Transcriptome analyses of porcine endometrium during the pre-implantation phase

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The porcine conceptus undergoes rapid differentiation and expansion of its trophoblastic membranes between days 11 and 12 of gestation. The production of estrogen, the porcine embryonic pregnancy recognition signal, by the conceptus increases with trophoblast elongation. A complex interplay of estrogen signaling and prostaglandin (PG) metabolism in the endometrium finally results in prevention of luteolysis. Conceptus attachment to the uterine surface epithelium starts around day 14 of pregnancy preceded by a pronounced vascularization at the implantation zones, initiating the epitheliochorial placentation. To characterize the transcriptome changes in the porcine endometrium in the course of maternal recognition of pregnancy (MRP) and initial placentation, several transcriptome analyses using DNA microarrays and RNA sequencing (RNA-Seq) have been performed. This review summarizes and compares the results of these studies. Particularly, the studies where RNA-Seq has been used, revealed more than 2,500 and 1,900 differentially expressed genes (DEGs) for days 12 and 14 of pregnancy, respectively, in comparison to corresponding cyclic controls. Analysis of the results of these two studies revealed distinct differential gene expression, reflecting the different functions of the endometrium during these stages. The comparison of RNA-Seq and microarray data for day 14 of pregnancy revealed a good agreement of the results. Moreover, results of microarray studies investigating local responses of the uterine horn to embryos in the blastocyst stage and effects of premature exposure of pregnant gilts to exogenous estrogen on endometrium during early pregnancy are discussed and compared to the results from day 12 and day 14 of pregnancy.

Introduction

Establishment of pregnancy in mammals requires prolongation of luteal life span for sustained progesterone (P4) production. P4 stimulates secretory functions of the endometrium required for conceptus development and implantation. Maternal recognition and establishment of pregnancy in pigs requires a biphasic pattern of estrogen (E2) secretion (Geisert *et al.* 1990). Between days 11 and 12 of gestation, the porcine conceptus undergoes rapid differentiation and expansion of its trophoblastic membranes (Geisert *et al.* 1982, Stroband & Van der Lende 1990, Yelich *et al.* 1997). Coordinate with trophoblastic elongation, conceptus secretion of E2 increases (Ford *et al.* 1982, Stroband & Van der Lende 1990). The second phase of increased E2 secretion is between days 15 and 30 of pregnancy. Luteoprotective action of E2 is complex.

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It stimulates luteal P4 secretion directly (Conley & Ford 1989). However, the basic model is that conceptus estrogen produced between days 11 and 16 elicits a change in the direction of prostaglandin F2 alpha (PGF2a) secretion from endocrine to exocrine resulting in sequestration of PGF2a in the uterine lumen and consequently in prevention of luteolysis (Bazer & Thatcher 1977). Furthermore, conceptus and endometrial prostaglandin E2 (PGE2) has been shown to influence prostaglandin (PG) metabolism in favor of luteoprotective PGE2 (Waclawik 2011, Ziecik *et al.* 2011). Endometrial luteoprotective PGE2 and luteolytic PGF2a are involved in reproduction processes in many species (Weems *et al.* 2006, Kennedy *et al.* 2007). It has been demonstrated that inhibition of PG synthesis before implantation causes pregnancy failure in different species, including the pig (Kennedy *et al.* 2007). Before implantation, the endometrium and trophoblast synthesize elevated amounts of PGE2, which results in high amounts of this PG in the uterine lumen and/or utero-ovarian circulation in pigs. It has been shown that expression of enzymes of the PG synthesis pathway is altered in the porcine conceptus and endometrium to favor luteoprotective PGE2 synthesis between days 10 and 13 of pregnancy (Waclawik *et al.* 2006, Waclawik & Ziecik 2007).

