Praca oryginalna *Original paper*

Effect of estradiol, progesterone and interleukin-1β on prostaglandin E2 and F2 secretion by porcine corpora lutea in vitro during the periimplantation period*)

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Summary

This study examines the influence of ovarian steroids and interleukin-1β on prostaglandin E2 (PGE2) and F2α (PGF2α) synthesis and secretion from corpora lutea (CL) slices obtained from ovaries of gilts on days 12-14 of pregnancy and days 12-14 of the estrous cycle. For this study the authors used a surgically-generated model in which one of the uterine horns was cut transversely and a in separated part of this horn embryos did not develop. Collected corpora lutea were cut into slices and treated for 6 h with estradiol (E2) (100 nM/ml), progesterone (P4) (50 nM/ml) and interleukin-1β (IL-1β) (2.5 ng/ml). Results indicated that P4 increased PGE2 synthesis in CL slices originating from the ovary ipsilateral to the uterine horn with the developing embryos. P4 did not influence PGF2α synthesis in cultured CL slices from the ovaries of pregnant gilts regardless of embryos present in the uterine horn. In contrast to the effect of P4, E2 increased PGF2α synthesis in cultured CL slices from the ovary ipsilateral to the uterine horn with the developing embryos. E2 had no effect on PGF2α content in CL slices from the contralateral ovary of pregnant gilts and CL slices from both ovaries of cyclic gilts but increased PGF2α release from CL slices from the ipsilateral ovary of pregnant gilts. IL-1β markedly increased PGE2 release from cultured CL slices from the ovary contralateral to the uterine horn with the developing embryos from pregnant gilts and cyclic gilts regardless of CL origin.

Keywords: prostaglandins, corpus luteum, early pregnancy

Prostaglandins belong to the most important factors involved in the process of maternal recognition of pregnancy and are crucial regulators of the corpus luteum lifespan. Prostaglandin (PG) F2α (8) is considered as a luteolysin, while PGE2 acts in a luteotrophic manner, promoting the maintenance of early pregnancy (1, 5, 24). Besides PGs of uterine origin, luteal PGE2 and PGF2α are involved in the autoregulation of CL functioning. It has been reported that luteal cells possess an inherent capacity to produce PGs in most mammalian species (2).

The luteal production of prostaglandins is regulated by many endocrine or paracrine mediators, including ovarian steroids. The effect of ovarian steroids on PGs synthesis in the tissues of the reproductive tract is well documented (17). It was indicated that estradiol (E2) and progesterone (P4) influence prostaglandins synthesis in luteal cells *in vitro* (9, 15, 16). Moreover, there is increasing evidence that the immune system participates in the regulation of gonadal functioning. Interleukin-1β (IL-1β) and other proinflammatory cytokines are potential factors involved in the modulation of luteal cells steroids secretion (12, 14). It was found that IL-1β is produced not only by macrophages infiltrating the ovary but also by other cell types of the ovarian tissue. IL-1β was found in follicular fluid and its receptor mRNA and protein were detected in granulosa cells (11, 18, 19, 22).

The aim of the present study was to determine the effect of E2, P4 and IL-1β on PGE2 and PGF2α secretion by cultured slices of corpora lutea from the ovaries ipsi- and contralateral to the uterine horn within the developing embryos on days 12-14 of pregnancy and days 12-14 of the estrous cycle using a surgically--generated model of porcine uterus.

