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STEROIDS IN PREGNANCY AND PARTURITION IN THE MARSUPIAL, *MACROPUS EUGENII*

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SUMMARY

Pregnancy in the tammar wallaby, *Macropus eugenii* may be prolonged by a long period of embryonic diapause of the 100-cell blastocyst; resumption of development occurs after removal of the suckling pouch young (RPY) or by exogenous progesterone injection. The corpus luteum is essential for the first 8 days of pregnancy, but is not required for normal development after this time. Normal parturition will only occur, however, if the corpus luteum is present up to at least day 21 of pregnancy.

The corpus luteum produces and contains progesterone, but peripheral plasma values are low (1 ng/ml). The peak progesterone content in the corpus luteum is attained at day 23 RPY, and is followed by a peak plasma concentration at day 25. Levels in both fall markedly by the day of birth, day 27 RPY. Ovariectomy will abolish the peak progesterone concentration in the peripheral plasma.

Contractile activity of the myometrium is inhibited throughout gestation, probably by progesterone, since exogenous progesterone delays parturition. The pregnant uterus shows less electrical activity than the contralateral uterus, but is more responsive to oxytocin. This implies the existence of local regulatory mechanisms within the pregnant uterus. Fetal cortisol production may be involved in the initiation of parturition, and progesterone withdrawal appears to be necessary for birth. These data are summarised into an hypothetical scheme showing possible control mechanisms for parturition in the tammar wallaby.

INTRODUCTION

(i) *The marsupial reproductive cycle*

Marsupial gestation is completed within the span of an oestrous cycle, thus the life of the corpus luteum is not prolonged during pregnancy as it is in the eutherian mammals. This means that the components of the oestrous cycle (the luteal and follicular phases) are not interrupted by pregnancy, although the influence of the suckling stimulus from a new born pouch young does suspend further reproductive activity.

In the kangaroos and wallabies (Macropodidae) pregnancy occupies the entire length of the cycle, but ovulation and oestrus are not inhibited [1]. In these marsupials, parturition is followed some 12–24 h later with a post-partum oestrus and fertilization. The fertilized egg passes quickly to the non-parturient uterus, and slowly develops over the next 7 days to a 100-cell blastocyst [2]. The corpus luteum forms from the follicle but does not complete its development or induce a luteal phase in the uterus so long as the neonate suckles in the pouch. The quiescent corpus luteum itself further inhibits the development of new follicles [3]. If the pouch young is lost, the corpus luteum and the blastocyst are released from suckling inhibition, and they resume development.

The tammar wallaby, *Macropus eugenii* is, like other macropodids, polyoestrus and monovular. However, it is a seasonal breeder, and while loss of the pouch young between January and June causes resumption of development of the delayed blastocyst (lactational quiescence), and the blastocyst remains quiescent until the summer solstice, December 22nd. The blastocyst resumes development on the longest day and the young is born about 27 days later, a new ovulation and oestrus occur, and the cycle begins again. Thus in the tammar, an embryo conceived at the end of January in one year is not born until the end of January in the following year [4]. The tammar reproductive cycle is therefore influenced by two types of control mechanism—that of the suckling stimulus in the first half of the year when the blastocyst is in lactational quiescence, and that of photoperiod which terminates seasonal quiescence in the second half of the year.

(ii) *The embryo and placenta*

The marsupial neonatus (Fig. 1) has well developed fore-limbs, and a functional respiratory and digestive system, but is naked, has poorly developed hind limbs, closed eyes and undifferentiated gonads [5]. The duration of pregnancy is relatively uniform in marsupials, ranging from 12 to 40 days, and is characterized by a relatively slow embryogenesis phase and a rapid organogenesis. During the embryogenesis phase, the embryo lies free and unattached in the uterus. The placenta of the tammar becomes closely apposed to the endometrium, but does not invade or erode it, and the placental attachment is formed by the yolk sac membrane, not the allantois as in most eutherians [5]. The placenta has long been dis-

