Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in gilts*

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Summary. Endometrial and conceptus tissues were obtained on Days 10·5, 11, 12, 16 and 25 of pregnancy and Day 25 of pseudopregnancy of gilts and incubated for 6 h in Minimal Essential Medium (5 ml) containing 35 ng [³H]progesterone. Metabolism of [³H]progesterone to oestrone, oestradiol and oestriol was determined by gas and high-pressure liquid chromatography and successive recrystallizations with unlabelled standards. Conceptuses collected between Days 10·5 and 12 were spherical, tubular or filamentous and incubated with 500 mg endometrium and [³H]progesterone. Production of oestrone by spherical conceptuses was not detected, but was 44–47 pg/tubular conceptus and 21 pg/filamentous conceptus. A similar trend was observed for oestradiol. Conceptus tissues from Days 16 and 25 (chorion) were most active in producing oestrone (123 and 520 pg/mg tissue, respectively) and oestradiol (277 and 876 pg/mg tissue, respectively). Endometrial oestrogen production was less than that for conceptus tissue for oestrone and oestradiol on Days 16 and 25 of gestation. Coincubations of endometrium and conceptus tissues had lower oestrogen production than conceptus alone. Endometrium from Day 25 of pseudopregnancy metabolized [³H]progesterone to several non-polar metabolites, but no oestrogens were detected. An unidentified phenolic metabolite of [³H]progesterone was detected in higher quantities than either oestrone or oestradiol; 445 to 461 pg/conceptus at the tubular stage. These results indicate temporal changes in the conversion of [³H]progesterone to oestrogens by conceptus and endometrial tissue from pregnant gilts, but not endometrium from pseudopregnant gilts.

Introduction

Conversion of androgens to oestrogens by porcine conceptus tissues is well documented (Perry, Heap & Amoroso, 1973; Gadsby, Heap & Burton, 1980), but there is less evidence for conversion of C₂₁ steroids to oestrogens by endometrial and conceptus tissues (Gadsby, 1978). There is a positive uterine arterial–venous difference in concentrations of progesterone in plasma of pigs, indicating net uptake of progesterone by the pregnant uterus (Knight, Bazer, Thatcher, Franke & Wallace, 1977). This progesterone is a potential substrate for enzymes in the endometrium and/or conceptus for metabolism to androgens and oestrogens. This study was designed to compare metabolism of progesterone to oestrogens by pig conceptus and endometrial tissues during the preimplantation period and determine at what point oestrogen synthesis begins. An additional goal was to determine whether endometrium from pseudopregnant gilts metabolizes progesterone to oestrogens.

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Materials and Methods

Animals. Sexually mature crossbred gilts (Duroc $\times$ Yorkshire $\times$ Hampshire) were observed daily for oestrous behaviour using intact mature boars. Following two oestrous cycles of normal duration (18–22 days) gilts were mated at detection of oestrus and 12 and 24 h later. Conceptus and endometrial tissues were obtained at surgery. Anaesthesia was induced with a 5% solution of thiamylal sodium (Vescor, Inc., Orlando, FL, U.S.A.) given intravenously and maintained with methoxyflurane (Pittman-Moore, Inc., Washington Crossing, NJ, U.S.A.). The reproductive tract was exposed by midventral laparotomy and gilts were ovario-hysterectomized on Days 10.5, 11, 12, 16 and 25 of pregnancy or Day 25 of pseudopregnancy. Conceptus tissue was flushed from each uterine horn using 20 ml sterile saline (9 g NaCl/l) except for Day 25 of pregnancy when conceptuses were collected by dissecting open the uterus so that chorion could be obtained. Endometrial tissue was obtained by dissection at several locations along each uterine horn.

Pseudopregnancy, i.e. prolonged maintenance of corpora lutea (CL), was induced by administering 5 mg oestradiol valerate subcutaneously on Days 11 through 15 of the oestrous cycle (Frank, Bazer, Thatcher & Wilcox, 1977).

