—Mini Review—

Changes in Gene Expression Associated with Conceptus Implantation to the Maternal Endometrium

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Abstract: Processes of conceptus implantation and placentation vary among mammalian species. However, differences in physiological and biochemical processes were once thought not to differ so much, particularly as to the kinds of genes expressed. In fact, recent progress has identified that in addition to the hormones, cytokines, proteases and cell adhesion molecules classically characterized, epithelial-mesenchymal transition (EMT), epigenetic regulation and the expression of endogenous retroviruses (ERV) are all required for the progression of conceptus implantation to placentation. Thus, continued research into EMT, epigenetic regulation and the expression of ERVs will aid in enhancing understanding of their impact on reproductive physiology in humans and domestic animals.

Key words: Implantation, Mammals, Gene expression, EMT, ERV

Introduction

The uterine structures in mammalian species as we know them today are the product of a long and complex evolutionary process. In a novel innovation, for the first time, not only fertilization but embryonic growth could be done inside the body [1]. The uterus could then provide an adequate environment for conceptus growth; however, this arrangement presented new challenges, most immediately immunogenic ones because the conceptus carries paternal genes allogenic to the mother. Although the exact sequences of events remains unclear, the means of protecting the conceptus took the form of the trophectodermal layer, while the ordeal of supporting and nourishing the conceptus was enabled by a tertiary structure called the placenta. However, extensive variation in trophectoderm (TE) and placental structures exists across different mammalian species. Trophectodermal cells also play a major role during the process of conceptus implantation to the maternal uterine endometrium. In this review, new information on TE and its gene regulation will be integrated.

Trophoblast Lineage Specification

In the mammalian preimplantation embryo, blastocyst formation marks the segregation of the first two cell lineages: the inner cell mass (ICM) that will form the embryo proper and the TE that gives rise to trophoblast lineages and all the specialized layers of the placenta. Commitment to ICM and TE is attributed to the reciprocal expression of OCT3/4 (encoded by *Pou5f1*) and the caudal-type homeodomain protein CDX2 [2]. In mice, deletion of either *Oct3/4* or *Cdx2* leads to the formation of abnormal blastocysts: ICM cells in *Oct3/4*-mutant blastocysts express trophectodermal markers and lose pluripotency [3], while *Cdx2* mutants undergo blastocyst formation but fail to maintain epithelial integrity, resulting in implantation failure [4]. Loss of *Cdx2* results in failure

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to down-regulate *Oct3/4* and another ICM transcription factor *Nanog* in outer cells of the blastocyst and subsequent death of these cells. Thus, *Cdx2* is essential for segregation of the TE from ICM lineages at the blastocyst stage by ensuring the repression of *Oct3/4* and *Nanog* in the TE [4, 5]. It was found in embryonic stem cells that GATA3 is capable of overriding pluripotency and directing the expression of a multitude of CDX2-independent trophoblast genes, whereas in trophoblast stem (TS) cells GATA3 promotes differentiation [6]. In addition to *Cdx2*, *Gata3* is now considered to be integral to trophoblast lineage specification.

Recently, Berg and coworkers [7] executed careful experimentations, examining both mouse and bovine *Cdx2* expression and their transcriptional regulation. They found that a mouse *Oct3/4* reporter repressed in mouse TE remained active in the cattle TE; additionally, bovine *Oct3/4* constructs were not repressed in the mouse TE. This was due to the presence of TCFAP2 binding sites in the mouse *Oct3/4* gene, but similar sites were not found in cattle, humans or rabbits, resulting in the maintenance of high OCT3/4 levels in the TE [7]. These data suggest that the reciprocal expression of *Oct3/4* and *Cdx2* established early on in mouse TE allows the rapid TE differentiation required for fast blastocyst implantation to the uterine endometrium.

Processes of Implantation

It is generally accepted that there are five phases of blastocyst implantation [8]: 1) Migration and Shedding of zona pellucida (ZP, hatching), 2) Pre-contact, blastocyst orientation and apposition, 3) Attachment, 4) Adhesion, and 5) Endometrial invasion. These processes are followed by placental formation. During Phase 1, the blastocyst enters and migrates within the uterus and shedding allows the expansion of the spherical blastocyst, or it may migrate and experience changes in its shape from spherical to tubular and filamentous form as in domestic animals. Phase 2 is a pre-contact period during which the blastocyst migrates or elongates without definitive contact between the TE and endometrial epithelium. In domestic animals, this is the period when the process of maternal recognition of pregnancy is initiated for the prevention of corpus luteum (CL) demise, resulting from biochemical communication between the developing conceptus and mother. Phase 3 is the attachment period, during which the TE establishes definitive contact with the uterine epithelium. Phase 4 is the time of firm adhesion between the TE and uterine epithelium and in some cases, superficial glandular epithelium, during which mononucleate TE cells differentiate into trophoblast binucleate cells. Phase 5 is when many mammalian species begin to diverge greatly in their development as invasive TE causes the formation of decidualized endometrium, whereas noninvasive does not. For the first four phases, however, implantation processes appear fairly similar among mammalian species [8].