Epitheliochorial placentation is starting on day 14 of pregnancy, and is associated with specific tissue remodeling at implantation sites, e.g. increased vascularization and vascular permeability at implantation zones and changes in the luminal epithelium (Keys & King 1988, Keys & King 1989, Keys & King 1990). Pigs show a true epitheliochorial placentation since the luminal epithelium remains completely intact throughout pregnancy, (Keys & King 1988). Several inflammatory mediators including cytokines and growth factors are associated with conceptus growth, implantation and establishment of pregnancy (Croy *et al.* 2009, Waclawik 2011). Porcine conceptuses and also the endometrium produce interferons (IFN), which do not have antiluteolytic functions (Johnson *et al.* 2009). The trophectoderm expresses both, type I IFNs (IFN delta, IFND) and type II IFNs (IFN gamma, IFNG) starting from Day 11/12 of pregnancy (Cross & Roberts 1989, Lefevre *et al.* 1990) with peak expression of IFND on Day 15, whereas natural killer (NK) cells are the endometrial source of IFNG (Murphy *et al.* 2009). This results in increased expression of IFN stimulated genes (ISGs) in the stroma and glandular epithelium (GE) which likely have a role in uterine remodeling to support placentation (Johnson *et al.* 2009). IFNG is thought to be involved in the initiation of endometrial vascular remodeling, angiogenesis at implantation sites and modulation of the maternal immune system (Murphy *et al.* 2009).

To obtain a systematic overview of gene expression changes in porcine endometrium in during the pre-implantation phase a number of transcriptome studies have been performed. This review summarizes the results of a microarray study on day 6 of pregnancy (Alminana *et al.* 2012), RNA-Seq studies on days 12 (Samborski et al. 2013, unpublished data) and 14 of pregnancy (Samborski *et al.* 2013) a microarray study on day 14 of pregnancy (Østrup *et al.* 2010) and a study of the effects of premature estrogen exposure on gene expression in pregnant endometrium (Ross *et al.* 2007).

Identification of early local endometrial responses to the presence of blastocysts

The porcine embryo enters the uterus around day 5 after estrus in the morula stage and reaches the blastocyst stage on day 6 (Rüsse & Sinowatz 1998). To study the effects of early embryo-maternal interactions on endometrial gene expression, Alminana et al. (Alminana *et al.* 2012) used laparoscopic insemination of one oviduct to obtain samples from the tip of the uterine horn containing either embryos ("pregnant horn") or oocytes ("control horn") from the same sow. Using this model, about 200 genes were identified as differentially expressed in the tip of the

uterine horn on day 6 of pregnancy, almost all genes with lower expression levels in the uterine horn containing embryos. The authors found a number of genes related to immune functions and postulated the down-regulation of immune-related genes as a mechanism to protect the early embryo from the maternal immune system. However, the analysis of overrepresented functional categories of the down-regulated genes (194 genes could be assigned to putative human orthologous genes) did not show overrepresentation of immune-related genes (Fig. 1). Four genes could be assigned to 'immune response' via SP_PIR keywords and 3 genes based on Gene Ontology. A number of other functional categories showing significant overrepresentation were identified, i.e. more genes were found for these categories than expected by chance. The highest overrepresentation was found for a group of categories related to 'vesicle membrane', particularly for 'Golgi-associated vesicle membrane' (Fig. 1). This could indicate an effect of early embryos on specific secretory processes in endometrial cells. Furthermore, functional categories related to the process 'RNA splicing' were found as overrepresented for the genes down-regulated on day 6 of pregnancy. Differential expression of a splicing factor during the cycle has been shown in human and mouse endometrium and suggested to play a role in preparation of a receptive uterus (Nie *et al.* 2002).

