Published by Australian Journal of Zoology

Shaw G & Renfree MB (2006) Parturition and perfect prematurity: Birth in marsupials. *Aust J Zool* **54**:139-149

http://www.publish.csiro.au/view/journals/dsp_journal_fulltext.cfm?nid=90&f=ZO05070

Parturition and perfect prematurity: Birth in marsupials

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Abstract

Marsupials are distinguished from eutherian mammals in their mode of reproduction and their greater dependence on the teat and mammary gland than on the placenta for their development. They give birth to a highly altricial young which completes its development whilst firmly attached to a teat, usually within the confines of a pouch. At birth, the marsupial neonate has relatively well-developed digestive, respiratory and circulatory systems but retains its fetal excretory system with a fully functional mesonephric kidney and undifferentiated gonads and genitalia. Despite this degree of developmental prematurity, we have evidence that this tiny (400mg) fetus of the tammar wallaby (*Macropus eugenii*) determines the time of its own delivery. Although plasma progesterone falls, and oestradiol associated with the postpartum oestrus typically rises, around the time of parturition, neither hormone seems to be essential for the timing of birth. However relaxin appears important in loosening the connective tissue of the cervix and vaginae for birth. Labour is sudden in onset and completes within minutes. Both prostaglandins and mesotocin are essential for the contractions that deliver the young. Prostaglandins from the reproductive tract act via the central nervous system to control the stereotyped parturient behaviour. In the last 2 days of gestation fetal adrenal glucocorticoid production increases and serves as a fetal signal that triggers labour. The increased cortisol also induces lung maturation and surfactant production in preparation for birth. The accessibility of the altricial neonatal marsupial provides a unique opportunity for experimental manipulation of organ development and maturation.

Introduction

Marsupials are distinguished from eutherian mammals by their mode of reproduction. Gestation is remarkably short in marsupials. The mouse sized *Sminthopsis macroura* give birth to neonates weighing under 14 mg after a gestation of only 10.7 days, the shortest of any mammal (Selwood and Woolley, 1991, Frigo & Woolley, 1997). Larger kangaroos such as the eastern gray kangaroo (*Macropus giganteus*), with a maternal weight of 27 kg give birth to neonates of only 700 mg while the honey possum, *Tarsipes rostratus*, gives birth to the smallest known mammalian neonate at <5mg (Renfree 1980). Whilst gestation in the kangaroo family is typically about 1 month, most of that gestation is spent as an expanding vesicle, and the phase of organogenesis is limited to the last week (Renfree, 1972; Tyndale-Biscoe and Renfree, 1987). In *Sminthopsis* organogenesis lasts only 1.5 days (Cruz et al., 2000). As a consequence of this condensed development phase many organ systems not essential to immediate post-partum survival are poorly developed, but there is more extensive development of structures such as heart and lungs that are essential for survival (Figure 1). Despite its altricial state, the neonate climbs unaided from the birth canal to the pouch where it then seeks and finds a teat and attaches. This climb is effected by powerful swimming motions of the well developed forelimbs. The hindlimbs are still at a paddle stage and play no role in this locomotion.

Figure 1 hereabouts.

If this level of coordination seems remarkable in the 400 mg tammar wallaby neonate in figure 1, consider the extremes in *Tarsipes*, where the rice-grain sized neonate weighing 5 mg, is still able make the unaided transition from birth canal to teat.

Marsupials provide still largely unexplored models to examine the physiological adaptations of premature young to extrauterine survival. In this review we will focus on this in the tammar and on some other unique aspects of marsupial reproduction that have provided useful experimental models.

Systemic hormones in pregnancy and parturition

Tammars normally mate at a post-partum oestrus and then carry the resulting early blastocyst in lactationally controlled embryonic diapause. Removal of the sucking pouch young (RPY) removes the inhibition, and initiates reactivation of the embryo from diapause, leading to birth about 26 days after RPY (Tyndale-Biscoe and Renfree, 1987). This makes tammars an excellent model species for pregnancy studies since it is particularly easy to obtain timed pregnancies by RPY.

