# Inhibition of luteolysis and embryo-uterine interactions during the peri-implantation period in pigs

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Inhibition of luteolysis and establishment of pregnancy in pigs results from oestrogen secretion by the conceptuses and requires progesterone produced by the corpus luteum (CL). An integral part of maternal recognition of pregnancy in the pig is the redirection of prostaglandin (PG) $F_{2\alpha}$ secretion from endocrine (blood) to exocrine (uterus) direction and an increase of $PGE_2$ synthesis in both the endometrium and conceptus. Uterine and conceptus $PGE_2$ synthases play an integrated role in establishing the $PGE_2$:$PGF_{2\alpha}$ ratio necessary for luteal maintenance. The luteolytic or luteotrophic changes in the CL are synchronised with the release of maternal pituitary and ovarian hormones. The presence of uterine oxytocin (OT) and luteinising hormone (LH) receptors are important for the luteolytic effect of $PGF_{2\alpha}$. Conceptus oestrogen secretion coincides with autocrine and paracrine dialogue between the multiple conceptuses and uterine biological compounds and their receptors in trophoblast and endometrium.

## Introduction

In pigs, the period from successful fertilisation to the process of initiation of implantation lasts about 14 days. Biochemical endometrial-blastocyst interactions occur by Day 11 of pregnancy (Bazer *et al.*, 1982). During this time some form of embryonic signalling is necessary not only for maternal recognition of pregnancy but also to prepare the uterine environment for implantation.

In the absence of fertilised ova and blastocyts endometrial secretion is limited to the period between the mid to late luteal phase of the oestrous cycle. This secretory activity is dependent upon maintenance of functional corpora lutea (CL). In the pig, as in other species, $PGF_{2\alpha}$ is the major signal for luteolysis. In order to maintain luteal function in early pregnancy, the effects of the uterine luteolysin are prevented. Recent concepts as to how this is brought about are presented in this review. An alternation of luteolytic or luteotrophic changes in the ovary are synchronised with secretion of pituitary/hypothalamus and ovarian hormones.

The developmental changes of embryos before implantation are dependent on biological molecules secreted by the endometrium and conceptuses. The aim of such maternal-conceptus dialogue is the synchronisation of blastocyst development with the preparation of the uterus for

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implantation. Biological molecules facilitating embryo-uterine cross talk include growth factors, cytokines, adhesion molecules, pregnancy-associated proteins, prostaglandins and hormones. The length of the article is limited and this review does not cover the role of cytokines, adhesion molecules and proteinases in the establishment of pregnancy. Some functions of these compounds have been previously reviewed (Lessey, 2002; Schäfer-Somi, 2003; Spencer and Bazer, 2004; Szafranska *et al.*, 2004).

## Hormones

### *Oestradiol*

Pig conceptuses begin to produce oestrogens on Day 11 of pregnancy (Geisert *et al.*, 1982). The pattern of release is biphasic, with increased secretion on Day 12 and Days 23-30 of pregnancy (Bazer *et al.*, 1982). It is generally believed that the luteotrophic effect of oestrogen in pigs is both indirect, resulting from a reduced uterine release of $PGF_{2\alpha}$ into peripheral circulation, and direct (Conley and Ford, 1989). Oestradiol also maintains high LH receptor levels both in the CL (Garverick *et al.*, 1982) and uterus (Ziecik *et al.*, 1992).

Interestingly, no differences in oestrogen receptor (ER) mRNA expression were found between cyclic and pregnant pigs on Days 10-15 after oestrus (Geisert *et al.*, 1993). In the same studies, immunocytochemical staining of ER in the luminal (LE) and glandular epithelium (GE) was readily detectable on Day 12 of pregnancy but reduced on Day 15. The intensity of ER staining in porcine endometrium was most prominent in deep lying glands (Sukjumlong *et al.*, 2004). Changes in endometrial ER expression are consistent with a physiological role for oestrogens produced by the conceptus in the maternal recognition of pregnancy in the pig.

### *Progesterone*

The establishment of normal endometrial receptivity appears to be associated with the down-regulation of epithelial progesterone receptors that coincides with the time of embryo implantation (Spencer and Bazer, 2002). In all mammalian uteri, progesterone receptors are expressed in the endometrial epithelium and stroma during the early luteal phase. Long exposure of the endometrium to progesterone negatively autoregulates progesterone receptor expression in the endometrial epithelium (Geisert *et al.*, 1994). In rodent, human and primate uterus progesterone regulates a number of genes including transcription factors, growth factors, homeobox genes and enzymes (Gray *et al.*, 2001). In pigs, progesterone increases expression of integrins α4, α5 and ß1 during the peri-implantation period, which may in part define the implantation window in this species (Burghardt *et al.*, 2002).

