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The Uterine Secretory Activity and its Physiological Changes in the Pig

Stanisław Okrasa*, Anita Franczak, Agata Zmijewska, Bartosz Wojciechowicz, Mariusz Dziekonski, Marcin Martyniak, Justyna Kolakowska, Kamila Zglejc and Genowefa Kotwica

University of Warmia and Mazury in Olsztyn, Department of Animal Physiology, 10-719 Olsztyn, Oczapowskiego 1A, Poland

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The uterine environment is subjected to precise multifactorial control during early gestation in relations to maternal recognition of pregnancy and implantation. In this paper a current knowledge concerning the role of uterine secretory activity in early pregnant pigs was reviewed.

Key words: uterus, pigs, the estrous cycle, pregnancy, hormones, cytokines, steroids, growth factors, neuropeptides, prostaglandins

INTRODUCTION

The uterus is an essential organ for reproduction in mammals. It ensures proper environment for developing organism during its prenatal life. The early pregnancy is the most critical period for successful reproduction due to maternal recognition of pregnancy, implantation and then placentation. During this period, the uterus exhibits high secretory activity to fulfill very specific requirements. Thereby, it produces and secretes into the uterine lumen a complex array of substances termed histotroph, which among others contains: enzymes, growth factors, transport proteins, chemokines, cytokines and different hormones (Bazer et al., 2012; Cooke et al., 2013). Disturbances of the uterine secretory activity may be a reason of reproductive failures. In pigs, the majority of embryonic mortality occurs between Day 10 and 30 of pregnancy and reaches even about 30% (Pope, 1994). Therefore, in this paper, we focus mainly on secretory activity of the pig uterus – an important determinant of its reproductive efficiency. We thus consider a developmental as-

*e-mail: okrasa@uwm.edu.pl

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pect of the uterine glands and biologically active uterine secretions, such as: a wide range of proteins, prostaglandins and steroid hormones, including results of our own research.

DEVELOPMENTAL CHANGES IN THE PORCINE UTERUS

In mammalian species, development of the female reproductive tract, including the uterus, begins pre-natally and is completed post-natally (Bartol et al., 2008). In the adult, the uterine wall is comprised of two functional compartments, the endometrium and myometrium, surrounded by perimetrium (Spencer et al., 2004). In the domestic pig, complete maturation of the uterus is reached by Day 120 from birth. This process includes developmental events, common to all mammalian uteri, such as: 1) organization and stratification of endometrial stroma, 2) differentiation and growth of myometrium, and 3) coordinated development of the glands (Gray et al. 2001; Spencer et al., 2004). At birth, in the porcine uterus, there is a simple columnar luminal epithelium (LE) supported by undifferentiated mesenchyme and encircled by a rudimentary myometrium, but endometrial glands are absent. Development of endometrial glands (adenogenesis), covered with glandular epithelium (GE), is undertaken soon after birth. At first, the bud formation occurs, then LE develops into epithelial tubes extending radially from the luminal surface into the endometrial stroma. Subsequently, tubular glands undergo coiling and branching within the stroma until they reach the adluminal border of the myometrium. The first step, the bud formation, takes place by postnatal Day (PND) 7, tubulogenesis – by PND 14, coiling – by PND 28, then branching and morphogenesis is continued to PND 120. Well-developed endometrial folds are observed on PND 28 and endometrial glands are numerous and extensive on PND 56 (Gray et al., 2001; Spencer et al., 2004; Cooke et al., 2013).

In adult, cyclic and pregnant pigs, particularly endometrium undergoes further histological remodeling. The tissue and cellular composition of the porcine uterus was studied by Blackwell et al. (2003) in relation to luteolysis, *i.e.* on Days 12 and 16 postestrus. They did not find any significant changes in mean weight and volume of the uterus between studied days, but some differences in the proportions of endometrium and myometrium were noted by day and uterine region. The proportion of endometrium was lower, but myometrium – higher on Day 16 than on Day 12. The anterior region of the uterus contained distinctively greater proportion of endometrium than myometrium in comparison to the middle or posterior regions. This study also revealed that the porcine endometrium is predominantly comprised of stromal and glandular epithelial cells. On Day 12, stromal, glandular epithelial and luminal epithelial cells constituted 47%, 37% and 16% of total cell quantity in the endometrium, respectively. By Day 16, only the number of glandular epithelial cells tended to increase, while the number of stromal and luminal cells remained relatively unchanged.

In pregnant pigs, the endometrial glands undergo hyperplasia and hypertrophy, and acquire high secretory activity (Perry and Crombie, 1982; Gray et al., 2001). During early pregnancy, the porcine endometrium is subjected to marked morphological and biochemical changes connected with its preparation to the implantation of embryos, involving three stages – the apposition, attachment and adhesion. This process starts on Day 13 and is completed between Days 18-24 after fertilization (Perry, 1981). Before implantation, the luminal uterine epithelium is transformed from a columnar epithelium with microvilli during the pre-receptive stage to an epithelium with a dome-like apical surface without microvilli (Keys and King, 1989). The epithelium then loses apico-basal polarity and its tight junctions become more complex and project towards the basal aspect of the cell (Johnson et al., 1988; Burghardt et al., 1998). It is thought that progesterone-dependent reduction or loss of glycoprotein Muc-1, demonstrating anti-adhesive properties, on the epithelial surface could contribute to the acquisition of uterine receptivity in the pig (Bowen et al., 1997; Bowen and Burghardt, 2000). The implantation is followed by placenta formation, classified in the pig as epitheliochorial, which is essentially completed by about Day 30 (King, 1993). After parturition, the uterine glands are subjected to a rapid involution in the pig (Perry and Crombie, 1982).

