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Steroid hormones in uterine washings and in plasma of gilts between Days 9 and 15 after oestrus and between Days 9 and 15 after coitus

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Summary. Between Days 9 and 15 after oestrus, concentrations of pregnenolone, pregnenolone sulphate, dehydroepiandrosterone (DHEA), DHEA sulphate, androstenedione, oestrone and oestrone sulphate in free uterine fluid collected from non-pregnant gilts were greater than respective values in plasma ($P < 0{\cdot}05$). The total contents of pregnenolone, progesterone, DHEA, testosterone, oestrone and oestradiol in washings from pregnant uteri exceeded ($P < 0{\cdot}05$) respective non-pregnancy levels during this same period.

Concentrations of pregnenolone, pregnenolone sulphate, DHEA, DHEA sulphate, androstenedione, oestrone, oestrone sulphate and oestradiol in free uterine fluid recovered from gravid uteri were also higher ($P < 0{\cdot}05$) than respective plasma values. By contrast, the progesterone concentration in uterine fluid from pregnant animals was lower ($P < 0{\cdot}001$) than the plasma value.

Concentrations of DHEA, DHEA sulphate, androstenedione and oestrone sulphate in plasma of pregnant gilts between Days 9 and 15 after mating exceeded ($P < 0{\cdot}05$) the respective concentrations in unmated gilts between Days 9 and 15 after oestrus. Plasma levels of pregnenolone sulphate were lower ($P < 0{\cdot}05$) in the pregnant animals.

We therefore suggest that the endometrium of the pig can concentrate steroid hormones in uterine fluid and that increases in steroid levels in this milieu between Days 9 and 15 after coitus reflect steroidogenesis by embryonic tissues and modification of enzyme activities within uterine tissues under the influence of progestagens. The pool of steroid sulphoconjugates present in uterine fluid between Days 9 and 15 *post coitum* could serve as an important precursor source for progestagen, androgen and oestrogen synthesis by tissues of pig embryos before implantation.

Introduction

Between Days 9 and 15 after fertilization, viable pig blastocysts change from spheres to filaments (Anderson, 1978); this change is associated with cellular remodelling in the endoderm and trophectoderm and an exponential increase in protein content (Geisert, Thatcher, Roberts & Bazer, 1982a; Wright, Grammer, Bondioli, Kuzan & Menino, 1983) and results in a higher ratio of trophoblast area/blastocyst volume. Trophoblast expansion increases the area of apposition of trophectoderm and endometrium at implantation (between Days 14 and 18 *post coitum*; Perry, 1981). Blastocysts that fail to elongate are then at a competitive disadvantage before and at implantation and it is proposed that this failure accounts for some of the embryonic loss that occurs between Days 9 and 15 of gestation in the pig (about 25%; Scofield, Clegg & Lamming, 1974). If determinants of blastocyst growth can be identified and corrected, this high level of early embryonic wastage in sows may be reduced.

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Because growth factors for protein anabolism in preimplantation blastocysts *in vivo* are provided from endometrial secretions via the uterine 'milk', we have attempted to identify steroidal determinants of preimplantation blastocyst growth by analysing uterine flushings collected from gilts between Days 9 and 15 after coitus. In this regard, steroids of particular interest include those that are known to stimulate release of proteins by the pig endometrium (predominantly progesterone and oestrogens; Schlosnagle, Bazer, Tsibris & Roberts, 1974; Basha, Bazer & Roberts, 1979; Aitken, 1979; Adams, Bazer & Roberts, 1981; Fazleabas, Bazer & Roberts, 1982; Geisert *et al.*, 1982a). Furthermore, because endometrial secretion is stimulated during pregnancy in the pig (Knight, Bazer & Wallace, 1973; Basha *et al.*, 1980; Geisert, Renegar, Thatcher, Roberts & Bazer, 1982b) and pig conceptuses have the ability to synthesize steroids *de novo* (Flint, Burton, Gadsby, Saunders & Heap, 1979; Heap, Flint, Gadsby & Rice, 1979), levels of steroids in washings of gravid uteri are compared with the values in uterine fluids collected from unmated gilts between Days 9 and 15 after oestrus, before the normal onset of luteolysis (Henricks, Guthrie & Handlin, 1972). Further comparison is made to the steroid content of plasma.

Materials and Methods

Animals

Large White sows (16 unmated and 16 mated) were slaughtered 9–15 days after the day of onset of the previous oestrus. Immediately after recovery of uteri, each uterine horn was cannulated above the bifurcation and flushed from the utero-tubal junction, towards the cannula (Stone, Whyte, Pointon, Quinn & Heap, 1984), with 10 ml 0·9% (w/v) NaCl at 4°C. Flushings were transported in ice to the laboratory and there centrifuged at low speed (56 ***g***, 20 min, 4°C) to precipitate conceptus tissue and/or other particulate matter. Supernatants were portioned and stored at −15°C. Precipitates from flushings of tracts of mated gilts were examined microscopically to confirm the presence of conceptus tissues consistent with stage of pregnancy (Anderson, 1978). Numbers of corpora lutea (CL) associated with individual uterine horns were recorded.

Blood samples (mixed arterial and venous) collected from all animals at exsanguination were centrifuged (2011 ***g***, 20 min, 4°C) and plasma samples were stored at −15°C.