Maternal Recognition of Pregnancy

In mammalian species, the maintenance of CL function and the continued secretion of a steroid hormone, progesterone (P4), are required for the establishment and maintenance of pregnancy. P4 is involved directly and/or indirectly in numerous uterine functions through endometrial secretions, alteration of blood flow at implantation sites and promotion of suitable physiological and/or immune environments for normal embryonic development. Despite critical importance, the biochemical mechanisms by which CL is maintained for continued P4 production differ from species to species. In humans, luteolysis is prevented by a luteotrophic factor, chorionic gonadotropin (CG), produced by the TE as it begins implantation to the uterine epithelium [9]. In rodents, CL is prolonged through the release of copulation-induced pituitary prolactin surges [10]. In ruminant species of cows, sheep and goats [11], interferon tau (IFNT), a major cytokine produced by the peri-implantation TE, is the anti-luteolytic factor essential for the prolongation of CL life span [12–16] (Fig. 1).

In a human pregnancy, hCG supports the CL to continuously produce P4, which regulates endometrial gene expression required for embryo implantation in the uterus. However, it has been shown that hCG may not be the only factor to maintain P4 production because the administration of hCG does not prevent CL regression in non-pregnant women [17]. But, to date a factor other than hCG has not been identified for CL maintenance in humans.

IFNT exhibits structural and functional similarities to those of type I IFNs such as IFNA and IFNB [18]. These include antiviral and anti-proliferative activities, but IFNT shows much less cytotoxic activity than do IFNA or IFNB [19–22]. It was found that type I IFNs bind to a common receptor complex with two polypeptide subunits (IFNAR1 and IFNAR2) [23], both of which are present in ovine uterine epithelial cells [24]. The surface epithelium of the uterine endometrium is the primary target for IFNT [25], but accumulated evidence suggests that IFNT can reach the stroma, and even the uterine myometrium [26–28]. It was characterized that upon binding to the receptor,

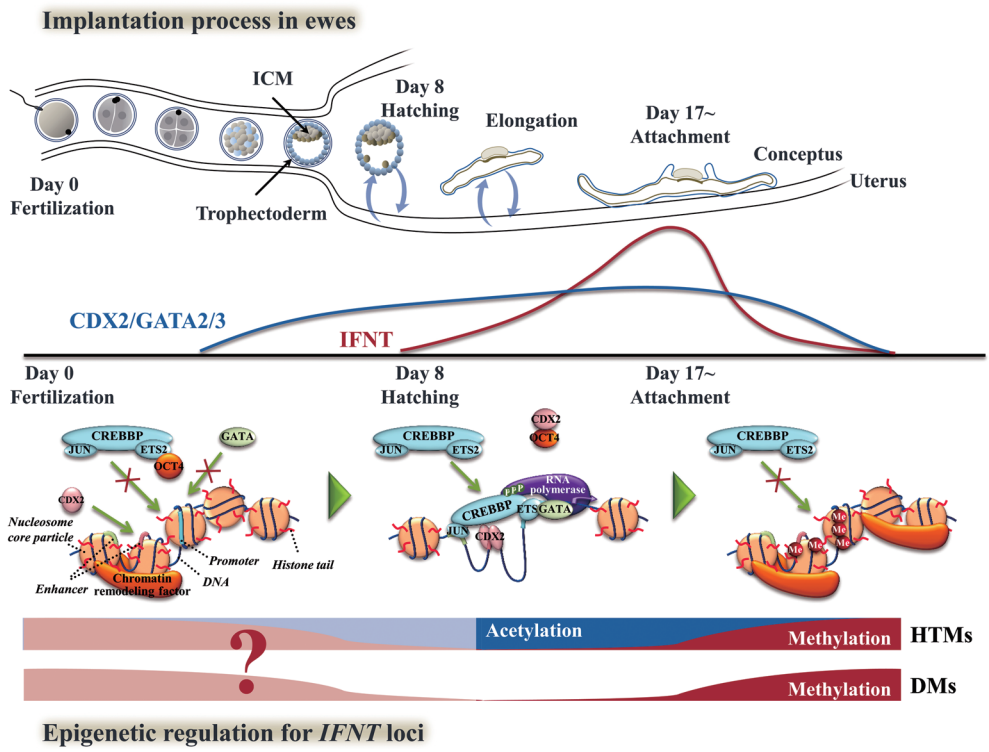

Fig. 1. Processes and gene expression associated with conceptus implantation to the uterine endometrium. Upper: Processes and gene expression during implantation period in ewes (female sheep). After entering the uterus, the conceptus goes through hatching, migration and elongation prior to the initiation of attachment to the uterine epithelium. Expression patterns of transcription factors determining trophectodermal lineages CDX2 and GATA2/3 and trophoblast cytokine IFNT are also shown. Lower: Epigenetic regulation during implantation period. Chromatin structures at the *IFNT* locus are shown. During the implantation period, histone proteins at the upstream region of *IFNT* gene are characterized by high acetylation and low methylation, allowing other transcription factor binding and higher transcriptional activity.

type I IFNs activate the janus kinase-signal transducer and activator of transcription-interferon regulatory factor (JAK-STAT-IRF) signaling pathway [29, 30], causing the activation of a group of interferon-stimulated genes (ISGs) [31, 32]. In addition to ISGs, wingless-type MMTV integration site family (WNTs) and LGALS gene expression [33, 34], IFNT induces several chemokines in endometrial tissues including chemokine ligand 10 (CXCL10) and CXCL9 [35, 36]. Endometrial CXCL10 in turn attracts immune cells, particularly NK cells, to the implantation site of the endometrium [37], and by acting through the CXCL10 receptor, CXCR3, this chemokine regulates TE cell migration and integrin expressions [38]. These changes result in conceptus migration, apposition and initial attachment to the uterine epithelial cells in ruminants [37, 38].