PROTEINS SECRETED BY PORCINE ENDOMETRIUM

The uterine cells produce a wide range of protein secretions, including: enzymes, transport proteins, growth factors, cytokines and peptide hormones. The dramatic changes in the proteome determined (by 2D-PAGE) in uterine flushing of the pig were demonstrated between Days 10 and 13 of the estrous cycle and pregnancy; 73% of 280 spots increased on Day 13 *vs.* Day 10 (Kayser et al., 2006). The changes found between considered days appeared to be mostly independent of the pregnancy. In turn, transcriptomic analysis of the porcine endometrium revealed changes in the expression of 2593 genes (with 1335 up-regulated and 1258 down-regulated) on Day 12 (pre-attachment phase) and 1933 genes (with 1229 up-regulated and 704 down-regulated) on Day 14 (initiation of implantation) of pregnancy in comparison to corresponding days of the estrous cycle (Samborski et al., 2013a; Samborski et al., 2013b). The comparison of the data sets showed a substantial overlap of genes differentially expressed on Days 12 and 14 of the pregnancy in comparison to the respective days of the estrous cycle. In addition, further analysis of the data revealed a greater distance between the samples from Day 12 and Day 14 of pregnancy than that between the corresponding days of the estrous cycle (Samborski et al., 2013b). Franczak et al., (2013c), studying the transcriptome of the porcine endometrium on Days 15-16 of pregnancy in comparison to Days 15-16 of the estrous cycle, found up-regulated expression of 266 genes and down-regulated expression of 323 genes out of 589 accurately annotated genes. In pregnant pigs, genes with the most significantly altered expression were involved in many biological processes, among others, related to metabolism, cell adhesion and communication, development and immune system activity. The chosen proteins secreted by the porcine uterus are characterized below.

Secreted phosphoprotein 1

Secreted phosphoprotein 1 (SPP1), named also osteopontin, is an extracellular matrix protein with Arg-Gly-Asp sequence bound by integrins. It influences a variety of physiological processes such as bone mineralization, cell-mediated immune responses, inflammation and angiogenesis (Johnson et al., 2003). In the pig uterus, SPP1 mRNA is initially expressed in discrete regions of uterine LE juxtaposed to the conceptus just prior to implantation (on Day 13), in response to E2 secreted by conceptuses (on Days 11 and 12) and then expands to the entire uterine LE by Day 20 when firm adhesion of conceptus trophectoderm to uterine LE is established (Johnson et al., 2003; Burghardt et al., 2002). In turn, SPP1 protein occupies the apical surface of uterine LE and trophectoderm cells along the entire maternal-placental interface (Burghardt et al., 2002; Johnson et al., 2003). The interaction between the integrin and SPP1 induces tissue remodeling at the conceptus-maternal interface and mediates adhesion between trophectoderm and uterine LE, which is essential for implantation and placentation. Moreover, it activates ion transporters to increase nutrient transport across the chorionic membrane to the placental vasculature (Bazer et al., 2009).

Transport proteins

Transport proteins constitute an essential part of histotroph proteins. This group of uterine proteins, among other, includes uteroferrin and retinol binding protein (RBP), which in pigs represent about 15% and 5% of the total proteins recovered from uterine flushings, respectively (Adams et al., 1981; Roberts and Bazer, 1988). Uteroferrin is an iron-binding protein, with acid phosphatase activity, produced by the uterine GE after Day 11 of pregnancy when embryos elongate and its secretion is maintained until late pregnancy. Entering the placental venous drainage, it delivers iron to the fetus (Roberts et al., 1986; Roberts and Bazer, 1988). In turn, RBP is synthesized in the liver and also in the uterus. In pigs, the uterine expression of RBP mRNA is generally much higher during pregnancy comparing to the estrous cycle (Harney et al., 1993). RBP proteins are detected in LE, GE, and placental areolae (Johansson et al., 2001). In the uterus, retinol bound to RBP is taken up by the developing conceptus through cellular RBP (Napoli et al., 1991). RBP is thus in-

volved in transport of retinol to the fetus and the development and growth of the porcine placenta (Johansson et al., 2001). Steroid hormones regulate synthesis of both proteins, uteroferrin and RBP in the porcine uterus (Simmen et al., 1991; Trout et al., 1992).

Growth factors

The insulin-like growth factor (IGF) system comprises two forms of IGF (IGF-1 and IGF-2), IGF receptors (IGF-RI and IGF-RII types) and family of IGF binding proteins (IGFBPs) (Jones and Clemmons, 1995). IGF-1 is expressed by endometrial GE of cyclic and pregnant pigs (Persson et al., 1997). IGF receptors were found in the porcine endometrium and myometrium as well as in conceptus (Hofig et al., 1991; Corps et al., 1990). The biological actions of IGFs can be modulated by IGFBPs (Jones and Clemmons, 1995), present also in the porcine uterus (Badinga et al., 1999). It has been proposed that increased uterine production of IGF on Days 11-12 of gestation is involved in the stimulation of conceptus aromatase ($P450_{arom}$) activity to enhance estrogen synthesis during the period of trophoblast elongation (Green et al., 1995). On the other hand, it was reported that early exposure of pregnant gilts (on Days 9-10) to estrogen causes premature loss of uterine IGFs (accompanied by proteolysis of the IGFBPs) during the period of conceptus elongation (Ashworth et al., 2005). Collectively, it might be stated that IGFs, acting through paracrine and autocrine manner within uterus, effectively participate in the preparation of its microenvironment to implantation and are responsible for communication with conceptuses.

Fibroblast growth factor 7 (FGF7), also known as keratinocyte growth factor, belongs to FGF superfamily, which has been reported to stimulate cell proliferation, differentiation, migration, and vascular angiogenesis in different tissues (Szebenyi and Fallon, 1999). In the pig uterus, FGF7 is expressed in the endometrial epithelium (Ka et al., 2000). The FGF7 expression appeared to be abundant between Days 12 and 15 of the estrous cycle and pregnancy with the highest levels on Day 12 in pregnant gilts and Day 15 in cyclic gilts (Ka et al., 2000). During pregnancy, the FGF7 expression in the porcine endometrial epithelium seems to be stimulated by E2 from developing conceptuses (Ka et al., 2007). FGF7 receptor, named FGF receptor 2IIIb (FGF2IIIb), was demonstrated in both endometrial epithelium and conceptus trophectoderm (Ka et al., 2000). There were some discrepancies concerning the target for the uterine FGF7 in pregnant pigs. The studies, based on the [^{3}H]thymidine incorporation assay and the proliferating cell nuclear antigen staining, suggest that FGF7 acts on conceptus trophectoderm in a paracrine manner rather than on endometrial epithelial cells in an autocrine manner (Ka and Bazer, 2005).