Material and methods

Animals and experimental protocol. All procedures involving animals were conducted in accordance with the Local Research Ethics Committee national guidelines for

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agricultural animal care. Prepubertal gilts (n = 6) with an average body weight of 100 kg and 7-months-of-age were used in our studies. The gilts were subjected to surgical procedure under general anesthesia. The uterine horns were presented by a midventral opening of the caudal part of the abdomen. One uterine horn of each gilt was cut transversely and the endings were closed by a suture. This way an experimental model was created in which the pig uterus consisted of a one whole uterine horn and a part of the second horn both connected with the uterine corpus. The remaining part of the second horn connected with the contiguous ovary was surgically detached from the uterine corpus. After a 10-day recovery from surgery, gilts were treated hormonally by an intramuscular injection of 750 I.U. PMSG (Folligon, Intervet) and 500 I.U. of hCG (Chorulon, Intervet) given 72 hours later. Subsequently, gilts (n = 6) were inseminated 24 h after hCG treatment. The insemination was repeated twice at 12 h intervals. The control group (n = 6) consisted of gilts subjected to the surgical procedure and hormonal treatment but not inseminated. At the days 12-14 of pregnancy or estrous cycle gilts were slaughtered. To confirm pregnancy, the uterine horns were flushed with 10 ml PBS (pH 7.4) to determine the presence of embryos in uterine flushings. Corpora lutea from both ovaries, ipsi- and contralateral to the uterine horn were collected with the developing embryos.

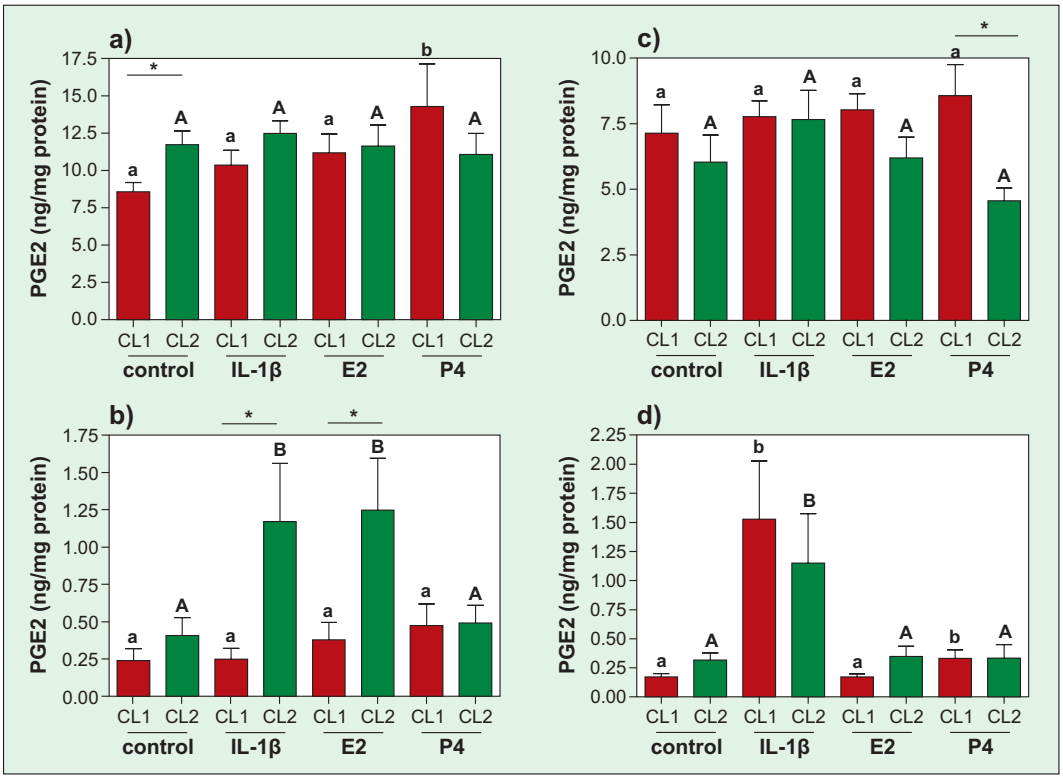

Fig. 1. PGE2 content in CL slices from the ovaries ipsi- (CL1) and contralateral (CL2) to the uterine horn with the developing embryos and in media after incubation of the slices of pregnant (a, c) and cyclic gilts (b, d) with E2, P4 and IL-1β. Figures: a and b – PGE2 content in homogenates of CL slices after incubation; c and d – PGE2 content in media after incubation of CL slices