Concentrations of steroid hormones in samples of plasma and in flushings from each uterine horn were determined by radioimmunoassay.

Days of slaughter were organized to provide post-mortem samples from a minimum of 2 gilts on each of Days 9 through 15 after oestrus/coitus.

General assay methods

Solvents for extraction were of analytical reagent grade and were redistilled before use. The solvent:sample ratio (v/v) exceeded 10:1 in all assays. Solvent was removed from sample extracts at 37°C under air.

Unconjugated steroid standards were purchased from Steraloids (Wilton, NH, U.S.A.) and pregnenolone sulphate (sodium salt) and oestrone-3-sulphate (potassium salt) from Sigma Chemical Company (St Louis, MO, U.S.A.). Standard steroids were not recrystallized further.

In the assays of pregnenolone sulphate, dehydroepiandrosterone (DHEA), DHEA sulphate and oestrone sulphate in plasma and in uterine washings, evaporated standards were equilibrated with steroid-free plasma or saline respectively (volumes equivalent to sample volume), then extracted and assayed with the uterine washing/plasma samples.

Before assay of pregnenolone, progesterone, androstenedione, oestrone, testosterone and oestradiol, solvent extracts of plasma and uterine flushings were fractionated by column chromatography (hydroxyalkoxypropyl Sephadex; Lipidex, Packard Instrument Company, IL, U.S.A.; column bed dimensions 80 mm × 3 mm), as detailed below.

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For pregnenolone and progesterone, samples (250 µl) were extracted with 2·5 ml hexane:ethyl acetate (9:1 v/v). The solvent was removed and residues redissolved in 200 µl hexane:ethyl acetate (9:1 v/v) and carefully loaded on to the column bed. Elution was made with 1·0 ml hexane (discard), 2·5 ml hexane (progesterone fraction, 77% recovery), a further 1·0 ml hexane (discard) and 2·5 ml hexane:ethyl acetate (9:1 v/v; pregnenolone fraction, 93% recovery).

For androstenedione, testosterone, oestrone and oestradiol, samples (400 µl) were extracted with 2·5 ml heptane:ethyl acetate (2:3 v/v), the solvent removed and residues redissolved in 200 µl hexane:ethyl acetate (9:1 v/v) and loaded. Elution was made with 0·5 ml hexane:ethyl acetate (9:1 v/v; discard), 2·0 ml hexane:ethyl acetate (9:1 v/v; androstenedione fraction, 71% recovery), 2·0 ml hexane:ethyl acetate (9:1 v/v; testosterone fraction, 93% recovery), 2·5 ml hexane:ethyl acetate (4:1 v/v; oestrone fraction, 84% recovery) and 2·0 ml ethyl acetate (oestradiol fraction, 96% recovery). Tritiated tracers were purchased from New England Nuclear (Boston, MA, U.S.A.). The assay buffer contained 6·1 g $NaH_2PO_4.2H_2O$, 10·9 g $Na_2HPO_4.2H_2O$, 9·0 g NaCl, 1·0 g NaN_3 and 1·0 g bovine serum albumin (BSA; Sigma) per litre, and was pH adjusted to 7·0. After incubation of sample/standard extracts with tracer and antiserum, antibody-bound steroid was precipitated by the addition of 1 mg gamma-globulin and polyethylene glycol 6000 (to provide a final concentration of 20·6%). After vortexing, tubes were centrifuged (4°C, 3574 ***g***, 20 min) and the supernatant, containing free steroid, was aspirated and discarded. Precipitated pellets, containing protein-bound steroid, were resuspended in 1·8 ml Scintisol (Isolab Incorporated, Akran, OH, U.S.A.), and radioactivity was measured in a Tracor Analytic 6891 liquid scintillation spectrometer.

Intra-assay coefficients of variation were < 10% in all assays. All uterine washing and plasma samples were assayed in single assays with blanks derived from saline or steroid-free pig plasma respectively.

Specific assays

Pregnenolone. After chromatography, solvent was removed from the pregnenolone fraction and pregnenolone was determined using an antiserum raised in goats against pregnenolone-3-carboxymethyloxime (CMO)–gamma globulin. The cross-reactivity of the antiserum, used at a final dilution of 1:16 000, was 37·5% with progesterone, 9·95% with 5α-pregnan-3-ol-20-one, 4·44% with 5α-pregnan-3,20-dione, 0·21% with 17α-hydroxyprogesterone, 0·18% with DHEA, 0·15% with DHEA sulphate, and < 0·10% with 17α-hydroxypregnenolone, 20α-hydroxypreg-4-ene-3-one, androstenedione, oestradiol, oestrone, oestrone sulphate and testosterone. The limit of sensitivity of the assay was 0·1 pmol per tube.

Pregnenolone sulphate. Unconjugated steroids were extracted from uterine washing plasma samples with 2·0 ml hexane:ethyl acetate (9:1 v/v) and discarded. The remaining pellet was saturated with NaCl and extracted with 2·5 ml ethyl acetate saturated with distilled water, the solvent was removed and the residue assayed using the same antiserum as employed in pregnenolone immunoassay. Levels of unconjugated steroids present in the pregnenolone sulphate-containing extracts were < 5% of levels in the original media. For assay, extracts were dispensed into polypropylene tubes, into which any unconjugated steroid would be adsorbed (Bruning, Jonker & Boerema-Baan, 1981), further diminishing any contribution of these compounds to levels of pregnenolone sulphate determined by this radioimmunoassay technique. The limit of sensitivity of the assay was 0·2 pmol pregnenolone sulphate per tube.

