.

Flynn, T. T., and J. P. Hill. 1947. "The development of the Monotremata- Part VI. The later stages of cleavage and the formation of the primary germ-layers." *Trans. Zool. Soc. Lond.* 26:1-151.

Frappell, P. B. 2008. "Ontogeny and allometry of metabolic rate and ventilation in the marsupial: matching supply and demand from ectothermy to endothermy." *Comp Biochem Physiol A Mol Integr Physiol* 150 (2):181-8. doi: 10.1016/j.cbpa.2008.02.017.

Freyer, C., U. Zeller, and M. B. Renfree. 2002. "Ultrastructure of the placenta of the tammar wallaby, Macropus eugenii: comparison with the grey short-tailed opossum, Monodelphis domestica." *J Anat* 201 (2):101-19. doi: 10.1046/j.1469-7580.2002.00084.x.

Freyer, C., U. Zeller, and M. B. Renfree. 2003. "The marsupial placenta: a phylogenetic analysis." *J Exp Zool A Comp Exp Biol* 299 (1):59-77. doi: 10.1002/jez.a.10291.

Freyer, C., U. Zeller, and M. B. Renfree. 2007. "Placental function in two distantly related marsupials." *Placenta* 28 (2-3):249-57. doi: 10.1016/j.placenta.2006.03.007.

Griffith, O. W., M. C. Brandley, C. M. Whittington, K. Belov, and M. B. Thompson. 2017. "Comparative genomics of hormonal signaling in the chorioallantoic membrane of oviparous and viviparous amniotes." *Gen Comp Endocrinol* 244:19-29. doi: 10.1016/j.ygcen.2016.04.017.

Griffith, O. W., A. R. Chavan, M. Pavlicev, S. Protopapas, R. Callahan, J. Maziarz, and G. P. Wagner. 2019. "Endometrial recognition of pregnancy occurs in the grey short-tailed opossum (Monodelphis domestica)." *Proc Biol Sci* 286 (1905):20190691. doi: 10.1098/rspb.2019.0691.

Griffith, O. W., A. R. Chavan, S. Protopapas, J. Maziarz, R. Romero, and G. P. Wagner. 2017. "Embryo implantation evolved from an ancestral inflammatory attachment reaction." *Proc Natl Acad Sci U S A* 114 (32):E6566-E6575. doi: 10.1073/pnas.1701129114.

Guernsey, M. W., E. B. Chuong, G. Cornelis, M. B. Renfree, and J. C. Baker. 2017. "Molecular conservation of marsupial and eutherian placentation and lactation." *Elife* 6:e27450. doi: 10.7554/eLife.27450.

Gulbis, B., E. Jauniaux, F. Cotton, and P. Stordeur. 1998. "Protein and enzyme patterns in the fluid cavities of the first trimester gestational sac: relevance to the absorptive role of secondary yolk sac." *Mol Hum Reprod* 4 (9):857-62. doi: 10.1093/molehr/4.9.857.

Heap, R. B., M. B. Renfree, and R. D. Burton. 1980. "Steroid metabolism in the yolk sac placenta and endometrium of the tammar wallaby, Macropus eugenii." *J Endocrinol* 87 (3):339-49. doi: 10.1677/joe.0.0870339.

Hempstock, J., T. Cindrova-Davies, E. Jauniaux, and G. J. Burton. 2004. "Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: a morphological and immunohistochemical study." *Reprod Biol Endocrinol* 2:58. doi: 10.1186/1477-7827-2-58.

Henty, K., R. M. Wells, and T. Brittain. 2008. "Characterization of the hemoglobins of the neonatal brushtailed possum Trichosurus vulpecula (Kerr): evidence for a highly cooperative, aggregated isoform of hemoglobin." *Comp Biochem Physiol A Mol Integr Physiol* 150 (1):52-7. doi: 10.1016/j.cbpa.2008.03.001.

Hertig, A. T., J. Rock, and E. C. Adams. 1956. "A description of 34 human ova within the first 17 days of development." *Am J Anat* 98 (3):435-93. doi: 10.1002/aja.1000980306.