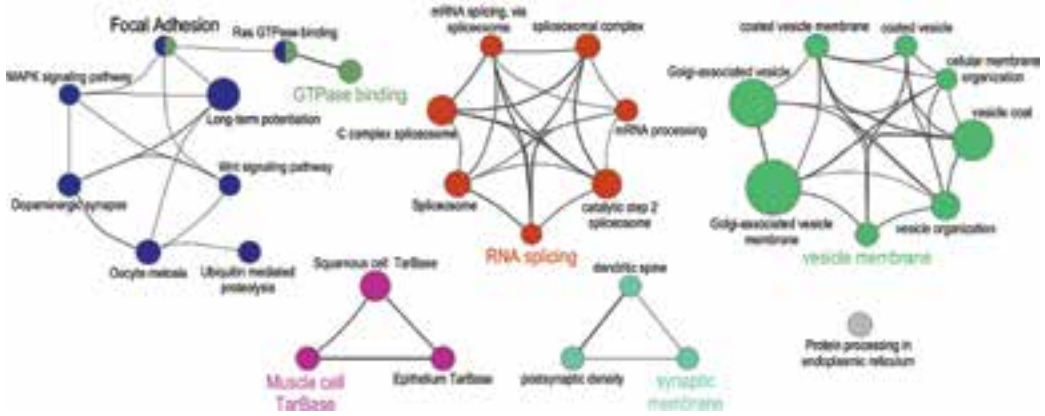

Fig. 1 Network of overrepresented Gene Ontology categories, KEGG and WIKI pathways for genes with lower transcript levels in the uterine horn harboring embryos compared to the control uterine horn without embryos on day 6 of pregnancy after laparoscopic insemination into one oviduct. All significant genes (human Entrez Gene IDs) with lower transcript levels in pregnant endometrium were used as input for the Cytoscape 3.0.0.beta1 application ClueGO 2.0.0.beta1. To limit the search results, the following parameters were used: GO tree levels: 2-15, minimal number of genes: 5, %genes: 3, p-value correction: 'Benjamini-Hochberg', terms with p-value <0.05, GO term fusion, GO term connection restriction (kappa score): 0.3, GO term grouping: initial group size = 3 and 50% for group merge. The resulting network was modified, i.e. redundant and non-informative terms were deleted and the network was manually rearranged. Node size was adjusted relative to % associated genes. Edge line width was assigned in relation to the kappa score.

Gene expression in porcine endometrium during the pre-implantation phase – RNA-Seq analysis on days 12 and 14 of pregnancy

Day 12 of pregnancy

In a recent study the response of the porcine endometrium to the presence of filamentous conceptuses on day 12 of pregnancy, the time of maternal recognition of pregnancy, was investigated using RNA sequencing (RNA-Seq) (Samborski et al. 2013, unpublished data).

This study resulted in the identification of 2,593 differentially expressed genes (DEG) (FDR 1%) indicating a complex response to conceptus signals. The search for significantly enriched functional categories for genes with higher mRNA concentration in pregnant endometrium revealed the highest overrepresentation for genes related to cell cycle, cell division and mitosis. Additional clearly overrepresented categories were 'cytoplasmic vesicle', 'metallopeptidase activity', 'apoptosis', and 'blood vessel development'. The genes contained in the latter category could have a role for vascular remodeling in the implantation zones that occurs during increase in conceptus estrogen release (Keys & King 1988).

For the down-regulated genes, overrepresented functional categories were related to 'intermediate filament', 'keratin filament', 'steroid metabolic process', 'phospholipase activity', 'icosanoid metabolic process', and 'unsaturated fatty acid metabolic process'. Particularly, the category 'intermediate filament' was highly overrepresented with 21 keratin genes and one keratin-associated gene (out of 23 genes in total). Keratins are typical intermediate filament proteins of epithelia and have an important role for the mechanical stability and integrity of epithelial cells. Furthermore, keratins are related to the epithelial type and stage of cellular differentiation (Moll *et al.* 2008).

The genes found as differentially expressed in the study of day 6 of pregnancy (Alminana *et al.* 2012) were compared to the DEGs found on day 12 of pregnancy. This revealed 35 genes differentially expressed on day 6 and on day 12 of pregnancy, which is slightly more than expected by chance. However, the overlapping genes were down-regulated on day 6 and up-regulated on day 12, except for two genes.

