Regulation of the porcine corpus luteum during pregnancy

Adam J Ziecik¹, Emilia Przygrodzka¹, Beenu M Jalali² and Monika M Kaczmarek¹

¹Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland and ²Department of Immunology and Pathology of Reproduction, Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland

Correspondence should be addressed to A J Ziecik; Email: a.ziecik@pan.olsztyn.pl

Abstract

The new corpora lutea (CLs) in pigs are formed from the preovulatory follicles after the luteinizing hormone (LH) surge. However, total autonomy and independence of CLs from LH up to Day 12 of cycle has recently been questioned. Transformation of estrous cycle CL to CL of pregnancy initiated by embryonic signals requires not only the cessation of prostaglandin F2 (PGF2α) supply to the luteal tissue but also needs the CL to overcome luteolytic acquisition and/or changing its sensitivity to PGF2α during Days 12–14 of pregnancy. The luteolytic cascade is prevented by inhibition of lymphocyte infiltration and leucocyte recruitment, limitation of cell apoptosis, upregulation of pregnancy-associated genes and an enhanced antiluteolytic role of PGE2. Our ‘two-signal switch hypothesis’ highlights the importance of post PGF2α and PGE2 receptor signaling pathways activation in CLs during luteolysis and rescue. The ‘luteolytic switch’ involves increased expression of many regression mediators and activation of the post PTGFR signaling pathway. The ‘rescue switch’ initiated by embryonic signals – estradiol 17β and PGE2 – induces post PTGER2/4 pathway, turning the ‘luteolytic switch’ off and triggering activity of genes responsible for CL maintenance. In mid and late pregnancy, CLs are maintained by LH and the synergistic action of metabolic hormones. This paper provides an outline of recent views on CL regression, rescue and maintenance during pregnancy in pigs that conflict with previous paradigms and highlights new findings regarding the actions of prostaglandins, role of microRNAs (miRNA) and immune system and signaling pathways governing the life cycle of porcine CL.


Introduction

The life cycle of the corpus luteum (CL) during the estrous cycle includes three phases: formation, maintenance and regression. However, the presence of embryo maintains the CL and instead of regression, CL undergoes rescue and the sustained maintenance during the 114 days long pregnancy in pigs. The presence of functional CLs is necessary not only for establishment but also for continuation of pregnancy until parturition. After Day 12 of each estrous cycle, when embryos are not present in the porcine uterus, CLs undergo regression that is crucial to prepare the ovaries for the next ovulation and to allow oocytes to be released and fertilized in the next cycle. This review is focused on the rescue and maintenance of porcine CLs during pregnancy, but some aspects of CL regression during the estrous cycle are also discussed.

The paradigmatic theories of maternal recognition of pregnancy, i.e. ‘endocrine vs exocrine’ (Bazer & Thatcher 1977) and/or ‘retrograde’ (Krzymowski & Stefanczyk-Krzymsowska 2004) transfer of prostaglandin F2α (PGF2α) in the porcine reproductive tract were focused on the redirection of PGF2α towards the ovary during the luteal phase or on PGF2α accumulation in the uterus during early pregnancy respectively. Fortunately, the ‘omics era’ brought a handful of evidence for crucial roles of several other factors in CL rescue, thereby broadening our knowledge beyond a simple role of circulating PGF2α in CL regression (Ostrup et al. 2010, Franczak et al. 2013, Zhang et al. 2013, Kiewisz et al. 2014). During pregnancy, CL rescue is possible due to the paracrine action of conceptuses on both the endometrium and luteal tissue (for review see: Waclawik et al. 2017). For many years, estrogen was recognized as the most important secretion of embryonic origin involved in the maintenance of luteal function during pregnancy; however, our studies clearly indicated a strong role of conceptuses and uterine PGE2 in the rescue of porcine CLs (Waclawik & Ziecik 2007, Przygrodzka et al. 2016). Here, we present our updated ‘two-signal switch hypothesis’ on the role of both PGF2α and PGE2 signaling pathways in CL regression and rescue during the maternal recognition of pregnancy in the pig.

The lack of global profiling studies on pregnancy-associated genes involved in rescue of the porcine CL during pregnancy forced us recently to evaluate the expression of over 50 genes potentially involved in the process of porcine CL maintenance (Przygrodzka et al. 2015, 2016). Moreover, due to increasing understanding of the regulatory role of non-coding RNAs and, on the other hand, the lack of evidence for their involvement...
in luteal function maintenance, we decided to identify miRNAs in porcine CLs to target genes associated with luteal function maintenance (unpublished). The suggested roles of the most important genes and miRNA–mRNA interactions are discussed in this review and contrasted with the available scientific literature.

Finally, a role of potential pituitary stimuli (LH and prolactin) affecting luteal function during the estrous cycle and pregnancy is summarized and known controversies are discussed in the light of new evidence.