The endocrinology of pregnancy and parturition has been studied in a number of marsupial species including tammars (reviewed in Renfree 1994, Renfree and Shaw 1996, and Shaw and Renfree, 2001). The hormones involved are essentially the same as those involved in eutherian mammals, although some marsupials have mesotocin (8-ile-oxytocin) instead of oxytocin. Mesotocin has uterotonic activity equal to oxytocin as assessed in a bioassay using uterine strips from a wallabies *in vitro*. The

roles of progesterone, oestradiol, relaxin, prolactin, prostaglandins and oxytocin/mesotocin in late pregnancy and parturition are well understood in one marsupial, the tammar wallaby, where most of the experimental studies have been conducted (reviewed in Shaw and Renfree, 2001).

Progesterone is essential for pregnancy in tammars. Reactivation from diapause is initiated by a transient pulse of progesterone about 5 days after RPY, and plasma progesterone concentrations are high from about day 10 to term. Curiously, once progesterone has initiated the uterine secretory pattern to establish growth of the blastocyst, progesterone is no longer needed to maintain pregnancy. Treatment of seasonally quiescent tammars with progesterone for 10 days initiated active pregnancy that proceeded to term (day 26-27) (Renfree and Tyndale-Biscoe, 1973), and removal of the corpus luteum after mid-gestation does not terminate pregnancy (Young and Renfree, 1979). Maintenance of pregnancy in the second half of gestation appears not to depend on placental progesterone, as the placenta has only incipient steroidogenic activity (Heap et al., 1980), and there is no difference in plasma progesterone profiles of late pregnant females and non-pregnant females at an equivalent stage of the oestrous cycle (reviewed in Tyndale-Biscoe and Renfree, 1987).

Progesterone levels then fall very suddenly at parturition (Figure 2) and in most females there is also a rise in oestradiol around parturition {refs in fig caption} in the lead up for the post-partum oestrus. Although a fall in progesterone and a rise in oestrogen is implicated in activation of parturition in some eutherian mammals, in

tammars neither a fall in progesterone nor a rise in oestrogen is needed for parturition
(reviewed in Shaw and Renfree, 2001)

Figure 2 hereabouts.

Relaxin like activity was identified in marsupials using bioassays as early as 1969
(Tyndale-Biscoe, 1969). It probably acts together with progesterone to prepare the
unusual birth canal of marsupials. Recent studies have provided more detailed
information the source of relaxin. Relaxin is made in the CL in electron dense
granules, with levels increasing towards term (Parry et al., 1997a; Bathgate et al.,
2002). Molecular analysis of the gene sequence shows conservation in the receptor
binding domains, and the gene is expressed in the late pregnant CL, placenta and
hypothalamus (Parry et al., 1997b). Besides its proposed role in preparing the
reproductive tract, relaxin may be important for development of the teats. Relaxin-
knockout mice have impaired teat development (Krajnc-Franken et al., 2004) and in
tammars the gene for the relaxin receptor LGR7 is upregulated in the teat and
mammary gland supporting the neonate 5-11 d postpartum (Gwyther et al., 2005).

Prolactin levels in plasma rise acutely about 8 hours before birth and remain high until
after delivery. Blocking the surge of prolactin using the dopamine agonist
bromocriptine does not affect the timing of birth nor prevent the abrupt fall of
progesterone in the peripartum. Thus prolactin is not essential for birth or luteolysis.
However, the surge of prolactin around birth may be needed for establishment of
lactation since the newborn pouch young died within 24 hours (Fletcher et al., 1990).

The key mediators of parturition are prostaglandins, notably PGF₂ α , and oxytocin/mesotocin in tammars, as in eutherian mammals (Liggins and Thorburn, 1994; Jenkin and Young, 2004). PGF₂ α , oxytocin and mesotocin are all potent activators of uterine contractions in wallabies (Shaw, 1983a,b; Parry et al., 1997c; Ingram et al., 2001). Prostaglandin production by the placenta and endometrium increases in the last 2 days of gestation (Shaw et al., 1999). Both hormones rise acutely within minutes before delivery of the neonate, then fall rapidly afterwards (Shaw, 1983b; Lewis et al., 1986; Parry et al., 1996). Blocking the rise in either hormone prevents or delays birth (Renfree et al., 1994, 1996a; Shaw and Renfree, 2001). Prostaglandins play a second role in parturition – they trigger parturient behaviour (Hinds et al., 1990; Shaw, 1990). This role is discussed later.

