Plasma concentrations of prolactin, progesterone, relaxin and oestradiol-17β in sows treated with progesterone, bromocriptine or indomethacin during late pregnancy

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Summary. Pregnant gilts (3/group) were given no treatment, 10 mg bromocriptine twice daily by mouth, from Day 111 of pregnancy to 1 day *post partum*, 25 mg progesterone s.c. at 6-h intervals from Days 111 to 116 inclusive or 400 mg indomethacin by mouth at 6-h intervals from Day 111 to 116 inclusive. Before spontaneous delivery maternal plasma prolactin and relaxin concentrations started to rise almost simultaneously between 57 and 47 h before the first piglet and both hormones reached peak values when the plasma progesterone concentration had started to decline rapidly (∼21–23 h). Suppression of prolactin levels by bromo-criptine prevented the onset of lactation completely but had no obvious influence on changes of the other hormone concentrations and the course of parturition. Pro-gesterone treatment delayed the onset of expulsion of the piglets but did not delay the simultaneous increase in prolactin and relaxin concentrations. These changes in hormone levels were prevented by indomethacin treatment but occurred essentially unchanged when the treatment was ended. The results support the concept that parturition in the pig is preceded by a biphasic increase of plasma prostaglandin levels.

Introduction

Parturition in the pig is preceded by changes in peripheral plasma concentrations of hormones originating from the ovaries (progesterone, relaxin), the placentas (oestrogens) and the maternal hypophysis (prolactin; reviews by Ellendorff *et al.*, 1979b; First & Bosc, 1979; Taverne, 1979). A decline in progesterone concentrations (Robertson & King, 1974; Ash & Heap, 1975), caused by the regression of the corpora lutea, seems to play an essential role in the onset of parturition because progesterone treatment after ovariectomy prevents premature delivery of piglets (First & Staigmiller, 1973), and induction of additional corpora lutea on Days 103 or 107 of pregnancy prolongs gestation (Bosc, du Mesnil du Buisson & Locatelli, 1974; Martin, Norton & Dziuk, 1977). Because both the pre-partum increase of the relaxin concentrations (Sherwood, Nara, Crnekovic & First, 1979) and the increase of the prolactin concentrations (Taverne, Willemse, Dieleman & Bevers, 1979) coincide with the final decrease of progesterone concentrations, it is possible that these hormonal changes are interdependent.

Experiments involving progesterone, indomethacin and prostaglandin treatment during late

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pregnancy in intact sows and data from sows with altered utero-ovarian relationships demonstrate that a common stimulus for luteolysis and relaxin release, in which prostaglandins are involved, must reach the ovaries by a systemic route (Sherwood, Chang, Bevier & Dziuk, 1975; Sherwood, Chang, Bevier, Diehl & Dziuk, 1976; Sherwood, Martin, Chang & Dziuk, 1977; Sherwood, Wilson, Edgerton & Chang, 1978; Sherwood et al., 1979). Release of prolactin from the hypophysis might be triggered by the same stimulus (Taverne, 1979).

The present study reports experiments designed to investigate (1) the sequence of changes in the plasma concentrations of prolactin, relaxin, progesterone and oestradiol-17β before spontaneous parturition, (2) whether an increase of maternal plasma prolactin concentration is an essential feature of the hormonal changes that lead to parturition, and (3) to what extent the increase of prolactin release before parturition is controlled by circulating progesterone levels.

Materials and Methods

Animals and experimental protocol

Twelve pregnant gilts (Dutch Landrace and Large White; 140–180 kg body weight) were used. They were each provided with an indwelling Silastic (Dow Corning) catheter in the external jugular vein, at least 5 days before the start of the experiment. After surgery the animals were placed in single farrowing cages. Antibiotics (1 g chloramphenicol) were given in the food (a standard pig chow) twice daily throughout the experiment. The gilts were randomly assigned to one of the four experimental groups (N = 3 for each group). The animals in Group A received no treatment and were controls. In Group B each gilt was treated with bromocriptine (Parlodel: Sandoz, Basel, Switzerland) twice daily by mouth from Day 111 to the day of parturition inclusive (Day 1 = day of mating). In Group C the gilts received 25 mg progesterone in oil s.c. (Progestine: Organon, Oss, The Netherlands) at 6-h intervals from Day 111 to Day 116 inclusive. Group D gilts received 400 mg indomethacin (Sigma, St Louis, Missouri, U.S.A.) by mouth at 6-h intervals from Days 111 to 116 inclusive.

