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EFFECT OF AN OXYTOCIN ANTAGONIST ON PROSTAGLANDIN $F_{2\alpha}$ SECRETION AND THE COURSE OF LUTEOLYSIS IN SOWS

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The role of oxytocin (OT) in the regulation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion during luteolysis in gilts was studied using a highly specific OT antagonist (CAP-581). In Experiment 1 gilts on Days 14 to 19 of the oestrous cycle in Latin square design were used, to determine the dose and time of application of OT and CAP. In Group I (n = 6) gilts were treated intravenously with saline or with 10, 20 and 30 IU of OT. Concentrations of the main $PGF_{2\alpha}$ metabolite i.e. 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) were measured in blood samples as uterine response to the treatment. Twenty IU of OT was the most effective to stimulate PGFM release and this dose was used after CAP treatment in gilts of Groups II, III and IV. Gilts of Group II (n = 3) were injected into the uterine horns (UH) with saline (5 ml/horn) or CAP (2 mg, 3 mg and 4 mg; half dose/horn) and OT was injected (i.v.) 30 min thereafter. Any of the CAP doses given into the UH affected PGFM plasma concentrations stimulated by OT. In Group III (n = 4) gilts were infused (i.v.) for 30 min with CAP (9 mg, 14 mg and 18 mg/gilt) followed by 20 IU of OT. All doses of CAP effectively inhibited OT-stimulated $PGF_{2\alpha}$ release, therefore 9 mg was selected for the further studies. Gilts of Group IV (n = 4) received OT 4, 6 and 8 h after CAP to define how long CAP blocks the OT receptors. Concentrations of PGFM increased after any of this period of time. Thus, we concluded that 9 mg of CAP infused every 4 h will effectively block OT receptors. In Experiment 2, gilts (n = 4) received CAP as a 30-min infusion every 4 h on Days 12–20 of the oestrous cycle. Control gilts (n = 3) were infused with saline. CAP infusions diminished the height of PGFM peaks ($P < 0.05$). Frequency of the PGFM ($P < 0.057$) and OT ($P < 0.082$) peaks only tended to be lower in the CAP-treated gilts. Peripheral plasma concentrations of progesterone (P_4) and oestradiol-17β (E_2) and the time of luteolysis initiation as measured by the decrease of P_4 concentration were the same in CAP- and saline-treated gilts. The macroscopic studies of the ovaries in gilts revealed lack of differences between groups. We conclude that OT is involved in the secretion of luteolytic $PGF_{2\alpha}$ peaks but its role is limited to controlling their height and frequency. Blocking of OT receptors did not prevent luteolysis in sows.

Key words: Oxytocin antagonist, oxytocin, prostaglandins, luteolysis, sows

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) of uterine origin is suggested to be necessary for luteal regression in sows (Gleeson et al., 1974; Moeljono et al., 1977). OT is assumed to be involved in the regulation of $PGF_{2\alpha}$ secretion at luteolysis (Kotwica et al., 1990; Mirando et al., 1996). (*i*) Treatment of sows with OT during late but not at early and mid-cycle increases $PGF_{2\alpha}$ secretion and largest OT responsiveness is at luteolysis (Kieborz et al., 1991; Carnahan et al., 1996; Edgerton et al., 1996). (*ii*) OT acts through the specific OT receptors the concentration of which is low in the endometrium during the early and mid-luteal phase, with a tendency to increase around luteolysis and it is the highest at oestrus (Whiteaker et al., 1994; Okano et al., 1996). However, increase of responsiveness to OT and subsequent $PGF_{2\alpha}$ secretion is weakly associated with changes in OT receptor numbers but it is strongly connected with coupling of OT receptors to G_q protein and their affinity to the ligand (Ludwig et al., 1998). (*iii*) Intracellular signalling pathway from the OT receptors depends on stimulation of phospholipase C activity to initiate phosphoinositide (PI) hydrolysis and formation of second messengers – inositol triphosphate (IP_3) and presumably diacylglycerol (DAG) (Whiteaker et al., 1995; Tysseling et al., 1996). In *in vitro* studies stimulation of endometrial PI hydrolysis by OT is positively correlated with $PGF_{2\alpha}$ release (Mirando et al., 1995; Whiteaker et al., 1995). Since $PGF_{2\alpha}$ and its metabolite PGFM are released during luteolysis in a pulsatile manner (Gleeson et al., 1974; Moeljono et al., 1977; Shille et al., 1979), the role of OT in the control of $PGF_{2\alpha}$ peaks secretion is possible, but this mechanism is unknown. Moreover, only pharmacological doses of OT, both in *in vitro* and *in vivo* experiments, are able to stimulate $PGF_{2\alpha}$ release in cyclic sows (Kieborz et al., 1991; Whiteaker et al., 1995). Thus, to assess the role of OT in $PGF_{2\alpha}$ release and the course of luteolysis, OT receptors were blocked by the continuous treatment of cyclic gilts with CAP, an OT antagonist (1-deamino-2-D-Tyr(Oet)-4-Thr-8-Orn-oxytocin, Ferring AB, Sweden). CAP binds directly to OT receptors and blocks PI hydrolysis (Lopez-Bernal et al., 1989; Melin, 1993).