Transforming growth factors β (TGFßs) include three isoforms (TGFß1, TGFß2 and TGFß3), acting via two types of receptors (TGFβRI and TGFβRII). TGFß is secreted in connection with latency-associated peptide (LAP). TGFß has to be released from its latent state to became active (Annes et al., 2003). LAP has the Arg-Gly-Asp (RGD) sequence and can interact with α_v-integrin receptors present on conceptus trophectoderm and uterine LE (Massuto et al., 2010), in turn liberated TGFß may affect TGFβRs. All forms of TGFß and TGFß receptors are expressed in the porcine endometrium and conceptuses (Gupta et al., 1998a, b). The endometrial expression of TGFßs is localized in epithelial and stroma cells, but TGFß receptors in apical side of epithelial cells and it increases on Days 10-14 of pregnancy (Gupta et al., 1998b). It was also indicated that porcine conceptuses may regulate TGFß1 synthesis in the endometrium during implantation as well as TGFß1 may promote conceptus development by increasing the proliferation of trophoblast cells (Blitek et al., 2013). Generally, it is thought that, within uterus, TGFßs are involved in the regulation of uterine secretory activity, tissue remodeling and immune responses as well as trophoblast invasion and early embryo development (Jones et al., 2006).

Epidermal growth factor (EGF) represents larger family of EGF factors, which participate in multiple developmental processes (Guzeloglu-Kayisili et al., 2009). The presence of EGF (Zhang et al., 1992; Kennedy et al., 1994; Kim et al., 2001) and EGF receptor (EGFR) (Zhang et al., 1992; Kennedy et al., 1994; Kim et al., 2001; Wollenhaupt et al., 2004) mRNAs was demonstrated in the porcine endometrium. The expression of EGF

was localized in the GE (Kennedy et al., 1994), but EGFR has been found at higher density in stroma cells and also in GE (Zhang et al., 1992). EGF and EGFR showed similar endometrial expression patterns in pregnant pigs, being markedly increased around implantation (Kim et al., 2001). Furthermore, the presence of EGF protein was confirmed in uterine fluid (Brigstock et al., 1996). EGF and EGFR were also detected in porcine embryos (Vaughan et al., 1992). The participation of EGF system in the preparation of endometrium to implantation (Wollenhaupt et al., 2004) and in the establishment of organ systems during the early pig development (Vaughan et al., 1992). In addition, EGF was found to increase PGE2 and PGF2α secretion by stromal cells (Zhang et al., 1992).

Vascular endothelial growth factor (VEGF) plays an important role in physiological as well as in pathological angiogenesis in a variety of tissues. It may induce proliferation and migration of target cells, and alter their gene expression (Ribatti, 2005). The presence of VEGF and its specific receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR), was demonstrated in the uterine structures of cyclic and pregnant pigs (Winther et al., 1999). VEGF and its receptors were found – depending on the physiological stage – in the uterine LE and GE, endothelial and smooth muscle cells of the vessel wall. During pregnancy, VEGF and its receptors were elevated in the endothelial cells, smooth muscle cells of the vessel wall and uterine GE throughout gestation, whereas in LE started to increase from Day 21. The expression of VEGF mRNA was constant during the cycle and significantly increased on Days 22-25 of gestation (*vs.* Days 9-17) (Kaczmarek et al., 2008b). In turn, the uterine content of VEGF protein is elevated during the periovulatory and periimplantation periods. VEGF receptors (type I and II) were localized in epithelial and stromal cells, blood vessels and myometrium, and their expression in the porcine uterus fluctuated during the cycle and pregnancy. Moreover, LH, IGF-I and relaxin displayed some potential to stimulate the uterine VEGF synthesis in pregnant pigs (Kaczmarek et al., 2008a). An abundance of VEGF system at the maternal-fetal interface in the pig was also documented (Charnock-Jones et al., 2001; Vonnahme et al., 2001).

Cytokines

Cytokines regulate many reproductive functions in pigs including embryo development, implantation, feto-maternal communication as well as endocrine activity of the uterus (Schäfer-Somi, 2003; Ross et al., 2003a, b; Blitek and Ziecik, 2006; van Mourik et al., 2009; Franczak et al., 2010; Jana et al., 2010; Seo et al., 2012). It is considered that cytokines involved in these processes, among others, are leukemia inhibitory factor (LIF), interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNFα).

Leukemia inhibitory factor is a pleiotropic cytokine participating in the regulation of many processes, including cell proliferation, differentiation and survival as well as different aspects of implantation (Sharkey, 1998; Dimitriadis et al., 2005; van Mourik et al., 2009). It exhibits proinflammatory properties with a potential to control the proportions and numbers of immune cells in the endometrium during implantation (Kimber, 2005). In the human endometrium, LIF mRNA and protein contents are low or undetectable during the proliferative phase, but significantly increase during mid-late secretory phase (Charnock-Jones et al., 1994; Dimitriadis et al., 2000). In pigs, the expression of LIF mRNA in the endometrium increases between Days 10 and 12 of the estrous cycle and pregnancy. The LIF protein content in the uterine lumen reaches higher values on Day 12 of pregnancy than on corresponding day of the cycle (Blitek et al., 2012). Expression of LIF receptor (LIF-R) was found in the human and mice embryos and luminal epithelium (Sharkey,1998). Endometrial expression of LIF can be stimulated by hCG, TNFß, IGF and leptin (Gonzales et al., 2004; Perrier et al., 2004; Kimber, 2005).

Interleukin 1 is a pro-inflammatory cytokine with multiple physiological functions in many tissues, including processes taking place in the uterus during early pregnancy (Krüssel et al., 2004). The IL-1 system includes two ligands, IL-1α and IL-1ß, two types of IL-1 cell-surface receptors (IL-1R1 and IL-1R2), a non-binding receptor accessory protein (IL1RAcP) and naturally occurring receptor antagonist (IL-1ra). IL-1ß is expressed in the porcine embryos and endometrium (Tuo et al., 1996; Ross et al., 2003a). Conceptus IL-1ß gene expression is enhanced

during the period of rapid trophoblastic elongation, followed by a dramatic decrease in elongated conceptuses on Day 15. The uterine lumenal content of IL-1ß increases during the trophoblastic elongation on Day 12 and then declines on Day 15 of pregnancy. IL-1ß gene expression in porcine conceptuses is temporally associated with increased endometrial IL-1R1 and IL-1RAcP gene expression in pregnant gilts. During Days 10–15 of pregnancy, IL-1ß gene expression in the porcine endometrium remains low (Ross et al., 2003a). On the basis of these observations, IL-1ß is thought to be implicated in the process of trophoblastic elongation and the establishment of pregnancy in the pig. Moreover, IL-1 also affects other activates of the pig uterus, including synthesis of prostaglandins and steroids as well as expression of genes coding for opioid precursors (Franczak et al., 2010, 2012, 2013a, b; 2014, Jana et al., 2010; Dziekonski et al., 2013a, b).

