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Novel Aspects of Cytokine Action in Porcine Uterus – Endometrial and Myometrial Production of Estrone (E_1) in the Presence of Interleukin 1β (IL1β), Interleukin 6 (IL6) and Tumor Necrosis Factor (TNFα) – *in Vitro* Study*

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Interleukin 1ß (IL-1ß), interleukin 6 (IL-6) and tumor necrosis factor α (TNFα) increased ($P<0.05$) estrone (E_1) release from endometrial explants of pregnant pigs on days 10 to 11 after 12 h of tissue incubation *in vitro* with cytokines and on days 12 to 13 after 6 h of incubation. After 12 h of incubation on days 12 to 13 and 15 to 16 of pregnancy only IL6 increased E_1 release. In non-gravid pigs IL1β, IL6 and TNFα increased endometrial E_1 release only on days 12 to 13 of the estrous cycle. The cytokines did not affect myometrial E_1 release on days 10 to 11 and 15 to 16 of pregnancy. On days 12 to 13 of pregnancy myometrial release of E_1 was markedly increased in response to IL1β and IL6. In cyclic pigs only IL6 after 6 h of *in vitro* incubation increased myometrial E_1 release on days 12 to 13 and 15 to 16. Progesterone (P_4) increased both endometrial and myometrial release of E_1 during the studied days of pregnancy and the estrous cycle, except for endometrial release on days 10 to 11 and 15 to 16 of the estrous cycle after 6 h of *in vitro* incubation. The results demonstrated that these cytokines may regulate the release of E_1 both from the endometrium and myometrium harvested from gravid and non-gravid pigs. The results showed a pivotal role of IL1β, IL6 and TNFα in the regulation of E_1 release in the porcine uterus *in vitro*.

Key words: Interleukin 1β, Interleukin 6, Tumor necrosis factor α, Estrone, Endometrium, Myometrium, Pregnancy, Pigs.

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In pigs estrone (E_1) and estradiol (E_2) produced by porcine embryos were shown to be signals for maternal recognition of pregnancy (BAZER & TCHATCHER 1977). Previously we have demonstrated that tissues of porcine uterus can produce estrogens (FRANCZAK 2008; FRANCZAK & KOTWICA 2008; FRANCZAK & KOTWICA 2010) and the endometrium together with the myometrium on days 14 to 16 of early pregnancy are an important source of estrone (E_1) (FRANCZAK 2008; FRANCZAK & KOTWICA 2008). E_1 was found to be a main contributor in endometrial and myometrial total steroid secretion (FRANCZAK 2008). On days 14 to 16 of pregnancy the endometrium releases more E_1 than on days 14 to 16 of the estrous cycle, while the myometrium apparently equally participated in the total uterine basal E_1 secretion. Thus, in pigs during early pregnancy the endometrium and the myometrium, while during luteolysis only the myometrium, may mostly contribute to the basal total secretion of E_1 (FRANCZAK 2008).

The uterus of pigs is known to be a source of estrogens, however the factors involved in the regulation of myometrial E_1 production remain unknown. In general, P_4 was shown to be a substrate for steroid synthesis in the porcine uterus (FRANCZAK 2008; FRANCZAK & KOTWICA 2008; FRANCZAK & KOTWICA 2010), but the hormone did not stimulate E_1 secretion on days 14 to 16 of pregnancy (FRANCZAK 2008). This phenomenon was not explained. Little is known about the hormones or other factors that influence uterine steroidogenesis.

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In the present study we have examined if interleukin 1β (IL1β), interleukin 6 (IL6) and tumor necrosis factor α (TNFα), which are produced by porcine embryos and the uterus, may be involved in the regulation of uterine E_1 production and release *in vitro*. Cytokines, acting through specific receptors, may regulate the secretory activity of the uterus in pigs (FRANCZAK *et al.* 2010; 2012). Peri-implantation porcine embryos and the endometrium express IL1β (TUO *et al.* 1996; ROSS *et al.* 2003a,b) and IL6 (MATHIALAGAN *et al.* 1992; ANEGON *et al.* 1994). Tumor necrosis factor α is also produced by the uterus of pregnant pigs (YU *et al.* 1998). In a recent study we documented that IL1β, IL6 and TNFα affect prostaglandin $F_2α$ ($PGF_2α$) release and activate $PGF_2α$ metabolism in the endometrium to protect the corpus luteum (CL) in pregnant pigs (FRANCZAK *et al.* 2012). IL1β induced synthesis and secretion of luteotrophic prostaglandin E_2 (PGE_2) to overcome luteolysis in pregnant pigs (FRANCZAK *et al.* 2010) and may be included in intraluteal luteotrophic regulation of CL functions in gravid and cyclic pigs (ŻMIJEWSKA *et al.* 2013). In the pig, the expression of IL1β at the site of embryo-maternal contact in the uterus is unique and species-specific (TUO *et al.* 1996). TNFα is expressed in embryos, ovaries, oviducts and uteri of human and rodents (HUNT 1993). In gravid pigs TNFα stimulates PGE_2 synthesis and secretion by luminal epithelial cells of the endometrium (WACLAWIK *et al*. 2010). It was found that during days 15 to 16 of pregnancy TNFα stimulates secretion of the prostaglandin F2α metabolite – 13,14-dihydro-15-keto PGF2α (PGFM) by the endometrium (FRANCZAK *et al*. 2012). In cyclic pigs TNFα is involved in the regulation of PGF2α synthase (PGFS) mRNA expression (FRANCZAK *et al*. 2012) and prostaglandin secretion (BLITEK & ZIECIK 2006). Because the ability of the uterine tissues to induce steroidogenesis has been confirmed, we hypothesized that IL1β, IL6 and TNFα may be involved in conditioning this process and may increase E_1 concentrations that enhance the effects of estrogens in the uterus.