Materials and methods

Animals and surgical procedure

Twenty-four gilts (103 ± 4.0 kg) during their second or third oestrous cycle were used in this study; 17 and 7 animals in Experiment 1 and Experiment 2, respectively. Four days before the experiments, polyvinyl chloride catheters were inserted into the right and left jugular veins (JV) in all gilts under general anaesthesia with pentabarbitone sodium (12 mg/kg b.w.) (Kotwica et al., 1978). Additionally, both uterine horns were cannulated in three gilts of Experiment 1 for saline and CAP infusions (Schneider et al., 1983). In the remaining gilts sa-

line, OT or CAP were given throughout the right JV cannulae. Blood samples were collected from the left vessel without restraining the animals.

Experiment 1

On Days 14–19 of the oestrous cycle gilts (n = 17) were used to establish the dose and way of the CAP infusion which would effectively block the function of OT receptors in the uterus. Gilts in the groups were treated in Latin square design. In Group I (n = 6) saline (2 ml) or OT (10 IU, 20 IU and 30 IU) were given intravenously (i.v.). In Group II (n = 3) saline (5 ml/horn) or CAP (2 mg, 3 mg and 4 mg) were injected into the uterine horns (the half dose/horn) and OT (20 IU) in bolus (i.v.) injection was given 30 min thereafter. In Group III (n = 4) CAP (9 mg, 14 mg and 18 mg/gilt) was infused for 30 min i.v. and OT (20 IU) in bolus injection was given immediately thereafter, while in Group IV (n = 4) CAP (9 mg) was given as in Group III and OT (20 IU) was injected 4 h, 6 h and 8 h after infusion of CAP, which was always dissolved in 10 ml of saline, 30 min before its infusion. Blood samples (6 ml) were taken every 10 min for 1 h before OT injection and every 5 to 20 min for 1 h after that. In Group III blood samples were collected every 5 to 10 min for two 3-h periods during saline or CAP infusions. Concentrations of PGFM were measured in plasma samples as uterine response to OT treatment.

Experiment 2

The model of CAP infusion established in Experiment 1 was applied in Experiment 2. Gilts (n = 4) were infused (i.v.) with 9 mg of CAP in 10 ml of saline for 30 min every 4 h from Day 12 to Day 20 of the oestrous cycle. Control gilts (n = 3) received saline in the same way. Blood samples were collected every hour during the experiment, starting four hours before the first infusion. Concentrations of PGFM, OT, progesterone and oestradiol-17 β were determined in blood samples. For macroscopic examination of the ovaries, gilts were slaughtered in the local slaughterhouse on the third and fourth day following the last infusion.