Heuser, C.H. 1927. "A study of the implantation of the ovum of the pig from the stage of the bilaminar blastocyst to the completion of the fetal membranes." *Contrib Embryol Carnegie Inst* 19:229-243.

Hill, J.P. 1900. "On the foetal membranes, placentation and parturition of the native cat (Dasyurus viverrinus)." *Anat Anz* 18:364-373.

Holland, R. A., and A. A. Gooley, 2nd. 1997. "Characterization of the embryonic globin chains of the marsupial Tammar wallaby, Macropus eugenii." *Eur J Biochem* 248 (3):864-71. doi: 10.1111/j.1432-1033.1997.00864.x.

Holland, R. A., A. A. Gooley, and R. M. Hope. 1998. "Embryonic globins of the marsupial the Tammar Wallaby (Macropus eugenii): bird like and mammal like." *Clin Exp Pharmacol Physiol* 25 (9):740-4. doi: 10.1111/j.1440-1681.1998.tb02288.x.

Hughes, R. L. 1974. "Morphological studies on implantation in marsupials." *J Reprod Fertil* 39 (1):173-86. doi: 10.1530/jrf.0.0390173.

Hughes, R. L. 1993. "Monotreme development with particular reference to the extraembryonic membranes." *J Exp Zool* 266 (6):480-94. doi: 10.1002/jez.1402660602.

Hughes, R. L., and L. S. Hall. 1998. "Early development and embryology of the platypus." *Philos Trans R Soc Lond B Biol Sci* 353 (1372):1101-14. doi: 10.1098/rstb.1998.0269.

Hughes, R.L. 1977. "Egg membranes and ovarian function during pregnancy in monotremes and marsupials." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J.H. Calaby and C. H. Tyndale-Biscoe, 281-291. Canberra: Australian Academy of Science.

Hughes, R.L., and R.H. Green. 1998. "Placentation and adaptations for survival at birth in the common wombat." In *Wombats*, edited by R.T. Wells and P. A. Pridmore, 86-108. Chipping Norton, Surrey: Beatty & Sons.

Hughes, R.L., and L.S. Hall. 1988. "Structural adaptations of the newborn marsupial." In *The developing marsupial: models for biomedical research*, edited by C.H. Tyndale-Biscoe and P. A. Janssens, 8-27. Berlin: Springer-Verlag.

Huttenlocker, A. K., D. M. Grossnickle, J. I. Kirkland, J. A. Schultz, and Z. X. Luo. 2018. "Late-surviving stem mammal links the lowermost Cretaceous of North America and Gondwana." *Nature* 558 (7708):108-112. doi: 10.1038/s41586-018-0126-y.

Haacke, W. 1885. "On the marsupial ovum, the mammary pouch, and the male milk glands of Echidna hystris." *Proc R Soc Lond B* 38:72-74.

James, J. L., A. M. Carter, and L. W. Chamley. 2012. "Human placentation from nidation to 5 weeks of gestation. Part I: What do we know about formative placental development following implantation?" *Placenta* 33 (5):327-34. doi: 10.1016/j.placenta.2012.01.020.

James, J. L., R. Saghian, R. Perwick, and A. R. Clark. 2018. "Trophoblast plugs: impact on utero-placental haemodynamics and spiral artery remodelling." *Hum Reprod* 33 (8):1430-1441. doi: 10.1093/humrep/dey225.

Jauniaux, E., T. Cindrova-Davies, J. Johns, C. Dunster, J. Hempstock, F. J. Kelly, and G. J. Burton. 2004. "Distribution and transfer pathways of antioxidant molecules inside the first trimester human gestational sac." *J Clin Endocrinol Metab* 89 (3):1452-8. doi: 10.1210/jc.2003-031332.

Jauniaux, E., and B. Gulbis. 2000. "Fluid compartments of the embryonic environment." *Hum Reprod Update* 6 (3):268-78. doi: 10.1093/humupd/6.3.268.

