Indomethacin blocks pre-partum nest building behaviour in the pig (*Sus scrofa*): effects on plasma prostaglandin F metabolite, oxytocin, cortisol and progesterone

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Abstract

In the pig, nest building occurs in the day preceding parturition (gestation=114–116 days). Nest building behaviour can be induced in pregnant, pseudopregnant and cyclic female pigs following injection of prostaglandin F_{2α}. Here we investigated behaviour and endocrine changes after the administration of indomethacin, which inhibits cyclo-oxygenase enzymes and thus prostaglandin synthesis.

In experiment 1, pregnant primiparous pigs (gilts) were blood sampled through jugular vein catheters every 20 min from 1000 h on day 113 of pregnancy and behaviour was recorded until birth. Two hours after pre-partum nest building began, animals received 4 mg/kg indomethacin ($n=7$) or control vehicle ($n=8$) intramuscularly. Indomethacin-treated animals showed less nest building than controls between 1 and 5 h after injection ($P<0.05$), during which time they were mostly inactive and lay down for longer than controls. From 5 h before birth until birth there was no significant treatment difference in nest

building behaviour. There was a tendency for the start of birth to be delayed in indomethacin-treated animals. Plasma 13,14-dihydro-15-keto-prostaglandin F_{2α} (a major metabolite of prostaglandin F_{2α}) rose during pre-injection nest building and then fell following indomethacin treatment, but was not significantly different between groups when behaviour differed. Plasma oxytocin, cortisol and progesterone were not significantly affected by treatment. In experiment 2, indomethacin-treated non-pregnant gilts ($n=7$) did not show any changes in activity or posture compared with vehicle-treated controls ($n=6$) between 90 and 150 min after treatment.

These results suggested that indomethacin treatment reversibly and specifically inhibits porcine pre-partum nest building by a mechanism that may involve endogenous prostaglandin F_{2α} synthesis inhibition but is independent of circulating oxytocin, cortisol and progesterone concentrations.

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Introduction

One to two days before giving birth, domestic pigs build a maternal nest (Arey 1992). Behaviours that constitute nest building in semi-natural environments include wandering to locate a secluded nest site, digging a hollow with paws and snout, gathering vegetation and arranging the bedding in the hollow (Jensen 1986, 1989). Elements of these behaviours are also seen in pre-partum sows housed in pens, and in space-restricting commercial environments such as farrowing crates (Castren *et al.* 1993, Cronin *et al.* 1993, Boulton *et al.* 1997a, Burne *et al.* 1999). The apparently strong motivation to build a maternal nest whatever the surroundings has led to suggestions that this behaviour results from internal (hormonal) factors with feedback from external factors (such as environment) superimposed on some components (Jensen 1993, Jensen

et al. 1993, Haskell & Hutson 1996). Internal factors are likely to be linked to the endocrinology of parturition induction, since the two events have a close temporal association.

Prolonged high levels of peripheral oestradiol and progesterone increase nest building behaviour in rabbits, and circulating prolactin also exerts an influence (González-Mariscal *et al.* 1996). However, manipulation of peripheral concentrations of neither oestradiol (Burne *et al.* 1999) nor prolactin (Boulton *et al.* 1998) affect nest building in the pig. The pre-partum rise in oxytocin (OT) secretion has been linked to the end of porcine nest building behaviour, which occurs 1–6 h before birth (Castren *et al.* 1993).

Prostaglandin (PG) F_{2α} has been proposed as a natural luteolytic agent in the pig (Nara & First 1981) and injections of PGF_{2α} and its analogues have been frequently used to control the time of parturition in pigs

through early and controlled induction of luteolysis (e.g. Gooneratne *et al.* 1979, Dial *et al.* 1987). PGF_{2α} treatment also results in behavioural changes, reported as similar to pre-partum nest building in late pregnant (Widowski & Curtis 1989), pseudopregnant (Boulton *et al.* 1997a) and post-parturient sows (Blackshaw 1983). Other behaviours including ataxia and an increase in scratching have been recorded (Blackshaw & Blackshaw 1982, Burne *et al.* 1999, Gilbert *et al.* 2000). Manufacturers of synthetic PGF_{2α} describe restlessness as one of a number of transient side-effects associated with its commercial application in pre-partum pigs (Pharmacia & Upjohn 2000).