Medium preparation. Eagles minimum essential medium (MEM) was prepared as described previously (Basha, Bazer, Geisert & Roberts, 1980). On the day of incubation, medium was transferred to a sterile beaker and bovine serum albumin (5 mg/ml) was added. The medium was filtered through a 0.2 μ m filter and pH was determined to ensure that it was between 7.3 and 7.5. Then 5 ml MEM were transferred into sterile 25-ml Erlenmeyer flasks. Progesterone, labelled with ^3H at the 1, 2, 6 and 7 positions (mean sp. act. 97.9 Ci/mmol), was obtained from New England Nuclear (Boston, MA, U.S.A.). The purity of each batch of [^3H]progesterone was checked using Sephadex LH-20 chromatography and contaminants, if present, were removed. The [^3H]progesterone was diluted in ethanol and added to the incubations (35 ng/ml) in 10% ethanol in MEM such that a maximum of 10 μ l ethanol was added to each incubation. In incubations containing reducing factors, NADPH was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) and added at a concentration of 0.2 mmol/ml. Tissues were incubated at 37°C in a Dubnoff shaking water bath under an atmosphere of 95% O_2 and 5% CO_2 for 6 h. Metabolism was stopped by addition of 5 ml acetone and each flask was corked and stored at -20°C until the MEM was extracted. All incubations were then subjected to the following procedures.

Extraction procedure. Oestrone, oestradiol-17 β , and oestriol-16 α ,17 β -labelled with ^{14}C at the four position were obtained from New England Nuclear and checked for purity. About 10 000 counts per minute (c.p.m.) were added to each incubation to aid in the determination of the identity of metabolites produced and the estimation of procedural losses. All solvents used were distilled once over glass. The tissue was homogenized with a Pyrex Model 7727 Ten Broeck Tissue Grinder with a 0.15 mm clearance between the two parts. Both the incubation flask and homogenizer were rinsed with a total of 15 ml acetone and transferred to a stainless-steel centrifuge tube. Samples were centrifuged at 12 000 g for 15 min to precipitate tissue residue and the supernatant transferred to a 40-ml conical tube. The pellet was washed three times with 80% acetone, supernatants were pooled, and the acetone was evaporated under nitrogen.

The remaining aqueous mixture was freeze dried almost to dryness, then resuspended in 5 ml 0.2 M-acetate buffer, pH 5. An enzyme preparation containing sulphatase (2000–5000 units/ml) and glucuronidase ($\sim 100\,000$ Sigma units/ml) from the snail (*Helix pomatia*) was obtained from Sigma Chemical Co. The enzyme preparation was added to the solution (5.8 U/ml) and incubated overnight at 37°C in the dark to convert conjugated oestrogens to free oestrogens. Therefore, all estimates of oestrogen production reflect both conjugated and free oestrogens. After incubation, the solution containing the free oestrogens was raised to a pH > 12 using 1 M-NaOH and extracted with 20 ml benzene:hexane (1:1, v/v). The organic phase was extracted twice more with 1 M-NaOH solution and the aqueous phases were combined. The pH of the aqueous phase was then lowered to

neutrality with 5 M-HCl and extracted twice with dichloromethane and once with ether. In this procedure, referred to as the Brown reaction (Brown, 1955), the strongly basic solution causes phenolic steroids to give up the hydrogen atom of the hydroxyl group on the aromatic ring. This makes the steroid very hydrophilic and it moves into the aqueous phase. Upon acidification to neutrality the hydrogen is replaced and the phenolic steroids move into the organic phase. All extractions with organic solvents were conducted in 125- or 250-ml separation funnels with polytetrafluoroethylene (Teflon) stopcocks and complete separation of the aqueous and organic phases was achieved. The pooled organic phases were dried under nitrogen and redissolved in benzene:methanol (90:10, v/v).