Marsupials as models for identifying local fetal effects in pregnancy and parturition

The basic anatomy of the reproductive tract in female marsupials differs strikingly from that of eutherians because the ureters pass medially rather than laterally, preventing the fusion of the mullerian ducts to form a single vagina and uterus as in eutherian mammals (Tyndale-Biscoe, 1973, 2005; Renfree et al., 1996b). Marsupials therefore have two completely separate uteri with separate cervixes, and a complicated vaginal system with two lateral vaginae and a median vagina that forms secondarily in later development (Tyndale-Biscoe 1966; Tyndale-Biscoe and Renfree, 1987).

In species such as the tammar wallaby, that has a single young, this means that one of the two uteri carries an embryo whilst the other is empty. The two uteri are exposed to the same systemic hormones, so differences between the gravid and non-gravid uteri reflect local influences of the embryo or placenta. Differences in uterine weight, secretory activity and histology have been recognised for a long time (Renfree, 1972; Renfree and Tyndale-Biscoe 1973; Renfree 1994; Rose 1992; reviewed in Renfree, 2000). Progesterone and oestradiol receptor concentrations are also different between the gravid and non-gravid uteri (Renfree and Blanden, 2000). More local effects have been identified recently that affect the control of parturition, notably in the control of uterine quiescence by nitric oxide during gestation and in the oxytocin receptor system that is essential for uterine contractions at parturition.

Spontaneous uterine activity during late gestation differs between the gravid and non-gravid uterus both in vivo (Shaw, 1983a) and in vitro (Ingram et al., 2001). The non-gravid uterus shows regular low-level contractile activity, whilst the gravid uterus shows little activity from day 21 of pregnancy until birth. In part this uterine quiescence may be mediated by nitric oxide (Figure 3) (Ingram et al., 2001), a paracrine smooth muscle relaxant that also mediates uterine relaxation in eutherian mammals in late gestation (Sladek et al., 1997; Yalampali et al., 1998). By using various inhibitors or agonists we were able to show in wallabies that nitric oxide relaxes the gravid myometrium through a classic NO/guanylate cyclase/cGMP system. However, although the non-gravid myometrium relaxed in response to cGMP, NO was without effect, suggesting that in late gestation guanylate cyclase was upregulated in gravid but not non-gravid uterus. A similar upregulation of guanylate cyclase, with attendant increased sensitivity to nitric oxide is seen in eutherian

mammals. Our studies in the tammar have shown this effect is mediated by local factors rather than systemic hormonal influences. It would be interesting to compare the situation in the monovular tammar with that in polyovular marsupials where both uteri carry embryos.

Figure 3 hereabouts:

In parallel with the quiescence mediated by upregulated nitric oxide, the potential for the myometrium to contract in response to mesotocin increases (Parry et al., 1997c; Sebastian et al., 1998; Ingram et al., 2001) (Figure 3). This change is due, again, to local influences. In the gravid, but not in the non-gravid uterus there is a dramatic upregulation of mesotocin receptor numbers and mesotocin receptor mRNA in the last few days of gestation (Figure 3) (Parry et al., 1997c; Sebastian et al., 1998; Siebel et al., 2005).