**CL function during the estrous cycle**

*Formation, early maintenance and role of LH*

During the late stages of follicular maturation under the influence of LH, the theca and granulosa cells of the ovulatory follicle undergo luteinization and differentiation into luteal cells. After luteinization and formation of new CLs, both the granulosa- and theca-derived cells can utilize cholesterol for *de novo* steroidogenesis and production of progesterone, the major functional steroid and product of CLs (LaVoie 2017). The high production of progesterone in pigs is necessary to prepare the uterus for implantation and early conceptus development. The presence of CLs in pigs is required during the entire pregnancy.

The early reports of Du Mesnil Buisson and Leglise (1963) and Anderson et al. (1967) suggested that after the initial preovulatory LH surge, the porcine CL can act autonomously up to Day 12 of the estrous cycle, so neither hypophysectomy nor decreased LH levels by progesterone administration (Woody et al. 1967) cause immediate regression of this endocrine gland.

The application of specific anti-LH antibodies produced in pigs (Szafranska & Ziecik 1989) significantly decreased progesterone concentrations in both non-pregnant gilts on Days 8–14 of the estrous cycle and pregnant animals on Days 42–46. Also, inhibition of LH secretion with a gonadoliberin (GnRH) antagonist during the early luteal phase reduced CL development and progesterone production (Brussow et al. 2001), indicating that the porcine CL appears to be not as autonomous during the estrous cycle as was believed earlier (Bazer et al. 1982). This was further supported by in vitro studies showing LH-induced secretion of progesterone by cultured large and small luteal cells (Buhr 1987) as well as slices of CLs collected at the mid-luteal phase (Przygodzka et al. 2014).

The amplitude of LH pulses in the mid-luteal phase of the estrous cycle and early gestation reaches 50% of the magnitude of the preovulatory LH surge (Ziecik et al. 1983), while their duration is 60–70 min with frequencies of 4–10 during 12 h (Hoover & Young 1979). Although LH is thought to be main luteotropic hormone in pigs, there is no correlation between pulses of LH and progesterone in their blood plasma (Brinkley 1981, Van de Wiel et al. 1981), except during late pregnancy (Parvizi et al. 1976).

Measurements of the luteal LH receptor (LHCGR) content in porcine CLs revealed dramatic changes during the cycle and early pregnancy. During the estrous cycle, its concentration peaks on Days 10 and 12 before declining as the CLs regress (Ziecik et al. 1980). Interestingly, administration of estrogen upregulates luteal LHCGR concentrations in non-pregnant animals (Garverick et al. 1982).

**Factors responsible for luteal regression during the luteal phase**

In the absence of embryonic signal(s) at the end of the luteal cycle, regression process in the pig is initiated; CL regression is mediated by pulsatile endometrial secretions of PGF₂α on Days 12–13 and 15–16 of the estrous cycle (Moeljono et al. 1977). The biological rationale for CL regression is preparation of the ovary for the next ovulation and to allow oocytes to be released and fertilized in the next cycle. The actions of PGF₂α include the reduction of steroidogenic enzyme activity and progesterone production within luteal tissue (Niswender 2000). In a number of studies, oxytocin (Camahan et al. 1996), tumor necrosis factor α (TNFα; Wuttke et al. 1998) and LH (Blitek & Ziecik 2005) were suggested as potential modulators of endometrial prostaglandin production within luteal tissue.

The role of LH in luteolysis was, however, reported to be limited to the late luteal phase of the estrous cycle (Ziecik et al. 2001). Interestingly, both components of the porcine uterus, endometrium and myometrium, also possess LHCGR (Ziecik et al. 1986). The appearance of relatively high amounts of LHCGR in the endometrium coincides with the elevation of PGF₂α secretion (Moeljono et al. 1977) and perhaps with decreased expression of progesterone receptors (PGRs). After the beginning of luteolytic PGF₂α release on Days 14–16, the amount of endometrial LHCGR declines. It was found that LH upregulates prostaglandin-endoperoxide synthase 2 (PTGS2) protein expression and PGF₂α secretion from endometrial cells in vitro (Stepien et al. 1999). Furthermore, intravenous administration (Ziecik et al. 2001) or intramuscular challenge with human chorionic gonadotropin (hCG; Guthrie & Bolt 1983) induces uterine PGF₂α release in vivo. It seems that a window of endometrial sensitivity to LH in vivo decreases during Days 15–17 of the estrous cycle. Additionally, there is a much higher correlation between peaks of LH and PGF₂α metabolite (PGFM; 75.5%; Ziecik et al. 2001) than between oxytocin and PGFM (30%; Kotwica et al. 1999). This means that the majority of the ‘luteolytic’ action of LH via its endometrial receptors contributes to destruction of unnecessary cyclic CLs to allow the next ovulation and another chance for successful conception. The luteal regression occurring on Days 13–14 of the
estrous cycle is also thought to be caused by an increase of luteolytic sensitivity (LS) by CLs to endometrial PGF$_{2\alpha}$

The mechanisms responsible for luteal regression are complex and still not well understood in pigs. There are older and some recent reviews summarizing the roles of several molecules involved in luteolysis (Zieck 2002, Wacławek et al. 2017, Zieck et al. 2017). Many of the identified factors associated with luteal regression, such as endothelin 1 (EDN1), hypoxia-inducible factor 1α (HIF1A), estrogen receptor 2 (ESR2) and PTGS2, undergo profound changes in their expression levels after Day 12 of the estrous cycle when CLs have acquired LS. Simultaneously, decreased expression of genes involved in lipid cleavage was observed (Przygrodzka et al. 2016; Fig. 1).

