

PLACENTAL STEROID METABOLISM IN LATE PREGNANCY

V.A. CRAIG

A.R.C. Institute of Animal Physiology, Babraham, Cambridge, UK

There are considerable differences between species in the contribution of the placenta to the concentration of steroids in the maternal circulation. This is particularly evident in the placental contribution to the maternal plasma concentration of progesterone, the hormone necessary for the maintenance of pregnancy. Some species depend predominantly on progesterone synthesized by the placenta for the maintenance of pregnancy, as in women (throughout the majority of gestation) and sheep (for the final two-thirds of gestation), while in other species, as in the goat and the pig, the placental contribution to maternal progesterone is relatively low and pregnancy is maintained by luteal progesterone secretion.

Changes in placental steroid metabolism have been implicated in the sequence of hormonal events in the maternal circulation which leads to the onset of labour in the goat and the sheep. In sheep these endocrine changes have been described in considerable detail (Anderson, Flint and Turnbull, 1975; Steele, Flint and Turnbull, 1976; Flint and Ricketts, 1979). However it is only in recent years that certain parallel mechanisms have become apparent in the goat. In this species, parturition occurs as a direct consequence of the cessation of luteal function which is associated with the release of uterine prostaglandin $F_{2\alpha}$ and which results in the removal of the 'progesterone block'. As in sheep, an early step in the chain of events leading to the release of prostaglandin $F_{2\alpha}$ is a rise in the concentration of cortisol in the foetal circulation which occurs over the last 4–10 days of pregnancy (Currie and Thorburn, 1977). This has been shown to induce the activity of the placental steroid metabolizing enzyme 17α -hydroxylase (Flint *et al.*, 1978) leading to the formation of increased amounts of substrate for the synthesis of oestrogens. Of the oestrogens thus formed, it has been demonstrated that oestradiol- 17β will induce the release of uterine prostaglandin $F_{2\alpha}$ into the uterine vein (Currie, Cox and Thorburn, 1976) thus providing an important link between the increase in foetal plasma cortisol and regression of the corpus luteum of pregnancy.

The pig is another species in which the corpora lutea are the major source of maternal progesterone and certain similarities exist between the pig and the goat in the hormonal changes occurring in both the foetal and the maternal circulation prior to parturition. In the pig, the peripheral plasma concentration of progesterone remains steady at approximately 10 ng/ml until about 48 hours prior to parturition when it declines rapidly

(Ash *et al.*, 1973; Robertson and King, 1974; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975). The plasma concentrations of oestrone (Figure 19.1) and oestradiol-17 β start to rise on or about 108 days of gestation and then fall rapidly once delivery is completed. The fall in the plasma concentration of progesterone is also associated with the release of uterine prostaglandin F_{2 α} into the maternal circulation (Nara and First, 1977; First and Bosc, 1979). Evidence has been obtained which suggests that increased foetal cortisol secretion presages the rise of oestrone and oestradiol-17 β

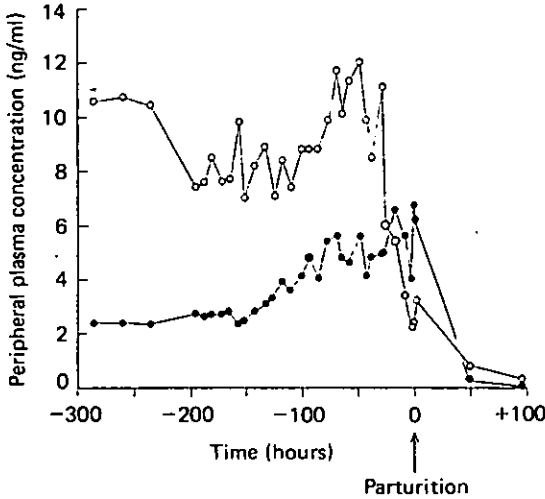


Figure 19.1 Changes in the peripheral plasma concentration of progesterone (○—○) and oestrone (●—●) over the last two weeks of gestation. The data were obtained from two animals and the peripheral plasma steroid concentration is expressed in hours relative to the delivery of the first piglet

seen in the maternal plasma over the last week of gestation; foetal cortisol rises concurrently with, or even precedes, that of oestrone and oestradiol-17 β , and dexamethasone or ACTH₁₋₂₄ administered to foetuses after 100 days of gestation will induce premature delivery accompanied by the expected changes in maternal plasma progesterone and oestrogens (North, Hauser and First, 1973; Bosc, 1973).

Although there is evidence to support the view that the porcine placenta synthesizes steroids, the possibility that the endometrium also contributes to the production of steroids by the gravid uterus should not be ignored. In order to provide a more comprehensive picture of steroid synthesis by the uterus in late pregnancy, the metabolism of steroids by both the placenta and endometrium will be discussed.