DHEA. Samples were extracted with 2·5 ml heptane:ethyl acetate (2:3 v/v), the solvent was removed and DHEA determined using an antiserum raised in goats against DHEA-15β-(3-thiopropionic acid)–BSA. The cross-reactivity of the antiserum, used at a final dilution of 1:5000, was 0·20% with androstenedione, 0·09% with DHEA sulphate, 0·06% with testosterone and < 0·04% with 5α-dihydrotestosterone, progesterone, oestradiol and pregnenolone. The limit of sensitivity of the assay was 0·1 pmol per tube.

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DHEA sulphate. Unconjugated steroids were extracted from samples with heptane:ethyl acetate (2:3 v/v) and discarded. The remaining pellet was saturated with NaCl and extracted with 2·5 ml ethyl acetate saturated with water. Dried extracts were assayed for DHEA sulphate using an antiserum raised in goats against DHEA-hemisuccinate–BSA. The cross-reactivity of the antiserum, used at a final dilution of 1:1000, was 67·3% with DHEA, 10% with androstenedione, 0·08% with testosterone, 0·07% with dihydrotestosterone and $< 0·02\%$ with progesterone and oestradiol. Levels of these unconjugated steroids in the DHEA sulphate-containing extracts were $< 2\%$ of levels in the original media. The limit of sensitivity of the assay was 0·1 pmol per tube.

Progesterone. After removal of solvent from the chromatography fractions, residues were assayed for progesterone using an antiserum raised in goats against progesterone-11α-hemisuccinate–gamma globulin. Used at a final dilution of 1:7000, the cross-reactivity of the antiserum was 1·2% with 17α-hydroxyprogesterone, 0·44% with pregnenolone, 0·17% with 20α-hydroxypreg-4-ene-3-one and $< 0·07\%$ with androstenedione, cortisol, testosterone and oestradiol. The limit of sensitivity of the assay was 0·1 pmol per tube.

Androstenedione. Solvent was removed from the chromatography fractions and residues were assayed for androstenedione using an antiserum raised in sheep against androstenedione-3-CMO–BSA. Used at a final dilution of 1:75 000, the cross-reactivity of the antiserum was 2·6% with DHEA, 1·18% with testosterone, 0·33% with 5α-dihydrotestosterone, 0·27% with progesterone and 17α-hydroxyprogesterone and $< 0·03\%$ with oestradiol and cortisol. The limit of sensitivity of the assay was 0·1 pmol androstenedione per tube.

Oestrone. Dried chromatography fractions containing oestrone were assayed for oestrone using an antiserum raised in sheep against oestrone-3-CMO–gelatin. Used at a final dilution of 1:30 000, the cross-reactivity of the antiserum was 83% with oestrone sulphate (not extracted into the organic phase), 4·2% with oestrone-3-glucosiduronate and $< 0·01\%$ with 21 other C_{19} and C_{21} steroids/conjugates tested. The limit of sensitivity of the assay was 0·05 pmol oestrone per tube.

Oestrone sulphate. After extraction of unconjugated steroids with ether, samples were saturated with NaCl, oestrone sulphate was extracted with 2·0 ml tetrahydrofuran and the solvent was removed. Residues were assayed for oestrone sulphate using the same antiserum as was used for oestrone, but at a final dilution of 1:33 000. The limit of sensitivity of the assay was 0·01 pmol oestrone sulphate per tube.

Oestradiol. After chromatography, solvent was removed from oestradiol fractions and residues were assayed for oestradiol using an antiserum raised in goats against oestradiol-6-CMO–gamma globulin. Used at a final dilution of 1:30 000, the cross-reactivity of this antiserum was 16·9% with oestrone, 1·1% with oestriol and $< 0·02\%$ with testosterone, androstenedione and progesterone. The limit of sensitivity of the assay was 0·04 pmol oestradiol per tube.

Testosterone. Dried chromatography fractions containing testosterone were assayed for testosterone using an antiserum raised in goats against testosterone-15β-(3-thiopropionic acid)–BSA. Used at a final dilution of 1:10 000, this antiserum cross-reacted 11·9% with 5α-dihydrotestosterone, 0·3% with androstenedione, 0·24% with progesterone, 0·05% with pregnenolone and $< 0·02\%$ with DHEA and oestradiol. The limit of sensitivity of the assay was 0·1 pmol testosterone per tube.

Statistics

For each steroid measured, statistical differences between the values for pregnant and non-pregnant animals, and between plasma and uterine fluid, were analysed by Student's t test (Clarke, 1969). For individual animals, relationships between numbers of CL and steroid properties of uterine washings (total steroid content and steroid concentration in free uterine fluid) and of

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plasma were examined by least squares linear regression analysis (Li, 1969). Relationships between these same steroid values and days *post coitum* and days after oestrus were analysed similarly.