Data on the role of inflammatory cytokines and immune cells in the control of luteolysis are also limited in pigs. Our recent results, however, have revealed that genes associated with infiltration of lymphocytes are induced during the beginning of functional regression in the pig (Przygrodzka et al. 2016). The role of macrophages in luteal regression is well established in pigs (Zhao et al. 1998, Chang et al. 2017). Macrophages are present in all phases of the porcine CL lifespan and their function depends on the luteal microenvironment and phase of the estrous cycle. However, their numbers are highest during the late luteal phase. Macrophages are the primary source of TNFα, an inflammatory cytokine involved in the regression process during the late luteal phase (Zhao et al. 1998). The LS to PGF$_{2\alpha}$ is also known to be associated with infiltration of macrophages into the porcine CL (Chang et al. 2017). Using flow cytometry, we showed that, whereas Tγδ (T-null) cells might have a role in CL regression, Treg (regulatory T cells) percentages were higher in CLs obtained from pregnant animals on Days 13–14. Although only a small proportion of T cells were Tγδ, their percentage was significantly higher on Day 14 of the estrous cycle compared to Day 9 (Andronowska A, Jalali BM, Liakszo P & Witek K unpublished data). Leukocyte-derived cytokines such as TNFα, interferon γ (IFNγ) and interleukins (IL-1, IL-4 and IL-6) are present in the CL at different stages of the luteal phase (Sakumoto et al. 2006, Zmijewska et al. 2012). Both TNFα and IFNγ are known to interact with PGF$_{2\alpha}$ leading to a decline in progesterone synthesis (Sakumoto & Okuda 2004). TNFα is also involved in structural luteolysis through its role in apoptosis (Okano et al. 2006). Sensitization of porcine CLs to the luteolytic action of PGF$_{2\alpha}$ involves upregulation of TNFα observed on Days 12–14 of the estrous cycle (Przygrodzka et al. 2014). The action of TNFα is mediated through two different receptors (TNFR1 and TNFR2) in porcine luteal and endothelial cells. TNFR1 mediates an apoptotic function because it possesses a death domain. On the other hand, TNFR2 is rather involved in the anti-apoptotic effects of TNFα. Indeed, a contribution of the TNFα pathway to survival, development and differentiation of porcine CLs during early gestation has been shown by Przygrodzka et al. (2015). In pigs, an increased presence of both TNFR1 mRNA and protein was found on Day 12 of the estrous cycle, but whether the luteolytic action of TNFα is dependent on the level of TNFα or the abundance of TNFR1 as proposed for bovine CLs (Skarzynski et al. 2003) needs further investigation.

Structural luteolysis in the porcine CL has been reported to be associated with apoptosis (Zorrilla et al. 2013, Przygrodzka et al. 2015). However, no change has been observed in the expression of apoptotic markers such as BAX and BCL2 or in BAX/BCL2 ratio during luteal regression (Przygrodzka et al. 2015). Similarly, the expression of caspase 3 and caspase 8 is also maintained at steady levels during the mid and late luteal phases of the estrous cycle (Zorrilla et al. 2013). However, mRNA expression of factors associated with extrinsic apoptosis (TNFA, TNFRSF1A, FAS and IFNG) are already elevated on Day 12 of the estrous cycle compared to the same day of pregnancy, while TNRSF1A showed higher abundance in luteal...

---

**Figure 1** Schematic presentation of mRNA expression of 21 genes potentially involved in the function of porcine CL on Day 14 of the estrous cycle and pregnancy. Arrows indicate decrease (↓) or increase (↑) of transcript when compared to Day 12 of the estrous cycle/pregnancy. CYP19A1, cytochrome P450 family 19 subfamily A member 1; ESR2, estrogen receptor 2; HSD3B1, 3 beta hydroxysteroid delta-isomerase 1; LHCCR, lutropin-choriongonadotrophic hormone receptor; NR5A1, nuclear receptor subfamily 5, group A, member 1; PTGS2, prostaglandin-endoperoxide synthase 2; PTGER4, prostaglandin E receptor 4; PTGFR, prostaglandin F receptor; EDN1, endothelin 1; HIF1A, hypoxia-inducible factor 1α; IFNG, interferon gamma; SCARB1, scavenger receptor class B, member 1; STAR, steroidogenic acute regulatory protein; 1 TNFA, tumor necrosis factor alpha; 1 TNFRSF1A and 1 TNFRSF1B, tumor necrosis factor receptor superfamily member 1A and 1B; VEGFA, vascular endothelial growth factor A.
tissue of pregnant gilts on Day 14 (Fig. 1). Two factors involved in apoptotic pathways (FOS, JUN) showed distinct upregulation on Day 14 of the estrous cycle compared to earlier days (Przygrodzka et al. 2015; Fig. 1). Our proteomic data showed higher abundance of N-ethylmaleimide-sensitive factor (NSFL1) and heat shock protein b6 (HSPb6) and a decrease in eukaryotic elongation factor 1 (eEF1) and lysosomal adaptor MTOR activator (LAMTOR3) on Day 16 of the estrous cycle relative to the corresponding day of gestation. Since NSFL1, HSPb6 and LAMTOR3 are involved in autophagy and eEF1 is a known modulator of apoptotic proteins, there is a possibility that luteolysis in pigs is associated with autophagy and apoptosis (Jalali BM, Likszo P & Skarzynski DJ 2017 unpublished observations).

