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Effects of progesterone on parturition in the tammar, *Macropus eugenii**

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Summary. Tammar wallabies were treated with progesterone injections or implants during late pregnancy to determine whether progesterone withdrawal was essential for parturition. Neither physiological (implanted group) nor pharmacological (injected group) levels of circulating progesterone prevented parturition occurring at about the expected time in about two-thirds of animals that were pregnant. The neonates of both groups were normal in size and weight, but about a third of treated pregnant animals retained their fetuses or aborted. The retained fetuses were retarded in development.

Therefore, progesterone treatment had no influence on the duration of gestation, or parturition, in the tammar wallaby, but high progesterone concentrations may interfere with the normal course of development and birth in a proportion of treated animals.

Introduction

A common feature amongst most mammals seems to be the indispensable role of progesterone in the maintenance of pregnancy. In the rabbit and rat progesterone inhibits uterine contractility (Davies & Ryan, 1972), and parturition is initiated by regression of the corpus luteum and a sharp fall in the circulating concentrations of progesterone (Csapo, 1956; Davies & Ryan, 1972). Progesterone withdrawal can also trigger the onset of parturition in cows, sheep and pigs, but there is no evidence for a pre-partum decline in progesterone in women, rhesus monkeys, horses, or guinea-pigs (Thorburn, 1983; Heap & Flint, 1984).

Very little is known about the onset of parturition in marsupials. They have relatively short gestations and a corpus luteum is not necessary for pregnancy maintenance; it can be dispensed with early in pregnancy in the quokka, tammar, brush possum and opossum (Tyndale-Biscoe, 1963, 1970; Sharman, 1965; Renfree, 1974). However, the marsupial ovary may have other important functions since parturition is inhibited after ovariectomy or excision of the corpus luteum (Renfree, 1974; Young & Renfree, 1979).

In the tammar, the corpus luteum is essential for the first 8 days of pregnancy (Tyndale-Biscoe, 1970) and normal parturition only occurs if the corpus luteum is present up to Day 23 of the 27-day gestation (Renfree & Young, 1979). Failure of parturition may be related to impaired vaginal transport, since normal delivery has to be preceded by preparation of the birth canal, or by opening or dilatation of the median vagina. Both progesterone and relaxin of luteal origin are thought to be involved in preparing the median vaginal canal for parturition (Tyndale-Biscoe, 1966, 1969, 1981; Tyndale-Biscoe, Hearn & Renfree, 1974), although direct evidence for the role of relaxin is scanty.

In the tammar there is a peak in the progesterone content of the corpus luteum at Day 23 of gestation followed by a peak plasma concentration at Day 25 (Renfree, Green & Young, 1979;

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Hinds & Tyndale-Biscoe, 1982). The levels in both decrease markedly just before birth on Day 26–27, and so progesterone withdrawal could initiate parturition in this species (Renfree & Young, 1979). Parturition also coincides with a transient high peak of prolactin (Tyndale-Biscoe, Hinds, Horn & Jenkin, 1983). Tyndale-Biscoe *et al.* (1983) have suggested that the occurrence and timing of post-partum oestrus and ovulation is a consequence of the pre-partum decline in progesterone, and that the fetus and/or placenta may be involved.

Materials and Methods

Animals. Tammar wallabies (*Macropus eugenii*) from Kangaroo Island (South Australia) and Garden Island (Western Australia) were maintained in a breeding colony at Murdoch University (Western Australia). Animals were kept in open grassed yards where they fed on the pasture available and were provided with lucerne hay, oats and water *ad libitum*.

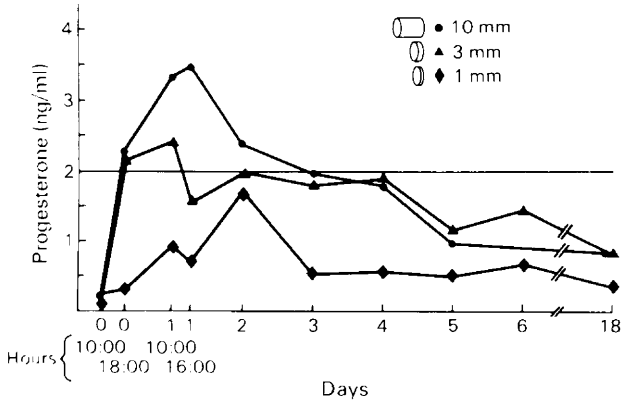
During the breeding season (late January to May), removal of pouch young from animals carrying diapausing blastocysts results in birth 26·9 $\pm$ 0·56 (s.d.) days later (Young & Renfree, 1979). Removal of pouch young was designated Day 0 of pregnancy. Animals not carrying a blastocyst returned to oestrus 28 days after removal of the pouch young. At all times, animal handling was kept to a minimum and animals were returned to outside yards whenever possible.