Uterine production of steroids *in vivo*

Studies of the uterine secretion of progesterone and oestrogens during late gestation in the pig are relatively few although there is evidence that the umbilical venous concentration of progesterone is greater than that of the

umbilical artery, which is consistent with intrauterine progesterone production (Godke and Day, 1973; Barnes, Comline and Silver, 1974; Silver *et al.*, 1979; MacDonald *et al.*, 1980). Evidence for an extra-ovarian source of oestrogens throughout gestation has been achieved by ovariectomizing sows in early pregnancy (the pregnancy being maintained by daily intramuscular injections of 300 mg of progesterone) and determining the amount of oestrogens excreted into the urine (Fèvre, Léglise and Rombauts, 1968). By using this technique it was found that the excretion of urinary oestrogens by ovariectomized animals did not differ from intact animals throughout the course of gestation.

A modification of this method has been utilized in the present study to determine the output of steroids by the uterus in late pregnancy. Four gilts were ovariectomized at 98 days of gestation and catheters were inserted into the uterine artery and vein. Pregnancy was maintained by intramuscular injection of 10 mg of medroxyprogesterone acetate every alternate day until 115 days post coitum (one day after the expected day of delivery) when the animals were killed. Daily paired samples were taken from the uterine artery and vein and the plasma concentration of progesterone, 17α -hydroxyprogesterone, androstenedione and unconjugated oestrogens determined using specific radioimmunoassays. Medroxyprogesterone acetate did not cross-react in any of the assays used.

A positive venous — arterial (V — A) difference was obtained for all the steroids assayed. There was an apparent increase in uterine progesterone

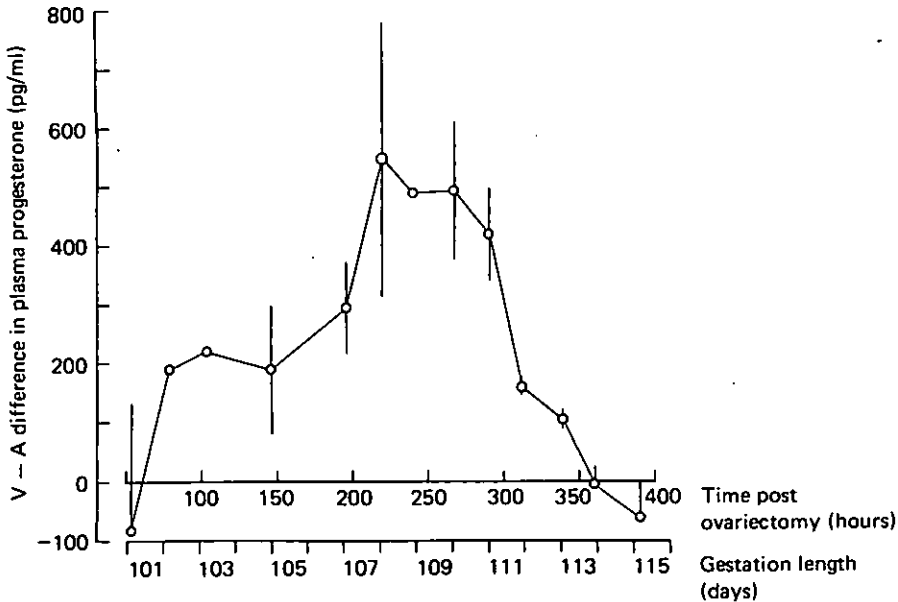


Figure 19.2 Uterine venous minus uterine arterial (V — A difference) plasma concentration of progesterone for gilts ovariectomized at 98 days post coitum in which pregnancy was maintained with medroxyprogesterone acetate. Each point represents the mean \pm S.E.M. V — A difference expressed in pg/ml for up to four animals and is plotted against both the time in hours after ovariectomy and the length of gestation in days

production between 104 days and 108 days post coitum with a peak V — A difference of 500 pg/ml (*Figure 19.2*). Thereafter the venous concentration fell until, by the expected day of delivery (114 days post coitum), the V — A difference had reached zero. The results obtained also demonstrated an increase in the output of 17 α -hydroxyprogesterone and androstenedione. In the case of androstenedione, this occurred from 108–110 days post coitum with a peak V — A difference of 115 pg/ml. In these animals a rise in the plasma concentration of unconjugated oestrogens occurred which was comparable to that obtained in intact animals thus indicating that the placenta or endometrium (or a combination of the two) is the major source of maternal circulating oestrogens in late pregnancy.