Sephadex LH-20 chromatography. Glass chromatography columns (30 × 1.5 cm) with sintered glass bottoms and polytetrafluoroethylene stopcocks were packed with Sephadex LH-20 and one of four solvent systems was used as the eluent. The sample was first loaded on a 12-cm benzene:methanol (90:10, v/v) column in 2 ml solvent: 1-ml fractions were collected and a 50 µl aliquant from each fraction was counted after addition of cocktail (Omni-scint, 4 g/l toluene; ICN Chemical and Radioisotope Division, Irvine, CA, U.S.A.). This procedure was used for Sephadex LH-20 column chromatography. Tubes containing detectable amounts of ¹⁴C-labelled oestrone, oestradiol or oestriol were pooled to isolate each of the respective steroids. Fractions containing ¹⁴C-labelled oestrone, oestradiol or oestriol were dried under nitrogen and redissolved in 1 ml cyclohexane:benzene:methanol (90:25:5, by vol.), benzene:methanol (90:10, v/v) and benzene:methanol (85:15, v/v), respectively.

The oestrone fraction was rechromatographed on a 12-cm column, equilibrated with cyclohexane:benzene:methanol (90:25:5, by vol.). The solvent system separated oestrone from most neutral steroids. The oestradiol fraction was loaded on a 17-cm column and the oestriol fraction on a 10-cm column with the appropriate solvent. After counting, tubes containing ¹⁴C-labelled oestrogens and appreciable ³H-label were saved. The tritiated compounds cochromatographing with [¹⁴C]oestrogen markers were tentatively identified as oestrone, oestradiol or oestriol. All columns were rinsed thoroughly between runs and periodically repacked.

If ³H-metabolites did not exactly comigrate with the ¹⁴C-standards, no further attempt was made to determine their identity, with the exception of compounds migrating near oestriol. Tritiated metabolites cochromatographing with ¹⁴C-markers were dissolved in 1 ml benzene. Then 300 µl were taken for analysis by high-pressure liquid chromatography (HPLC). Another 300 µl were used on a gas chromatography system (GC) and the remaining 400 µl were subjected to recrystallization with unlabelled steroid.

High-pressure liquid chromatography (HPLC). A Water's Associates HPLC system was used to study the oestrogens. A Water's octadecylsilica (C-18) reverse-phase column was mounted in a radial compressor (Water's Model RCM100). The solvents used were HPLC-grade acetonitrile and distilled water filtered through 0.5 µm Teflon and 0.45 µm cellulose filters, respectively. The solvent systems which yielded maximum resolution were 50:50, 42:58 and 27:73 acetonitrile:water (v/v) for oestrone, oestradiol and oestriol, respectively. With this system a ³H-labelled compound could be separated from the same compound labelled with ¹⁴C (Text-fig. 1). Therefore, exact cochromatography could not be demonstrated, but ³H steroids migrating in a manner similar to the ³H standards and migrating near radioinert standards were assumed to be identical. Additionally, separation of oestradiol-17β and oestradiol-17α was possible (Text-fig. 2) but oestradiol-17α was not identified in any incubation medium.

Gas chromatography. A Varian gas chromatograph (Model 2700) was used with a trifluoropropyl silicone column. The oven temperature was 240°C and nitrogen gas pressure was 179.4 kPa. A flame ionization detector (FID) and a splitter were used and about 10% of the effluent went to the FID and 90% to small glass collecting tubes. As with HPLC, the gas chromatography column was characterized by using radioinert oestrogens. Retention times for oestrone, oestradiol-17β, and oestriol were 11.5, 7.5 and 14 min, respectively. The samples were injected and 30-sec fractions were collected over the area where the steroids eluted. The small collecting tubes, open at both ends,

Text-fig. 1. Elution profile of [1,2,6,7- ^3H]oestrone (---) and [^{14}C]oestrone (—) from a reverse-phase HPLC column using a solvent system of 50% acetonitrile and 50% water (v/v). Although these are the same compound they are separated using this system.

Text-fig. 2. Elution profile of unlabelled oestradiol-17 α and -17 β from an ultraviolet light detector after a reverse-phase HPLC column. The solvents used were 42% acetonitrile and 58% water (v/v). These two compounds are separated. Oestradiol-17 β appears first followed by oestradiol-17 α ~1.5 min later.

were rinsed with acetone into scintillation vials. The acetone was evaporated, cocktail (3 ml) was added, and the radioactivity in the vials was counted. Tritium-labelled oestrogens comigrated with ^{14}C -labelled and radioinert oestrogens.