Unlike in other species such as humans and cattle, the level of vascular endothelial growth factor A (VEGFA) mRNA is maintained almost constant until Day 15 of the estrous cycle in pigs (Kaczmarek et al. 2007, Przygrodzka et al. 2016). There is a decrease in the expression of VEGFA and its receptors only during the late estrous cycle (Days 16–17; Kaczmarek et al. 2007). Luteal expression of VEGFA during pregnancy (Days 16–25) is similar to Days 5–12 of the estrous cycle. Other angiogenic factors such as angiopoietins (ANGPT1, ANGPT2) do not seem to be associated with luteal regression in pigs (Przygrodzka et al. 2016).

Rescue and maintenance of CLs for pregnancy

Old theories and new aspects of maternal recognition of pregnancy

The luteolytic effect of PGF$_{2\alpha}$ on structural and functional luteolysis needs to be prevented during early pregnancy in order to rescue the CLs, since the maintenance of luteal function ensures continued release of progesterone needed for the secretion of specific endometrial products and constituents of the histotroph required for blastocyst growth and development. It is believed that at least 4 ng/mL progesterone in the circulation is critical to maintain pregnancy in the pig (Ellicott & Dziuk 1973) and removal of CLs during pregnancy results in its termination (Belt et al. 1971).

According to the original theory of maternal recognition of pregnancy in pigs created by Bazer and Thatcher (1977), the main role in the process of CL rescue is played by conceptus-driven redirection of endometrial PGF$_{2\alpha}$ secretion after Day 12 post coitum (p.c.) from endocrine to exocrine, i.e., into the uterine lumen. Thus, the threshold level of PGF$_{2\alpha}$ concentration in the peripheral blood reaching the luteal tissues is too low to begin luteolysis. An alternative antiluteolytic mechanism was proposed by Krzymowski and Stefanczyk-Krzymska (2004), based on the retrograde transfer of PGF$_{2\alpha}$ from the uterine venous blood and lymph into the uterus, causing PGF$_{2\alpha}$ accumulation in the veins and arterial walls of the uterus instead of reaching the late luteal CLs.

A closer look into CL characteristics helped us to understand that the prolonged insensitivity to the luteolytic action of PGF$_{2\alpha}$ during early pregnancy was also explained by decreased numbers of PGF$_{2\alpha}$-binding sites in freshly isolated luteal cells obtained from porcine CL on Day 14 of pregnancy in comparison to Day 14 of the estrous cycle (Gadsby et al. 1990, 1993). However, more recent ex situ studies performed on whole porcine luteal tissue even showed an increase of PTGFR protein and mRNA in CL on Days 12 and 14 of pregnancy relative to the same days of the estrous cycle (Przygrodzka et al. 2016). A similar abundance of PTGFR mRNA was reported in cycling and early pregnant sheep (Wiepza et al. 1992). Davis and Rueda (2002) also suggested the presence of alternatively spliced PTGFR in ovine CLs, leading to either their reinforcement or destruction.

This discrepancy between the earlier pioneering work of Gadsby et al. (1990, 1993) and our recent results (Przyzgrodzka et al. 2014, 2016) could be due to at least two reasons: application of completely different methods, i.e., receptor binding sites vs Western blot and/or PCR, and use of isolated luteal cells instead of whole luteal tissue. Worth mentioning is the presence of PTGFR not only on luteal but also on endothelial cells (Zannoni et al. 2007) responsible for building vascular network of the CL. Interestingly, Przygrodzka et al. (2016) found an elevated intraluteal content of PGF$_{2\alpha}$ on Day 14 of the estrous cycle vs the corresponding day of gestation, confirming earlier suggestions of possible PGF$_{2\alpha}$ production in CLs with acquired LS (Diaz et al. 2000, Wasielak et al. 2008). It seems that protein expression of PTGFR is decreased in CLs on Day 14 of the estrous cycle due to a negative correlation between high levels of luteal PGF$_{2\alpha}$ and PTGFR abundance (Diaz et al. 2000). In contrast to reports by Gadsby et al. (1990, 1993), the above-mentioned recent observations and earlier report by Zorrilla et al. (2009) suggest that induction of different post PTGFR signaling networks including specific kinase C (PKC) expression is more important than PTGFR abundance during the course of luteolysis.