Placental and endometrial metabolism of steroids in the pig; studies *in vitro*

Histochemical evidence has been obtained for the presence of $\Delta^5\beta$ - and 17 β -hydroxysteroid dehydrogenases in placental tissue from the fourth week of gestation until term (Christie, 1968; Dufour and Raeside, 1969). Earlier studies by Bloch and Newman (1966) which demonstrated the conversion of dehydroepiandrosterone to androstenedione by porcine placental tissue obtained at the end of gestation also provide evidence for the presence of an active $\Delta^5\beta$ -hydroxysteroid dehydrogenase. An *in vitro* study of placental tissue taken in late pregnancy (112 days post coitum) revealed the presence of an active aromatase complex (Ainsworth and Ryan, 1966). Placental preparations were shown to convert [7 α -³H]dehydroepiandrosterone and [4-¹⁴C]androstenedione to oestrogens with oestrone being the major oestrogen formed. More recently, Choong and Raeside (1974) reported the presence of high concentrations of unconjugated and conjugated oestrogens in placental tissue and suggested that the placenta was the site of synthesis. High concentrations of unconjugated oestrogens have also been found in allantoic and amniotic fluid; Knight *et al.* (1977) showed that from 60–100 days the concentration of oestrone present in allantoic fluid increased from 0.9 ng/ml to 537.7 ng/ml, which greatly exceeds the concentration of oestrone in the maternal circulation and again indicates that the placenta is the probable site of synthesis. Although Ainsworth and Ryan (1966) demonstrated that the porcine placenta could utilize androgen substrates for oestrogen synthesis, no synthesis of oestrogens from either pregnenolone or progesterone could be detected by these authors. From these results it was concluded that the porcine placenta did not possess all the enzymes of the Δ^4 pathway, in particular C-17,20-lyase, necessary for the conversion of pregnenolone to oestrogens. An outline of the enzymes of the Δ^4 pathway which have been shown to be present in late pregnancy is given in *Figure 19.3*.

In vitro studies have demonstrated that both blastocyst (Gadsby and Heap, 1980) and endometrial tissue (Dueben *et al.*, 1980) taken in early pregnancy are capable of synthesizing oestrogens from the C-21-precursors pregnenolone or progesterone respectively. However, relatively little is known about the metabolism of steroids by the placenta and endometrium in late gestation in the pig. In view of the accumulating evidence for the

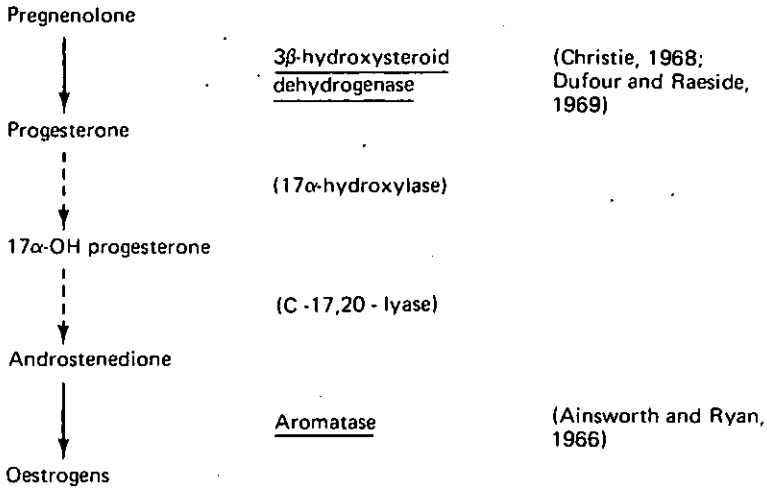


Figure 19.3 The Δ^4 -3-keto pathway from pregnenolone to oestrogens. Enzymes whose presence has been confirmed by histochemical or *in vitro* incubation techniques are underlined.

placental synthesis of oestrogens, it was decided to re-examine the possibility that these tissues might be capable of synthesizing oestrogens from C-21 steroids. Furthermore, the metabolism of steroids by the endometrium in late pregnancy has not been studied extensively although there is some histochemical evidence that endometrial tissue possesses both Δ^5 3 β - and 17 β -hydroxysteroid dehydrogenase activity (Christie, 1968; Dufour and Raeside, 1969). The enzyme Δ^5 3 β -hydroxysteroid dehydrogenase occupies a key position in the synthesis of steroid hormones and its presence in endometrial tissue gives rise to the possibility that the endometrium may be able to synthesize progesterone from its immediate precursor, pregnenolone.