The initial step in PG synthesis (the conversion of arachidonic acid to PGG₂) is catalysed by the enzyme cyclo-oxygenase (Shimizu & Wolfe 1990) which occurs in two isoforms (COX 1 and COX 2). COX 1, the constitutive form, is widely distributed in the body and COX 2, the inducible form, is also found in many tissues, including placenta (Mitchell *et al.* 1995), brain neurones (Yamagata *et al.* 1993) and porcine choroid plexus (Vellucci & Parrott 1998). Plasma concentrations of PGF_{2α} measured as the major metabolite 13,14-dihydro-15-keto-PGF_{2α} (PGFM) rise dramatically in the day before parturition in the pig (Watts *et al.* 1988, Whately *et al.* 1990). Porcine endometrial (Uzumcu *et al.* 1998) and placental (Burchard *et al.* 1992) tissues have been shown to secrete PGF_{2α}. Unlike sheep, in which 99% of circulating PGF_{2α} is removed by a single pass through the lungs, in the pig only 18% is removed (Davis *et al.* 1979). Permeability of the blood-brain barrier of the rat to PGs has been demonstrated (Eguchi *et al.* 1992). Given the behavioural changes that follow exogenous PGF_{2α} injection, it is possible that endogenous PGF_{2α} (or a metabolite) acts on the brain to initiate the behavioural changes necessary for nest building to occur at the correct time in relation to parturition.

In these experiments we used indomethacin to inhibit the activity of both COX isoforms (Frölich 1997). In experiment 1, we report behavioural and endocrine responses of pre-partum nest building animals following indomethacin treatment. In experiment 2, we studied the behaviour of indomethacin-treated, non-pregnant, non-nest building female pigs to examine the specificity of the changes to nest building observed in experiment 1. Preliminary data from experiment 1 on OT secretion immediately before birth have been reported elsewhere (Gilbert 1999).

Materials and Methods

Experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, using Large White primiparous pigs (gilts) from the Babraham Institute herd.

Experiment 1

Animal housing The experimental area was six concrete-floored pens (2.6 × 2.2 m) arranged on both sides of a corridor in a heated, fan-ventilated room with thermostatic control set to 20 °C. Overhead strip lighting was on throughout the experiment. Pens were cleaned and supplied with 5 kg clean wheat straw, and animals were fed 3–4 kg of a standard commercial sow diet daily between 0800 h and 0900 h with water available *ad libitum*.

Synchronisation of pregnancy Oestrous cycles were synchronised in three batches of eight animals using 18 days oral treatment with 20 mg/day altrenogest (Regumate porcine; Hoescht UK Ltd, Milton Keynes, Bucks, UK). At the ensuing oestrus, animals were mated with a fertile Large White boar or artificially inseminated on 2 consecutive days (first day of mating = day 0 of gestation). A gap of 8 weeks occurred between each batch. Six gilts from each batch were then selected for the experiment, one of which was later found to be not pregnant, reducing that batch to five individuals. Animals from within each batch were randomly assigned to treatment group, balanced for each batch. On the expected date of parturition the gilts' age was 429 ± 5.3 days (means ± S.E.M.).

Implantation of jugular catheters The day of surgery ranged from 8 to 12 days before expected parturition (means ± S.E.M. = 106.1 ± 0.3 days of pregnancy). Food was withheld for 24 h before induction of anaesthesia (Gilbert *et al.* 1994) and surgical placement of catheters (Burne *et al.* 1999). Catheter patency and sterility were maintained as described (Burne *et al.* 1999). On the next day animals were moved to their experimental housing.

Experimental plan and measurements of behaviour

Video cameras (Panasonic WV-PB100; Fineplan Video Services, Harrow, Middx, UK) were placed above each pen and connected to monitors in an adjacent laboratory, where behaviour was observed and recorded onto videotapes for later analysis using time-lapse video recorders (Hitachi VT-L1000E set to record ten pictures/s) from 1000 h on day 113 until parturition. In addition, gilt behaviours were scored every 10 min continually by eye from this time to determine the start of nest building, defined as when one or more relevant behaviours (Table 1) were observed in eight or more 10-min blocks within any 2-h period. Once these criteria were satisfied for each animal, local anaesthetic cream (EMLA; Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK) was applied to an area of the gilt's neck. Fifteen minutes later, gilts were injected intramuscularly at this site with 4 mg/kg indomethacin (Sigma Ltd, Poole, Dorset, UK) as a suspension in 10 ml 0.9% NaCl containing 10% (v/v) ethanol

Table 1 Behavioural ethogram used to define measured behaviours

Behaviour	Activity
Stand	Standing on all four legs
Sit	Sitting upright with two front legs on the ground
Lie on belly	Lying with belly concealed
Lie on side	Lying with belly exposed
Carry*	Grasping straw in the mouth without chewing and raising the head with or without locomotion
Bite/chew	Material in mouth chewed and/or eaten
Circle*	Turning through 360° within a 2-min period
Paw fixture*	Raking movement of either foreleg at the walls or fixtures within the pen
Paw floor*	Raking movement of either foreleg at straw/floor
Root fixture	Oro-nasal contact with the walls or fixtures within the pen and head movement
Root floor*	Oro-nasal contact with straw/floor and head movement
Drink	Oro-nasal contact with drinker
Defaecate/urinate	Either voiding behaviour
Object scratch	Scratch or rub any part of the body against another object
Leg scratch	Scratch, or attempt to scratch, any part of the body with the rear leg
Shake head	Vigorous sideways movement of the head

*These behaviours were used to determine the time of onset of nest building and hence individual injection times.

