Maternal recognition of pregnancy in marsupials

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Pregnancy in kangaroos and wallabies (macropodid marsupials) induces multiple unilateral responses in the reproductive system that override those related to proximity to the single corpus luteum on one ovary or to the follicle on the contralateral ovary. This situation is in contrast to most other non-macropodid marsupials, in which the responses are dependent on the corpus luteum. There is now good evidence that these unilateral responses in macropods are controlled by the fetoplacental unit acting locally to stimulate the endometrium and myometrium. Pregnancy also influences the duration of the oestrous cycle and maternal behaviour. The stimuli responsible for these effects probably include paracrine, endocrine and mechanical stimuli resulting from uterine stretch. Taken together, these unilateral responses demonstrate that there is a refined maternal recognition of pregnancy in at least the macropodid marsupials.

The concept of maternal recognition of pregnancy, as described by Short (1969) focused attention on the diverse ways by which the maternal organism first becomes aware of the presence of an embryo in the uterus. The earliest maternal recognition may occur in the differential tubal transport of fertilized versus unfertilized eggs, as described by von Niekerk and Gerneke (1966) in horses, but the best studied response is the way in which the lifespan and function of the corpus luteum is prolonged by the presence of an embryo: originally almost all attention was directed to this aspect of maternal recognition. However, advances in understanding of embryo-maternal interactions, including the local responses of the uterus and the developmental changes in the embryo, have broadened the concept of maternal recognition to include almost all aspects of the ways by which pregnancy can redirect maternal physiology (Heap, 1979).

In many mammals, the secretory activity of the corpus luteum is unaffected by pregnancy and, in mammals that have embryonic diapause, maternal recognition must be delayed. Marsupials (Table 1) provide examples of both of these strategies. The life of the corpus luteum is not extended by pregnancy in most marsupials and because pregnancy is accommodated within the oestrous cycle in all but one marsupial, these mammals had been thought to lack any maternal recognition of pregnancy. Indeed, Hill and O’Donaghue (1913) coined the term ‘pseudopregnancy’ to describe the similarity of the changes that occurred in the uterus and mammary glands of unmated and mated female marsupials. Despite these superficial similarities, it is now known that there are changes in maternal physiology that are specific to pregnancy in marsupials.

Marsupials offer certain advantages for the study of maternal recognition of pregnancy because of their unusual reproductive anatomy. All marsupials possess two completely separate uteri, each with a separate cervix but, in the monovular species, only one of these two uteri becomes gravid, and ovulation usually occurs from alternate ovaries, thus providing researchers with an inbuilt ‘control’ uterus at all stages of pregnancy. In macropodid marsupials (the kangaroos and wallabies) many unilateral responses of the pregnant uterus are now well defined, particularly for tammar wallabies, which have been the subjects of the most detailed studies.

Pregnancy and the oestrous cycle

The duration of the marsupial oestrous cycle varies among species from 22 to 45 days, and the lifespan of the corpus luteum is not prolonged by the presence of a conceptus, although in the bandicoot family, Peramelidae, the corpus luteum persists during lactation (for review, see Tyndale-Biscoe and Renfree, 1987). There is no apparent unilaterally active (but systemically inactive) luteolysin derived from the uterus since hysterectomy has no effect on opossums or on the corpus luteum of brushtail possums. The corpus luteum reaches maximum size during the mid-luteal phase, and decreases in size toward the end of pregnancy. This pattern is mirrored by the proliferation and secretory activity of the endometrium. The histological changes in the gravid uterus during the follicular and luteal phases are similar to those of the non-gravid uterus in monovular and polyovular species (Tyndale-Biscoe and Renfree, 1987). However, closer examination of the endometrial responses provided the first example of maternal recognition of pregnancy in marsupials.