Transcriptional Regulation of *IFNT*

Expression of IFNT is not induced by viruses or double stranded RNA [39], but produced by the early trophoblast [12, 40]. Minute expression of IFNT can be detectable from the first day following hatching [39] (Fig. 1). The production of IFNT increases remarkably on day 13, when the blastocyst starts to elongate [41] and reaches the maximum level on day 16 of pregnancy, 100 μg per cultured conceptus during 24 h, while the blastocyst initiates its attachment to the uterine epithelial cells [12, 40]. Following this event, however, IFNT expression decreases rapidly as the process of implantation proceeds and at day 22, IFNT is no longer detected [12].

Intensive experimentations have been conducted to elucidate molecular mechanisms by which *IFNT* transcription is regulated. Although IFNT production could

be initiated after *in vitro* fertilization and maturation [42, 43], substantial production of IFNT seen *in utero* could not be achieved without interaction with the uterine environment [42]. It has been demonstrated that endometrial cytokines, GM-CSF, IL3 and FGF2, of which expression increases in the pregnant endometrium [44, 45], enhance IFNT expression in conceptus tissues and bovine trophoblast CT-1 cells [44–47].

Numerous transcription factors thus far found as potential regulators of the *IFNT* gene are ETS2 [48, 49], activating protein 1 (AP-1, official symbol JUN) [50], CDX2 [51, 52], homeobox protein distal-less 3 (DLX3) [53], and co-activators cAMP-response element binding protein (CREB)-binding protein (CREBBP) and p300 [54, 55] (Fig. 1). While identifying Gata3 as another factor for trophoblast lineage specification, we additionally found that GATA2/3 could enhance *IFNT* gene transcription [56].

Epigenetic Regulation of IFNT

Epigenetic alterations such as variation in covalent histone modification and DNA methylation regulate gene expression by altering chromatin conformation. While it is known that IFNT production is normally limited to TE or trophoblast BT-1 and CT-1 cells [16, 57, 58], Sakurai and coworkers investigated whether or not *IFNT* gene transcription could be induced in a cell type not related to trophoblast cells [52]. These investigators demonstrated that significant increases in endogenous *IFNT* transcription in non-IFNT producing, bovine kidney epithelial MDBK cells could be induced through CDX2 over-expression and high H3K18 acetylation. They also noted that lowering H3K9 methylation appears to be another condition required for the degree of *IFNT* transcription seen in trophoblast cells. In addition, co-activator CREBBP and p300 with their intrinsic histone acetyl-transferase (HAT) activity are recruited to enhance *IFNT* transcription [59]. However, the observation that the use of HAT inhibitor reduced histone acetylation at the *IFNT* gene even after *CDX* transfection indicates that CDX2-facilitated histone acetylation could be a triggering event necessary for gene expression unique to TE (Fig. 1). Furthermore, CREBBP/p300 recruitment is known to be associated with greater acetylation of the gene [59]. These results suggest that induction of endogenous *IFNT* transcription in bovine trophoblast cells results from partial decondensation of chromosomal domains by histone acetylation and sufficient CDX2 expression, allowing other transcription factor bindings to the upstream region of *IFNT* genes for higher transcription of the gene.

Ovine genomic DNAs extracted from uterine endometrium (no IFNT production), white blood cells (WBC, no IFNT production), day 14 trophoblast (high IFNT production) and day 20 trophoblast (low IFNT production) were examined for methylation status of the *IFNT*'s upstream region containing 14 CpG sites [60]. Genomic DNA from uterine endometrium and WBC displayed higher methylation than day 14 and 20 trophoblasts. Day 14 trophoblasts, which had highest *IFNT* transcription, were less methylated than day 20 trophoblasts, which possessed minute amounts of *IFNT* mRNAs, and day 17 trophoblasts contain half as much *IFNT* mRNA as in day 14 trophoblasts. When cultured *in vitro* with demethylation reagent 5-aza-dC, amounts of *IFNT* mRNA in day 17 trophoblasts became similar to those of day 14 *IFNT* mRNAs. These findings suggest that changes in the degree of DNA methylation in the upstream sequences of the *IFNT* gene could be one of the major mechanisms leading to down-regulation of its expression and possibly its silencing in non-trophoblast tissues [60].

Epithelial and Mesenchymal Transition

The TE forms epithelial structure of the blastocyst and possesses epithelial characteristics, including apicobasal cell polarity, lateral junctions with neighboring cells and basal contact with the basement membrane proteins [61–63]. Despite the fact that the apical plasma membranes of simple epithelia normally lack adhesive properties, the TE still manages to adhere to the uterine epithelium through its apical domains as part of the preimplantation process. Thus, the adhesion between TE and uterine epithelium has long been considered a cell biological paradox [64]. With the exception of rodents, in which the conceptus enters a receptive uterus and attaches immediately to the uterine epithelium, primates and most domestic animals have a prereceptive phase during which the conceptus does not physically interact with the uterine epithelium. In the bovine species, attachment between trophectodermal epithelium and endometrial epithelium is first seen on day 20 of gestation, and subsequent stable adhesion occurs between days 20 and 22 [65].

Another surprising finding was that changes in gene expression associated with epithelial-mesenchymal transition (EMT) occurred not before attachment, but rather on day 22, two to three days after the initiation of conceptus attachment to the uterine epithelium [66]. Positive signals for both the epithelial marker cytokeratin and the mesenchymal marker vimentin were seen in the elongated TE on day 22. Increased transcripts of N-cadherin, vimentin, matrix metalloproteinase 2 (*MMP2*), and *MMP9*

were also found on day 22, concurrent with E-cadherin mRNA and protein down-regulation. These observations indicate that after the conceptus-endometrium attachment, EMT-related transcripts as well as cytokeratin are present in the bovine TE, and suggest that in addition to extracellular matrix expression, partial EMT is required for proper adhesion of trophoblasts in noninvasive implantation.