Day 14 of pregnancy

To characterize transcriptome changes in the endometrium associated with initial conceptus attachment deep sequencing of endometrial RNA samples was performed (Samborski *et al.* 2013) on day 14 in comparison of pregnant and corresponding cyclic gilts. This study revealed 1,933 differentially expressed genes (FDR 1%), 1,229 with higher and 704 with lower mRNA concentration in the samples from pregnant gilts. The comparison of the results to the previous microarray study of day 14 of pregnancy (Østrup *et al.* 2010) using Gene Set Enrichment Analysis (GSEA) (Subramanian *et al.* 2005) revealed a good overlap even though different experimental settings and different platforms for gene expression analysis were used (Samborski *et al.* 2013). Furthermore, a substantial overlap of the day 14 RNA-Seq data set was also found for the comparison to results of a microarray study of bovine endometrium on day 18 of pregnancy and the response of bovine endometrium to IFN alpha (Bauersachs *et al.* 2012). Most of these genes were known IFN-stimulated genes (ISGs) probably induced by conceptus IFND and IFNG. Correspondingly, bioinformatics analysis revealed for the genes with higher mRNA concentration in day 14 pregnant porcine endometrium strong overrepresentation for immune-related functional terms, but also for functions related to apoptosis and cell adhesion. For the process of cell adhesion a number of members of the integrin family were found as differentially expressed (Samborski *et al.* 2013). According to non-invasive placentation in pigs, integrins with functions in cell-cell adhesion were found with higher mRNA concentration in day 14 pregnant porcine endometrium, whereas integrins functioning in cell migration and invasion were found to have lower mRNA concentration in pregnant endometrium. The analysis of the genes with lower mRNA concentration in pregnant endometrium revealed strong overrepresentation of members of the EPH-ephrin system. The EPH-ephrin system has an important function in cell-cell and cell-matrix adhesion and in the regulation of cell migration through interaction with a variety of proteins involved in other signaling pathways such as integrins, claudins, cadherins, and connexins (Arvanitis & Davy 2008). In porcine endometrium down-regulation

of the EPH-ephrin system could be a mechanism to control trophoblast attachment and prevent invasion through the endometrial epithelium. An additional process important in the context of placentation is the transport of nutrients and ions. The transport of ions was also highly overrepresented for the down-regulated genes. Most of the identified transporters were related to sodium, potassium and calcium transport (Samborski *et al.* 2013). Comparing transporter genes of the up-regulated and the down-regulated genes, very different types of transporters with respect to their substrates were obtained, indicating specific regulation by conceptus signals of endometrial secretions according to the requirements of the developing conceptus.

Comparison of differential gene expression on days 12 and 14 of pregnancy

The comparison of the RNA-Seq results obtained for days 12 and 14 (overlap of DEG pregnant vs. non-pregnant) revealed only a moderate overlap (Samborski et al. 2013, unpublished data). This reflects the different functions of the endometrium during these stages, i.e. recognition of pregnancy on day 12 with the corresponding response to conceptus estrogen and preparation for conceptus implantation on day 14 associated with the response to IFNs. Selected functional categories, which are specifically overrepresented for up-regulated genes either on day 12 or on day 14 are listed in Table 1. On day 12 for example, specific overrepresentation was obtained for genes related to cell division and vasculature development and genes coding for serine/threonine-protein kinases. Several typical genes related to angiogenesis and vascular remodeling were found such as fms-related tyrosine kinase 1 (*FLT1*, alias *VEGFR1*), kinase insert domain receptor (a type III receptor tyrosine kinase) (*KDR*, alias *VEGFR2*) placental growth factor (*PGF*) vascular endothelial growth factor C (*VEGFC*), angiopoietin 2 (*ANGPT2*), and TEK tyrosine kinase, endothelial (*TEK*). Most of the functional categories specifically overrepresented on day 14 were related to immune functions. In addition, specific up- or down-regulation of genes with functions involved in cell adhesion and cell migration was found for day 14. These genes could play a role in regulation of conceptus attachment and prevention of trophoblast invasion.