Interlukin 6 is a multifunctional cytokine with pro-inflammatory properties, produced in many tissues. As shown in humans, it is also expressed by endometrial epithelium and stromal cells at higher levels during the peri-implantation period (Tabibzadeh et al., 1995; Cork et al., 2002). Receptors for IL-6 (IL-6R) are localized in GE and stromal cells of the human endometrium (Tabibzadeh et al., 1995), and also in preimplantation embryos (Sharkey et al., 1995). In the pig, the endometrial expression of IL-6 mRNA and protein was higher on Day 12 of pregnancy in comparison to Day 12 of the estrous cycle. In turn, expression of IL-6R mRNA in the porcine conceptuses was increased on Days 10, 12 and 14 than on Days 16 and 18 of pregnancy. Furthermore, IL-6 stimulated an attachment and proliferation of the porcine trophoblast cells under *in vitro* conditions (Blitek et al, 2012). These results indicate that IL-6 is an important component of embryo-uterine interactions during early pregnancy in the pig. Moreover, IL-6 also appeared to affect the uterine secretion of prostaglandins and steroids as well as expression of genes coding for opioid precursors in pigs (Franczak et al., 2012, 2013a,b, 2014; Jana et al., 2010; Dziekonski et al., 2013b).

Tumor necrosis factor α is a pluripotent cytokine expressed in many tissues/cells, including immune cells and structures of the female reproductive system, such as: ovaries, oviducts and uteri as well as in embryos and placentae (Hunt, 1993). Its expression was found in the porcine endometrium during early pregnancy (Yu et al., 1998). However, the role of TNFα in the uterus is still ambiguous. The involvement of TNFα in the regulation of uterine PG secretion (Blitek and Ziecik, 2006; Franczak et al., 2012; Jana et al., 2010; Waclawik et al., 2010) and steroidogenesis (Franczak et al., 2013a, b, 2014) in cyclic and pregnant pigs was documented. The overexpression of TNFα mRNA in the endometrium, caused by estrogen administration to gilts on Days 9-10 of gestation, was associated with embryonic degeneration (Yu et al., 1998). It is thought that abnormal expression of TNFα and/or its receptors (TNFR1 or TNFR2) may be connected with pregnancy failures (Calleja et al., 2012).

Neuropeptides

Studies performed with different species, indicate capability of uterine tissues to produce different neuropeptides, among others: endogenous opioid peptides (EOP), corticotrophin-releasing hormone (CRH) and gonadotrophin-releasing hormone (GnRH) (Li et al., 1987, 1992, 1993a, b; Makrigiannakis et al., 1995, 2001; Gravanis et al., 2001; Okrasa et al., 2003; Giammarino et al., 2009). The expression of genes coding for three major opioid precursors (i.e. proopiomelancortin, proenkephalin and prodynorphin) was confirmed in the uterus of the pig and other species during the estrous cycle and pregnancy (Jin et al., 1988; Low et al., 1989; Zhu and Pinta, 1998; Dziekonski et al., 2013a, b). In addition, our studies revealed that uterine expression of these genes in pigs remains under influence of IL-1ß, TNFα and IL-6 (Dziekonski et al., 2013a, b). Among EOP, the presence of ß-endorphin (proopiomelancortin derivate) in the porcine uterus was the best documented. Namely, it was localized in GE and LE, and in the uterine fluids. Within chosen periods of the estrous cycle and early pregnancy (Days 8-14/15), its concentration in the endometrium was higher on Days 14-15 than on Days 8-12, but total content in the uterine fluids appeared to be the highest on Days 10-11 (in Meishan gilts on Day 8) (Li et al., 1992, 1993a). The treatment of OVX gilts with estradiol benzoate stimulated ß-endorphin content in the uterine tissues (Okrasa et al., 2003). It is consi-

dered that EOP, depending on the type of opioid receptor involved in their action, may affect many different processes in the uterus, including: cell proliferation (Vertes 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., 1987; 1992, 1993a). In turn, endometrial CRH may participate in intrauterine inflammatory processes, blastocyst implantation and early maternal tolerance as suggested on the basis of studies mostly performed in rodents (Gravanis et al., 2001; Makrigiannakis et al., 2006). The presence of GnRH in porcine uterus was noted in several studies (Li et al., 1993a, b; Okrasa et al., 2003) and an inhibitory influence of this peptide on uterine contractility was demonstrated in rats (Margioris et al., 1988). Collectively, it seems that biologically active peptides are implicated in the paracrine and autocrine regulations of uterine functions, but their contribution needs further experiments.

PROSTAGLANDINS

Prostaglandins (PGs) are involved in the regulation of several reproductive processes in females such as ovulation, luteal regression, the implantation and maintenance of pregnancy, parturition and postpartum physiology (Weems et al., 2006). Prostaglandins derive from arachidonic acid, which is at first converted to PGH2 by cyclooxygenase-1 and -2 (PTGS1 and PTGS2) and then to various forms of PGs, including PGE2 and PGF2α. PGE synthases (PTGES1, PTGES2, and PTGES3) and PGF synthase (PGFS or AKR1B1) convert PGH to PGE2 and PGF2α, respectively. PGE2 can be converted to PGF2α by PGE2-9-oxoreductase (CBR1) (Choi et al., 2014). Presence of these enzymes and/or their mRNAs in the pig uterus was confirmed in many studies (Dubois et al., 1993; Smith and DeWitt, 1996; Franczak et al., 2004; 2006, 2010; Ashworth et al., 2006; Geisert et al., 2006; Waclawik et al., 2006; Waclawik and Ziecik, 2007; Seo et al., 2008; Wasielak et al., 2009; Blitek et al., 2010; Jana et al., 2010). Both prostaglandins are produced in the porcine uterus; mainly by the endometrial tissue (Zhang and Davis, 1991; Blitek et al., 2006), as well as by myometrium (Franczak et al., 2004, 2006; Jana et al., 2010). In pigs, PGF2α exhibits luteolytic properties (Christenson et al., 1994; de Rensis et al., 2012), whereas PGE2 is luteoprotective with luteotrophic and antiluteolytic effects (Christenson et al., 1994; Stefanczyk-Krzymowska et al., 2006). Porcine conceptuses also express PTGES and AKR1B1 (Waclawik et al., 2005; Ziecik et al., 2006). In pigs, during the estrous cycle, PGF2α pulses in the utero-ovarian venous blood associated with luteolysis appear between Days 13-18 (Dusza et al., 1988; Christernson et al., 1994; Kotwica et al., 1999). In turn, during early pregnancy, prostaglandin secretion reaches the highest values on Days 11-12 with predominance of PGE2 (Christenson et al., 1994). Thus, in cyclic gilts, the uterine production/secretion of PGF2α is privileged, but in pregnant animals it is effectively restricted, what changes the PGE2/PGF2α ratio in a favor of luteoprotective action (Zhang and Davis, 1991). Intrauterine infusion of PGE2 to cyclic gilts resulted in comparable increases in concentrations of both PGE2 and PGF2α in the utero-ovarian venous blood (Okrasa et al., 1985). This might arise from immediate uterine conversion of PGE2 to PGF2α, since the endometrial CBR1 protein expression in cyclic gilts is upregulated, in contrast to its downregulation observed in gilts on Days 10-11 of pregnancy (Waclawik and Ziecik, 2007). Moreover, the expression profile of enzymes involved in prostaglandin synthesis (relatively high mPGES-1 *vs.* low PGFS and CBR1) in the porcine endometrial tissue on Days 10-13 of pregnancy is favorable for increasing the PGE2/PGF2α ratio (Waclawik et al., 2006). During early pregnancy, the protection of corpora lutea from luteolytic pulses of PGF2α possibly is also provided by the changed direction of its secretion from the uterine venous drainage to the uterine lumen (Bazer and Thatcher, 1977) and the mechanism of retrograde PGF2α transfer from the uterine blood and lymph outflow to the uterine lumen through arterial blood supplying the uterus, including the PGF2α accumulation in arterial walls (Krzymowski and Stefanczyk-Krzymowska, 2004). It is thought that the changes in uterine production/secretion and distribution of PGs in pigs during early pregnancy, leading to maintenance of corpora lutea beyond the luteal phase, are triggered by conceptus estrogen (Bazer and Thatcher, 1977; Wasielak et al., 2009). In addition, it has

