Original article

Luteinising hormone receptor kinetic and LH-induced prostaglandin production throughout the oestrous cycle in porcine endometrium

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Abstract — The present studies were performed to determine the LH/hCG receptor concentration and to evaluate the LH effect on prostaglandin production in porcine endometrium throughout the oestrous cycle. LH/hCG receptors in cell membrane preparations of the endometrium were found from days 12–14 and 15–16 of the oestrous cycle but not in preparations from days 6–7 and 18–20 using the ligand radioreceptor assay. Western blot analysis revealed, however, that the endometrium from all stages of the oestrous cycle contains a 75-kDa immunoreactive LH receptor protein similar to corpora lutea. The incubation of endometrial explants with LH (0, 1, 10 and 100 ng·mL^{-1}) resulted in an increase of 13,14-dihydro-15-keto-PGF$_{2\alpha}$ accumulation in a dose-dependent manner on days 5, 10, 14 and 16 of the oestrous cycle. The most effective dose was 10 ng LH·mL^{-1} on days 5–16, but the strongest effect was found on days 14 and 16 (7.40 ± 0.14 versus 12.75 ± 1.40 and 5.67 ± 0.35 versus 9.4 ± 1.25 ng·100 mg^{-1} tissue/6 h, respectively; $P < 0.01$). It was also observed that 10 and 100 ng·mL^{-1} of LH significantly increased cyclo-oxygenase expression to 135.2 and 123.5 % respectively, above the control value ($P < 0.01$) on day 16 of the oestrous cycle. Our data suggest that LH receptors are of physiological significance in the porcine endometrium, since LH induces cyclo-oxygenase synthesis and increases prostaglandin production. © Inra/Elsevier, Paris

pig / endometrium / LH / hCG receptor / prostaglandin / cyclo-oxygenase

Résumé — Des récepteurs à LH fonctionnels sont présents dans l'endyomètre de truie. L'objectif de cette étude était d'étudier les fluctuations du nombre de récepteurs à LH présents dans l'endomètre de truie au cours du cycle et d'évaluer les effets de LH sur la production de prostaglandines par ce tissu. Un dosage radiorécepteur a permis de montrer la présence de récepteurs à LH dans des préparations membranaires d'endomètre obtenues à j12–14 et j15–16 du cycle mais ni à j6–7, ni à j18–20. En revanche, quand l'analyse est effectuée en *western blot*, une bande à 75 kDa est détectable à tous les stades du cycle. L'incubation d'explants d'endomètre avec des doses variables de LH

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(0, 1, 10 et 100 ng·mL^{-1}) produit une augmentation des concentrations de 13,14-dihydro-15-keto-PGF$_{2\alpha}$. La dose de LH la plus efficace est 10 ng·mL^{-1} et la stimulation la plus forte est observée pour des explants obtenus à j14 et j16 (172 et 166 % d'augmentation respectivement). De plus, l'addition de 10 et 100 ng·mL^{-1} de LH induit une augmentation (de 135 et 123 % respectivement) de l'expression de la cyclooxygénase à j16 du cycle. Ces résultats indiquent que les récepteurs à LH présents dans l'endomètre porcin sont fonctionnels puisque la LH est capable d'affecter la synthèse de cyclo-oxygénase et d'augmenter la production de prostaglandines. © Inra/Elsevier, Paris

truie / endomètre / récepteur à LH / prostaglandines / cyclo-oxygénase

1. INTRODUCTION

The regulation of the oestrous cycle is a uterine-dependent event in farm animals. Luteal regression occurring at the end of the oestrous cycle is caused by episodes of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) release from the uterus [32]. Lysis of the corpora lutea and the concomitant decline in progesterone production allows further follicular development leading to oestrus, ovulation and the opportunity for mating and pregnancy to occur [38]. In ruminants, the mechanism of luteolysis has been extensively studied. It is thought that oxytocin (OT) secreted from the corpus luteum and released into the ovarian vein, binds to endometrial OT receptors and stimulates a pulsatile secretion of uterine PGF$_{2\alpha}$ [35, 40]. Pulses of endometrial PGF$_{2\alpha}$ also occur during the late luteal phase in swine [28, 34], but the mechanism regulating this process is not clearly elucidated. In pigs it is suggested that OT can be involved in the secretion of luteolytic PGF$_{2\alpha}$ peaks but its role is limited only to controlling their height and frequency, since PGF$_{2\alpha}$ release and luteolysis in sows was not prevented by blocking the OT receptors [23].