Recrystallization. After tentative identification of an oestrogen using Sephadex LH-20 chromatography, 30 mg recrystallized radioinert standards (oestrone and oestradiol) were added to the sample. The sample and ^{14}C -labelled standard were dissolved in toluene with a small amount of methanol in boiling water. Most of the methanol was removed (boiling point 65°C), leaving the oestrogen in toluene. If the sample contained oestrone a small amount of warm hexane was added, but this was not done for samples containing oestradiol. Oestriol was not conclusively identified using Sephadex LH-20 and was not, therefore, recrystallized. Tubes containing samples were left in hot water and allowed to cool to 4°C . Crystals were grown for 2–7 days, then the solvent was removed and about 1 mg of the crystals was dissolved in toluene cocktail, counted, and the ^3H : ^{14}C ratio was calculated. This procedure was repeated until three consecutive crystallizations had ratios within 5% of each other. This was considered final confirmation of the synthesis of a particular

oestrogen from the [^3H]progesterone substrate. The remainder of each sample from the last crystallization was then counted to calculate the amount of steroid produced. In making these calculations it was assumed that ^3H atoms behaved as a hydrogen molecule. There are two ^3H atoms on the A ring of progesterone at the 1 and 2 positions. Both are in the α -orientation (M. Cabell, New England Nuclear, Boston, MA, U.S.A., personal communication) and neither is lost during aromatization (Fishman, 1982). The factor of radioactive decay for [^3H]progesterone was accounted for when necessary.

Statistical analysis. Statistical analyses were not performed on the results. Because of low oestrogen production in some incubates it was necessary to pool similar samples to permit accurate measurement of oestrogen production. This pooling eliminated the possibility of measuring the variability between different samples. Additionally, time and cost involved in obtaining repeated samples to provide a valid statistical model were prohibitive. Therefore, when the amount of oestrogen production is said to be higher, it does not reflect a statistically significant difference, but an apparent quantitative difference.

Results

Conceptuses recovered from 4 gilts between Days 10.5 and 12 of pregnancy were spherical, tubular or filamentous (Table 1). They were incubated with 500 mg endometrium in medium containing NADPH. Conversion of progesterone to oestrone by 5 mm or 7 mm spherical conceptuses could not be detected. Conversion of progesterone to oestradiol was demonstrated for 7 mm spherical, tubular and filamentous conceptuses. A phenolic unknown compound was produced from progesterone by conceptuses at all stages studied. As summarized in Table 1, oestradiol production was initiated at the late spherical stage, oestrone and oestradiol production increased through the tubular stages and then decreased to the filamentous stage. The dramatic change in oestrone

Table 1. Production of oestrone, oestradiol and phenolic unknown compound by conceptuses and endometrial tissue coincubations obtained from gilts between Days 10.5 and 12 of pregnancy

	Conceptus shape				
	Spherical		Tubular		Filamentous
	5 mm	7 mm	10 mm	17 mm	
No. of conceptuses	7	4	4	8	6*
Oestrone					
pg/incubate	ND	ND	293	347	99
pg/conceptus	ND	ND	73	44	21
% progesterone conversion	ND	ND	0.7	0.9	0.3
Oestradiol					
pg/incubate	ND	153	167	NA	101
pg/conceptus	ND	24	41	NA	17
% progesterone conversion	ND	0.4	0.4	NA	0.3
Phenolic unknown compound					
pg/incubate	23	1733	1847	3560	2200
pg/conceptus	3	433	461	445	373
% progesterone conversion	0.05	4.1	4.3	8.3	5.1

* Estimated by dividing number of dilated ends of trophoblast detected by two.

ND, not detected; NA, samples were lost and, therefore, not available for assay.

production is demonstrated in Text-figs 3 and 4. One uterine horn was removed on Day 11 and spherical conceptuses were recovered. From the same gilt, 24 h later, tubular conceptuses were recovered. No oestrone production by spherical conceptuses was detected, but oestrone was produced by tubular conceptuses. Other unidentified metabolites of [^3H]progesterone were also increased in incubations with tubular vs spherical conceptuses.