Hormone analysis

Concentrations of hormone were determined by RIA described for progesterone (P_4) and oestradiol-17 β (E_2) (Hotchkiss et al., 1971), OT (Schams et al., 1979) and PGFM (Homanics and Silvia, 1988). The specificity of the antibodies used was given earlier: for P_4 (Dziadkowiec et al., 1982), E_2 (Szafranska and Tilton, 1993), OT (Kotwica and Skarzynski, 1993) and PGFM (Homanics and Silvia, 1988). OT antibodies used in our study did not cross-react with CAP. Efficiency of extraction for E_2 and OT was 89.0 ± 1.1 and 85.0 ± 0.8%, respectively. Final data were corrected for the procedural losses. The sensitivity of the assay was: P_4 –

0.1 ng/ml, E_2 – 2.5 pg/ml, OT – 3 pg/ml and PGFM – 60 pg/ml. The coefficient of correlation (r) between added and recovered amounts of different hormone concentrations (2–4 concentrations in blank plasma) were: P_4 – 0.991, E_2 – 0.988, OT – 0.836 and PGFM – 0.992. The intra- and interassay CVs were: P_4 – 6.9% and 8%, E_2 – 8.9% and 12.3%, OT – 8.6% and 14% and PGFM – 4.2% and 6.0%.

Statistical analysis

The baselines for PGFM in Experiment 1 were calculated on the basis of its seven determinations before OT or saline treatment and then compared by *t*-test with the post-treatment PGFM concentrations. The peaks for OT and PGFM in Experiment 2 were defined when any 3 consecutive concentrations of hormones exceeded the baselines by 50%. The baselines of hormones were calculated on the basis of the four determinations before the first infusion of CAP or saline. The mean (± SEM) values between groups were compared by *t*-test using PRISM software (GraphPAD, San Diego, CA, USA).

Results

Experiment 1

In Group I the effective dose of OT for stimulation of PGFM secretion was established. OT given to gilts at the dose of 20 IU most effectively stimulated PGFM release ($P < 0.001$ *vs.* baseline; Fig. 1 and Table 1). Ten IU of OT had weaker influence ($P < 0.05$) and 30 IU and saline did not increase plasma PGFM concentrations ($P > 0.05$). Thus, in gilts of the next groups the dose of 20 IU was used. Any of CAP doses given into uterine horns (Group II) were effective and OT (20 IU) stimulated PGFM release ($P < 0.001$, Fig. 1, Fig. 2, Table 1). Oxytocin (20 IU) injected immediately after infusion of CAP at doses of 9 mg, 14 mg or 18 mg did not increase PGFM concentration (Group III; $P > 0.05$). When OT (20 IU) was administered 4 h, 6 h or 8 h following infusion of 9 mg CAP the post-treatment PGFM plasma level also did not exceed the baseline ($P > 0.05$, Fig. 1 and Table 1).

Experiment 2

Administration of CAP diminished ($P < 0.05$) the height of the PGFM peaks compared to saline infusion and tended to reduce the number of PGFM peaks ($P < 0.057$; Table 2). The first PGFM peak related to the luteolytic release of $PGF_{2\alpha}$ (accepted arbitrarily as > 500 pg/ml) occurred at 122.8 ± 11.3 h and at 72.0 ± 11.8 h from the first infusion of CAP and saline, respectively, and these data were statistically different ($P < 0.03$). The height, amplitude, and number of OT peaks were the same ($P > 0.05$) in CAP- and in saline-treated gilts (Table 2).

