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The expression of mRNAs for opioid precursors in endometrium of cyclic and early pregnant pigs; effects of IL-1β, IL-6 and TNFα

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ABSTRACT. The expression of genes encoding opioid precursors: proopiomelanocortin (*POMC*), proenkephalin (*PENK*) and prodynorphin (*PDYN*) was studied in the porcine endometrial explants in the presence or absence of selected cytokines (interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα)) on days 10–11, 12–13, 15–16 of the oestrous cycle and pregnancy. In pregnant pigs, these days among others are important for 1. migration of the embryos, 2. maternal recognition of pregnancy and 3. the onset of implantation, respectively. During the oestrous cycle, the basal (in the absence of cytokines) mRNA expression of *PDYN*, but not *POMC* or *PENK* was increased on days 15–16 compared to days 10–11. During pregnancy, the gene expression of *POMC* increased on days 10–11 and 15–16 (vs days 12–13), but *PENK* – on days 12–13 and 15-16 (vs days 10–11) as well as *PDYN* – on days 15-16 (vs days 12–13) decreased. The endometrial expression of studied genes was influenced by cytokines. The expression of *PENK* was stimulated by IL-6 in all studied days of the cycle as well as on days 10–11 and 12–13 of pregnancy. In turn, the *PENK* mRNA expression was increased by TNFα on days 10–11 and decreased by IL-1β on days 15–16 of pregnancy. The *POMC* expression was decreased by IL-1β on days 12–13, but increased by both IL-6 and TNFα on days 15–16 of the cycle. The *PDYN* mRNA expression in endometrial explants was increased by IL-1β on days 10–11 of the cycle. These results indicate that the opioid system is involved in the local regulation of uterine functions during the oestrous cycle end early pregnancy in pigs.

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Introduction

Endogenous opioid system involves endogenous opioid peptides (EOPs), divided into three families: endorphins, enkephalins, dynorphins and their receptors (mu, delta or kappa). EOPs derive from three main opioid precursors: proopiomelanocortin (*POMC*), proenkephalin (*PENK*) and prodynorphin (*PDYN*). Peptides originating from these precursors – β-endorphin (from *POMC*), enkephalins (from *PENK*) and dynorphins (from *PDYN*) preferentially act through one type of opioid receptors – i.e. mu, delta or kappa, respectively (Simon, 1991).

Studies performed with different species have proved a widespread distribution of EOPs throughout the central nervous system and peripherial organs/tissues (Simon, 1991), including the uterus (Wahlström et al., 1985; Li et al., 1991ab,1992, 1993; Okrasa et al., 2003). Our previous studies have indicated that all genes coding for opioid

precursors are expressed in porcine pituitary (Wylot et al., 2008) and steroidogenic tissues such as ovary (Staszkiewicz et al., 2007a,b) and adrenal cortex (Krazinski et al., 2011). Their expression has been also found in the uterus of different species (Douglass et al., 1987; Jin et al., 1988; Low et al., 1989; Rosen et al., 1990; Zhu and Pintar, 1998).

Endogenous opioid peptides are involved in the regulation of many physiological processes (Simon, 1991), including those related to reproduction. They affect the reproductive axis in females at all levels. In studies performed *in vitr*o, with the use of porcine tissues/cells, selected opioid agonists appeared to influence gonadotropin releasing hormone release from the stalk median eminence (Okrasa et al., 1995), gonadotropin secretion by pituitary cells (Wylot et al., 2013) and steroid hormones by ovarian cells (Kaminski et al., 2003, 2004). It was also reported that EOPs may affect many different processes in the uterus, including: cell proliferation (Vértes et al., 1996), apoptosis (Chatzaki et al., 2001), immunological interactions and myometrial contractility (Zoumakis et al., 1997) as well as the early pregnancy events (Li et al., 1992, 1993).