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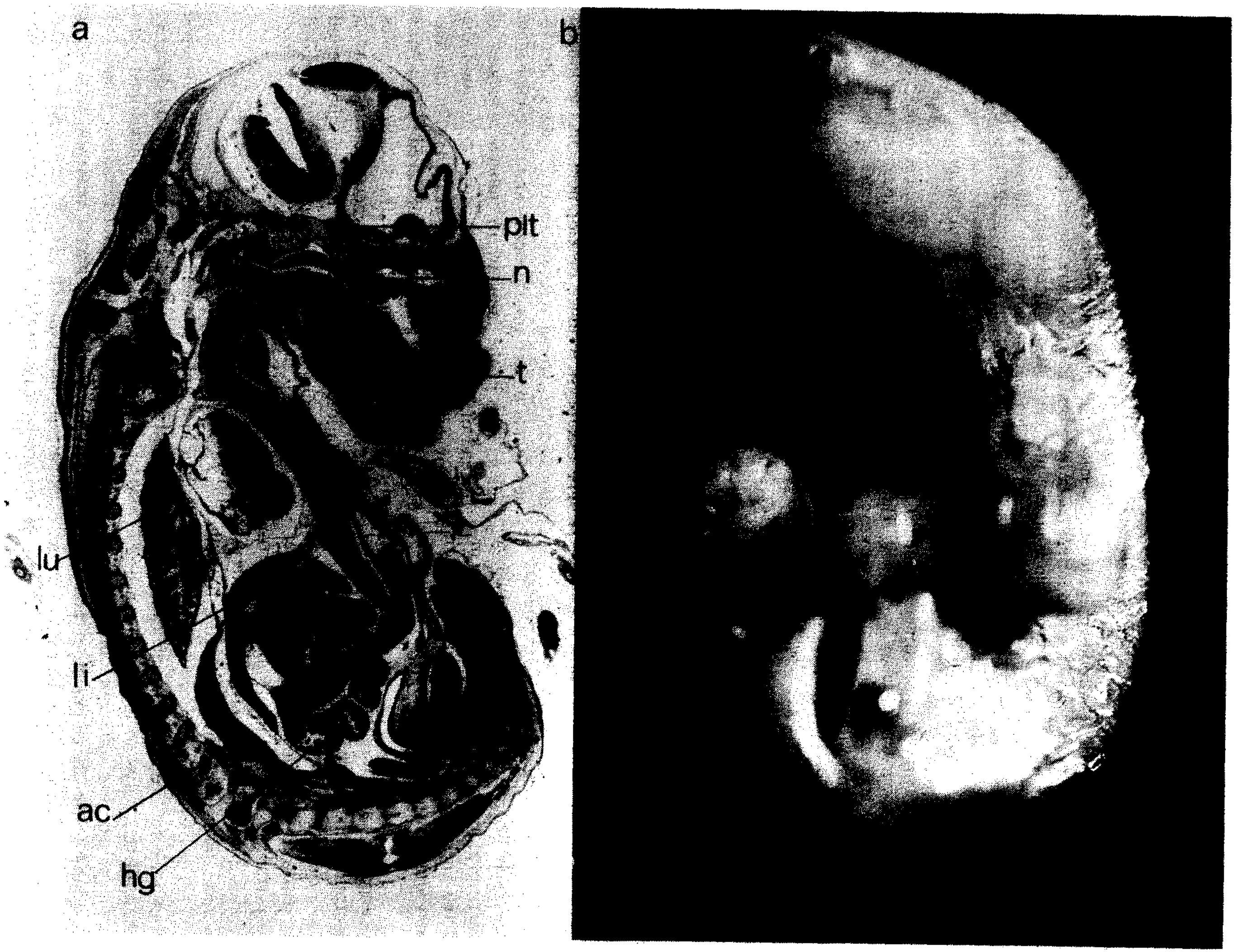

Fig. 1(a). Longitudinal section of a full-term fetus of *Macropus eugenii* 24 h before birth showing the adrenal cortex (a.c.), hind gut (h.g.), liver (li), lungs (lu), nasal passage (n), pituitary (pit) and tongue (t). (b) Neonatus of *M. eugenii* 24 h after birth (approx. 350 mg).

counted as a potential source of hormones in the marsupial, and the endocrine control of pregnancy and parturition have been poorly studied until recently. It is the purpose of this paper to review the most recent data on the role of steroid hormones during pregnancy and to suggest mechanisms for the control of parturition in the tammar wallaby.