(IND; $n=7$) or with vehicle alone (CON; $n=8$). (Two animals from the original 17 began to nest build before 1400 on day 113 and were excluded.)

Video recordings were analysed from 4 h pre-injection until the birth of the first piglet, using a computerised event recorder (Noldus 1991). Behaviours measured are shown in Table 1. Behaviour was scored using one-zero scoring (i.e. occurrence vs non-occurrence) within 2-min time bins. The percentage of time bins/20 min in which a behaviour occurred was determined and averaged over each hour. The timing of birth onset was also recorded.

Blood sampling At 1400 h on day 113, a 5 m length of vinyl tubing (internal diameter 1.4 mm, outside diameter 1.9 mm; Dural Plastics, Auburn, NSW, Australia) was fitted to each jugular catheter. These extensions were taken via leads hanging from the roof to the room's central passageway, where a system of counterweights held the tubing clear of the animals without impeding movement. Blood samples (4 ml) were taken into heparinised tubes every 20 min from 1400 h on day 113 until one sample following the birth of the first piglet. Samples were taken and the catheter filled with 50 IU/ml heparin sodium in 0.9% NaCl from outside the animal pens, causing minimal disturbance. Blood was centrifuged immediately (3000 *g* at 4 °C for 10 min) and plasma stored at −20 °C for OT,

cortisol and progesterone assay, and at −20 °C initially and then −80 °C for PGFM. Each 1 ml aliquot of plasma stored for OT assay was first acidified with 10 µl 10 M acetic acid.

Radioimmunoassays (RIA) All samples were assayed for PGFM and OT. Cortisol and progesterone were measured in every third sample (i.e. hourly). Detection limits were calculated using mean c.p.m. minus 1 s.d. from the zero standard correlated dose value interpolated from the curve using Packard counter software.

PGFM The assay was based on the method supplied by Sigma Immuno Chemicals (Poole, Dorset, UK) with their antiserum product number D-3417. The 13,14-dihydro-15-keto-[³H]PGF2α was obtained from Amersham International plc, Amersham, Bucks, UK (code TRK 517 – 6.88 TBq/mmol). A 200 µl mixture of diluted anti-PGFM antiserum and labelled PGFM (10 000 c.p.m. – 0.16 KBq) in 0.01 M phosphate-buffered saline (pH 7.4), containing 0.01% sodium azide was added to each duplicate 50 µl plasma sample and incubated overnight at 4 °C. Free and bound counts were separated using 0.5% dextran-coated charcoal. Standard quantities of PGFM (0.39–100 nmol/l) were made up in unhaemolysed female pig double charcoal-stripped plasma and run simultaneously with the samples. Validations of the assay included comparing logit transformed dose–response curves for standards made up in buffer, gilt unhaemolysed double charcoal-stripped plasma and gilt unstripped, unhaemolysed plasma from pseudopregnant gilts containing no detectable PGFM. All curves showed good parallelism. The detection limit of the assay was 0.1 nmol/l and the inter- and intra-assay coefficients of variation were 9.05% and 6.52% respectively. The cross-reactivities were taken as given on the product sheet for the antisera: PGFM, 100%; prostaglandin F1α, <0.02%; dihydro-prostaglandin F1α, 1.5%; ketoprostaglandin F1α, 23%; dihydroketoprostaglandin F1α, 83%; prostaglandin B₂, <0.02%; dihydroprostaglandin E₂, 0.04%; prostaglandin F2α, 0.02%; dihydroprostaglandin F2α, 1.3%; keto-prostaglandin F2α, 4.4%; ketoprostaglandin H₂, 0.15%.

Oxytocin Extraction from plasma using C₁₈ SepPak Cartridges (Waters Chromatography, Milford, MA, USA) and the RIA were carried out as described (Thornton *et al.* 1986, Gilbert *et al.* 1994, Boulton *et al.* 1997b) using an antiserum previously described (Sheldrick & Flint 1981). The extraction recovery was 87%. The intra- and inter-assay coefficients of variation were 3.14 and 10.62% respectively. The detection limit for the assay was 1.39 pmol/l.

Cortisol Measurements used an RIA previously described (Parrott & Goode 1992) with changes for use in the pig (Boulton *et al.* 1997a). The detection limit was

0.88 nmol/l, and intra- and interassay coefficients of variations were 5.3 and 6.5% respectively.