The expression of genes coding for opioid precursors and/or opioid peptide secretion seem to be controlled by many factors, including: corticotropin-releasing hormone (CRH) (Parsadaniantz et al., 1997), adrenocorticotropic hormone (ACTH) (Krazinski et al., 2011), steroid hormones (Li et al., 1987; Muffly et al., 1988; Low et al., 1989; Okrasa et al., 2003) and cytokines (Ruzicka and Akil, 1995, 1997; Parsadaniantz et al., 1997; Sun et al., 2006; Takayasu et al., 2010). Nevertheless, the data concerning the regulation of uterine expression of genes encoding opioid precursors are limited and the role of proinflammatory cytokines (interleukin-1β (IL-1β), (interleukin-6 (IL-6) and tumor necrosis factor α (TNFα)) in this process has not been studied. Collectively, basing on the above cited data, we hypothesize that genes encoding opioid precursors are expressed in the porcine uterus and the proinflammatory cytokines (IL-1β, IL-6 and TNFα) modulate their expression. Therefore the aim of this study was to determine: 1. the expression of genes coding for the opioid peptide prcursors (*POMC*, *PENK* and *PDYN*) at mRNA level in porcine endometrium during the oestrous cycle and early pregnancy and 2. potential influence of selected cytokines (IL-1β, IL-6 and TNFα) on the expression of these genes. For the study, days 10–11, 12–13 and 15–16 of the oestrous cycle and pregnancy were selected since they are important for 1. migration of the embryos to and within the uterus, 2. maternal recognition of pregnancy and 3. corpus luteum (CL) protection against luteolysis and the onset of implantation, respectively (Geisert et al., 1982).

Material and methods

Animals

All experiments were performed in accordance with the principles and procedures of the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (Poland). Tissues were collected from mature cross-bred pigs (Large White × Polish Landrance, weight 90–110 kg) during the oestrous cycle (days 10–11, 12–13, 15–16; n = 5 for each group) and early pregnancy (days 10–11, 12–13, 15–16; n = 5 for each group). Gilts were observed for oestrus behaviour in the presence of an intact boar. The onset of the second oestrus was designated as day 0 of the oestrous cycle. Gilts intended for the early pregnancy group were naturally bred on the second day of oestrus. Animals were slaughtered in commercial slaughterhouse. The stage of the oestrous cycle was confirmed by testing morphology of the ovaries (Akins and Morrissette, 1968). Pregnancy in mated gilts was confirmed by the presence of embryos in flushings of uterine horns rinsed with 20 ml of sterile saline. Uterine horns were harvested and placed in ice-cold phosphate buffered saline (PBS) with 100 IU · ml^{-1} of penicillin and 100 μg · ml^{-1} of streptomycin and transported to the laboratory for endometrial tissue isolation.

Preparation and incubation of endometrial explants

Endometrial tissue was harvested from the middle part of the uterine horn. Endometrium was separated from the myometrium by careful scraping using a scalpel blade. Subsequently, the tissue was sliced into 200–210 mg explants, washed twice with PBS and preincubated in 2 ml of Medium 199 (Sigma-Aldrich, Germany) with the addition of 0.1% bovine serum albumin fraction V (ICN, USA) and 20 μg of gentamycin (Sigma-Aldrich, Germany) for 18 h at 37°C under an atmosphere of 95% O_2 and 5% CO_2. Then the medium was replaced and explants were incubated for 12 h without or with addition of IL-1β, IL-6 or TNFα in amounts (10 ng · ml^{-1}) previously used by Franczak et al. (2012). All cytokines have been purchased from Biomol, GmbH, Germany. After incubation, the tissue slices were recovered, washed in PBS and stored at –80°C for further analysis.

RNA isolation and reverse transcription

Total RNA was extracted using QiagenRNeasy columns (Qiagen, The Netherlands) in accordance with manufacturer's protocol. The quantity and purity of RNA were determined spectrophotometrically (TECAN, Switzerland), then randomly selected RNAs were additionaly tested by 1.5% agarose gel electrophoresis. Reverse transcription (RT) was performed using QuantiTect Reverse Transcription Kit (Qiagen, The Netherlands). For this purpose, RNA template (1 µg) and gDNA Wipeout Buffer (2 µl) were added to the tube and filled up with RNase free water to the final volume of 14 µl. Subsequently, 1 µl of Quantiscript Reverse Transcriptase, 4 µl of Quantiscript 5×RT buffer and 1 µl of RT primers were added to the tube. Samples were then incubated at 42°C for 15 min and next at 93°C for 3 min to inactivate reverse transcriptase. The RT product was kept frozen at –20°C for PCR analysis.