Table 1: Comparison of overrepresented Gene Ontology categories between days 12 and 14 of pregnancy for up-regulated genes

	Day 12 of pregnancy			*Day 14 of pregnancy*		
Functional category	*Genes*	*FE*	*FDR %*	*Genes*	*FE*	*FDR %*
Specific enrichment on day 12 of pregnancy						
cell cycle	107	2.1	0.0	40	0.9	100.0
centrosome	37	2.5	0.0	9	0.7	100.0
vasculature development	32	1.9	1.0	21	1.4	88.8
metallopeptidase activity	26	2.2	0.4	11	1.0	100.0
serine/threonine-protein kinase	46	2.0	0.0	18	0.9	100.0
Specific enrichment on day 14 of pregnancy						
defense response	49	1.2	91.8	115	3.1	0.0
leukocyte activation	20	1.2	99.5	63	4.4	0.0
integrin-mediated signaling pathway	10	2.2	52.6	17	4.1	0.0
regulation of apoptosis	70	1.3	23.6	91	1.9	0.0
chemotaxis	11	1.0	100.0	30	3.1	0.0

FE: fold enrichment; FDR: false discovery rate

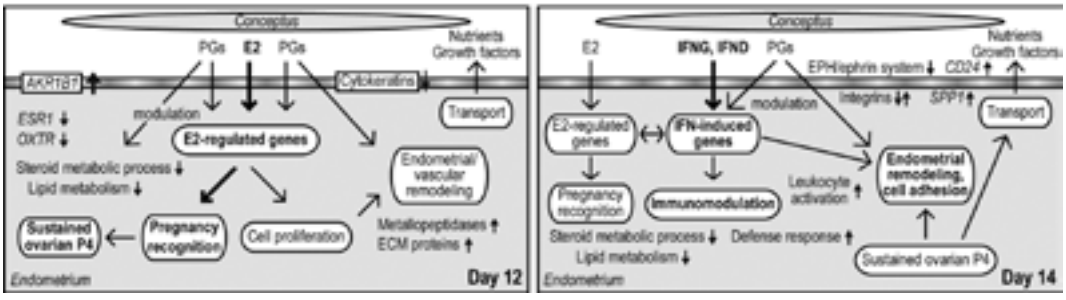

Fig. 2 Schematic overview of important processes on days 12 and 14 of pregnancy deduced from results of transcriptome studies. E2: estradiol, IFNG: interferon gamma, IFND: interferon delta, PGs: prostaglandins, P4: progesterone, *ESR1*: estrogen receptor 1 (ER alpha), *OXTR*: oxytocin receptor, *CD24*: CD24 molecule, *SPP1*: secreted phosphoprotein 1 (osteopontin).

A schematic overview of important processes on days 12 and 14 of pregnancy revealed by the transcriptome analyses is shown in Fig. 2.

Expression of genes related to prostaglandin metabolism and signaling

Prostaglandin metabolism and signaling plays a particular role in recognition of pregnancy, endometrial and conceptus development. A number of genes related to these pathways have been found as differentially expressed during the pre-implantation period. Table 2 shows selected genes found as differentially expressed in porcine endometrium on day 12 and/or on day 14 of pregnancy in comparison to non-pregnant control endometria. The highest fold up-regulation on day 12 was found for aldo-keto reductase family 1, member B1 (aldose reductase) (*AKR1B1*). First identified as a more general detoxifying enzyme, AKR1B1 has been shown to exert also more specific functions and modifies or generates signal molecules (Pastel *et al.* 2012). With regard to reproductive functions, AKR1B1 has been shown to function as a highly efficient and physiologically relevant PGF synthase that is expressed during the secretory phase in both epithelial and stromal cells in human endometrium (Bresson *et al.* 2011). The expression in porcine endometrium was 32-fold higher on day 12 of pregnancy with very high expression levels as indicated by very high read counts found in the RNA-Seq data. Interestingly, *AKR1B1* mRNA expression is also 8-fold lower in day 14 cyclic compared to day 12 cyclic endometrium and decreases 86-fold from day 12 to day 14 of pregnancy, indicating a specific short-term up-regulation of *AKR1B1* that coincides with the time of recognition of pregnancy. Localization of *AKR1B1* mRNA expression in porcine endometrium has been shown in the luminal epithelium on days 12 and 13 of pregnancy (Ross *et al.* 2007). The highest expression difference for day 14 was found for phospholipase A2, group IID (*PLA2G2D*) with 53-fold higher mRNA concentration in pregnant compared to cyclic endometrium. Expression was not detectable on day 12. This agrees well with the finding that *PLA2G2D* expression is induced by IFNG (Lindbom *et al.* 2002). Most of the genes involved in PG synthesis showed lower mRNA concentrations in day 12 pregnant endometrium, particularly hydroxyprostaglandin dehydrogenase 15-(NAD) (*HPGD*) and prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) (*PTGS2*). In addition, lower mRNA concentration in pregnant endometrium on day 14 were found for genes involved in the synthesis of unsaturated fatty acids (Samborski *et al.* 2013). One of the end products of this pathway is arachidonic acid, the precursor for PG synthesis, further suggesting a general down-regulation of the PG synthesis pathway, which could be a general mechanism to regulate PG synthesis and to prevent luteolysis.