to be mentioned that the uterine synthesis/secretion of PGs remains under influence of many factors (e.g. growth factors, cytokines) and they are briefly presented in Table 1.

TABLE 1. Factors affecting prostaglandin synthesis and/or secretion by the porcine uterus

Factor	Effects	References
Estrogens	↑ PGE2 synthesis and PGE2/PGF2α ratio ↑ Prostaglandin E2 receptor (PTGER2) expression ↓ PGE2 secretion to peripherial circulation	Waclawik et al., 2009 Geisert et al., 2006 Bazer and Thatcher, 1977
Progesterone	↓ PGE2 and PGF2α production *in vitro* by endometrium (Day 9 of the cycle) Modulation of PGs secretion by the uterine tissues in response to OT	Blitek et al., 2010 Franczak et al., 2006 Kotwica et al., 2010
OT	↑ PGF2α pulsatile secretion *in vivo* during luteolysis (Days 14-19) ↑ PGF2α secretion *in vitro* by endometrial explants during luteolysis and pregnancy (Days 14-16) and PGE2 during luteolysis (Days 14-16) ↑ PGE2 synthesis and secretion by endometrial LE cells (Days 10-11 of pregnancy)	Kotwica et al., 1999 Kotwica et al., 2010 Waclawik et al., 2010
LH	↑ PGE2 and PGF2α *in vitro* secretion by endometrial cells	Blitek and Ziecik, 2006
PGE2	↑ mRNA and protein expression of PTGS2 and mPGES-1 ↑ PTGER2 mRNA expression and PGE2/PGF2α ratio during the peri-implantation period (uterine autoregulation loop)	Waclawik et al., 2009
Tumor necrosis factor α	↑ PGF2α secretion from endometrial stromal and LE cells ↑ PTGS2 and mPGEs-1 expression and PGE2 release (Days 11-12 of the cycle and pregnancy) ↑ PGFS mRNA expression (Days 12-16 of the cycle) ↑ PGFM (metabolite PGF2α) secretion (Days 15-16 of pregnancy)	Blitek and Ziecik, 2006 Waclawik et al., 2010 Franczak et al., 2012
Interleukin 1ß	↑ PTGS1/2 mRNA expression in endometrium ↑ COX-2 *in vitro* expression in endometrial (Days 10-13 of the cycle and pregnancy) and miometrial explants (Days 10-11 and 15-16 of the cycle and pregnancy) ↑ PGE2 *in vitro* synthesis and secretion during early pregnancy (Days 10-13) and the cycle (Days 15-16) ↑ PGFS mRNA expression (Days 12-16 of the cycle) and ↓ (Days 12-13 of pregnancy) ↑ PGF2α endometrial release (Days 12-13 of pregnancy and 15-16 of the cycle) ↑ PGFM secretion (Days 10-11 and 15-16 of the cycle and 12-16 of pregnancy)	Seo et al., 2012 Franczak et al., 2010 Franczak et al., 2012
Interleukin 6	↑ PGFS mRNA expression (Days 12-16 of the cycle) ↑ PGF2α *in vitro* release (Days 12-16 of pregnancy) by endometrium ↑ PGFM secretion (Days 15-16 of pregnancy) by endometrium	Franczak et al., 2012
Peroxisome proliferator activated receptors (PPARs)	↑ PGE2 *in vitro* secretion after activation: PPARγ (Days 10-12 and 14-16 of the cycle and pregnancy), PPARß (Days 14-16 of the cycle and pregnancy), PPARα (Days 10-12 of the cycle and 14-16 of pregnancy). ↑ PGF2α *in vitro* secretion after activation: PPARs (α, ß and γ) (Days 10-12 and 14-16 of the cycle)	Bogacka et al., 2013a Bogacka et al., 2013b
Lysophosphatidic acid (LPA)	↑ PTGS2 endometrial expression	Seo et al., 2008
Seminal plasma	↑ PGE2/PGF2α ratio in uterine lumen	Kaczmarek et. al., 2009

STEROIDOGENESIS IN THE PORCINE UTERUS – BASED ON OUR OWN STUDIES

The classical organs of sex steroids synthesis and secretion in females, including pigs, are ovaries (Niswender et al., 2000; Schams and Berisha, 2002). Therefore, the role of ovarian steroids: progesterone (P4), androgens – androstenedione (A4) and testosterone (T) and estrogens – estrone (E1) and estradiol-17ß (E2) in the regulation of reproductive processes is well known (Gregoraszczuk et al., 1983; Schams and Berisha, 2002; Jammongjit and Hammes, 2006). It was found however, that uterine tissues in pigs are rich sources of steroid hormones (Franczak, 2008; Franczak and Kotwica, 2008, 2010).

