Influence of the fetal pituitary–adrenal axis on fetal and maternal progesterone and unconjugated oestrogen concentrations in the pig

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Summary. Fetal and maternal plasma progesterone and unconjugated oestrone and oestradiol-17 β concentrations were measured in intact pig fetuses and those in which the pituitary had been destroyed. Progesterone concentrations were significantly higher ($P < 0.05$) and oestrogen concentrations significantly lower ($P < 0.01$) in hypophysectomized fetuses than in intact fetuses. When fetuses in one uterine horn only were hypophysectomized, oestrogen concentrations in the uterine vein draining this horn were lower than those from the contralateral vein. The results indicate that both fetal and maternal oestrogen concentrations are influenced by the fetal pituitary. When dexamethasone was infused (at 27 $\mu\text{g/h}$ for 96 h) into 5 chronically-catheterized hypophysectomized fetuses no changes in peripheral fetal progesterone or oestrone were observed.

Introduction

Fetal progesterone concentrations in the pig are considerably higher than maternal concentrations during late pregnancy (Barnes, Comline & Silver, 1974; Silver *et al.*, 1979; Hagen, Houtz, Griel & Kavanaugh, 1983) although they appear to decrease during the last week of gestation (Silver *et al.*, 1979; Macdonald *et al.*, 1979). The major sources of fetal progesterone are the placenta (Silver *et al.*, 1979; Craig, 1982) or active transfer from the maternal circulation (Barnes *et al.*, 1974), whereas little is produced by the fetal adrenal during late gestation (Macdonald, Colenbrander, Elsaesser & Heilhecker, 1980).

Oestrogen concentrations increase in the maternal (Robertson & King, 1974) and fetal (Macdonald *et al.*, 1979) circulations during the last month of gestation, with a more rapid increase occurring in the last week. Maternal oestradiol-17 β concentrations have been shown to be lower in gilts carrying small numbers of hypophysectomized fetuses than in those carrying small numbers of normal fetuses (Kendall, Dziuk, Nelson & Thurmon, 1985). The placenta is the major source of oestrogens during gestation in the pig (Fèvre, Léglise & Rombauts, 1968; Robertson & King, 1974) and Craig (1982) showed that the placenta has limited enzymic ability to synthesize oestrogens from pregnenolone by the Δ^4 -pathway *in vitro*. Moreover, the activity of those enzymes essential for the metabolism of progesterone to oestrone increased at term. Activation of one of these enzymes, 17 α -hydroxylase, is mediated by fetal cortisol in sheep and goats (Anderson, Flint & Turnbull, 1975; Flint, Kingston, Robinson & Thorburn, 1978) and forms part of the cascade by which parturition is induced by the fetal pituitary–adrenal axis. A similar mechanism has been suggested for the pig (Craig, 1982). Fetal decapitation (Stryker & Dziuk, 1975) or hypophysectomy (Bosc, du Mesnil du Buisson & Locatelli, 1974) are known to prolong gestation in the pig and

during the development of these procedures we found that progesterone concentrations were higher in hypophysectomized than in intact fetuses. The present study was carried out to determine whether the fetal pituitary influenced progesterone and unconjugated oestrogen concentrations in fetal plasma and whether this might be mediated through glucocorticosteroids.

Materials and Methods

Surgical procedures. Yorkshire gilts weighing 92–164 kg were used; pre- and post-operative handling and anaesthetic regimens have been described previously (Randall, 1977). All surgical procedures were performed under general anaesthesia. The study was carried out in three experiments.

Experiment 1. In this experiment, all fetuses in a litter were decapitated or hypophysectomy was attempted on all fetuses in the litter. Decapitation was performed between Days 40 and 45 (Stryker & Dziuk, 1975) and hypophysectomy between Days 74 and 84 of gestation as described in detail elsewhere (Randall, 1986). Briefly, the pituitary was destroyed by electrocoagulation following blind passage of a probe through the uterine wall and fetal skull to the sella turcica. Pregnancy was then allowed to continue until 119–122 days of gestation when the fetuses were removed at Caesarian section and blood samples were collected from the umbilical artery and vein. Similar samples were collected at 112 and 114 days of gestation from unoperated fetuses (intact) in 2 gilts. Samples from 8 fetuses (4 male; 4 female) were assayed in each group. These were selected randomly from 3 'decapitated', 4 'hypophysectomized' and the 2 intact litters.