Table 2: Selected genes related to prostaglandin and estrogen metabolism and signaling

Ssc Gene symbol	*Ssc Gene ID*	*Hsa Gene symbol*	*Hsa Gene ID*	*base mean C D12*	*base mean P D12*	*base mean C D14*	*base mean P D14*	*D12 FC P/C*	*P adj*	*D14 FC P/C*	*P adj*	*Affy FC P/C*	*P adj*
AKR1B1	396816	AKR1B1	231	8168	260115	1012	3024	31.8	< 0.001	3.0	0.002	4.9	0.007
LOC100620614	100620614	ALOX12	239	764	986	328	3019	1.3	0.750	9.2	< 0.001		
ALOX15B	100525835	ALOX15B	247	167	65	121	13	-2.6	0.351	-9.6	< 0.001		
ESR1	397435	ESR1	2099	1757	583	615	248	-3.0	< 0.001	-2.5	< 0.001	-2.1	0.004
HPGD	100156186	HPGD	3248	4676	474	1770	890	-9.9	< 0.001	-2.0	0.092	-3.3	0.004
HSD11B2	396948	HSD11B2	3291	640	412	669	363	-1.6	0.299	-1.8	0.041		
OXTR	397092	OXTR	5021	251	74	745	160	-3.4	< 0.001	-4.7	0.039		
LOC100513930	100513930	PLA2G2D	26279			11	597			53.1	< 0.001		
LOC100520687	100520687	PLA2G4A	5321	3276	1788	1466	707	-1.8	0.004	-2.1	< 0.001		
PLA2G4B	100152927	PLA2G4B	100137049	1374	631	807	753	-2.2	< 0.001	-1.1	0.828		
PLB1	100519306	PLB1	151056	168	66	112	24	-2.6	0.050	-4.7	0.003		
LOC100510947	100510947	PTGDR2	11251	345	195	810	115	-1.8	0.549	-7.0	< 0.001		
LOC100127164	100127164	PTGER2	5732	680	1790	1938	1500	2.6	0.051	-1.3	0.670		
PTGES	654407	PTGES	9536	2226	621	1118	503	-3.6	< 0.001	-2.2	0.041	-2.2	0.003
PTGFR	397126	PTGFR	5737	232	135	40	35	-1.7	0.224	-1.1	0.892		
PTGR1	397678	PTGR1	22949	87	755	43	141	8.7	< 0.001	3.3	< 0.001	2.2	0.002
PTGS1	397541	PTGS1	5742	109	308	134	539	2.8	< 0.001	4.0	< 0.001		
PTGS2	397590	PTGS2	5743	4258	2579	4745	1464	-1.7	0.246	-3.2	< 0.001		