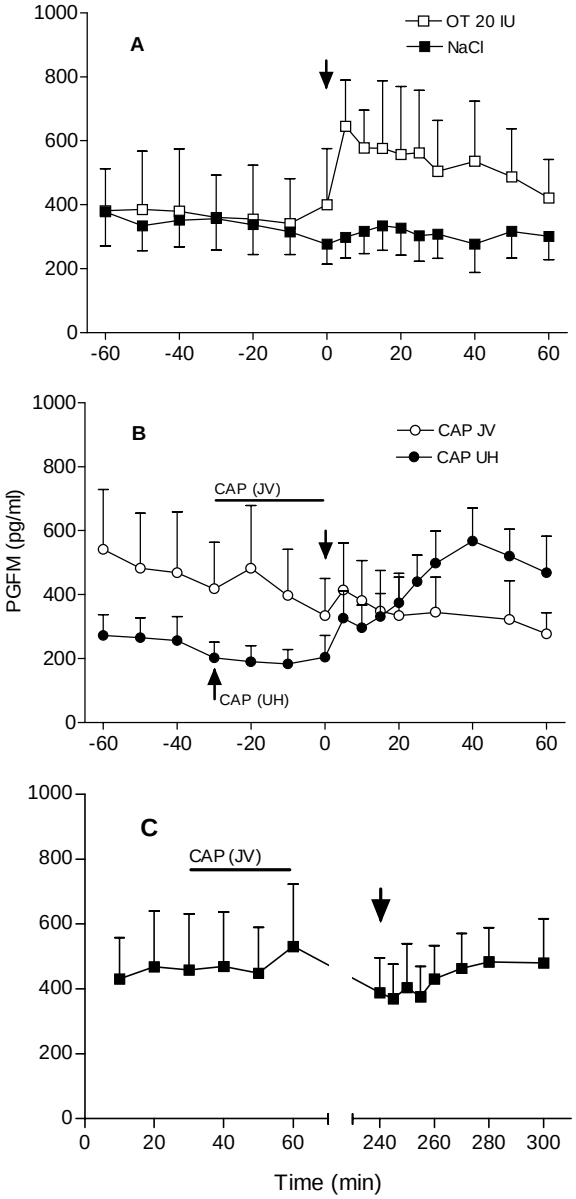

Fig. 1. Plasma concentrations of PGFM (mean ± SEM) in gilts of Experiment 1. (A) – gilts of Group I (n = 6) received bolus injection (i.v.) of saline or OT. (B) – gilts of Group II (n = 3) were injected into the uterine horns (UH) with CAP (2 mg–4 mg – since there was no difference between treatments, data were combined) and OT (20 IU; i.v.) was administered 30 min later; in Group III (n = 4) CAP (9 mg) was infused (30 min) into JV and OT (20 IU; i.v.) was given immediately thereafter. (C) – gilts of Group IV (n = 4) received a 30-min infusion of 9 mg of CAP at 4 h before OT (20 IU)

Table 1

Influence of saline (2 ml) and OT (20 IU) i.v. injections and CAP (2–4 mg) injection into the uterine horns (UH) 30 min before OT (20 IU), and CAP (9 mg) infusions into the jugular vein (JV) immediately before or 4 h prior to OT (20 IU i.v.) on PGFM plasma concentrations (pg/ml; means ± SEM) in gilts

Treatment	Baseline (–60–0 min)	After OT (0–60 min)
Saline (n = 6)	336 ± 30	310 ± 7
OT (n = 6)	372 ± 23[a]	541 ± 21[b]
CAP (UH) + OT (n = 3)	225 ± 22[a]	425 ± 32[b]
CAP (JV) + OT (n = 4)	446 ± 58	346 ± 41
CAP (JV) + OT 4 h later (n = 4)	483 ± 58	424 ± 41

[a,b]Values in the same row with different superscripts are significantly different (P < 0.001)

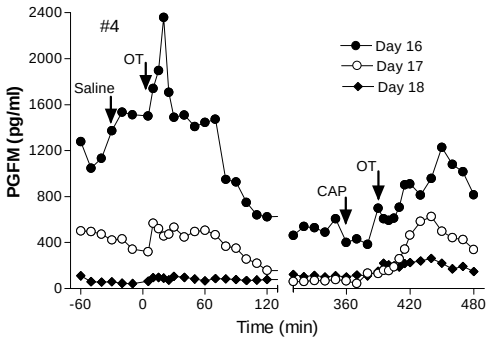

Fig. 2. Plasma PGFM concentrations in an individual gilt in response to bolus injections of OT (20 IU) into JV preceded by injection of saline or CAP (2 mg - Day 16, 3 mg - Day 17 and 4 mg - Day 18) into uterine horns