THE OVARY IN PREGNANCY AND PARTURITION

(i) *The role of the corpus luteum*

The corpus luteum is essential for early development in the tammar, but by day 8 it is not required for gestation to continue to full term [6]. The single corpus luteum reaches a peak size [4] and weight [7] by about day 15 after removal of the pouch young (RPY). The peak in corpus luteum weight corresponds closely to the peak endometrial weight attained in the pregnant uterus (Fig. 2). In the macropodids, the endometria of pregnant uteri become much heavier whereas, in non-macropodid marsupials, both gravid and non-gravid uteri respond to a similar extent [5]. In the tammar, the proliferation of the endometrium and resumption of development of the diapausing blastocyst can be initiated and maintained by injections of exogenous progesterone (10 mg daily) but in this case both gravid and non-gravid endometria respond [4, 8]. If progesterone administration ceases, only the uterus containing the developing embryo will continue to show the secretory, enlarged endometrium and the non-pregnant uterus declines to levels usually observed in the non-pregnant cycle. The conclusion from these experiments is that the essential factor for resumption of early development originates in the corpus luteum, and that its function is to initiate secretory activity by the endometrium. The secretions provided by the uterine glands appear to be critical for the provision of nutrients to the embryo during the long, pre-attachment embryogenesis phase [9, 10]. Since progesterone can initiate the same response, it seems reasonable to suggest that this is the factor produced by the corpus luteum.

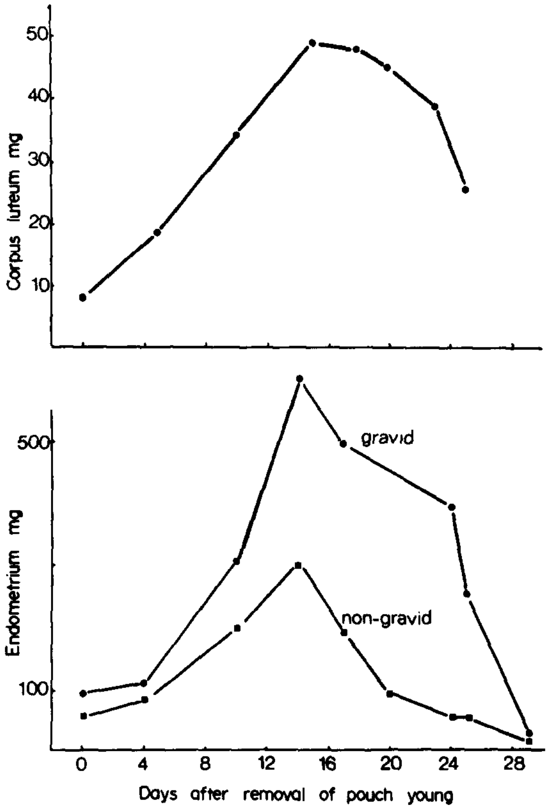

Fig. 2. Wet weights of corpora lutea during pregnancy (top) and endometria (bottom) from gravid (●) and non-gravid (■) uteri of the tammar wallaby *Macropus eugenii*. Corpora lutea values redrawn from Young and Renfree (1979) and endometrial values redrawn from Renfree and Tyndale-Biscoe (1973).