From Day 110 of pregnancy (= start of the experiments), blood samples (10 ml) were taken at 6-h intervals (05:00, 11:00, 17:00 and 23:00 h) immediately before each experimental treatment. Blood sampling stopped within 24 h after completion of parturition. The duration of labour and the numbers of live and stillborn piglets were noted.

Blood samples were centrifuged immediately and the plasma was stored at −20°C in two separate portions (one for the relaxin determination, the other for prolactin, progesterone and oestradiol-17β determination).

Hormone analysis

Prolactin. Plasma prolactin concentrations were measured by a specific, sensitive and homologous double-antibody radioimmunoassay, using a highly purified porcine prolactin preparation (PRL A-7). Details of the purification of prolactin, the antibody production and the assay procedure are given by Bevers, Willemse & Kruip (1978) and can be summarized as follows: antibody, raised in rabbits to the prolactin A-7 preparation, was used in a dilution of 1 : 10 000, which resulted in about 50% binding of the radioiodinated (according to Greenwood, Hunter & Glover (1963) with slight modifications) PRL A-7. A standard curve of 0·1–5·0 ng PRL A-7 in triplicate was employed routinely in each assay. Plasma samples were assayed at three different dilutions. Samples with a relative binding of <10% or >90% or samples which differed by more than 10% from the average of the 3 determinations were discarded and reassayed. The sensitivity of the assay was 0·4 ng/ml plasma. The intra-assay coefficient of variation was 1·8% for the intercept 46·1% bound, 5·3% at 12·8% relative binding and 3·1% at 88·1% relative binding ($n = 10$). The interassay coefficient of variation, assessed by replicate

measurement of the same plasma sample within separate assays, was 15·1% ($n = 20$). The recovery percentage of 10–50 ng PRL A-7, added to the plasma from sows in the post-weaning period, was 99·12 ± 5·47% (mean ± s.d., $n = 50$). Cross-reaction with porcine LH, FSH, ACTH and GH was <0·01%, while the cross-reaction with the SP 162C (NIAMDD) porcine prolactin preparation was 36%.

Relaxin. The radioimmunoassay was performed essentially by the method of Bryant-Greenwood (1977). Porcine relaxin was prepared from frozen ovaries of pregnant sows by extraction and purification according to the method of Sherwood & O'Byrne (1974). Highly purified relaxin (CMy) was eluted from CM-cellulose at a position close to that of the CM-B relaxin of Sherwood & O'Byrne (1974). It has an activity of 2000–3000 G.P.U./mg in the mouse pubic symphysis bioassay of Steinetz *et al.* (1960).

The antiserum used was raised by injecting 1 mg partly pure (G2) relaxin intradermally into a male rabbit in saline/Freund's complete adjuvant. This was followed by further 0·5 mg injections (i.m. in 0·9%, w/v, NaCl) at 2–3-week intervals for 2 months. Several months later 3 booster injections were given at fortnightly intervals and the rabbit was bled out 1 month after the last injection under pentobarbitone sodium anaesthesia. The serum was stored in 1 ml sealed ampoules and at 1/50 dilution in aliquots of assay buffer, all at −20°C. The final dilution used in the assay was between 1:30 000 and 1:50 000 depending on the radioactive relaxin preparation.

The CMy relaxin was iodinated by the method of Bolton & Hunter (1973) as modified for relaxin by Kwok, McMurtry & Bryant (1976): 1 μg *N*-succinimidyl 3-(hydroxyphenyl) propionate (Fluka Chemicals, Buchs, Switzerland) was reacted with 25 μg CMy relaxin in 20 μl 0·1 M-borate buffer, pH 8·5, in ice for 40–45 min. The mixture was iodinated with 1·5 mCi ^{125}I (I.M.S. 30: Radiochemical Centre, Amersham, U.K.) by the chloramine T method. The reaction mixture was diluted with KI solution, transferred to a Sephadex G25 column (18·5 × 1·5 cm) equilibrated with 0·05 M-phosphate buffer, pH 7·5, containing 10 mg bovine serum albumin (BSA, Sigma)/ml and eluted with the same buffer. Two peaks of radioactivity were obtained. The most active 2–3 tubes of the first peak were pooled, diluted 10 times in 0·05 M-barbitone buffer, pH 8·5, containing 10 mg BSA/ml and stored in the refrigerator or deep freeze in aliquots. Since the iodinated low molecular weight byproducts of the reaction sequence bind to BSA (Bolton & Hunter, 1973) the ^{125}I-labelled relaxin solution was purified further 1–2 days before use. Some (1·5 ml) of the ^{125}I-labelled relaxin solution was layered onto a 20 × 1·0 cm column of Sephadex G50 superfine equilibrated with 0·05 M-barbitone buffer, pH 8·5, containing 10 mg BSA/ml, and eluted with the same buffer. Three peaks were obtained of which the middle peak (equivalent to the G2-relaxin position) was highly pure ^{125}I-labelled relaxin. This peak was diluted further as required for use in the radioimmunoassay. The specific activities of the iodinated relaxin were in the range of 5–10 μCi/μg.