Real-Time PCR and sequencing

The expression of genes coding for opioid precursors was determined by Real-Time PCR method (7500 Real-Time PCR System, Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as a normalization control. Primers for this gene (Table 1) were applied following Bogacka et al. (2006). Other primers specific for target genes (Table 1) were designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Each sample prepared for Real-Time PCR analysis (25 µl) contained: SYBR Green MIX (12.5 µl) (Applied Biosystems, USA), specific primers at the concentration 10 nM (1 µl for *POMC* and *PDYN* and 0.25 µl for *PENK*), cDNA (2 µl) and filled up with RN-ase free water to the final volume. The PCR programme was started with an initial denaturation step (10 min at 95°C) and then comprised 40 cycles. Each cycle consisted of the following steps: denaturation (95°C) for 15 sec, primers annealing at a specific temperatures (see Table 1) for 1 min and elongation (1 min at 72°C). The last PCR cycle included a final extension step (10 min at 72°C). All runs included negative controls which were performed without RT product. After Real-Time PCR reaction, randomly selected samples for each gene were sequenced (Genomed, Poland) to confirm the specificity of amplicons for studied genes.

Statistical analysis

All data were expressed as means ± SEM. The data were analysed using Statistica 10 software (StatSoft Inc., 2011). The mRNA expression for studied genes was presented as a ratio of their products to the reference *(GAPDH*) gene product. The obtained results, after logarytmical transformation were submitted to one-way analysis of variance (ANOVA) followed by NIR Fisher's test. Differences with $P < 0.05$ were considered to be statistically significant.

Results

Basal expression of opioid precursor genes in porcine endometrium

POMC. The basal expression of *POMC* mRNA in the porcine endometrial explants was maintained at almost the same level during the oestrous cycle (Figure 1A). Differences in this gene expression however appeared during pregnancy. Its expression was the lowest on days 12–13 of pregnancy and significantly different ($P < 0.05$) from those on days 10–11 and 15–16. In turn, differences between parallel days of the oestrous cycle and pregnancy appeared to be significant on days 10–11 ($P < 0.05$) and 15–16 ($P < 0.05$).

Table 1. Primers specific for genes encoding EOPs precursors (*POMC, PENK, PDYN*) and *GAPDH* used for Real-Time PCR

Gene	Forward primer	Reverse primer	T_A, °C	Product, size/T_M	NCBI
GAPDH	CCTTCATTGACCTCCACTACATGGT	CCACAACATACGTAGCACCACGAT	59	183	U48832
POMC	AAGGACGAAGGGCCCTATAA	CTTCTCGGAGGTCATGAAGC	60	90	NM_213858.1
PENK	AGTGAGGACGAAGAGGTGAG	CTTGAGGAAGCCACCGTACC	63	177	XM_003125621.2
PDYN	GAGAGGGAGGGTGGAGATTC	CAAGACGTCCACCTGGATTC	62	137	NM_001004040.1

EOP – endogenous opioid peptides, *GAPDH* – glyceraldehyde-3-phosphate dehydrogenase, *POMC* – proopiomelanocortin, *PENK* – proenkephalin, *PDYN* prodynorphin, NCBI – mRNA sequence accession number, T_A – the temperature of annealing, T_M – melting point of PCR product

PENK. The basal expression of *PENK* mRNA in the endometrial explants did not significantly differ between studied days of the oestrous cycle (Figure 1B). During pregnancy, the expression of *PENK* mRNA was lower on days 12–13 and 15–16 than on days 10–11 ($P < 0.05$).

