Pre-partum changes in the plasma concentrations of progesterone, relaxin, prostaglandin F-2 α and 13,14-dihydro-15-keto prostaglandin F-2 α in meclofenamic acid-treated sows

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Summary. The pre-partum plasma concentrations of progesterone, relaxin, PGF-2 α and 13,14-dihydro-15-keto PGF-2 α (PGFM) were determined in 6 sows treated with meclofenamic acid (5.0 mg/kg body wt daily, from Day 109 to Day 113 of pregnancy), and 2 sows which farrowed prematurely. The inhibition of PG synthesis with meclofenamic acid did not prevent either the decline in plasma progesterone or the release of relaxin. Three distinct stages in the progressive changes of the above hormones were apparent before parturition: (1) the gradual decline in the concentrations of progesterone from about 7 days *pre partum* to reach <4.5 ng/ml at farrowing; (2) a surge in relaxin levels (>100 ng/ml) by 2 days *pre partum*; and (3) sharp increases in the concentrations of PGF-2 α and PGFM within 24 h of parturition. Maximal concentrations of PGF-2 α (>0.5 ng/ml) and PGFM (>20.0 ng/ml) occurred during farrowing. It is suggested that PG from an extra-ovarian source may not be responsible for initiating functional regression of the corpora lutea in the sow.

Introduction

There is considerable evidence that the prostaglandins (PG) play an important role in the control of initiation of parturition in the pig. PG concentrations remain low in the maternal circulation until the day of parturition when both PGF-2 α (Silver *et al.*, 1979) and its metabolite 13,14 dihydro-15-keto-PGF-2 α (PGFM) (Silver *et al.*, 1979; First & Bosc, 1979; Liptrap, 1980; Nara & First, 1981) increase 10–20-fold. The administration of PGF-2 α to late pregnant gilts and sows induces parturition within 30 h (see First & Bosc, 1979), and administration of PG synthesis inhibitors delays the onset of parturition in the sow (Nara & First, 1981; Gooneratne, Hartmann & Barker, 1982) until withdrawal of treatment.

Nara & First (1981) concluded that PGF-2 α was the natural luteolytic agent at parturition in the sow. However, the findings from studies using meclofenamic acid, a potent PG synthesis inhibitor (Flower, 1974), are consistent with a two-phase luteolysis in the sow—the first being independent of PG, the second requiring large amounts of PG (Gooneratne *et al.*, 1982). There is, in addition, no conclusive evidence in the pre-partum sow, that a rise in plasma concentrations of PG occurs before the changes in plasma progesterone or relaxin.

This study was undertaken to determine the relationship between progesterone, relaxin and PG in sows in which PG synthesis was temporarily inhibited in late pregnancy by the administration of meclofenamic acid, in order to clarify the hormonal mechanisms initiating parturition and lactation.

Materials and Methods

Animals and treatment

Landrace, Large White and Middle White cross-bred sows were housed under intensive husbandry conditions at Medina Pig Research Station, Western Australia. The mean ($\pm$ s.e.m.) gestation length of the sows at this piggery during the 3 preceding years was 113.31 ± 0.13 days ($n = 408$). Eight sows (98–105 days pregnant) were selected at random for surgical cannulation of the jugular vein and carotid artery (Heap *et al.*, 1981). The sows were brought into individual farrowing crates at least 3 days before surgery and maintained in the crates until weaning.

Meclofenamic acid was given orally (5.0 mg/kg body weight/day) to 6 sows (Nos 1–6). Treatment was from Day 109 to 113 of pregnancy with the morning and afternoon feeds (08:30 and 15:30 h). On Day 114 of pregnancy 200 μ g cloprostenol (Estrumate, ICI) was injected intramuscularly. Sows 7 and 8 did not receive any treatment because they farrowed prematurely.

Collection of plasma and mammary secretions

Plasma. Blood samples (20 ml) were collected daily through the indwelling cannulae from the day of surgery until 7 days *post partum*. Samples were collected at 4-h intervals after cloprostenol injection until 24 h after parturition; from the time of birth of the first piglet, blood was collected hourly for a period of 4 h. From Day 2 *post partum* daily sampling was resumed. Blood was collected simultaneously from the carotid artery and the jugular vein, placed into heparinized tubes and immediately plasma was separated from the cells and frozen at -20°C . Thereafter the samples to be assayed for prostaglandins and relaxin were stored at -80°C . Carotid artery samples were used for relaxin assay, and venous blood was used for progesterone and prostaglandin assays.