The assay buffer was 0·05 M-sodium barbitone, pH 8·5, containing 50% inactivated horse serum (Wellcome Reagents, London, U.K.) to minimize differences in protein concentration between some samples. Plasma samples were diluted into an equal volume of barbitone buffer as their first dilution. They were assayed in triplicate at 4 or 6 different dilutions. Solutions of standard and unknowns (0·2 ml) were incubated with 0·05 ml antiserum diluted in barbitone buffer for 22–24 h at 4°C. Then 0·05 ml ^{125}I-labelled relaxin solution was added to each tube and the incubation was continued for a further 22–24 h at 4°C. Bound and free ^{125}I-labelled relaxin were separated by double-antibody precipitation using an anti-rabbit γ-globulin antiserum raised in sheep: 0·05 ml normal rabbit serum (Wellcome Reagents; 1:80 dilution) and 0·1 ml double-antibody solution (1:20 dilution), both in barbitone buffer, were added to each assay tube. Incubation was continued for 16–18 h when all tubes were centrifuged for 40 min at 2000 *g*, 4°C. In each assay 6–12 tubes were run with no sample but with the usual amount of antiserum to determine maximum binding (45%) and 6–12 tubes were run with no sample and with 0·05 ml barbitone buffer instead of antiserum to determine the assay blank, which was

2–3%. A standard curve of 15–1800 pg CMy relaxin per tube in triplicate was employed routinely in each assay. Cross-reactivity was 100% with CMa′ relaxin (18–28 AAE-CMa′ kindly donated by Dr B. G. Steinetz), 20% with NIH relaxin standard, Lot 0147 (442 G.P.U./ mg), <0·02% with porcine prolactin A-7 and <0·01% with porcine insulin (Sigma Chemicals). Parallelism was shown between CMy relaxin, the CMa′ relaxin of Steinetz, NIH relaxin (442 G.P.U./mg, Lot 0147) standard and sow plasma samples. No immunoassayable relaxin was detected in the plasma of boars or ovariectomized sows. Recovery of CMy relaxin added to boar plasma was 99·6 ± 1·4% ($n = 4$).

The sensitivity of the assay was 30–80 pg/tube. It was estimated from the standard curve, assuming that differences in percentage binding within 3 standard deviations of the maximum binding were not significant. The interassay coefficient of variation, determined by measuring a sample from a pool of pregnant sow plasma in several assays, was 11·2% ($n = 8$). The intra-assay coefficient of variation, determined from replicate samples measured in the same assay, was 5·6% at 74·2% relative binding; 4·2% at 54·2% relative binding and 9·8% at 26·7% relative binding ($n = 10$).

Steroids. Progesterone levels were estimated by radioimmunoassay similar to the method described by Dieleman & Schoenmakers (1979). The antiserum S74 B12 against an 11α-hydroxyprogesterone-hemisuccinate–BSA conjugate was raised in sheep. Oestradiol-17β levels were estimated by a radioimmunoassay largely similar to the procedure for oestrone as described by Dieleman & Schoenmakers (1979). The antiserum OR-500 against an oestradiol-17β-6-keto-oxime–BSA conjugate was raised in sheep and generously supplied by Dr R. J. Scaramuzzi (Scaramuzzi, Corker, Young & Baird, 1975). Fresh diethyl ether (Merck, Darmstadt, West Germany) and *n*-hexane 96% (Baker Chemicals, The Netherlands) were used for the extraction of oestradiol-17β and progesterone respectively. Steroids were purchased from Steraloids Inc. (Wilton, New Hampshire, U.S.A.). All other chemicals were of analytical reagent grade.