Explanations: Values are expressed as means ± SEM. Different letters above the bars indicate significant differences ($P < 0.05$); a, b – mean differences in comparison to the control of CL1, A, B – mean differences in comparison to the control of CL2, * – means differences between CL1 and CL2

Corpora lutea (CLs) were cut into 50 mg slices that were placed in individual vials containing 1 ml of Medium 199 (Sigma, USA) supplemented with 0.1% BSA fraction V (Sigma, USA). CL slices were pre-incubated in a water bath at 37°C for 18 h in an atmosphere of 5% CO_2 in the air and then treated for 6 h with E2 (100 nM/ml), P4 (50 nM/ml) and IL-1β (2.5 ng/ml). After incubation culture medium was collected and CL slices were homogenized and stored at –70°C for further analysis of PGE2 and PGF2α content in homogenates and medium.

Homogenization of the tissues. The homogenization of the CL slices was performed with the use of Ultra-Turax T25 homogenizer (IKA, Sweden). Frozen tissue was cut with a scalpel on the Petri dish and transferred into tubes containing a homogenization buffer composed of the following components: 150 mM NaCl, 1 mM EDTA, 0.02% NaN_3 (POCH, Poland), 1% Triton X-100 (Sigma, USA), proteases inhibitors – aprotinin 10 µg/ml, leupeptin 25 µg/ml pepstatin, 1 µM/ml and PMSF 100 µM/ml (Sigma, USA) and 10 µl/ml of stabilization buffer (0.3 M EDTA and 1% acetylosalicylic acid, pH = 7.4); pH 8.0. Each sample was homogenized three times for 20 sec. The suspension was then centrifuged at 5000 rpm at 4°C for 20 min (Beckman, UK). After centrifugation the supernatant was collected and kept at –70°C for further hormonal analyses.

PGE2 and PGF2α determination. PGE2 content in CL homogenates and media was determined by a direct EIA test according to Skarzynski and Okuda (20). To determine the content of PGF2α, a direct enzyme immunoassay (EIA) test described by Uenoyama et al. (21) was used. The dilutions of anti-sera were 1 : 150 for PGF2α and 1 : 300 and 1 : 200 for PGE2 in homogenates and medium, respectively. Standard curves for PGE2 and PGF2α ranged from 0.19-100 ng/ml and 1.9-60 ng/ml, respectively. The intra- and interassay coefficients of variation for PGE2 were 7.6 and 14.9, and 7.5 and 11.4 for PGF2α. The sensitivity of the assay was 0.19 ng/ml and 1.9 ng/ml for PGE2 and PGF2α, respectively. Mean concentrations of hormones were presented as ng of a hormone per mg of protein. Total tissue protein in homogenates was determined with the Bradford method 1976 (4).