### *Oxytocin (OT)*

In ruminants, OT from the CL binds to uterine OT receptors to elicit pulses of $PGF_{2\alpha}$, but this mechanism is not well-defined for pigs (Ziecik, 2002). The porcine CL synthesises OT, although to a lesser extent than in ruminants (Choy and Watkins, 1988). The increase in circulating concentrations of OT during luteolysis is associated with an elevation in uterine secretion of $PGF_{2\alpha}$ (Kotwica *et al.*, 1990). Moreover, exogenous OT stimulates $PGF_{2\alpha}$ secretion in cyclic and early pregnant pigs (Carnahan *et al.*, 1996). Whiteaker *et al.* (1994) proposed that OT binds to its endometrial receptors and utilises the phosphoinositide pathway to initiate luteolytic secretion of $PGF_{2\alpha}$ in the pig. The low concentration of OT receptors in the endometrium of early pregnant pigs (Okano *et al.*, 1996) could indicate that this suppression is an important component of the mechanism of the recognition of pregnancy in the pig. However, Ludwig *et al.* (1998) did not support the hypothesis that endometrial responsiveness to OT is regulated by the amount of OT receptors in swine, but rather with the level of OT receptors coupling to G protein and phospholipase C pathway.

The question of whether OT causes $PGF_{2\alpha}$ release in intact gilts remains open, since agreement between OT and PGFM peaks is only about 30% and blocking of OT receptors neither prevents luteolysis nor changes the duration of the oestrous cycle in swine (Kotwica *et al.*, 1999). It may suggest that OT is not mandatory to induce endometrial release of $PGF_{2\alpha}$.

*Luteinising hormone (LH)*

An early report by Du Mesnil du Buisson and Legise (1963) suggested that after an initial LH trigger, the porcine CL can function without pituitary support until Day 12 of the oestrous cycle. However, passive immunisation of the gilt with anti-pLH serum on Day 8 of the oestrous cycle dramatically inhibited progesterone secretion (Szafranska and Ziecik, 1989). Inhibition of LH secretion with GnRH antagonist during the early luteal period reduces CL size and progesterone production (Brussow *et al.*, 2001). These data indicate that during the oestrous cycle the porcine CL appears not as autonomous as was believed earlier (Bazer *et al.*, 1982). The porcine uterus, including the endometrium, possesses LH receptors (Ziecik *et al.*, 1986). The appearance of relatively high amounts of LH receptors in the endometrium coincides with the increase of $PGF_{2\alpha}$ secretion and perhaps with the down-regulation of progesterone receptors. Following the initiation of luteolytic $PGF_{2\alpha}$ secretion on Days 14-16, LH receptors decline in the endometrium. LH up-regulates COX-2 protein expression and $PGF_{2\alpha}$ secretion from endometrium *in vitro* (Stepien *et al.*, 1999). Furthermore, the systemic infusion (Ziecik *et al.*, 2001) or intra-muscular injection of hCG (Guthrie and Bolt, 1983) induces $PGF_{2\alpha}$ release *in vivo*. It seems that a window of endometrial responsiveness to LH *in vivo* falls within the period between Days 15 and 17 of the oestrous cycle. Additionally, there is a much higher correlation between LH and PGFM peaks (75.5%; Ziecik *et al.*, 2001) than between OT and PGFM (30%; Kotwica *et al.*, 1999).

The uterine LH receptors may also be involved in the maintenance of early pregnancy in the pig, since LH induced $PGE_2$ release from endometrium on Days 14-16 of the oestrous cycle (Blitek and Ziecik, 2005) or early pregnancy (Ziecik *et al.*, 2000). Moreover, LH in contrast to OT, affects secretion of the known angiogenic factor vascular endothelial growth factor (VEGF) from endometrial cells in culture (M.M. Kaczmarek, A. Blitek, D. Schams, A.J. Ziecik, unpublished).

*Prolactin (PRL)*

The function of PRL in the maintenance of pregnancy in the pig has not been completely defined (Dusza and Tilton, 1990). Circulating concentrations of PRL in early pregnancy do not differ from the basal concentrations during the oestrous cycle (Dusza and Krzymowska, 1981). It was suggested that PRL cooperates with oestrogen in the exocrine secretion of $PGF_{2\alpha}$ during establishment of pregnancy in pigs (Gross *et al.*, 1990). Since administration of oestrogen to Day 11 cyclic gilts up-regulates endometrial PRL receptors and a significant increase in the number of these receptors was observed in the Day 12 pregnant uterus (Young *et al.*, 1990), PRL may also play a role in maternal recognition of pregnancy. However, administration of PRL during the luteal phase of the oestrous cycle in gilts had no effect on the basal levels of progesterone or oestradiol (Dusza *et al.*, 1986).