The key-enzyme in prostaglandin production is prostaglandin G/H synthase (PGHS), also known as cyclo-oxygenase (COX), which catalyses the conversion of arachidonic acid to PGH$_2$ – a precursor of prostaglandin synthesis. Two isoforms of prostaglandin synthase have been identified. The first one, referred to as cyclo-oxygenase-1 (COX-1), is expressed constitutively and is relatively unresponsive to stimuli. The second one, cyclo-oxygenase-2 (COX-2), can be induced by a wide variety of factors including cytokines, growth factors, tumour promoters and gonadotropins [5, 8, 15, 16, 18].

LH receptor presence in porcine endometrium has already been reported by Ziecik et al. [42] in 1986. Using the radioligand receptor assay, they localised these receptors in the endometrium from the luteal but not from the follicular phase. To our knowledge, there is currently no information available about the role of LH receptors in endometrial processes of pigs. The luteal phase lasts nearly three-quarters of the cycle and consists of the early-luteal phase (CL formation), mid-luteal phase (functional CL) and late-luteal phase (luteolysis). It is not known at which stage of the CL life span that LH receptors appear and can be activated. Also, the biological effect of endometrial LH receptors in pigs is still unknown. In bovine endometrium, LH receptors were detected at the time of luteolysis [13] and LH has been shown to increase COX expression and prostaglandin production [33].

The objectives of the present investigation were: 1) to determine the level of LH/hCG receptors in the porcine endometrium throughout the oestrous cycle using two methods of receptor localisation; and 2) to examine the possible physiological role of these receptors by measuring the effect of LH on COX expression and prostaglandin production in the porcine endometrium.

2. MATERIALS AND METHODS

2.1. Chemicals

Purified hCG (CR-127: 14 900 IU·mg⁻¹) was provided by the National Hormone and Pituitary Program (NIDDKD). Polyclonal antibodies raised against the synthetic N-terminal rat LH receptor peptide sequence 15–38 (anti-LHR 15–38) were obtained from Dr Patrick Roche of the Mayo Clinic (Rochester, MN). Antiserum against 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) was donated by Dr William Silvia (Department of Animal Science, University of Kentucky, Lexington, KY). Biotinylated goat anti-rabbit IgG was purchased from Vector Laboratories Inc. (Burlingame, CA).

2.2. Animals and tissues

Twenty crossbred gilts (8 months of age) with observed oestruses were used. The first day of behavioural oestrus was established as day 0 of the oestrous cycle. Days of the oestrous cycle were additionally confirmed by the morphological appearance of the ovaries after slaughter [2, 25]. These parameters were the basis for the classification of the uterine tissues into four major groups (n = 4–6): 1) pro-oestrus (days 18–20); 2) early luteal phase (days 6–7); 3) pre-luteolysis (days 12–14); and 4) luteolysis (days 15–16). To examine LH-induced prostaglandin production in vitro, endometrial tissue was collected at the following days of the oestrous cycle: 5, 10, 14, 16 and 19. Uteri were obtained immediately after slaughter and transported on ice to the laboratory within 5 min.

2.3. Preparation of membrane fractions for LH/hCG binding sites

Cell membrane fractions were obtained by the procedure described by Ziecik et al. [42]. Briefly, tissues were homogenised four times in bursts of 7 s at 4 °C with an Ultra-Torax homogeniser in six volumes (vol/wt) of 25 mM Tris-HCl buffer pH 7.4, containing 250 mM of sucrose. The homogenate was then filtered through four layers of cheesecloth, and the filtrate centrifuged for 20 min at 800 g at 4 °C. The resulting supernatant was further centrifuged for 1 h at 25 000 g at 4 °C and the sediment suspended in 1 mL of ice-cold 25 mM Tris-HCl buffer, pH 7.2 containing 0.1 % BSA and 5 mM $MgCl_2$. The result-

ing suspension was stored at –70 °C for further experiments. A fraction of the membrane preparation without BSA was assayed for protein determination by the method of Lowry et al. [26].

2.4. LH/hCG receptor assay

A ligand binding assay in endometrial tissue, corpora lutea and skeletal muscle and kidney (negative control) was performed as previously described [42]. Human CG (CR-127) was labelled according to the Iodogen method [11]. Na[¹²⁵I]iodite was purchased from Amersham International (Amersham, UK). Separation of protein-bound and free ¹²⁵I was performed by chromatography on Sephadex G-50 (Pharmacia, Uppsala, Sweden) columns (1 cm × 15 cm). The specific activity of labelled hCG was determined by self displacement analysis on CL membrane preparations in the radioligand receptor assay [19] and varied from 50 to 70 × 10³ cpm/ng.