Progesterone The ELISA used has recently been described (Boulton *et al.* 1997b). The detection limit was 1.81 nmol/l. Intra- and interassay coefficients of variation were 3.56 and 11.05% respectively.

Experiment 2

Animals and housing Gilts of 28 months of age and weighing 120–131 kg were selected and housed in two social groups of ten in open-sided pens (8 × 4.7 m with half of the pen on straw bedding and half as a concrete-floored run). When the gilts showed signs of oestrus, six from the first group and eight from the second were selected for use in the trial and the remaining animals were removed from the pens. They were kept under artificial light from fluorescent strip lights (0700–1800 h) in addition to natural daylight, and the temperature varied between 10 and 25 °C. Throughout the duration of the experiment all animals were fed 3 kg of a standard commercial sow diet daily and water was available *ad libitum*. Pens were cleaned out daily at 0800–0830 h.

Experimental plan On the test day (day 5 after oestrus) half of the animals in each group ($n=7$ in total) were given 4 mg/kg IND (Indocid; Merck Sharp & Dohme Ltd, Harlow, Herts, UK), which was given by mouth hidden within foodstuff (tinned cat food), at 1000 h. The remaining animals were given cat food only. All animals were observed closely to determine the presence of any behavioural side-effects immediately after treatment and on the following day. In addition, from 1.5 h after administration, 1 h of behaviour was systematically recorded (one-zero scan sampling every min) simultaneously on animals within their social group and home pen using the ethogram shown in Table 1. One gilt from the control group was seen to be lame on the test day and data from this animal were not used in the analysis.

Statistical analyses

Behaviour Treatment effects in experiments 1 and 2 were analysed using Mann–Whitney U tests on pooled hourly data. For longitudinal effects in experiment 1, the following time points were selected: –4 to –3 h (control); –1 to 0 h (pre-injection nesting); 3 to 4 h (post-injection behavioural change) and 3–4 h before birth. Within treatment between time point differences were analysed using a Friedman (F_r) test (Siegel & Castellan 1988). Pre-treatment time effects in experiment 1 were analysed using a Friedman test on pooled data. Significant differences ($P<0.05$) were further analysed with post hoc Wilcoxon matched-pairs signed-ranks test.

Plasma hormone concentrations relative to injection

Mean PGFM ($\log_{10}$ transformed to remove heterogeneity of variance) and OT concentrations were blocked

into four periods: –8 to –4 h (control); –2 to 0 h (pre-injection nesting); 0 to 2 h (post-treatment nest building) and 2–4 h (post-treatment when behaviour differed). These periods are longer than their equivalents for behaviour since fewer measurements per hour were available from hormone assays. Treatment effects were compared using unpaired t -tests with Welch's correction. Time effects were compared using a repeated measures ANOVA followed by Tukey's multiple comparison test on pairs of data sets. In addition, since the 2-h period before test injection was defined by an increase in nest building, we determined whether this was related to endocrine change. Mean measurements pooled between treatments for PGFM and OT 8–4 h before injection were compared with those 2–0 h before injection for each individual using paired t -tests.

Pre-treatment differences between treatment groups were present in plasma concentrations for cortisol and progesterone ($P<0.05$, two-tailed t -test on averaged pre-treatment data). To test for treatment differences in plasma concentrations when behavioural differences occurred, mean values 2–4 h post-treatment were first subtracted from mean 8–4 h pre-treatment values for each individual before comparison using two-tailed unpaired t -tests with Welch's correction.

To examine relationships between nest building behaviour and plasma hormone concentrations, regression analysis was used. The behaviours termed root floor, paw floor, circle and carry (see Table 1) were combined into a nest building score. A score of 0 or 1 was given, depending on whether any of these behaviours occurred in a 2-min time bin. Scores were averaged over the 20-min period before and, separately, the 20-min period after withdrawal of hourly blood samples from 6 h pre-injection until 6 h post-injection. These scores were then regressed separately (response variables) on the different hormone concentrations (explanatory variables) measured in the hourly blood samples (Genstat 5 1997).

Events relative to birth Gestation, time from injection to birth ($\log_{10}$ transformed to remove heterogeneity of variance) and litter size were compared using t -tests with Welch's correction. Owing to possible confounding effects between treatment and the variable time to birth, treatment effects between cortisol and progesterone relative to birth were not analysed. However, the raw data for OT and PGFM plasma concentrations showed clear pre-partum increases, which were analysed by fitting bicubic splines (mathematical best lines of fit) to the data for each animal separately. Times at which fitted concentrations reached 4 pmol/l above minimum fitted values were calculated, and taken to be the start of the pre-birth surge. Rise times were compared using t -tests with Welch's correction.