Mammary secretion. At farrowing 2–3 ml colostrum were expressed from the 4 anterior glands into separate sterile containers. Mammary secretion was collected from the same glands for 5 days *post partum*, and frozen at -20°C until analysis. From Day 2 *post partum* an i.v. injection of oxytocin (1 i.u. Pitocin; ICI) was required to induce milk let-down. Mammary secretion was collected at the same time as plasma.

Analysis of plasma

Progesterone assay. The radioimmunoassay used antibodies raised in rabbits against progesterone-11-succinyl-bovine serum albumin, as described by Martin, Cake & Hartmann (1980). All samples were extracted with 7 volumes of *n*-hexane and duplicate aliquants were assayed. Free and bound progesterone were separated with dextran-coated charcoal in 0.1% (w/v) gelatin. The intra- and inter-assay coefficients of variation were 5.5 and 8.4% respectively. The standards ranged from 0 to 500 pg, and a 5.0 pg amount was significantly different from zero.

[1,2- ^3H]Progesterone (sp. act. > 50 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, U.K., and the non-radioactive progesterone (grade A) was obtained from Calbiochem, Los Angeles, California, U.S.A.

Prostaglandin assays. The concentrations of PGF-2 α and PGFM in plasma were measured by radioimmunoassay as described by Maule Walker & Peaker (1980). [5,6,8,9,11,12,14,15,(n)- ^3H]-PGF-2 α and 13,14 dihydro-15-keto [5,6,8,11,12,14,(n)- ^3H]PGF-2 α were obtained from the

Radiochemical Centre. The plasma was extracted in peroxide-free diethyl ether and the residues were resuspended in tricine buffer containing 0.9% sodium chloride, 0.1% sodium azide and 0.1% gelatin, pH 8.0 for assay. Free and bound fractions were separated using dextran-coated charcoal. The intra- and inter-assay coefficients of variation were 8.4 and 12.5% for the PGF-2 α assay and 9.1 and 13.6% for the PGFM assay, respectively.

The sensitivity of each assay, estimated as the smallest mass of PG significantly different from zero standard binding, was 2.0 pg for PGF-2 α and 21.0 pg for PGFM. The specificity of the antisera and general validation of the assays have been described previously (Maule Walker & Peaker, 1980).

Relaxin assay. The radioimmunoassay was performed as described by Afele, Bryant-Greenwood, Chamley & Dax (1979). Relaxin was prepared from pregnant sow ovaries and radioiodinated (McMurtry, Kwok & Bryant-Greenwood, 1978). Separation of bound and free antibody was carried out by a conventional double-antibody procedure. The inter-assay coefficient of variation during a 6-month period for the lowest (26.0 pg) and highest (1250.0 pg) standards ranged between $44.0 \pm 8.9\%$ and $15.1 \pm 7.3\%$ respectively. The intra-assay coefficient of variation was 7.7%, and the quantitative sensitivity of the assay was 212.0 pg/ml. The specificity of the assay has been described previously (Bryant-Greenwood & Greenwood, 1979).

Analysis of mammary secretion

The concentration of lactose in milk was measured by the method of Kuhn & Lowenstein (1967). The milk protein content was estimated by the method described by Lowry, Rosebrough, Farr & Randall (1951). Milk fat was estimated as total esterified fatty acids (Hartmann, 1973), assuming the mean molecular weight of sow milk fatty acids to be 266 (De Man & Bowland, 1963). Na⁺ and K⁺ determinations were carried out on a Varian Techtron atomic absorption spectrophotometer model 1200 as described by Gooneratne, Hartmann, McCauley & Martin (1979).

Statistical analysis

All results were analysed statistically using a one-way analysis of variation and Student's *t* test (Snedecor & Cochran, 1978).

Results

Parturition was initiated in 4 sows (Sows 1–4) between 21.0 and 36.5 h after the withdrawal of meclofenamic acid administration, and between 4.0 and 20.5 h after PG injection. Sows 5 and 6 farrowed on Day 113 before the completion of meclofenamic acid treatment, while Sows 7 and 8 farrowed prematurely on Days 106 and 108 of pregnancy before meclofenamic acid treatment. The meclofenamic acid-treated sows (Sows 1–6) had 9.33 ± 0.2 (s.e.m.) piglets per litter and the mean $\pm$ s.e.m. birth weight of a piglet was 1.45 ± 0.04 kg. Sows 7 and 8 which farrowed prematurely had increased numbers of stillborn piglets: Sow 7 had only 3 live piglets of which none survived beyond 3 days *post partum*: 6 piglets of the same age were fostered onto her during lactation.

