Uterine and Placental Growth: RNA and Protein Metabolism and Steroid Hormone Receptor Levels in the Endometrium, Whole Uterus and Cotyledons during Pregnancy in the Ewe

B. G. Miller[A] and G. M. Stone[B]

[A] Department of Animal Husbandry, University of Sydney, N.S.W. 2006.
[B] Department of Veterinary Physiology, University of Sydney, N.S.W. 2006.

Abstract

Some aspects of uterine and placental growth have been examined during pregnancy in the ewe. Changes in *in vitro* rates of protein synthesis, RNA:DNA and protein:DNA ratios and the tissue concentration of DNA in intercaruncular endometrium and caruncles/cotyledons between days 0 (oestrus) and 112 of pregnancy were compared with corresponding changes in the concentrations of high-affinity cytosol receptors for oestradiol and progesterone in whole uterus and caruncles/maternal cotyledons. Rapid growth of the intercaruncular endometrium between days 28 and 112 and of the developing cotyledons between days 28 and 84 occur in the presence of tissue levels of both steroid receptors that are extremely low in relation to the corresponding levels seen in the uterus at oestrus. If uterine responses to steroid hormones are regulated by the amounts of specific receptor present in the tissue, the results support the concept that uterine growth after day 28 of pregnancy results primarily from the physical stimulus of the growing conceptus rather than from the actions of endogenous steroid sex hormones.

Introduction

In a previous study we examined some changes that occur in the uterus during the oestrous cycle of the ewe (Miller *et al*. 1977). The *in vitro* rate of protein synthesis and RNA:DNA ratio in caruncular endometrium were maximal at or shortly after oestrus (days 0–2) and declined to low levels during the late luteal phase of the cycle (days 10–14). Similarly, the concentrations of both oestradiol (E_2) and progesterone (P) cytosol receptors in caruncles and in whole uterus were maximal at or shortly after oestrus and declined to minimal values on day 14. During pregnancy the weight of the ewe's uterus increases dramatically, and the weight of the cotyledons increases steadily until about day 95 of pregnancy (Alexander 1964). This uterine growth may result from the physical stimulus of the growing conceptus or changing uterine tissue levels of female sex hormones or both. The capacity of the uterus to grow and differentiate in response to circulating ovarian steroid hormones may be determined, in part, by the level of high-affinity E_2 and P receptors in uterine cytoplasm (see Clark and Peck 1979 for a review). Except during the first and last 4–7 days of pregnancy, gestation in the ewe is characterized by (1) maternal peripheral serum P levels which are not less than those seen during the luteal phase of the oestrous cycle and which between days 50 and 120 increase to levels 2–5 times those seen during the luteal phase of the cycle; and (2) extremely low maternal peripheral serum levels of unconjugated oestrogens (Bassett *et al*. 1969; Chamley *et al*. 1973; Robertson and Smeaton 1973; Tsang 1978).

In this study we have sought to determine mechanisms of uterine and placental growth in the ewe. We have examined *in vitro* rates of protein synthesis and RNA:DNA and protein:DNA ratios in intercaruncular endometrium and in caruncles or cotyledons during days 0–112 of pregnancy, and compared these data with corresponding results for levels of high-affinity E_2 and P cytosol receptors in whole uterus and caruncles or cotyledons.

Materials and Methods

Twenty-seven mature Merino ewes were joined with crayon-bearing rams on pasture and observed daily. The day on which mating first occurred was designated day 0 of pregnancy. Mated ewes were allotted at random to be killed on days 0, 28, 56, 84 and 112 of pregnancy. Each ewe was killed by exsanguination and sectioning of the cervical spinal chord at about 0900 h, and the genital tract and ovaries were promptly dissected and packed in crushed ice. The uteri remained in crushed ice for no more than 3 h before initiation of the various incubation procedures. The weight of the uterus and foetal weight and crown–rump length were recorded.