Results

Despite efforts to avoid rupture of the fragile trophectoderm tissues during collection of uterine flushings, disrupted membranes were seen when precipitated conceptus tissues were examined microscopically. Uterine washings collected from pregnant gilts in this study therefore include blastocoele fluids and, while the volume of fluid contributed from this source would be low, concentrations of steroids in blastocoele fluid may be high (Seamark & Lutwak-Mann, 1972; Borland, Erickson & Ducibella, 1977; Gadsby & Heap, 1978) and at variance to concentrations in the uterine fluid which bathes blastocysts *in vivo*. Regardless, all steroidal components of the fluid intrauterine milieu (or precursors to these steroids) will have originated from maternal pools and differences between steroidal properties of uterine fluids collected from pregnant and from unmated gilts can be attributed to the presence of conceptus tissues. Analysis of these differences is the primary objective of this study. The admixture of histotroph with blastocoele fluid is hereafter referred to as free uterine fluid.

The total recovered flush volume exceeded 10 ml in 24/32 non-pregnant and in 20/32 pregnant uterine horns. Despite earlier evidence that endometrial secretion is enhanced during early pregnancy in sows (Murray, Bazer, Wallace & Warnick, 1972; Knight *et al.*, 1973; Bazer, 1975; Zavy, Bazer, Thatcher & Wilcox, 1980; Basha *et al.*, 1980; Geisert *et al.*, 1982b), the overall average volume of free uterine fluid ($\pm$ s.e.m.) recovered from the gravid uterine horns ($11 \cdot 7 \pm 4 \cdot 3$ ml) was not significantly higher than the respective non-pregnant value ($10 \cdot 1 \pm 3 \cdot 6$ ml; *t* test).

Total levels of pregnenolone, progesterone, DHEA, testosterone, oestrone and oestradiol were higher ($P < 0 \cdot 05$) in washings of pregnant uteri than of non-pregnant uteri between Days 9 and 15 after oestrus (Table 1), reflecting rising values for pregnant gilts after Day 10 *post coitum* (Text-fig. 1). Similarly, significant ($P < 0 \cdot 05$) differences between pregnant and non-pregnant animals in regard to concentrations of pregnenolone, DHEA, DHEA sulphate, androstenedione, oestrone, oestrone sulphate and oestradiol in free uterine fluids (total volume of intrauterine fluid recovered, minus the volume of saline flush; Tables 2 & 3) can be attributed to divergence of pregnant and non-pregnant values after Day 9 *post oestrus* (Text-fig. 2).

Table 1. Total content of steroids in washes of single uterine horns of mated (pregnant, $n = 32$) and of unmated (non-pregnant, $n = 32$) gilts between Days 9 and 15 after oestrus

	Steroid	Mean ± s.e.m. steroid content (pmol/horn) Non-pregnant	Pregnant	
C_{21}	Pregnenolone	26·9 ± 6·5	202·1 ± 46·6	***
	Pregnenolone sulphate	65017 ± 25583	106865 ± 41874	
	Progesterone	86·8 ± 27·6	337·0 ± 108·6	*
C_{19}	DHEA	3·70 ± 1·44	18·7 ± 2·1	***
	DHEA sulphate	2959 ± 1290	937 ± 469	
	Androstenedione	14·1 ± 5·1	20·4 ± 6·6	
	Testosterone	2·10 ± 1·08	9·40 ± 3·34	*
C_{18}	Oestrone	2·92 ± 0·72	13·92 ± 4·05	**
	Oestrone sulphate	1234 ± 453	404 ± 199	
	Oestradiol	0·69 ± 0·21	62·96 ± 32·44	*

Significantly different from value for non-pregnant sows: $^{*}P < 0 \cdot 05$; $^{**}P < 0 \cdot 01$; $^{***}P < 0 \cdot 001$ (Student's *t* test).

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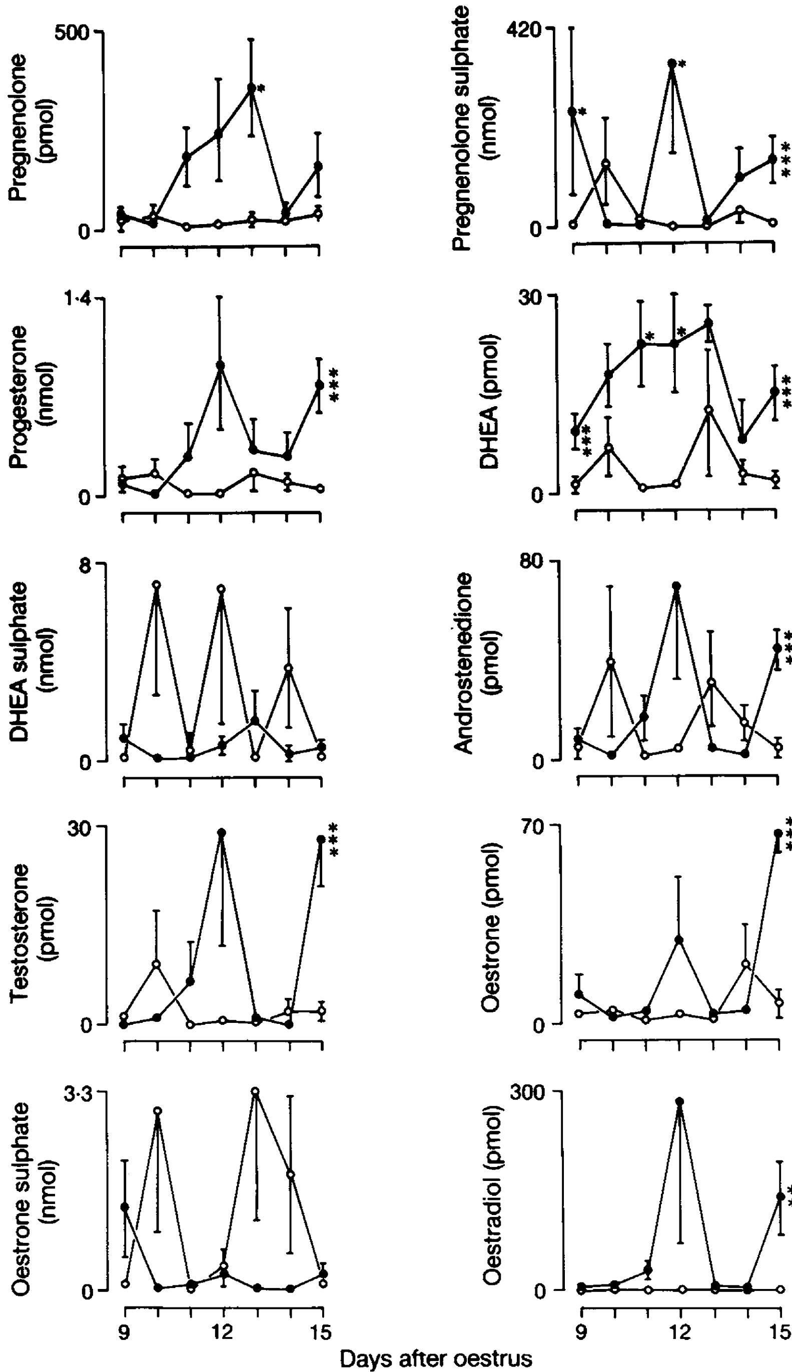