The changes in the plasma concentrations of progesterone, relaxin, PGF-2 α and PGFM for all the sows are presented in Text-figs 1 and 2. The concentration of progesterone varied between 4.8 and 10.5 ng/ml before meclofenamic acid treatment. A gradual decline in progesterone was observed during meclofenamic acid treatment (Text-fig. 1; Table 1). At parturition plasma progesterone dropped to low levels (< 2.7 ng/ml in 3 of the sows, < 4.0 ng/ml in the other). Relaxin levels were low before the administration of meclofenamic acid, but the concentration increased during treatment (Text-fig. 1) to reach maximum levels between 12 and 24 h before farrowing in all sows, thereafter declining. At parturition the concentration of relaxin had dropped to less than 24%

Text-fig. 1. The progressive changes in the peripheral plasma concentrations of progesterone (Δ), relaxin ($\blacktriangle$), PGF-2 α ($\bullet$) and PGFM ($\circ$) in Sows 1–4 treated with meclofenamic acid (5.0 mg/kg body wt/day, from Day 109 to 113 of pregnancy) and cloprostenol (200 μ g on Day 114 of pregnancy). Parturition = Day 0.

Table 1. The peripheral plasma concentrations of progesterone, relaxin, prostaglandin F-2 α and 13,14-dihydro-15-keto prostaglandin F-2 α in the maternal plasma before meclofenamic acid treatment (Day 109 of gestation) and at the end of treatment (Day 113)

Sow	Time of treatment	Progesterone (ng/ml)	Relaxin (ng/ml)	PGF-2 α (ng/ml)	PGFM (ng/ml)
1	Before	5.88	11.9	0.068	0.82
	After	3.27	434.7	0.048	1.97
2	Before	8.85	32.1	0.149	0.71
	After	6.99	73.7	0.090	1.01
3	Before	10.59	5.7	0.028	0.15
	After	8.34	40.4	0.058	1.52
4	Before	4.80	2.1	0.040	0.11
	After	0.98	172.7	0.044	1.46

of the peak values and low concentrations of plasma relaxin were observed by Day 1 *post partum*. PGF-2 α concentrations in maternal plasma remained low throughout meclofenamic acid administration except in Sow 3 in which there appeared to be an increase on Day 4 *pre partum*. However, PGFM increased 2–10-fold by the end of meclofenamic acid treatment. The concentrations of PGF-2 α and PGFM increased in the 24 h before parturition, maximum levels being

Text-fig. 2. The progressive changes in the peripheral plasma concentrations of progesterone (Δ), relaxin ($\blacktriangle$), PGF-2 α ($\bullet$) and PGFM ($\circ$) in (a) Sows 5 and 6 treated with meclofenamic acid (5.0 mg/kg body wt/day), from Day 109 of pregnancy until parturition on Day 113, and (b) Sows 7 and 8 which farrowed prematurely on Days 106 and 108 respectively. Parturition = Day 0.

reached within 12 h of the start of parturition, and decreased thereafter to low levels by Day 1 *post partum* (Text-fig. 1). Similar hormonal changes during the pre- and post-partum periods were observed in the 2 sows which farrowed prematurely (Text-fig. 2b).

The changes in the major constituents of mammary secretion (lactose, protein, fat, Na^+ , K^+ and Ca^{++}) were similar in all the sows (Text-fig. 3). The concentration of lactose increased by Day 1 *post partum* and continued to rise thereafter for the next 5 days. Milk protein concentrations dropped sharply from > 150 g/l at parturition to < 100 g/l on Day 1 *post partum*. The total fat content in the mammary secretion increased for 2–3 days after farrowing and then declined. The Na^+ in milk decreased after parturition while Ca^{++} increased. K^+ increased at first and then declined to steady levels.

Text-fig. 3. The progressive changes in the concentrations of constituents in the mammary secretion of 6 sows (Sows 1–6) treated with meclofenamic acid ($\bullet$) and 2 sows (Sows 7 and 8) farrowing prematurely ($\circ$).

Discussion

Parturition did not occur before withdrawal of meclofenamic acid treatment in 4 sows (Sows 1–4). The length of gestation was extended to 114.25 ± 0.23 days in these sows compared to the mean gestation length observed in the piggery (113.31 ± 0.13 days). In the two sows operated on at an earlier stage of gestation (Sows 7 and 8) parturition occurred before meclofenamic acid treatment was started. In Sows 5 and 6 parturition occurred during the meclofenamic acid treatment. These results suggest that surgery would normally precipitate premature parturition in the absence of meclofenamic acid treatment. The dose of meclofenamic acid used was sufficient to prolong gestation, in the absence of surgical interference, in an earlier experiment (Gooneratne *et al.*, 1982) but the result obtained for Sows 5 and 6 suggests that a larger dose of meclofenamic acid may be required to maintain pregnancy after surgery in late gestation.