Table 2

Characterisation of PGFM and OT peaks in the saline and OT antagonist (CAP) treated gilts from Day 12 to 20 of the oestrous cycle (mean ± SEM)

Treatment	Hormone	Highest value in peak (pg/ml)	Amplitude (pg/ml)	No. of peaks per gilt	No. of peaks per 24 h
Saline	PGFM	940 ± 129[a]	503 ± 85	14.7 ± 0.9[a]	1.63 ± 0.1
(n = 3)	OT	30.4 ± 1.5	12.0 ± 1.3	12.7 ± 0.9	1.4 ± 0.2
CAP	PGFM	663 ± 55[b]	402 ± 45	11.5 ± 1.3[c]	1.28 ± 0.1
(n = 4)	OT	33.7 ± 1.4	11.4 ± 1.2	8.8 ± 1.3	1.0 ± 0.1

Means in the same column for the same hormone with different superscripts differ [a,b](P < 0.05); [a,c](P < 0.057)

Agreement between OT and PGFM peaks was similar (P = 0.23) in CAP- and saline-treated gilts and it was 17% and 30%, respectively. The mean concentrations of P_4 before luteolysis did not differ (P < 0.83) in CAP- (25.5 ± 0.4 ng/ml) and saline- (25.0 ± 0.5 ng/ml) treated gilts. The continuous decline of plasma P_4 (below 10 ng/ml) was observed at 117.8 ± 16.2 h and 99.0 ± 7.0 h from the first infusion of CAP and saline, respectively (P < 0.39). The mean concentrations of E_2 before luteolysis did not differ (P = 0.23) in gilts treated with CAP (36.0 ± 0.9 pg/ml) and saline (42.4 ± 1.0 pg/ml). The ovaries from gilts receiving CAP and saline were similar and they were typical of the periovulatory period of the oestrous cycle. Eleven to 16 follicles near ovulation (∅ 0.7–1 cm) were observed in CAP- (#25 and #32) and saline-treated gilts (#27, #28 and #34) and corpora lutea in haemorrhagicum stage in CAP-treated gilts (#26 and #33) (Figs 3 and 4).

Discussion

Injections of OT at doses of 10 (P < 0.05) and 20 IU (P < 0.001) increased plasma PGFM concentrations above baseline, but 30 IU was ineffective. An explanation can be the age of gilts we used, since in multiparous sows 30 IU of OT stimulated PGFM secretion (Kieborz et al., 1991; Edgerton et al., 1996). After administration of 20 IU of OT the mean plasma concentration of OT increased nine-fold (from 20.3 ± 2.0 pg/ml to 179.0 ± 29 pg/ml) and this high level lasted for about one hour. It was accompanied by an increase of PGFM concentration (r = 0.78; P = 0.0009).

The CAP infusion into JV at the dose of 9 mg suppressed PGFM secretion when OT (20 IU) was injected 4 h later (after CAP treatment). Thus, we assumed that 9 mg of CAP given every 4 h effectively blocked $PGF_{2\alpha}$ release by OT and this regimen was used in Experiment 2.

The concentration of OT receptors in swine endometrium is constant between Days 10 to 16 post-oestrus but at this time their affinity increased markedly (Ludwig et al., 1998). Responsiveness to exogenous OT develops before the onset of functional luteolysis (Days 12 to 14), but treatment with OT increased $PGF_{2\alpha}$ secretion on Days 14 to 18 of the cycle with maximal release of hormone on Day 16 (Kieborz et al., 1991; Edgerton et al., 1996; this study). Therefore, the CAP infusion in this study was started before the time of the maximal responsiveness of OT receptors on OT (Day 12) and lasted beyond the time of spontaneous luteolysis (Day 20). However, this did not prevent the pulsatile release of PGFM, though the height of PGFM peaks was statistically lower (P < 0.05) in gilts receiving CAP compared to the control. The frequency of PGFM peaks also tended to be smaller (P = 0.057) in the CAP group. The first luteolytic peak of PGFM occurred later in CAP-treated than in saline-treated