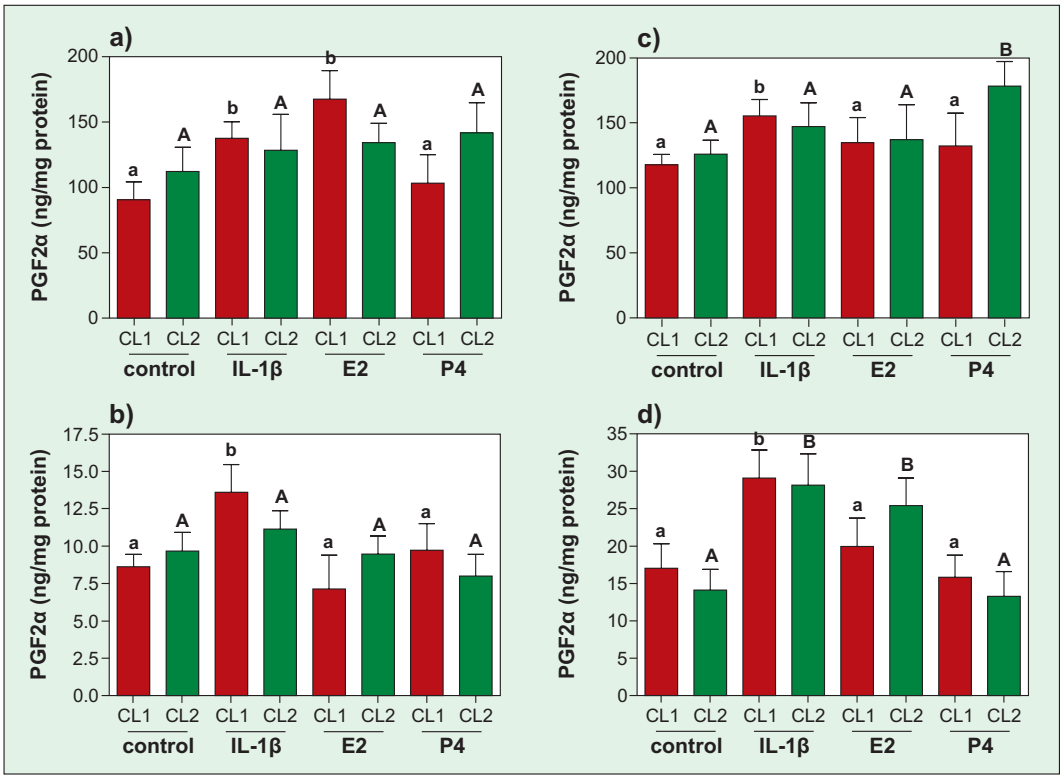

Fig. 2. PGF2α content in CL slices from the ovaries ipsi- (CL1) and contralateral (CL2) to the uterine horn with the developing embryos and in media after incubation of the slices of pregnant (a, c) and cyclic gilts (b, d) with E2, P4 and IL-1β. Figures: a and b – PGF2α content in homogenates of CL slices after incubation; c and d – PGF2α content in media after incubation of the slices

Explanations: as in fig. 1.

Results and discussion

Exposure of CL slices from the ovary ipsilateral to the uterine horn with the developing embryos to P4 *in vitro* resulted in enhanced PGE2 synthesis on days 12-14 of pregnancy in comparison to non-treated slices ($p < 0.05$; fig. 1a). Only the tendency to increase PGE2 synthesis after the incubation of CL slices from the ipsilateral ovary with E2 was observed ($p = 0.08$, fig. 1a), whereas E2 significantly elevated PGF2α synthesis ($p < 0.05$; fig. 2a). IL-1β treatment had no effect on PGE2 synthesis in CL slices from both ovaries of gilts on days 12-14 of pregnancy ($p > 0.05$; fig. 1a); however, IL-1β stimulated PGF2α synthesis but only in CL slices from the ovary ipsilateral to the uterine horn with the developing embryos ($p < 0.05$; fig. 2a). Basal PGE2 synthesis was significantly higher in CL slices from the ovary contralateral to the uterine horn with the developing embryos in comparison to CL slices from the ipsilateral ovary ($p < 0.05$; fig. 1a). There were no significant differences in PGF2α synthesis between CL slices from the ipsi- and contralateral ovaries of pregnant gilts ($p > 0.05$; fig. 2a).

IL-1β and E2 significantly increased PGE2 release to the medium after incubation of CL slices from the ovary contralateral to the uterine horn with the developing embryos on days 12-14 of pregnancy ($p < 0.05$; fig. 1b). Moreover, IL-1β treatment resulted in elevated release of PGF2α from CL slices from the ovary ipsilateral to the uterine horn with the developing embryos ($p < 0.05$; fig. 2b). PGE2 release was significantly higher after incubation with IL-1β from CL slices from the ovary contralateral to the uterine horn with the developing embryos ($p < 0.05$; fig. 1b) in comparison to CL slices from the ipsilateral ovary. In the authors' study E2 increased PGE2 release from CL slices from the contralateral ovary in comparison to its secretion from CL slices from the ipsilateral ovary ($p < 0.05$; fig. 1b).