The objective of this investigation was to test the hypothesis that endometrial and myometrial production of E_1 is regulated by cytokines. To address this issue, we have determined if the endometrium and the myometrium of pigs can produce E_1 *in vitro* in response to IL1β, IL6 and TNFα on days 10 to 11, i.e. before the time of maternal recognition of pregnancy, on days 12 to 13, i.e. during the time of maternal recognition of pregnancy and on days 15 to 16, i.e. during the time of corpus luteum maintenance, beginning of implantation and prevention of luteolysis. The effects observed in pregnant pigs were compared with effects on corresponding days of the estrous cycle.

Material and Methods

Animals and collection of endometrial and myometrial tissue

All experiments were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn, Poland. Post-pubertal, six month old crossbred pigs (Large White × Polish Landrace), weighing 90-110 kg were used during early pregnancy or the estrous cycle. Gilts were observed for estrus behavior in the presence of an intact boar. The onset of the second estrus was designated as day 0 of the estrous cycle. Gilts assigned to the early pregnancy group were naturally bred on the second day of estrus. The animals on days 10 to 11 (n = 5), 12 to 13 (n = 5) and 15 to 16 (n = 5) of pregnancy, or days 10 to 11 (n = 5), 12 to 13 (n = 5) and 15 to 16 (n = 5) of the estrous cycle were slaughtered in a commercial slaughterhouse. Pregnancy in mated gilts was confirmed by the presence of embryos after flushing each uterine horn with 20 ml sterile saline. The stage of the estrous cycle was also confirmed by morphological changes of the ovaries and CL quality (AKINS & MORRISSETTE 1968). Uterine horns from early-pregnant or cyclic gilts were placed immediately in ice-cold PBS supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin and transported to the laboratory on ice.

Preparation of endometrial and myometrial slices

The middle part of the uterine horns collected from experimental gilts was opened longitudinally on the mesometrial surface and the endometrium and the myometrium were separated using a scalpel blade. The endometrium and the myometrium were sliced thinly (200-210 mg, 3 mm thick) and washed twice with PBS supplemented with antibiotics.

In vitro incubation of endometrial and myometrial slices

Individual fresh endometrial and myometrial slices were placed separately in culture vials containing 2 ml of Medium 199 (Sigma, Germany) supplemented with 0.1% BSA fraction V (ICN, USA) and 20 μg gentamycin (Sigma, Germany). These tissue cultures were pre-incubated in a water bath for 18 h at 37°C in an atmosphere of 95% O_2 and 5% CO_2. After preincubation, endometrial and myometrial slices were incubated for 6 and 12 h in control medium or in medium supplemented with progesterone (P_4, 10^{-5}M), IL1β (1 ng/ml and 10 ng/ml), IL6 (1 ng/ml and 10 ng/ml) or TNFα (10 ng/ml). P_4 was used as a control and substrate for

E_1 production. Cytokines were obtained from Biomol, GmbH, Germany. The doses of cytokines were selected according to earlier studies (FRANCZAK *et al.* 2010, 2012). After incubation, culture vials were placed in an ice bath, culture medium was collected and frozen at -20°C until E_1 assay with radioimmunoassay (RIA).

Estrone (E_1) determination

Concentration of E_1 was determined by the RIA method (CIERESZKO 1999). Cross-reactivity of antisera against E_1 has been reported (SZAFRANSKA et al. 2002). The efficiency of extraction for E_1 was 85.3 ± 0.07%. E_1 assay sensitivity was 1 pg/ml. The coefficient of correlation between the added and recovered amount of E_1 concentrations was 0.975. The intra- and interassay coefficients of variation were 0.5% and 1.9%, respectively.

Statistical analysis

Mean concentrations of E_1 released in response to cytokines were log-transformed to reduce heterogeneity of variance and were analyzed by multi-way ANOVA with the dose of treatments, time of incubation, reproductive status and days of pregnancy or the estrous cycle as the main effects followed by a LSD post-hoc test (Statistica, StatSoft Inc, Tulsa, OK, USA).