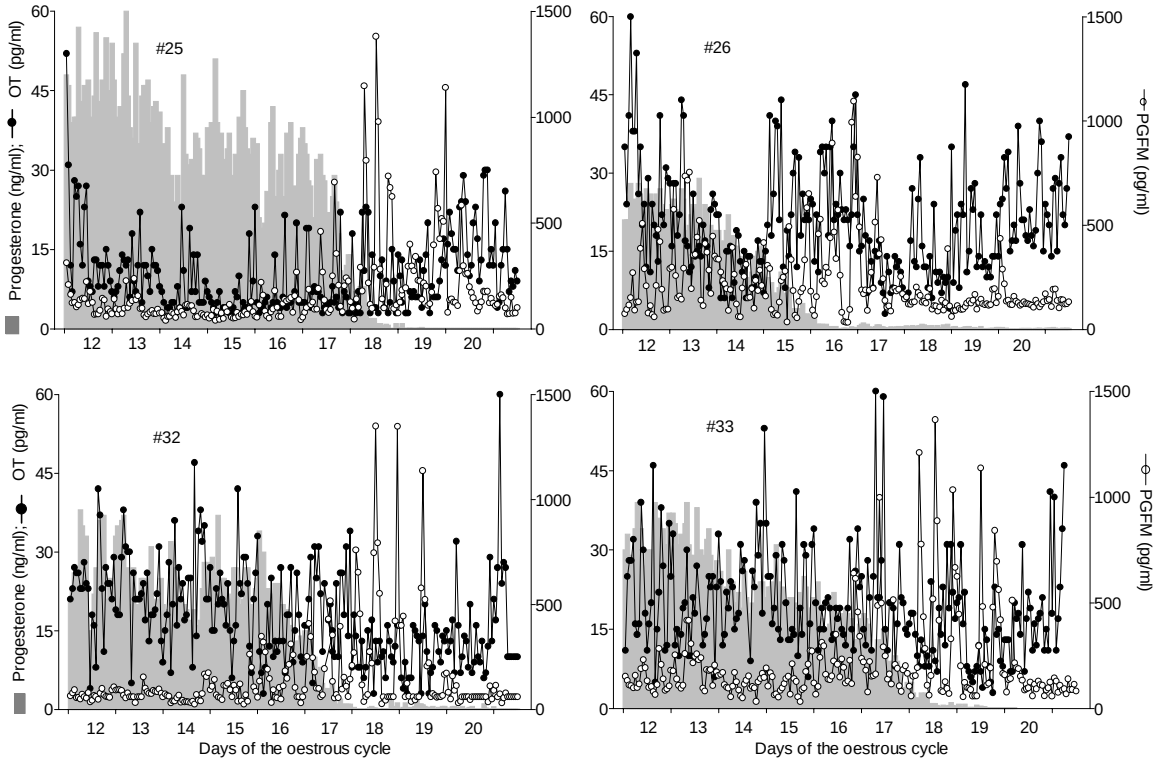

Fig. 3. Concentrations of progesterone, oxytocin and PGFM in blood plasma samples collected every hour in four CAP-treated gilts between Days 12 and 20 of the oestrous cycle. Gilts received a 30-min infusion of 9 mg CAP every 4 h