Gestation periods in control animals. The length of gestation in untreated tammars was determined in 30 animals. The pouches were examined daily from Day 26 after removal of pouch young until birth, or to Day 30 if no young was found. Weight and head length were recorded for all newborn pouch young.

Progesterone treatment. To achieve a pharmacological level of progesterone, 23 animals were injected intramuscularly with 15 mg progesterone (Steraloids Inc., Wilton, NH, U.S.A.) in 1 ml peanut oil twice daily from Day 24 until the evening of Day 29 or until birth. Treatment was begun on Day 24 (11 animals) or Day 25 (12 animals) because this is when plasma progesterone levels are highest (Lemon, 1972; Renfree *et al.*, 1979; Hinds & Tyndale-Biscoe, 1982).

To achieve a physiological dose of progesterone, groups of animals were treated with progesterone-impregnated Silicone rubber implants (Abbott Laboratories Pty Ltd, Kurnell, NSW, Australia). These were surgically implanted beneath the pouch skin on Day 24 after removal of pouch young, and the small incision closed by a single suture. Surgical anaesthesia was induced by the administration of a 6% solution of pentobarbitone sodium (Abbott) in 0·9% (w/v) sterile NaCl. The entire operation took less than 10 min and animals had recovered from anaesthesia by 15 min. Three ovariectomized animals were used to determine the size of an implant 9 mm in diameter required to elevate the plasma progesterone level to 2 ng/ml for approximately 7 days; this was found to be between 3 and 10 mm in length (see Text-fig. 1). One group of 5 intact animals received a 3-mm implant, and 13 intact animals received a 10-mm implant. The pattern of circulating progesterone shown by the three ovariectomized animals was reproduced in the experimental animals, with no noticeable differences in progesterone concentrations between the two groups (see Text-fig. 1).

Collection of samples and progesterone assay. Pouches were examined daily between Days 24 and 30 for the presence of any neonates. Newborn young were weighed immediately after removal from the pouch and the head length was measured with vernier calipers. Crown–rump length of retained fetuses was measured. The onset of oestrus (determined by assessment of vaginal smears) was only monitored on one day in one group of 6 animals with implants. This was discontinued due to the additional stress and disturbance it caused the animals. Non-parturient animals were examined by laparotomy on Day 30 to assess their reproductive status. The diagnosis of pregnant (*post partum*) or not pregnant was based upon the appearance of the uterus, which is enlarged for several days if



Text-fig. 1. Plasma progesterone concentrations in ovariectomized tammars with progesterone implants of different lengths (9 mm diam.) given on Day 0.

recently gravid, and the ipsilateral corpus luteum of pregnancy. Anaesthesia was induced by pentobarbitone sodium and was maintained by a mixture of halothane (Fluothane, I.C.I., Australia Ltd, Villawood, NSW, Australia) in oxygen administered via mask. Animals that had retained a fetus were killed and the entire reproductive tract was removed.