Although the onset of uterine secretory activity requires an active corpus luteum, intra-uterine development will proceed to full-term after lutectomy or ovariectomy on day 8, but parturition fails [1]. Similarly, ovariectomy performed between days 6 and 10 in three other marsupial species (the American opossum, *Didelphis virginiana*, the brush-tailed possum, *Trichosurus vulpecula*, and the quokka, *Setonix brachyurus*) has no effect on pregnancy and the embryo continues to grow to full term [11–14]. The uterus remains secretory and embryonic development is not impaired, although parturition is inhibited. However, in tammars lutectomized after attachment (or "implantation") of the yolk sac placenta has occurred, parturition can occur in a proportion of the animals [15]. In this case it seems probable that there has been sufficient stimulation of the genital tract by the secretions of the corpus luteum to allow parturition (Table 1). Again, progesterone is implicated as at least one of the essential factors, possibly acting in conjunction with relaxin.

Relaxin has been found in the corpus luteum of the brush-tailed possum [16, 17] and recently bioassayed in the corpus luteum of the tammar, reaching a maximum concentration at day 17 (C.H. Tyndale-Biscoe, personal communication). However, successful parturition was not achieved after administration of relaxin to animals with full term fetuses but inactive corpora lutea [18].

In the tammar, just as development will proceed to full term after ovariectomy on day 8 or later, development can be initiated during the period of seasonal

Table 1. Numbers of tammar wallabies giving birth after lutectomy or sham operation before (days 6–15) or after (days 17–25) attachment of the yolk placenta

Day of operation	Lutectomy	Sham control
6–15	0/19	9/15
17–25	7/20	9/16

Derived from Young and Renfree (1970).

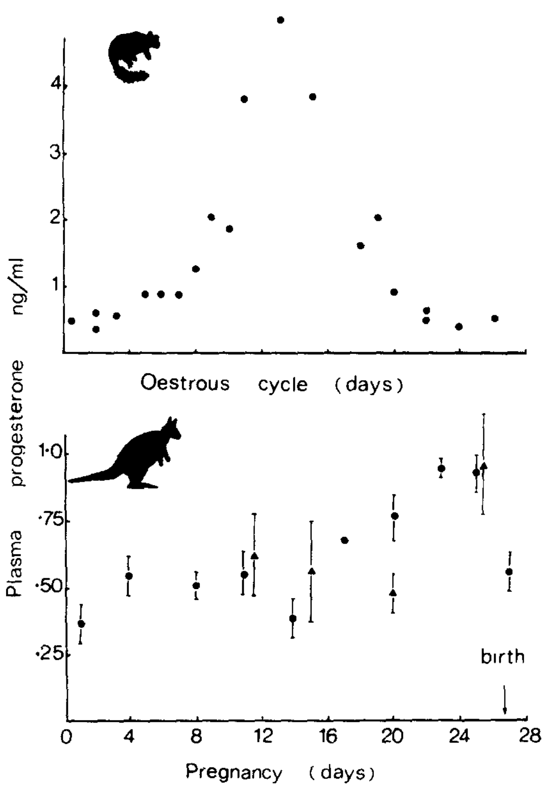

Fig. 3. Top: Peripheral plasma progesterone levels in the brush-tailed possum, *Trichosurus vulpecula* during the oestrous cycle (redrawn from Tyndale-Biscoe, 1973; data derived from Thorburn *et al.*, 1971). Bottom: Peripheral plasma progesterone levels in the tammar, *Macropus eugenii* during pregnancy. Data redrawn from Lemon (1972) (●) and Renfree and Heap (1977) (▲). Values given are means ± S.E.

quiescence by administration of exogenous progesterone for 10 days [4]. In both cases, parturition is inhibited; in seasonally quiescent animals the progesterone stimulates the uterus directly, and the corpus luteum remains inactive.

(ii) *Progesterone concentrations in plasma and corpora lutea*

Levels of circulating steroids are not well defined in marsupials. Peripheral plasma progesterone during the oestrous cycle of the brush-tailed possum, and during pregnancy in the tammar show two distinct patterns (Fig. 3). In the possum, levels change from 0.5 ng/ml before day 8, peak at day 12 with 3–4 ng/ml, and decline by day 17 when the corpus luteum involutes [19]. Birth in this species occurs at day 17.5, so parturition is preceded by a fall in progesterone levels. In the tammar, peak progesterone levels occur at day 23 subsequent to the peak size of the corpus luteum at day 15 (Figs 2 and 3). Total concentrations are far lower than in the possum, with 0.4 ng/ml at day 17 and a maximum of only 1 ng/ml at around day 25 [20, 21]. Parturition, at day 27, is once again preceded by a fall in plasma progesterone concentration.