The uterine steroidogenesis is particularly crucial in control of the groundbreaking periods of early pregnancy and the estrous cycle in pigs. These periods include the maternal recognition of pregnancy and the beginning of the implantation as well as luteolysis (Geisert et al., 1990; Ziecik, 2002; Krzymowski and Stefanczyk-Krzymowska, 2004). The maternal recognition of pregnancy comes after receiving hormonal signals from embryos. In pigs, estrogens secreted by blastocysts on Days 12-13 of pregnancy play a role of such signal (Bazer and Thatcher, 1977; Heap et al., 1981; Geisert et al., 1982, 1990). This leads to maintenance of corpora lutea (CLs) with continuation of high P4 secretion and simultaneously the process of implantation is initiated on Days 15-16 of pregnancy (Bowen and Burghardt, 2000; Franczak and Bogacki, 2009). In contrast, during the estrous cycle, CLs undergo regression on Days 15-16 (Moeljono et al., 1976; Ziecik, 2002). Our recent studies provided new data, pointing at the uterine tissues as an important source of estrogens during early pregnancy, including the period of its maternal recognition (Franczak, 2008; Franczak and Kotwica, 2008).

In our study we focused on different aspects of steroidogenesis in the porcine uterus during the estrous cycle and early pregnancy. The experiments aimed to determine in both the endometrium and myometrium: 1) the presence of mRNAs and proteins of the main steroidogenic enzymes, 2) secretion of androgens and estrogens *in vitro* under basal conditions and in the presence of P4 and cytokines on Days 10-16 of pregnancy and the estrous cycle.

Steroidogenic enzymes in the porcine uterus

The expression of mRNAs and proteins of the main steroidogenic enzymes: 3ß-hydroxysteroid dehydrogenase/Δ^5-Δ^4isomerase (3ß-HSD), cytochrome P450 17 α-hydroxylase/C_{17-20}lyase (CYP17) and aromatase cytochrome P450 ($P450_{arom}$) were determined in the endometrium and myometrium of pigs on Days 10-11, 12-13 and 15-16 of pregnancy and the estrous cycle (Franczak and Kotwica, 2008, 2010; Franczak et al., 2013a; Wojciechowicz et al., 2013).

We found that the porcine uterus possesses active 3ß-HSD (Wojciechowicz et al., 2013), responsible for P4 and A4 production (Simard et al., 2005). Endometrial and myometrial expression of 3ß-HSD mRNA did not change during early pregnancy, but in the estrous cycle, it decreased in the endometrium on Days 12-16 and the myometrium on Days 15-16 (Table 2B). The high activity of 3ß-HSD was established in the tested tissues during maternal recognition of pregnancy (Days 12-13, Table 1B). This proves that uterine tissues of gravid and cyclic pigs have a potential for synthesis of P4 and A4.

CYP17, encoded by *CYP 17* gene, is the enzyme that controls the entry of P4 and pregnenolone into steroid biosynthetic pathway (Hall, 1986). The uterine expression of *CYP 17* was demonstrated in the endometrium and myometrium on Days 14-16 of pregnancy and the estrous cycle (Franczak and Kotwica, 2008). Thus, it means that porcine uterus produces itself pregnenolone and P4 for further intrauterine synthesis of steroid hormones.

The endometrial expression of *CYP 19* gene, encoding $P450_{arom}$ enzyme catalyzing synthesis of estrogens from androgens (Nelson et al., 1996), in pregnant and cyclic pigs was higher on Days 10-11 and 12-13 than on Days 15-16 (Table 2A) (Franczak et al., 2013a). In turn, the expression of *CYP19* in the myometrium was higher on Days 10-11 *vs.* Days 12-13 and 15-16 of pregnancy, and remained stable in cyclic pigs (Franczak et al., 2014). However, the quantity of $P450_{arom}$ protein in the endometrium and myometrium of gravid pigs was the highest on Days 15-16 (Franczak et al., 2013a; 2014). During the estrous cycle, the content of $P450_{arom}$ protein did not change in the endometrium, but in the myometrium was higher on Days 12-13 and 15-16 than on Days 10-11.

TABLE 2. The changes in expression of 3βHSD and CYP19 mRNAs (A), and 3βHSD and P450$_{arom}$ proteins (B) in the endometrium and myometrium of pigs during chosen days of early pregnancy and the estrous cycle.
NC no changes, ↑ increased, ↓ decreased.

A/ mRNAs expression

Tissue / Studied days	Pregnancy		The estrous cycle	
	Endometrium	Miometrium	Endometrium	Miometrium
	3βHSD – androstenedione as a product			
10-11	NC	NC	↑	↓
12-13	NC	NC	↓	↑
15-16	NC	NC	↓	↓
	3βHSD – progesterone as a product			
10-11	NC	NC	↑	↓
12-13	NC	NC	↓	↑
15-16	NC	NC	↓	↓
	CYP19			
10-11	↑	↑	↑	NC
12-13	↑	↓	↑	NC
15-16	↓	↓	↓	NC

B/ protein expression

Tissue / Studied days	Pregnancy		The estrous cycle	
	Endometrium	Miometrium	Endometrium	Miometrium
	3βHSD – androstenedione as a product			
10-11	↑	↑	↑	↑
12-13	↑	↑	↓	↑
15-16	↓	↓	↓	↓
	3βHSD – progesterone as a product			
10-11	↑	↓	↑	↑
12-13	↑	↑	↓	↑
15-16	↓	↑	↓	↓
	P450arom			
10-11	↓	↓	NC	↓
12-13	↓	↓	NC	↑
15-16	↑	↑	NC	↑

The data clearly prove the possibility of estrogen synthesis by endometrial and myometrial tissues in pigs.

Immunohistochemical studies confirmed the localization of 3βHSD and P450$_{arom}$ proteins in the stromal, glandular and luminal cells of the endometrium and in myocytes (Wojciechowicz et al., 2013; Franczak et al., 2013a, 2014). Furthermore, the secretion of A4, T and E2 by isolated endometrial (epithelial and stromal) and myometrial cells of gravid and cyclic pigs has been demonstrated (Franczak and Kotwica, 2010). The results indicate that in pigs all types of functional uterine cells possess enzymes responsible for steroidogenic pathway.

Basal secretion of steroid hormones by uterine tissues

In order to investigate the role of uterine steroids in the regulation of early pregnancy and the estrous cycle in pigs, the incubation of endometrial and myometrial slices (200-210 mg w/w) was performed for 12 h. This procedure allowed to examine basal and induced secretion of steroid hormones by the uterine tissues of gravid and cyclic pigs (Franczak et al., 2006).