In this study, we also identified that transcription factor *SNAI2*, *ZEB1*, *ZEB2*, *TWIST1*, *TWIST2*, and *KLF8* transcripts were up-regulated concurrent with cytokeratin expression in the TE [66]. It has been characterized that SNAIL, ZEB, and KLF8 factors bind to and repress E-cadherin promoter activity [67, 68], whereas TWIST1 and TWIST2 repress E-cadherin transcription indirectly [69]. In addition, SNAIL and ZEB factors are known to induce the expression of MMPs that can degrade basement membrane, thereby favoring invasion [70]. Although the bovine trophoblasts do not penetrate into the endometrium, the confirmation that *MMP2* and *MMP9* transcripts are up-regulated not only suggests that they play a role in noninvasive trophoblasts, but also confirms further the similarity between invasive and non-invasive modes of implantation.

Endogenous Retroviruses and Pregnancy

Endogenous retroviruses (ERVs) are now appreciated as factors implicated in development and differentiation of TEs in humans, rodents, sheep and possibly rabbits [71–75]. During the course of evolution, all vertebrates have been exposed to multiple waves of cross-species infection by exogenous retroviruses, some of which infected germ cells and are inherited in an integrated, proviral form [76]. They were once considered junk DNAs, however, it is now realized that ERVs play biological roles in protection against retroviral infection [77] and in placental development [78, 79]. Recently, it was found that high levels of transcripts found in ES cells, most of which are expressed in two-cell stage embryos, are induced by long terminal repeats of ERVs, suggesting the possibility that the foreign sequences have helped to drive cell-fate regulation in placental mammals [80].

Trophectoderm cells are very invasive in nature, and as uncontrolled invasiveness could destroy the uterine structures, this aggression must be regulated for the protection of uterine endometrium. When the cell cycles of TE cells are restricted, these cells go through endoreduplication, resulting in the formation of giant trophoblast cells. Although human syncytiotrophoblast cells result from cell fusion, these cells do not go through cell cycles, and thereby their invasiveness is held under control [81]. There is no doubt that tissue inhibitors for MMPs (TIMPs) play a role in controlling the activity of MMPs *in utero* [82, 83]. However, inhibition of cell cycles through cell fusion and/or endoreduplication may also contribute to the regulation of TE invasiveness.

Syncytin-1 and -2 are products of the two human ERV envelop (*env*) genes, and are involved in the fusion of trophoblast cells, resulting in multinucleated syncytiotrophoblast formation [71, 72]. It was determined that Syncytin-2 entered the primate lineage more than 40 million years ago (MYA) while Syncytin-1 entered the lineage 25–40 MYA [72]. In rodents, there are Syncytin A and Syncytin B, both of which are homologous to those of human Syncytin-1 and -2 [73]. Recent study has shown that syncytin-like putative fusogenic proteins are also expressed in the placenta of rabbits [75]. In humans, cytotrophoblast cell fusion starts on day 7–11 pregnancy, the time corresponding to the implantation period [76].

In sheep, Jaagsiekte sheep retrovirus (JSRV) is a pathogenic exogenous retrovirus and is known as the causative agent of ovine pulmonary adenocarcinoma [74, 84]. The sheep genome contains a minimum of 27 copies of endogenous JSRV (*enJSRV*), some transcripts of which are found to be abundant in reproductive tracts, particularly in the uterine luminal and glandular epithelium, and epithelial regions of oviducts and cervix [85]. In the conceptus, expression of enJSRV *env* begins on day 12 of pregnancy, coincident with the onset of conceptus elongation, the increase in IFNT production and the period of maternal recognition of pregnancy [84]. Transcripts for *enJSRV* are detectable in mononucleate TE, but more abundant in trophoblast binucleate cells located at the fetal side of placentomes, and multinucleated syncytia located in the uterine endometrium [85, 86]. In addition, a cell surface receptor for the exogenous enJSRV and enJSRV envelope protein is hyaluronidase 2 (HYAL2) [87], which is expressed by binucleate trophoblast cells and syncytial plaques in the ovine placenta, but not in uterine epithelia, stroma or myometrium [88].

While it has not been determined whether binucleate cells result from cell fusion or endoreduplication, it is clear that trinucleate cells or syncytia are products of fusion between binucleate cells and uterine epithelial cells [88–90]. Unlike primates and rodents, TE cells of ruminants are not invasive, and thus do not penetrate deep into uterine stroma or spiral arteries; however, the facts that binucleate cells from bovine placenta possess *BERV-K1* [89] with fusogenic activity (Nakaya *et al.*, 2013, Manuscript in preparation), and that trinucleate cells and syncytia are located in the endometrium [90,

91] suggest that they may strengthen the adhesion between conceptus and uterine endometrium at the placentomes. Perhaps more importantly, these cells represent the foremost trophoblast population, which faces maternal immune cells, for the protection of allogenic embryo during the course of pregnancy.