Although the corpus luteum at day 25 has a degenerate appearance histologically [4], the corpus luteum is still capable of conversion of [^{3}H]-pregnenolone to [^{3}H]-progesterone *in vitro* (Table 2), showing that 5-ene-3β-hydroxysteroid dehydrogenase activity is high in this tissue even at day 25. Non-luteal ovarian tissue is also able to synthesize progesterone from pregnenolone *in vitro*, and this may contribute to the progesterone pool ([21] and R. B. Heap and M. B. Renfree unpublished results).

The corpus luteum of the tammar contains appreciable quantities of progesterone throughout gestation [7]. The total amount present in each corpus luteum correlates well with the size of the gland, but by day 24 both the size and progesterone content drop markedly (Fig. 4). As in the peripheral plasma, the highest total concentration is observed towards the end of pregnancy at day 23, and there is a fall in progesterone content immediately prior to parturition. The drop in peripheral plasma levels occurs about 2 days after the fall in corpus luteum content.

In animals ovariectomized at day 10 RPY, the peak in peripheral plasma progesterone was abolished [20]. This, together with the content of progesterone in the corpus luteum, supports the idea that all measurable plasma progesterone is derived from the corpus luteum.

Since the myometrium remains quiescent during pregnancy under the influence of progesterone ([22] and see below), it is not surprising to observe a fall in corpus luteum progesterone content by day 25 RPY. Recently, parturition has been delayed in a small group of tammars by injections of progesterone on day 26 [18] implying that a fall in progesterone concentration is essential for normal parturition.

Table 2. Incorporation of [^{3}H]-pregnenolone into [^{3}H]-progesterone *in vitro* by the corpus luteum of the tammar wallaby at various stages of pregnancy

Stage of pregnancy (days RPY)	No. of animals	% Incorporation
11	3	44.1
15	4	57.0
20	3	55.0
25	3	58.3

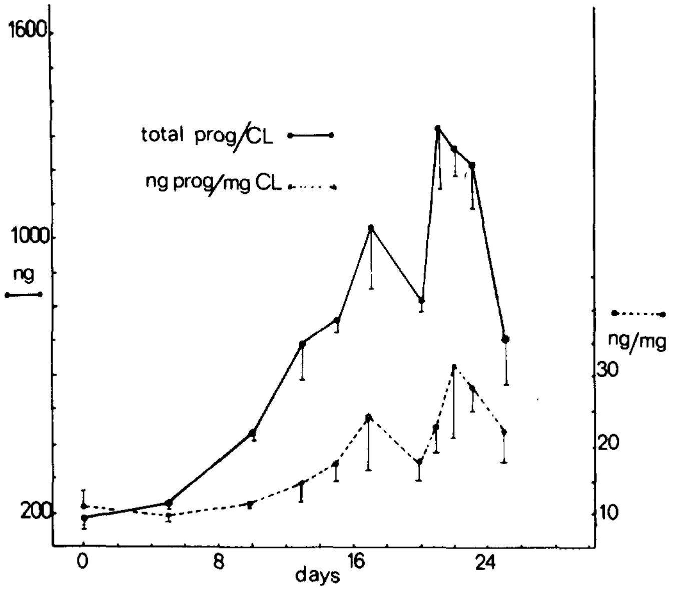

Fig. 4. Progesterone content of corpora lutea from tammar wallabies during pregnancy. Total progesterone per corpus luteum and ng progesterone/mg corpus luteum both increase to reach a peak at day 23, but concentrations fall by day 25. Data redrawn from Renfree *et al.* (1979). Values given are means ± S.E.