In tammars in late gestation, the gravid side ovary contains a CL that is producing high levels of progesterone, whilst the non-gravid uterus is associated with an ovary that usually has a large oestradiol producing follicle, growing in preparation for the post-partum oestrus. However the differences in uterine MT receptors cannot be due to local transfer of steroids from the ovary to the ipsilateral uterus (Towers et al., 1986; Harder et al., 1984). Embryo transfer experiments have shown that the differential growth of the uterus is related to the location of the embryo regardless of whether it was ipsilateral or contralateral to the CL (reviewed in Renfree, 2000). In non-pregnant females the profile of hormone production by the CL is identical to that in pregnant females, but no upregulation of MTR is seen in the uterus ipsilateral to the

CL (Siebel et al., 2002a). Unilateral ovariectomy, that removed either the CL bearing ovary or the follicle bearing ovary in late gestation, also failed to affect the subsequent upregulation of MTR (Siebel et al., 2004). Uterine distension also is probably not the critical factor in the upregulation (Siebel et al., 2002b). This data therefore supports the role of local hormonal influences from the fetus or placenta in the differential upregulation of mesotocin receptors.

Behavioural effects of systemic prostaglandins

Prostaglandins are usually regarded as locally active rather than systemic hormones because most prostaglandins are rapidly inactivated whilst passing through the pulmonary circulation (Granstrom and Hansson, 1976). However marsupials provide a dramatic model system in which prostaglandins do act as systemic hormones.

At birth marsupials display a characteristic stereotyped parturient behaviour (Renfree et al., 1989). In wallabies the females sit on their rump with their tails thrust forward between their legs, and the hips rotated to elevate the urogenital opening. The female becomes very attentive to licking the urogenital opening, the pouch and the fur in between. This behaviour starts minutes before delivery of the young, and lasts until a few minutes after delivery, by which time the neonate is in the pouch.

Besides their role in mediating uterine contractions at birth, prostaglandins also mediate parturient behaviour (Hinds et al., 1990; Shaw, 1990). Intramuscular injections of prostaglandin F₂ α into adult female tammar in various reproductive stages or into juvenile females initiates parturient behaviour within minutes (Hinds et al., 1990; Shaw, 1990). Surprisingly, males respond in a similar manner (Shaw,

1990), though they lick their scrotum and abdominal fur rather than the pouch that they do not have (figure 4). This effect is not restricted to tammars, but is seen in a diverse range of other marsupials including bettongs (Rose and McFadyen, 1997), opossums (Rose and Fadem, 2000) and bandicoots (Smith et al., 2001). Although the behavioural effects of prostaglandins in the central nervous system are well recognised, prostaglandins are not typically thought of as systemically active hormones. Prostaglandins are cleared from the peripheral circulation with great rapidity. Rapid metabolism of PGF₂ α occurs on passage through the lungs, and this also seems to be true in marsupials (Shaw, 1983a,b). Nonetheless, these studies on marsupials have shown clearly that this view is incorrect and that systemic prostaglandins can have dramatic behavioural effects. Prostaglandins can pass through the blood-brain barrier (Eguchi et al., 1992; Hayashi et al., 1989), and there is now strong evidence that systemic prostaglandins are important regulators of farrowing behaviour in pigs (Walton et al., 2002).

Figure 4 hereabouts

The trigger for birth – fetal cortisol

The very rapid, coordinated set of hormonal changes that cause uterine contractions, fetal delivery, and parturient behaviour occur in a matter of minutes. However the nature of the trigger for parturition is a key question. We have direct evidence from just a few eutherian species (reviewed in Liggins and Thorburn, 1994 and Jenkin and Young, 2004), of the cascade of hormones that lead to birth. Understanding the endocrine pathway in marsupials, that have very different timing of birth relative to

developmental stage, and a long evolutionary separation from eutherians, will point to the most conserved pathways that are likely to be common to all mammals.

In eutherian mammals where sufficient data is available, the signal comes from the fetus and/or placenta, a source that ensures prepartum changes and parturition are coordinated with fetal developmental stage. We now have good evidence that this is also true in marsupials despite their small size and relative immaturity.

Evidence that the maternal system is not the timekeeper for parturition has come from some elegant studies. Eastern grey kangaroos (*Macropus giganteus*) have a gestation length of 36.4 days, whilst western grey kangaroos (*M. fuliginosus*) have a gestation of 30.5 days. It is possible to hybridise eastern and western grey kangaroos. When eastern females were mated to western males the resulting pregnancies of the F1 hybrid embryos lasted 34 days, 2½ days shorter than would be expected if the mother determined gestation length (Kirsch and Poole, 1972; Poole, 1975). Furthermore, when female F1 hybrids were mated to western males, the resulting gestations were even shorter, at 32 days. This clearly demonstrated the importance of fetal genotype for setting gestation length in marsupials, as it is in eutherians.