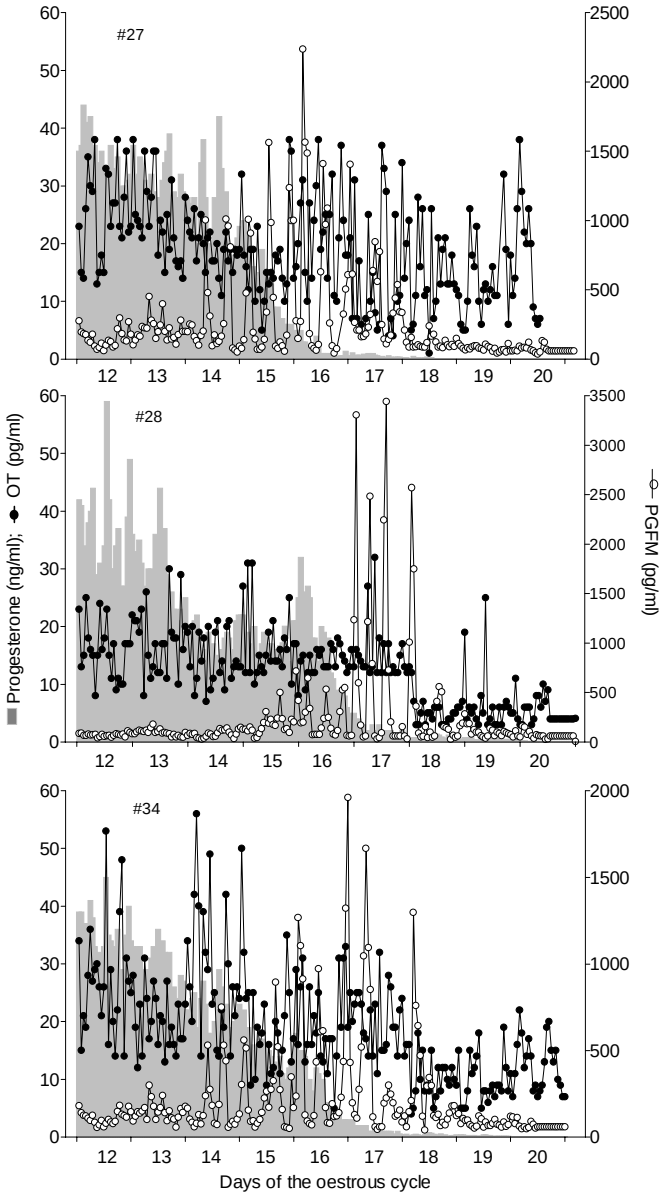

Fig. 4. Concentrations of progesterone, oxytocin and PGFM in blood plasma samples collected every hour in three saline-treated gilts between Days 12 and 20 of the oestrous cycle. Gilts received a 30-min infusion of 10 ml saline every 4 h

gilts (P = 0.03). The decrease of PGFM secretion had no influence on the initiation of luteolysis. Ovaries, examined after slaughter of gilts, were similar in both groups. Thus, the lower $PGF_{2\alpha}$ secretion was sufficient to cause the luteolysis anyway. Similar data were obtained by Rampacek et al. (1979) who could induce ovulation in prepubertal gilts, though they had lower $PGF_{2\alpha}$ peaks and these peaks were observed later as compared to mature sows. Collectively, our results indicate that OT may be the only one of the many factors which control the initiation, quantity and duration of pulsatile $PGF_{2\alpha}$ release in sows.

The uterus is known to be necessary for $PGF_{2\alpha}$ release by OT, because in hysterectomized sows OT injections did not increase plasma hormone concentrations (Carnahan et al., 1996). In swine endometrium, glandular epithelial cells and luminal surface produce greater quantities of $PGF_{2\alpha}$ than stromal cells (Davis and Blair, 1993). However, in *in vitro* studies OT-stimulated $PGF_{2\alpha}$ secretion was highest from the stromal cells, much lower from glandular epithelial cells, and the luminal surface was unresponsive to OT (Uzumcu and Mirando, 1996; Uzumcu et al., 1997). Thus, in swine OT seems to stimulate $PGF_{2\alpha}$ release from a small part of endometrial cells (stromal cells), synthesising only low amount of $PGF_{2\alpha}$. Therefore, it is possible in our study that in CAP-infused gilts only stromal cells (able to respond to OT) were blocked, whereas the other types of endometrial cells were still able to produce $PGF_{2\alpha}$ in spite of CAP infusion due to the lack of OT receptors. Deficiency of CAP influence on luminal surface (unresponsive to OT) is also suggested by results of our studies in Group II. CAP at doses from 2 to 4 mg/gilt given directly into both uterine horns did not suppress OT-induced $PGF_{2\alpha}$ secretion.