The [1,2,6,7(n)-³H]progesterone (sp. act. 90 Ci/mmol) and [2,4,6,7(n)-³H]oestradiol-17β (sp. act. 104 Ci/mmol) used as tracers (10 000 d.p.m.) in the radioimmunoassay were obtained from the Radiochemical Centre, Amersham, U.K. The tracers were subjected to routine checks for purity by thin-layer or gas chromatography with radio detection and, if necessary, purified by gel filtration on Sephadex LH20, according to the method described by Mikhail, Wu, Ferin & Van de Wiele (1970). A dextran T70-coated charcoal procedure was applied for separating bound and free fractions.

The specificity of the antisera was expressed as the percentage cross-reaction. For the progesterone assay the main cross-reactions were: 1·35% for 17α-hydroxyprogesterone, 1·90% for 20β-dihydroprogesterone, 77·5% for 11α-hydroxyprogesterone, 23·8% for 11β-hydroxyprogesterone, 0·52% for pregnenolone, 2·27% for 5α-pregnane-3β-ol,20-one, 0·81% for corticosterone, and <0·5% for other steroids tested (Dieleman & Schoenmakers, 1979). For the oestradiol-17β assay cross-reactions of 2·4%, 0·22% and 0·19% were found for oestrone, oestradiol-17α and oestriol respectively and <0·1% for other steroids. The intra- and inter-assay coefficients of variation were 11·0 and 12·2% respectively for the progesterone assay and 8·0 and 9·6% respectively for the oestradiol-17β assay.

Analysis of data

Because of the differences in absolute values of the individual hormone concentrations in the 3 animals of each group, it was decided not to analyse these data statistically. The individual measurements are presented in Text-fig. 1 and the main results are summarized in Table 2. The start of the first prolactin increase, the start of the relaxin increase and the start of the final progesterone decrease (expressed in hours before the birth of the first piglet) are defined as the time of the first sample out of a series of at least 4 consecutive samples with increased, increased and decreased hormone concentrations respectively. Differences between means of intervals were tested with Student's *t* test.

Results

The results are illustrated in Tables 1 and 2 and Text-fig. 1.