For the studies of RNA and protein metabolism small portions of intercaruncular (or inter-cotyledonary) endometrium each weighing 100–200 mg were collected with fine scissors. On days 28–112 care was taken to peel away the loosely adherent foetal trophoblast prior to tissue collection. A varying portion of epithelium is denuded from the intercotyledonary endometrium by the trophoblast (Amoroso 1952). At days 0 and 28 slices of caruncular endometrium were collected using a Stadie–Riggs microtome (Miller *et al.* 1977). The wall of the chorionic sac could be easily peeled away from caruncles at day 28. At day 56 and later times full separation of maternal and foetal placental tissues was not possible. The spongy foetal cotyledon was bluntly dissected from the embracing and more fibrous endometrial capsule or maternal cotyledon. Small portions of tissue each weighing 150–300 mg were collected with scissors from the surface of the foetal cotyledon which faced the endometrium. Such tissue contains cells of both maternal and foetal origin (for discussions of the gross and fine structure of the ovine placenta, see Amoroso 1952; Battaglia *et al.* 1961; Davies and Wimsatt 1966; Steven 1975 and Teasdale 1976). The tissue concentration of DNA, RNA:DNA and protein:DNA ratios and *in vitro* rates of synthesis of protein were determined in these tissue samples as previously described (Miller 1976; Stone *et al.* 1978). Briefly, rates of protein synthesis were determined by incubating tissue samples for 2 h at 37°C in 5 ml Eagle's HeLa medium supplemented with 1·0 mM glutamine and 1 μCi L-[4,5-^{3}H] leucine (1 Ci/mmol, Radiochemical Centre, Amersham, U.K.) under an atmosphere of 95% O_2:5% CO_2.

Cytosol levels of E_2 and P receptors were estimated as described previously (Miller *et al.* 1977; Stone and Miller 1978). In brief, cytosol preparations were initially treated with charcoal/dextran to remove free or weakly bound steroid. The stripped cytosols were then incubated with the appropriate labelled steroid with or without a 100-fold excess of unlabelled steroid. Exchange with endogenous steroid bound to the receptors was achieved either by incubation at 30°C for 20 min (E_2 receptor) or 16 h at 0°C (P receptor). Cytosols were treated again with charcoal/dextran and the receptors measured following agar gel electrophoresis at low temperature. Measurements were made on 'whole uterus' at each time. This tissue comprised that remaining after removal of the trophoblast from day 28 onwards and, in addition, of the foetal cotyledons from day 56 onwards as described above. The uterine horns were slit lengthwise and portions for assay were selected to contain, as far as could be observed, similar and representative portions of caruncular and intercaruncular endometrium and myometrium. Caruncular endometrium (days 0 and 28) and maternal cotyledons (day 56 onwards) were also assayed.

For each parameter the significance of differences between stages of pregnancy was determined by Duncan's multiple-range test (Steel and Torrie 1960).

Results

Data were obtained from four mature ewes on day 0, and from four pregnant ewes at each of days 28, 56 and 84, and three pregnant ewes at day 112. One ewe killed at each of days 56 and 84 had twin foetuses; the remainder had single foetuses. Uterine weights (mean $\pm$ s.e.) at days 0 and 28 were 63·7 $\pm$ 4·9 g and 58·4 $\pm$ 3·1 g,