UTERUS, MYOMETRIUM AND MEDIAN VAGINA

(i) *Steroid metabolism*

There is evidence of steroid metabolism in the endometria of pregnant tammars. In incubation studies, Heap and Renfree[21] have found that progesterone can be converted to androstenedione. Androstenedione and dehydroepiandrosterone were metabolized to more polar compounds, but it is unclear whether these were oestrogens. Some differences occur between synthetic abilities of pregnant and non pregnant uteri. Yolk sac placenta exhibited low (<2%) conversion of pregnenelone to progesterone. The finding of Renfree[8] that pregnancy, and possibly the yolk sac placenta, induces greater endometrial proliferation in the pregnant uterus than in the non-pregnant (Fig. 2), and the findings of steroid metabolism by fetal tissue [21, 23] refute the contention that the endocrinology of pregnancy is identical to that of the oestrous cycle in marsupials.

(*ii*) *Myometrial activity*

Myometrial activity has been investigated by electromyography in seasonal quiescence and during the breeding season [18, 22]. In seasonal quiescence spontaneous activity is low but the uterus is sensitive to stimulation with oxytocin and a prostaglandin analogue (cloprostenol: I.C.I. 80996). Priming for 2 days with oestradiol benzoate (10 μg/day in oil by intramuscular (i.m.) injection) significantly elevates spontaneous myometrial activity whereas progesterone priming (10 mg/day in oil, i.m.) does not. Neither treatment affects the response to oxytocin or cloprostenol.

In the early stages of the oestrous cycle and pregnancy, following removal of pouch young, myometrial activity is low but by day 14 of the oestrous cycle spontaneous activity is elevated. This change does not occur in the pregnant uterus which remains electrically quiescent up to full term at day 27. Simultaneous recording of activity in the pregnant and non-pregnant uteri of gestating animals shows that the non-pregnant uterus is the more active in terms of spontaneous activity while the pregnant uterus is the more responsive to oxytocin after mid-gestation (Table 3). The findings of unilateral effects on the myometria are interesting, especially in regard to the unilateral endometrial response, and demand explanation.

The selective inhibition of activity in the pregnant uterus could be due to locally elevated levels of progesterone from the placenta or the corpus luteum of pregnancy. While placental progesterone synthesis is believed to be minor, it could account for locally elevated levels in the uterine tissue by direct diffusion. Another source of progesterone is the corpus luteum. Von der Borch[24] and Renfree[8] have shown that there is preferential endometrial proliferation in the uterus ipsilateral to the corpus luteum in non-pregnant brush-tailed possums and tammars. This suggests there is a selective distribution of corpus luteum secretions between the two uteri. The vascular anatomy of the reproductive tracts of both species is consistent with the existence of a countercurrent mechanism for the transfer of hormones from the ovarian

Table 3. Relative myometrial activity at different stages of pregnancy and the oestrous cycle in the tammar wallaby

	Early	Mid	Late	Full Term	Post partum
Spontaneous activity during pregnancy	+	+	+		–
Spontaneous activity during the oestrous cycle	+	+ + +	+ +		
Oxytocin induced activity during pregnancy		+ +	+ + +	+ + +	–

– indicates no activity; + + + very high activity.
Derived from Young (1978).

vein to the ovarian artery which supplies the ipsilateral uterus [18, 25]. The relative importance of these two proposed mechanisms requires elucidation.

The enhanced sensitivity of the pregnant uterus to oxytocin implies the existence of a stimulatory factor. This could be mechanical (uterine wall tension) or hormonal. The possible conversion by endometrium of androstenedione and DHA to oestrogens could be significant. Furthermore, the gravid and non-gravid uteri differ in their ability to synthesize more polar compounds from these precursors.

(iii) *The median vaginal canal*

The median vagina through which the fetus passes at birth is long and narrow with fibromuscular walls [16]. There is evidence that some loosening of vaginal tissue is necessary to permit birth of an undamaged fetus [13, 16, 18]. Tyndale-Biscoe found that administration of progesterone to brush-tailed possums caused loosening of the median vaginal tissue and Sharman[12] obtained live births of lutectomized pregnant animals in the same species.