Group A

Hormonal changes relative to the moment of expulsion of the first piglet were very similar in all 3 sows (Text-fig. 1a). From basal levels of <10 ng/ml, prolactin and relaxin concentrations started to increase between 57 and 47 h before the first piglet, reaching peak values at the same time, i.e. 21–23 h before the first piglet. At about this time the progesterone concentration, which
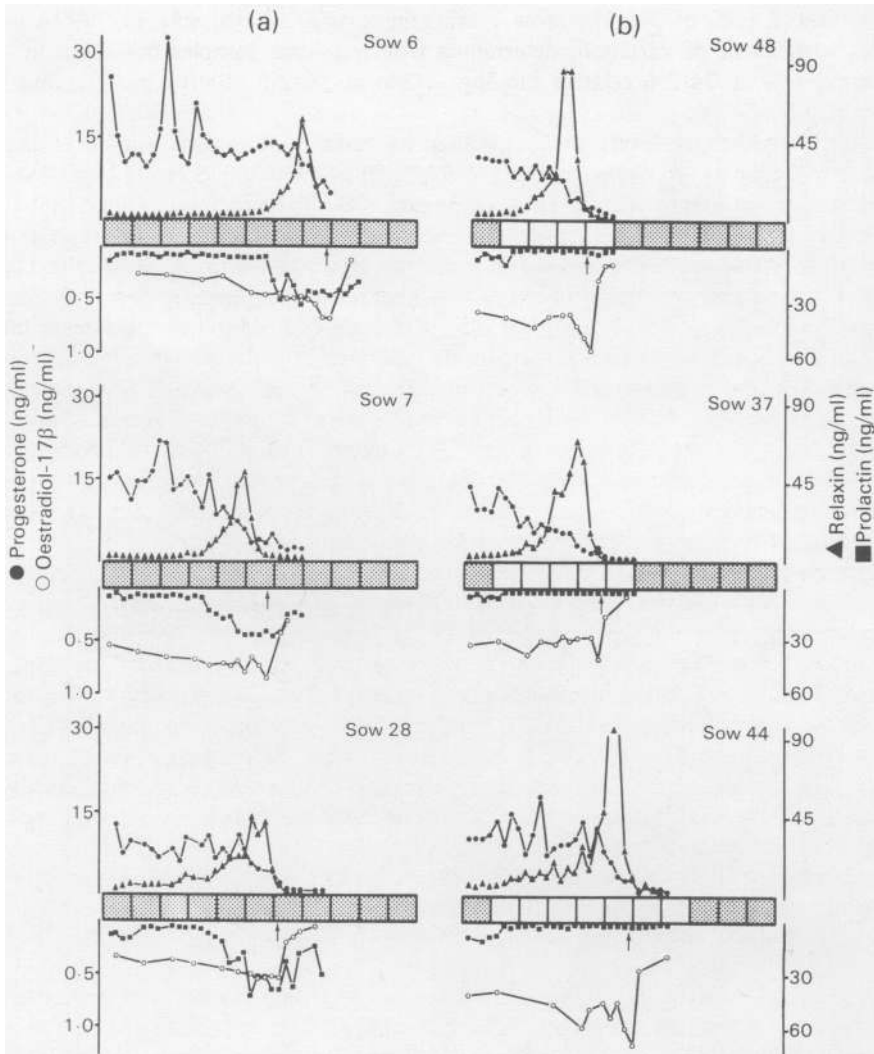
Text-fig. 1. Plasma concentration of prolactin, relaxin, progesterone and oestradiol-17β during late pregnancy and parturition in (a) control sows and sows treated with (b) bromocriptine, (c) progesterone and (d) indomethacin. The days of treatment are indicated by the white squares of the horizontal bars. The first (shaded) square is the start of the experiment at Day 110 of pregnancy. The arrows indicate the time of expulsion of the first piglet. Note the different scale of the progesterone concentrations in the progesterone-treated animals (c).
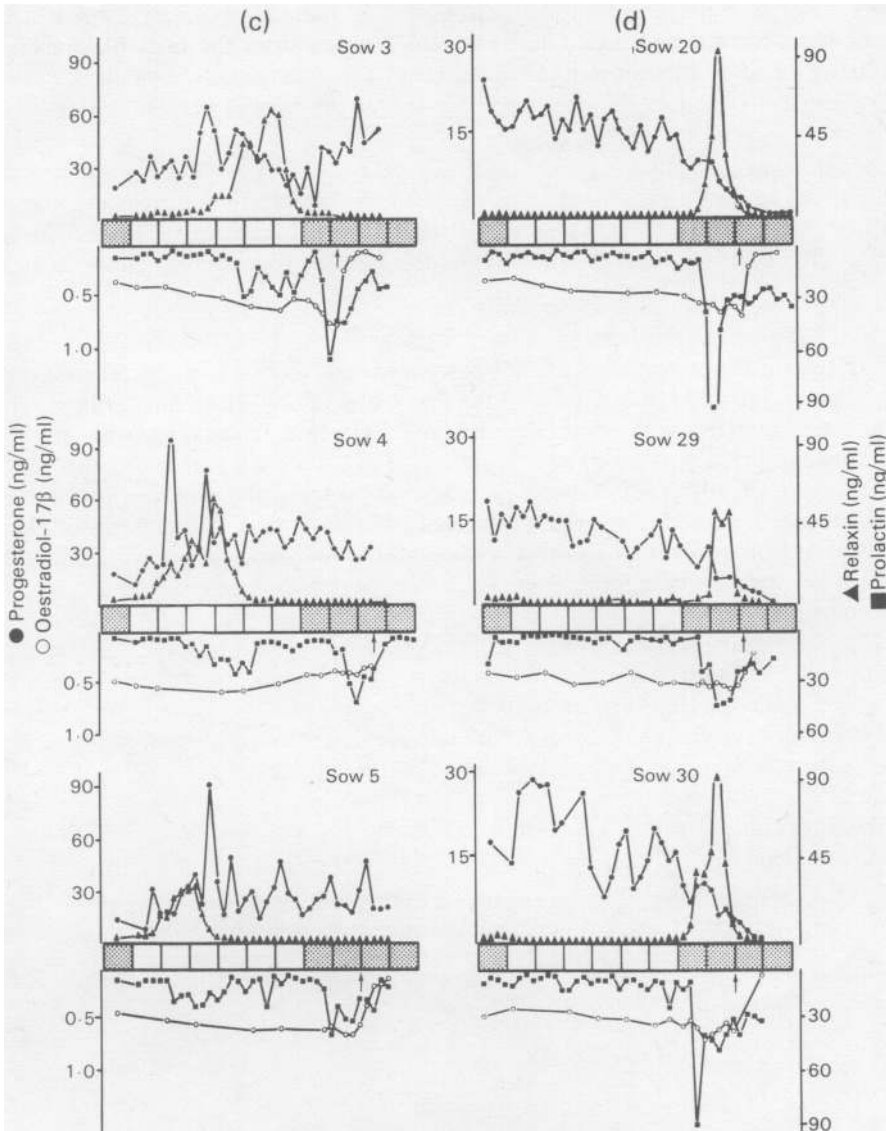
Text-fig. 1—continued.

had shown marked fluctuations hitherto, started to decline. Oestradiol-17β concentrations gradually increased during the last week of gestation, reaching peak values of 0·58–0·90 ng/ml shortly before or during the expulsive stage of parturition.