Text-fig. 1. Average (± s.e.m.) total amounts of steroids in washings of single uterine horns of unmated (○) and mated (●) gilts on individual days between Days 9 and 15 after oestrus. For each day, non-pregnancy and pregnancy values were compared using a t test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

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Table 2. Concentrations of steroids in free uterine fluid and in plasma of unmated and of mated gilts between Days 9 and 15 after oestrus

	Steroid	Mean ± s.e.m. steroid concentration (nM)						
		Unmated gilts			Mated gilts			
		Uterine fluid ($n = 24$)	Plasma ($n = 16$)		Uterine fluid ($n = 20$)	Plasma ($n = 16$)		
C_{21}	Pregnenolone	8·04 ± 1·75	3·36 ± 0·99	*	39·37 ± 13·39	3·88 ± 0·84	*	
	Pregnenolone sulphate	1973 ± 533	37·3 ± 8·4	***	3812 ± 1109	25·0 ± 1·9	**	
	Progesterone	14·6 ± 5·8	72·7 ± 12·0	***	17·1 ± 3·9	89·2 ± 6·4	***	
C_{19}	DHEA	0·24 ± 0·06	0·10 ± 0·02	*	10·37 ± 3·24	0·44 ± 0·10	**	
	DHEA sulphate	95·2 ± 26·1	0·61 ± 0·10	***	13·3 ± 3·2	1·34 ± 0·27	***	
	Androstenedione	1·26 ± 0·09	0·45 ± 0·04	***	2·38 ± 0·40	1·15 ± 0·14	**	
	Testosterone	0·39 ± 0·12	0·85 ± 0·18	*	0·76 ± 0·23	0·80 ± 0·16		
C_{18}	Oestrone	0·92 ± 0·26	0·05 ± 0·01	**	3·07 ± 0·73	0·10 ± 0·05	***	
	Oestrone sulphate	47·3 ± 12·1	0·32 ± 0·04	***	12·9 ± 3·4	0·68 ± 0·16	***	
	Oestradiol	0·26 ± 0·12	0·03 ± 0·01		4·31 ± 1·03	0·03 ± 0·01	***	

Significantly different from value for free uterine fluid: $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$ (Student's t test).

Table 3. Analysis of differences between pregnant and non-pregnant gilts in respect to concentrations of steroids in free uterine fluid and in plasma

	Steroid	Significance of difference between concentrations of pregnant and non-pregnant gilts		Pregnant/non-pregnant ratio	
		Uterine fluid	Plasma	Uterine fluid	Plasma
C_{21}	Pregnenolone	*	NS	4·90	1·15
	Pregnenolone sulphate	NS	*	1·93	0·67
	Progesterone	NS	NS	1·17	1·23
C_{19}	DHEA	**	**	43·2	4·40
	DHEA sulphate	**	**	0·14	2·20
	Androstenedione	**	***	1·89	2·56
	Testosterone	NS	NS	1·95	0·94
C_{18}	Oestrone	**	NS	3·34	2·00
	Oestrone sulphate	*	*	0·27	2·13
	Oestradiol	***	NS	16·6	1·00

NS, not significant; $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$ (Student's t test).

Between Days 9 and 15 after oestrus, average levels of DHEA, DHEA sulphate, androstenedione and oestrone sulphate in plasma of pregnant gilts exceeded non-pregnant concentrations while pregnenolone sulphate levels were diminished ($P < 0{\cdot}05$; Tables 2 & 3). Daily plasma steroid levels between Days 9 and 15 after oestrus/coitus are summarized in Text-fig. 3.