Besides the well-known and described role of conceptus estrogens in the rescue of porcine CLs from luteolysis during establishment of pregnancy (Ford et al. 1982), PGE$_2$ started to be recognized as another or second conceptus signal in the pig (Waclawik et al. 2017). The antiluteolytic role of PGE$_2$ in the pig was known for decades, ever since an increased ratio of PGE$_2$/PGF$_{2\alpha}$ content in the uterus was observed during the period of maternal recognition of pregnancy (Christenson et al. 1994). The expression of microsomal PGE$_2$ synthase-1 (mPGES-1) is observed in spherical conceptuses since Day 10 p.c. and dramatically increases in tubular conceptuses on Day 13.
(Waclawik & Ziecik 2007). The production of PGE\textsubscript{2} in embryos and the endometrium seems to be involved in the escape of porcine CLs from luteolysis due to (1) a much higher luteal concentration of PGE\textsubscript{2} on Day 14 of pregnancy than on the same day of the estrous cycle (Przygrodzka et al. 2016) and (2) higher PGE\textsubscript{2} content only in CLs ipsilateral to the gravid horn of unilaterally pregnant gilts (Wasielak et al. 2008), not accompanied by increased mPGES-1 expression. In addition, a local transfer of PGE\textsubscript{2} via blood and lymph vessels from the uterus to the ovary (Stefanczyk-Krzymowska et al. 2006) can aid this process. The porcine CL possesses at least two forms of PGE\textsubscript{2} receptors: PGE\textsubscript{2} receptor 2 (PTGER2) and PGE\textsubscript{2} receptor 4 (PTGER4), both upregulated on Day 14 of pregnancy and participating in the production of prostaglandin in cultured luteal slices through a cAMP-mediated pathway (Waclawik et al. 2010). Our recent study revealed an increased phosphorylation of protein kinase A (PKA) at the position of Threonine 197 (Thr197), cAMP response element-binding protein (CREB) at the position of Serine 133 (Ser133) and protein kinase B (PKB) at the position of Serine 473 (Ser473) in porcine CL on Day 14 of pregnancy compared to the same day of the estrous cycle. Simultaneously, we observed an increase in levels of PGE\textsubscript{2} and elevated content of steroidogenic acute regulatory protein (STAR), aromatase CYP19A1 (cytochrome P450 family 19 subfamily A member 1) and VEGFA in luteal tissue slices on Day 14 of pregnancy (Przygrodzka, A Waclawik & A J Ziecik, unpublished observations). These results suggest that binding of PGE\textsubscript{2} to PTGER2/4 may activate PKA and PKB signaling pathways as well as enhance the expression of proteins involved in processes determining maintenance of luteal function during early pregnancy in pigs. Moreover, PGE\textsubscript{2} significantly stimulated VEGFA secretion by luteal cells on Days 10–12 of gestation (Kowalczyk et al. 2008). Additionally, downregulation of endogenous antagonist of VEGFA-soluble receptor (sFLT1) in porcine CLs during early pregnancy may serve to enhance their content of bioavailable VEGFA. Consequently, prolonged progesterone secretion appears to be possible due to increased permeability of luteal small blood vessels and supply of cholesterol to the cells as well as easier transfer of prostaglandins from the peripheral circulation (Kaczmarek et al. 2009). The evidence that PGF\textsubscript{2\alpha} may stimulate progesterone secretion in early CLs of the estrous cycle and during early gestation or inhibit it in CLs approaching luteolysis was documented in early \textit{in vivo} experiments employing a microdialysis system (Wuttke et al. 1998). Also the ‘luteolytic’ PGF\textsubscript{2\alpha} elevated production of cAMP previously stimulated \textit{via} both increased Ca\textsuperscript{2+} release and PKC activation in bovine CLs (Mamluk et al. 1999). Recently, it was shown that PGF\textsubscript{2\alpha} increased the content of CREB in CL slices of early pregnant pigs and progesterone secretion in mid-luteal phase CLs, but decreased progesterone production by luteal slices from CLs with acquired LS at the late-luteal phase (Przygrodzka et al. 2014, 2016).

The aforementioned studies show the dual and opposing roles of PGF\textsubscript{2\alpha} in the porcine CL, depending on the phase of the estrous cycle and pregnancy, i.e., its luteotropic role in CLs without acquired LS (early CLs during the estrous cycle and CLs of early pregnancy) and its luteolytic role in CLs with acquired LS (the late luteal phase of the estrous cycle, approaching luteolysis).