Endometrial responses in pregnancy

As long ago as 1834, Richard Owen recorded that the endometrium of the gravid uterus of the Eastern grey kangaroo was twice as thick as the endometrium of the contralateral, non-gravid uterus. Almost 100 years later, Flynn (1930) described marked histological differences between the gravid and non-gravid uteri of Tasmanian bettongs. Both these observations were largely overlooked until the finding, in tammar wallabies, that the wet weight of the endometrium of the gravid uterus was consistently and significantly greater than that of the adjacent non-gravid uterus (Renfree, 1972). The proliferation of the endometrium is always greater in the uterus adjacent to...
the corpus luteum, whether or not pregnancy occurs, but the effect is enhanced in the second half of pregnancy compared with the oestrous cycle (Renfree, 1972; Renfree and Tyndale-Biscoe, 1973; Tyndale-Biscoe, 1979). That this enhancement is dependent on the presence of the embryo or its fetal membranes was unequivocally demonstrated by the transfer of the blastocyst to the uterus of a cyclic, non-pregnant animal, or to the uterus contralateral to the corpus luteum. In all cases, similar endometrial proliferation occurred in the gravid uterus, regardless of its position relative to the corpus luteum (Fig. 1). In animals in which blastocysts develop in both uteri, both sides had enhanced endometrial responses. In addition to these morphogenetic effects, the composition of the uterine secretions differs between the two uteri (Renfree, 1973a; Renfree and Tyndale-Biscoe, 1973; Tyndale-Biscoe, 1979) and the rate of protein synthesis is greater in the gravid uterus (Shaw and Renfree, 1986). The synthesis of specific uterine secretions (Renfree, 1973a) and growth factors, such as platelet-activating factor (PAF) (Kojima et al., 1993), are also likely to reflect these unilateral influences. Thus, the importance of the feto-placental unit overrides the local effects of proximity to the corpus luteum.

A similar endometrial response occurs in the potoroo (Shaw and Rose, 1979), the quokka (Wallace, 1981) and in Bennett’s wallaby (Walker and Rose, 1981). So far, these effects have been observed only in macropodids. In monovular non-macropodids, such as the brushtail possum, both gravid and non-gravid uteri respond to a similar extent, although there is also a local effect of the adjacent corpus luteum in brushtail possums (Von der Borch, 1963; Curlew and Stone, 1986). In polyovular species, such as opossums, both uteri are gravid, but a few values from non-pregnant animals (Renfree, 1975; Fleming and Harder, 1981a,b) indicate an identical endometrial response during the oestrous cycle and pregnancy. Likewise, there is a similar pattern of histological changes in the gravid and non-gravid uteri in two species of polyovular, dasyurid marsupials, the dunnart and the antechinus (Cruz and Selwood, 1993, 1997), but there are distinct uterine differences in pregnancy and non-pregnancy at some (dunnart) or at most (antechinus) time points. These differences may be due to direct embryonic signals affecting the corpus luteum since these differences are correlated with the rate of embryonic development and with progesterone concentration. The results of further studies of these species will be awaited with interest.

**Urogenital vasculature**

The local effects of ovarian stimulation are possible because of the close association between the ovarian and uterine vascular supplies. The ovarian venous plexus is a fusion of many small veins draining the ovary, and the multiple uterine branches of the ovarian artery almost completely surround the plexus. This arrangement is similar in brushtail possums (Lee and O’Shea, 1977) and tammar wallabies (Towers et al., 1986), and probably occurs in the potoroo (Shaw and Rose, 1979). There is no portal blood supply from the ovary to the uterus, but the proximity of the branched uterine arterial supply to the ovarian drainage provides an anatomical arrangement whereby hormones of luteal or follicular origin can diffuse from the ovarian vein via a countercurrent system into the arteries of the uterine branch of the respective ovarian veins. Evidence to support this suggestion is provided by the differential concentrations of progesterone (Towers et al., 1986; Kojima et al., 1993) and oestradiol (Harder et al., 1984) sampled from the uterine branch of the ovarian vein in tammar wallabies (sometimes labelled erroneously the ‘utero-ovarian vein’) (Fig. 2). Similar unequal concentrations of progesterone and oestradiol occur in the left and right ovarian veins of brushtail possums (Curlew and Stone, 1986) but not of opossums (Harder and Fleming, 1981).