Jauniaux, E., B. Gulbis, and G. J. Burton. 2003. "The human first trimester gestational sac limits rather than facilitates oxygen transfer to the foetus--a review." *Placenta* 24 Suppl A:S86-93. doi: 10.1053/plac.2002.0932.

Jones, C. J. 1997. "The life and death of the embryonic yolk sac." In *Embryonic medicine and therapy*, edited by E. Jauniaux, E. R. Barnea and R. G. Edwards, 180-196. Oxford: Oxford University Press.

Jones, C. J., and E. Jauniaux. 1995. "Ultrastructure of the materno-embryonic interface in the first trimester of pregnancy." *Micron* 26 (2):145-73. doi: 10.1016/0968-4328(95)00002-l.

Jones, C. J., J. N. Skepper, M. B. Renfree, and J. D. Aplin. 2014. "Trophoblast specialisations during pregnancy in the tammar wallaby, Macropus eugenii: a morphological and lectin histochemical study." *Placenta* 35 (7):467-75. doi: 10.1016/j.placenta.2014.03.018.

King, B. F., and A. C. Enders. 1970. "Protein absorption and transport by the guinea pig visceral yolk sac placenta." *Am J Anat* 129 (3):261-87. doi: 10.1002/aja.1001290303.

Kitchen, H., and I. Brett. 1974. "Embryonic and fetal hemoglobin in animals." *Ann N Y Acad Sci* 241:653-71. doi: 10.1111/j.1749-6632.1974.tb21921.x.

Laird, M. K., J. R. Dargan, L. Paterson, C. R. Murphy, B. M. McAllan, G. Shaw, M. B. Renfree, and M. B. Thompson. 2017. "Uterine molecular changes for non-invasive embryonic attachment in the marsupials Macropus eugenii (Macropodidae) and Trichosurus vulpecula (Phalangeridae)." *Mol Reprod Dev* 84 (10):1076-1085. doi: 10.1002/mrd.22861.

Laird, M. K., V. L. Hansen, B. M. McAllan, C. R. Murphy, and M. B. Thompson. 2020. "Uterine epithelial remodelling during pregnancy in the marsupial Monodelphis domestica (Didelphidae): Implications for mammalian placental evolution." *J Anat*. doi: 10.1111/joa.13162.

Laird, M. K., H. McShea, C. R. Murphy, B. M. McAllan, G. Shaw, M. B. Renfree, and M. B. Thompson. 2018. "Non-invasive placentation in the marsupials Macropus eugenii (Macropodidae) and Trichosurus vulpecula (Phalangeridae) involves redistribution of uterine Desmoglein-2." *Mol Reprod Dev* 85 (1):72-82. doi: 10.1002/mrd.22940.

Laird, M. K., M. Turancova, B. M. McAllan, C. R. Murphy, and M. B. Thompson. 2017. "Uterine focal adhesion dynamics during pregnancy in a marsupial (Sminthopsis crassicaudata; Dasyuridae)." *Anat Rec (Hoboken)* 300 (6):1150-1159. doi: 10.1002/ar.23535.

Luckett, W. P. 1974. "Comparative development and evolution of the placenta in primates " In *Reproductive biology of the primates*, edited by W. P. Luckett, 142-234. Basel: Karger.

Lyne, A. G. 1974. "Gestation period and birth in the marsupial Isoodon macrourus." *Aust J Zool* 22 (3):303-309.

Lyne, A.G., and D.E. Hollis. 1977. "The early development of marsupials, with special reference to bandicoots." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J. H. Calaby and C. H. Tyndale-Biscoe, 293-302. Canberra: Australian Academy of Science.

Ma, H., J. Zhai, H. Wan, X. Jiang, X. Wang, L. Wang, Y. Xiang, X. He, Z. A. Zhao, B. Zhao, P. Zheng, L. Li, and H. Wang. 2019. "In vitro culture of cynomolgus monkey embryos beyond early gastrulation." *Science* 366 (6467):eaax7890. doi: 10.1126/science.aax7890.