## Growth factors

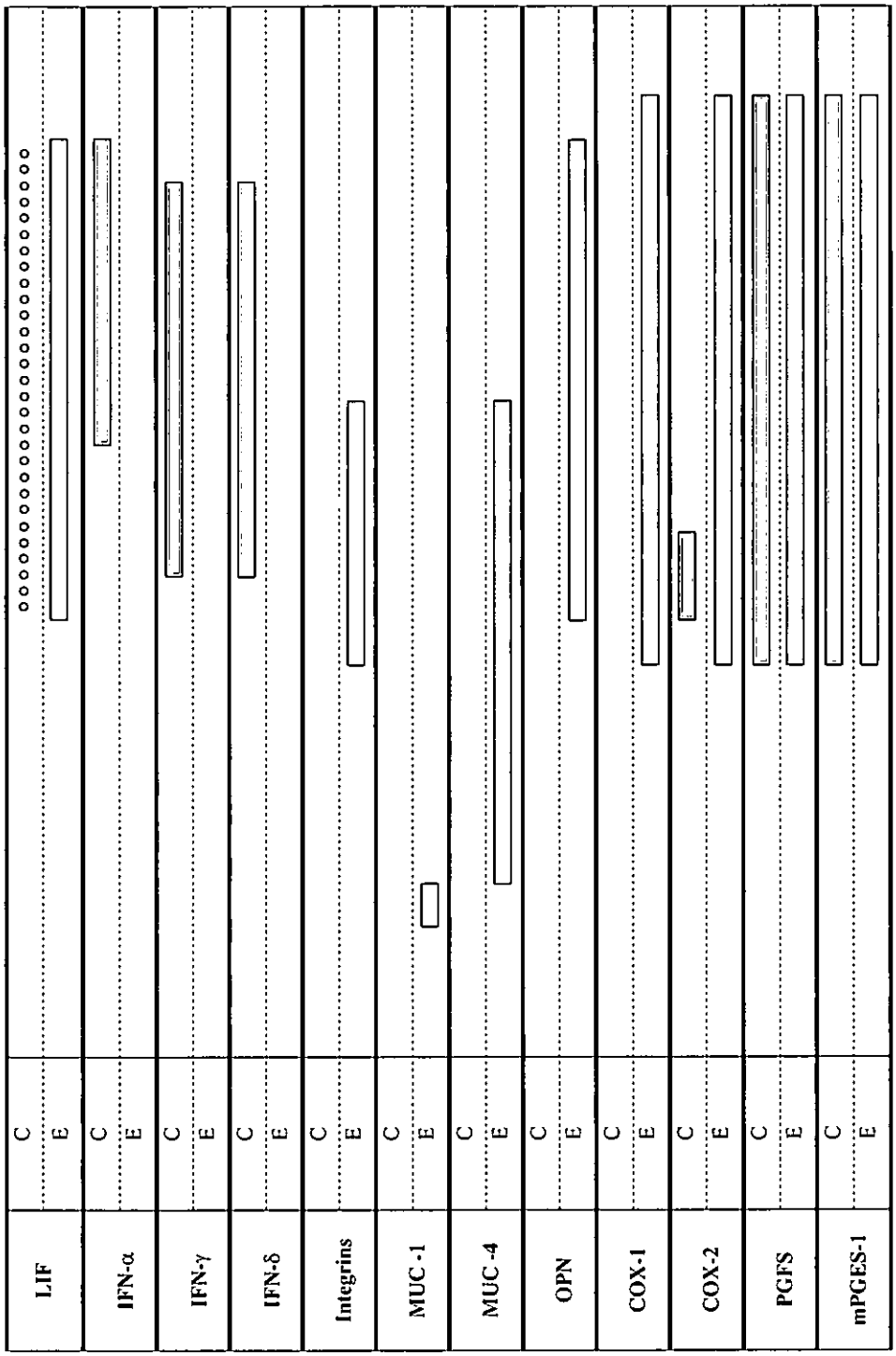
*Insulin-like growth factors (IGFs)*

The IGFs are implicated in the control of proliferation and differentiation of the uterus in preparation for blastocyst implantation and during later foeto-placental development. In pigs, endometrial IGF-I mRNA was detected on Days 8-14 of pregnancy (Letcher *et al.*, 1989) and IGF-II mRNA after implantation (Simmen *et al.*, 1992). Thus, in the porcine uterus IGF-I, rather than IGF-II, appears to dominate in early pregnancy (Table 1).

Table 1. Markers of peri-implantation uterine development in the pig[a]

| Biological molecules | Conceptus / Endometrium | Days of pregnancy: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oestrogens | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| IGF-I | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| EGF | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| TGF-α | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| Ar | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| FGF | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| TGF-ß | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| KGF | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| VEGF | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| IL-1ß | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |
| IL-6 | C | | | | | | | | | | | | | | | | | | | | | | |
| | E | | | | | | | | | | | | | | | | | | | | | | |

Abbreviations used: C, conceptus; E, endometrium; IGF-I, insulin-like growth factor; EGF, epidermal growth factor; TGF-α, TGF-ß, transforming growth factor -α and -ß; Ar, amphiregulin; KGF, keratinocyte growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; IL-1ß, IL-6, interleukins - 1ß and –6; IFN-α, IFN-δ, IFN-γ, interferons -α, -δ and γ; LIF, leukemia inhibitory factor; OPN, osteoponin; MUC-1, MUC-4,




mucosins –1 and –4; COX-1, COX-2, cyclooxygenases – 1 and –2; PGFS, PGF synthase; mPGES-1, microsomal PGE synthase-1. The presence of markers and receptors is based on the available literature. The absence of markers or receptors indicates that they have not yet been measured, or that they have been studied and not found

The endometrial expression of IGF-I mRNA and secretion of IGF-I peptide into the uterine lumen peaks at Day 12 of pregnancy, concomitant with maximal oestrogen production by the conceptuses (Simmen *et al.*, 1989; 1992). Moreover, densities of immunoreactive $P450_{arom}$ in porcine conceptuses were highly correlated with the ratio of IGF-I/IGF-II in the uterine lumen (Ko *et al.*, 1994). A role for IGF-I in conceptus development is supported by the presence of IGF-I receptor mRNA in the peri-implantation porcine blastocyst (Green *et al.*, 1995).