Conclusion

The placenta is considered to be a fairly recent invention in mammals, of which the conceptus side consists of TE cells. These cells play an important role in preventing rejection from the beginning of implantation process, hatching, when paternal gene products are directly exposed to the maternal system. Until recently, processes of conceptus implantation to the maternal endometrium have been studied from the standpoint of attachment and invasion through extracellular matrices, cell adhesion molecules, cytokines, and/or proteinases and their inhibitor expression. Recent progress suggests that although implantation is still a complex phenomenon, it can be analyzed as whole as well as in specific events. In particular, the implantation study must include ERV genes and their specific expression in genital tracts. However, ERV research in reproduction is fairly new and with various ERV genes yet to be found, our current understanding of implantation and placental formation may be far from finalized. We must then treat these processes, therefore, as a work still in progress, and prepare for much work ahead in the elucidation of implantation and placentation innovated in mammalian reproduction.

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References

1) Amoroso, E.C. (1968): The evolution of viviparity. Proc. R. Soc. Med., 61, 1188–1200.
2) Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R. and Rossant, J. (2005): Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell, 123, 917–929.
3) Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H. and Smith, A. (1998): Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell, 95, 379–391.
4) Strumpf, D., Mao, C.A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F. and Rossant, J. (2005): Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development, 132, 2093–2102.
5) Niwa, H., Miyazaki, J. and Smith, A.G. (2000): Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet., 24, 372–376.
6) Ralston, A., Cox, B.J., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., Guo, G., Robson, P., Draper, J.S. and Rossant, J. (2010): Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. Development, 137, 395–403.
7) Berg, D.K., Smith, C.S., Pearton, D.J., Wells, D.N., Broadhurst, R., Donnison, M. and Pfeffer, P.L. (2011): Tropectoderm lineage determination in cattle. Dev. Cell, 20, 244–255.
8) Bazer, F.W., Spencer, T.E., Johnson, G.A., Burghardt, R.C. and Wu, G. (2009): Comparative aspects of implantation. Reproduction, 138, 195–209.
9) Hearn, J.P., Webley, G.E. and Gidley-Baird, A.A. (1991): Chorionic gonadotrophin and embryo-maternal recognition during the peri-implantation period in primates. J. Reprod. Fertil., 92, 497–509.
10) Soares, M.J., Faria, T.N., Roby, K.F. and Deb, S. (1991): Pregnancy and the prolactin family of hormones: coordination of anterior pituitary, uterine, and placental expression. Endocr. Rev., 12, 402–423.
11) Short, R.V. (1969): Implantation and the maternal recognition of pregnancy. In "Foetal Anatomy", Ciba Foundation Symposium (Wolstenhome, G.E.W. and O'Connor, M., eds.), pp. 2–26, J&A Churchill, London.
12) Godkin, J.D., Bazer, F.W., Moffatt, J., Sessions, F. and Roberts, R.M. (1982): Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13–21. J. Reprod. Fertil., 65, 141–150.
13) Imakawa, K., Anthony, R.V., Kazami, M., Marotti, K.R., Polites, H.G. and Roberts, R.M. (1987): Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. Nature, 330, 377–379.
14) Stewart, H.J., McCann, S.H.E., Barker, P.J., Lee, K.E., Lamming, G.E. and Flint, A.P.F. (1987): Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. J. Endocrinol., 115, R13–R15.
15) Charpigny, G., Reinard, P., Huet, J.C., Guillomot, M., Charlier, M., Pernollet, J.C. and Martal, J. (1988): High homology between a trophoblastic protein (trophoblastin) isolated from ovine embryo and α-interferon. FEBS Lett., 228, 12–16.
16) Roberts, R.M., Cross, J.C. and Leaman, D.W. (1992): Interferons as hormones of pregnancy. Endocr. Rev., 13, 432–452.
17) Quagliarello, J., Goldsmith, L., Steinetz, B., Lustig, D.S. and Weiss, G. (1980): Induction of relaxin secretion in nonpregnant woman by human chorionic gonadotropin. J. Clin.