All numerical data reported in the results are means $\pm$ S.E.M.

Table 2 Mean ($\pm$ S.E.M.) scores for behaviour of vehicle (CON) and indomethacin (IND)-treated sows

Behaviour	3–4 h before injection	1–0 h before injection	3–4 h after injection	3–4 h before birth	F _r	P
CON (n=7)						
Stand	0.41 $\pm$ 0.11	0.72 $\pm$ 0.07	0.46 $\pm$ 0.10	0.31 $\pm$ 0.08	6.8	0.08†
Sit	0.07 $\pm$ 0.03	0.15 $\pm$ 0.04	0.10 $\pm$ 0.05	0.14 $\pm$ 0.03	3.2	0.4
Lie on belly	0.37 $\pm$ 0.12	0.22 $\pm$ 0.06	0.23 $\pm$ 0.08	0.27 $\pm$ 0.04	2.4	0.5
Lie on side	0.46 $\pm$ 0.14	0.41 $\pm$ 0.10	0.37 $\pm$ 0.08	0.75 $\pm$ 0.07	5.4	0.1
Carry	0.03 $\pm$ 0.03	0.03 $\pm$ 0.02	0.10 $\pm$ 0.10	0.02 $\pm$ 0.02	0.6	0.9
Paw floor	0.16 $\pm$ 0.06	0.37 $\pm$ 0.05	0.24 $\pm$ 0.06	0.20 $\pm$ 0.05	5.2	0.2
Root floor	0.36 $\pm$ 0.10	0.67 $\pm$ 0.08	0.42 $\pm$ 0.10	0.27 $\pm$ 0.08	6.8	0.08†
Paw fixture	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.0	1.0
Root fixture	0.12 $\pm$ 0.05	0.20 $\pm$ 0.08	0.15 $\pm$ 0.07	0.06 $\pm$ 0.05	1.4	0.7
Circle	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.4	0.7
Drink	0.07 $\pm$ 0.02 ^b	0.10 $\pm$ 0.02 ^b	0.08 $\pm$ 0.02 ^b	0.01 $\pm$ 0.01 ^a	10.0	0.02*
Leg scratch	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.3	0.9
Object scratch	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	3.0	0.4
Shake head	0.09 $\pm$ 0.03 ^a	0.17 $\pm$ 0.05 ^b	0.11 $\pm$ 0.05 ^{ab}	0.03 $\pm$ 0.01 ^a	7.8	0.05*
IND (n=7)						
Stand	0.36 $\pm$ 0.05 ^b	0.72 $\pm$ 0.08 ^c	0.10 $\pm$ 0.07 ^a	0.48 $\pm$ 0.14 ^{bc}	12.8	0.005*
Sit	0.12 $\pm$ 0.03 ^b	0.22 $\pm$ 0.02 ^c	0.01 $\pm$ 0.01 ^a	0.13 $\pm$ 0.02 ^{bc}	12.5	0.006*
Lie on belly	0.32 $\pm$ 0.07 ^{bc}	0.30 $\pm$ 0.07 ^b	0.06 $\pm$ 0.03 ^a	0.47 $\pm$ 0.11 ^c	11.1	0.01*
Lie on side	0.50 $\pm$ 0.06 ^a	0.35 $\pm$ 0.07 ^a	0.90 $\pm$ 0.07 ^b	0.74 $\pm$ 0.33 ^{ab}	7.6	0.05*
Carry	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.9	0.8
Paw floor	0.24 $\pm$ 0.04 ^{bc}	0.40 $\pm$ 0.09 ^c	0.05 $\pm$ 0.05 ^a	0.18 $\pm$ 0.06 ^b	12.7	0.005*
Root floor	0.35 $\pm$ 0.05 ^b	0.60 $\pm$ 0.09 ^c	0.08 $\pm$ 0.07 ^a	0.48 $\pm$ 0.15 ^{bc}	11.7	0.008*
Paw fixture	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.0	1.0
Root fixture	0.06 $\pm$ 0.03 ^a	0.18 $\pm$ 0.08 ^b	0.00 $\pm$ 0.00 ^a	0.01 $\pm$ 0.01 ^a	13.9	0.003*
Circle	0.02 $\pm$ 0.01	0.09 $\pm$ 0.07	0.02 $\pm$ 0.01	0.03 $\pm$ 0.02	2.2	0.5
Drink	0.09 $\pm$ 0.02 ^b	0.10 $\pm$ 0.03 ^b	0.01 $\pm$ 0.01 ^a	0.01 $\pm$ 0.01 ^a	12.4	0.006*
Leg scratch	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.3	0.9
Object scratch	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.0	1.0
Shake head	0.07 $\pm$ 0.02 ^{ab}	0.11 $\pm$ 0.03 ^b	0.02 $\pm$ 0.02 ^a	0.04 $\pm$ 0.02 ^{ab}	8.5	0.04*

The data are the mean proportion of observations in 1 h in which each behaviour had been observed in separate 2-min time bins, and were used for analysis of time-effects on behaviour using Friedman's test (F_r).