respectively. Uterine weights at later times were not recorded, as it was not practicable to separate the conceptus from the uterus after day 28. Foetal weights and crown–rump lengths (mean $\pm$ s.e.) at days 28, 56, 84 and 112 were $0 \cdot 46 \pm 0 \cdot 08$ g and $1 \cdot 83 \pm 0 \cdot 63$ cm, $29 \cdot 5 \pm 2 \cdot 2$ g and $8 \cdot 83 \pm 3 \cdot 0$ cm, 322 ± 21 g and $19 \cdot 3 \pm 1 \cdot 5$ cm, and $1 \cdot 33 \pm 0 \cdot 06$ kg and $32 \cdot 5 \pm 10 \cdot 5$ cm, respectively. These data are given simply to indicate that the pregnancies were normal.
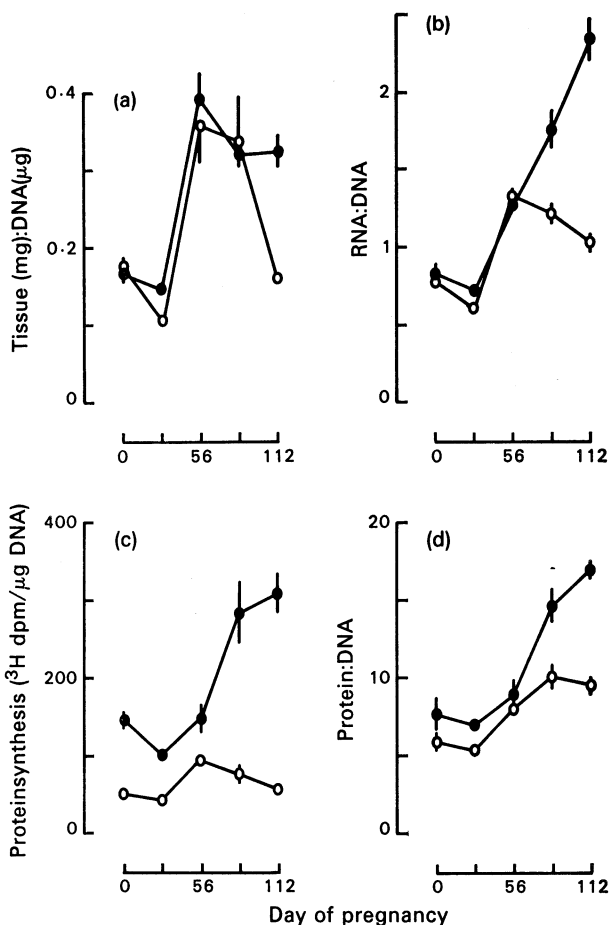

Fig. 1. Effect of stage of pregnancy on metabolism in the intercaruncular endometrium ($\bullet$) and caruncle ($\circ$, days 0, 28) and cotyledon ($\circ$, days 56, 84, 112). Day 0 is the day on which mating was first observed (oestrus). Results for *in vitro* protein synthesis are expressed as the incorporation of L-[4,5^{3}H]leucine into protein per microgram of DNA. Vertical bars indicate 1 s.e. Standard errors not shown fall within the points as drawn.

The amount of tissue per microgram of DNA in both intercaruncular endometrium and caruncle/cotyledon increased markedly between days 28 and 56 ($P < 0 \cdot 01$, Fig. 1). Thereafter the ratio in intercaruncular tissue decreased slightly (day 112 *v.* day 56, $P < 0 \cdot 05$), whereas in cotyledons it fell markedly between days 84 and 112 ($P < 0 \cdot 01$). At each of days 0, 28, 56 and 84 the amount of tissue per microgram of DNA was similar in these different tissues.

In the intercaruncular endometrium the rate of protein synthesis and RNA:DNA and protein:DNA ratios all declined slightly but not significantly between oestrus and day 28, then increased markedly between days 28 and 112 to values at day 112 which were at least twice those observed at oestrus. The increases above the levels seen at oestrus first became significant at day 56 (RNA:DNA, $P < 0.01$) or day 84 (protein synthesis and protein:DNA, $P < 0.01$). The pattern of changes in RNA:DNA and protein:DNA ratios and in vitro rate of synthesis of protein in caruncle/cotyledon between oestrus and day 56 was very similar to that seen in intercaruncular endometrium. The decrease in RNA:DNA ratio in caruncle between oestrus and day 28 was significant ($P < 0.01$). However, the RNA:DNA ratio and rate of synthesis of protein were maximal at day 56 and declined thereafter (day 112 v. day 56, $P < 0.01$), while the protein:DNA ratio in cotyledons continued to increase until day 84 (day 84 v. day 56, $P < 0.05$). The reason for this apparent discrepancy is not clear, but presumably net protein accumulation in cotyledons per unit of DNA continues to increase for some weeks after the times of maximal cell content of RNA and of rate of protein synthesis per unit of DNA.
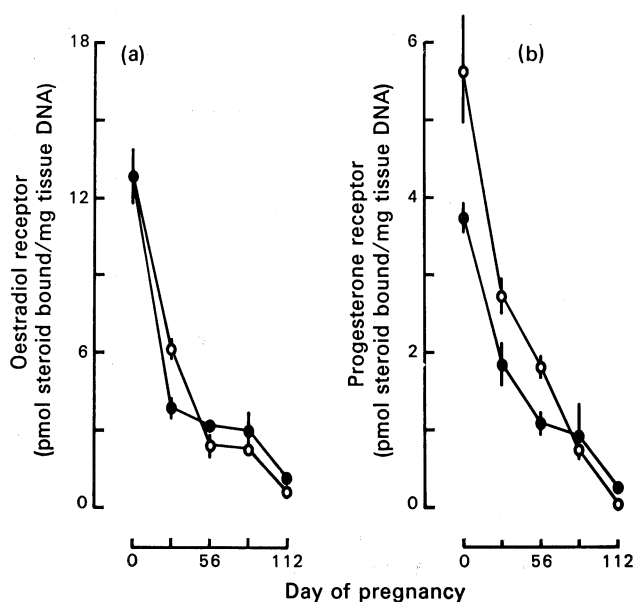