Experiment 2. Since fetal hypophysectomy resulted in prolonged gestation and differences in gestational ages between groups in Exp. 1, a second experiment was designed to compare fetuses at the same gestational age and within the same litter. In 4 gilts all the fetuses in one uterine horn were hypophysectomized whereas those in the other horn were left intact. At a second operation (Days 103–105 of gestation) polyvinyl catheters were inserted into branches of the uterine vein of each horn and the middle uterine artery in one horn (Randall, 1977). To remove the contribution of maternal progesterone, one of these gilts was bilaterally ovariectomized when the catheters were inserted, and pregnancy was maintained by feeding medroxyprogesterone acetate (100 mg/day; Sigma Chemical Co., St Louis, MO, U.S.A.). Maternal blood samples were collected from each vessel until Day 112–113 of gestation when Caesarian section was performed and samples collected as in Exp. 1. The completeness of the hypophysectomy was assessed by gross examination of the pituitary fossa for pituitary remnants and by determining fetal cortisol concentrations in arterial plasma. This assessment was subsequently confirmed by determination of plasma LH concentrations (Ponzilius, Sanford, Phillips & Palmer, 1983).

Experiment 3. In 3 gilts, fetuses in one uterine horn were hypophysectomized on Days 70, 73 and 76 of gestation. On Day 105 of gestation two polyvinyl catheters were inserted into the jugular veins (or into one vein and a carotid artery) of 1 or 2 hypophysectomized fetuses in each gilt by previously described techniques (Randall, 1986). At this time, the gilts were bilaterally ovariectomized and pregnancy was maintained as in Exp. 2. Fetal blood samples (1.5 ml) were collected daily from the fetal jugular catheter until Caesarian section. Beginning 2–5 days after fetal catheterization, all 5 fetuses were infused continuously through the other catheter with dexamethasone (Dexagen, Rogar/STB, Pointe Claire, Quebec) at a rate of 27 µg/h. This rate was approximately 25% higher than that shown to suppress endogenous cortisol production in intact fetuses (Randall, Kendall, Tsang & Taverne, 1984). The infusion was continued for 96 h and the total volume infused per day was ~3.0 ml. The gilts were then anaesthetized and fetal blood samples were collected as before.

In all experiments, blood samples were collected into pre-chilled tubes containing EDTA (Vacutainers, Becton-Dickinson Canada, Mississauga, Ontario) and immediately centrifuged at 4°C. The separated plasma was stored at –20°C until assayed.

Hormone assays. Progesterone was estimated in duplicate by radioimmunoassay (RIA) by procedures described previously (Randall, Taverne, Challis, Kendall & Tsang, 1986). Plasma volumes of 25 and 50 μl were used for fetal and 100 and 200 μl for maternal determinations. The intra- and inter-assay coefficients of variation were 6.8% and 17.2% respectively. In 5 fetal samples, progesterone was estimated with and without prior LH-20 chromatography (Murphy, 1971) and concentrations in both were in close agreement. Unconjugated oestrone and oestradiol-17 β concentrations were estimated using RIA procedures previously described (Tsang, Armstrong & Whitfield, 1980). Combined intra- and inter-assay coefficients of variation were < 10% for oestrone and < 15% for oestradiol-17 β . Cortisol was estimated by RIA using an antibody produced in a rabbit against cortisol 3-CMO-BSA. The main cross-reacting steroids were cortisone (4.4%), corticosterone (2.2%), 17-hydroxyprogesterone (2.2%) and 11-desoxycortisol (2.2%). All other steroids tested cross-reacted at < 0.2%. Plasma samples (10 or 20 μl) were diluted to 0.22 ml with distilled water, prewashed twice with 2 ml hexane-carbon tetrachloride (1.5:1, v/v) to remove progesterone and extracted twice with redistilled dichloromethane (1.0 ml). Combined organic extracts were evaporated to dryness and reconstituted (as were standards) in 0.5 ml 0.1 M-phosphate buffer, pH 7.0, containing 0.1% gelatin. To each tube, 100 μl of a 1:3000 dilution of antibody were added and the tubes were incubated at room temperature for 30 min after which time 100 μl [^3H]cortisol trace solution were added. The tubes were then vortexed and incubated overnight at 4°C. Bound and free steroids were separated by adding 300 μl of the buffer containing 0.5% charcoal and 0.05% Dextran T-70, vortexing and centrifuging the mixture at 4°C; 500 μl were then removed for scintillation counting. The average recovery of [^3H]cortisol added to three aliquants of plasma in each assay was 80.4% and results were corrected for extraction losses. When cortisol was added to pig plasma (previously stripped of endogenous steroids with charcoal) to provide concentrations of 0, 20, 50 and 100 ng/ml, 10 μl samples handled as above yielded 0.46, 20.3, 52.6 and 108.4 ng/ml ($n = 11$). Intra- and inter-assay coefficients of variation were 8.2% and 7.5% respectively.