To determine which enzymes of the Δ^4 pathway were present in the placenta and endometrium in late pregnancy, samples of these tissues were taken from gilts slaughtered at 100–112 days post coitum. Placental tissue obtained after both prostaglandin F_{2 α} -induced and spontaneous delivery

Table 19.1 INTERMEDIATES OF THE Δ^4 PATHWAY ISOLATED FROM INCUBATIONS OF PLACENTAL OR ENDOMETRIAL TISSUE OBTAINED AT 100 DAYS POST COITUM

Substrate	Product	Enzyme	Tissue	
			Placenta	Endometrium
Pregnenolone	progesterone	3 β -hydroxysteroid dehydrogenase	✓	✓
Progesterone	17 α -hydroxy progesterone	17 α -hydroxylase	✓	×
17 α -hydroxy progesterone	androstenedione	C-17,20-lyase	✓	×
Androstenedione	oestrone	aromatase	✓	✓

Tissues incubated with appropriate tritium-labelled precursor steroid in presence of excess co-factor.
 ✓ conversion demonstrated; × conversion undetectable.

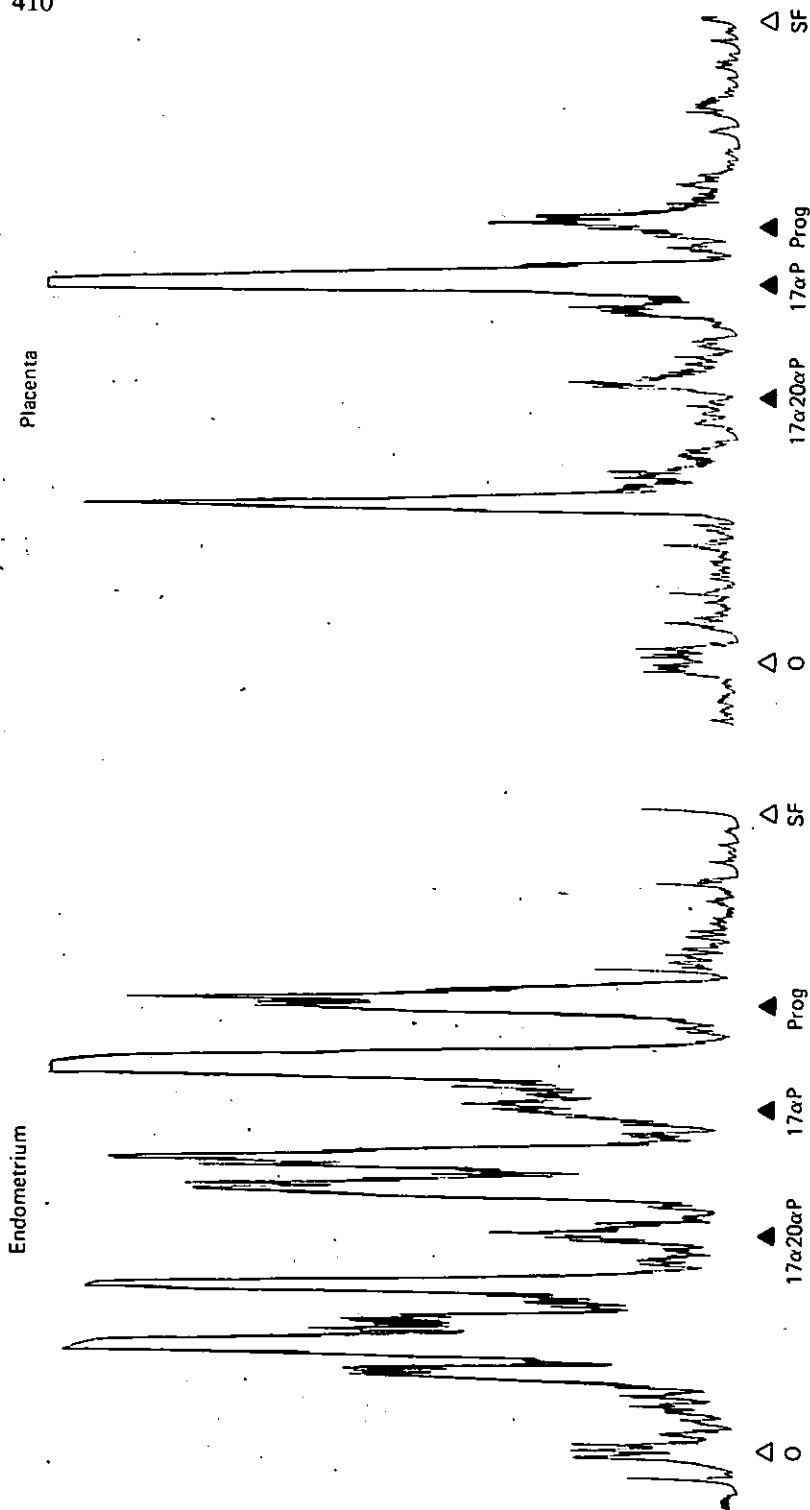


Figure 19.4 Radiochromatogram scan illustrating the metabolism of $[1\alpha,2\alpha\text{-}^3\text{H}]$ progesterone (in the presence of excess NADPH) by homogenates of placental and endometrial tissue obtained at 100 days post coitum. Open triangles indicate the position of the origin (O) and solvent front (SF). Closed triangles indicate the position of the authentic marker steroids, progesterone (Prog), 17α -hydroxyprogesterone ($17\alpha\text{P}$) and $17\alpha,20\alpha$ -dihydroprogesterone ($17\alpha,20\alpha\text{P}$). 5β -pregnanolone co-chromatographs with $17\alpha\text{P}$ in the solvent system used.