CONTROL OF PREGNANCY AND PARTURITION

(i) *Resumption of development after diapause and maintenance of pregnancy*

The sequence of events leading to and following blastocyst activation can now be reasonably clearly defined in the tammar. Removal of the suckling young during lactational quiescence causes immediate resumption of development of the embryo, and results in cessation of lactation within 2 days. The role of the pituitary in diapause and the maintenance of pregancy is an interesting one. Hypophysectomy during diapause results in the reactivation of the corpus luteum and the blastocyst, although the full term fetuses are found dead in the uterus 2 days after the expected birth date [26, 27]. Subsequently it has been shown that reactivation results from a reduction in prolactin secretion by the anterior pituitary which releases the corpus luteum from inhibition; prolactin is apparently the tonic inhibitor of corpus luteum development [2, 28]. The corpus luteum secretions, as described above, stimulate the uterus to produce secretions necessary for the blastocyst.

It was not clear whether the hormonal milieu associated with suckling, or the neural stimulus from the mammary gland, was responsible for the maintenance of delay. However, after denervation of the mammary gland, the blastocyst will resume development although the pouch young are not affected and continue to suckle [29]. This points to a direct neural pathway between mammary gland and the higher centres as the proximate factors.

Once initiated, development will continue in the absence of the ovaries and pituitary, but the characteristic endometrial response of the pregnant uterus is apparently dependent on the presence of the embryo or the fetal placenta [8]. This effect must be a local response, since it is only observed unilaterally. If the factor is progesterone, as suggested, it must be produced in very low concentrations as ovariectomy abolishes the rise in peripheral plasma progesterone seen at the end of pregnancy [20]. Although the yolk sac placenta has low progesterone synthetic ability *in vitro*, it actively metabolises other steroids: 5α-androstanedione and androsterone have been identified from androstenedione precursor [21].

(ii) *Initiation of parturition*

Production of steroids by the fetal adrenal is a common step in the early stages of initiation of parturition in many species and typically occurs some days before birth. Fetal steroid levels usually rise until birth while maternal progesterone levels fall. In many species oestrogen levels rise before birth.

In the tammar, presumptive adrenal tissue is evident by day 20–21 [30] and the cortex is differentiated by day 22 [31]. By this stage the tissue has $3\text{-}\beta$ steroid dehydrogenase activity and corticosteroids have been identified in the plasma of a day 24 fetus [30, 32].

Maternal injection of 0.5 g of metapyrone, an inhibitor of 11β steroid dehydrogenase, at day 22 did not affect the time of parturition in two tammars whereas injection at days 20 and 22 significantly delayed birth in three other animals [18]. This result could have been due to suppression of maternal adrenal function but this would be hard to explain in terms of accepted theories of parturition. A more acceptable explanation is that metapyrone crossed the placenta and inhibited fetal adrenal function. The metapyrone molecule is small and Renfree's findings [9, 31] suggest that it could readily cross the placenta. If this explanation is correct it would imply that fetal corticosteroids have already activated the mechanisms leading to parturition by day 22.

Progesterone levels fall shortly after the proposed time of action of fetal cortisol, and there may be a causal relationship. Most of the circulating progesterone is secreted by the corpus luteum [1, 20] and Merchant[33] has demonstrated that pregnancy reduces the time from RPY to oestrus. These results suggest the possibility that there is a pregnancy-specific luteolytic mechanism responsible for the decline in progesterone synthesis. The timing of this decline suggests that the luteolysin may be secreted by uterine tissue as a result of fetal cortisol synthesis. This hypothesis is highly speculative and untested but agrees with the findings in domestic species. However, hysterectomy has no effect on the life span of the corpus luteum in the oestrous cycles of two other marsupial species [34, 35].

(iii) *Proposed control mechanism*

It is clear from these various studies that steroid hormones play as critical a role in marsupial pregnancy and parturition as they do in the eutherian mammals. While much more information needs to be collected before a definitive scheme to describe parturition can be proposed, the evidence suggests the following working hypothesis.