Menkhorst, E., A. Nation, S. Cui, and L. Selwood. 2009. "Evolution of the shell coat and yolk in amniotes: a marsupial perspective." *J Exp Zool B Mol Dev Evol* 312 (6):625-38. doi: 10.1002/jez.b.21235.

Menzies, B. R., T. B. Hildebrandt, and M. B. Renfree. 2020. "Unique reproduction strategy in the swamp wallaby." *Proc Natl Acad Sci U S A*. doi: 10.1073/pnas.1922678117.

Menzies, B. R., A. J. Pask, and M. B. Renfree. 2011. "Placental expression of pituitary hormones is an ancestral feature of therian mammals." *Evodevo* 2:16. doi: 10.1186/2041-9139-2-16.

Mess, A. M., and K. J. Ferner. 2010. "Evolution and development of gas exchange structures in Mammalia: the placenta and the lung." *Respir Physiol Neurobiol* 173 Suppl:S74-82. doi: 10.1016/j.resp.2010.01.005.

Moser, G., G. Weiss, M. Gauster, M. Sundl, and B. Huppertz. 2015. "Evidence from the very beginning: endoglandular trophoblasts penetrate and replace uterine glands in situ and in vitro." *Hum Reprod* 30 (12):2747-57. doi: 10.1093/humrep/dev266.

Mossman, H. W. 1987. *Vertebrate fetal membranes : comparative ontogeny and morphology; evolution; phylogenetic significance; basic functions; research opportunities*. New Brunswick: Rutgers University Press.

Murphy, W. J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W. de Jong, and M. S. Springer. 2001. "Resolution of the early placental mammal radiation using Bayesian phylogenetics." *Science* 294 (5550):2348-51. doi: 10.1126/science.1067179.

Niu, Y., N. Sun, C. Li, Y. Lei, Z. Huang, J. Wu, C. Si, X. Dai, C. Liu, J. Wei, L. Liu, S. Feng, Y. Kang, W. Si, H. Wang, E. Zhang, L. Zhao, Z. Li, X. Luo, G. Cui, G. Peng, J. C. Izpisua Belmonte, W. Ji, and T. Tan. 2019. "Dissecting primate early post-implantation development using long-term in vitro embryo culture." *Science* 366 (6467):eaaw5754. doi: 10.1126/science.aaw5754.

Opazo, J. C., F. G. Hoffmann, and J. F. Storz. 2008. "Genomic evidence for independent origins of beta-like globin genes in monotremes and therian mammals." *Proc Natl Acad Sci U S A* 105 (5):1590-5. doi: 10.1073/pnas.0710531105.

Oriol, J. G., F. J. Sharom, and K. J. Betteridge. 1993. "Developmentally regulated changes in the glycoproteins of the equine embryonic capsule." *J Reprod Fertil* 99 (2):653-64. doi: 10.1530/jrf.0.0990653.

Padykula, H. A., and J. M. Taylor. 1976. "Ultrastructural evidence for loss of the trophoblastic layer in the chorioallantoic placenta of Australian bandicoots (Marsupialia: Peramelidae)." *Anat Rec* 186 (3):357-85. doi: 10.1002/ar.1091860303.

Padykula, H.A., and J.M. Taylor. 1977. "Uniqueness of the bandicoot chorioallantoic placenta (Marsupialia: Peramelidae) cytological and evolutionary interpretations." In *Reproduction and evolution : proceedings of the Fourth Symposium on Comparative Biology of Reproduction, held in Canberra, December, 1976*, edited by J. H. Calaby and C. H. Tyndale-Biscoe, 303-311. Canberra: Australian Academy of Science.

Perry, J. S. 1981. "The mammalian fetal membranes." *J Reprod Fertil* 62 (2):321-35. doi: 10.1530/jrf.0.0620321.

Perry, J. S., and I. W. Rowlands. 1962. "Early pregnancy in the pig." *J Reprod Fert* 4 (2):175-188.