Results

Basal endometrial and myometrial release of E_1 during days 10 to 11, 12 to 13 and 15 to 16 of pregnancy and the estrous cycle

The results of the basal endometrial and myometrial production of E_1 are presented in Table 1. During pregnancy basal endometrial release of E_1 after 6 h of incubation did not differ among days 10 to 11, 12 to 13 and 15 to 16 ($P>0.05$). After 12 h of incubation *in vitro* basal E_1 release was higher on days 12 to 13 and 15 to 16 than on days 10 to 11 of pregnancy ($P<0.05$). During the estrous cycle E_1 production by the endometrium was about two-fold lower ($P<0.05$) on days 12 to 13 than that observed during days 10 to 11 and 15 to 16, both after 6 and 12 h of incubation. Basal myometrial E_1 production did not differ among the studied days of pregnancy ($P>0.05$) after 6 and 12 h of incubation and did not differ among the studied days of the estrous cycle after 6 h of incubation. After 12 h of incubation myometrial production of E_1 in cyclic pigs was about two fold higher on days 15 to 16 of

Table 1

Basal estrone (pg / ml) secretion (mean ± S.E.M.) from endometrial and myometrial explants harvested on days 10 to 11, 12 to 13 and 15 to 16 of pregnancy and the estrous cycle and incubated *in vitro* within 6 and 12 hours

Endometrium				
Days	Pregnancy		The estrous cycle	
	6 hours of incubation *in vitro*	12 hours of incubation *in vitro*	6 hours of incubation *in vitro*	12 hours of incubation *in vitro*
10 to 11	55.5 ± 9.6^{Aa}	23.1 ± 2.8^{Aa}	58.0 ± 8.0^{Aa}	58.9 ± 14.3^{Ab}
12 to13	32.8 ± 7.1^{Aa}	39.1 ± 7.6^{Ba}	27.4 ± 6.7^{Ba}	23.9 ± 4.9^{Ba}
15 to 16	39.6 ± 5.2^{Aa}	39.9 ± 11.3^{Ba}	50.2 ± 8.1^{Aa}	55.2 ± 18.8^{Aa}
Myometrium				
Days	Pregnancy		The estrous cycle	
	6 hours of incubation *in vitro*	12 hours of incubation *in vitro*	6 hours of incubation *in vitro*	12 hours of incubation *in vitro*
10 to 11	40.5 ± 6.2^{Aa}	44.9 ± 13.1^{Aa}	55.0 ± 14.7^{Aa}	40.9 ± 14.8^{Aa}
12 to 13	33.7 ± 8.1^{Aa}	40.4 ± 6.8^{Aa}	41.7 ± 7.9^{Aa}	40.2 ± 8.5^{Aa}
15 to 16	37.7 ± 5.8^{Aa}	48.0 ± 19.8^{Aa}	46.3 ± 15.3^{Aa}	76.5 ± 14.0^{Ba}

[A,B] Different uppercase letters designate significant differences among the studied days of pregnancy or the estrous cycle within the same time of incubation (data in columns).

[a,b] Different lowercase letters designate significant differences between corresponding days of pregnancy and the estrous cycle after the same time of incubation (data in rows).