Another experiment in tammars confirmed this conclusion. Tyndale-Biscoe *et al.* (1988) treated a group of tammars with progesterone at the time of RPY, to initiate earlier reactivation of the embryo. These females gave birth several days early to a normal full term embryo. However the timing of other pregnancy-associated endocrine changes in the mother, such as corpus luteum regression and oestrus,

occurred at the normal time about 27 days after RPY, long after birth had actually occurred.

In most eutherian mammals where there is data, activation of the fetal adrenal appears to regulate the timing of birth and the fetal maturation that precedes it (Liggins and Thorburn 1994; Thorburn and Liggins, 1994; Jenkin and Young, 2004). Despite their small size and immaturity at birth, the same is true in tammars. The fetal adrenal is morphologically developing from day 21 of gestation (Renfree, 1972; Call et al., 1980) and by day 24 cortisol is easily measurable in fetal blood. Fetal adrenal cortisol production then increases in the last 2 days of gestation (Ingram et al., 1999) before falling post-partum (Figure 5). The fetal adrenal is capable of acute responses to ACTH and PGE₂ with increased production of cortisol. This cortisol plays a role in fetal maturation (see later) as it does in eutherians. It also provides the trigger for parturition. Exogenous administration of the potent glucocorticoid agonist on day 24 of gestation initiates delivery of small, premature young 23 hours later, significantly earlier than normal (Shaw et al., 1996).

Figure 5 hereabouts.

Adaptations for premature birth

Parturition imposes dramatic physiological stresses on the offspring as it needs to adapt to the challenging extrauterine environment. Prematurity at birth is a major cause of neonatal death. However marsupials give birth to young that by eutherian standards are supremely immature and yet have no difficulties in postnatal survival.

Lung development and gas exchange in neonatal marsupials have been investigated in a range of marsupials including macropodids (tammar: Runciman et al., 1996, 1998a,b, 1999; red-necked wallaby: Walker and Gemmell, 1983; quokka, Makanya et al., 2001; Burri et al., 2003) dasyurids (Gemmell and Nelson, 1988; Mortola et al., 1999; Frappell and Mortola, 2000), possums (Gemmell and Nelson, 1988), bandicoots (Gemmell, 1986; Gemmell and Little, 1982) and opossums (Zavala et al., 1974). At birth, lung development in marsupials is significantly less advanced than is seen in eutherian mammals (Figure 5).

Figure 6 hereabouts.

Before birth, gas exchange occurs via the placenta. Whilst most marsupials have a superficial yolk sac placentation, in contrast to the invasive chorioallantoic placentation seen in a number of well-studied eutherian mammals, there is a large area of intricately vascularised choriovitelline membrane associated with an intricately folded and vascular endometrial epithelium (Freyer et al., 2002, 2003), and this is patently capable of supplying the respiratory needs of the tiny embryo up to term. Indeed, it can even supply sufficient respiratory gases for several days after normal term, but if gestation is prolonged too long, they die (Renfree, 1972; Renfree et al., 1994). As in eutherian mammals, prenatal cardiovascular function involves central cardiovascular shunts to direct blood away from the lungs and towards the systemic and placental circulation. Tammar have a patent ductus arteriosus which closes shortly after birth, and a fenestrated septum between the atria, performing the function of the foramen ovale in eutherians, that closes as tissue proliferates in the several days after birth (Runciman et al., 1995). In addition, in quolls, that give birth to much

314 smaller young than tammars, neonates have a large interventricular communication
315 and septation of the outflow tract is incomplete. Despite their small size and relative
316 immaturity of the central nervous systems, marsupial neonates also have well
317 established cardiovascular control mechanisms, as evidenced by responses to carotid
318 arterial occlusion and vagal stimulation in neonatal opossums (House et al., 1979).
319 Thus, despite anatomical and temporal differences, marsupials have adopted similar
320 cardiovascular adaptations to cope with the transition from intrauterine to postnatal
321 life.