Ssc: Sus scrofa; Hsa: Homo sapiens; base mean: normalized read count from DESeq; C: cyclic control gilts; P: pregnant gilts; FC: fold change; P adj: P value corrected for multiple testing; Affy: data from Affymetrix microarray study on day 14 of pregnancy

Effects of premature exposure of pregnant gilts to exogenous estrogen on endometrium during early pregnancy

Premature estrogen exposure disrupts the establishment of a viable pregnancy in pigs since it interferes with conceptus estrogen signaling (Morgan *et al.* 1987). In a previous microarray study, the effects of premature estrogen exposure to pregnant gilts on endometrial gene expression during the peri-implantation phase have been investigated (Ross *et al.* 2007). A number of genes were identified to have aberrant expression on days 10, 13 and 15 of pregnancy in comparison of the treatment with estradiol and corn oil as a control on days 9 and 10 of pregnancy. The comparison to the results of our RNA-Seq studies on days 12 and 14 of pregnancy showed, that many of these genes were differentially expressed between pregnant and cyclic endometrium. Genes with at least twofold change between pregnant and cyclic endometrium are listed in Table 3. The gene retinol binding protein 4, plasma (*RBP4*) was found as up-regulated on day 10 of pregnancy after estrogen treatment on day 9 (8-fold) and on day 12 of pregnancy in comparison to day 12 of the estrous cycle (7-fold). Genetic studies have shown an association of *RBP4* gene variants with litter size (Munoz *et al.* 2010). The expression of 4 genes (*AKR1B1*, *CD24*, *NMB* and *SPP1*) was localized to the luminal epithelium suggesting a role in maternal-conceptus interaction (Ross *et al.* 2007). CD24 is normally expressed on mature granulocytes and in many B cells but was also found in decidual stromal cells (Montes *et al.* 1996). Expression in porcine endometrium on days 12 and 14 of the estrous cycle was very low but increased strikingly on days 12 and 14 of pregnancy (108-fold and 668-fold, respectively, in comparison to corresponding days of the cycle). *AKR1B1* expression on day 13 was 14-fold lower after premature estrogen treatment. Together with the specific up-regulation found on day 12 of pregnancy this suggests an important role of *AKR1B1* for recognition and establishment of pregnancy in the pig. Likewise, for secreted phosphoprotein 1 (osteopontin) (*SPP1*) a wrong timing of regulation due to estrogen exposure on days 9 and 10 of pregnancy is obvious, with 3.4-fold higher expression on day 13 and 1.9-fold lower expression on day 15 of pregnancy after estrogen treatment, whereas similar expression on days 12 and 14 of the estrous cycle and 5.5-fold higher expression on day 12 and 13-fold higher expression on day 14 of pregnancy was found. Altogether, this comparison revealed a number of genes, which could have a particular role in maternal recognition of pregnancy.

In conclusion, transcriptome studies during the pre- and peri-implantation period revealed a number of new molecular pathways and biological processes associated with and important for recognition of pregnancy and preparation for embryo implantation in the pig. Although these studies have their limitations since endometrial tissue samples have a complex and dynamic composition regarding different endometrial cell types and changes in mRNA concentrations do not always correlate with changes in abundance of the corresponding protein, they can deliver new hypotheses and find new molecular pathways which never have been considered before to be involved in these biological processes.

Declaration of interest and funding

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Table 3: Genes with altered expression after premature estrogen exposure to pregnant gilts and differential expression on day 12 and/or on day 14 of pregnancy compared to cyclic controls