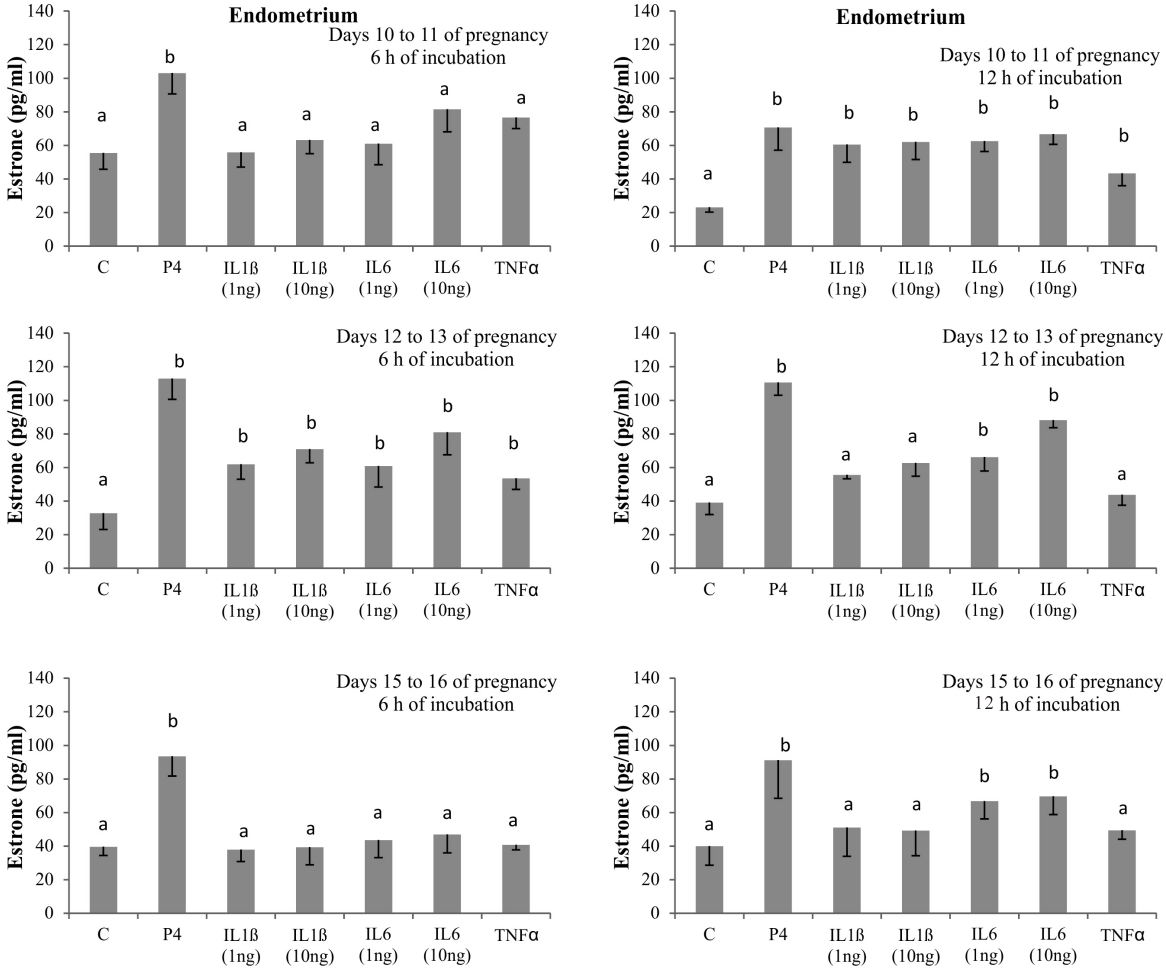

Fig. 1. Release of E_1 *in vitro* (mean ± S.E.M.) by endometrial explants obtained from gilts during early pregnancy (days 10 to 11, 12 to 13 and 15 to 16) after stimulation with progesterone (P_4, 10^{-5} M), interleukin 1β (IL1β, 1 and 10 ng / ml), interleukin 6 (IL6, 1 and 10 ng / ml) or tumor necrosis factor α (TNFα, 10 ng / ml). After preincubation (18 h; 37°C, 95% O_2 + 5% CO_2) endometrial explants (200-210 mg) were incubated for the next 6 and 12 h in control medium (C) or the presence of P_4 and the studied cytokines. Different letters indicate significant differences between each treatment and control samples within 6 and 12 h of incubation ($P<0.05$).

the estrous cycle than on days 10 to 11 and 12 to 13 ($P<0.05$).

IL1β, IL6 and TNFα-induced endometrial E_1 release *in vitro* during early pregnancy and the estrous cycle

IL1β, IL6 and TNFα increased E_1 release *in vitro* ($P<0.05$) in endometrial explants harvested on days 10 to 11 from gravid pigs and incubated *in vitro* for 12 h as well as in endometrial explants harvested on days 12 to 13 of pregnancy and incubated for 6 h (Fig. 1). After 12 h *in vitro* incubation on days 12 to 13 of pregnancy only IL6 (1 ng/ml and 10 ng/ml) increased E_1 release ($P<0.05$), while an effect of IL1β or TNFα was not observed ($P>0.05$). During days 15 to 16 of pregnancy the enhanced release of E_1 was observed only in the presence of IL6 after 12 h of *in vitro* incubation (Fig. 1). Neither IL1β, nor TNFα affected endometrial E_1 release during days 15 to 16 of pregnancy ($P>0.05$). In non-gravid pigs the stimulatory effect of IL1β, IL6 and TNFα on E_1 release was observed only on days 12 to 13 of the estrous cycle, both after 6 and 12 h incubation (Fig. 2).

IL1β, IL6 and TNFα-induced myometrial E_1 release *in vitro* during early pregnancy and the estrous cycle

On days 10 to 11 of pregnancy IL1β, IL6 and TNFα did not affect E_1 release from myometrial explants ($P>0.05$) (Fig. 3). Myometrial E_1 production was increased on days 12 to 13 of pregnancy in the presence of IL1β and IL6 after 6 and 12 h of *in vitro* incubation (Fig. 3, $P<0.05$). An effect of TNFα during days 12 to 13 of pregnancy was not observed ($P>0.05$). The cytokines did not affect E_1