* $P < 0.05$, † $P < 0.1$, followed post-hoc by Wilcoxon matched-pairs signed-rank test (superscript letters within any row are significantly different, $P < 0.05$).

Results

Experiment 1

Behaviour relative to test injection All animals fulfilled the criteria used to define the onset of nest building behaviour. There was no significant difference between IND and CON treatment groups in the time at which criteria were fulfilled and injections given (114.9 ± 0.32 vs 115.0 ± 0.45 days of gestation for IND and CON respectively). Characteristic nest building behaviours included standing and pawing with the forepaws, predominantly at straw-covered areas of the floor. Animals also rooted with the snout and carried straw by mouth, depositing it in a chosen area in the pen. Circling behaviour around the chosen area was also seen and maternal nests took shape. Chewing or eating straw was rare.

Treatment effects No significant differences in behaviour were seen between treatment groups before treatment.

However, between 1 and 5 h post-treatment, IND animals rooted and pawed the floor less and lay down for longer than CON animals. Other behaviours did not differ between treatment groups at this time. After 5 h post-treatment, there were no significant treatment differences. Nest building in the IND group appeared to be restored (Fig. 1A, C and D).

Within group time effects For the two pre-injection periods (3–4 and 0–1 h pre-treatment), analysis of pooled IND and CON animal data showed that both paw floor ($P < 0.01$) and root floor behaviour ($P < 0.002$) were significantly greater during the latter period and circle behaviour showed a tendency to be greater ($P < 0.07$). No other behaviours were significantly different using this pooled analysis.

In the overall within group time analysis, all postures, paw floor, root floor and root fixture behaviour varied significantly with time in IND animals (Table 2). These

Figure 1 Behaviour of pregnant primiparous pigs treated with 4 mg/kg indomethacin (IND) (■) or vehicle (○) during pre-partum nest building. Animals were injected after the first 2-h pre-partum period during which nest building criteria were satisfied. Data in the left panels (A–D) are zeroed around injection time, and those in the right panels (E–H) are zeroed around birth. * $P < 0.05$, treatment difference in behaviour at time points shown (Mann–Whitney U test). For longitudinal (time) effects see Table 2.

behaviours were reduced at 3–4 h after injection, except for lie on side behaviour, which was increased relative to pre-injection values. CON animals did not show significant time effects for these behaviours, although root floor and stand behaviour showed time-effect tendencies. Both treatment groups showed significant time effects for drink and shake head behaviour (Table 2). No other significant time effects were seen.

Plasma hormone concentrations relative to test injection

Treatment effects OT and PGFM concentrations did not differ between treatment groups (Fig. 2A and B) either in the pre-treatment period or up to 4 h post-treatment. PGFM values (nmol/l) for IND and CON groups respectively were 1.54 ± 0.46 and 1.85 ± 1.03 (–8 to –4 h), 3.30 ± 0.88 and 9.72 ± 6.52 (–2 to 0 h), 6.32 ± 2.92 and 8.73 ± 5.54 (0 to 2 h) and 2.4 ± 1.06 and 10.19 ± 6.54 (2 to 4 h). Concentrations of cortisol and progesterone were significantly different between groups (all $P < 0.01$) before treatment (Fig. 2C and D). However, post-treatment plasma concentrations, corrected by subtraction from baseline values, were not significantly affected by treatment.

Regression analysis showed no significant relationships between any hormones measured and nest building scores determined in either the 20 min before or after blood samples were withdrawn.

Time effects Pooled (for treatment) plasma PGFM concentrations rose during the 2-h pre-injection phase, when nest building criteria were satisfied (3.53 ± 1.07 vs 13.67 ± 6.91 nmol/l for –8 to –4 and –2 to 0 h respectively, $P < 0.05$; Fig. 2A), whereas other hormones measured did not (Fig. 2B–D). Repeated measures ANOVA on within group time effects for PGFM was significant for IND ($P < 0.01$) but not CON animals. IND plasma concentrations were increased between –8 to –4 h and –2 to 2 h. Subsequently, concentrations between 2 to 4 h declined (Fig. 2A). No significant time effects were seen for OT.