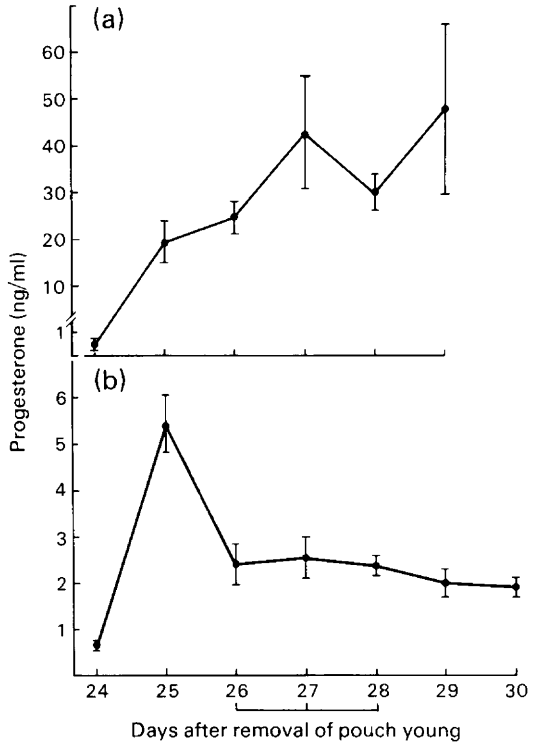
Blood was collected (before progesterone injection in the injection group (11 animals) and from all 18 implanted animals) from a lateral tail vein daily from Day 24. Duplicate aliquants of 500 µl of each plasma sample were extracted in 5 ml redistilled 40–60°C petroleum ether (British Drug Houses Chemicals, Port Fairy, Victoria, Australia) in 12 × 100 mm teflon-capped culture tubes by vigorous shaking at 4°C for 20 min. Separation of the solvent phase and the subsequent radioimmunoassay were as previously described for tammar corpora lutea (Renfree *et al.*, 1979), using a sheep antiserum (No. 230) provided by Dr R. I. Cox, Hormone Assay Group, C.S.I.R.O. Division of Animal Production, Sydney, NSW, Australia. The assay sensitivity was 5·9 ± 0·9 (s.e.m.) pg/tube and the intra- and inter-assay coefficients of variation were 12·2% and 23·87% respectively. These coefficients are higher than normal because all post-treatment plasma samples fell within the high (and therefore least accurate) region of the assay standard curve. Accuracy was determined by the addition of a range of concentrations of progesterone to plasma and the measured concentration closely correlated with the actual amount added (linear correlation coefficient $r = -0{\cdot}999$, $P < 0{\cdot}001$). Data are represented as mean ± s.e.m. and were analysed by parametric statistics when appropriate.

Results

Effects of progesterone treatment on plasma progesterone concentrations

Progesterone injections resulted in a dramatic elevation of the plasma progesterone to 10–50 ng/ml in all animals (Text-fig. 2a), significantly greater than the maximum peripheral plasma concentration in late pregnancy of about 1 ng/ml (Lemon, 1972; Hinds & Tyndale-Biscoe, 1982). These levels were maintained until injections were discontinued. Progesterone implants resulted in a peak of plasma progesterone 24 h after insertion (5·44 ± 0·67 (s.e.m.) ng/ml, N = 7) (Text-fig. 2b). By Day 26 the levels had stabilized to about 2 ng/ml and were maintained until Day 30 (Text-fig. 2b).

On the day of birth, the injected animals had progesterone concentrations that were about 10 times higher (20–30 ng/ml) than those of the implanted animals (2 ng/ml) (Table 1). Progesterone concentration was measured in 17 of the 22 animals that gave birth; in 11 of these 17 animals there



Text-fig. 2. Plasma progesterone concentrations (mean ± s.e.m.) of (a) 11 animals injected with progesterone from Day 24 until birth (Days 26–28) or Day 29 after removal of pouch young, and (b) 18 animals treated with progesterone implants. Bar shows the normal expected time range for birth. In (a) births occurred at Day 26 (N = 4), Day 27 (N = 2) and Day 28 (N = 1); 4 animals did not give birth. In (b) births occurred at Day 26 (N = 4), Day 27 (N = 3), Day 28 (N = 2) and Day 29 (N = 1); 8 animals did not give birth.

Table 1. Plasma progesterone levels (mean ± s.e.m. for the no. of animals indicated in parentheses) on the day of birth (Day 26–29 after removal of pouch young) in tammar wallabies treated with progesterone by injection or implant

Day of birth/sampling	Progesterone conc. (ng/ml)	
	Injection	Implant
26	20·45 ± 4·13 (4)	2·39 ± 0·28 (4)
27	32·89 ± 8·84 (2)	2·56 ± 1·10 (3)
28	19·44 (1)	2·23 ± 0·71 (2)
29	—	1·046 (1)

was a drop of variable magnitude in peripheral plasma progesterone concentrations just before birth or on the day of birth.