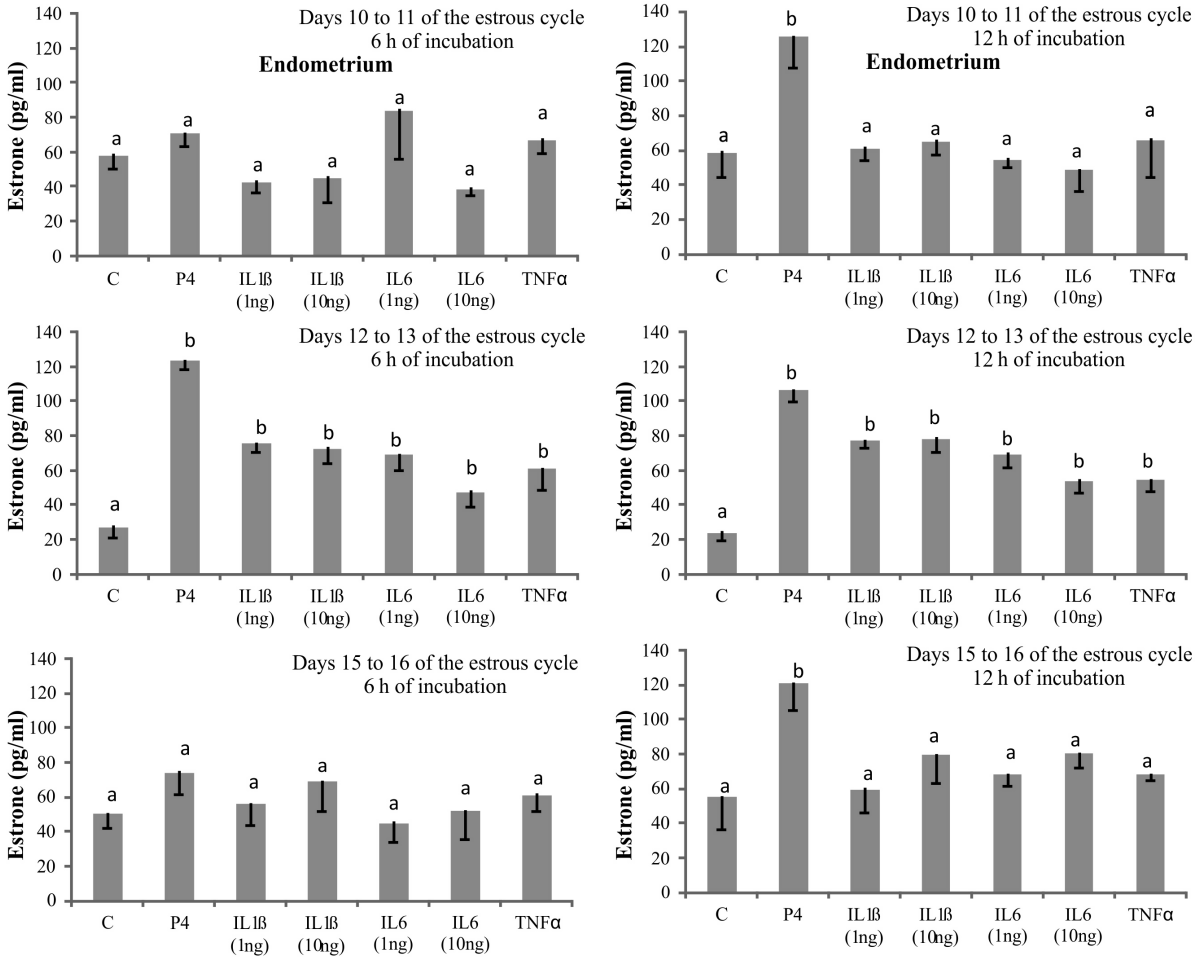

Fig. 2. Release of E_1 *in vitro* (mean ± S.E.M.) by endometrial explants obtained from gilts during the estrous cycle (days 10 to 11, 12 to 13 and 15 to 16) after stimulation with progesterone (P_4, 10^{-5} M), interleukin 1β (IL1β, 1 and 10 ng / ml), interleukin 6 (IL6, 1 and 10 ng / ml) or tumor necrosis factor α (TNFα, 10 ng / ml). After preincubation (18 h; 37°C, 95% O_2 + 5% CO_2) endometrial explants (200-210 mg) were incubated for the next 6 and 12 h in control medium (C) or presence of P_4 and the studied cytokines. Different letters indicate significant differences between each treatment and control samples within 6 and 12 h of incubation ($P<0.05$).

release during days 15 to 16 of pregnancy (Fig. 3, $P>0.05$). In cyclic pigs (Fig. 4) only IL6 increased myometrial E_1 release after 6 h of *in vitro* culture on days 12 to 13 and 15 to 16 of the estrous cycle (Fig. 4). Neither IL1β, nor TNFα affected myometrial E_1 release in cyclic pigs ($P>0.05$, Fig. 4).

The effect of P_4 on endometrial and myometrial E_1 release *in vitro*

In gravid pigs P_4 (10^{-5}M) increased endometrial release of E_1 on the studied days of pregnancy ($P<0.05$, Fig. 1) both after 6 and 12 h of incubation. In non-gravid pigs a stimulatory effect of P_4 was observed after 6 h of incubation on days 12 to 13 and after 12 h of incubation on days 10 to 11, 12 to 13 and 15 to 16 of the estrous cycle (Fig. 2). Myometrial production of E_1 in the presence of P_4 was increased during all studied days of pregnancy and the estrous cycle ($P<0.05$) (Figs 3 & 4).

