Involvement of gonadotropins in induction of luteolysis in pigs

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SUMMARY

In our previous study we demonstrated that treatment of endometrial explants with LH increased 13,14-dihydro-15-ketoprostaglandin F\textsubscript{2α} (PGFM) accumulation in pigs. This was particularly visible on Days 14-16 of the estrous cycle. The action of gonadotropin in porcine endometrium appears to be mediated by LH/hCG receptors whose number is dependent on the day of the estrous cycle. In the current study i.v. infusion (1 hour) of hCG (200 IU) performed on Days 10 (n=4 gilts) and 12-14 (n=4 gilts) of the porcine estrous cycle did not affect plasma PGFM (pg/ml±SEM) concentrations. In contrast, administration of hCG on Days 15-17 – depending on plasma PGFM level before the infusion period – produced three different types of response: I. plasma PGFM surge was observed when the mean basal pre-infusion PGFM plasma level was 233±52.1 (n=6 gilts); II. the delayed PGFM surge was observed when basal pre-infusion PGFM level was 801±112.7 (n=6 gilts); and III. lack of PGFM response to hCG was found when basal pre-infusion PGFM level was 1271±480.8 (n=6 gilts). Concentrations of plasma PGFM before and after saline infusion did not differ on Days 12-14 and 16 of the estrous cycle. In the next experiment blood samples were collected every one hour on Days 12-18 of the estrous cycle to determine concentrations of LH, PGFM and progesterone in four gilts. In particular gilts, plasma peaks of LH closely preceded surges of PGFM in 72.7, 84.6, 75.0 and 66.6% of cases, respectively. The highest PGFM surges followed a decline in plasma progesterone concentration. In conclusion, we demonstrated that concentration of PGF\textsubscript{2α} metabolite was increased after

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hCG infusion during the late luteal phase of the porcine estrous cycle. Moreover, we observed close relationship between plasma LH and PGFM peaks in gilts around the time of luteolysis. These facts may suggest the LH involvement in the elevation of endometrial PGF$_{2\alpha}$ secretion in pigs, and, in consequence, induction of luteolysis. Reproductive Biology 2001 1 (2): 33-50

**Key words:** LH, hCG, PGFM, gilts

**INTRODUCTION**

In pigs, as in many other domestic species, pulsatile endometrial secretion of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) is thought to be responsible for cyclic luteolysis. Luteinizing hormone (LH), in turn, is considered to be the principal luteotropin in pigs. However, our recent *in vitro* study revealed that LH may also participate in luteal regression by increasing the PGF$_{2\alpha}$ production in the porcine endometrium [28]. The action of gonadotropin appears to be mediated by endometrial LH receptors whose number is dependent on the day of the estrous cycle. Treatment of endometrial explants with LH resulted in a dose dependent increase of 13,14-dihydro-15-ketoprostaglandin F$_{2\alpha}$ (PGFM) accumulation. This was particularly evident on Days 14-16 of the estrous cycle [28]. Since LH, similar to PGF$_{2\alpha}$, during the luteal phase is released in a pulsatile manner it is possible that LH can be involved in the initiation and/or maintenance of luteolysis by direct effect on production of PGF$_{2\alpha}$.

The objective of the present study was to determine the effect of the LH agonist – hCG on peripheral plasma PGFM concentration and to examine a relationship between LH and PGF$_{2\alpha}$ secretion during the mid- and late luteal phase of the estrous cycle in gilts.