In blood and other extracellular fluids IGFs are complexed to specific binding proteins (IGFBP). In human, IGFBP-1 is the major IGFBP of the endometrium and decidua. In the pig, however, IGFBP-1 mRNA was not detected in porcine endometrium. Of the five IGFBP genes found in the uterus of pregnant pigs, only IGFBP-2 exhibited obvious tissue-specificity of expression. This protein was localised in GE and LE, with much greater intensity of immunostaining during mid- than early pregnancy. Also IGFBP-2 mRNA expression was higher after the implantation period (Days 30-60; Song *et al.*, 1996).

In the pig, uterine luminal flushings collected on Days 10 and 11 of gestation contain several IGFPBs, with IGFBP-3 being most abundant. By day 12, however, IGFBPs were substantially diminished or undetectable. Examination of the morphology of flushed conceptuses showed that the loss of IGFBPs in uterine fluid was associated with the transition from spherical to filamentous forms. Since the concentration of IGFBP-3 mRNA in endometrial tissue was not altered in a similar way, it may indicate that the lack of IGFBP-3 in filamentous conceptuses is a result of proteolysis rather than decreased expression of IGFBP-3 gene. Thus, elongating porcine conceptuses induce IGFBP protease activity, which may increase the intrauterine bioavailability of IGFs (Lee *et al.*, 1998).

*Epidermal Growth Factors (EGFs)*

The EGF family includes EGF itself, transforming growth factor-α (TGFα), heparin binding EGF-like factor (HB-EGF) and amphiregulin (Ar). All of these molecules bind to the same cell surface tyrosine kinase receptor (Prigent and Lemoine, 1992). The possible function of EGFs during pregnancy is to stimulate embryonic growth and development.

Porcine embryos possess EGF receptors (EGF-R) as indicated by their binding of labelled EGF (Corps *et al.*, 1990). Moreover, EGF-R mRNA has been demonstrated in both pre- and post-elongation blastocyst (Days 7-12 and 15-22, respectively). On the other hand, EGF mRNA expression is limited to post-elongation conceptuses. EGF is present predominately in the embryo and amnion (Vaughan *et al.*, 1992). Since the amnion synthesises $PGE_2$, and $PGE_2$ concentration in amniotic fluid increases as pregnancy progresses, it is possible that endogenous EGF may regulate $PGE_2$ synthesis by the amnion. TGFα, which has significant sequence homology to EGF, was detected only in the developing blastocyst at Days 8-12, with the highest mRNA levels at Day 10. Since the porcine blastocyst begins to elongate from Day 10.5 of pregnancy (Stroband and Van der Lende, 1990), TGFα may be involved in the complex developmental reorganisation of the conceptus.

EGF-R in the porcine uterus was detected on Day 13 of pregnancy and the binding capacity of labelled EGF was higher (5-fold) for stromal, than for glandular epithelial cells (Zhang *et al.*, 1992). Endometrial EGF-R mRNA expression was lowest on Day 10 of pregnancy and increased significantly from Days 12 to 22. EGF, TGFα and Ar are also present in the porcine endometrium, but with different patterns of expression. EGF mRNA level was similar on all days of pregnancy studied (Days 10-22), but TGFα and Ar mRNA concentrations were increased on Day 12 of pregnancy (Kennedy *et al.*, 1994).

*Fibroblast Growth Factors (FGFs)*

FGFs are structurally related proteins able to stimulate fibroblast proliferation. FGFs may also

influence cell differentiation, matrix formation and cell movement. These peptides affect extracellular matrix deposition, which is important for embryonic development, suggesting that FGFs could be critical for embryogenesis (Baird and Böhlen, 1991).

In pigs, the presence of acidic and basic FGF (aFGF and bFGF respectively) was studied in conceptuses and uterine endometrium on Days 10-14 of pregnancy (Gupta *et al.*, 1997). Differential expression of both aFGF and bFGF was observed according to pregnancy status. Positive immunostaining for bFGF was shown in both LE and GE and in stromal cells of uterus, with stronger positive staining from Day 12. This pattern of bFGF expression could be an effect of conceptus-derived oestrogens. The intense immunostaining for bFGF was also localised in cells on the embryonic disc and visceral endoderm on Days 10 and 11. Mesoderm cells were positively stained for bFGF on Days 11-12 of pregnancy. Acidic FGF was localised only in stromal cells of porcine endometrium. These data indicate that bFGF, but not aFGF, may directly influence the development and/or differentiation of porcine conceptuses (Gupta *et al.*, 1997). Moreover, FGF peptides were extracted from porcine uterine tissues and luminal fluids during early pregnancy (Brigstock *et al.*, 1989).

*Transforming growth factor (TGF) ß*

The regulatory roles of TGFß in the uterus during pregnancy include decidualisation, apoptosis, trophoblast attachment, growth, invasion and differentiation, immunotolerance, cytokine and hormone production and embryogenesis (Godkin and Doré, 1998). During early pregnancy in pigs, mRNA expression for TGFß 1, 2 and 3 increases progressively in LE and underlying stroma from Days 10 to Day 12 (2-fold) and from Day 12 to Day 14 (4-fold; Gupta *et al.*, 1998a). TGFß proteins were also localised in uterine tissues during pregnancy and the pattern of protein expression was similar to mRNA for TGFß (Gupta *et al.*, 1998b). The increased uterine TGFß expression may be due to the effect of conceptus-secreted oestrogens.