Discussion

The objective of this investigation was to determine if endometrial and myometrial production of E_1 is up-regulated by cytokines. To address this issue, we used porcine endometrial and myometrial explants to study the effects of IL1β, IL6 and TNFα on E_1 release. The effects of the cytokines were determined during three important periods of early pregnancy in pigs, e.g. on days 10 to 11, 12 to 13 and 15 to 16, and compared with the effects that occurred on the respective days of the estrous cycle. The tissues explants were treated with IL1β, IL6 and TNFα during 6 h or 12 h *in vitro* incubation. The main findings showed that in gravid pigs IL1β, IL6 and TNFα increased endometrial production of E_1 on days 10 to 11 only after 12 h of *in vitro* incubation and after 6 h of *in vitro* incubation on days 12 to 13 of pregnancy. Moreover, after 12 h of incubation *in vitro* IL6 increased endometrial E_1

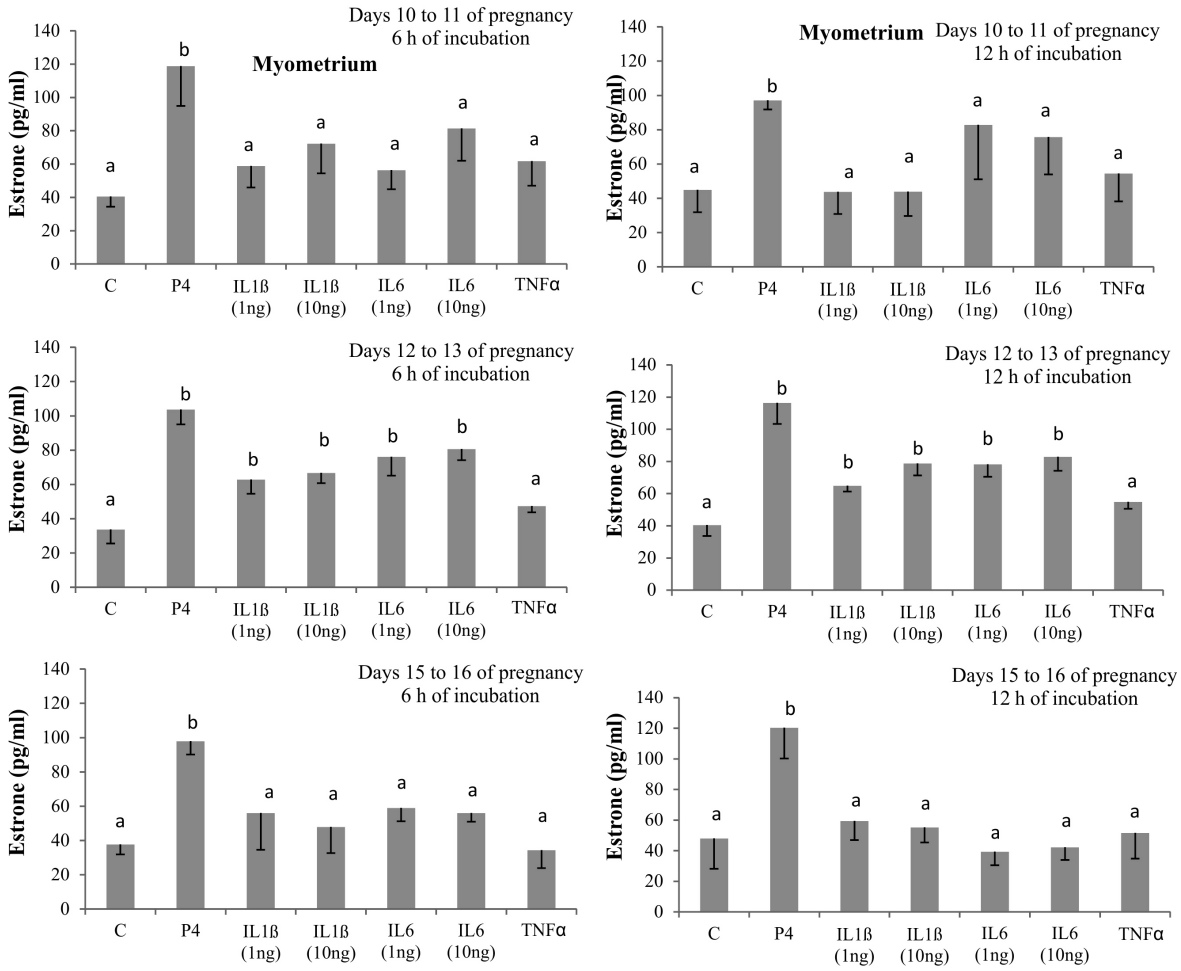

Fig. 3. Release of E_1 *in vitro* (mean ± S.E.M.) by myometrial explants obtained from gilts during early pregnancy (days 10 to 11, 12 to 13 and 15 to 16) after stimulation with progesterone (P_4, 10^{-5} M), interleukin 1β (IL1β, 1 and 10 ng / ml), interleukin 6 (IL6, 1 and 10 ng / ml) or tumor necrosis factor α (TNFα, 10 ng / ml). After preincubation (18 h; 37°C, 95% O_2 + 5% CO_2) myometrial explants (200-210 mg) were incubated for the next 6 and 12 h in control medium (C) or presence of P_4 and the studied cytokines. Different letters indicate significant differences between each treatment and control samples within 6 and 12 h of incubation ($P<0.05$).

production on days 12 to 13 and 15 to 16 of pregnancy. In non-gravid pigs IL1β, IL6 and TNFα increased E_1 release *in vitro* from the endometrium only during days 12 to 13, both after 6 h and 12 h of exposure to cytokines. Myometrial production of E_1 *in vitro* was specifically enhanced in the presence of IL1β and IL6 on days 12 to 13 of pregnancy. TNFα did not affect myometrial E_1 production in pregnant and cyclic pigs. Interestingly, in cyclic pigs IL6 increased myometrial E_1 release only on days 12 to 13 and 15 to 16 after 6 h of incubation *in vitro*.