Linear regressions between steroid properties of uterine fluids and plasma, numbers of CL, days after oestrus and days *post coitum*, were not statistically significant ($P > 0{\cdot}05$).

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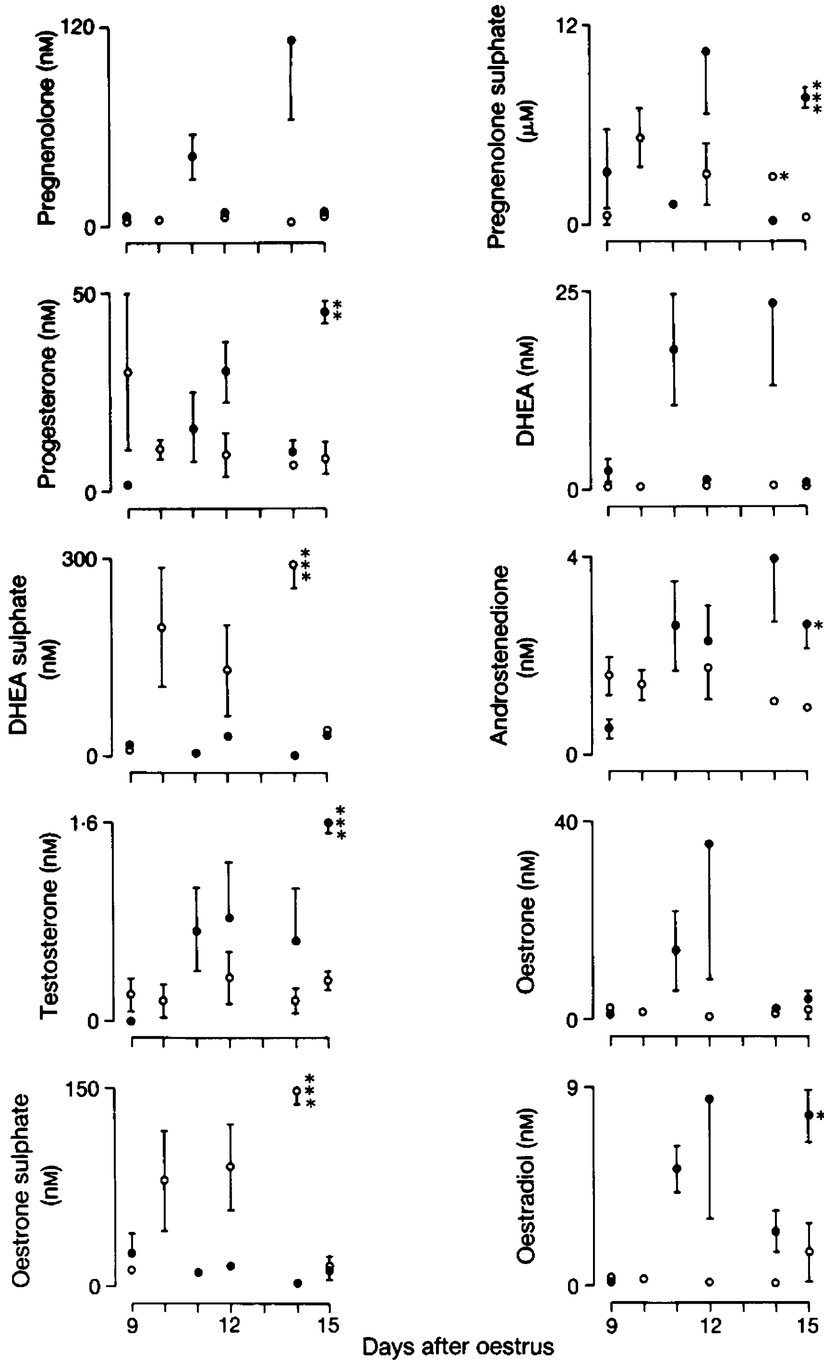

Text-fig. 2. Average (± s.e.m.) concentrations of steroids in free uterine fluid recovered from single uterine horns of unmated (○) and mated (●) gilts on individual days between Days 9 and 15 after oestrus. The data represent only those uterine horns from which free fluid could be recovered (non-pregnant, $n = 24$; pregnant, $n = 20$). For each day, non-pregnancy and pregnancy values were compared using a t test: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