Group B

Treatment twice daily with bromocriptine reduced peripheral plasma prolactin concentrations to values below 2 ng/ml in all 3 animals (Text-fig. 1b). In 2 of the 3 animals (Nos 48 and 37) parturition seemed to be advanced by the treatment (Table 1). The final drop in progesterone concentrations and the changes in relaxin levels occurred at times, before the expulsion of the first piglet, which were comparable with those of the control animals. Oestradiol concentrations were within the same range as those of the control animals.

Lactogenesis was prevented by the treatment. The normal swelling and pink flush of the udder was not observed and milk could not be expressed from the teats of any of the sows before, during or after expulsion of the piglets and the placentas. Nevertheless, the course of parturition was not obviously different from that seen in the control sows in Group A and only 2 of the 28 piglets were stillborn (7·1%).

Group C

Progesterone treatment postponed the time of delivery of the first piglet (Table 1). The mean interval between the start of the experiments at 00:00 h on Day 110 and the birth of the first piglet (214 h, N = 3) was significantly different ($P < 0.025$) from that of the control animals (158 h, N = 3, Table 1). In contrast, the start of the prolactin and relaxin increases, and the time of the peak relaxin concentration, were not postponed, and there was an increase in the interval between these events and the expulsion of the first piglet (Table 2). In all 3 animals changes in prolactin concentrations were biphasic: an initial increase, coinciding with the period of increasing relaxin titres, was followed by a decrease at a time when relaxin levels had reached basal levels again (Text-fig. 1c). A second sustained increase of the prolactin concentration was measured between 6 and 25 h before the first piglet. Plasma progesterone concentrations rose to high levels during progesterone treatment. Values >30 ng/ml were frequently measured but there were large variations in titres in each of the 3 animals. Progesterone concentrations, although variable, remained elevated even after the last progesterone injection on Day 116 of pregnancy at 23:00 h. Around the time of delivery of the first piglet plasma progesterone concentrations measured 31·2, 32·0 and 28·8 ng/ml in Sows 3, 4 and 5 respectively. In Sows 3 and 5 plasma oestradiol-17β concentrations continued to rise during and after progesterone treatment, reaching maximal values at about the time of expulsion of the first piglet. However, in Sow 4, the oestradiol concentration was maximal on Day 114 of pregnancy (i.e. on the 4th day of the progesterone treatment) and thereafter gradually declined, reaching a value of 0·34 ng/ml when the experiment was ended to prepare the sow for laparotomy.

Table 1. Details relating to parturition in sows receiving various treatments (see text)

		Expulsion of first piglet		
Sow	Treatment	Day*	Hours†	Litter
6	None	117	190	11 living
7		115	140	9 living, 1 dead
28		116	146	11 living
48	Bromocriptine	114	99	8 living, 1 dead
37		114	116	7 living, 1 dead
44		115	142	13 living
3	Progesterone	118	197 (30)	9 living
4		119‡	229‡ (62)	8 dead
5		118	216 (49)	5 living, 4 dead
20	Indomethacin	119	221 (54)	8 living
29		119	218 (51)	8 living
30		118	216 (49)	10 living

* Day 1 = day of mating.
† 0 h = start of the experiment at the beginning of Day 110 (00:00 h). The interval (h) between the last treatment on Day 116 and the moment of expulsion of the first piglet is indicated in parentheses.
‡ The start of preparation of the sow for a laparotomy (see text).