Previously it was established that E_1 together with E_2 produced by porcine embryos provide a signal for pregnancy maintenance and successful implantation (BAZER *et al.* 1977; HEAP *et al.* 1981; GEISERT *et al.* 1982). During early pregnancy in pigs the production of estrogens is the result of androgen conversion (BAZER & TCHATCHER 1977; RYAN 1982). Androstenedione (A_4), a principal circulating androgen in pigs (SIMPSON *et al.* 2001), is converted to E_1 (RYAN 1982). Basal production of E_1 indicates the aromatization of A_4 to E_1 in the porcine uterus both during early pregnancy and the estrous cycle.

We have determined that in gravid pigs on days 10 to 11 IL1β, IL6 and TNFα increased endometrial production of E_1 after 12 h of *in vitro* stimulation, while on days 12 to 13 after 6 h of *in vitro* stimulation. IL6 increased E_1 production on days 12 to 13 and 15 to 16 of pregnancy only after 12 h of incubation *in vitro*. Thus, the effect of the cytokines depends on the stage of pregnancy and the duration of exposure to cytokines. From days 12 to 16 of pregnancy E_1 production in the endometrium is only IL6 dependent. Previously it was found that in postmenopausal women formation of estrogens in the adipose tissue and skin is controlled primary by IL6, IL1β and TNFα (for a review see BULUN *et al.* 2002).