The decline in the concentration of progesterone before parturition in sows is generally interpreted to reflect the termination of steroidogenesis in the corpus luteum and its functional luteolysis (First & Bosc, 1979). Furthermore, the pre-partum surge of relaxin is thought to be associated with the termination of luteal function (Sherwood, Nara, Crnekovic & First, 1979). The hormonal changes observed in the peripheral plasma of meclofenamic acid-treated sows in this study suggest that there are 3 distinct stages in the sequence of hormonal changes preceding parturition in the sow. The first stage is characterized by a decline in the concentration of plasma progesterone from 7 days *pre partum* (Text-figs 1 & 2); this decline in progesterone continued during meclofenamic acid treatment (Text-fig. 1; Gooneratne *et al.*, 1982). By 2 days *pre partum* relaxin was released into the peripheral circulation (stage 2) and this was also observed in meclofenamic acid-treated sows (Text-figs 1 and 2). The concurrence of the relaxin surge and a drop in progesterone levels was similar to that reported for normally farrowing sows (Sherwood, Chang, Bevier & Dziuk, 1975). Maximal concentrations of relaxin appeared in the plasma before any significant increase in the PG concentration (Text-figs 1 & 2). Coinciding with the drop in relaxin by 12 h *pre partum*, there was an increase in the concentration of PGF-2 α (stage 3) in the peripheral plasma (Text-figs 1 & 2). PG concentrations reached peak values at or after the start of parturition in all the sows (Text-figs 1 & 2). Indeed, in Sows 1–4 the peak PG levels were reached during the process of parturition (i.e. after the birth of 1 or more piglets) (Text-figs 1a, b, c), and may be as late as 12 h *post partum* (Text-fig. 1d).

The increase in the concentration of PGFM before the rise in PG levels may be a reflection of the slower rate of clearance of the metabolite compared to the parent compound, PGF-2 α . PGFM is not formed during collection of blood although PGF-2 α may be synthesized by platelets (Green, Bygdeman, Topozada & Wiquist, 1974). However, particular care was taken during sampling to ensure that cells were not damaged, and this is supported by the low basal values of PGF-2 α before and after parturition (Text-figs 1 & 2). Davis, Fleet, Harrison & Maule Walker (1980) demonstrated that the pulmonary metabolism of PGF-2 α in the sow was not as efficient as in the sheep; only 18% of injected PGF-2 α was metabolized during first passage through the lungs, and even after five circulations 19% of the injected PGF-2 α remained unchanged. Therefore, the concentrations measured are believed to reflect true endogenous values.

Surgical separation of the normal anatomical relationship between the ovary and uterus did not prevent natural regression of the corpora lutea in cyclic (Harrison & Heap, 1972; Torres & First, 1975) and late pregnant (Martin, Bevier & Dziuk, 1978) sows, and led to the belief that the message initiating luteolysis travelled from the uterus to the ovary through the systemic circulation. However, the findings from the present study, in which both stages 1 and 2 (decline in plasma progesterone and release of relaxin into the maternal circulation) occurred before any significant increase in PG concentration in the peripheral circulation, suggest that PGs from an extra-ovarian source may not be the 'natural' luteolytic agent at parturition as reported by Nara & First (1981). Nevertheless, the involvement of intraluteal PG in the initiation of luteal regression (Rothchild, 1981) cannot be discounted, as in-vitro studies have shown that the porcine corpus luteum is capable of synthesizing PGF-2 α (Patek & Watson, 1976; Guthrie, Rexroad & Bolt, 1978).

It is the third stage (release of PGF-2 α) which seems to override all other factors and initiate parturition. Regression of the corpora lutea by the injection of exogenous PGF-2 α seems to mimic this stage and abolish the previous two stages. PGs, together with relaxin (Zarrow, Neher, Sikes, Brennen & Bullard, 1956; Kertiles & Anderson, 1979), may cause cervical dilatation (Liggins, Fairclough, Grieves, Forster & Knox, 1977). The presence of elevated levels of PG during farrowing, and sometimes up to 10 h *post partum* (Text-fig. 1), is consistent with the belief that PGs play an important role in the expulsion of the fetuses. These observed pre-partum endocrine changes need to be confirmed in the normal parturient sow. However, it is remarkable that the sequence of endocrine changes during the pre-partum period is similar in all the sows whether parturition occurred prematurely (Text-fig. 2b), during meclofenamic acid treatment (Text-fig. 2a) or after the withdrawal of treatment (Text-figs 1 a-d).