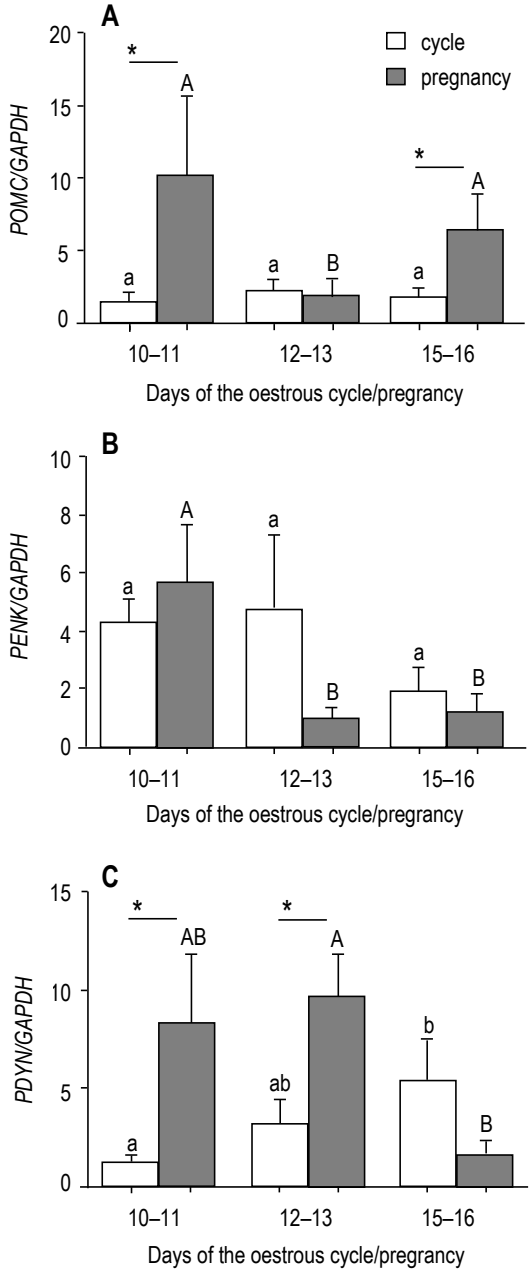

Figure 1. Changes in the basal expression of A. proopiomelanocortin (*POMC*), B. proenkephalin (*PENK*) and C. prodynorphin (*PDYN*) genes in porcine endometrium during the oestrous cycle and pregnancy (days 10–11, 12–13, 15–16). Various letters indicate significant differences between different days ($P < 0.05$) of the ^{a,b} oestrous cycle or ^{A,B} pregnancy. Significant differences between comparable days of the cycle and pregnancy are marked with horizontal lines and (*).

PDYN. The basal expression of *PDYN* mRNA in the endometrial explants during the oestrous cycle was the greatest on days 15–16 and it was significantly greater ($P < 0.05$) than on days 10–11 (Figure 1C). During pregnancy, the expression of *PDYN* mRNA was significantly lower on days 15–16 than on days 12–13 ($P < 0.05$). Comparison between parallel days of the oestrous cycle and pregnancy has demonstrated significant differences on days 10–11 ($P < 0.05$) and 12–13 ($P < 0.05$).

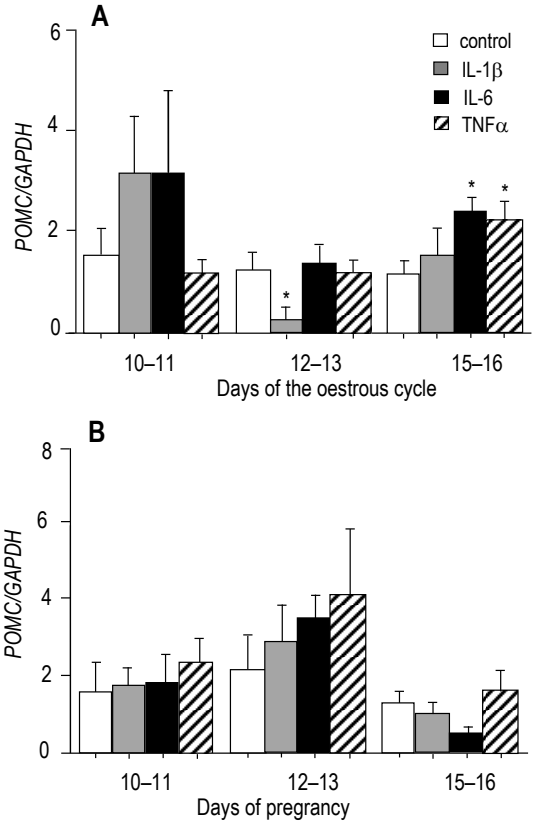

Figure 2. The influence of interleukin-1β (10 ng · ml^{-1}), interleukin-6 (10 ng · ml^{-1}) or tumor necrosis factor α (10 ng · ml^{-1}) on proopiomelanocortin (*POMC*) gene expression in porcine endometrium during A. the estrous cycle and B. pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n=5). Significant differences in comparison to the respective control value are marked with asterisks (* $P < 0.05$)