Birth effects There was a non-significant tendency for time from injection to birth of the first piglet to be delayed in IND animals (CON: 10.43 ± 1.73 h; IND: 20.91 ± 6.51 h; $P = 0.09$ on $\log_{10}$ transformed data). There was no significant difference between groups in gestation length (CON: 115.3 ± 0.51 days; IND: 115.7 ± 0.29 days, $P = 0.46$). No consistent treatment effects in behaviour relative to birth occurred, although IND animals showed greater rooting at birth –9 h and pawing at birth –6 h than CON (Fig. 1 G and H). No treatment differences in other behaviours were observed.

Plasma concentrations for PGFM and OT, zeroed around birth, are shown in Fig. 3. There were no differences between treatment groups in the 8-h period before birth in either OT or PGFM concentrations.

Figure 2 Plasma hormone concentrations in pregnant primiparous pigs zeroed around injection of either 4 mg/kg IND (■) or control vehicle (CON: ○) which was given after the first 2-h pre-partum period in which behavioural criteria of nest building were fulfilled. Data for each point are shown as means $\pm$ S.E.M. * $P < 0.05$, IND values significantly different during the timespans indicated by arrows. PGFM, PGF 2α metabolite.

Figure 3 Plasma hormone concentrations in pregnant primiparous pigs (zeroed around the birth of the first piglet) that had received either 4 mg/kg IND (■) or vehicle (○) during pre-partum nest building. Data for each point are shown as means ± S.E.M. Data on rise times above a 4 pmol/l threshold are given in the text.

However, clear trends over time were seen, with both hormone plasma concentrations increasing as birth approached. There were no differences between treatment groups in the rise times for either hormone. However, for CON animals, PGFM rise times preceded those of OT (8.39 ± 0.62 h vs 5.84 ± 0.84 h respectively, $P < 0.01$) whereas for IND no significant difference was seen (7.06 ± 1.24 h vs 5.83 ± 0.86 h respectively).

Experiment 2

Mean ± S.E.M. frequencies of behaviour for CON vs IND animals between 1.5 and 2.5 h following treatment are shown in Table 3. There were no differences between treatment groups in the frequency with which any measured behavioural activity or posture was recorded. When not lying down and resting, both groups were mostly engaged in foraging, reflected as high proportions of time spent in rooting behaviour with associated biting, chewing and apparent eating of materials amongst the

Table 3 Percentage frequencies ± S.E.M. with which behaviours were observed in animals in experiment 2

	CON (n=6)	IND (n=7)
Behaviour		
Stand	65.03 ± 12.38	83.61 ± 3.98
Lie	21.04 ± 14.08	5.15 ± 4.39
Walk	13.93 ± 2.93	11.24 ± 1.21
Root	22.95 ± 5.37	25.06 ± 2.55
Paw	0 ± 0	0.47 ± 0.30
Bite/chew	59.56 ± 12.72	69.32 ± 5.74
Carry straw	1.64 ± 0.85	1.87 ± 0.98
Drink	3.01 ± 0.99	6.56 ± 1.79
Defaecate/urinate	1.09 ± 0.69	1.64 ± 0.72
Scratch	0.54 ± 0.54	0.00 ± 0.00

Behaviour was recorded for 1 h, starting 1.5 h after treatment. No significant difference in behaviour between treatment groups were found ($P > 0.1$ in all cases, Mann–Whitney U test).

bedding. Pawing behaviour was very rare. In addition, close observation during the treatment period and on the following day for signs of toxicity associated with COX antagonist treatment (such as lassitude, inappetence, ataxia, vomiting and diarrhoea) failed to show any animals with such signs at any time.

Discussion

The results of experiment 1 showed that IND inhibited pre-partum nest building behaviour in the pig. IND animals became inactive and generally lay down between 1 and 5 h post-injection whereas their CON counterparts continued to show typical pre-partum nest building behaviour, resulting in the construction of recognisable nests. By contrast, IND treatment of non-pregnant gilts in experiment 2 did not produce any behavioural changes from CON animals at an equivalent time. Overt toxic effects associated with prolonged treatment or high doses of COX antagonists (Frölich 1997) were not seen in either experiment. Both treatment groups in experiment 1 went on to deliver normally, with no significant difference in time to delivery, although there was a tendency ($P < 0.1$) for IND to delay birth as previously reported (Nara & First 1981). All gilts had successfully reared litters at 1 month post-partum. Taken together, these separate experiments suggest that the observed inhibition of nest building in pregnant animals is less likely to be caused by non-specific drug side-effects than by a specific inhibition of pre-partum behaviours that are induced by endogenous PGs. Other studies strongly support this hypothesis. For example, exogenous $\text{PGF}_{2\alpha}$ treatment generated nest building in pre-partum sows (Widowski & Curtis 1989). Data from Table 2 show that time effects for many behaviours in experiment 1 directly relating to nest building were markedly different after injection,

reflecting the change produced by IND. In addition, some behaviours not apparently related to nest building (oral contact with the drinker, shake head) were also different, with both being reduced during the period of reduced nest building in IND animals. Both these findings may be a secondary consequence of the IND animals after treatment showing a reduced motivation to nest build and therefore being generally less active. In particular, a reduction in activity is likely to reduce thirst. This hypothesis is supported by data from experiment 2, in which treatment differences in drinking behaviour were not observed and where there was also no difference in overall activity.