Renfree, M. B. 1973. "The composition of fetal fluids of the marsupial Macropus eugenii." *Dev Biol* 33 (1):62-79. doi: 10.1016/0012-1606(73)90165-6.

Renfree, M. B. 2010. "Review: Marsupials: placental mammals with a difference." *Placenta* 31 Suppl:S21-6. doi: 10.1016/j.placenta.2009.12.023.

Renfree, M. B., and J. C. Fenelon. 2017. "The enigma of embryonic diapause." *Development* 144 (18):3199-3210. doi: 10.1242/dev.148213.

Renfree, M. B., and C. H. Tyndale-Biscoe. 1973. "Transferrin variation between mother and fetus in the marsupial, Macropus eugenii." *J Reprod Fertil* 32 (1):113-5. doi: 10.1530/jrf.0.0320113.

Roberts, C. T., and W. G. Breed. 1994a. "Embryonic-maternal cell interactions at implantation in the fat-tailed dunnart, a dasyurid marsupial." *Anat Rec* 240 (1):59-76. doi: 10.1002/ar.1092400107.

Roberts, C. T., and W. G. Breed. 1994b. "Placentation in the dasyurid marsupial, Sminthopsis crassicaudata, the fat-tailed dunnart, and notes on placentation of the didelphid, Monodelphis domestica." *J Reprod Fertil* 100 (1):105-13. doi: 10.1530/jrf.0.1000105.

Roberts, C. T., and W. G. Breed. 1996. "The marsupial shell membrane: an ultrastructural and immunogold localization study." *Cell Tissue Res* 284 (1):99-110. doi: 10.1007/s004410050570.

Roberts, V. H. J., T. K. Morgan, P. Bednarek, M. Morita, G. J. Burton, J. O. Lo, and A. E. Frias. 2017. "Early first trimester uteroplacental flow and the progressive disintegration of spiral artery plugs: new insights from contrast-enhanced ultrasound and tissue histopathology." *Hum Reprod* 32 (12):2382-2393. doi: 10.1093/humrep/dex301.

Romanoff, A.L., and A.J.S. Romanoff. 1967. *Biochemistry of the avian embryo : a quantitative analysis of prenatal development*. New York: Interscience.

Rossant, J., and J. C. Cross. 2001. "Placental development: lessons from mouse mutants." *Nat Rev Genet* 2 (7):538-48. doi: 10.1038/35080570.

Rowe, T., T. H. Rich, P. Vickers-Rich, M. Springer, and M. O. Woodburne. 2008. "The oldest platypus and its bearing on divergence timing of the platypus and echidna clades." *Proc Natl Acad Sci U S A* 105 (4):1238-42. doi: 10.1073/pnas.0706385105.

Rüsse, I., F. Sinowatz, L. Richter, M. Lehmann, and E. Schallenberger. 1992. "Die Entwicklung des Dottersackes beim Wiederkäuer (Schaf und Rind)." *Anat Histol Embryol* 21 (4):324-47. doi: 10.1111/j.1439-0264.1992.tb00464.x.

Selwood, L. 2000. "Marsupial egg and embryo coats." *Cells Tissues Organs* 166 (2):208-19. doi: 10.1159/000016733.

Semon, R. 1894. "Die Embryonalhüllen der Monotremen und Marsupialer " In *Zoologische Forschungsreisen in Australien und dem Malayischen Archipel Band 2*, edited by R. Semon, 19-58. Jena: Gustav Fischer.

Shahbazi, M. N., A. Jedrusik, S. Vuoristo, G. Recher, A. Hupalowska, V. Bolton, N. N. M. Fogarty, A. Campbell, L. Devito, D. Ilic, Y. Khalaf, K. K. Niakan, S. Fishel, and M. Zernicka-Goetz. 2016. "Self-organization of the human embryo in the absence of maternal tissues." *Nat Cell Biol* 18 (6):700-708. doi: 10.1038/ncb3347.

Sharman, G. B. 1961. "The embryonic membranes and placentation in five genera of diprotodont marsupials." *Proc Zool Soc Lond* 137:197-220.