The influence of IL-1β, IL-6 and TNFα on the expression of opioid precursor genes in porcine endometrium

POMC. All studied cytokines affected the endometrial *POMC* mRNA expression on specific days of the oestrous cycle (Figure 2). IL-1β decreased the expression of *POMC* mRNA in comparison to the control on days 12–13 ($P < 0.05$), but IL-6 and TNFα increased it on days 15–16 ($P < 0.05$). During pregnancy, IL-6 tended to decrease *POMC* mRNA expression on days 15–16 ($P = 0.058$).

PENK. Compared to control, IL-6 significantly increased *PENK* mRNA expression in the endometrial explants during all studied days of the cycle: 10–11 ($P < 0.01$), 12–13 ($P < 0.05$), 15–16 ($P < 0.01$) as well as on days 10–11 ($P < 0.01$) and 12–13 ($P < 0.01$) of pregnancy (Figure 3). TNFα increased the expression of *PENK* mRNA on days 10–11 of pregnancy ($P < 0.05$). IL-1β decreased the expression of *PENK* mRNA on days 15–16 of pregnancy ($P < 0.05$).

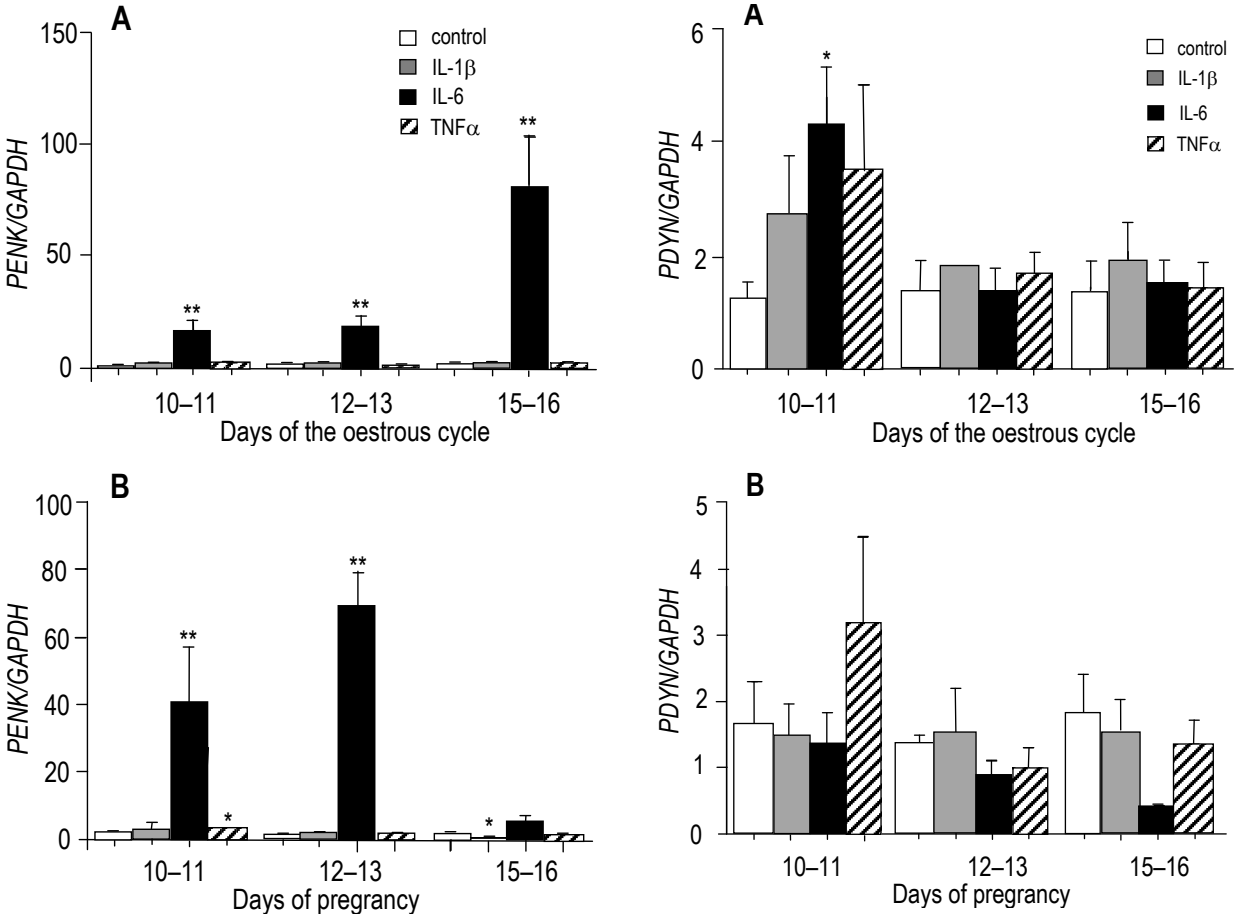

Figure 3. The influence of interleukin-1β (10 ng · ml^{-1}), interleukin-6 (10 ng · ml^{-1}) or tumor necrosis factor α (10 ng · ml^{-1}) on proenkephalin (*PENK*) gene expression in porcine endometrium during A. the oestrous cycle and B. pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n = 5). Significant differences in comparison to the respective control value are marked with asterisks (* $P < 0.05$; ** $P < 0.01$)

Figure 4. The influence of interleukin-1β (10 ng · ml^{-1}), interleukin-6 (10 ng · ml^{-1}) or tumor necrosis factor α (10 ng · ml^{-1}) on prodynorphin (*PDYN*) gene expression in porcine endometrium during A. the oestrous cycle and B. pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n = 5). Significant difference in comparison to the respective control value is marked with the asterisk (* $P < 0.05$)