Incubations of conceptus and endometrial tissues from Day 16 were performed in triplicate from 2 gilts. Tissues from one gilt were incubated in the absence of NADPH and no oestrogen production from [^3H]progesterone was detected. Data from incubations containing NADPH indicated that conceptus tissue alone was most active in producing oestrone, oestradiol and the phenolic unknown compound (data not shown).

Text-fig. 3. Elution profile of [^{14}C]oestrone (---) and [^3H]oestrone (—) (1 ml fractions) from a cyclohexane:benzene:methanol (90:25:5 by vol.) LH-20 column. The ^3H -metabolites were synthesized, from [^3H]progesterone, by incubates containing spherical embryos collected from a gilt on Day 11 of pregnancy. The profile of metabolites is markedly different following incubation of blastocysts obtained from the opposite uterine horn of this same gilt 24 h later (see Text-fig. 4).

Text-fig. 4. Elution profile of ^3H -labelled unknown metabolites (---) and [^{14}C]oestrone (—) (1 ml fractions) from a cyclohexane:benzene:methanol (90:25:5 by vol.) LH-20 column. The ^3H -metabolites were synthesized from [^3H]progesterone by incubates containing tubular blastocysts collected from a gilt on Day 12 of pregnancy. Note the phenolic unknown compound eluting between fractions 35 and 45.

Table 2. Production of oestrone, oestradiol and phenolic unknown compound by conceptus and endometrial tissues from gilts at Days 6 and 25 of pregnancy

		Endometrium	Conceptus	Coincubate
Day 16	Oestrone			
	pg/incubate	22	46	34
	pg/mg tissue	43	92	34
	% progesterone conversion	0.07	0.15	0.11
	Oestradiol			
	pg/incubate	30	104	22
	pg/mg tissue	60	208	22
	% progesterone conversion	0.10	0.34	0.07
	Unknown			
	pg/incubate	18	1155	152
Day 25	pg/mg tissue	35	2316	152
	% progesterone conversion	0.06	3.60	0.47
	Oestrone			
	pg/incubate	183	192	208
	pg/mg tissue	363	390	211
	% progesterone conversion	0.60	0.63	0.69
	Oestradiol			
	pg/incubate	41	324	57
	pg/mg tissue	80	657	57
	% progesterone conversion	0.14	1.07	0.19
Unknown	pg/incubate	46	505	170
	pg/mg tissue	91	1025	172
	% progesterone conversion	0.14	1.57	0.53

Table 3. The ^3H counts from compounds that migrated with ^{14}C markers in Day 25 tissue incubates and without added NADPH

Incubate	No.	NADPH	^3H (c.p.m.)	
			Oestrone	Oestradiol-17 β
Endometrium	2	—	5 449	2 031
	2	+	6 241	2 325
Conceptus	2	—	19 558	11 799
	2	+	24 921	12 330
Endometrium plus conceptus	2	—	11 654	25 548
	2	+	15 455	37 940

Incubations from 2 gilts on Day 25 of gestation were in quadruplicate with 2 replicates incubated with and 2 without NADPH. At this stage of gestation there was oestrone and oestradiol production by incubations of chorion alone, endometrium alone or chorion plus endometrium in the presence and absence of NADPH (Table 2). The trend, however, was for greater oestrone and oestradiol production in incubations containing NADPH (Table 3). This difference was more apparent for conceptus incubations and coincubations compared to endometrial incubations. As

with Day 16 tissues, chorion (conceptus) alone was most active in converting [^3H]progesterone to oestrone, oestradiol and the unknown phenolic steroid. The Day 25 tissues were all more active in producing oestrogens than tissues from Day 16 of pregnancy (Table 2).

Endometrial tissue was cultured in triplicate from two Day 25 pseudopregnant gilts in the presence and absence of NADPH. No oestrogen production from [^3H]progesterone was detected and the unknown phenolic steroid was absent (data not shown).