**MATERIALS AND METHODS**

**Experiment 1**

Thirty-four crossbred cyclic gilts, each weighing approximately 110 kg, were prepared for estrus synchronization by individual feeding of 15 mg active progestin (Regumate, Hoechst) for 15 days. On Days 5-6 of the estrous cycle, a cannula was inserted into the vena cava via a cephalic vein and exteriorized by passage under the skin to the back to facilitate blood collection and hCG/saline infusion [19]. Gilts were infused i.v. for 60 min (20 ml/hr in saline) with 200 IU hCG (Werft Chemie) on Days 10 (n=4; fig. 1), 12-14 (n=4; fig. 1) and 15-17 (n=18; figs. 2 and 3) of the estrous cycle. Control gilts (n=8) were infused with 20 ml saline/hr on days 12-14 (n=4) and 16 (n=4) of the estrous cycle.
Fig. 1. Mean plasma concentration of PGFM (mean±SEM) before and after hCG infusion on (A) Day 10 (n=4 gilts) and (B) Days 12-14 (n=4 gilts) of the porcine estrous cycle. The shaded area represents the period of hCG administration (200 units; 60 min).
Fig. 24. Mean plasma concentration of PGFM (mean±SEM) before and after hCG infusion on Days 15-17 of the porcine estrous cycle. Horizontal segments represent respectively: A – pre-infusion period, B – first post-infusion period (90-240 min), C – second post-infusion period (270-420 min). The shaded area represents the time of hCG administration (200 units; 60 min). We observed three different patterns of response to gonadotropin: I – PGFM surge during period B (n=6 gilts); II – delayed surge of PGFM (n=6 gilts) and III – lack of PGFM response (n=6 gilts).
Fig. 2B. Mean plasma concentration of PGFM (mean±SEM) before (period A) and after (periods B and C) hCG infusion in gilts from groups I, II and III treated with gonadotropin on Days 15-17 of the estrous cycle. For details see description of fig. 2A. Bars with different superscripts are significantly different (particular p values are given in Materials and Methods.)
Fig. 3. Mean plasma concentrations of LH (mean±SEM) before and after hCG infusion on Days 15-17 of the porcine estrous cycle. The shaded area represents the period of hCG administration (200 units; 60 min).
Blood samples (4 ml) were collected every 30 min for 2 hrs before and 6 hrs after the period of hCG or saline infusion. Heparinized plasma samples were stored at -20°C until PGFM and LH concentrations were determined by radioimmunoassay (RIA).

Gilts receiving hCG on Days 15-17 were divided into 3 groups (I, II and III) depending on basal concentrations of PGFM before hCG infusion (pre-infusion level) and whether or not they responded to gonadotropin. To visualise the effect of hCG administration on plasma PGFM concentrations in these gilts, time of the experiment was divided into three following periods: period A – the pre-infusion period (90 min → 0 min), period B – the first post-infusion period (90 min → 240 min) and period C – the second post-infusion period (240 min → 420 min). Time point "0 min" designated the beginning of hCG infusion.

**Experiment 2**

Four gilts (103±4.0 kg) during their second or third estrous cycle were used to determine the releasing pattern of LH and PGFM. Four days before the experiment polyvinyl chloride catheter was inserted into the jugular vein under general anaesthesia according to the method of Kotwica et al. [19]. Blood samples (4 ml) were collected every one hour on Days 12-18 of the estrous cycle to determine concentrations of LH, PGFM and progesterone (figs. 4A-D).

**Hormone analyses**

Concentrations of prostaglandin 13,14-dihydro-15-keto-PGF2α (PGFM) in 100 µl of blood plasma were quantified by RIA without extraction using PGFM (Sigma) for standards, 13, 14-dihydro-15-keto[3H]PGF2α (183 Ci/mmol; Amersham LIFE SCIENCE) and antiserum to PGFM (WS 4468-7; 1:1500 final dilution). Cross reactions of PGF2α, PGE2, PGA2, and 6-keto-PGF1α with antiserum to PGFM were <1% [14]. Standards and samples (100 µl) were incubated with 100 µl anti-PGFM and 200 µl [3H]PGFM for 18-24 h at 4°C. Separation of free and antibody-bound PGFM was achieved by incubating samples 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% NaN3. After centrifugation (20 min, 800xg, 4°C) the supernatant was transferred to counting vials to determine the antibody-bound [3H]PGFM by counting for one min in a beta counter. Sensitivity of the assay was 60 pg/ml and intra- and interassay coefficients of variation were 4.2 and 8.5%, respectively.
A) Pig No. 25