322

323 Marsupials also provide excellent models for the study of lung development because
324 they are at a relatively early stage of development at birth. This allows easy access
325 for experimental functional studies at early stages of development in a way that
326 cannot be achieved with fetal eutherian mammals with non-functional fluid filled
327 lungs.

328

329 The least developed lungs of any mammal at birth are seen in marsupials where the
330 lungs essentially comprise a pair of highly vascularised air sacs at birth (quolls: Hill
331 and Hill, 1955; Gemmell and Nelson, 1988; Tasmanian devil: Hughes and Hall, 1988;
332 opossum: Krause and Leeson, 1975). In marsupials with larger young the lungs have
333 developed a small number of thick walled septae dividing the air sacs (eg tammar:
334 Runciman et al., 1999; quokka Makanya et al., 2001), and development of the full
335 alveolar structure seen in the adult occurs gradually over several months. In tammars,
336 the lungs are at a terminal air sac stage until about day 70 after birth, at which time
337 they start to undergo a transition to alveolar lung structure (Runcimann et al., 1999). It
338 is only by about day 180 that a fully alveolar structure is developed, and after this

stage further maturation continues to establish the mature capillary systems of the adult.

The small size of marsupial neonates and the immaturity of the lung structure limits the functionality of the neonatal lung. In the smallest neonates, such as the Julia Creek dunnart, with a 15 mg neonate 4 mm long, the tiny size of the airways means that breathing actions are extremely inefficient due to the resistance of the airways (Frappell and Mortola, 1989; Mortola et al., 1999). In fact breathing actions in these neonates are sporadic and associated with gross body movements which probably don't involve much movement of air in the airways. They do not establish regular breathing patterns until 2-3 weeks after birth. Despite this, the neonates still have established some of the central reflexes needed for breathing such as the Hering Breuer reflex, which halts respiratory action if the lungs are inflated (Frappell and Mortola 2000). At birth the young rely almost entirely on cutaneous respiration for gas exchange, and even at 3 weeks of age a third of the oxygen transfer occurs across the skin (Mortola et al., 1999; Frappell and Mortola 2000). In tammar, that weigh about 400 mg at birth, the lungs are better developed and regular breathing movements occur, but still 30% of oxygen transfer occurs across the highly vascular skin, and even at day 6, 14% of oxygen is taken up across the skin (MacFarlane et al., 2002).

Postnatal function of the lungs depends not only on gross structural development of the gas exchange surfaces, but also requires a surfactant system to allow the gas-fluid interface to function. In eutherian mammals, the surfactant secreting Type II pneumocytes develop and become functional close to birth as a result of increasing

action of glucocorticoids. Despite their relatively poorly developed lungs, Type II pneumocytes are present and increase in number before birth in marsupials (Gemmell and Little 1982; Walker and Gemmell, 1983; Miller et al., 2001; Krause et al., 1976). The onset of surfactant production coincides with increasing evidence of adrenal activation, and dexamethasone is a potent inducer of surfactant production by Type II pneumocytes from neonatal tammar lungs (Renfree et al., 1995; Miller et al., 2001) suggesting that control of surfactant production is similar to that in eutherians.

Conclusions

Marsupials, because of their reproductive features, make excellent experimental models for studies on pregnancy, parturition and especially the postnatal adaptations that make delivery and survival of such an altricial young possible. The differential growth and function of the gravid versus non-gravid uterus provides opportunities to unravel some of the intricate dialogue that occurs between the embryo, its placenta and the uterus. The need to synchronise fetal development with maternal changes in altricial marsupial neonates provides a valuable model system for understanding prematurity. The central role of glucocorticoids in achieving this synchrony, as they do in eutherians, argues strongly that this is a fundamental mechanism in all mammals. The synchronisation of parturient behaviour with delivery through systemic release of prostaglandins provides a striking example showing that prostaglandins are also important as behavioural regulators. The possible roles of systemic prostaglandin has been much neglected, but the clear demonstration of a behavioural effect in tammar suggests that further study may be valuable. One example is in the control of premenstrual syndrome, when mood changes are