The swine myometrium contains $PGF_{2\alpha}$ during luteolysis (Stefańczyk-Krzymowska et al., 1994). CAP can prevent myometrial contractions induced by OT (Melin, 1993). However, it is not known if OT receptors are involved in $PGF_{2\alpha}$ release by the myometrium.

The lack of full inhibition of PGFM release by CAP in our experiment may also be connected with the ability of the uterus to synthesise OT (Trout et al., 1995; Boulton et al., 1996). Whether this endogenous OT can compete for the uterine receptor and thus impair the effect of CAP may be only speculated.

The results of this study suggest the lack of involvement of OT in regulating the length of oestrous cycle in gilts. In contrast to our data, exogenous OT given on Days 10 to 16 of the cycle shortened the inter-oestrus interval by 1–2 days (Prince et al., 1995). This divergence is difficult to elucidate. Prince et al. (1995) started to give OT on Day 10, *i.e.* 2 days earlier than CAP was infused in our study. Ludwig et al. (1998) found that the secretory pathway between protein G_q and $PGF_{2\alpha}$ release from endometrial cells is fully functional from Day 10; however, $PGF_{2\alpha}$ secretion after OT treatment is not stimulated before

Day 16. Thus, the question about the mechanism of luteolysis initiation in pigs is still open. One of the candidates in this mechanism can be P_4. This hormone seems to play an important role as a regulator of pulsatile release of $PGF_{2\alpha}$ in ewes and cattle (Ottobre et al., 1980; Lafrance and Goff, 1988). In sows that received exogenous P_4 on Days 1 to 5 after oestrus, the premature basal and OT-induced elevation of PGFM plasma concentration was also observed (Printz et al., 1994), although this affected neither the length of oestrous cycle nor endometrial OT receptor concentration (Printz et al., 1994; Uenoyama et al., 1997). It is suggested that the duration of P_4 exposure of the porcine uterus may be important for determination of the time when luteolytic $PGF_{2\alpha}$ secretion will start.

OT antagonists were used for studies on the role of OT in controlling luteolysis in ruminants, but the results are not consistent. It prolonged the duration of the cycle, elevated P_4 concentration until Day 23 post-oestrus and diminished peaks of $PGF_{2\alpha}$ observed during luteolysis in ewes (Jenkin et al., 1994). Similarly, an OT antagonist [dET_2 Tyr (Et)OVT] suppressed the increase in PGFM concentration and extended the length of the oestrous cycle in goats (Homeida and Khalafalla, 1987). However, in cattle CAP treatment did not change the duration of the cycle and P_4 and PGFM pulsatile secretion (Kotwica et al., 1997).

The agreement between OT and PGFM peaks in our study ranged between 17% (CAP-treated gilts) and 30% (saline-treated gilts). It is possible that sampling at 1-h intervals may have failed to detect some hormone peaks. Supposedly, therefore, the correlation between OT and PGFM peaks in this study was less evident than found in sheep (Hooper et al., 1986). On the other hand, the lower frequency of OT peaks in CAP-treated gilts as compared to saline-treated ones was accompanied with the lower frequency of PGFM surges. Recent studies by Walker et al. (1997) have revealed in ewes that pulses of $PGF_{2\alpha}$ which were linked to OT secretion ranged from 36% to 53%. These pulses had a longer duration and a higher amplitude than pulses that were not linked. In our study, peaks of PGFM were lower and less frequent in CAP-infused gilts. This confirms that OT affects the shape of the luteolytic $PGF_{2\alpha}$ peaks in sows and ewes.

In conclusion, data obtained in this study show that OT may be involved in the control of pulsatile release of $PGF_{2\alpha}$ at luteolysis in sows. However, its role is not mandatory, since the blockade of OT uterine receptors diminished only the height and frequency of PGFM peaks. Moreover, the blockade of OT receptors neither prevents luteolysis nor changes the duration of the oestrous cycle in swine.

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