Endocrinol. Metab., 51, 74–77.
18) Imakawa, K., Hansen, T.R., Malathy, P.V., Anthony, R.V., Polites, H.G., Marotti, K.R. and Roberts, R.M. (1989): Molecular cloning and characterization of complementary deoxyribonucleic acids corresponding to bovine trophoblast-1: a comparison with ovine trophoblast protein-1 and bovine interferon-alpha II. Mol. Endocrinol., 3, 127–139.
19) Pestka, S., Langer, J.A., Zoon, K.C. and Samuel, C.E. (1987): Interferons and their actions. Annu. Rev. Biochem., 56, 727–777.
20) Roberts, R.M., Imakawa, K., Niwano, Y., Kazemi, M., Malathy, P.V., Hansen, T.R., Glass, A.A. and Kronenberg, L.H. (1989): Interferon production by the preimplantation sheep embryo. J. Interferon Res., 9, 175–187.
21) Pontzer, C.H., Bazer, F.W. and Johnson, H.M. (1991): Antiproliferative activity of a pregnancy recognition hormone, ovine trohoblast protein-1. Cancer Res., 51, 5304–5307.
22) Pontzer, C.H., Yamamoto, J.K., Bazer, F.W., Ott, T.L. and Johnson, H.M. (1997): Potent anti-feline immunodeficiency virus and anti-human immunodeficiency virus effect of IFN-τ. J. Immunol., 158, 4351–4357.
23) Pestka, S., Krause, C.D. and Walter, M.R. (2004): Interferons, interferon-like cytokines, and their receptors. Immunol. Rev., 202, 8–32.
24) Rosenfeld, C.S., Han, C.S., Alexenko, A.P., Spencer, T.E. and Roberts, R.M. (2002): Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. Biol. Reprod., 67, 847–853.
25) Imakawa, K., Tamura, K., Lee, R.S., Ji, Y., Kogo, H., Sakai, S. and Christenson, R.K. (2002): Temporal expression of type I interferon receptor in the peri-implantation ovine extra-embryonic membranes: demonstration that human IFN-alpha can bind to this receptor. Endocr. J., 49, 195–205.
26) Ott, T.L., Yin, J., Wiley, A.A., Kim, H.T., Gerami-Naini, B., Spencer, T.E., Bartol, F.F., Burghardt, R.C. and Bazer, F.W. (1998): Effects of the estrous cycle and early pregnancy on uterine expression of Mx protein in sheep (*Ovis aries*). Biol. Reprod., 59, 784–794.
27) Johnson, G.A., Spencer, T.E., Hansen, T.R., Austin, K.J., Burghardt, R.C. and Bazer, F.W. (1999): Expression of the interferon tau inducible ubiquitin cross-reactive protein in the ovine uterus. Biol. Reprod., 61, 312–318.
28) Hicks, B.A., Etter, S.J., Carnahan, K.G., Joyce, M.M., Assiri, A.A., Carling, S.J., Kodali, K., Johnson, G.A., Hansen, T.R., Mirando, M.A., Woods, G.L., Vanderwall, D.K. and Ott, T.L. (2003): Expression of the uterine Mx protein in cyclic and pregnant cows, gilts, and mares. J. Anim. Sci., 81, 1552–1561.
29) Stark, G.R., Kerr, I.M., Williams, B.R., Silverman, R.H. and Schreiber, R.D. (1998): How cells respond to interferons. Annu. Rev. Biochem., 67, 227–264.
30) Kim, S., Choi, Y., Bazer, F.W. and Spencer, T.E. (2003): Identification of genes in the ovine endometrium regulated by interferon tau independent of signal transducer and activator of transcription 1. Endocrinology, 144, 5203–5214.
31) Chen, Y., Antoniou, E., Liu, Z., Hearne, L.B. and Roberts, R.M. (2007): A microarray analysis for genes regulated by interferon-τ in ovine luminal epithelial cells. Reproduction, 134, 123–135.
32) Spencer, T.E., Sandra, O. and Wolf, E. (2008): Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction, 135, 165–179.
33) Gray, C.A., Adelson, D.L., Bazer, F.W., Burghardt, R.C., Meeusen, E.N. and Spencer, T.E. (2004): Discovery and characterization of an epithelial-specific galectin in the endometrium that forms crystals in the trophectoderm. Proc. Natl. Acad. Sci. USA, 101, 7982–7987.
34) Mohamed, O.A., Jonaert, M., Labelle-Dumais, C., Kuroda, K., Clarke, H.J. and Dufort, D. (2005): Uterine Wnt/*β*-catenin signaling is required for implantation. Proc. Natl. Acad. Sci. USA, 102, 8579–8584.
35) Nagaoka, K., Sakai, A., Nojima, H., Suda, Y., Yokomizo, Y., Imakawa, K., Sakai, S. and Christenson, R.K. (2003): Expression of a chemokine, IFN-gamma-inducible protein 10 kDa, is stimulated by IFN-τ in the ovine endometrium. Biol. Reprod., 68, 1413–1421.
36) Imakawa, K., Imai, M., Sakai, A., Suzuki, M., Nagaoka, K., Sakai, S., Lee, S.R., Chang, K.T., Echterrnkamp, S.E. and Christenson, R.K. (2006): Regulation of conceptus adhesion by endometrial CXC chemokines during the implantation period in sheep. Mol. Reprod. Dev., 73, 850–858.
37) Nagaoka, K., Nojima, H., Watanabe, F., Christenson, R.K., Sakai, S. and Imakawa, K. (2003): Regulation of blastocyst migration, apposition and initial adhesion by a chemokine, IFN-γ-inducible protein 10 kDa (IP-10), during early gestation. J. Biol. Chem., 278, 29048–29056.
38) Imakawa, K., Nagaoka, K., Nojima, H., Hara, Y. and Christenson, R.K. (2005): Changes in immune cell distribution and IL-10 production are regulated through endometrial IP-10 expression in the goat uterus. Am. J. Reprod. Immunol., 53, 54–64.
39) Farin, C.E., Imakawa, K. and Roberts, R.M. (1989): In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Mol. Endocrinol., 3, 1099–1107.
40) Imakawa, K., Tamura, K., McGuire, W.J., Khan, S., Harbison, L.A., Stanga, J.P., Helmer, S.D. and Christenson, R.K. (1995): Effect of interleukin-3 on ovine trophoblast interferon during early conceptus development. Endocrine, 3, 511–517.
41) Ashworth, C.J. and Bazer, F.W. (1989): Changes in ovine conceptus and endometrial function following asynchronous embryo transfer or administration of progesterone. Biol. Reprod., 40, 425–433.
42) Hernandez-Ledezma, J.J., Sikes, J.D., Murphy, C.N., Watson, A.J., Schultz, G.A. and Roberts, R.M. (1992): Expression of bovine trophoblast interferon in conceptuses derived by in vitro technique. Biol. Reprod., 47, 374–380.
43) Stojkovic, M., Buttner, M., Zakhartchenko, V., Riedl, J., Reichenbach, H.D., Wenigerkind, H., Brem, G. and Wolf, E. (1999): Secretion of interferon-tau by bovine embryos in long-term culture: comparison of in vivo derived, in vitro produced, nuclear transfer and demi-embryos. Anim. Reprod. Sci., 55, 151–162.
44) Imakawa, K., Helmer, S.D., Nephew, K.P., Meka, C.S.R.