Table 2. Summary of hormonal changes in sows at about parturition

	Prolactin		Relaxin		Progesterone
Sow	Start of increase	Peak value	Start of increase	Peak value	Start of final decrease
6	143 (−47)	167 (−23)	137 (−53)	167 (−23)	167 (−23)
7	89 (−51)	119 (−21)	89 (−51)	119 (−21)	107 (−33)
28	89 (−57)	125 (−21)	89 (−57)	125 (−21)	119 (−27)
48	—	—	41 (−58)	77 (−22)	83 (−16)
37	—	—	53 (−63)	95 (−21)	95 (−21)
44	—	—	41 (−101)	125 (−17)	125 (−17)
3	113 (−84)	191 (−6)	89 (−108)	143 (−54)	—
4*	71 (−158)	215 (−14)	47 (−182)	96 (−133)	—
5	59 (−157)	191 (−25)	41 (−175)	77 (−139)	—
20	191 (−30)	197 (−24)	185 (−36)	203 (−18)	203 (−18)
29	185 (−33)	197 (−21)	191 (−27)	197 (−21)	197 (−21)
30	185 (−31)	185 (−31)	179 (−37)	203 (−13)	203 (−13)

Values indicate the time (in hours) after the start of the experiment at Day 110 (00:00 h). The parenthetic values give the time (in hours) before the expulsion of the first piglet.

* The time that the sow was prepared for a laparotomy was taken as the time of expulsion of the first piglet.

The course of parturition was different in each of the 3 sows of this group. Sow 3 delivered 9 live piglets within 3 h. Parturient behaviour and milk ejection were first observed in Sow 4 at Day 117 but delivery of piglets did not occur despite mucous discharge from the vulva and straining movements observed during Day 119. At laparotomy on Day 119, 9 dead piglets were found in the uterus with three placentas still attached to the uterine wall. In Sow 5, 6 piglets (5 alive and 1 dead) were delivered within 2·5 h but another 3 piglets were born dead 3–6 h after the 6th piglet.

Group D

Indomethacin treatment postponed delivery of the first piglet; the mean interval between the start of the experiment and the birth of the first piglet (218 h, N = 3) was significantly different ($P < 0.01$) from that of the control Group A animals (Table 1). In addition the start of the increase of the prolactin concentrations was significantly postponed (187 h, N = 3, for Group D gilts compared with 107 h for the control animals; $P < 0.005$). The same was true for the onset of the relaxin increase (185 and 105 h, $P < 0.005$). Consequently, peak values of prolactin and relaxin concentrations (Text-fig. 1d) occurred at times before the birth of the first piglets which were not significantly different from those of the control animals. Also, the final drop of the progesterone concentration occurred within the same time period before the first piglet as found in Group A controls. The delivery of the piglets was completed within 3–5 h in all 3 animals and none of the piglets was born dead.

Discussion

Four major conclusions can be drawn from the above results. (1) Before spontaneous parturition in the sow maternal plasma prolactin and relaxin concentrations start to rise at about the same time and reach peak values by the time that plasma progesterone concentrations start

to decline rapidly. (2) The pre-partum increase in prolactin concentrations does not seem to be an essential feature of the hormonal changes that induce parturition although the normal onset of lactation was prevented by decreasing the maternal plasma prolactin concentrations. (3) Progesterone treatment delays the onset of expulsion of the piglets but not the concurrent increase in prolactin and relaxin concentrations. (4) These pre-partum changes in the plasma prolactin, relaxin and progesterone concentrations are prevented by indomethacin treatment, but occur apparently unchanged after the treatment is ended.

Although an increase of the prolactin concentration immediately before parturition has been reported before (van Landeghem & van de Wiel, 1978; Taverne *et al.*, 1979; Smith & Wagner, 1980; Dusza & Krzymowska, 1981), the present study indicates that this increase is not initiated by a decrease of the peripheral plasma progesterone concentration. On the contrary, the early onset of luteolysis in 2 of the animals that were treated with bromocriptine suggests that prolactin might be involved in the maintenance of luteal function during late gestation. An increasing density of prolactin binding sites in the luteal cells has been reported for the first half of pregnancy in the sow (Rolland, Gunsalus & Hammond, 1976) and injections of bovine prolactin maintained luteal function (weight and progesterone content of corpora lutea) in hypophysectomized sows between Days 70 and 80 of gestation (du Mesnil du Buisson & Denamur, 1969). However, Cook, Kaltenbach, Norton & Nalbandov (1967) found that LH, but not porcine prolactin or FSH, stimulated steroid synthesis in porcine corpora lutea *in vitro*. Therefore the evidence for a role of prolactin in luteal maintenance during pregnancy in the sow is inconclusive.