Fig. 2. Effect of stage of pregnancy on oestradiol and progesterone cytosol receptor concentrations in whole uterus (●) and caruncle (○, days 0, 28) and cotyledon (○, days 56, 84, 112). Day 0 is the day on which mating was first observed (oestrus). Vertical bars indicate 1 s.e. Standard errors not shown fall within the points as drawn.

The concentrations of high-affinity cytosol receptors for both E_2 and P decreased steadily between oestrus and day 112 of pregnancy ($P < 0.01$), so that in whole uterus the level of each receptor was only about 7–9 % of that seen at oestrus (Fig. 2). The decline in receptor levels in the whole uterus occurred primarily during the first 28 days of pregnancy, but the further decreases between days 28 and 112 were highly significant ($P < 0.01$). The levels of both E_2 and P receptors in maternal cotyledons were much lower than the corresponding levels in caruncles at oestrus. In maternal cotyledons the level of each receptor declined from day 56 to extremely low values at day 112 ($P < 0.01$).

Discussion

In the ewe the cotyledons grow steadily after implantation to maximal weights at around day 95 (Alexander 1964). Total placental DNA reaches a plateau value at the same stage, so that the functional maturation of the cotyledons that occurs during the last third of gestation is not accompanied by an increase in total numbers of placental nuclei (Kulhanek *et al.* 1974). From day 80 to term the number of foetal mesenchymal nuclei in foetal cotyledons decreases and there is a concomitant increase in foetal endothelial nuclei. Maternal stromal nuclei comprise only about 7–17% of total nuclei in the foetal cotyledon (Teasdale 1976). Our results for RNA:DNA ratio and *in vitro* rate of protein synthesis suggest that the rate of growth of the foetal cotyledon is maximal at around day 56 and declines thereafter. A surprising aspect of our findings was that even though the caruncles and loosely adherent trophoblast at day 28 undergo a transformation to become established cotyledons by day 56 the changes occurring in the placenta during this interval that are depicted in Fig. 1 are very similar to the corresponding changes observed in intercaruncular endometrium. As was expected, our results for RNA and protein metabolism in intercaruncular endometrium suggest that extensive tissue growth and cell hypertrophy occur between days 28 and 112 of pregnancy. This growth and that of the cotyledons up to day 95 occur during a period when cytosol levels of E_2 and P receptors in both whole uterus and maternal cotyledon are low. We do not know how these low levels of E_2 and P receptors are distributed between cells of uterine and foetal origin. In the case of data for whole uterus, most of the tissue sample used must presumably have been of maternal origin, being either myometrium, intercotyledonary endometrium, or cotyledonary tissue of maternal origin.