The significance of differences between concentrations of steroids in different groups was assessed by one-way analysis of variance using the Newman-Keuls procedure to identify significant differences or by Student's *t* test when appropriate.

Results

Experiment 1

Gestation was prolonged in all gilts carrying decapitated or hypophysectomized fetuses. Progesterone concentrations were significantly higher and oestrogen significantly lower in decapitated or hypophysectomized fetuses than in intact fetuses (Table 1). Individual values for both progesterone and oestrone, however, varied considerably within groups although there were no consistent differences between males and females. There was a negative arterio-venous difference for all three steroids ($P < 0.05$) in all fetal groups. Mean fetal cortisol concentrations were 6.7, 8.9, and 47.3 ng/ml for decapitated, hypophysectomized and intact fetuses respectively.

Experiment 2

In 1 of the 13 hypophysectomized fetuses small remnants of pituitary tissue remained and this fetus had an LH concentration of 0.78 ng/ml. Concentrations of progesterone and oestrone in the umbilical artery and vein were 40.3 and 78.1 ng/ml and 2122 and 3412 pg/ml respectively. In one other fetus the pituitary was missed, a small lesion being present on the latero-ventral surface of the left cerebral hemisphere, and data from this fetus are included in the 'intact' group. In all other hypophysectomized fetuses LH concentrations were below assay sensitivity (0.13 ng/ml). As in Exp. 1, oestrone and progesterone concentrations in hypophysectomized fetuses were significantly

Table 1. Mean concentrations ($\pm$ s.e.m.) of progesterone, unconjugated oestrone and oestradiol-17 β in the umbilical blood of pig fetuses at Caesarian section

Group	Gestational age (days)	Progesterone (ng/ml)		Oestrone (pg/ml)		Oestradiol-17 β (pg/ml)	
		Artery	Vein	Artery	Vein	Artery	Vein
Decapitated (<i>n</i> = 8)	119–122	*31.2 $\pm$ 7.4	**77.6 $\pm$ 9.2	**995.5 $\pm$ 169.9	**1956.6 $\pm$ 416.0	**151 $\pm$ 10.8	**195 $\pm$ 15.3
Hypophysectomized (<i>n</i> = 8)	119–120	*33.8 $\pm$ 5.4	**76.5 $\pm$ 16.9	**847.9 $\pm$ 181.9	**1953.4 $\pm$ 377.6	**127 $\pm$ 11.1	**165 $\pm$ 3.5
Intact (<i>n</i> = 8)	112–114	10.0 $\pm$ 1.4	25.5 $\pm$ 2.5	2699.5 $\pm$ 284.0	8552.0 $\pm$ 1412.0	250 $\pm$ 17.3	431 $\pm$ 18.7

n = number of fetuses in group.

Values significantly different from those of intact fetuses: **P* < 0.05; ***P* < 0.01.

Table 2. Mean concentrations ($\pm$ s.e.m.) of progesterone, unconjugated oestrone and oestradiol-17 β in the umbilical blood of hypophysectomized pig fetuses and their intact litter mates

Group	Progesterone (ng/ml)		Oestrone (pg/ml)		Oestradiol-17 β (pg/ml)	
	Artery	Vein	Artery	Vein	Artery	Vein
Hypophysectomized (<i>n</i> = 12)	**22.6 $\pm$ 2.3	**55.0 $\pm$ 4.5	*1786.4 $\pm$ 190.6	**3745.6 $\pm$ 519.2	134.1 $\pm$ 9.2	*167.1 $\pm$ 13.3
Intact (<i>n</i> = 13)	13.3 $\pm$ 1.82	30.2 $\pm$ 2.8	3418.7 $\pm$ 624.9	7627.4 $\pm$ 927.1	168.2 $\pm$ 19.5	234.4 $\pm$ 20.3

n = number of fetuses in group.