Endometrium

Placenta

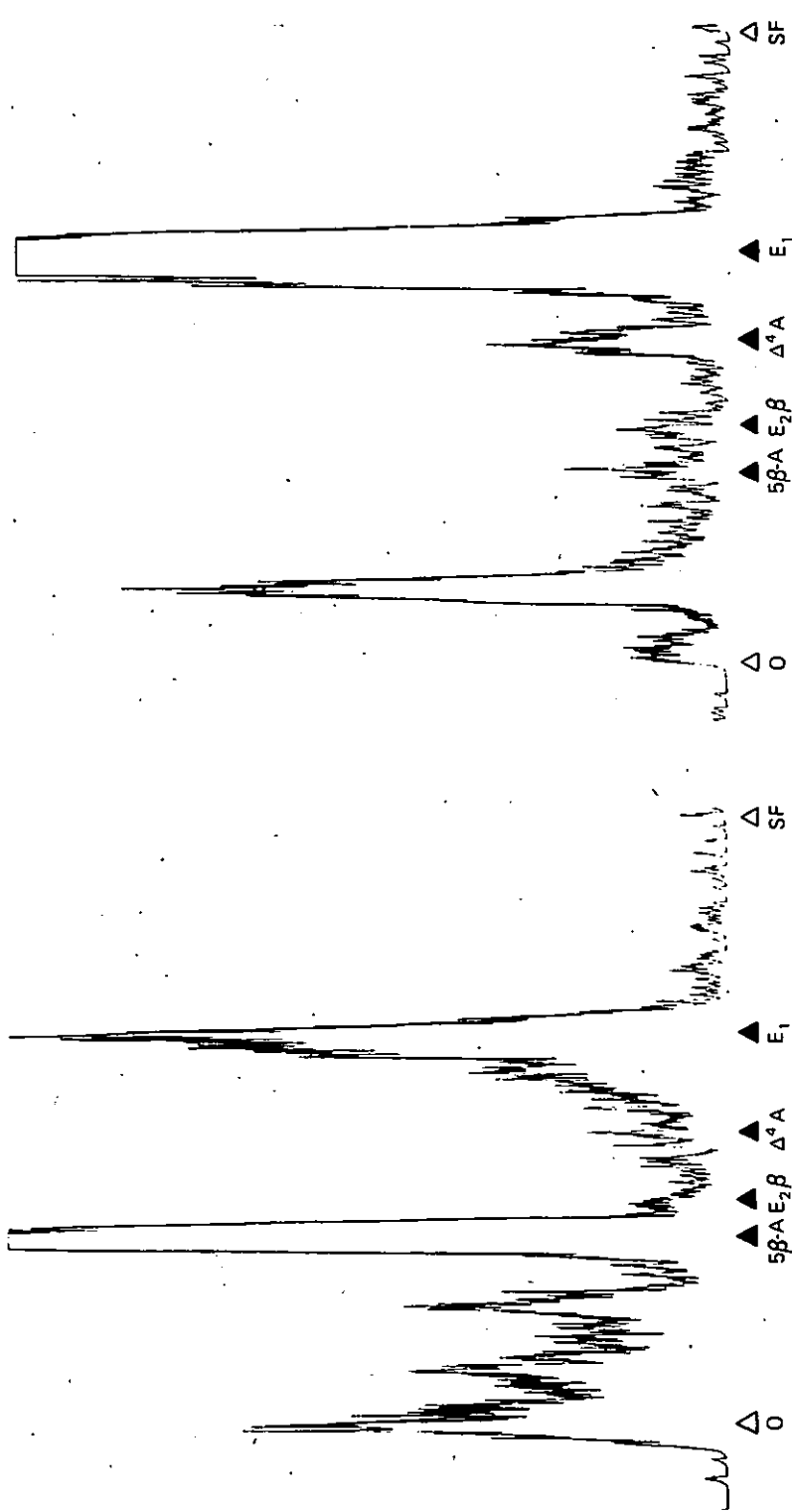


Figure 19.5 Radiochromatogram scans illustrating the metabolism of [1,2,6,7-³H] androstenedione (in the presence of excess NADPH) by microsomal preparations of placental and endometrial tissue obtained at 100 days post coitum. Open triangles indicate the position of the origin (O) and solvent front (SF). Closed triangles indicate the position of the authentic marker steroids, oestrone (E₁), androstenedione (Δ⁴A), oestradiol-17β (E₂β), 3α-hydroxy 5β-androstane-17-one (5β-A).

was also studied. Homogenates of the tissue were incubated with the following tritium-labelled precursors, [7 α -³H]pregnenolone, [1 α ,2 α -³H]progesterone and [7 α -³H]17 α -hydroxyprogesterone in the presence of excess cofactor. Aromatase activity was determined by incubating microsomal preparations of the tissue with [1,2,6,7-³H]androstenedione. At the end of the incubation period the unconjugated metabolites were extracted from the medium with diethyl ether. These ether-soluble metabolites were then separated by thin layer chromatography and detected by using a radiochromatogram scanner. Identification of the metabolites was achieved by derivative formation and recrystallization with authentic carrier steroid to constant specific activity.

Table 19.1 indicates which enzymes of the Δ^4 pathway were shown to be present in endometrial and placental tissue at 100 days post coitum. The major products of progesterone metabolism by the endometrium were 5 β -reduced pregnanediols and pregnanetriols together with 5 β -pregnan-3 α -ol-20-one (5 β -pregnanolone). Less than 10% of the metabolites were 5 α -reduced. In contrast to this, the products of placental progesterone metabolism were predominantly 5 α - and 5 β -pregnanolones. 17 α -hydroxylated products accounted for less than 5% of the metabolites isolated (see *Figure 19.4*). The major product of androstenedione metabolism by placental tissue was oestrone, while the endometrium synthesized predominantly 5 β -reduced androstanolones and 5 β -androstanediols (see *Figure 19.5*).

Changes in the activity of the enzymes of the Δ^4 pathway before term

Δ^5 3 β -hydroxysteroid dehydrogenase