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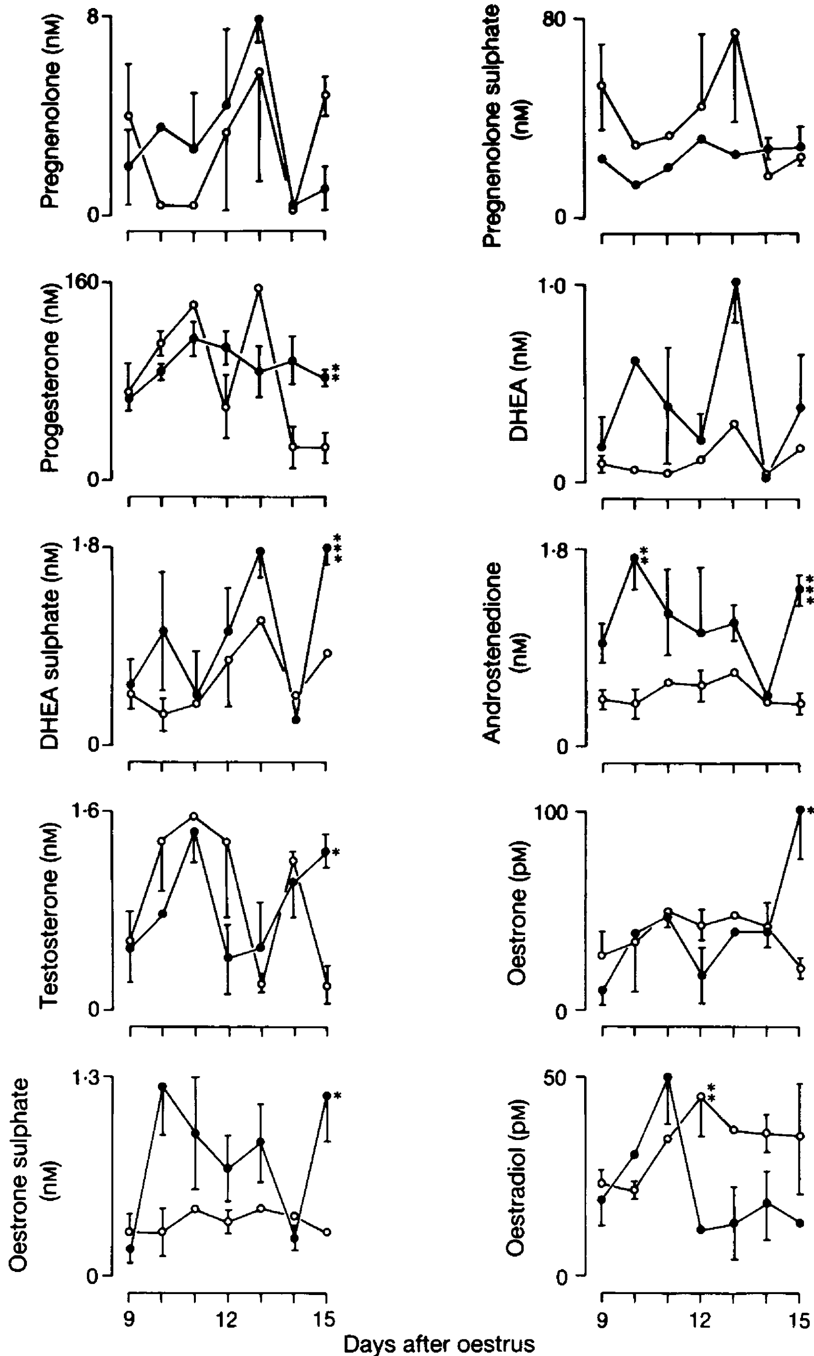

Text-fig. 3. Average (± s.e.m.) concentrations of steroids in plasma of unmated (○) and mated (●) gilts on individual days between Days 9 and 15 after oestrus. For each day, non-pregnancy and pregnancy values were compared using a *t* test: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

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Discussion

Significant amounts of steroid hormones were present in uterine washings recovered from unmated sows between Days 9 and 15 after oestrus (Table 1), with evidence for a marked (50- to 150-fold) concentration of the sulphoconjugates of pregnenolone, DHEA and oestrone in free uterine fluid compared with plasma levels (Table 2). Further accumulation of pregnenolone, progesterone, DHEA, testosterone, oestrone and oestradiol occurred in uterine fluid during pregnancy ($P < 0{\cdot}05$; Table 1; Text-fig. 1).

The higher levels of oestrone and oestradiol in uterine fluids of gilts between Days 9 and 15 of pregnancy (Table 1; Text-fig. 1) are consistent with oestrogen synthesis by dispersed pig blastocyst tissues (Heap *et al.*, 1981) and the results concur with those of Ford, Christenson & Ford (1982) in respect to the predominance of oestradiol in uterine fluid (Tables 1 & 2) and their evidence for concentration of oestradiol in uterine venous plasma. Levels of oestrone and oestradiol in the washings of gravid uteri increased after about Day 11 of pregnancy, and were significantly higher ($P < 0{\cdot}05$) than non-pregnancy values by Day 15 after oestrus (Text-fig. 1). This result is in accord with previous reports that oestrogen synthesis by pig blastocyst tissues *in vivo* is initiated between Days 10 and 12 of gestation (Heap, Perry, Gadsby & Burton, 1975).

Oestrone sulphate levels in uterine fluid were similar for pregnant and non-pregnant animals (Table 1). However, concentrations of oestrone sulphate in plasma were elevated in pregnancy ($P < 0{\cdot}05$; Table 2; see Text-fig. 3), this being consistent with oestrone secretion by blastocyst tissues as proposed by Heap *et al.* (1981), its accumulation in histotroph ($P < 0{\cdot}001$; Table 2; Text-fig. 1), sulphation in uterine or other maternal tissues (Pack & Brooks, 1974; Pack, Brooks, Dukelow & Brooks, 1979; Meyers *et al.*, 1983), and release to the maternal circulation as the sulphoconjugate.