The relationship of normal changes in relaxin and progesterone concentrations to the moment of expulsion of the first piglet in the bromocriptine-treated sows together with the lack of effect of the drug on parturition is consistent with data from the cow (Schams, 1974) and sheep (Burd, Ascherman, Dowers, Scommegna & Auletta, 1978) that increasing prolactin levels are not an essential component of the mechanism of parturition. Our data appeared to be the first to indicate that reduction of the prolactin concentrations before parturition impedes the onset of lactation, although suppression of an already established lactation with bromocriptine has been reported before in sows (Smith & Wagner, 1980). Lactation in pigs was also impeded when pigs were given porcine relaxin during an extended period in late pregnancy (Kertiles & Anderson, 1979) and when premature parturition was induced by ovariectomy (Nara, Darmadja & First, 1981). It would be interesting to determine the changes in prolactin concentration in these experimental conditions.

The relaxin data in our study confirm the results of Sherwood *et al.* (1975, 1978, 1979) who used similar experimental protocols, although in our study the peak values of relaxin occurred about 6 h earlier and appeared to be somewhat lower. Although a common stimulus may evoke the prolactin and relaxin spikes, they are independent because abolition of the prolactin spike with bromocriptine did not prevent the occurrence of a relaxin spike. Conversely, in the sows treated with progesterone there was a second prolactin spike after cessation of treatment which was not accompanied by a release of relaxin. The significance of the pre-partum relaxin spike is not clear although involvement in the softening of the cervix (Kertiles & Anderson, 1979) and in myometrial co-ordination (Downing, Bradshaw & Porter, 1980) suggest that it may be important in ensuring efficient and safe deliveries.

In several studies in which pregnancy was prolonged by exogenous progesterone (Nellor, Daniels, Hoefer, Wildt & Dukelow, 1975; Sherwood *et al.*, 1978) or endogenous progesterone (Bosc *et al.*, 1974) milk appeared in the teats at about the normal expected time of parturition, and may have been due to a rise in the plasma prolactin concentration, as observed during progesterone treatment in this study.

The persistence of high progesterone concentrations in the blood of sows after cessation of progesterone treatment was an unexpected finding in this study. One explanation is that pro-gesterone treatment induces the production of steroid metabolites that persist in the plasma for

several days after the end of treatment and cross-react with the antiserum of our radioimmuno-assay. The most likely candidates for such cross-reactivity appear to be 11α- and 11β-hydroxy-progesterone and 5α- and 5β-pregnane-3,20 dione and we cannot as yet eliminate this possibility. However, the need for such metabolites to persist for at least 85 h after the last progesterone injection and for at least 33 h after delivery make it unlikely. Another possibility is that sub-cutaneous injections of 25 mg progesterone in oil establish depots of progesterone which are only depleted very gradually. The rather fluctuating levels of progesterone in treated sows may reflect variations in the vascularization and hence absorption rates at the injection sites. Sig-nificantly elevated plasma progesterone concentrations have also been reported even after cessation of intramuscular injections of progesterone during late pregnancy (Gooneratne, Hartmann, McCauley & Martin, 1979). If the latter explanation is true the high progesterone levels measured during parturition in animals from Group C suggest that progesterone may not block myometrial activity in the sow (Taverne, 1982) and its role in parturition in the pig is as problematic as it is in the ewe (Liggins, Fairclough, Grieves, Forster & Knox, 1977).

The results obtained from the progesterone-treated animals provide additional evidence that the pre-partum prolactin spike is not dependent upon a changing progesterone/oestrogen ratio. Indeed, both prolactin and relaxin release seemed to be triggered by a common stimulus rather than by the withdrawal of an inhibitor. The observation that final changes in the concentration of the two hormones occurred only after indomethacin treatment was ended indicates that prosta-glandins are at least involved in the triggering process. A single injection of PGF-2α (Taverne *et al.*, 1979) or its synthetic analogue, cloprostenol (Taverne, 1979), cause an immediate release of prolactin in the late pregnant sow well before the luteolytic action of the drug becomes evident.

The results of this study are consistent with the reports that parturition in the sow is preceded by a biphasic increase of prostaglandin levels (Nara & First, 1977; Martin, 1980). The first small rise might be responsible for the simultaneous increase of the prolactin and relaxin concen-trations, whereas luteolysis requires a somewhat longer period of increased PGF-2α release. The latter has been reported during luteolysis in the cyclic sow (Moeljono *et al.*, 1977; Shille *et al.*, 1979). A second and larger increase of PGF-2α concentrations, close to the onset of delivery (Silver *et al.*, 1979; First & Bosc, 1979; Martin, 1980), might also be responsible both for the sustained rise of the prolactin values and the onset of uterine contractions either by direct stimulation of the myometrium (Taverne, 1982) and/or by provoking oxytocin release (Ellendorff *et al.*, 1979a).

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