The concentration of TGFß receptors (type I and II) in porcine uterine tissue was also dependent on the day of pregnancy. Immunostaining for both receptors was very weak on Days 10 and 11 and detected only in apical membranes of LE and GE, but later (Days 12-14 of gestation) the intensity of staining increased and was present in both membranous and cytoplasmic components of the epithelial cells (Gupta *et al.*, 1998b).

Porcine conceptuses express mRNA and immunoreactive protein for both TGFß and TGFß-R, indicating that they are not completely dependent on a maternal source of TGFß (Gupta *et al.*, 1996; 1998b).

Bioactive TGFß at the conceptus-maternal interface may be associated with conceptus attachment. In pigs, active forms of TGFß were detected in luminal fluid on Days 12-14, but not on Days 10 and 11 of gestation (Gupta *et al.*, 1998b). Additionally, TGFß was shown to stimulate extracellular matrix proteins like fibronectin, collagens, proteoglycans, and their integrin receptor subunits (Roberts *et al.*, 1990).

*Keratinocyte growth factor (KGF)*

Keratinocyte growth factor/fibroblast growth factor-7 (KGF/FGF-7) is a paracrine mediator of epithelial-mesenchymal interactions in the female reproductive tract. In the pig, however, KGF is expressed predominantly in the endometrial epithelium (Ka *et al.*, 2000). KGF mRNA was detected in the endometrium throughout pregnancy with very high levels on Days 12-15. Messenger RNA concentrations were about 3-fold higher on Day 12 of gestation in comparison to Day 12 of the oestrous cycle. Receptors for KGF (KGF-R) were localised only in LE and GE of porcine endometrium. The trophectoderm of conceptuses also expresses KGF-R, but not KGF. These data suggest that in the pig, which has a diffuse, epitheliochorial placentation, KGF

may play a role in paracrine epithelial-epithelial interactions between conceptus and uterus during early pregnancy (Ka *et al.*, 2000). Moreover, the expression of uPA (a marker of differentiation) in pig conceptuses was stimulated by KGF, suggesting that KGF affects trophectoderm cell differentiation (Ka *et al.*, 2001).

## VEGF ligand-receptor system

*Expression of VEGF ligand-receptor system in the CL during luteolysis*

Cyclic changes of vascularity in the CL are tightly regulated by several angiogenic factors among which vascular endothelial growth factor (VEGF) seems to play a paramount role. Previous results indicate constant expression of VEGF mRNA in the porcine CL. However VEGF receptor (VEGFR) populations vary during the cycle, with reports of high levels of VEGFR-1 and low levels of VEGFR-2 during luteolysis (Boonyaprakob *et al.*, 2003). Recently, we have evaluated in detail the mRNA and protein expression of VEGF, VEGFR-1 and VEGFR-2 in the porcine CL during the oestrous cycle (Kaczmarek *et al.*, 2005). Interestingly, mRNA and protein expression of VEGF and its binding sites decreased as luteolysis progressed. Therefore, taking account of other physiological features of luteolysis in pigs such as increasing $PGF_{2\alpha}$ secretion and gradual dissolution of small blood vessels in the CL, our results suggest that the VEGF ligand-receptor system may be regulated by $PGF_{2\alpha}$ during CL regression. Recent results show a sharp decrease of VEGF mRNA and protein concentrations after $PGF_{2\alpha}$ induced luteolysis in mares (Al-zi'abi *et al.*, 2003) and cows (Neuvians *et al.*, 2004), suggesting that $PGF_{2\alpha}$ may be responsible for cessation of VEGF support for the luteal vasculature during luteolysis.

On the other hand, when pregnancy occurs, the lifespan of the CL must be extended to maintain pregnancy to term. Sufficient CL vascularity appears essential for the maintenance of luteal function, such as progesterone production during gestation. Our recent results show relatively high protein and mRNA levels of VEGF and its receptors in CL from pregnant pigs, which were comparable with mid-luteal phase and significantly higher than in regressed CL (Kaczmarek *et al.*, 2005). It seems that extension of the luteal vascular lifespan during gestation in the pig is connected with prolonged survival and stabilisation of vessels that maintain CL function and structure for longer than in the non-fertile cycle.

*Expression and control of VEGF expression in endometrium*

An important stage in embryo implantation in all mammals includes the close apposition of the foetal and maternal blood supplies. For this connection to occur there must be dramatic growth and remodelling of the endometrial vasculature (Lee and DeMayo, 2004). The uterine expression of VEGF has been well characterised in a number of animal models, and it is tempting to suggest that VEGF may be important for the establishment of sufficient vascular sheath within the endometrium during pregnancy. VEGF ligand-receptor system expression was localised in GE and LE of the gravid and non-gravid porcine uterus (Winther *et al.*, 1999). Recently, we have observed increased protein levels of VEGF in the porcine endometrium before ovulation and in early luteal phase that suggests an important role of VEGF in the development and remodelling of uterine vasculature before implantation in pigs (Kaczmarek *et al.*, 2004).