and Christenson, R.K. (1993): A novel role for GM-CSF: Enhancement of pregnancy specific interferon production, ovine trophoblast protein-1. Endocrinology, 132, 1869–1871.
45) Ocón-Grove, O.M., Cooke, F.N., Alvarez, I.M., Johnson, S.E., Ott, T.L. and Ealy, A.D. (2008): Ovine endometrial expression of fibroblast growth factor (FGF) 2 and conceptus expression of FGF receptors during early pregnancy. Domest. Anim. Endocrinol., 34, 135–145.
46) Rooke, J., Ewen, M., McEvoy, T., Entrican, G. and Ashworth, C. (2005): Effect of inclusion of serum and granulocyte-macrophage colony stimulating factor on secretion of interferon-tau during the in vitro culture of ovine embryos. Reprod. Fertil. Dev., 17, 513–521.
47) Michael, D.D., Alvarez, I.M., Ocon, O.M., Powell, A.M., Talbot, N.C., Johnson, S.E. and Ealy, A.D. (2006): Fibroblast growth factor-2 is expressed by the bovine uterus and stimulates interferon-tau production in bovine trophectoderm. Endocrinology, 147, 3571–3579.
48) Ezashi, T., Ealy, A.D., Ostrowski, M.C. and Roberts, R.M. (1998): Control of interferon gene expression by Ets-2. Proc. Natl. Acad. Sci. USA, 95, 7882–7887.
49) Ezashi, T., Ghosh, D. and Roberts, R.M. (2001): Repression of Ets-2-induced transactivation of the tau interferon promoter by Oct-4. Mol. Cell. Biol., 21, 7883–7891.
50) Yamaguchi, H., Ikeda, Y., Moreno, J.I., Katsumura, M., Miyazawa, T., Takahashi, E., Imakawa, K., Sakai, S. and Christenson, R.K. (1999): Identification of a functional transcriptional factor AP-1 site in the sheep interferon-τ gene that mediates a response to PMA in JEG3 cells. Biochem. J., 340, 767–773.
51) Imakawa, K., Kim, M.S., Matsuda-Minehata, F., Ishida, S., Iizuka, M., Suzuki, M., Chang, K.T., Echternkamp, S.E. and Christenson, R.K. (2006): Regulation of the ovine interferon-tau gene by a blastocyst-specific transcription factor, Cdx2. Mol. Reprod. Dev., 73, 559–567.
52) Sakurai, T., Sakamoto, A., Muroi, Y., Bai, H., Nagaoka, K., Tamura, K., Takahashi, T., Hashizume, K., Sakatani, M., Takahashi, M., Godkin, J.D. and Imakawa, K. (2009): Induction of endogenous Tau interferon gene transcription by CDX2 and high acetylation in bovine non-trophoblast cells. Biol. Reprod., 80, 1223–1231.
53) Ezashi, T., Das, P., Gupta, R., Walker, A. and Roberts, R.M. (2008): The role of Homeobox protein distal-less 3 and its interaction with ETS2 in regulating bovine interferon-tau gene expression-synergistic transcriptional activation with ETS2. Biol. Reprod., 79, 115–124.
54) Xu, N., Takahashi, Y., Matsuda, F., Sakai, S., Christenson, R.K. and Imakawa, K. (2003): Involvement of coactivator CBP in the regulation of conceptus IFNτ gene expression. Mol. Reprod. Dev., 65, 23–29.
55) Das, P., Ezashi, T., Gupta, R. and Roberts, R.M. (2008): Combinational roles of protein kinase A, Ets2, and 3′,5′-cyclic-adenosine monophosphate response element-binding protein-binding protein/p300 in the transcriptional control of interferon-tau expression in a trophoblast cell line. Mol. Endocrinol., 22, 331–343.
56) Bai, H., Sakurai, T., Kim, M.S., Muroi, Y., Ideta, A., Aoyagi, Y., Nakajima, H., Takahashi, M., Nagaoka, K. and Imakawa, K. (2009): Involvement of GATA transcription factors in the regulation of endogenous bovine interferon-tau gene transcription. Mol. Reprod. Dev., 76, 1143–1152.
57) Talbot, N.C., Caperna, T.J., Edwards, J.L., Garrett, W., Wells, K.D. and Ealy, A.D. (2000): Bovine blastocyst-derived trophectoderm and endoderm cell cultures: interferon tau and transferrin expression as respective in vitro markers. Biol. Reprod., 62, 235–247.
58) Shimada, A., Nakano, H., Takahashi, T., Imai, K. and Hashizume, K. (2001): Isolation and characterization of a bovine blastocyst-derived trophoblastic cell line, BT-1: development of a culture system in the absence of feeder cell. Placenta, 22, 652–662.
59) Sakurai, T., Bai, H., Konno, T., Ideta, A., Aoyagi, Y., Godkin, J.D. and Imakawa, K. (2010): Function of a transcription factor CDX2 beyond its trophectoderm lineage specification. Endocrinology, 151, 5873–5881.
60) Nojima, H., Nagaoka, K., Christenson, R.K., Shiota, K. and Imakawa, K. (2004): Increase in DNA methylation down-regulates conceptus interferon-tau gene expression. Mol. Reprod. Dev., 67, 396–405.
61) Biggers, J.D., Bell, J.E. and Benos, D.L. (1988): Mammalian blastocyst: transport functions in a developing epithelium. Am. J. Physiol., 255, C419–C432.
62) Kang, H.M., Kim, K., Kwon, H.B. and Cho, W.K. (1990): regulation of laminin gene expression in the expansion of mouse blastocysts. Mol. Reprod. Dev., 27, 191–199.
63) Fleming, T.P., Sheth, B. and Fesenko, I. (2001): Cell adhesion in the preimplantation mammalian embryo and its role in trophectodermal differentiation and blastocyst morphogenesis. Front. Biosci., 6, D1000–D1007.
64) Denker, H.W. (1993): Implantation: a cell biological paradox. J. Exp. Zool., 266, 541–558.
65) Wathes, D.C. and Wooding, F.B. (1980): An electron microscopic study of implantation in the cow. Am. J. Anat., 159, 285–306.
66) Yamakoshi, S., Bai, R., Chaen, T., Ideta, A., Aoyagi, Y., Sakurai, T., Konno, T. and Imakawa, K. (2012): Expression of mesenchymal-related genes by the bovine trophectoderm following conceptus attachment to the endometrial epithelium. Reproduction, 143, 377–387.
67) Peinado, H., Olmeda, D. and Cano, A. (2007): Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype. Nat. Rev. Cancer, 7, 415–428.
68) Wang, X., Zheng, M., Liu, G., Xia, W., McKeown-Longo, P.J., Hung, M.C. and Zhao, I. (2007): Küppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion. Cancer Res., 67, 7184–7193.
69) Yang, J. and Weinberg, R.A. (2008): Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev. Cell, 14, 818–829.
70) Thiery, J.P., Acloque, H., Huang, R.Y. and Nieto, M.A. (2009): Epithelium-mesenchymal transitions in development and disease. Cell, 139, 871–890.
71) Mi, S., Lee, X., Li, X., Veldman, G.M., Finnerty, H., Racie, L., LaVallie, E., Tang, X.Y., Edouard, P., Howes, S., Keith, J.C. and McCoy, J.M. (2000): Syncytin is a captive retroviral envelop protein involved in human placental morphgenesis.