Values significantly different from those of intact fetuses: **P* < 0.025; ***P* < 0.005.

Table 3. Individual maternal unconjugated oestrogen concentrations (pg/ml) at Caesarian section in sows in which fetuses in one uterine horn were hypophysectomized

	Maternal uterine artery	Maternal uterine vein	
		Intact fetuses*	Hypophysectomized fetuses†
Oestrone	1960	3359 (6)	2503 (5/5)
	2603	5106 (2)	2638 (4/4)
	—	10611 (5)	6214 (4/5)
	1047‡	5524 (1)	3431 (3/3)
Oestradiol-17 β	170	191	164
	160	191	169
	—	276	198
	141‡	172	160

*Number of fetuses in parentheses.

†Number of fetuses successfully hypophysectomized in horn in parentheses.

‡Ovariectomized sow.

different from those of their intact littermates (Table 2) and these differences were more marked in the umbilical vein. Differences between arterial oestradiol-17 β concentrations were not significant although those in the umbilical vein were. Marked variations in the steroid concentrations of individual fetuses were present and this was more marked in the intact fetuses. Mean fetal arterial cortisol concentrations were 5.7 and 42.6 ng/ml for hypophysectomized and intact fetuses respectively.

In all 4 sows, unconjugated oestrone and oestradiol-17 β concentrations were higher in the uterine vein draining the horn containing intact fetuses than in that draining the horn containing hypophysectomized fetuses (Table 3). However, concentrations in both veins were generally higher than those in the uterine artery.

Experiment 3

Fetal packed cell volumes were unchanged during the experimental period and, when measured, fetal pH values were within previously described values (Randall, 1977) for chronically catheterized fetuses.

Concentrations of progesterone and unconjugated oestrone in the fetal jugular vein did not change significantly during the 4-day period when dexamethasone was infused into the fetuses (Fig. 1). Oestrone concentrations in samples taken at Caesarian section from the umbilical vein of infused fetuses tended to be higher than those of non-infused fetuses, although small numbers precluded exact comparisons being made.

Fig. 1. Concentrations of progesterone $\pm$ s.e.m. and unconjugated oestrone in 5 catheterized, hypophysectomized fetuses infused with dexamethasone for 96 h.

In 3 of the 5 fetuses, from 2 sows, fetal oestrone concentrations were considerably higher in the samples taken on the first 2 days after surgery (804–3476 pg/ml) than on subsequent days. In addition, oestrone concentrations were higher in those samples taken at Caesarian section than in those taken through the catheter 1 h before anaesthesia.

Discussion

Although it has been known for some time that fetal progesterone concentrations are higher than maternal concentrations (Barnes *et al.*, 1974; Silver *et al.*, 1979; Hagen *et al.*, 1983), there is relatively little information on the sources and function of the high fetal concentrations. Craig

(1982) has demonstrated that the fetal placenta can produce progesterone *in vitro* and earlier suggestions that the placenta is an important source of progesterone in the fetus (Hagen *et al.*, 1983; Nase, Hagen, Kavanaugh & Griel, 1985) are supported in our study by the maintenance of high fetal concentrations and negative arterio-venous differences in umbilical vessels of fetuses in ovariectomized sows. However, there is also evidence that progesterone might be transferred from the maternal to the fetal circulation (Barnes *et al.*, 1974), in which case the relative proportions arising from placental synthesis or maternal transfer remain to be determined.

In fetuses in which the pituitary was absent, fetal progesterone concentrations were higher than those in intact fetuses. Whether this reflected increased (or maintained) placental production, decreased metabolism or altered placental transfer could not be determined. Higher concentrations of unconjugated oestrogens were present in the intact fetuses: the use of progesterone as a substrate for their production (as discussed below) might explain, in part, differences between the two groups. However, other metabolic pathways for progesterone, which might also be affected by the pituitary, are present in the pig, e.g. 5α - and 5β -reduction (Craig, 1982). That the differences were due to variations in maternal concentrations seems unlikely since the intact and hypophysectomized fetuses in Exp. 2 shared a common maternal environment.