IGF-I and relaxin (RLX) stimulate VEGF secretion by porcine endometrial stromal cells obtained on Days 10-12 of pregnancy (M.M. Kaczmarek, A. Blitek, D. Schams, A.J. Ziecik, unpublished). The stimulating action of RLX on VEGF expression has already been demonstrated in human endometrial cells (Palejwala *et al.*, 2002). Therefore it is possible that both IGF-I and RLX, may play an important role in angiogenesis and the maintenance of vascular

function during implantation and placentation in pigs. Furthermore, we observed that LH may also stimulate VEGF output from porcine endometrial stromal cells *in vitro* (M.M. Kaczmarek, A. Blitek, D. Schams, A.J. Ziecik, unpublished), however its action was not as strong as in the case of IGF-I and RLX.

## Prostaglandins (PGs)

Prostaglandins are key mediators of several female reproductive functions including ovulation, fertilisation, implantation and parturition. Cyclooxygenases (COX-1 and COX-2) convert arachidonic acid into $PGH_2$, the common precursor of various forms of prostaglandins, including $PGE_2$ and $PGF_{2\alpha}$. The downstream enzymes, PGE synthase (PGES) and PGF synthase (PGFS), catalyse the transformation of $PGH_2$ to $PGE_2$ and $PGF_{2\alpha}$, respectively (Smith and Dewitt, 1996). $PGE_2$ can be also converted to $PGF_{2\alpha}$ by $PGE_2$-9-oxoreductase. There are three forms of PGES, among them microsomal PGES-1 (mPGES-1), a highly inducible form, along with COX-2 (Thoren *et al.*, 2003). Prostaglandins are thought to be critical for establishment of pregnancy in the pig, since inhibition of prostaglandin synthesis results in pregnancy failure (Kraeling *et al.*, 1985). In pigs, prostaglandins reach the CL by a combination of both local and/or systemic mechanisms (Flint *et al.*, 1982). The major luteolysin in pigs is $PGF_{2\alpha}$. Administration of $PGF_{2\alpha}$ *in vivo* initiates luteal regression and reduces serum progesterone concentration, but only after Day 12 of the oestrous cycle (Krzymowski *et al.*, 1978). The mechanism of insensitivity of early porcine CL to exogenous $PGF_{2\alpha}$ was partially explained by Gadsby *et al.* (1990), who found that the number of $PGF_{2\alpha}$ high affinity receptors was low on Days 6-8, increased gradually up to Day 12, then increased dramatically on Day 13 and remained high on Days 14-17. A change in direction of $PGF_{2\alpha}$ secretion is the basis of the 'endocrine versus exocrine' theory of maternal recognition of pregnancy in the pig (Bazer and Thatcher, 1977). These authors proposed that secretion of $PGF_{2\alpha}$ in non-pregnant animals during the mid- to late-luteal phase of the oestrous cycle is directed primarily toward uterine venous drainage (endocrine direction). This hypothesis was supported by the observation that $PGF_{2\alpha}$ concentration in utero-ovarian vein is highest between Days 13 and 17-18 of the oestrous cycle, i.e. when events leading to luteolysis are initiated, while its accumulation in the uterine lumen during the oestrous cycle is low (Bazer *et al.*, 1982). In non-pregnant pigs $PGF_{2\alpha}$ and some components of histotroph, e.g. uteroferrin, move towards the endometrial stroma (endocrine direction) during the period of CL regression. $PGF_{2\alpha}$ is secreted into the uterine venous system and transported, possibly by counter-current exchange, to the ovarian artery and the CL, where it exerts its luteolytic effect. In pregnant gilts, however, there were no significant changes in utero-ovarian vein $PGF_{2\alpha}$ concentrations between Days 12 and 25 of pregnancy but the number of $PGF_{2\alpha}$ peaks and their frequency were higher in non-pregnant gilts.

Krzymowski *et al.* (1990) and Stefanczyk-Krzymowska *et al.* (1990) suggested that the mechanism of CL protection is based on the uptake of $PGF_{2\alpha}$ originating from the uterus by the mesometrium. Then, $PGF_{2\alpha}$ is transferred to the uterus in arterial blood by retrograde transfer (via the counter current system; Fig. 1). It has been suggested that the retrograde transfer of $PGF_{2\alpha}$ from uterine venous to arterial vessels and then to the uterine lumen may strongly reduce the peak concentrations during the pulsatile release of $PGF_{2\alpha}$ from the uterus. Furthermore, the high $PGF_{2\alpha}$ concentrations found in the uterine lumen during early pregnancy (Bazer and Thatcher, 1977; Stefanczyk-Krzymowska *et al.*, 1990) could also be a consequence of $PGF_{2\alpha}$ uptake from arterial blood supplying the uterus and removal into the uterine lumen.