There was a decrease in the percentage of progesterone synthesized by placental tissue over the last two weeks of gestation while synthesis of progesterone by the endometrium showed little change. These findings contrast with the histochemical studies of Dufour and Raeside (1969) which suggested that a moderate increase in placental Δ^5 3 β -hydroxysteroid dehydrogenase activity occurred near term and with those of Christie (1968) who found only trace activity of this enzyme from 101–112 days of pregnancy.

17 α -hydroxylase

The two major products of progesterone metabolism by placental tissue obtained after spontaneous delivery at term (114 days post coitum) were 17 α -hydroxyprogesterone and 17 α ,20 α -dihydroxy-4-pregnen-3-one (17 α ,20 α -progesterone). These two 17 α -hydroxylated metabolites accounted for 42% of the substrate added (see *Table 19.2*), with 50% of the radioactivity being associated with unmetabolized progesterone. These findings may represent an increase in 17 α -hydroxylase activity. An alternative explanation which needs to be investigated is the apparent loss of 5 β -reductase activity, since no 5 β -reduced products were obtained by

Table 19.2 PLACENTAL STEROID SYNTHESIS: CHANGES BEFORE TERM

Substrate	Enzyme	Product	Stage of gestation (days)		Term (114)
			100	106	
Progesterone	17 α -hydroxylase	17 α -hydroxyprogesterone	<5	<5	19.8 \pm 4.8 (n = 6)
17 α -hydroxyprogesterone		17 α ,20 α -progesterone	<5	<5	22.5 \pm 2.9 (n = 6)
Androstenedione	C-17,20-lyase	Oestrone	18.4 \pm 4.4 (n = 3)	30.6 \pm 1.8 (n = 4)	34.4 \pm 3.6 (n = 7)
	Aromatase	Oestrone	43.8 \pm 7.9 (n = 4)	48.8 \pm 11.8 (n = 3)	74.0 \pm 4.4 (n = 4)

Values given as mean percentage conversions (\pm S.E.M.)

Figures in parentheses indicate the number of animals (at least three observations, in duplicate, were made for each animal)

metabolism of progesterone by placental tissue obtained post delivery. Whether this loss of 5β -reductase activity occurred prior to delivery or was the result of anoxia arising from cessation of placental blood flow at term remains uncertain. No 17α -hydroxyprogesterone could be isolated from incubations of endometrial tissue with progesterone at any of the stages of pregnancy studied.