Discussion

Conversion of C_{19} steroids to oestrogens by pig conceptus tissues is well documented (Gadsby, Heap & Burton, 1980); however, there is less evidence for conversion of C_{21} steroids to oestrogens by these tissues. The results from this study indicate that [^3H]progesterone can be converted to oestrogens. The amount of oestrogens synthesized from [^3H]progesterone by Day-16 conceptus tissues was similar to that reported by Gadsby (1978). The efficiency of conversion of [^3H]progesterone to oestrogens appears less than that for conversion of androgens to oestrogens (Gadsby, 1978). It is possible that: (1) enzymes required for conversion of progesterone to androgens are relatively inactive; (2) the Δ^5 pathway from pregnenolone to dehydroepiandrosterone to oestrogens is preferred; (3) progesterone may be converted to 5α - and/or 5β -reduced metabolites that are non-aromatizable; or (4) more time is required for additional enzymic steps. It is evident from this study, however, that [^3H]progesterone can be converted to oestrogens which are essential for the establishment and maintenance of pregnancy in pigs (Bazer & Thatcher, 1977).

The present results demonstrate that oestradiol production is begun by large (7 mm) spherical conceptuses and then oestrone and oestradiol are produced by tubular conceptuses in amounts greater than are produced by Day-12 filamentous conceptuses. These results are consistent with previous results of Geisert, Renegar, Thatcher, Roberts & Bazer (1982) which indicated that there was a transient increase in oestrogens in uterine flushings collected from gilts between Days 10.5 and 12 of gestation. It is during this period that oestrogen induces release of uterine epithelial cell secretory vesicle contents into the uterine lumen (Geisert *et al.*, 1982). There is also a transient increase in uterine blood flow (Ford & Christenson, 1979) and oestrogen-mediated events which inhibit secretion of luteolytic amounts of prostaglandin $\text{F-}2\alpha$ (Bazer & Thatcher, 1977). The lower production of oestrogens from [^3H]progesterone on Days 12 and 16 in this study is consistent with lower quantities of oestrogens in uterine flushings, as well as in maternal peripheral and utero-ovarian vein plasma (see Bazer, Geisert, Thatcher & Roberts, 1982). The mechanism(s) responsible for the rapid increase in oestrogen production by tubular conceptuses, decreased production between Days 12 and 16 and then increased oestrogen production between Days 16 and 25 of pregnancy is not known.

The hypothesis that endometrial tissue metabolizes progesterone to precursors for oestrogen production by the conceptus (Bazer *et al.*, 1982) was not substantiated in this study. Coincubations of endometrium and conceptus tissues consistently produced less oestrone and oestradiol from [^3H]progesterone than conceptus tissue alone. Endometrium does convert progesterone to 5α -reduced metabolites (Henricks & Tindal, 1971) which are not aromatizable. The 5α -reductase may, therefore, rapidly deplete the pool of progesterone and progesterone-metabolites available for conversion to oestrogens.

In this study, we proposed that treatment of non-pregnant gilts with oestradiol-valerate between Days 11 and 15 of the oestrous cycle activates the endometrial aromatase enzyme system. If true, the endometrium from pseudopregnant gilts would produce adequate oestrogens to allow maintenance of CL and pseudopregnancy. Evidence to support this hypothesis was not found. In fact, there was no evidence for conversion of [^3H]progesterone to oestrone and oestradiol by endometrium from pseudopregnant gilts. Comparison of the results from pseudopregnant and pregnant gilts suggests that the conceptus may influence metabolism of [^3H]progesterone to oestrone and

oestradiol by endometrium. It is possible that endometrial incubations, especially from Day 25 of pregnancy, were contaminated with some trophoctoderm (chorion). However, it seems unlikely that such contamination would be extensive enough to account for the substantial oestrone and oestradiol produced by endometrium from Days 16 and 25 of pregnancy.

An unidentified polar steroid was produced from [^3H]progesterone by all tissues that were incubated from pregnant gilts. It was found in the phenolic fraction after the Brown reaction (Brown, 1955), it eluted chromatographically near oestriol and was produced in large quantity. This unknown compound could be an isomer of the classic $16\alpha,17\beta$ -oestriol. It did migrate very close to $16\alpha,17\beta$ -oestriol and could be, for example, $16\beta,17\alpha$ -oestriol. Terqui (1978) reported that all four isomers of oestriol were in urine of pregnant ewes and that $16\alpha,17\beta$ -oestriol migrated closest to $16\beta,17\alpha$ -oestriol. It is also possible that the unknown compound is a catecholestrogen; however, the unknown compound is very stable while catecholestrogens are not stable.