The cell membrane fraction was incubated with a tracer in polystyrene tubes for 16 h at room temperature. The incubation buffer consisted of 25 mM Tris-HCl (pH 7.2), 5 mM $MgCl_2$ and 0.1 % BSA. The incubation mixture was prepared with 0.1 mL incubation buffer, 0.1 mL incubation buffer containing 25 000 cpm ¹²⁵I-hCG and 0.2 mL receptor preparation. Preliminary experiments showed that binding of labelled hCG was proportional to the amount of the receptor added from 0.6 to 2 mg protein of endometrial cell membrane preparation. Nonetheless, 1.0 mg of membrane extract protein was used consistently to determine binding parameters in the endometrium and 0.4 mg in the CL. These values were normalised to a per-milligram basis for data presentation.

Two millilitres of ice-cold incubation buffer were added at the end of the incubation period and the tubes were centrifuged at 1 000 g for 30 min. The supernatants were removed by aspiration and the radioactivity of the pellets was determined in a γ-spectrometer with a counting efficiency of 75 %. Non-specific binding was determined in the presence of 0.5 µg hCG and was usually less than 5 % of the total ¹²⁵I label added.

The receptor concentration and equilibrium association constant (K_a) of unoccupied binding sites were determined by Scatchard analysis [31]. Six to eight subsaturating doses (0.03–5.00 ng) of unlabelled hCG were used in duplicate for each receptor preparation.

The sensitivity of the assay (0.15 fmol·mg^{-1} protein) was the lowest concentration of the receptor per mg of protein of crude membrane preparation which responded to a change in ligand concentration.

2.5. Preparation of membrane fractions for western blot

Membrane fractions for immunoblotting were obtained using the procedure described by Ziecik et al. [42] with some modification. Briefly, tissue was homogenised four times in bursts of 7 s at 4 °C with an Ultra-Torax homogeniser in four volumes (v/w) of lysis buffer (50 mM Tris-HCl buffer pH 8.0, containing 150 mM NaCl, 100 mM EDTA, 0.02 % NaN$_3$, 1 % Triton X-100, 100 µg·mL^{-1} phenylmethylsulfonyl fluoride and 1 µg·mL^{-1} aprotinin). The homogenate was then filtered through four layers of cheesecloth, and the filtrate was centrifuged for 20 min at 800 g at 4 °C. The resulting supernatant was then centrifuged for 1 h at 25 000 g at 4 °C and the sediment was suspended in 2 mL of ice-cold lysis buffer. The resulting suspension was stored at –70 °C for further experimentation. The protein level was determined by the method of Lowry et al. [26].

2.6. Incubation of endometrial explants

The endometrium was obtained immediately after slaughter and transported to the laboratory in ice-cold phosphate-buffered saline (PBS; Ca, Mg-free), pH 7.4. The endometrial strips (100–110 mg) were placed in glass tubes containing 2 mL of Dulbecco Modified Eagle Medium (DMEM, Sigma) with 10 % of FCS, 1 000 IU·mL^{-1} penicillin, 100 µg·mL^{-1} streptomycin and increasing amounts of LH: 0, 1, 10 and 100 ng·mL^{-1}. Samples were incubated in quadruplicate for 6 h at 37 °C, gently shaken in a water bath with controlled gas distribution (95 % O$_2$ and 5 % CO$_2$). After the time of incubation, the medium was collected and frozen at –20 °C until analysis for PGFM and/or explants were immediately homogenised for COX western blot analysis.

2.7. The estimation of the incubation time

Endometrial explants were incubated as described above with 0 or 10 ng·mL^{-1} LH for 3 and 6 h. The medium was then collected and frozen in –20 °C until PGFM analysis.

2.8. Radioimmunoassay of PGFM

Concentrations of prostaglandin 13,14-dihydro-15-keto-PGF$_{2\alpha}$ (PGFM) in 100 µL of the incubation medium were quantified by RIA without extraction using 13,14-dihydro-15-keto[^{3}H]PGF$_{2\alpha}$ (183 Ci/mmol; Amersham LIFE SCIENCE), antiserum to PGFM (WS 4468-7; 1:1 500 final dilution) and PGFM (Sigma) for standards. Cross reactivities of PGF$_{2\alpha}$, PGE$_2$, PGA$_2$, and 6-keto-PGF$_{1\alpha}$ with antiserum to PGFM were <1 % [35]. Standards and samples (100 mL) were incubated with 100 µL anti-PGFM and 200 µL [^{3}H]PGFM for 18–24 h at 4 °C. Separation of free and antibody-bound PGFM was achieved by incubation for 20 min at 4 °C with 0.63 % Norit A charcoal and 0.063 % dextran in PBS-EDTA buffer (pH 7.0), containing 0.1 % gelatin and 0.1 % NaN$_3$. After centrifugation for 20 min at 3 500 r.p.m. at 4 °C the supernatant was transferred to counting vials for determination of antibody-bound [^{3}H]PGFM for 1 min on a beta counter. Concentrations of PGFM in the medium were calculated using the log-logit regression programme. The release of PGFM was expressed as ng/100 mg of wet tissue. The sensitivity of this assay was 15 pg/tube. Intra-assay and inter-assay coefficients of variation were 8.5 and 15.1 %, respectively.