**Feto-placental influences**

Blastocyst development can be initiated experimentally during seasonal quiescence in tammar wallabies with daily exogenous progesterone injection, which also induces endometrial growth of both uteri for the duration of the treatment (Renfree and Tyndale-Biscoe, 1973). However, after the injections cease on day 10, only the uterus containing the developing embryo continues to enlarge, while the non-gravid uterus reduces to a size typical of the non-pregnant cycle. In these animals, the corpus luteum remains inactive, so the response of the gravid uterus after progesterone withdrawal must be due to the presence of the fetus or its placenta. The choriovitelline placenta, which is never invasive and only makes a chorionic–epithelial interdigitation with the uterine epithelium (Tyndale-Biscoe and Renfree, 1987), appears to be responsible for this effect because some treated animals produced a vesicle that lacked any embryonic or vascular mesodermal tissue (Renfree and Tyndale-Biscoe, 1973). These expanded vesicles, which consisted only of non-vascular yolk sac tissue (trophoblast plus endoderm), nevertheless stimulated the endometrium and the uterine secretions as in a normal pregnancy. These uterine responses on the gravid side may be due to either uterine stretch, or to hormonal or

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**Table 1. Maternal recognition of pregnancy in marsupials**

<table>
<thead>
<tr>
<th>Species with clear evidence of maternal recognition of pregnancy</th>
<th>Species with some evidence of maternal recognition of pregnancy</th>
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</thead>
<tbody>
<tr>
<td>Kangaroos and wallabies (Macropodidae)</td>
<td>Possums (Phalangeridae)</td>
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<tr>
<td>Quokka</td>
<td>Antechinus agilis (stuartii)</td>
</tr>
<tr>
<td>Tammar</td>
<td>Trichosurus vulpecula</td>
</tr>
<tr>
<td>Bennett’s wallaby</td>
<td>American opossum (Didelphidae)</td>
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<tr>
<td>Eastern grey kangaroo</td>
<td>Virginia opossum</td>
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<tr>
<td>Western grey kangaroo</td>
<td>Grey short-tailed opossum</td>
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<tr>
<td>Potoroo</td>
<td>Monodelphis domestica</td>
</tr>
<tr>
<td>Tasmanian bettong</td>
<td>Sminthopsis macroura</td>
</tr>
</tbody>
</table>

**Species that apparently lack a maternal recognition of pregnancy**

- Dasyurids (Dasyuridae)
- Monodelphis domestica
- Virginia opossum
- Grey short-tailed opossum
- Bandicoots (Peramelidae)
cytokine stimulation by the placenta (Renfree, 1972; Young and Renfree, 1979; Shaw, 1983).

Although hormone production by the marsupial placenta has not been well studied, and the tammar placenta has only incipient steroid synthetic activity (Heap et al., 1980), it is known to have considerable capacity to synthesize prostaglandins (Shaw et al., 1999). The tammar placenta also expresses the peptide relaxin (Parry and Renfree, 1999). The fetal fluids are also rich in prostaglandins (Shaw et al., 1999) and cortisol (Ingram et al., 1999), and the placenta maintains high concentrations of these hormones against a concentration gradient, as it does with the wide variety of amino acids, proteins and carbohydrates secreted into the fetal fluids (Renfree, 1973b).

Clearly, the marsupial placenta has considerable endocrine and paracrine capacity, and probably autocrine activity, much of which is as yet undefined.

Uterine receptors

The fact that the endometrium of the non-gravid uterus decreases in mass and secretory activity during the second half of pregnancy or the oestrous cycle, even though peripheral progesterone concentrations are at their highest, indicates that there is a decreasing sensitivity to progesterone. It was suggested that the local stimulation of the gravid uterus reverses sensitivity to progesterone by stimulating increased progesterone receptor synthesis, but recent evidence does not support this hypothesis. There are differences in progesterone receptor concentration between the gravid and non-gravid uteri of quokkas (Owen et al., 1982) and tammar wallabies (M. B. Renfree and D. R. Blanden, unpublished). However, both progesterone and oestriadiol receptor concentrations are downregulated after day 10 in early pregnancy, concomitant with the increase in progesterone in the peripheral plasma. Another unilateral effect occurs at term, in that there is an increase in oestrogen receptor concentration in the endometrium of the non-gravid uterus and immediately after birth, indicating that oestrogen receptors are upregulated selectively in the non-gravid endometrium before birth, in response to the local effects of follicular oestradiol from the ipsilateral ovary (Fig. 3).