In non-pregnant, ovariectomized ewes, oestrogen administration increases the levels of E_2 and P receptors in endometrial and whole uterine cytosol preparations, whereas the administration of P for several days to E_2-treated ewes diminishes the cytosol levels of both receptors (Koligian and Stormshak 1977a; Stone *et al.* 1978, 1979; Miller *et al.* 1979). Similarly, the levels of both E_2 and P receptors decline during the luteal phase of the oestrous cycle when serum P levels are high (Koligian and Stormshak 1977b; Miller *et al.* 1977). Hence the decline in E_2 and P receptor levels in whole uterus and caruncles between oestrus and day 28 in this study is probably explained, at least in part, by the continuing high serum P levels after the first 4–7 days of pregnancy. The reasons for the continuing decline in the level of each receptor after day 28 are not clear. The further increase in maternal serum P levels that occurs after day 50 may be relevant, but the inclusion of varying proportions of cells of foetal origin in samples of whole uterus and especially of maternal cotyledon probably also diminished the average quantity of each receptor per unit of DNA.

There are relatively few reports of E_2 and P receptor levels in the uterus of other species during pregnancy. In the rat, E_2 receptor concentration declines soon after mating and remains at a relatively low level until parturition (Feherty *et al.* 1970). Davies and Ryan (1973) found a progressive rise in the myometrial P receptor level until about day 9 of pregnancy in the rat followed by a progressive fall until parturition. Vu Hai *et al.* (1978), however, observed a general rise in uterine P receptor level from about day 6 until parturition. In the mouse, P receptor level

declined after mating and showed little change during the remainder of pregnancy (Philibert and Raynaud 1977). In no case was the pattern similar to that seen here in the ewe nor was any decline as dramatic as that shown in our data.

Despite considerable difficulties in interpreting the significance of results for levels of E_2 and P receptors, the data do not suggest that female sex hormones have any substantial role in promoting uterine growth during pregnancy in the ewe. The principal cause of myometrial and endometrial growth is probably the physical stimulus of the developing conceptus. Chronic stretch induced with fluid-filled balloons elicits vigorous growth and a doubling of RNA:DNA ratios in the uteri of rabbits receiving large doses of P daily (Csapo *et al.* 1965). Similarly, the distension of one uterine horn with fluid in ovariectomized rats receiving no hormones initiates epithelial growth and an increase in RNA and DNA synthesis in all tissues of the uterus (Sartor 1976). However, a similar gradual doubling of the uterine RNA:DNA ratio over an extended period has been observed in ovariectomized mice receiving constant daily injections of P and a low dose of E_2 in the absence of any such physical stimulus (Miller 1979). In summary, the factors which regulate uterine growth during pregnancy in the ewe remain unclear: although the levels of high-affinity cytosol E_2 and P receptors in whole uterus and maternal cotyledons after day 28 of pregnancy are very low in relation to those seen in the uterus at oestrus, important roles for E_2 or P or both cannot presently be excluded.

Acknowledgments

References

Alexander, G. (1964). Studies on the placenta of the sheep (*Ovis aries* L.). Placental size. *J. Reprod. Fertil.* **7**, 289–305.

Amoroso, E. C. (1952). Placentation. In 'Marshall's Physiology of Reproduction'. (Ed. A. S. Parkes.) Vol. II. pp. 127–300. (Longmans, Green & Co.: London.)

Bassett, J. M., Oxborrow, T. J., Smith, I. D., and Thorburn, G. D. (1969). The concentration of progesterone in the peripheral plasma of the pregnant ewe. *J. Endocrinol.* **45**, 449–57.

Battaglia, F. C., Meschia, G., Blechner, J., and Barron, D. H. (1961). Effect of insulin and poisons on glucose uptake of sheep and goat placenta. *Am. J. Physiol.* **200**, 64–6.

Chamley, W. A., Buckmaster, J. M., Cerini, M. E., Cumming, I. A., Goding, J. R., Obst, J. M., Williams, A., and Winfield, C. (1973). Changes in the levels of progesterone, corticosteroids, estrone, estradiol-17β, luteinizing hormone and prolactin in the peripheral plasma of the ewe during late pregnancy and at parturition. *Biol. Reprod.* **9**, 30–5.

Clark, J. H., and Peck, E. J. (1979). 'Female Sex Steroids. Receptors and Function.' (Springer-Verlag: Berlin.)

Csapo, A., Erdos, T., de Mattos, C. R., Gramss, E., and Moscowitz, C. (1965). Stretch-induced uterine growth, protein synthesis and function. *Nature (London)* **207**, 1378–9.