Length of gestation

Normal gestation periods. The mean gestation length after removal of pouch young for 30 untreated animals was 27·30 ± 0·95 (s.d.) days. This was not significantly different from the

Table 2. The effect of removal of pouch young (RPY) on control and progesterone-treated tammars

Day after RPY	Result of RPY	No (%) of animals	
		Controls	Progesterone-treated
26, 27, 28	Birth	25 (61)	20 (49)
29	Birth	4 (10)	1 (2)
30	Birth	1 (2)	1 (2)
30	Retained fetus	0 (0)	5 (12·5)
30	Aborted fetus	0 (0)	5 (12·5)
30	Not pregnant	11 (27)	9 (22)
Total		41 (100)	41 (100)

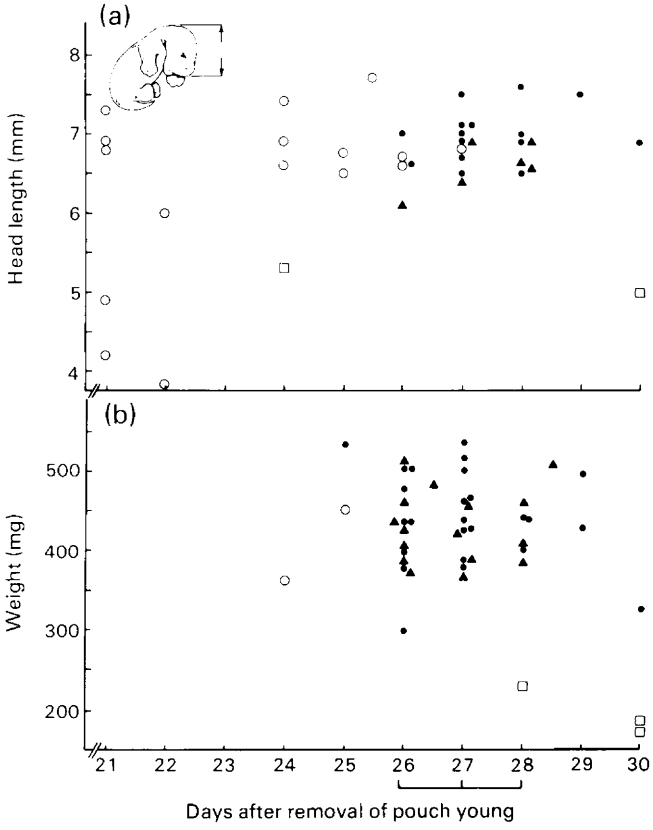
previously calculated value of 26·9 ± 0·56 days (N = 10) for tammars in the Murdoch colony (Young & Renfree, 1979). On the basis of these data, the normal expected range for birth in this study was taken as Days 26–28 after removal of pouch young. Of the 30 control animals that gave birth, 83% did so within this range (Table 2). Of the total number of animals checked, 73% gave birth after removal of pouch young. This compares well with the 75% of animals that normally give birth after removal of pouch young and that carry a diapausing blastocyst in the wild (73%) (Renfree & Tyndale-Biscoe, 1973). It also is similar to the 75% of animals that gave birth after laparotomy on Day 23 or Day 25 after removal of pouch young (Young & Renfree, 1979).

Gestation periods after progesterone treatment. The mean gestation length of the 22 progesterone-treated animals that gave birth was 26·82 ± 0·23 days; this did not differ from the control values. All but 2 (i.e. 91%) of the progesterone-treated animals that gave birth did so within the normal expected range (Table 2) and were classed as having had a normal pregnancy; the neonates appeared normal and were all attached to teats when found in the pouch. Of the 10 other progesterone-treated and pregnant tammars, 5 retained a fetus until Day 30, and 5 were diagnosed as having aborted (Table 2). There were no obvious differences between the two types of treatment, injection and implant, despite the marked differences in progesterone concentrations (Table 1) (injected: births 12, retained fetus 3, abortion 3, non-pregnant 5; implanted: births 10, retained fetus 2, abortion 2, non-pregnant 4). Post-partum oestrus was not prevented by the progesterone treatment; 2 of 6 animals with implants came into oestrus, one on Day 28 and the other on Day 29. These two animals gave birth on Days 27 and 28 respectively, indicating that oestrus had not been unduly delayed. However, none of the animals had any signs of a semen plug up to Day 30 after removal of pouch young.