Days of the estrous cycle

LH (pg/ml) 0 10 20 30 40

PGF2α (pg/ml) 0 250 500 750 1000 1250 1500

progesterone (ng/ml) 0 5 10 15 20 25 30 35 40

peak LH

peak PGF2α
Fig. 4A-D. Concentrations of progesterone, LH and PGFM in peripheral blood plasma samples collected every hour in four individual gilts between Days 12 and 18 of the estrous cycle. Note the occurrence of LH and PGFM peaks.
Plasma concentrations of LH were determined by the double-antibody RIA method described by Ziecik et al. [32] with modifications [17] using our own antiserum (SZ/Z/89/396). Purified pig LH (USDA-pLH-1) was used for the preparation of the radiiodinated antigen and USDA-pLH-B-1 as a standard. There was little competition between pLH and hCG, because hCG (0.1 to 100 mIU) failed to inhibit binding of the assay tracer by 50%. Concentrations of 10 and 100 mIU hCG/tube displaced iodinated LH at a rate of 97% and 73%, respectively. The assay sensitivity at 95% binding was 0.15 ng/ml and intra- and interassay coefficients of variation were 9.7 and 12.1%, respectively.

The concentration of progesterone was determined in duplicates by RIA described by Hotchkiss et al. [15]. The antibody used for this assay [6] was tested for cross-reactivity with six closely related steroids, all of which exhibited cross-reactions of <1%. The sensitivity of the assay was 0.1 ng/ml. Intra- and interassay coefficients of variation were 6.9 and 15.2%, respectively, based upon the analysis of a selected sample which was run with each assay.

**Statistical analysis**

All results are expressed as mean±SEM. The PGFM surges in gilts receiving hCG on Days 15-17 (experiment 1) were identified when three consecutive concentrations of hormone exceeded the baseline by 50% (fig. 2A). In group I the PGFM baseline was calculated on the basis of hormone concentrations determined at five time points before the beginning of hCG treatment. An average of five post-treatment concentrations of PGFM was used to create baselines for groups II and III.

To analyze PGFM response to hCG (fig. 2B), mean plasma concentrations of PGFM during the pre-infusion period A and the post-infusion periods B and C were subjected to one-way ANOVA for repeated measurements followed by Sheffe test for multiple comparisons (StatSoft Inc., Tulsa, OK, USA).

"Area under curve" subprogram of GraphPAD Prism (GraphPad Software, Inc., San Diego, USA) was used as an objective method to determine the frequency of episodic release of LH and peaks of PGFM between Days 12-18 of the porcine estrous cycle (experiment 2; figs. 4A-D).

**RESULTS**

**Experiment I**

Infusion of hCG did not affect plasma PGFM concentrations and profiles on Days 10 and 12-14 of the porcine estrous cycle (fig. 1). The basal concentrations of PGFM (pg/ml±SEM) before the beginning of hCG infusion were 110±10 and 140±40, respectively. In contrast, hCG administration on Days 15-17 of
the estrous cycle produced three different responses. The type of response depended on the PGFM level during the pre-infusion period A (fig. 2A). In group I (n=6 gilts), characterised by a low plasma pre-infusion level of PGFM, a surge of plasma PGFM was determined during the first post-infusion period B. The mean plasma PGFM concentration (fig. 2B) increased from 233.3±52.1 pg/ml (the pre-infusion period A) to 441.2±64.5 pg/ml (period B; p<0.01) and then dropped to 266.3±94 pg/ml (period C; p<0.01). In group II (n=6 gilts) characterised by medium plasma PGFM pre-infusion level (801±112.7 pg/ml), the post-treatment surge of PGFM was delayed. The mean plasma PGFM concentration decreased from 801±112.7 pg/ml during period A to 289.7±95.4 pg/ml during period B (p<0.01) and then increased again to 648.3±150.1 pg/ml (period C; p<0.05). No surge of plasma PGFM was observed in group III (n=6 gilts) which had the highest pre-infusion level of the prostaglandin metabolite (1271±480.8 pg/ml). The mean plasma PGFM concentration during periods B and C were very low (260.2±105.7 and 180.4±50.6 pg/ml, respectively). The pre-treatment level of plasma PGFM in group III (lack of PGFM response) was significantly higher (p<0.001) than that of group I (presence of PGFM surge during period B).