The receptor for the oxytocic peptide mesotocin is also up-regulated preferentially in late pregnancy in the gravid myometrium and downregulated in the non-gravid myometrium (Parry et al., 1997; Sebastian et al., 1998). The increase in mesotocin receptors in the gravid but not in the non-gravid myometrium indicates that, at the end of gestation, there may be a paracrine control of mesotocin receptors by the feto–placental unit (Fig. 3). This suggestion is supported by the demonstration that neither uterus of non-pregnant animals shows these increases in mesotocin receptor concentrations (Siebel, Bathgate and Parry, 1999), highlighting this specific maternal recognition of pregnancy.

Myometrial responses

Myometrial activity is quiescent during pregnancy in tammar wallabies and, as the gravid myometrium grows, the gravid myometrium, unlike the non-gravid myometrium, becomes increasingly sensitive to oxytocin and prostaglandin (Tyndale-Biscoe and Renfree, 1987). In quokkas, isotonic contractile activity also changes through the oestrous cycle, and pregnancy influences uterine contractility. Myometrial activity in vivo has been recorded in tammar wallabies by electromyography (Young and Renfree, 1979; Shaw, 1983). Activity remains low and cyclic in pregnant uteri at all stages of gestation, and sensitivity increases in the gravid uterus in late pregnancy, but by day 1 after birth both uteri are inactive and refractory (Shaw, 1983). Since these effects differ between gravid and adjacent non-gravid uteri, they reflect a local rather than systemic influence. Prostaglandin F20 concentrations increase sharply at parturition. Myometrial responsiveness to the PGF20 analogue Cloprostenol increases markedly towards the end of gestation, but the myometrium is also refractory after birth. There is an increase in uterine PGF20, PGE2 and 6-keto-PGF1 concentrations at term, but these concentrations decrease markedly by day 1 after birth (Shaw et al., 1999).
Effects of pregnancy on the duration of the oestrous cycle

The duration of reproductive cycles in macropodids is affected by the presence of the embryo and by preceding lactation. The duration of gestation in the Eastern and Western species of grey kangaroos is 36 and 31 days, respectively, but the gestation of Eastern grey females mated to Western grey males is intermediate at 34 days (for review, see Tyndale-Biscoe and Renfree, 1987), reflecting the hybrid genotype of the fetuses. The duration of the oestrous cycle of female progeny was intermediate between the parent species. In the agile wallaby, the interval from one oestrus to the next is shorter in pregnant females than in non-pregnant females (Merchant, 1976), a phenomenon that has been confirmed in tammar (Merchant, 1979) and Bennett’s wallabies (Merchant and Calaby, 1981). These results are unequivocal demonstrations of maternal recognition of pregnancy. It is clear that the feto–placental unit of macropodid marsupials is influencing ovarian function by systemic signals, a characteristic that marsupials had been thought to lack.

Behavioural recognition of pregnancy

In most marsupials, it is essential that the mother recognizes the impending birth, to ensure that the tiny, altricial neonate is able to reach the pouch or mammary area to complete its development. Most species studied so far achieve this by adopting a specific birth posture. In macropodids, the birth posture
is characterized by the female sitting or resting against a tree or support with the tail passed forward between her legs. The female licks her pouch and urogenital opening especially vigorously in the minute immediately preceding birth (Renfree et al., 1989). This behaviour is now known to be under the influence of prostaglandins (Shaw, 1990; Hinds et al., 1990), and even males treated with the prostaglandin analogue Cloprostenol will adopt the birth posture and lick their scrotum (Shaw, 1990). Oxytocin, if given in pharmacological doses, can also induce adoption of the birth position in the potoroo, bettong, bandicoot and the grey short-tailed opossum (Rose and McFadyen, 1997; Rose and Fadem, 1999).

Conclusions
Numerous unilateral effects are imposed by the anatomy of the marsupial reproductive tract and, in monovular species, by the proximity of the corpus luteum of pregnancy or the developing follicle. These effects can be over-ridden by paracrine and endocrine signals from the feto–placental unit, and some of these responses may also be due to local physical reactions to the presence of a developing fetus. The vascular anatomy of the reproductive tract allows preferential unilateral delivery of these signals regardless of their origin. However, the feto–placental unit in marsupials, as in all other mammals, can redirect maternal physiology to ensure the success of pregnancy and parturition. Macropodid marsupials have a refined maternal recognition of pregnancy, which is manifest by unilateral responses within the female reproductive tract.

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