C-17,20-lyase

Placental C-17,20-lyase activity as determined by conversion of 17α -hydroxyprogesterone to oestrone was found to increase on or about day 105 post coitum. At 100 days post coitum conversion to oestrone was 18.5%, by 106 days 30% and by the end of pregnancy had reached 30–35% (see *Table 19.2*). Conversion of 17α -hydroxyprogesterone to oestrone by endometrial tissue remained at less than 3% throughout the last two weeks of gestation.

Aromatase

An increase in the activity of both placental (see *Table 19.2*) and endometrial aromatase was seen from 105–114 days post coitum. The increase in oestrone produced from androstenedione could, in part, be due to a decrease in the synthesis of 5β -reduced metabolites.

A composite diagram of the pathways of steroid metabolism present *in vitro* at 100 days and 114 days post coitum in both placental and endometrial tissue is given in *Figure 19.6*.

Conclusions

From the above studies, the following points may be made.

- (a) Ovariectomy and medroxyprogesterone acetate replacement therapy allow the study of the uterine contribution to circulating steroids in late pregnancy. Using this technique, it has been demonstrated that the gravid uterus is capable of producing progesterone, 17α -hydroxyprogesterone and androstenedione although the plasma concentration of these steroids is 5–10% that of intact, untreated animals. In contrast, plasma concentrations of unconjugated oestrogens were unchanged by ovariectomy thus providing further evidence for an extra-ovarian source of oestrogens in late pregnancy.
- (b) *In vitro* investigations have demonstrated that both endometrial and placental tissue will actively metabolize steroids of the Δ^4 pathway, although from these studies it would appear that only the placenta possesses all the enzymes necessary for the synthesis of oestrogens from pregnenolone by the Δ^4 pathway.

There is, however, an important species difference in the role of oestradiol-17 β in the control of parturition. Whereas in the goat the rising plasma concentration of oestradiol-17 β plays an important role in controlling uterine prostaglandin F_{2 α} production and is therefore indirectly luteolytic, no such role for oestradiol-17 β has been demonstrated in the control of parturition in the pig. Infusion of oestradiol-17 β into the maternal circulation (Flint, Ricketts and Craig, 1979) or intramuscular injection of oestradiol-17 β (Coggins, 1975; First and Bosc, 1979) in late pregnancy has no effect on the length of gestation. It may be postulated however that oestrogens play a facilitatory rather than obligatory role in the pig, possibly by raising the concentration of the myometrial oxytocin receptor and thereby preparing the uterus for oxytocin release during labour.

Acknowledgements

The author acknowledges the support of an M.R.C. studentship.

References

- AINSWORTH, L. and RYAN, K.J. (1966). Steroid hormone transformations by endocrine organs from pregnant mammals. I. Oestrogen biosynthesis by mammalian placental preparations *in vitro*. *Endocrinology* **79**, 875–883
- ANDERSON, A.B.M., FLINT, A.P.F. and TURNBULL, A.C. (1975). Mechanism of action of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. *J. Endocr.* **66**, 61–70
- ASH, R.W. and HEAP, R.B. (1975). Oestrogen, progesterone and corticosteroid concentration in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. *J. Endocr.* **64**, 141–154
- ASH, R.W., BANKS, P., BAILES, G., BROAD, S. and HEAP, R.B. (1973). Plasma oestrogen, progesterone and corticoid concentrations in the pregnant, parturient and lactating sow. *J. Reprod. Fert.* **33**, 359–360
- BALDWIN, D.M. and STABENFELDT, G.H. (1975). Endocrine changes in the pig during late pregnancy, parturition and lactation. *Biol. Reprod.* **12**, 508–515
- BARNES, R.J., COMLINE, R.S. and SILVER, M. (1974). Foetal and maternal progesterone concentrations in the pig. *J. Endocr.* **62**, 419–420
- BLOCH, E. and NEWMAN, E. (1966). Comparative placental steroid synthesis. I. Conversion of (7-³H)-dehydroepiandrosterone to (³H)-androst-4-ene 3,17-dione. *Endocrinology* **79**, 524–530
- BOSC, M.J. (1973). Modification de la durée de gestation de la Truie après administration d'ACTH aux foetus. *C. r. hebd. Seanc. Acad. Sci., Paris* **276**, 3183
- CHOONG, C.H. and RAESIDE, J.I. (1974). Chemical determination of oestrogen distribution in the foetus and placenta of the domestic pig. *Acta endocr.* **77**, 171–185
- CHRISTIE, G.A. (1968). Distribution of hydroxysteroid dehydrogenase in the placenta of the pig. *J. Endocr.* **40**, 285–291