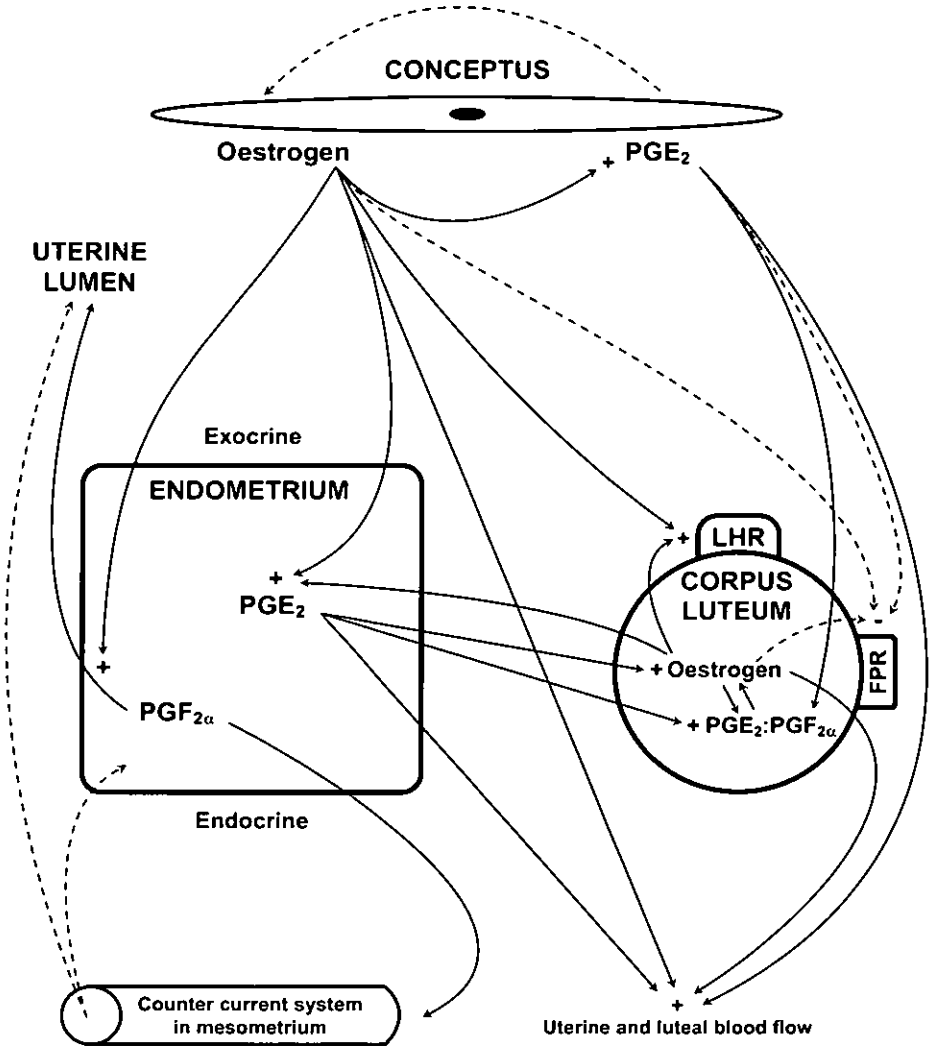



**Fig. 1** Inhibition of luteolysis in pigs - a recent integrated concept. Oestrogens produced by the conceptus provide the first signal for establishment of pregnancy altering the direction of $PGF_{2\alpha}$ from endocrine to exocrine. Additionally, $PGF_{2\alpha}$ of uterine origin is taken up by the mesometrium and transferred to the uterus in arterial blood by the counter current system operating in the broad ligament of the uterus. Oestrogens also cause $PGE_2$ secretion by endometrium and conceptus to predominate (up-regulation of $PGE_2$ synthase and possible inhibition of $PGE_2$-9-oxoreductase) by changing $PGE_2$:$PGF_{2\alpha}$ ratio, which is crucial for protection of the CL from the luteolytic actions of $PGF_{2\alpha}$. A putative feedback loop exists between oestrogen secretion by conceptus and CL, and $PGE_2$ release from conceptus, endometrium and CL. The elevated $PGE_2$:$PGF_{2\alpha}$ ratio increases oestradiol production by luteal cells, which serves as an additional source of oestrogens. Oestrogens also maintain LH receptors in the CL and have a direct luteotrophic effect and, together with $PGE_2$, ensure the proper blood flow to endometrium and the CL. Finally, we propose that a conceptus product (oestrogen, $PGE_2$ or other) acts on the CL to reduce the concentrations of $PGF_{2\alpha}$ receptors (Gadsby *et al.*, 1993), providing an added level of luteal protection. This figure was modified from Ziecik (2002) and Spencer and Bazer (2004).

A luteotrophic/antiluteolytic effect of $PGE_2$ in the pig has been demonstrated (see review: Ziecik, 2002). The failure of attempts to maintain luteal function with intra-uterine infusions of $PGE_2$ in cyclic gilts could be a consequence of its conversion to $PGF_{2\alpha}$ (Okrasa *et al.*, 1985). During the oestrous cycle pulsatile secretion of $PGF_{2\alpha}$ increased markedly on Day 13 and continued to increase through Days 16-18 (Christenson *et al.*, 1994; Kotwica *et al.*, 1999). The secretion of $PGE_2$ also increases from Day 13 to 16 of the oestrous cycle but remains threefold lower than $PGF_{2\alpha}$ (Christenson *et al.*, 1994). In contrast, prostaglandin secretion in mated gilts peaked earlier (Day 11-12) with $PGE_2$ being the predominant eicosanoid. Furthermore, according to Christenson *et al.* (1994), concentrations of $PGE_2$ were higher in utero-ovarian venous blood draining the gravid compared with the non-gravid uterine horn, and the ratio of secreted PGE:PGF is increased in harvested stromal cells from endometrium of pregnant pigs compared to cyclic gilts (Zhang and Davis, 1991).