PDYN. Out of the tested cytokines (Figure 4), only IL-1β increased the expression of *PDYN* mRNA in the endometrial explants on days 10–11 of the oestrous cycle ($P < 0.05$). During pregnancy, IL-6 tended to decrease *PDYN* mRNA expression (like in the case of *POMC* mRNA) on days 15–16 ($P = 0.056$).

Discussion

The present study has documented changes in the expression of mRNAs for opioid precursors (*POMC*, *PENK* and *PDYN*) in the examined tissue under basal conditons and in response to selected cytokines (IL-1β, IL-6 and TNFα) on days 10–11, 12–13, 15–16 of the oestrous cycle and early pregnancy.

In previous studies, EOPs and the expression of opioid precursor genes were demonstrated in the uterine tissues of different species, including the pig. Immunoreactive β-endorphin has been shown to be present in the endometrium of pigs (Li et al., 1991a; Okrasa et al., 2003), women (Wahlström et al., 1985) and in the uterine fluid of pigs (Li et al., 1993), cows and women (Petraglia et al., 1986). In pigs, β-endorphin was localized to the surface and glandular epithelial cells of the endometrium (Li et al., 1992). Immunoreactive methionine-enkephalin was identified in rabbit (Li et al., 1991a), porcine (Li et al., 1991b), cow and woman (Petraglia et al., 1986) uterine fluids. In turn, the *POMC* mRNA has been found in the uterus of rats and hamsters (Jin et al., 1988), *PENK* mRNA – in the uterus of mice (Rosen et al., 1990; Zhu and Pintar, 1998), rats, hamsters (Jin et al., 1988) and primates (Low et al., 1989; Quezada et al., 2006) and *PDYN* mRNA – in the rat uterus (Douglass et al., 1987). The uterine expression of *PENK* mRNA in mice was mostly localized to the deep glandular layer of endometrium (Rosen et al., 1990). The presence of mRNAs for all opioid precursors in the endometrium documented in

the present and other studies suggests that EOPs are *de novo* synthesized in the uterus.