Figure 3 presents mean plasma concentrations of LH in gilts with a different type of response to gonadotropin (hCG administration on Days 15-17). Group III had the highest basal level of LH (ng/ml) before hCG infusion (0.31±0.06), did not differ from group I (0.21±0.06) and was higher (p<0.05) in comparison to group II (0.8±0.04). Administration of hCG caused a 2-3 fold increase in plasma LH level determined at the end of the infusion period. Such an increase, in LH determined by RIA, might be caused by a cross reaction with exogenous hCG. Treatment with hCG had no effect on LH concentrations after the hCG infusion. Infusion of saline did not affect plasma profiles or concentrations of LH.

The mean concentrations of PGFM and progesterone (data not presented) before and after saline infusion did not differ on Days 12-14 and 16 of the porcine estrous cycle.

**Experiment 2**

Figure 4 presents profiles of plasma progesterone, LH and PGFM concentrations in peripheral blood of four individual gilts (A, B, C, D) on Days 12-18 of the estrous cycle. LH was secreted and PGFM accumulated in blood in a pulsatile manner. Peaks of LH closely preceded surges of PGFM in 72.7% (A), 84.6% (B), 75.0% (C) and 66.6% (D) of cases. In all examined animals the highest peaks of PGFM were noticed on Days 16-17 of the estrous cycle when a decline in progesterone secretion occurred.
DISCUSSION

Peripheral plasma concentration of PGFM in studied gilts during the luteal phase as well as the time of luteolysis were comparable with those presented by Guthrie and Bolt [11], Printz et al. [24] and Kotwica et al. [18]. Typically, luteolysis in pigs is well underway by Days 14-16 of the estrous cycle [5, 13, 21]. Moreover, our current study confirmed the previous reports [11, 24] that porcine uterus is "tonically" secreting PGF$_{2\alpha}$ on Days 15-17 at a higher rate compared to earlier days of the estrous cycle. Treatment of gilts with hCG during Days 10 and 12-14 of the estrous cycle had no effect on plasma PGFM concentrations. It is of interest that in gilts a lack of PGFM response to an oxytocin challenge was also found on Days 10 and 12 of the estrous cycle [24]. On Day 15, however, the magnitude of the PGFM response to oxytocin was high [24]. In our study the PGFM response to hCG at the time of luteolysis depended on the pre-treatment level of prostaglandin metabolite in peripheral blood. The significant increase in prostaglandin F$_{2\alpha}$ secretion was found when PGFM concentration before the hCG infusion was low. The high pre-treatment PGFM level resulted in either lack of response or delayed surge of PGFM. Interestingly, the early work of Guthrie and Bolt [11] reported an increase in plasma PGFM after a single i.m. injection of low dose of hCG on Day 12 of the estrous cycle of gilts.

The high plasma concentration of PGFM before the beginning of hCG infusion during the time of luteolysis probably caused refractory or delayed responses of porcine endometrium. Oxytocin (OT)-induced refractoriness to subsequent stimulation has been demonstrated in pigs [24] and cattle [9, 16]. The question still remains open whether oxytocin causes PGF$_{2\alpha}$ release in intact gilts since the agreement between oxytocin and PGFM peaks was only about 30%. The blocking of oxytocin receptors neither prevented luteolysis nor changed the duration of the estrous cycle in swine [18]. It can be suggested that oxytocin may not be mandatory to induce endometrial release of PGF$_{2\alpha}$ in pigs. However, Whiteaker et al. [31, 32] showed a close relationship among OT, OT receptors and PGF$_{2\alpha}$ in vitro secretion from pig endometrium on 15-16 Days of the estrous cycle. On the other hand oxytocin caused similar releases of both PGF$_{2\alpha}$ and PGE$_2$ from porcine endometrium on Days 14-16 of the estrous cycle in vitro [35]. If oxytocin acts in the same way in vivo, then PGE$_2$ would neutralize the luteolytic effect of PGF$_{2\alpha}$ [1].