Although both indomethacin (Nara & First, 1981; Taverne *et al.*, 1982) and meclofenamic acid (Gooneratne *et al.*, 1982) can cause a delay in parturition, indomethacin prevented the decline in progesterone and a rise in relaxin concentrations during treatment, but meclofenamic acid in the present study did not. The reasons for these differences remain unknown. However, Flower (1974) has emphasized the differences in the specificity, potency and required dosages between the various PG synthetase inhibitors, and Barden, Bergeron & Betteridge (1976) have shown that the fenamates inhibit both LH-RH-dependent cyclic AMP release and LH secretion, whereas indomethacin does not. It would be interesting to determine the changes in prolactin concentrations in meclofenamic acid-treated sows because a close relationship between the concentrations of relaxin and prolactin is apparent before parturition (Taverne *et al.*, 1982).

Lactation was initiated at parturition in all the sows (treated and untreated), and the changes in the amounts of the major constituents (Text-fig. 3) are in agreement with those previously reported for normal sows (Gooneratne *et al.*, 1979). The influence of the endocrine changes in late pregnancy on lactogenesis is not well understood. Both parturition and lactogenesis are closely associated in time, and therefore it is probable that the hormones which control the start of parturition also control the initiation of lactation (Fulkerson, 1979). The increase in the concentration of lactose in mammary secretion was observed after the pre-partum decline in plasma progesterone and relaxin values (Text-figs 1 & 2), but further studies are required to ascertain the role of the pre-partum endocrine changes in the initiation of lactation in the sow.

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References

- Afele, S., Bryant-Greenwood, G.D., Chamley, W.A. & Dax, E.M. (1979) Plasma relaxin immunoactivity in the pig at parturition and during nuzzling and suckling. *J. Reprod. Fert.* **56**, 451-457.
- Barden, N., Bergeron, L. & Betteridge, A. (1976) Effects of prostaglandin synthetase inhibitors and prostaglandin precursors on anterior pituitary cyclic AMP and hormone secretion. *Adv. Prostaglandin Thromboxane Res.* **1**, 341-344.
- Bryant-Greenwood, G.D. & Greenwood, F.C. (1979) Specificity of radioimmunoassays for relaxin. *J. Endocr.* **81**, 239-247.
- Davis, A.J., Fleet, I.R., Harrison, F.A. & Maule Walker, F.M. (1980) Pulmonary metabolism of prostaglandin F2 α in the conscious non-pregnant ewe and sow. *J. Physiol., Lond.* **301**, 86P.
- De Man, J.M. & Bowland, J.P. (1963) Fatty acid composition of sows colostrum, milk and body fat as determined by gas liquid chromatography. *J. Dairy Res.* **30**, 339-343.
- First, N.L. & Bosc, M.J. (1979) Proposed mechanisms controlling parturition and the induction of parturition in swine. *J. Anim. Sci.* **48**, 1407-1421.
- Flower, R.J. (1974) Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* **26**, 33-67.
- Fulkerson, W.J. (1979) Hormonal control of lactation. *Ann. Res. Rev.* **1**, 22-35.
- Gooneratne, A., Hartmann, P.E., McCauley, I. & Martin, C.E. (1979) Control of parturition in the sow using progesterone and prostaglandin. *Aust. J. Biol. Sci.* **32**, 587-595.