Shaw, G., H. M. Gehring, and E. C. Bell. 1999. "Production of prostaglandin f2alpha and its metabolite by endometrium and yolk sac placenta in late gestation in the tammar wallaby, Macropus Eugenii." *Biol Reprod* 60 (3):611-4. doi: 10.1095/biolreprod60.3.611.

Smith, K. K., and A. L. Keyte. 2018. "Adaptations of the marsupial newborn: birth as an extreme environment." *Anat Rec (Hoboken)*. doi: 10.1002/ar.24049.

Sobotta, J. 1911. "Die Entwicklung des Eies der Maus vom ersten Auftreten des Mesoderms an bis zur Ausbildung der Embryonalanlage und dem Auftreten der Allantois. I. Teil: Die Keimblase." *Arch Mikrosk Anat* 78:271-352.

Stringer, J. M., S. Suzuki, A. J. Pask, G. Shaw, and M. B. Renfree. 2012a. "Promoter-specific expression and imprint status of marsupial IGF2." *PLoS One* 7 (7):e41690. doi: 10.1371/journal.pone.0041690.

Stringer, J. M., S. Suzuki, A. J. Pask, G. Shaw, and M. B. Renfree. 2012b. "Selected imprinting of INS in the marsupial." *Epigenetics Chromatin* 5 (1):14. doi: 10.1186/1756-8935-5-14.

Tavian, M., K. Biasch, L. Sinka, J. Vallet, and B. Peault. 2010. "Embryonic origin of human hematopoiesis." *Int J Dev Biol* 54 (6-7):1061-5. doi: 10.1387/ijdb.103097mt.

Terasawa, Y., S. J. Cases, J. S. Wong, H. Jamil, S. Jothi, M. G. Traber, L. Packer, D. A. Gordon, R. L. Hamilton, and R. V. Farese, Jr. 1999. "Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development." *J Lipid Res* 40 (11):1967-77.

Tyndale-Biscoe, C. H., and M. Renfree. 1987. *Reproductive Physiology of Marsupials*. Cambridge: Cambridge University Press.

Waelchli, R. O., and K. J. Betteridge. 1996. "Osmolality of equine blastocyst fluid from day 11 to day 25 of pregnancy." *Reprod Fertil Dev* 8 (6):981-8. doi: 10.1071/rd9960981.

Waelchli, R. O., and K. J. Betteridge. 2013. "Morphology of twin and triplet equine conceptuses during weeks 3 and 4 of pregnancy." *Reprod Fertil Dev* 25 (5):762-74. doi: 10.1071/RD12067.

Werneburg, I., M. Laurin, D. Koyabu, and M. R. Sanchez-Villagra. 2016. "Evolution of organogenesis and the origin of altriciality in mammals." *Evol Dev* 18 (4):229-44. doi: 10.1111/ede.12194.

Whittington, C. M., D. O'Meally, M. K. Laird, K. Belov, M. B. Thompson, and B. M. McAllan. 2018. "Transcriptomic changes in the pre-implantation uterus highlight histotrophic nutrition of the developing marsupial embryo." *Sci Rep* 8 (1):2412. doi: 10.1038/s41598-018-20744-z.

Wilson, D. E., and D. M. Reeder. 2005. *Mammal species of the world : a taxonomic and geographic reference*. 3rd ed. Baltimore, Md.: Johns Hopkins University Press.

Young, D., A. R. Klemm, D. A. Beckman, R. L. Brent, and J. B. Lloyd. 1997. "Uptake and processing of 59Fe-labelled and 125I-labelled rat transferrin by early organogenesis rat conceptuses in vitro." *Placenta* 18 (7):553-62. doi: 10.1016/0143-4004(77)90010-8.

Zohn, I. E., and A. A. Sarkar. 2010. "The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation." *Birth Defects Res A Clin Mol Teratol* 88 (8):593-600. doi: 10.1002/bdra.20705.

Yolk sac and chorioallantoic placentas exist side by side in the hedgehog tenrec