Progesterone from the corpus luteum is essential for the initiation of uterine secretory activity and resumption of blastocyst development [4, 10]. Once initiated it may be maintained by a local effect of the placenta [8]. Progesterone inhibits myometrial activity, but oestrogen produced by the endometrium may explain the higher sensitivity of the myometrium to oxytocin and prostaglandin [18, 22].

External stimuli such as handling the animals may influence the timing of parturition but detailed studies need to be made [18]. These factors may influence the release of oxytocin by the maternal pituitary. Pro-

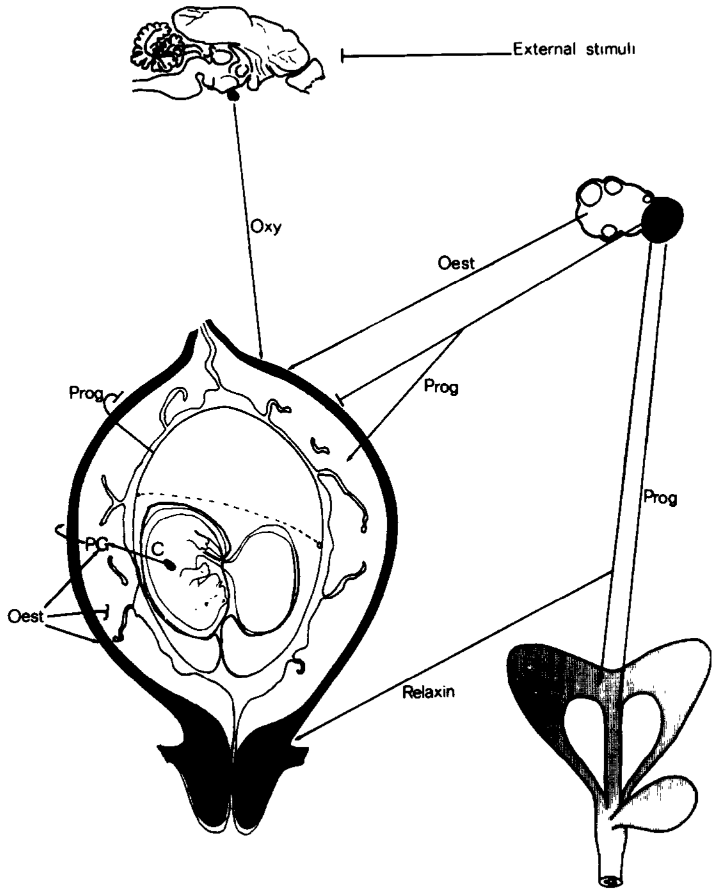

Fig. 5. An hypothetical scheme consistent with present evidence, to describe the possible control of parturition in the tammar wallaby. During pregnancy progesterone stimulates uterine secretory activity. Fetal cortisol may stimulate prostaglandin synthesis in the myometrium. This could also be stimulated by oestrogens produced by the endometrium. External stimuli act through the hypothalamus on pituitary oxytocin; prostaglandin causes myometrial contraction in an oestrogen-sensitized uterus, but progesterone inhibits this effect. Placental progesterone production may contribute to the observed unilateral response. Finally, progesterone and relaxin may be necessary for the preparation of the cervix and the median vaginal canal to allow passage of the fetus to the outside. (See text for further details and references.); C: cortisol, oest: oestrogens, oxy: oxytocin, PG: prostaglandins, prog: progestins. Arrows show proposed stimulatory actions; T show proposed inhibitory actions.

gesterone levels need to decline since progesterone inhibits myometrial activity [18, 22]. Fetal adrenal cortisol [30, 32] may induce prostaglandin synthesis by the endometrium, and placental progesterone may counteract this effect of cortisol until the end of gestation [18]. Progesterone, and possibly relaxin, appear to be required to allow passage of the fetus from the uterus through the cervix and median vaginal canal [15, 16]. These concepts are summarized in the final figure (Fig. 5) to provide a basis for further research.

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