2.9. Western blot analysis for LH receptor and COX

2.9.1. LH receptor protein

Membrane fractions of endometrium from different days of the oestrus cycle were used. Aliquots of 70 µg of protein were dissolved in double-strength loading buffer consisting of 50 mM Tris-HCl (pH 6.8), 4 % SDS, 20 % glycerol and 2 % 2-mercaptoethanol and separated on 10 % SDS-PAGE (polyacrylamide gel electrophoresis in the presence of SDS). The separated proteins were electroblotted onto 0.45-µm nitrocellulose membranes for 2 h at 250 mA in a 25 mM Tris-HCl buffer (pH 8.2), 192 mM glycine, containing 0.1 % SDS. The non-specific binding sites were blocked overnight at 4 °C with 5 % non-fat dry milk in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1 % Tween-20 (TBST buffer). The membranes were then incubated with 1:1 000 dilution of the LH receptor

antibody (PR) for 1.5 h at 22 °C and washed three times for 10 min each with TBST. The washed blots were reincubated for 1 h at 22 °C with a 1:3 000 dilution of biotinylated antirabbit IgG, washed with TBST and incubated for 1 h at 22 °C with avidin-biotin-HRPO complex (1:3 000 dilution; Vectastain ABC kit, Vector Laboratories, Inc?, Burlingame, CA). To develop colour, TBS containing 0.01 % H_2O_2 and 0.04 % 3, 3'-diaminobenzidine was used.

2.9.2. COX

After incubation endometrial explant extracts (40 µg protein) were homogenised in lysis buffer, centrifuged at 5 000 g for 20 min at 4 °C and the supernatant was then centrifuged at 25 000 g for 1 h at 4 °C. The pellet was resuspended in lysis buffer, separated by SDS-PAGE and electroblotted as described above. The antibody for COX [17] was used in a dilution of 1:200. In the preliminary experiments a 72-kDa band was observed to be the most sensitive to LH in the porcine endometrium. Densitometric scans were obtained by the use of the HPScanJet T scanner (Hewlett-Packard, Lorvallis, OR) and processed with the Photostyler Image Version 2.0. The results are presented as arbitrary units of relative optical density.

2.10. Statistical analysis

Data were analysed by ANOVA followed by the Duncan new range test. The results are expressed as mean ± SEM.

3. RESULTS

3.1. Concentration of LH/hCG receptor in endometrium

The estimation of the number and affinity constant (Ka) of LH/hCG receptors in porcine endometrium at days 12–14 and 15–16 of the cycle are shown in *figure 1*. Scatchard analysis of the data obtained at equilibrium gave a Ka of $1.62 \pm 0.20 \times 10^{10}$ M^{-1} at days 12–14, and of $1.87 \pm 0.52 \times 10^{10}$ M^{-1} at days 15–16 (*table I*). These values were three times lower than the Ka for mid-cycle luteal cells ($P < 0.05$). Receptors for LH/hCG were not detectable (<0.15 $fmol \cdot mg^{-1}$ protein) in the endometrium from early luteal phase (days 7–8) and proestrus (days 18–20).
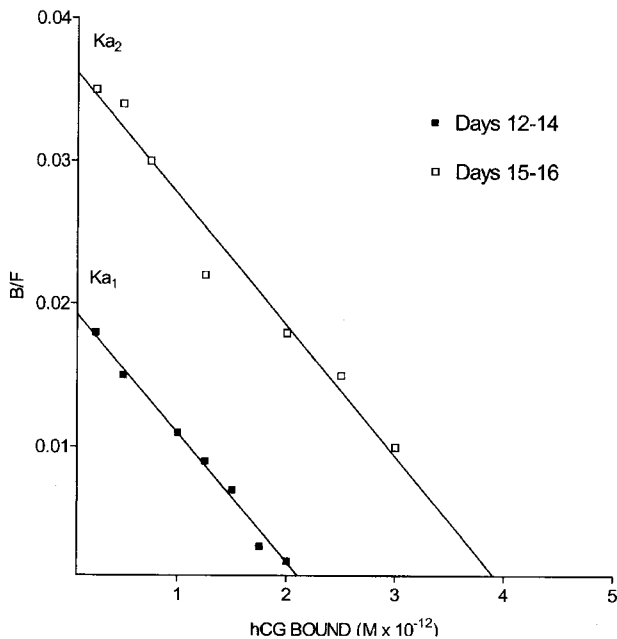