The preferred precursor and pathway for oestrogen synthesis by pig conceptuses have not previously been identified. However, since ovarian progesterone is not an obligatory precursor, Flint *et al.* (1979) and Heap *et al.* (1981) have proposed that oestrogens are synthesized by the blastocyst from adrenal progesterone or pregnenolone, or from cholesterol or acetate. The immediate oestrogen precursor measured in the present study, androstenedione, was concentrated in uterine fluid relative to plasma in pregnant and in non-pregnant gilts ($P < 0{\cdot}01$; Table 2), although mechanisms inducing the pregnancy-associated increases in concentration of this steroid in plasma and in uterine fluid ($P < 0{\cdot}001$ and $P < 0{\cdot}01$ respectively; Tables 2 & 3; see Text-figs 2 & 3) remain to be determined. Furthermore, the accumulation and concentration of androgens in uterine fluid between Days 9 and 15 of pregnancy (Tables 1, 2 & 3; Text-figs 1 & 2) provide a potentially important source of oestrogen precursor, but whether these events reflect changes in enzyme activity in maternal or embryonic tissues remains unresolved. Besides being potential oestrogen precursors, androstenedione and testosterone may be important in their own right, as both are potent protein anabolic agents which may play a role in the exponential increase in protein content of pig embryos between Days 9 and 15 after coitus (Anderson, 1978; Wright *et al.*, 1983).

Pig blastocyst tissues show arylsulphatase activity (Flint *et al.*, 1979) and are able to synthesize oestrogen from pregnenolone and DHEA *in vitro* (Heap *et al.*, 1981). The steroid sulphoconjugates are also, therefore, potential precursors for blastocyst steroidogenesis and, with the high concentration of pregnenolone sulphate (3·81 μM) and of DHEA sulphate (13·3 nM) in free uterine fluid of pregnant gilts (Table 2), oestrogen synthesis from these sources must be considered.

The accumulation and concentration of the sulphoconjugates of pregnenolone and of DHEA in uterine fluid of unmated animals ($P < 0{\cdot}001$; Tables 1 & 2) indicates a concentrating mechanism for sulphoconjugates which is specific to maternal tissues. Further accumulation of pregnenolone sulphate in uterine fluid during pregnancy (Table 1; Text-fig. 1) could then be attributable to induction of further sulphotransferase activity during early pregnancy (Dwyer & Robertson, 1980) by progestagens or other pregnancy-associated hormones, as previously demonstrated in cultured human endometrium (Clarke, Adams & Wren, 1982). As average pregnenolone sulphate

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concentrations were lower in plasma of pregnant compared with non-pregnant sows between Days 9 and 15 after oestrus ($P < 0.05$, Table 3), while pregnenolone concentrations were similar (Table 3), we propose that pregnenolone sulphate in uterine fluid derives from the pool of pregnenolone sulphate in maternal plasma, rather than from the plasma pregnenolone pool. Furthermore, as there is no evidence to suggest that steroid production rates of pregnant and non-pregnant gilts differ between Days 9 and 12 after oestrus, the plasma pregnenolone sulphate values shown in Text-fig. 3 indicate that the rate of clearance of this steroid sulphate from maternal plasma is higher in pregnant animals before Day 12 after oestrus. Precise mechanisms of transfer of steroids and/or steroid conjugates across the pig endometrium have not yet been elucidated.

Our own in-vitro studies have identified sulphokinase activity in intact pig blastocysts, and have shown that pregnenolone sulphate can be metabolized to pregnenolone and to progesterone by blastocysts cultured between Days 3 and 8 after fertilization (B. A. Stone & R. F. Seamark, unpublished data). If pregnenolone sulphate concentrated in uterine luminal fluids is metabolized to progesterone by pig blastocysts *in vivo*, this progesterone may be concentrated within the blastocoele cavity (Heap *et al.*, 1981) and at the trophectoderm boundary layer where it can then provide immune cytoprotection for the embryo (Siiteri *et al.*, 1977) or, if released, stimulate secretory activity by the endometrium (Knight *et al.*, 1973; Schlosnagle *et al.*, 1974; Roberts, Bazer, Baldwin & Pollard, 1976; Adams *et al.*, 1981; Fazleabas *et al.*, 1982). Higher levels of progesterone in fluids recovered from pregnant tracts in the present study ($P < 0.05$; Table 1) are consistent with progesterone synthesis and secretion by preimplantation pig blastocysts, although concentrations of progesterone in uterine fluid and in plasma of pregnant and of non-pregnant gilts were similar between Days 9 and 15 after oestrus ($P > 0.05$; Table 3). Uterine metabolism of progesterone released by blastocysts is thus indicated, with no direct contribution by conceptus tissue to the maternal progesterone pool (see Robertson & King, 1974). Similarly, large quantities of progesterone which are synthesized by placental tissues of pigs later in gestation appear to be metabolized locally, and do not increase systemic progesterone levels (Kukoly, Knight & Notter, 1984).

Higher levels of progesterone in plasma of pregnant gilts at Day 15 after oestrus ($P < 0.05$; Text-fig. 3) are consistent with luteotrophic effects of oestrogens (Gardner, First & Casida, 1963; Bazer & Thatcher, 1977; Frank, Bazer, Thatcher & Wilcox, 1978; Ford & Magness, 1980) which are secreted into the uterine lumen of pregnant sows at this time (Text-fig. 1).

On the basis of our results, we suggest that the high levels of the sulphoconjugates of pregnenolone and of DHEA in uterine fluid of the early pregnant pig serve as an important precursor pool for steroid synthesis by preimplantation pig embryos *in vivo*.

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