Present results confirmed that LH is released in a pulsatile manner during the luteal phase of the estrous cycle [23, 29]. Furthermore, a much higher agreement was found between LH and PGFM peaks (75.5%) than between oxytocin and PGFM (30%; [18]). Since both systemic infusion (our study) and i.m. injection of hCG [11] are able to induce PGF$_{2\alpha}$ release in vivo we suggest that the endogenous LH pulses may provoke prostaglandin secretion from
endometrium in pigs. The marked agreement between LH and PGFM pulses clearly supports such a notion.

This hypothesis is also supported by the results of our previous *in vitro* studies. Stepień et al. [28] demonstrated that the incubation of porcine endometrial explants with LH caused, in a dose dependent manner, an increase in PGFM accumulation. This was particularly evident at the time of luteolysis when the number of endometrial LH receptors was also the highest. The LH action in endometrium is due to the stimulation of cyclooxygenase (prostaglandin H synthase) expression [26, 27, 28]. The appearance of a relatively high amount of LH receptors in endometrium coincides with an increase of PGF₂α output [28] and perhaps with a down-regulation of progesterone receptors. Endometrial PGF release *in vitro* increased slightly from Days 8 to 14, then between Days 14 and 16 increased significantly [12]. In the same study progesterone release from luteal tissue was found to decrease from 18.0 ng/ml on Day 8 to 2.7 ng/ml tissue on Day 16. After initiating luteolytic secretion of PGF₂α on Days 14-16, endometrial LH receptors started to decline [28]. During the same period of the estrous cycle the number of LH receptors in porcine corpora lutea also decreases [33].

The "luteolytic" role of LH is limited only to the period of the late luteal phase of the porcine estrous cycle. Presence of blastocysts appears to overcomes this effect and brings back classical i.e. luteotrophic action of LH. Maternal recognition of pregnancy in pigs occurs on Days 11-12 and it is thought to be dependent on the production of estrogen by the blastocysts. Estrogen acts as an anti-luteolysin by causing a re-orientation of uterine PGF₂α secretion away from the vasculature into the uterine lumen [2]. In addition, the presence of blastocysts probably alters the release of the final product of prostaglandin synthesis, secreting PGE₂ instead of PGF₂α [1, 22] or at least changes the ratio PGF₂α:PGE₂ [4, 35]. Moreover, Gregoraszczuk and Michas [10] showed that increasing the ratio PGE₂:PGF₂α abrogated the luteolytic effect of PGF₂α on luteal cells collected from mature and regressing corpora lutea.

Since LH receptors have been found in endometrium of pregnant pigs [34] we suggest that estrogen produced by the blastocysts and/or early embryos increases and maintains LH receptors in the endometrium, which also ensures a higher output of PGE₂ to counteract the luteolytic action of PGF₂α. Recently, we have also found that exogenous estrogen administered between Days 11 and 15 after the onset of estrus caused an increase in LH induced PGE₂ output from porcine endometrium *in vitro* (G.Bodek and A.J. Ziecik – unpublished data). This is in agreement with data of Christensen et al. [3] who demonstrated that, in contrast to non-mated gilts, prostaglandin secretion in mated gilts peaked earlier with PGE₂ predominating. Endometrial LH receptors could be also involved in the production of vascular endothelial growth factor (VEGF). VEGF, in turn, may be involved in the process of implantation. The beneficial
effect of hCG on endometrial synthesis of VEGF was already shown in women during the late luteal phase by Licht et al. [20]. It is also possible that estrogen secreted by the conceptus is an additional stimulus for maintenance of luteal LH receptors by mechanism that is independent of the uterus in pigs [8].

In conclusion, we demonstrated that production of PGF$_{2\alpha}$ metabolite was increased after hCG infusion during the late luteal phase of the porcine estrous cycle. Moreover, we observed close relationship between plasma LH and PGFM peaks in gilts around the time of luteolysis. These facts suggest the LH involvement in the elevation of endometrial PGF$_{2\alpha}$ secretion in pigs, and, in consequence, induction of luteolysis.

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