In the pig, COX-1 and COX-2 were localised in the uterine stromal and epithelial cells. Quantification of COX-2 mRNA and protein expression revealed a significant increase at the time of luteolysis. In pregnant gilts, endometrial mRNA and protein concentrations of COX-2 were up-regulated at the time of implantation (A. Blitek, A. Waclawik, M.M. Kaczmarek, A.J. Ziecik, unpublished). Thus, COX-2 seems to be involved in elevated prostaglandin production at the time of luteolysis and during implantation in pregnant animals.

The terminal prostaglandin synthases PGFS and PGES have been most studied in the bovine species (Arosh *et al.*, 2004). Since porcine PGFS and mPGES-1 transcripts had not been previously characterised, we have recently cloned their cDNAs and found them to be highly similar to known mammalian homologues (Waclawik *et al.*, 2004). Our studies on endometrial expression of PGFS throughout the oestrous cycle and early pregnancy indicate a potential role of PGFS in regulation of luteolysis in the pig. We also detected modulation of mPGES-1 mRNA and protein concentrations in porcine endometrium during the oestrous cycle and early pregnancy. High expression of mPGES-1 in endometrium before implantation may be involved in the change of $PGE_2$:$PGF_{2\alpha}$ ratio necessary for maternal recognition of pregnancy. Another enzyme able to change the proportion of $PGE_2$:$PGF_{2\alpha}$, is $PGE_2$-9-oxoreductase converting $PGE_2$ to $PGF_{2\alpha}$. Until now, control of $PGE_2$-9-oxoreductase activity in porcine endometrium and placenta has not been studied extensively.

$PGE_2$ alone increased, but $PGF_{2\alpha}$ decreased, progesterone secretion by luteal cells (Gregoraszczuk and Michas, 1999). Treatment of cells collected from regressing CL with $PGE_2$ and $PGF_{2\alpha}$ in ratio 2:1 and 4:1 increased oestradiol production. Thus, oestradiol released under the influence of $PGE_2$ by luteal cells may serve as an additional source of oestradiol to blastocysts during early pregnancy in the pig. It also supports an earlier suggestion that oestradiol has a direct luteotrophic effect in the pig (Conley and Ford, 1989). Pig conceptuses have tremendous prostaglandin synthetic ability (Geisert *et al.*, 1982; Rosenkrans *et al.*, 1992). Wilson *et al.* (2002) showed, for the first time, that COX-2 expression is developmentally regulated during elongation of porcine conceptuses. COX-2 was not expressed in spherical or transitional conceptuses, but was up-regulated by the time a conceptus reaches a filamentous morphology.

Patterns of mPGES-1 and PGFS expression in the pig trophoblast have been recently determined (Waclawik *et al.*, 2005). Profiles of mPGES-1 and PGFS mRNA and protein concentrations in trophoblasts/conceptuses correlated with changes in expression of these enzymes in endometrium. High expression of mPGES-1 in Day 10-12 conceptuses corresponds to elevated $PGE_2$ concentrations in the uterine lumen and vein, and may be important in modulation of the $PGE_2$:$PGF_{2\alpha}$ ratio necessary for the establishment of pregnancy.

## Conclusion

Oestrogens and prostaglandins are critical components of the inhibition of luteolysis and main-

tenance of CL function during early pregnancy in pigs (Fig. 1). Expression of oestrogen receptors in the uterus coincides with the secretion of oestrogen from the conceptus. Autocrine and paracrine dialogue between multiple conceptuses and uterine biological compounds and their receptors begins with a rapid morphological transformation from a spherical to filamentous form of trophoblast. Expression of many proteins (growth factors, integrins, inhibitors, etc.) that take part in trophoblast elongation or attachment to the uterine surface and in implantation coincides with oestrogen secretion by the conceptus (some of them are up-regulated by oestrogen). The determination of prostaglandins $E_2$ and $F_{2\alpha}$ downstream enzymes (PGES and PGFS) in endometrium and spherical/elongated conceptuses highlights the integrated role of uterine/conceptus PGES in the inhibition of luteolysis by changing $PGE_2$:$PGF_{2\alpha}$ ratio. New emerging concepts also emphasise the autocrine and paracrine roles of luteal prostaglandins and oestrogen in CL function. Luteotrophic $PGE_2$ action in pigs probably requires oestrogen for inhibition of $PGE_2$ 9-oxoreductase in maternal and conceptus prostaglandins producing tissues. This may explain the inability to maintain luteal function following intrauterine infusions of $PGE_2$, as such treatment would stimulate $PGF_{2\alpha}$ release by the intact pig uterus.

## Acknowledgement

Some research reported in this chapter was supported by the State Committee for Scientific Research in Poland as a solicited project PBZ-KBN-084/P06/2002 and project 2 P06D041 26. M.M. Kaczmarek was awarded with Domestic Grant for Young Scientist from the Foundation for Polish Science.

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