**Lessons learned from gene expression studies**

Unfortunately, so far, there is a lack of global profiling studies on pregnancy-associated genes contributing to the antiluteolytic mechanisms in the porcine CL. Such data are available for ovine CLs (Romero et al. 2013) and revealed 734 genes differentially expressed on Day 14 of pregnancy. On the other hand, early stages of luteolysis (Days 12–14) in the sheep were associated with altered expression of 682 genes in the luteal tissue. However, different embryo signaling systems for CL rescue in ruminants (IFNT) and pigs (mainly E\textsubscript{2} and PGE\textsubscript{2}) make it difficult to find genes universal for antiluteolytic mechanisms in these animal species.

Nevertheless, an examination of over 50 genes potentially involved in the process of porcine CL maintenance during early pregnancy helped to identify 14 genes with increased expression in CLs on Day 14 of pregnancy involved in steroidogenesis (i.e. scavenger receptor class B, member 1 (SCARB1); STAR; hydroxy-delta-5-steroid dehydrogenase, 3 beta and steroid delta-isomerase 1 (HSD3B1); LHCGR; nuclear receptor subfamily 5, group A, member 1 (NR5A1); estrogen receptor 1 (ESR1); PGR; PGR membrane components 1/2 (PGRMC1/2) and in angiogenesis (i.e. angiopoietin 2 (ANGPT2); prostaglandin F synthase (PTGSF); hydroxyprostaglandin dehydrogenase 15-(NAP) (HPGD); kinase insert domain receptor (KDR); VEGFR2; low density lipoprotein receptor (LDLR); nuclear factor of kappa light polypeptide gene enhancer in B cells (NFkB1) and pentraxin 3 (PTX3)). Downregulated genes on Day 14 of pregnancy included EDN1, CYP19A1, ESR2, PTGS2, JUN and FOS (Fig. 1; Przygrodzka et al. 2015, 2016).

Factors involved in progesterone synthesis and metabolism, i.e., LHCGR, SCARB1, STAR and HSD3B1 were upregulated on Day 14 vs Day 12 of gestation and the respective day of the estrous cycle. Also, expression of NR5A1, a transcription activator of steroidogenic genes important for maintenance of progesterone secretion in gilts, was upregulated in CLs on Day 14 of gestation. These changes together with elevated abundance of PGRMC1 on the same day of pregnancy seem to be essential to support an anti-apoptotic mechanism mediated by progesterone in luteal tissue (Kowalcik & Kotwica 2008, Diaz et al. 2011).
The observed relatively low expression of genes related to metabolism and action of E\textsubscript{2} (CYP19A1 and ESR2) in CLs during early pregnancy is due to their paradoxical high expression in porcine CLs with acquired LS during induced (Diaz & Wiltbank 2005) or spontaneous luteolysis (Przygrodzka et al. 2016). However, the abundance of CYP19A1 protein, sufficient to increase intraluteal content of E\textsubscript{2} and enhance conversion of testosterone to E\textsubscript{2} on Days 12 and 14 of gestation, suggest that luteal tissue of early pregnant pigs can produce E\textsubscript{2}.

Although increased EDN1 (Fig. 1) levels on Day 14 of the estrous cycle were suggested to play a role in development of LS in the pig (Zorrilla et al. 2010), a high abundance of HIF1A (Fig. 1) seems to act in the opposite way. Our recent in vitro study on the involvement of hypoxia and HIF1A in maintenance of porcine CL function revealed that CoCl\textsubscript{2}, a HIF1A activator, stimulated HIF1A, VEGFA and STAR expression only in CLs of cycling gilts. In addition, in silico analysis revealed the presence of HIF1A transcription-binding sites within the promoters of VEGFA and STAR genes. Other genes associated with luteal function maintenance (e.g., PGRMC1, EDN1) were also found to be potential targets of HIF1A. Thus, it seems likely that HIF1A can be another modulator of luteal function maintenance (E Przygrodzka, K Myszczynski & AJ Ziecik, unpublished observations).