The pattern of uterine expression of genes encoding opioid precursors in cyclic and pregnant females has not been studied in detail. Assuming that our *in vitro* studies reflect physiological potential of endometrium for the transcription of these genes, some of arisen observations are worthy of attention. Namely, during the cycle – increased endometrial expression of *PDYN* gene on days 15–16 (vs days 10–11), and during pregnancy – increased expression of *POMC* gene on days 10–11 and 15–16 (vs days 12–13) but decreased expression of *PENK* gene on days 12–13 and 15–16 (vs days 10–11) and *PDYN* on days 15–16 (vs days 12–13). This suggests that basal endometrial expression of opioid precursors in the pig is submmited to greater changes during early pregnancy than the oestrous cycle. The uterine expression of genes coding for opioid precursors was mainly studied in rodents so far, but not in large domestic animals. Using *in situ* hybridization technique, Zhu and Pintar (1998) found that the expression of opioid system in the mouse uterus begins soon after implantations and is continued at least until late gestation (day 18). Interestingly, uterine tissues (endometrium and myometrium) of pregnant mouse exhibited only *PENK* mRNA, but not those of *POMC* or *PDYN*. The *POMC* mRNA was localized in trophoblast giant cells, but *PDYN* mRNA was detectable neither in the uterus nor in placenta during studied period of pregnancy (from day 3.5 to day 18). In rats, the highest uterine expression of *PENK* gene during the oestrous cycle was established at metoestrus and dioestrus, whereas the abundance of *POMC* mRNA remained relatively constant throughout the cycle. In turn, during pregnancy, the uterine content of *PENK* transcript markedly increased, but that of *POMC* did not change substantially (Jin et al., 1988). The uterine β-endorphin production/secretion was studied in several previous experiments performed with pigs. In the endometrium of crossbred gilts, higher concentration of β-endorphin was found on days 14–15 than on days 8–12 of both – the oestrous cycle and pregnancy (Li et al., 1992). In turn, within days 8-14 of the oestrous cycle and pregnancy, the total content of β-endorphin in uterine fluid was increased on day 8 in Meishan gilts and on days 10–11 in Large White gilts, without significant differences between studied periods (Li et al., 1993). Authors of this study suggested that increased uterine β-endorphin content on days 14–15 had at least partially resulted from decreased secretion of the peptide. In the present research, the endometrial expression of *POMC* mRNA was different in studied periods; similar during the cycle, but increased on days 10–11 and 14–15 of pregnancy. Therefore, we hypothesize that the composition and profile of the uterine expression of genes encoding opioid precursors as well as the uterine content and/or secretion of their protein products undergo changes during the oestrous cycle and early pregnancy depending on animal species and/or breeds.

The present study has demonstrated some differences in the endometrial expression of genes coding for opioid precursors (except *PENK*) between cyclic and pregnant gilts. The endometrial expression of *POMC* gene on days 10–11 and 15–16 as well as *PDYN* on days 10–11 and 12–13 of pregnancy appeared to be significantly higher than on the respective days of the cycle. The increased potency of porcine endometrium to produce the *POMC* and *PDYN* transcripts during early pregnancy suggest an implication of opioid peptides – deriving from these precursors – in preparation of uterine environment to the embryo implantation. However, the role of *PENK* derivatives during early pregnancy in pigs can not be excluded. In mice, the endometrial expression of *PENK* mRNA dramatically increased during pregnancy, especially around the implantation site (Rosen et al., 1990). In rats, the uterine content of *PENK* mRNA was higher during pregnancy than the oestrous cycle, but *POMC* transcript did not vary markedly (Jin et al., 1988). It was reported that steroid hormones may affect the uterine expression of genes encoding opioid precursors. The expression *PENK* mRNA was stimulated by progesterone (P4) in the rat and hamster uterus (Muffly et al., 1988). In turn, estradiol (E2) inhibited this effect of P4 in rats, while in hamsters both steroids acted synergistically. In contrast, E2 treatment of ovarectomized primate (rhesus macaques) stimulated the endometrial expression of *PENK* gene, but P4 antagonized this effect of E2 (Low et al., 1989). Microarray analysis revealed down-regulation of *PENK* gene in the rhesus monkey endometrium on days 21–23 (secretory stage) vs day 13 (proliferative stage) of the menstrual cycle (Ace and Okulicz, 2004). In women, a substantial reduction of *PENK* gene expression in endometrium was noted during the transition between the early luteal and mid-luteal phase (Carson et al., 2002; Quezada et al., 2006). In pigs, P4 given to ovarectomized gilts stimulated uterine secretion of β-endorphin (*POMC*-derivative) and met-enkephalin, whereas concomitant treatment with E2 abrogated these effects of P4, but E2 administered alone did not affect β-endorphin secretion (Li et al., 1991b). These data suggest that different