Analysis of time effects in experiment 1 showed that the start of pre-partum nest building coincided with a rise in endogenous PGFM concentrations. Furthermore, the reduction in nest building seen following IND treatment was associated with a within group decrease in plasma PGFM, supporting the view that peripheral PGF2 α could be an endogenous stimulator of nest building. However, no significant treatment difference in PGFM between IND and CON animals was seen, but PGFM error bars were large during nest building (Fig. 2A), suggesting that secretion is variable and possibly pulsatile during this time. A sample rate of 20 min and use of a metabolite assay may have been inadequate to show differences between treatments particularly if secretion was pulsatile. Furthermore, regression analysis failed to reveal a relationship between overall nest building activity and plasma PGFM. Gooneratne *et al.* (1983) found pre-partum increases in plasma PGFM but not PGF2 α and suggested that these differences could be due to the slower rate of plasma PGFM clearance compared with the parent compound. Such an effect could offset a relationship between behaviour and PGs in experiment 1.

It is also possible that IND inhibited nest building by central inhibition of PG synthesis not wholly reflected in changing peripheral concentrations of PGFM. Inducible COX 2 activity in hippocampal and cortical regions has been observed following peripheral stimuli in rats (Yamagata *et al.* 1993) and central COX 2 mRNA increases following ischaemia can be attenuated by IND in piglets (Dégè *et al.* 1998). A report of mRNA specific to PGF2 α receptors in hypothalamic nuclei (Burne 2000) suggests a central site of action for PGF2 α . Furthermore, PGF2 α -induced nest building is not inhibited by prior ovariectomy (Burne *et al.* 2001), suggesting that mediators from peripheral reproductive tissues are not required for this process.

In addition to its actions on COX, IND also blocks the activity of 3 α -hydroxysteroid oxo-reductase (Penning *et al.* 1985) and behavioural changes in rats (on grooming, locomotion and sexual receptivity) have been attributed to this mode of action (Beyer *et al.* 1999). Other reported effects of IND include blockade of the lipoxygenase pathway (Downey *et al.* 1998), inhibition of cyclic

AMP-dependent protein kinase (Kantor & Hampton 1978) and inhibition of mitochondrial Ca²⁺ accumulation (Burch *et al.* 1983). Non-PG-mediated mechanisms could therefore account for the behavioural changes seen.

Plasma concentrations of OT were unaffected by treatment and not significantly related to nest building behaviour. This finding supports earlier work suggesting that peripheral OT is not involved in nest building behaviour (Boulton *et al.* 1997b). However, OT is released in response to PGF2 α treatment (Ellendorf *et al.* 1979, Boulton *et al.* 1997b) and is a powerful uterotonic agent. PGF2 α therefore appears to be able to initiate both nest building behaviour (Blackshaw 1983, Widowski & Curtis 1989, Boulton *et al.* 1997a, Burne *et al.* 1999, 2000) and, separately, processes that lead to uterine contractions and foetal expulsion. The pre-partum rise in plasma PGFM before OT seen in CON animals supports this view (Fig. 3). The absence of this effect in IND animals and their tendency to have delayed births suggests an effect of IND to delay PGF2 α stimulation of OT release that is longer term than effects on behaviour.

Plasma concentrations of cortisol and progesterone were unaffected by treatment and not significantly related to nest building behaviour. Previous studies found that plasma cortisol became elevated during the pre-partum period and was further increased by space restriction in a farrowing crate (Lawrence *et al.* 1994). In the present experiment, environment was unaltered and cortisol unaffected by the level of nest building. These results suggest that cortisol is not involved in the regulation of nest building but may be influenced by external factors such as environment. Although there is evidence for progesterone being involved in aspects of nest building regulation in rabbits (González-Mariscal *et al.* 1996) and straw carrying in pigs (Castren *et al.* 1993), the present experiments have not shown any relationship between plasma progesterone and nest building.

In conclusion, these experiments provide further evidence to support the hypothesis that pre-partum behaviour in the pig is regulated by endogenous PGF2 α . In some environments, the creation of a suitable maternal nest is likely to make an important contribution to neonatal survival. Initiation of these behaviours and, separately, the endocrine cascade leading to birth through luteolysis by a single substance would allow for the co-ordination of these events in time.

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