**MicroRNAs as new players in CL maintenance**

Since it was found that almost two-thirds of the genomic DNA is transcribed in eukaryotes, but less than 2% is translated into proteins, appreciation of the role of ncRNAs in regulating processes occurring in eukaryotes has dramatically increased (Rinn & Chang 2012). However, the role of ncRNAs in luteal function maintenance remains poorly investigated. Among various types of ncRNAs the most studied are the miRNAs defined as short ~22-nt long RNA molecules that alter transcription and/or translation of mRNA molecules by binding mainly to its 3’ untranslated region (3’UTR). The general role of miRNAs in ovarian functions was stressed in studies performed on mice with deletion of Dicer, an endonuclease responsible for cleavage of pre-miRNA to mature miRNA. This genetic modification led to inhibited follicle growth and reduced ovulation rate as well as faulty oocyte development and oviductal and uterine defects (Tang et al. 2007, Hong et al. 2008, Nagaraja et al. 2008). Global profiling of miRNAs during bovine and ovine CL development (Fiedler et al. 2008, McBride et al. 2012) and CL regression and rescue during pregnancy in cows (Ma et al. 2011, Maalouf et al. 2014) showed a number of differentially expressed miRNAs, but the specific roles of selected miRNAs have rarely been demonstrated. For instance, miR-378 was found to regulate apoptosis in bovine CLs (Ma et al. 2011), miR-17-5p and miR-7p can regulate luteal angiogenesis in mice (Otsuka et al. 2008), while miR-96 was associated with luteal survival and steroidogenesis in human CLs (Mohammed et al. 2017). Our latest studies showed that miRNAs can also be involved in molecular and consequently hormonal changes in the porcine CL. Using microarrays, we identified several differentially expressed miRNAs in porcine CLs collected during the period of luteolysis (Day 14 of the estrous cycle) and maternal recognition of pregnancy (Day 14 of pregnancy). Among predicted targets of miRNAs upregulated during pregnancy, we found genes previously described as markers of luteolysis, e.g., EDN1, FOS, JUN, PTGS2, FAS, ESR2. In contrast, miRNAs upregulated in CLs during luteolysis were found to target genes associated with luteal function maintenance, e.g., PGR, PGRMC2, IGF2, FLI1, CREB, PTGER2 (Przygrodzka & Kaczmarek 2016 unpublished data). Considering results of global studies performed in other animal species and with porcine CLs, it is likely that some miRNAs can have a supportive role in the luteolytic or luteotropic actions of primary factors regulating luteal function. That conclusion is further supported by our in vitro studies on luteal tissue slices, showing E\textsubscript{2}, a luteotropic conceptus-derived signal in pigs, to be a factor able to upregulate the expression of miRNAs targeting genes involved in progesterone metabolism (Przygrodzka & Kaczmarek 2017 unpublished data). These results shed new light on the role of miRNAs in a novel molecular mechanism supporting the luteotropic action of conceptus-derived signals during early pregnancy.

**Two-signal switch hypothesis**

Recently, we proposed the ‘two-signal switch hypothesis’ to highlight the importance of post PGF\textsubscript{2a} and PGE\textsubscript{2} receptor signaling pathways activation in CLs during luteolysis and CL rescue during maternal recognition of pregnancy in the pig (Ziecik et al. 2017). The updated version of the hypothesis is presented in Fig. 2. After acquisition of LS by CLs on Days 12–14 of the estrus cycle due to the influence of cytokines, e.g., TNFa, IFNy and EDN1 (forming the ‘luteolytic switch’), luteal cells begin to be sensitive to the luteolytic activity of PGF\textsubscript{2a}. As a result, the post PTGFR signaling pathway activates two phospholipase C (PLC) pathways through: 1) diacylglycerol (DAG) and PKC, leading to inhibition of cAMP production and 2) inositol 1,4,5-triphosphate (IP3) and Ca\textsuperscript{2+} activating proto-oncogene protein-serine threonine kinase (RAF1) and the downstream RAF/MEK/ERK signaling cascade in the cytoplasm. Phosphorylated ERK1/2 proteins are translocated into the nucleus and induce both FOS and JUN and other proto-oncogenes, contributing to AP1 transcription factor dimers formation. Furthermore, this leads to transcription activation of genes involved in functional and structural luteolysis. Before the ‘luteolytic switch’ is triggered, the porcine
CL rescue and maintenance during pregnancy in pigs

reducing cholesterol availability for progesterone synthesis (Talbott & Davis 2017) and (2) RAF1 (Bos et al. 2004). Together, LH and PGE₂ increase the availability of cholesterol for progesterone synthesis. At the same time, phosphorylation of PKA, PKB and CREB by PGE supports maintenance of angiogenesis, steroidogenesis and cell survival in the CL. Interestingly, the results of recent report on mechanisms for rescue of bovine CL during early pregnancy (Ochoa et al. 2018) also strongly support a luteoprotective role of PGE₂.

**CL function during pregnancy**

**Role of LH and prolactin**

It was suggested that later in pregnancy, prolactin can maintain luteal function in hypophysectomized gilts (Du Mesnil du Buisson & Denamur 1968, Li et al. 1989). However, it is not clear whether the influence of LH on CL function is ever lost during pregnancy, since temporal interrelationships between pulses in circulating LH and progesterone levels have been demonstrated as late as on Day 90 (Parvizi et al. 1976).

The first reports on LH concentrations during various periods of pregnancy in pigs were fragmentary and contradictory (Tilson et al. 1970, Guthrie et al. 1972, Parvizi et al. 1976) until the profile of plasma LH during the course of pregnancy in the pig was described in detail (Ziecik et al. 1983). It was shown that from the first 6–7 days of gestation until parturition, LH release showed rhythmic pulses throughout pregnancy with decreasing amplitudes from Days 12 to 24 until parturition. The levels and patterns of LH secretion in early and mid-gestation were similar to the mid-luteal phase of the estrous cycle.