- Gooneratne, A.D., Hartmann, P.E. & Barker, I. (1982) The influence of meclofenamic acid on the initiation of parturition and lactation in the sow. *J. Reprod. Fert.* **65**, 157–162.
- Green, K., Bygdeman, M., Topozada, M. & Wqvist, N. (1974) The role of prostaglandin F_{2α} in human parturition. *Am. J. Obstet. Gynec.* **120**, 25–31.
- Guthrie, H.D., Rexroad, C.R., Jr & Bolt, D.J. (1978) *In vitro* synthesis of progesterone and prostaglandin F by luteal tissue and prostaglandin F by endometrial tissue from the pig. *Prostaglandins* **16**, 433–440.
- Harrison, F.A. & Heap, R.B. (1972) Ovarian activity in a pig after autotransplantation of an ovary. *J. Physiol., Lond.* **226**, 39.
- Hartmann, P.E. (1973) Changes in the composition and yield of mammary secretion of cows during the initiation of lactation. *J. Endocr.* **59**, 231–247.
- Heap, R.B., Flint, A.P.F., Hartmann, P.E., Gadsby, J.E., Staples, L.D., Ackland, N. & Hamon, M. (1981) Oestrogen production in early pregnancy. *J. Endocr.* **89**, 77P–94P.
- Kertiles, L.P. & Anderson, L.L. (1979) Effect of relaxin on cervical dilatation, parturition and lactation in the pig. *Biol. Reprod.* **21**, 57–68.
- Kuhn, N.J. & Lowenstein, J.M. (1967) Lactogenesis in the rat: changes in metabolic parameters at parturition. *Biochem. J.* **105**, 995–1002.
- Liggins, G.C., Fairclough, R.J., Grieves, C.S., Forster, C.S. & Knox, B.S. (1977) Parturition in the sheep. In *The Fetus and Birth* (Ciba Fdn Symp. No. 47), pp. 5–25. Eds J. Knight & M. O'Connor. Elsevier, Amsterdam.
- Liptrap, R.M. (1980) Prostaglandin F_{2α} and progesterone in experimental hypogalactia in sows. *Res. Vet. Sci.* **29**, 240–247.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Martin, C.E., Cake, M.H. & Hartmann, P.E. (1980) Corticosteroids and progesterone in amniotic fluid and maternal plasma and their relation to parturition. *J. devl Physiol.* **2**, 1–9.
- Martin, P.A., Bevier, G.W. & Dziuk, P.J. (1978) The effects of disconnecting the uterus and ovary on the length of gestation in the pig. *Biol. Reprod.* **18**, 428–433.
- Maule Walker, F.M. & Peaker, M. (1980) Local production of prostaglandins in relation to mammary function at the onset of lactation in the goat. *J. Physiol., Lond.* **309**, 65–79.
- McMurtry, P.J., Kwok, S.M. & Bryant-Greenwood, G.D. (1978) Target tissues for relaxin identified *in vitro* with ¹²⁵I-labelled porcine relaxin. *J. Reprod. Fert.* **53**, 209–216.
- Nara, B.S. & First, N.L. (1981) Effect of indomethacin and prostaglandin F_{2α} on parturition in the sow. *J. Anim. Sci.* **52**, 1360–1370.
- Patek, C.E. & Watson, J. (1976) Prostaglandin F and progesterone secretion by porcine endometrium and corpora lutea *in vitro*. *Prostaglandins* **12**, 97–111.
- Rothchild, I. (1981) The regulation of the mammalian corpus luteum. *Recent Prog. Horm. Res.* **37**, 183–283.
- Sherwood, O.D., Chang, C.C., Bevier, G.W. & Dziuk, P.J. (1975) Radioimmunoassay of plasma relaxin levels throughout pregnancy and parturition in the pig. *Endocrinology* **97**, 834–837.
- Sherwood, O.D., Nara, B.S., Crnekovic, V.E. & First, N.L. (1979) Relaxin concentrations in pig plasma after the administration of indomethacin and prostaglandin F_{2α} during late pregnancy. *Endocrinology* **104**, 1716–1721.
- Silver, M., Barnes, R.J., Comline, R.S., Fowden, A.L., Clover, L. & Mitchell, M.D. (1979) Prostaglandins in the foetal pig and prepartum endocrine changes in mother and foetus. *Anim. Reprod. Sci.* **2**, 305–322.
- Snedecor, G.W. & Cochran, W.G. (1978) *Statistical Methods*, 6th edn. Iowa State University Press, Ames.
- Taverne, M., Bevers, M., Bradshaw, J.M.C., Dieleman, S.J., Willemse, A.H. & Porter, D.G. (1982) Plasma concentrations of prolactin, progesterone, relaxin and oestradiol-17β in sows treated with progesterone, bromocriptine or indomethacin during late pregnancy. *J. Reprod. Fert.* **65**, 85–96.
- Torres, C.A.A. & First, N.L. (1975) The effect of surgical separation of uterus and ovaries on estrous cycle length in swine. *J. Anim. Sci.* **40**, 905–910.
- Zarrow, M.X., Neher, G.M., Sikes, D., Brennen, D.M. & Bullard, J.F. (1956) Dilatation of the uterine cervix of the sow following treatment with relaxin. *Am. J. Obstet. Gynec.* **72**, 260–265.

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