The present results therefore provide the first indication of the exact developmental stage when oestrone and oestradiol production by conceptuses begins and indicate that conceptus and endometrial tissues can convert [^3H]progesterone to oestrone and oestradiol. Evidence was not found to indicate that the endometrium converts [^3H]progesterone to precursors which are readily aromatizable to oestrogens or that endometrium from oestradiol-valerate treated, pseudopregnant, gilts converts [^3H]progesterone to oestrone and oestradiol. The ability of both conceptus and pregnant endometrial tissue to convert progesterone to oestrogens may allow each tissue to affect events such as uterine secretory activity, uterine blood flow, nutrient transport, conceptus elongation and placentation which are critical for establishment and maintenance of pregnancy in pigs (see Bazer *et al.*, 1982).

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References

- Basha, S.M., Bazer, F.W., Geisert, R.D. & Roberts, R.M. (1980) Progesterone-induced uterine secretions in pigs. Recovery from pseudopregnant and unilaterally pregnant gilts. *J. Anim. Sci.* **50**, 113–123.
- Bazer, F.W. & Thatcher, W.W. (1977) Theory of maternal recognition of pregnancy in swine based on oestrogen controlled endocrine versus exocrine secretion of prostaglandin $\text{F}_{2\alpha}$ by the uterine endometrium. *Prostaglandins* **14**, 397–401.
- Bazer, F.W., Geisert, R.D., Thatcher, W.W. & Roberts, R.M. (1982) The establishment and maintenance of pregnancy. In *Control of Pig Reproduction*, pp. 227–252. Eds D. J. A. Cole & G. R. Foxcroft. Butterworth Scientific, London.
- Brown, J.B. (1955) A chemical method for determination of oestriol, oestrone and oestradiol in human urine. *Biochem. J.* **60**, 185–193.
- Fishman, J. (1982) Biochemical mechanism of aromatization. *Cancer Res. (Suppl.)* **42**, 3277s–3280s.
- Ford, S.P. & Christenson, R.K. (1979) Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. *Biol. Reprod.* **21**, 617–624.
- Frank, M., Bazer, F.W., Thatcher, W.W. & Wilcox, C.J. (1977) A study of prostaglandin $\text{F}_{2\alpha}$ as a luteolysin in swine: III. Effects of estradiol valerate on prostaglandin F , progestins, estrone and estradiol concentrations in utero-ovarian vein of nonpregnant gilts. *Prostaglandins* **14**, 1183–1196.
- Gadsby, J.E. (1978) *Oestrogen synthesis and steroid metabolism by blastocyst, embryonic and maternal tissues of the pig, cow and sheep during early pregnancy*. Ph.D. thesis, University of Cambridge.
- Gadsby, J.E., Heap, R.B. & Burton, R.D. (1980) Oestrogen production by blastocyst and early embryonic tissue of various species. *J. Reprod. Fert.* **60**, 409–417.
- Geisert, R.D., Renegar, R.H., Thatcher, W.W., Roberts, R.M. & Bazer, F.W. (1982) Establishment of pregnancy in the pig. I. Interrelationships between pre-implantation development of the pig blastocyst and uterine endometrial secretions. *Biol. Reprod.* **27**, 925–939.
- Henricks, D.M. & Tindal, D.J. (1971) Metabolism of progesterone-4- ^{14}C in porcine uterine endometrium. *Endocrinology* **89**, 920–924.
- Knight, J.W., Bazer, F.W., Thatcher, W.W., Franke,

- D.E. & Wallace, H.D.** (1977) Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. *J. Anim. Sci.* **44**, 620–637.
- Perry, J.S., Heap, R.B. & Amoroso, E.C.** (1973) Steroid hormone production by pig blastocysts. *Nature, Lond.* **245**, 45–47.
- Terqui, M.** (1978) *Contribution a l'étude des oestrogènes chez la brebis et la truie*. Thèse de doctorat detat et sciences naturelles, Le Université Paris VI.

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