Hsa Entrez Gene ID	*Hsa Gene symbol*	*EC/CO FC*	*P value*	*base mean C*	*base mean P*	*D12 FC P/C*	*P adj*	*base mean C*	*base mean P*	*D14 FC P/C*	*P adj*
D10 after exogenous EC on d9 of gestation											
5950	RBP4	8.3	0.028	23640	173649	7.3	<0.001	111862	199800	1.8	0.003
D13 after exogenous EC on d9 and d10 of gestation											
57406	ABHD6	1.9	0.083	1216	4067	3.3	0.001	779	2058	2.6	<0.001
199	AIF1	-2.4	0.100	213	282	1.3	0.352	102	939	9.2	<0.001
231	AKR1B1	-13.9	0.001	8168	260115	31.8	<0.001	1012	3024	3.0	0.002
307	ANXA4	-2.1	0.015	43908	51138	1.2	0.503	10151	5003	-2.0	<0.001
245973	ATP6V1C2	-1.9	0.056	24315	10681	-2.3	0.002	4798	3371	-1.4	0.674
79161	C7orf23	-3.5	0.015	5016	15860	3.2	<0.001	2013	1461	-1.4	0.156
100133941	CD24	4.6	0.001	97	10479	107.6	<0.001	62	41726	667.7	<0.001
29126	CD274	-4.1	0.013	16	87	5.5	<0.001	9	189	20.3	<0.001
1075	CTSC	2.0	0.056	1835	11766	6.4	<0.001	2946	10822	3.7	<0.001
2224	FDPS	-2.7	0.013	109960	55144	-2.0	<0.001	38788	6761	-5.7	<0.001
2938	GSTA1	2.0	0.019	1195	518	-2.3	0.327	3855	1129	-3.4	0.003
51451	LCMT1	2.3	0.062	172	45	-3.9	0.009	38	27	-1.4	0.553
9448	MAP4K4	-2.5	0.003	8015	28078	3.5	<0.001	4468	5831	1.3	0.186
4233	MET	-2.3	0.006	1329	5970	4.5	<0.001	1367	960	-1.4	0.378
10874	NMU	-2.7	0.004	52080	28756	-1.8	0.097	39169	1861	-21.0	0.000
26227	PHGDH	-1.8	0.016	5844	3222	-1.8	0.066	7154	1702	-4.2	0.000
51316	PLAC8	3.3	0.015	490	460	-1.1	0.905	264	4281	16.2	0.000
5357	PLS1	-4.4	0.004	18270	37315	2.0	<0.001	6243	2410	-2.6	0.000
29968	PSAT1	2.3	0.007	17963	5236	-3.4	0.001	5501	6899	1.3	0.533
25914	RTTN	1.8	0.072	196	94	-2.1	0.014	94	150	1.6	0.111
23623	RUSC1	-1.8	0.089	11288	4453	-2.5	<0.001	4939	2022	-2.4	0.004
6288	SAA1	2.9	0.005	70	663	9.5	<0.001	40	540	13.5	<0.001
6446	SGK1	2.0	0.023	6811	22148	3.3	0.006	5892	10866	1.8	<0.001
6696	SPP1	3.4	0.008	701	3855	5.5	0.016	721	9261	12.8	0.001
6822	SULT2A1	2.9	0.083	2099	9665	4.6	0.004	2379	14420	6.1	<0.001
8875	VNN2	-2.0	0.018	1114	4860	4.4	0.003	7811	2110	-3.7	0.216
79971	WLS	-1.8	0.065	23483	15407	-1.5	0.036	10810	5428	-2.0	<0.001
D15 after exogenous EC on d9 and d10 of gestation											
203054	ADCK5	-1.9	0.073	15881	8348	-1.9	<0.001	9395	2810	-3.3	<0.001
567	B2M	-1.9	0.064	50308	55252	1.1	0.710	47584	165175	3.5	<0.001
6288	SAA1	-2.1	0.056	70	663	9.5	<0.001	40	540	13.5	<0.001
6696	SPP1	-1.9	0.062	701	3855	5.5	0.016	721	9261	12.8	0.001
6772	STAT1	-2.1	0.064	2445	7537	3.1	<0.001	714	5019	7.0	<0.001
57169	ZNFX1	-1.8	0.064	5621	5108	-1.1	0.691	3504	15424	4.4	<0.001

Hsa: Homo sapiens; EC: estradiol cypionate; CO: corn oil; FC: fold change; base mean: normalized read count from DESeq; C: cyclic control gilts; P: pregnant gilts; P adj: P value corrected for multiple testing

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