The basal concentrations of P4 in media after incubation of the endometrium and myometrium ranged from 110±6.0 pg/ml to 393±40 pg/ml and from 65±23 pg/ml to 360±38 pg/ml, respectively (Wojciechowicz et al., 2013). The relative secretion of P4 (Fig. 1) by the endometrium did not differ on studied days of pregnancy and was higher by the tissue harvested on Days 10-12 than on Days 15-16 of the estrous cycle. The myometrium of pregnant pigs secreted relatively lower amounts of P4 on Days 15-16 than on Days 10-14. The myometrial P4 secretion was gradually increasing during studied period of the estrous cycle (Fig. 1).

The basal concentrations of A4 in culture media after incubation of the endometrium and myometrium ranged from 17.0±7 pg/ml to 110.5±27.3 pg/ml and from 20.8±6.4 pg/ml to 96.3±9.5 pg/ml, respectively (Wojciechowicz et al., 2013). The relative secretion of A4 by both uterine tissues was the highest on Days 12-13 of pregnancy and the

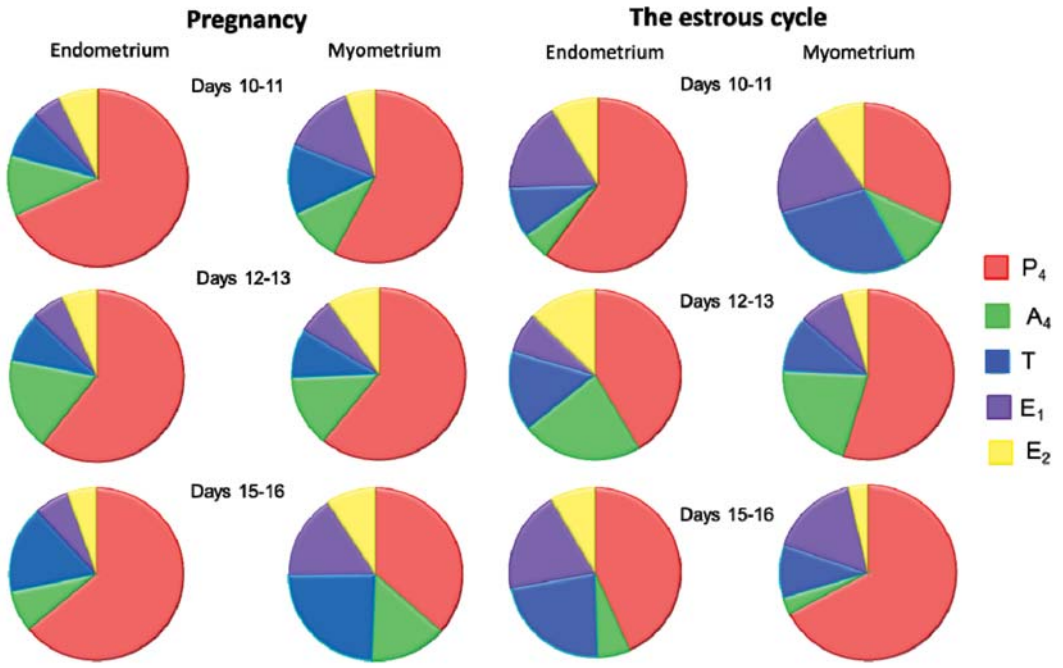

Fig. 1. The relative basal amounts of progesterone (P4), androstenedione (A4), testosterone (T), estrone (E1) and estradiol-17β (E2) secreted by the endometrial and myometrial slices (200-210 mg w/w) *in vitro* during 12 h-incubation under an atmosphere of 95% O_2 and 5% CO_2 at 37°C. The slices were harvested from gravid and cyclic pigs on Days 10-11, 12-13 and 15-16 [Franczak et al., 2013a,b, 2014; Wojciechowicz et al., 2013; data of T were unpublished].

estrous cycle (Fig. 1). On Days 15-16, the gravid myometrium released markedly higher amounts of A4 than non-gravid.

The basal concentrations of T in media after incubation of the endometrium and myometrium ranged from 32.8±3.9 to 100.9±5.6 pg/ml and from 40.5±6.4 to 73.3±18.5 pg/ml, respectively (Franczak, 2008). The highest relative secretion of T by the endometrium of pregnant gilts was noted on Days 15-16, but gradual increase occurred during studied period of the estrous cycle (Fig. 1). The myometrium of both gravid and non-gravid pigs secreted similar amounts of T (except Days 15-16 of pregnancy and 10-11 of the cycle).

The basal concentrations of E1 in media following incubation of the endometrium and myometrium ranged from 23.1±2.8 to 58.0±8.0 pg/ml and from 40.2±8.5 to 76.5±14.0 pg/ml, respectively (Franczak et al. 2013b). The relative secretion of E1 by the gravid endometrium was stable, but during the cycle, it was reduced on Days 12-13 (Fig. 1). The myometrium released relatively lower amounts of E1 on Days 12-13 than on other days of pregnancy and higher on Days 15-16 than on Days 10-14 the estrous cycle.

The basal concentrations of E2 in media after incubation of the endometrium and myometrium ranged from 23.3±11.0 to 41.0±14.7 pg/ml and from 16.6±3.6 to 56.9±6.5 pg/ml, respectively (Franczak et al., 2013a; 2014). The relative basal secretion of E2 by the endometrium was stable on studied days of pregnancy and the estrous cycle (Fig. 1). The myometrium released higher amounts of E2 on Days 12-16 than Days 10-11 of pregnancy. The relative amounts of E2 secreted by myometrium exhibited decreasing tendency during studied days of the estrous cycle.

The above data clearly confirm the existence of functional steroidogenic pathway in the porcine uterine tissues, which is active during both the estrous cycle and pregnancy. However, contribution of the endometrium and myometrium to the uterine steroidogenesis varies depending on the physiological stage. Since E2 is implicated in the maintenance of early pregnancy and the regulation of luteal activity in pigs, we estimated an engagement of the uterine tissues in its secretion to the uterine fluid (Franczak and Kotwica, 2008, 2010). During early pregnancy (Days 14-16), the endometrium and myometrium delivers about 65% and 35% of the total E2 content in uterine fluid, respectively. In turn, during Days

14-16 of the estrous cycle, the endometrial and myometrial basal input of E2 constitutes about 41% and 59%, respectively. This indicates that endometrium is preferential source of E2 during maintenance of early pregnancy, while the myometrium during luteolysis. We calculated also that daily uterine production of E2 in pregnant pigs is about 1 μg. This amount of E2 is significant, in comparison to about 1.5 μg of estrogens daily leaving the pig uterus on Day 13 of pregnancy, as reported by Ford et al., (1982). Therefore, we have documented that uterine tissues are important sources of E2 in pigs.