Figure 1. Estimation of number and affinity constant (K_a) of LH/hCG receptors in porcine endometrium. Human CG binding sites with seven different subsaturating amounts of unlabelled hCG from two different crude membrane preparations were tested. K_{a1} = 12–14; K_{a2} = 15–16 days of the estrous cycle.

Table I. The affinity and capacity of gonadotropin binding sites in the endometrium and a corpus luteum of the pig (skeletal muscle and kidney are negative control tissue). Values are mean ± SEM.

Tissue	Days of cycle	Affinity constant $(\times 10^{10} \text{ M}^{-1})$	Capacity $(\text{fmo} \cdot \text{mg}^{-1} \text{ protein})$
Corpus luteum	8–10	4.8 ± 1.0^a	8.92 ± 1.61^a
Endometrium	7–8	–	< 0.15
	12–14	1.62 ± 0.20^b	0.56 ± 0.11^c
	15–16	1.87 ± 0.52^b	0.31 ± 0.04^c
	18–20	–	< 0.15
Skeletal muscle	6–20	–	< 0.15
Kidney	6–20	–	< 0.15

a > b, $P < 0.05$; a > c, $P < 0.001$.

The number of hCG-binding sites in the endometrial tissue was 0.56 ± 0.11 fmol·mg^{-1} protein at days 12–14 and 0.31 ± 0.04 fmol·mg^{-1} protein at days 15–16 of the oestrous cycle. The binding capacity for hCG by luteal cells was 8.92 ± 1.61 fmol·mg^{-1} protein ($P < 0.01$). LH/hCG receptors were not found in the negative control tissues – skeletal muscle and kidney.

3.2. LH/hCG receptor protein

Western blotting with a polyclonal LH/hCG receptor antibody showed that the endometrium from all stages of the oestrous cycle contained a 75-kDa protein (*figure 2*). The level of LH/hCG receptors was higher before and during the time of luteolysis (days 12–14 and 15–16) than in the early luteal phase (days 6–7) and follicular phase (days 18–20).

3.3. Determination of incubation time

LH caused a significant increase of PGFM concentration (ng·100 mg^{-1} wet tissue) in the medium after 6 h of incubation when compared to the control ($P < 0.01$). Such a difference was not observed after a 3 h incubation period (*figure 3*).

The basic secretion of prostaglandins also increased after 6 h of incubation ($P < 0.01$; *figure 3*).

3.4. Effect of LH on endometrial PG production during the oestrous cycle

In preliminary experiments, the effect of LH on the concentration of PGF$_{2\alpha}$ and PGFM in ten samples of incubation medium was measured. The concentration of PGF$_{2\alpha}$ and PGFM in response to LH action was
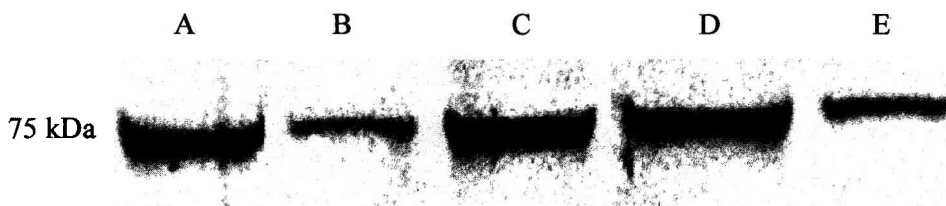