associated with elevated uterine production of prostaglandins. The altricial young are a curious mixture of developmental stages – those features not essential for postnatal survival are typically poorly developed, whilst those needed for survival are relatively well developed. This heterochrony in organ and system development has already proven valuable as models in the study of sex determination and sexual differentiation (Renfree et al., this volume). Given the clinical significance of fetal control of the onset of labour and the necessity of inducing lung and cardiovascular maturation for the survival of premature infants, it is surprising that these issues have not been explored in more detail using this experimental model where prematurity has reached such a peak of perfection.

Acknowledgments

We thank Professor Lynne Selwood and Dr Graeme Coulson for their efforts in organizing this symposium. We thank Professor Richard Behringer for permission to use the image in figure 1d. We thank Dr Terry Fletcher, Mr James Ingram, Ms Sonia Cehun, Dr Claudia Freyer and Dr Laura Parry for extensive discussions and assistance with laboratory work over the years, and numerous students, and colleagues and staff in the wallaby research group who have contributed to the work reviewed here.

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648

Figure Captions

Figure 1. Altricial development in marsupials. Neonatal marsupials are tiny relative to maternal size, as illustrated here by *Antechinus agilis* (a) and tammar (b) newborn young in the pouch. Some structures are relatively advanced in development, such as the forelimbs (fl), which are used in the climb from the urogenital opening to the pouch. The hindlimbs (hl), by contrast are still rudimentary. In longitudinal section (c) the advanced development of the tongue (to) is evident whilst structures such as the cerebral cortex (cc) and reproductive tract (see Renfree et al., this volume) () are still poorly differentiated. The Wolffian duct (wd) is visible in this section. Alcian blue staining of a cleared tammar neonate reveals the disparity in skeletal development around the fore and hind limbs. (d) kindly provided by Professor Richard Behringer.

Figure 2. Hormonal profiles in tammars in the peripartum. See text for details. Adapted from Renfree and Shaw 1996, based on data from Tyndale-Biscoe et al. (1983), Shaw and Renfree (1984), Fletcher et al. (1990), Hinds et al. (1990) and Parry et al. (1996).

Figure 3. Differences between gravid and non-gravid uteri are highlighted here by (a) the differential response of uterine strips from the gravid and non-gravid uteri to stimulation by mesotocin (MT) and inhibition by nitric oxide (NO), and (b) the upregulation of mesotocin receptors in the gravid uterus (open squares) compared to the non-gravid uterus (shaded circles) in the days before birth. In (a) the photograph of the reproductive tract at the right shows the differential growth of the gravid uterus

compared to the adjacent but totally separate non-gravid uterus The complex vaginal tract below the uteri is also shown. Adapted in part from Ingram et al. (2001) and Sebastian et al. (1998).

Figure 4. Parturient behaviour in a female (a) and a male treated with PGF₂ α (b). (a) from Renfree et al, (1989).

Figure 5. By day 24 of gestation the fetal adrenal is well developed, with differentiation of steroidogenic cells rich in smooth ER (S) visible in electron micrographs. Cortisol levels in the adrenal glands (b) and plasma (b) rise to high levels in the last day of gestation then fall post partum. EM in (a) from Renfree, 1994. Data in b and c from Ingram et al. (1999).

Figure 6. Timing of development of lungs in relation to birth and development in tammar (top), mouse (centre) and human (bottom).

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689 Figure 1

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Figure 2

694 Figure 3

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697 Figure 4

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Figure 5

Figure 6

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Title:

Parturition and perfect prematurity: birth in marsupials

Date:

2006-01-01

Citation:

Shaw, G. & Renfree, M. B. (2006). Parturition and perfect prematurity: birth in marsupials. AUSTRALIAN JOURNAL OF ZOOLOGY, 54 (3), pp.139-149. <https://doi.org/10.1071/ZO05070>.

Publication Status:

Published

Persistent Link:

<http://hdl.handle.net/11343/35022>