Davies, I. J., and Ryan, K. J. (1973). The modulation of progesterone concentration in the myometrium of the pregnant rat, by changes in cytoplasmic 'receptor' protein activity. *Endocrinology* **92**, 394–401.

Davies, J., and Wimsatt, W. A. (1966). Observation on the fine structure of the sheep placenta. *Acta Anat.* **65**, 182–223.

Feherty, P., Robertson, D. M., Waynforth, H. B., and Kellie, A. E. (1970). Changes in the concentration of high-affinity oestradiol receptors in rat uterine supernatant preparations during the oestrous cycle, pseudopregnancy, pregnancy, maturation and after ovariectomy. *Biochem. J.* **120**, 837–44.

Koligian, K. B., and Stormshak, F. (1977a). Progesterone inhibition of estrogen receptor replenishment in ovine endometrium. *Biol. Reprod.* **17**, 412–16.

Koligian, K. B., and Stormshak, F. (1977b). Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. *Endocrinology* **101**, 524–33.

Kulhanek, J. F., Meschia, G., Makowski, E. L., and Battaglia, F. C. (1974). Changes in DNA content and urea permeability of the sheep placenta. *Am. J. Physiol.* **226**, 1257–63.

Miller, B. G. (1976). RNA and protein metabolism in the ewe oviduct and endometrium at pro-oestrus; regulation by oestradiol and progesterone. *J. Endocrinol.* **69**, 57–66.

Miller, B. G. (1979). Delayed interactions between progesterone and low doses of 17β-estradiol in the mouse uterus. *Endocrinology* **104**, 26–33.

Miller, B. G., Murphy, L., and Stone, G. M. (1977). Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle of the ewe. *J. Endocrinol.* **73**, 91–8.

Miller, B. G., Wild, J., and Stone, G. M. (1979). Effects of progesterone on the oestrogen-stimulated uterus: a comparative study of the mouse, guinea pig, rabbit and sheep. *Aust. J. Biol. Sci.* **32** 549–60.

Philibert, D., and Raynaud, J. P. (1977). Cytoplasmic progestin receptors in mouse uterus. In 'Progesterone Receptors in Normal and Neoplastic Tissues'. (Raven Press: New York.)

Robertson, H. A., and Smeaton, T. C. (1973). The concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β in the maternal plasma of the pregnant ewe in relation to the initiation of parturition and lactation. *J. Reprod. Fertil.* **35**, 461–8.

Sartor, P. (1976). Action de la distension sur la croissance utérine. *Ann. Endocrinol. (Paris)* **37**, 257–70.

Steel, R. G. D., and Torrie, J. H. (1960). 'Principles and Procedures of Statistics.' (McGraw Hill: New York.)

Steven, D. H. (1975). 'Comparative Placentation. Essays in Structure and Function.' (Academic Press: London.)

Stone, G. M., and Miller, B. G. (1978). The isthmic oviduct of the ewe: what is the biological significance of high affinity cytosol receptors for oestradiol and progesterone. *Biol. Reprod.* **19** 653–6.

Stone, G. M., Murphy, L., and Miller, B. G. (1978). Hormone receptor levels and metabolic activity in the uterus of the ewe: regulation by oestradiol and progesterone. *Aust. J. Biol. Sci.* **31**, 395–403.

Stone, G. M., Wild, J., and Miller, B. G. (1979). The uterine endometrium and isthmic oviduct of the ewe: does progesterone act as an antiestrogen? *Biol. Reprod.* **21**, 273–80.

Teasdale, F. (1976). Numerical density of nuclei in the sheep placenta. *Anat. Rec.* **185**, 187–96.

Tsang, C. P. W. (1978). Plasma levels of estrone sulfate, free estrogens and progesterone in the pregnant ewe throughout gestation. *Theriogenology* **10**, 97–110.

Vu Hai, M. T., Longeat, F., and Milgrom, E. (1978). Progesterone receptors in the rat uterus: variations in cytosol and nuclei during the oestrous cycle and pregnancy. *J. Endocrinol.* **76**, 43–8.