Figure 2. Western immunoblotting for LH/hCG receptors in the endometrium on day 5 (B), 10 (C), 15 (D) and 18 (E) of the oestrous cycle. Line A represents the corpus luteum. Forty micrograms of protein of each tissue were used.
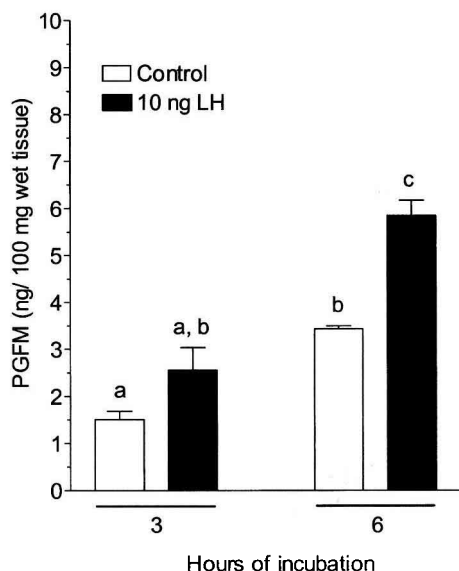
Figure 3. The time-dependent effect of LH on PG production in endometrial tissue. Endometrial tissue from day 10 of the oestrous cycle (preparations from four gilts, four replicates for each point) were incubated for 3 and 6 h in the presence (closed bars) or absence (open bars) of LH (10 ng·mL^{-1}). PGFM was determined in the media by RIA. Bars represent mean ± SEM. Means with no superscripts in common are different (a < b, b < c; $P < 0.01$).

highly correlated (r = 0.9; $P < 0.001$). The PGFM level was, however, about 100 times lower than the level of PGF$_{2\alpha}$.

The ability of LH to modulate PG production by the endometrium was examined at five stages of the oestrous cycle. LH increased PG production in a dose-dependent manner on days 5, 10, 14 and 16 of the oestrous cycle (*figure 4*). The most effective dose was 10 ng LH·mL^{-1} on days 5–16. On day 5, both basal (3.06 ± 0.23) and LH stimulated (4.74 ± 0.20) PG release was, however, low when compared to corresponding values on day 14 (control – 7.40 ± 0.14 and LH stimulated – 12.75 ± 1.40 ng·100 mg^{-1}·6 h^{-1}) and day 16 (control – 5.67 ± 0.35 and LH stimulated – 9.42 ± 1.25 ng·100 mg^{-1}·6 h^{-1}; $P < 0.01$, respec-

tively). A dose of 100 ng·mL^{-1} did not show a higher effect. The lowest basal (without LH stimulation) PG production was found on day 19 of the oestrous cycle and only the highest dose of LH (100 ng·mL^{-1}) was able to stimulate PG secretion at this time (basal – 1.28 ± 0.17 versus LH stimulated – 3.27 ± 0.21 ng·100 mg^{-1}·6 h^{-1}; $P < 0.01$).

3.5. Effect of LH on the induction of endometrial COX

To determine whether LH-stimulated PG secretion was modulated by COX endometrial strips (100 mg) collected on day 16 of the oestrous cycle, gilts were incubated in the absence or presence of increasing doses of LH. After a 6-h incubation period in the presence of 1, 10 and 100 ng LH·mL^{-1}, there was an increase in COX expression, above the control values of 101.1, 135.2 and 123.5 %, respectively ($P < 0.01$) (*figure 5*).

4. DISCUSSION

The data presented show that porcine endometrium contains LH/hCG receptors on all studied days of the oestrous cycle. When Ziecik et al. [42] reported for the first time the presence of uterine gonadotropin receptors in female mammals, using pigs as a model, it was not known whether the endometrium contained LH/hCG receptors for only a specific short period of the oestrous cycle. Our recent results obtained using a ligand radioreceptor assay revealed that endometrial LH/hCG receptors, contrary to the myometrium [42], appear temporarily during the oestrous cycle in pigs. We found these receptors in preparations from days 12 to 14 (pre-luteolysis) and 15 to 16 (luteolysis) but not in preparations from other stages – from days 18 to 20 (pro-oestrus) and 6 to 7 (early luteal phase). These findings support those of Freidman et al. [13] who reported the presence of LH receptors in bovine endometrium only on days 2–4 and 15–17 of the oestrous cycle.
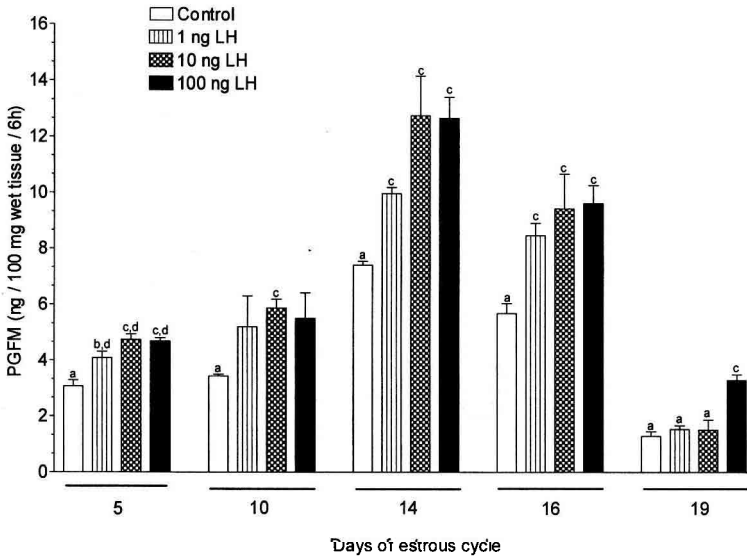
Figure 4. Dose response of LH (0–100 ng·mL^{-1}) on endometrial PG production. Tissues from four to five individual gilts on different days of the oestrous cycle were used and all treatments were conducted in quadruplicate. Values are mean ± SEM. For particular days, means with no superscripts in common are different (a < b, $P < 0.05$; a < c, $P < 0.01$; b < c, $P < 0.001$).