The regulation of steroid secretion by uterine tissues

We have also studied the role of P4 and cytokines in the regulation of steroid secretion from porcine uterine tissues. It was assumed that P4 may be a direct substrate for uterine synthesis of androgens and estrogens, because both the endometrium and myometrium are rich source of P4 (Wojciechowicz et al., 2013). The presence of P4 in culture media resulted in significant increases (several-fold) in production of androgens (A4 and T) and estrogens (E1 and E2) by the endometrium and myometrium harvested from both early pregnant and cyclic pigs (Table 3) (Franczak and Kotwica, 2008, 2010; Franczak et al., 2013a, 2013b, 2014). These results were confirmed in experiments with both isolated cells and slices of the uterine tissues. The exception was only the lack of P4 stimulatory effect on E2 secretion by endometrium during Days 10-13 of the cycle (Franczak et al., 2013a). Thus, we have shown that P4 may serve as a substrate for the uterine synthesis of androgens and estrogens in pigs.

The cytokines (IL-1ß, IL-6 and TNFα) are important regulators of peri-implantation events and luteolysis in pigs (Anegon et al., 1994; Yu et al., 1998; Ross et al., 2003a, Zmijewska et al., 2013). Specific receptors for these cytokines are present on the porcine endometrial and myometrial cells (Anegon et al., 1994; Yu et al., 1998; Ross et al., 2003a; Franczak et al., 2014). Therefore, we studied the role of IL-1ß, IL-6 and TNFα as a potential regulators of estrogen production in the endometrium and the myometrium of early pregnant and cyclic pigs.

The cytokines, IL-1ß, IL-6 and TNFα, increased E1 secretion by the endometrium of gravid pigs on Days 10-13 and in cyclic pigs on Days 12-13 (Franczak et al., 2013b). Interleukin 6 specifically increased E1 secretion by gravid endometrium only on Days 15-16. The stimulatory effect of cytokines on E1 production by the myometrium was less pronounced than by the endometrium. On Days 12-13 of pregnancy, the myometrial tissue increased E1 production in response to IL-1ß and IL-6. In turn, the cyclic myometrium responded only to IL-6 on Days 12-16. These results provide evidence for a pivotal role of IL-1ß, IL-6 and TNFα in the regulation of E1 production in the porcine uterus. They allow to suggest that physiological significance of increased uterine E1 secretion by cytokines can rely on: 1) supplementing the pool of estrogens produced by embryos during maternal recognition of pregnancy (on Days 12-13), as well as 2) remodeling of uterine tissues on Days 10-16 of pregnancy and Days 12-13 of the estrous cycle.

TABLE 3. The effects of progesterone (P4) on secretion of androgens (A4 and T) and estrogens (E1 and E2), as well as interleukin 1ß (IL-1ß), interleukin 6 (IL-6) and tumor necrosis factor α (TNFα) on secretion of E1 and E2 by incubated (12 h) endometrial and myometrial slices harvested from pregnant and cyclic pigs on Days 10-11, 12-13 and 15-16. A4 concentration in culture media was determined only on Days 14-16. *NC no changes,* ↑ *increased,* ↓ *decreased, - not studied.*

Studied hormones P4 and cytokines	Pregnancy								The estrous cycle							
	Endometrium				Myometrium				Endometrium				Myometrium			
	A4	T	E1	E2	A4	T	E1	E2	A4	T	E1	E2	A4	T	E1	E2
10-11																
P4	-	↑	↑	↑	-	↑	↑	↑	-	↑	↑	NC	-	↑	↑	↑
IL-1β	-	-	↑	NC	-	-	NC	NC	-	-	NC	NC	-	-	NC	NC
IL-6	-	-	↑	NC	-	-	NC	NC	-	-	NC	NC	-	-	NC	NC
TNF-α	-	-	↑	-	-	-	NC	NC	-	-	NC	-	-	-	NC	NC
12-13																
P4	-	NC	↑	↑	-	↑	↑	↑	-	NC	↑	NC	-	↑	↑	↑
IL-1β	-	-	↑	NC	-	-	↑	NC	-	-	↑	NC	-	-	NC	NC
IL-6	-	-	↑	NC	-	-	↑	NC	-	-	↑	NC	-	-	↑	NC
TNF-α	-	-	↑	-	-	-	NC	NC	-	-	↑	NC	-	-	NC	NC
15-16																
P4	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
IL-1β	-	-	NC	↑	-	-	NC	↑	-	-	NC	NC	-	-	NC	NC
IL-6	-	-	↑	↑	-	-	NC	↑	-	-	NC	NC	-	-	↑	NC
TNF-α	-	-	NC	-	-	-	NC	↑	-	-	NC	-	-	-	NC	NC

SUMMARY

The uterus is an essential organ for reproduction in mammals. In the pig, its development begins pre-natally and is continued post-natally, reaching complete maturation by Day 120. In the adult, the uterine wall is comprised of two functional compartments, the endometrium and myometrium, surrounded by perimetrium. During the estrous cycle and pregnancy, it undergoes specific remodeling. The main function of the uterus is to ensure proper reception of embryos and to provide further support for the save development of embryos/fetuses. These very complex processes require an adequate intrauterine microenvironment and embryo-maternal interactions. Therefore, the uterus produces and secretes a wide array of substances, among others numerous proteins, prostaglandins and steroid hormones. The uterine protein secretions involve enzymes, growth factors, transport proteins, chemokines, cytokines and hormones. Prostaglandins, mainly involving PGF2α and PGE2, exhibit several opposite actions and the ratio between them is important for their regulatory effects. The uterus is well known as a target organ for ovarian steroids, but it is also predisposed to produces steroid hormones, including progesterone, androgens and estrogens. The porcine endometrium was known to be responsible for production of the above secretions. Our recent studies provided evidences that porcine myometrium is also involved in synthesis of substantial amounts of steroids and prostaglandins. Collectively, the uterine secretory activity is subjected to precise control to make it well-adjusted to reproductive events connected with gestation period, including the extension of CL life-span, maintenance of pregnancy, implantation, placentation.

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