types of steroid effects on the expression of opioid genes are possible depending on animal species. Moreover, the differences in the endometrial expression of the opioid genes between the oestrous cycle and pregnancy (on days 10–11 and 12–13) imply a participation of non-steroid factors in their regulation since steroid concentrations during these periods are similar in pigs (Franczak et al., 2010).

In the present study, the effects of selected cytokines (IL-1β, IL-6 and TNFα) on the expression of genes encoding opioid precursors in the porcine endometrial explants on days 10–11, 12–13, 15–16 of the oestrous cycle and early pregnancy were observed. The strongest effects of cytokines were noted in the case of *PENK* gene expression. A stimulation of this gene expression by IL-6 was observed in all studied days of the cycle as well as on days 10–11 and 12–13 of pregnancy. Furthermore, TNFα increased the *PENK* gene expression on days 10–11, but IL-1β decreased it on days 15–16 of pregnancy. The most notable issue concerning the action of cytokines in question, it seems to be the exclusion of PENK gene expression from stimulation by cytokines (especially by IL-6) on days 15–16 of pregnancy, which coincides with the implantation of embryos in pigs (Geisert et al., 1982). The other intriguing observations concern the modulation of the endometrial expression of POMC gene by cytokines in cyclic gilts. The inhibition of *POMC* mRNA expression by IL-1β on days 12–13 and stimulation by IL-6 and TNFα on days 15–16 of the cycle suggest a connection of these relationships with changes in the porcine endometrium during final days of the secretory stage. Nevertheless, physiological implications of denoted interactions between cytokines and opioid system require better elucidation in further experiments. Currently, data concerning a role of cytokines in the regulation of uterine transcription of opioid genes so far are not available. However, in other tissues, proinflammatory cytokines were found to stimulate the *POMC* gene expression; IL-1β – in the rat anterior pituitary (Parsadaniantz et al., 1997), IL-1β and TNFα – in the mouse corticotroph tumor cell line AtT20 (Ruzicka and Akil, 1995; Takayasu et al., 2010). The *in vitro* studies revealed an inhibitory effect of IL-1β on *PENK* mRNA expression in astrocytes derived from hippocampus, but not from other structures (i.e. cortex, striatum, cerebellum or hypothalamus) of the rat brain (Ruzicka and Akil, 1997). The expression of *PDYN* gene was down-regulated by IL-6 and TNFα in the human macrophage U-937 line (Sun et al., 2006), however, IL-1 was not effective in this study (at concentration 10 ng · ml^{-1}). The above data indicate a potential of proinflammatory cytokines to affect the expression of opioid genes in a cell/tissue type-dependent fashion. On the basis of our study, it can be concluded that influence of cytokines on the endometrial expression of opioid genes transiently appears and strongly depends on the physiological status of animal, e.g., day of the oestrous cycle or pregnancy.

Conclusions

In summary, the study has demonstrated the expression of mRNA for three opioid precursors (proopiomelanocortin (*POMC*), proenkephalin (*PENK*) and prodynorphin (*PDYN*)) in the porcine endometrial explants from the oestrous cycle and early pregnancy. The basal expression of genes coding for these precursors changed during studied periods; *PDYN* during the oestrous cycle and early pregnancy, but *POMC* and *PENK* during pregnancy. The expression of *POMC* gene on days 10–11 and 15–16 as well as *PDYN* on days 10–11 and 12–13 of pregnancy was up-regulated in comparison to the respective days of the cycle. The cytokines primarily affected *PENK* mRNA expression in the endometrial explants. Overall, these results indicate that the opioid system plays a role in the regulation of uterine functions in pigs, including maternal recognition of pregnancy and implantation.

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