Surprisingly, using western blot analysis LH receptors were detected in all stages of the oestrous cycle in pigs. Our studies showed that, like the corpora lutea, myometrium and cervix, porcine endometrium contain a main 75-kDa receptor species [37]. The highest level of LH receptors was observed before and during the time of luteolysis. Porcine endometrium expressed the LH receptor gene and Southern blot revealed the presence of main 740- and 470-bp products [37].

With the radioligand receptor assay and immunoblotting, we did not observe the biological activity of LH receptors in the endometrium, but only the presence of the protein. To explain the possible physiological role of these receptors we turned our attention to the luteolysis process, which is still not clearly elucidated despite many attempts undertaken in the past.

It is believed that in swine, as in ruminants, the episodic release of prostaglandin

$F_{2\alpha}$ is responsible for corpus luteum regression. It has been shown that oxytocin stimulates $PGF_{2\alpha}$ release from bovine [24], ovine [35, 36] and porcine [39] endometrium at the time of luteolysis. Although the ovaries are the main source of oxytocin during luteolysis in ruminants [32], the role of ovarian oxytocin in pigs is still unclear. Since the concentration of oxytocin in porcine corpora lutea is many times lower than in ovine and bovine corpus luteum, and its level is maximal during the early luteal phase [6, 29] it is thought that oxytocin may play a role as an intraovarian mediator necessary for the control of steroidogenesis [9, 14, 30] or corpora lutea development [6, 29]. Einspannier et al. [10], using a microdialysis system implanted after ovulation into early CL, demonstrated that OT stimulates progesterone release from freshly formed CL. This indicates the possibility of the luteotropic action of OT in the early luteal phase. Furthermore, oxytocin in pigs is
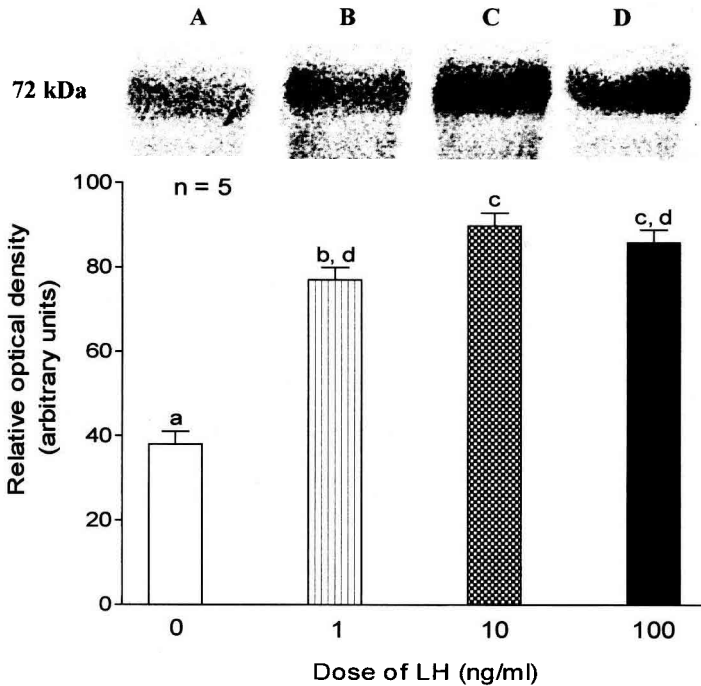
Figure 5. Effect of LH on the induction of cyclo-oxygenase by endometrial tissue in gilts on day 16 of the oestrous cycle. The immunoblot is representative of tissue from five individual gilts. The lower panel represents the mean ± SEM of samples from separate experiments expressed as the relative optical density. Lane A, control; lane B, 1 ng $LH \cdot mL^{-1}$; lane C, 10 ng $LH \cdot mL^{-1}$; lane D, 100 ng $LH \cdot mL^{-1}$. Means with no superscripts in common are different (a < b, c, $P < 0.001$; b < c, $P < 0.05$).