- COGGINS, E.G. (1975). Mechanisms controlling parturition in swine. PhD Thesis. University of Wisconsin, Madison, USA
- CURRIE, W.B. and THORBURN, G.D. (1977). The foetal role in timing the initiation of parturition in the goat. In *The Fetus and Birth*, pp. 49–72. Amsterdam, Elsevier/Excerpta Medica/North Holland
- CURRIE, W.B., COX, R.I. and THORBURN, G.D. (1976). Release of prostaglandin F, regression of corpora lutea and induction of premature parturition in goats treated with oestradiol-17 β . *Prostaglandins* **12**, 1093–1103
- DUEBEN, B.D., WISE, T.H., BAZER, F.W., FIELDS, M.J. and KALRA, P.S. (1980). Metabolism of H³-progesterone to estrogens by pregnant gilt endometrium and conceptus. *J. Anim. Sci.* **49**, Suppl. **1**, 293(Abstract 375)
- DUFOUR, J. and RAESIDE, J.I. (1969). Hydroxysteroid dehydrogenase activity in the placenta of the domestic pig. *Endocrinology* **84**, 426–431
- FÈVRE, J., LÉGLISE, P.-C and ROMBAUTS, P. (1968). Du rôle de l'hypophyse et des ovaires dans la biosynthèse des oestrogènes aux cours de la gestation chez la truie. *Annls Biol. anim. Biochim. Biophys.* **8**, 225–233
- FIRST, N.L. and BOSCH, M.J. (1979). Proposed mechanisms controlling parturition and the induction of parturition in swine. *J. Anim. Sci.* **48**, 1407–1421
- FLINT, A.P.F. and RICKETTS, A.P. (1979). Control of placental endocrine function: role of enzyme activation in the onset of labour. *J. Steroid Biochem.* **11**, 493–500
- FLINT, A.P.F., RICKETTS, A.P. and CRAIG, V.A. (1979). The control of placental steroid synthesis at parturition in domestic animals. *Anim. Reprod. Sci.* **2**, 239–251
- FLINT, A.P.F., KINGSTON, E.J., ROBINSON, J.S. and THORBURN, G.D. (1978). Initiation of parturition in the goat: evidence for control by foetal glucocorticoid through activation of placental C₂₁-steroid 17 α -hydroxylase. *J. Endocr.* **78**, 367–378
- GADSBY, J.E. and HEAP, R.B. (1980). Oestrogen synthesis by embryos of the pig, cow and sheep. *J. Anim. Sci.* **49**, Suppl. **1**, 299 (Abstract 389)
- GODKE, R.A. and DAY, B.N. (1973). Maternal and fetal plasma progesterone levels in swine. *J. Anim. Sci.* **37**, 313
- KNIGHT, J.W., BAZER, F.W., THATCHER, W.W., FRANK, D.E. and WALLACE, M.D. (1977). Conceptus development in intact and unilaterally hysterectomized and ovariectomized gilts: inter-relations among hormonal status, placental development, fetal fluids and fetal growth. *J. Anim. Sci.* **44**, 620–637
- MACDONALD, A.A., COLENBRANDER, B., ELSAESSER, F. and HEILHECKER, A. (1980). Progesterone production by the pig fetus and the response to stimulation by adrenocorticotrophin. *J. Endocr.* **85**, 34P
- NARA, B.S. and FIRST, N.L. (1977). Effect of indomethacin and PGF_{2 α} on porcine parturition. *J. Anim. Sci.* **45**, Suppl. **1**, 191
- NORTH, S.A., HAUSER, E.R. and FIRST, N.L. (1973). Induction of parturition in swine and rabbits with the corticosteroid dexamethasone. *J. Anim. Sci.* **36**, 1170–1174
- ROBERTSON, H.A. and KING, G.J. (1974). Plasma concentrations of progesterone, oestrone, oestradiol-17 β and oestrone sulphate in the pig at implantation during pregnancy and at parturition. *J. Reprod. Fert.* **40**, 133–141

418 *Placental steroid metabolism in late pregnancy*

- SILVER, M., BARNES, R.J., COMLINE, R.S., FOWDEN, A.L., CLOVER, L. and MITCHELL, M.D. (1979). Prostaglandins in the foetal pig and parturition endocrine changes in mother and foetus. *Anim. Reprod. Sci.* **2**, 305-322
- STEELE, P.A., FLINT, A.P.F. and TURNBULL, A.C. (1976). Increased utero-ovarian androstenedione production before parturition in sheep. *J. Reprod. Fert.* **46**, 443-445