The plasma LH fluctuated in a pulsatile manner throughout gestation with declining amplitude toward parturition and a significant correlation between the decrease in LH concentrations and the day of gestation was determined: \( y = 1.758 - 0.0088x \), where variable ‘\( x \)’ is a day of gestation (Ziecik et al. 1983). It is possible that increasing levels of estrogens during pregnancy (Robertson & King 1974) can negatively influence the basal LH level and the magnitude of its fluctuations. The reduction in LH levels may cause a simultaneous decrease in the concentration of progesterone (Guthrie et al. 1972).

The onset of prolactin dependency is supposed to be associated with an increased content of prolactin receptors in CLs during early pregnancy (Jammes et al. 1985). Our results indicated that decreasing the concentration of blood plasma prolactin by treatment with bromocriptine (inhibitor of prolactin secretion) is not mandatory for the weakening of CL steroidogenesis during mid-pregnancy (Zafranska & Ziecik 1990a) and in the second half of pregnancy in pigs (Zafranska & Ziecik 1990b). It is possible that pregnant gilts have a
specific substitute mechanism of compensation at the level of the hypothalamus and/or pituitary, developed to maintain CL function during pregnancy if LH or prolactin deficiency occurs. It seems likely that pregnancy stage has no effect on prolactin concentrations until shortly before term, when a dramatic surge of prolactin occurs presumably due to lactogenesis (Duszka & Krzymowska 1981). Prolactin together with other metabolic hormones, growth factor (GH) and insulin-like growth factor 1 (IGF1), may act synergistically to increase progesterone secretion by porcine luteal cells (Yuan & Lucy 1996).

**Strategies to support enhanced CL function during pregnancy**

The majority of studies concerning CL function during pregnancy were focused on the period of maternal recognition of pregnancy and implantation, i.e., Days 12–18 of pregnancy. Some reports extended observations to Day 30 of pregnancy, since the highest embryonic mortality (20–30%) in pigs happens between Days 12 and 30 of pregnancy (Lambert et al. 1991). According to Jindal et al. (1997), progesterone is important in mediating the supply of nutrients influencing embryonic survival in gilts. Thus, to date, many attempts have been undertaken to stimulate luteal function in order to reduce early embryo mortality by application of various hormones that enhance luteal function in pigs. However, multiple injections of hCG, a known analog of LH, during the first 8 days of gestation (Stone et al. 1987) or a single administration of hCG after Day 12 (Tilton et al. 1989, Bolzan et al. 2013) did not affect the level of progesterone in the blood plasma of pigs. A single injection of hCG on Day 12 of pregnancy elevated E₂ concentration and increased expression of STAR, accompanied by reduced levels of early apoptotic luteal cells and elevated percentages of luteal cells in CLs on Day 30 of pregnancy (Bolzan et al. 2013). It is worth mentioning that resorption of all fetuses on Day 30 of pregnancy did not affect the level of progesterone in blood until Day 60 of pregnancy, which may suggest that fetus-derived signals are not necessary to maintain CL function between Day 30 and 60 of pregnancy in pigs (Webel et al. 1975).

**Concluding remarks**

The earlier fundamental theories of maternal recognition of pregnancy based on redirection and cessation of PGF₂α supply to CLs have not considered the overcoming of LS acquisition during the process of CL rescue after Day 12 p.c. This complex process initiated by embryo signals, mainly E₂ and PGE₂, depends on cooperation between many pleiotropic factors determining luteal function maintenance. Overcoming of LS to PGF₂α also relies on the change of the ‘luteolytic’ to the ‘luteotropic’ role of this prostaglandin to support CL function, besides the proven and already known actions of LH and PGE₂ during early pregnancy.

Our ‘two signal switch’ hypothesis concerning participation of PGF₂α and PGE₂ and their post receptor signaling systems, activated in CLs during luteolysis and during the process of luteal rescue, is based on available knowledge and our recent studies at the molecular level. It also shows the complexity of the interactions driving luteal control during the most sensitive periods of the life cycle of CLs: regression and rescue.

Complete understanding of the mechanisms driving luteal function in pigs and other species requires establishment of a sequence hierarchy and particular timing of molecular interrelationship between many mediators of the CL life span (development, regression, rescue and maintenance) as well as their endocrine and paracrine actions at the systemic, local and subcellular levels.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

**Funding**

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Acknowledgments**

Some research described in the review was supported by the statutory Fund of IARFR in Olsztyn (Poland). The authors thank J Murawska-Kempa for her helpful assistance in typing the manuscript.

**References**


www.reproduction-online.org


Yuan W & Lucy MC 1996 Evidence for the presence of luteinizing hormone receptor, luteinizing hormone receptor, and steroidogenic enzymes during the estrous cycle and pregnancy in porcine and bovine corpora lutea. Domestic Animal Endocrinology 13 431-444. (https://doi.org/10.1001/0378-4320(96)0007-002)


Received 30 October 2017
First decision 4 December 2017
Revised manuscript received 27 April 2018
Accepted 22 May 2018