released at the time of luteolysis from the pituitary but not from the ovary. Around the time of luteolysis, pulses of OT are present in the peripheral circulation and concentrations of OT in the plasma increase. Moreover, OT pulses frequently occur simultaneously with episodes of $PGF_{2\alpha}$ secretion indicating that a temporal association between $PGF_{2\alpha}$ and OT may exist [22]. Endometrial responsiveness to OT develops between days 12 and 14 post-oestrus and occurs before the onset of functional luteolysis [4]. Recent studies of Kotwica et al. [23] showed, however, that blocking of OT receptors neither prevented luteolysis nor changed the duration of the oestrous cycle in swine. Furthermore, the above authors did not find any correlation between

OT and PGFM pulses. Thus, the question about the mechanism of luteolysis initiation in pigs is still open. Our recent studies indicate that the luteinising hormone could be involved in prostaglandin production in the porcine endometrium. The incubation of porcine endometrial explants with different doses of LH resulted in an increased PGFM secretion in a dose-dependent manner on days 5–16 of the oestrous cycle. The strongest effect was observed at the time of luteolysis. The mechanism of LH action on endometrial cells is due to the stimulation of COX expression. Similarly, Shemesh et al. [33] demonstrated the ability of LH to simulate COX expression and $PGF_{2\alpha}$ production by bovine endometrial cells. The interaction between LH and PGFM pulses should

be investigated in vivo to clarify the physiological role of LH on PGF$_{2\alpha}$ release in pigs.

There is no doubt that a direct temporal relationship among the presence of LH receptors, the induction of COX and the production of PGF$_{2\alpha}$ exists. The mechanism of induction and control of the porcine endometrial LH/hCG binding sites is, however, unknown, but it is possible that, as with the myometrium, oestradiol and progesterone could be involved in the synthesis and action of LH receptors [21, 44]. LH is released in a pulsatile manner during the luteal phase of the oestrous cycle [39]. We hypothesise that those pulses can induce PGF$_{2\alpha}$ release from the endometrium. This indicates a possible role for LH in the initiation of luteolysis in pigs and cows. After the initiation of the luteolytic endometrial PGF$_{2\alpha}$ secretion, however, LH/hCG receptors decline in both species. We suggest that PGF$_{2\alpha}$ could indirectly affect endometrial gonadotropin binding sites at the follicular stage by progesterone level regulation. This concept is supported by experiments showing that the injection of PGF$_{2a}$ induced a rapid inhibition of bovine CL progesterone production followed by a decrease in the concentration of luteal LH/hCG binding sites [3].

It is interesting that in our earlier papers [41, 43], we reported the presence of LH/hCG receptors in the endometrium of pregnant pigs. It could be suggested that LH may also stimulate endometrial COX and prostaglandin synthesis during the early stages of pregnancy. Maternal recognition of pregnancy in pigs occurs from days 11 to 12 and is thought to be dependent on the production of oestrogen by the blastocyst which acts in an anti-luteolytic way to cause a reorientation of uterine PGF$_{2\alpha}$ secretion away from the vasculature and into the uterine lumen [12]. The presence of the blastocyst probably also acts to alter the final product such that the primary prostaglandin released is PGE$_2$ rather than PGF$_{2\alpha}$ [1, 28]. As a matter of fact, PGE$_2$ neutralised the

luteolytic effect of simultaneously infused PGF$_{2\alpha}$ in indomethacin-treated gilts [1] with fewer PGF$_{2\alpha}$ secretion spikes, and with a lower amplitude than has been reported for pregnant, as compared to cyclic gilts [28].

In the ovaries, the LH/hCG receptor activates two intracellular signalling pathways: one leading to the stimulation of adenyl cyclase and resulting in an increase of cellular cyclic AMP [27] and the other stimulating phospholipase C, resulting in the formation of inositol phosphates and raising the intracellular calcium level [7]. The same second-messenger systems were described for LH receptors in the porcine myometrium [20]. LH binding to its endometrial receptors could affect many systems, since LH-dependent cAMP or calcium second-messenger systems activate a variety of enzymes. The induction of COX by LH may represent just one of several enzymes induced by LH to regulate the course of the oestrous cycle and pregnancy.

In conclusion, data obtained in this study show that an LH receptor protein is present in the endometrium during the course of the oestrous cycle and provide evidence of the existence of functional LH/hCG receptors in porcine endometrium since LH can stimulate COX expression and PG production by endometrial cells.

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