Fetal and neonatal size

Between Days 25 and 30, the head lengths of neonates from progesterone-treated (6·7 ± 0·2 (s.d.) mm, N = 5) and control (7·0 ± 0·5 mm, N = 15) animals were similar (Text-fig. 3). One retained fetus on Day 30 had a head length of 5 mm (Text-fig. 3a). Two other retained fetuses on Day 30 were estimated to be 22–23 days after pouch young removal using the growth curve of Renfree & Tyndale-Biscoe (1973), but the head lengths of these 2 or the remaining 2 tammars could not be measured accurately because they were slightly squashed during removal from the tract.

The weights of neonates did not vary by day of birth or between control and progesterone-treated animals (Text-fig. 3b). The mean weight of control neonates (444·2 ± 61·0 s.d. mg, N = 25) was very close to the mean weight of neonates of progesterone-treated animals (432·7 ± 47·0 mg, N = 16). By contrast, the retained fetuses of 3 progesterone-treated animals weighed substantially



Text-fig. 3. Head lengths (a) and weights (b) of embryos and newborn pouch young of control and progesterone-treated tammars. ○, Control embryo; □, retained embryo; ●, control pouch young; ▲, progesterone-treated pouch young. Bar shows the expected time range for birth.

less than all other neonates (Text-fig. 3b), presumably partly due to a lack of any milk in the stomach, but also because they were less developed (Text-fig. 3a).

Discussion

Progesterone treatment did not prevent a normal duration of gestation, and parturition proceeded unimpaired between Days 26 and 28 in 22 of 32 animals that were pregnant. Those animals that gave birth had young that were of a similar size (whether measured by weights or head length) to those of untreated control animals. However, one-third of the treated animals that were pregnant had an abnormal pregnancy. Fetal development was retarded in 5 animals, and the fetus was retained up to the day of autopsy, Day 30 after removal of pouch young. The weights of the retained fetuses were half those of the control animals. High progesterone concentrations therefore appear to be embryotoxic (or embryostatic) at later stages of gestation. This effect of progesterone has been suggested previously to explain early losses or retardation of blastocyst development (Renfree & Tyndale-Biscoe, 1973), and has also been shown for mouse and rabbit embryos *in vitro*, in which cleavage is inhibited (Whitten, 1957; Daniel & Levy, 1964).

Injected as well as implanted animals gave birth, despite the circulating concentrations of progesterone at the time of parturition in the injection group being far in excess of the normal

peripheral plasma progesterone concentration in late pregnancy (Hinds & Tyndale-Biscoe, 1982). However, progesterone concentrations fell slightly before birth or before Day 30 in most (11/17) of the implanted animals, and this relative progesterone withdrawal could perhaps have been of some significance. Progesterone inhibits myometrial contractility *in vivo* in the tammar (Renfree & Young, 1979), and so some degree of progesterone withdrawal may be necessary for myometrial reactivation, as in other species (Thorburn, 1983). In sheep, exogenous progesterone administered in physiological amounts fails to blocks parturition, and only when high doses (up to 200 mg/day) are given is uterine activity blocked and normal or induced delivery prevented (Thorburn, 1983). In the tammar, however, neither physiological nor pharmacological doses of progesterone prevented births.

Several workers have suggested that the oestrogen : progesterone ratio may be more important at parturition than the actual level of either hormone (Bedford, Challis, Harrison & Heap, 1972; Liggins, Fairclough, Grieves, Forster & Knox, 1977; Thorburn & Challis, 1979). Oestrogen formed by uterine or fetal tissue could act directly on the myometrium to stimulate actinomyosin synthesis and indirectly to cause prostaglandin synthesis (Young, 1978). An oestrogen-dominated uterus would then be more sensitive to prostaglandins and oxytocin. Prostaglandin is found in low concentrations in the peripheral plasma of tammars at parturition with only an occasional high value observed (Tyndale-Biscoe *et al.*, 1983; Shaw, 1983). The hormonal ratio theory is not supported by the present results for progesterone-injected tammars in which a massive increase in oestrogen production would be required to compensate for the high circulating levels of progesterone in these animals; oestrogen levels in intact animals are very low during pregnancy and even at oestrus rise to only 20 pg/ml (Flint & Renfree, 1982; Shaw & Renfree, 1984). In sheep, parturition can occur without a fall in progesterone levels but only if large doses of oestrogen are given (Thorburn, 1983).

There is therefore no evidence for a fall in progesterone being essential for parturition to occur in tammars, but high concentrations interfere with normal delivery in a proportion of animals and may be embryotoxic in late pregnancy.

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