There was no effect of the investigated steroids on PGE2 synthesis in CL slices collected on days 12-14 of the estrous cycle ($p > 0.05$; fig. 1c), while PGF2α synthesis was enhanced significantly by IL-1β in CL slices from the ipsilateral ovary, as well as by P4 from CL slices from the contralateral ovary ($p > 0.05$; fig. 2c). PGE2 and PGF2α contents in the medium after incubation of CL slices both from the ipsi- and contralateral ovaries on days 12-14 of the estrous cycle were significantly higher after IL-1β treatment ($p < 0.05$; fig. 1d, fig. 2d, respectively). In the present study it was indicated that PGE2 release from CL slices from the ipsilateral ovary was elevated after incubation with P4 ($p < 0.05$; fig. 1d). E2 significantly increased PGF2α release from CL slices from the contralateral ovary in comparison to its basal secretion ($p > 0.05$; fig. 2d).

In the present study P4 increased PGE2 synthesis in CL slices from the ovary ipsilateral to the uterine horn with the developing embryos, which indicates that embryos and products of their secretion enhanced sensitivity of CL to P4 supporting its luteotrophic activity. This effect was not observed in case of PGF2α synthesis in CL slices obtained from the ovaries of pregnant gilts cultured with P4. Moreover, Farina et al. (6) found that P4 not only increased PGF2α but significantly inhibited uterine production of PGF2α *in vivo* in rats. In the studies of Franczak et al. (7) P4 was without effect on myometrial PGE2 and PGF2α secretion regardless of the reproductive status (estrous cycle and pregnancy). In this study in contrast to the effect of P4, E2 increased PGF2α synthesis in CL

slices from the ovary ipsilateral to the uterine horn with developing embryos. E2 had no effect on PGF2α content in CL slices from the contralateral ovary of pregnant gilts and CL slices of both ovaries of cyclic gilts but increased PGF2α release from CL slices from the ipsilateral ovary of pregnant gilts. Johnson et al. (10) found that E2 and P4 had no effect on PGs production (in any tested dose) and thus did not influence luteal function *in vitro* in rhesus monkeys during the mid-luteal phase of the menstrual cycle. Moreover, in studies of Blitek et al. (3) it was indicated that both E2 and P4 did not enhance PGE2 and PGF2α secretion by cultured endometrial cells, while E2 and P4 were added together, the secretion of both PGs was stimulated. In the present study CL slices were not treated with combined P4 and E2, however the authors observed their opposite stimulation on PGE2 and PGF2α, respectively. Supposedly P4 alone was able to stimulate PGE2 synthesis due to the high concentration of endogenous E2 of embryonic origin in CL. The fact that exogenous E2 alone stimulated PGF2α synthesis might be caused by enhanced luteotrophic capacity of so-called „pregnant" CL and its higher P4 content. However, why E2 increased PGF2α synthesis in CL slices from ipsilateral ovary of cyclic gilts is unclear and needs further investigation. In the present study IL-1β markedly increased PGE2 release from cultured CL slices from the ovary contralateral to the uterine horn with developing embryos of pregnant gilts and in cyclic gilts regardless of CL origin. This observation indicates that cultured CL slices responded to IL-1β only when they originated from the ovaries connected to the uterine horn without embryos or both ovaries of non-pregnant gilts. Similarly, Miceli et al. (12) observed a significant increase in prostaglandins release from cultured human luteal cells to the incubation medium after IL-1β treatment and the cytokine was more effective on PGE2 than PGF2α release. It was found that in primate and bovine luteal cells *in vitro* this stimulatory effect is mediated by the cyclooxygenase activity (COX) pathway (13, 14, 23) rather than release of intracellular prostaglandins.

Concluding, some regulations are associated with embryos presence in the uterine horn such as the stimulatory effect of IL-1β on CL slices that originated from the ovary connected to the uterine horn without embryos independently whether gilts were pregnant or cyclic. It is possible that embryonic signals affect CL and modify its sensitivity to gonadal steroids and cytokine.

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