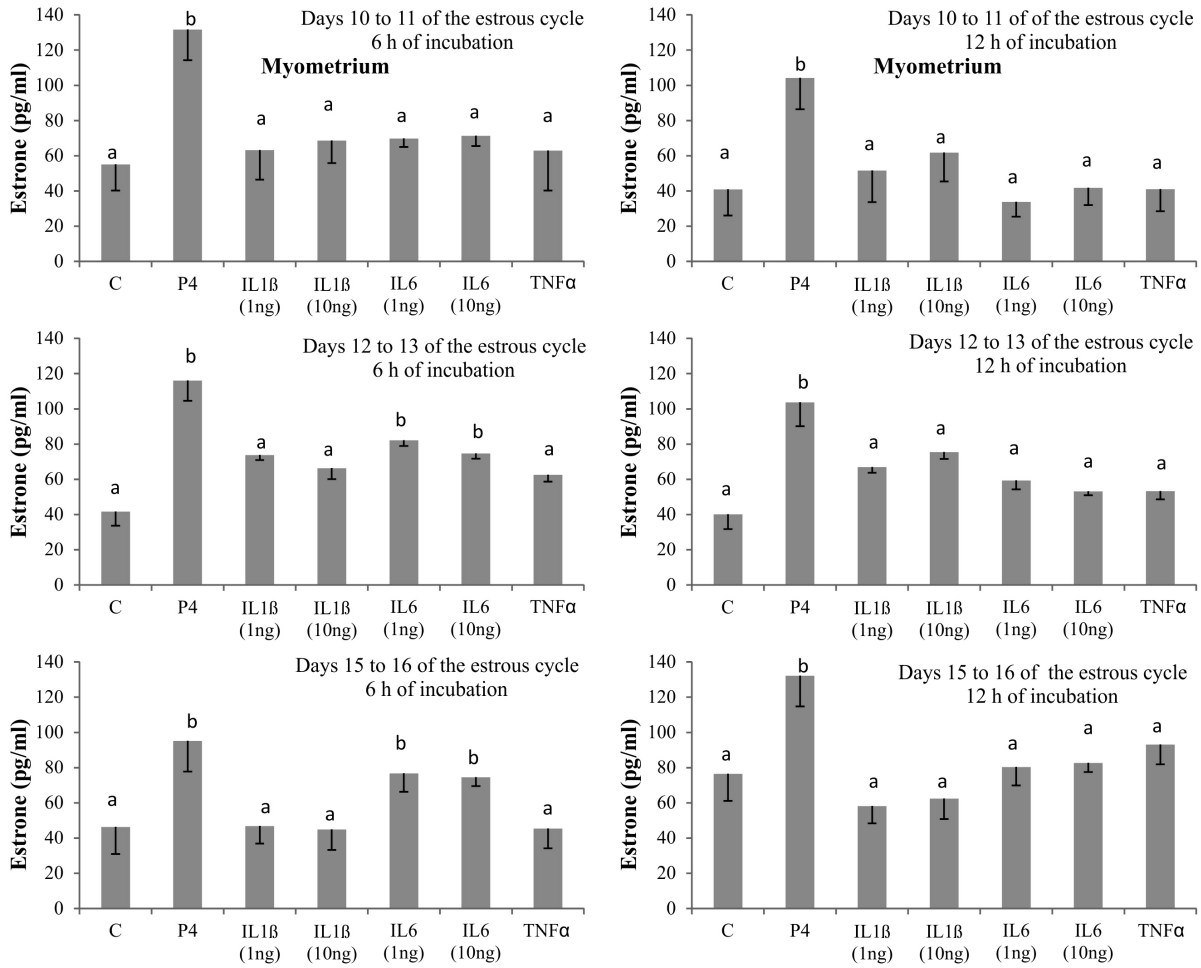

Fig. 4. Release of E_1 *in vitro* (mean ± S.E.M.) by myometrial explants obtained from gilts during the estrous cycle (days 10 to 11, 12 to 13 and 15 to 16) after stimulation with progesterone (P_4, 10^{-5} M), interleukin 1β (IL1β, 1 and 10 ng / ml), interleukin 6 (IL6, 1 and 10 ng / ml) or tumor necrosis factor α (TNFα, 10 ng / ml). After preincubation (18 h; 37°C, 95% O_2 + 5% CO_2) myometrial explants (200-210 mg) were incubated for the next 6 and 12 h in control medium (C) or presence of P_4 and the studied cytokines. Different letters indicate significant differences between each treatment and control samples within 6 and 12 h of incubation ($P<0.05$).

The present study showed for the first time that IL1β and IL6, not TNFα acting exactly during maternal recognition of pregnancy, e.g. on day 12 to 13 of pregnancy caused the release of E_1 by myometrial explants cultured *in vitro*. This time is accompanied by the highest expression of IL1β and IL6 by porcine conceptuses (MATHIALAGAN *et al.* 1992; ANEGON *et al.* 1994; TUO *et al*. 1996; ROSS *et al.* 2003a, b). Interestingly, after this time, on days 15 to 16 of pregnancy, when the process of implantation begins, IL1β, IL6 and TNFα did not affect E_1. This is indicative of a specific responsiveness of the myometrial tissue and the myometrial ability to produce E_1 in the presence of cytokines. We have found that the response of pregnant endometrium was dependent on the time of IL1β, IL6 and TNFα exposure and it developed earlier than the myometrial response, e.g. on days 10 to 11 of pregnancy, after 12 h of *in vitro* incubation. On days 12 to 13 of pregnancy the endometrium after 6 h of *in vitro* incubation responded positively in the presence of all cytokines, while after 12 h of incubation increased concentration of E1 was observed only in the presence of IL6. Interestingly, on days 15 to 16 of pregnancy, endometrial E_1 was induced only by IL6 after 12 h of *in vitro* incubation.

Comparing uterine responsiveness to cytokines in pregnant and cyclic pigs we have found that pregnant myometrium as well as pregnant and cyclic endometrium harvested on days 12 to 13 of pregnancy or the estrous cycle enhanced production of E_1 in response to IL1β and IL6. This period of pregnancy and the estrous cycle is accompanied by similar, high concentrations of P_4 and E_2 in peripheral blood plasma (FRANCZAK *et al.* 2010). We suggest that uterine responsiveness to cytokines may depend on the concentrations of endogenous P_4.

It is important to note that factors affecting uterine (endometrial and myometrial) secretion may influence the efficiency of conceptus proliferation, growth and development as well as uterine activity and capacity (VALLET & CHRISTENSON 1996). Despite the fact that E_1 is a less active estrogen than estradiol (VALLET & CHRISTENSON 1996), E_1 can be converted to more potent and active estradiol. Moreover E_1 can be converted to catecholestrogens (CHAKRABORTY *et al.* 1989) which may have high biological activity (ROSENKRANS *et al.* 1990). Thus, the level of E_1 production by the uterus in general may be very important for modulation of endometrial protein secretion (TROUT *et al.* 1992) and for proper communication between embryos and the endometrium (ROBERTS & BAZER 1988). It was found that treatment of pregnant gilts with E_1 resulted in increased endometrial secretion of nondialyzable macromolecules in culture, reflecting total protein synthesis in the tissue (VALLET & CHRISTENSON 1996). It is important to note that the proper level of uterine estrogen synthesis and metabolism during early pregnancy may be a prerequisite for successful implantation. Previously, we have found in pregnant endometrium increased expression of genes encoding sulfotransferase 1E1 and sulfotransferase 2A1 (FRANCZAK *et al.* 2013). Sulfotransferases convert estrogens into non-active estrogen sulphates. Thus, the mechanism of E1 metabolism may allow quick adjustment of steroid concentration in the uterus.

In conclusion: 1) endometrial and myometrial explants of pigs release E_1 *in vitro* in response to cytokines; 2) in pregnant pigs IL1β, IL6 and TNFα regulate endometrial production of E_1 on days 10 to 11 and on days 12 to 13 in a time of culture dependent manner; 3) on days 15 to 16 of pregnancy IL6 caused E_1 release from the endometrium; 4) in cyclic pigs IL1β, IL6 and TNFα increase E_1 release *in vitro* from the endometrium only during days 12 to 13 of the estrous cycle; 5) in the presence of IL1β and IL6 in gravid pigs myometrial production of E_1 *in vitro* was enhanced specifically on days 12 to 13 of pregnancy.

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