Concentrations of oestrogens increase during the last month of gestation in the fetal (Macdonald *et al.*, 1979) and maternal (Robertson & King, 1974) blood. The rapid decrease in oestrogens after parturition suggests that they are produced by the gravid uterus (Fèvre *et al.*, 1968; Robertson & King, 1979) and the negative arterio-venous difference in the uterine and umbilical circulations (Silver *et al.*, 1979) indicates their placental origin. The high oestrogen content of placental tissue (Choong & Raeside, 1974) and the demonstration that fetal placental tissue can convert androstenedione (Ainsworth & Ryan, 1966) or pregnenolone (Craig, 1982) to oestrogens are additional evidence for the placental synthesis of oestrogens in the pig.

In the present study, a negative arterio-venous difference for unconjugated oestrone and oestradiol- 17β was found in umbilical and uterine plasma, confirming earlier studies. Furthermore, it was found that the concentrations of these steroids were reduced in fetal and maternal plasma in the absence of a fetal pituitary. This extends the findings of Kendall *et al.* (1985) that lower oestradiol- 17β concentrations were present in gilts carrying small numbers of hypophysectomized fetuses than in those carrying small numbers of intact fetuses. However, despite the lower concentrations, the negative arterio-venous difference persisted in the umbilical circulation for both steroids even when all fetuses in the litter were decapitated or hypophysectomized, suggesting that the placenta may produce 'base-line' quantities of oestrogens but that these are increased in a normally maturing fetus. Whether progesterone is a major substrate for oestrogen production before any increased 17α -hydroxylase activity is not clear. The high oestrone concentrations in chronically-catheterized hypophysectomized fetuses in the days after surgery would suggest that other steroids may be converted to oestrogens.

Craig (1982) demonstrated that the fetal placenta has some ability to convert progesterone to oestrogens and that the 17α -hydroxylase activity increased between Days 106 and 114 of gestation, a period when fetal cortisol concentrations are increasing (Randall, 1983). The activation of this enzyme by cortisol is an essential step in the increased oestrogen production in sheep and goats (Anderson *et al.*, 1975; Flint *et al.*, 1978), and Craig (1982) suggested that a similar mechanism may be present in the pig. However, infusion of dexamethasone into catheterized hypophysectomized fetuses for 96 h did not cause significant alterations in either progesterone or oestrone in our study. The concentration infused was higher than that previously shown to suppress endogenous cortisol production in intact fetuses (Randall *et al.*, 1984). It is possible that a longer period of infusion might be required to induce changes in oestrogen production. When ACTH or dexamethasone were infused into intact fetuses progesterone concentrations did fall, although only after about 6 days of infusion (G. C. B. Randall, J. Z. Kendall, B. K. Tsang & M. A. M. Taverne, unpublished data), but oestrone concentrations did not increase markedly. Infusion of ACTH or dexamethasone into all fetuses in litters of reduced size (Randall *et al.*, 1984) did not result in premature

delivery and this may argue against a central role for corticosteroids as the trigger for parturition in the pig. However, Coggins & First (1977) have also shown that maternally-administered dexamethasone did not induce parturition in gilts carrying decapitated fetuses and it is possible that the placentas of our hypophysectomized fetuses were unable to respond to dexamethasone in the absence of some other pituitary factor. The elevated oestrone concentrations in the plasma of 3 catheterized fetuses on the day after surgery are of interest in that they suggest that the placentas of these hypophysectomized fetuses were able to produce oestrone, presumably from some substrate other than progesterone since its levels were unaltered. These sows were ovariectomized and other possible substrates might therefore be steroids produced by the maternal adrenal gland after the stress of surgery.

In the current study we examined only unconjugated oestrogens and whether the differences are also present for total oestrogens is not known. Dwyer & Robertson (1980) showed that endometrial oestrogen sulphatase activity increased whereas sulphotransferase decreased in late pregnancy. Whether the maturation of the fetus or its placenta can influence the relative activity of these enzymes in late gestation in the pig is not known. The role of oestrogens in the onset of parturition itself is still equivocal. Oestradiol-17 β plays an important role in controlling prostaglandin production in sheep and goats but does not appear to play such a role in pigs (First & Bosc, 1979). It is possible that it plays a less obligatory role in this species, e.g. by raising oxytocin receptor concentrations, or in lactation.

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