Nature, 403, 785–789.

72) Blaise, S., de Parseval, N., Benit, L. and Heidmann, T. (2003): Genomewide screening for fusogenic human endogenous retrovirus envelops identifies syncytins 2, a gene conserved on primate evolution. Proc. Natl. Acad. Sci. USA, 100, 13013–13018.

73) Be, L., Kanellopoulos, C., Sapin, V., Dupressoir, A., Marceau, G. and Heidmann, T. (2005): Placenta-specific murine envelop genes of retroviral origin conserved in Muridae. Science, 102, 725–730.

74) Arnaud, F., Caporale, M., Varela, M., Biek, R., Chessa, B., Alberti, A., Golder, M., Mura, M., Zhang, Y.P., Yu, L., Pereira, F., Demartini, J.C., Leymaster, K., Spencer, T.E. and Palmarini, M. (2007): A paradigm for virus-host coevolution: sequential counter-adaptations between endogenous and exogenous retroviruses. PLoS Pathog., 3, e170.

75) Heidmann, O., Vernochet, C., Dupressoir, A. and Heidmann, T. (2009): Identification of an endogenous retroviral envelop gene with fusogenic activity and placenta-specific expression in the rabbit: a new "syncytins" in a third order of mammals. Retrovirology, 6, 107.

76) Boeke, J.D. and Stoye, J.P. (1997): Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In "Retroviruses", (Coffin, J.M., Hughes, S.H. and Varmus, H.E., eds.), Cold Spring Harbor, New York.

77) Best, S., Le Tissier, P.R. and Stoye, J.P. (1997): Endogenous retroviruses and the evolution of resistance to retroviral infection. Trends Microbiol., 5, 313–318.

78) Harris, J.R. (1998): Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance. Bioessays, 20, 307–316.

79) Rawn, S.M. and Cross, J.C. (2008): The evolution, regulation, and function of placenta-specific genes. Annu. Rev. Cell Dev. Biol., 24, 159–181.

80) Macfarlan, T.S., Gifford, W.D., Driscoll, S., Lettieri, K., Rowe, H.M., Bonanomi, D., Firth, A., Singer, O., Trono, D. and Pfaff, S.L. (2012): Embryonic stem cell potency fluctuates with endogenous retrovirus activity. Nature, 487, 57–63.

81) Huppertz, B., Kaufmann, P. and Kingdom, J. (2002): Trophoblast turnover in health and diseases. Fetal. Matern. Med. Rev., 13, 103–118.

82) Das, S.K., Yano, S., Wang, J., Edwards, D.R., Nagase, H. and Dey, S.K. (1997): Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinses in the mouse uterus during the peri-implantation period. Dev. Genet., 21, 44–54.

83) Itoh, Y., Iwata, K., Tanzawa, K., Mori, Y. and Nagase, H. (1998): Plasma membrane-bound tissue inhibitor of metalloproteinases (TIMP)-2 specifically inhibits matrix metalloproteinase 2 (gelatinase A) activated on the cell surface. J. Biol. Chem., 273, 24360–24367.

84) Pötgens, A.J.G., Schmitz, U., Bose, P., Versmold, A., Kaufmann, P. and Frank, H.G. (2002): Mechanisms of syncytial fusion: a review. Placenta 23,(Suppl A): S107–S113.

85) Dunlap, K.A., Palmarini, M. and Spencer, T.E. (2006): Ovine endogenous betaretroviruses (enJSRVs) and placental morphogenesis. Placenta 27,(Suppl A): S135–S140.

86) Palmarini, M., Mura, M. and Spencer, T.E. (2004): Endogenous betartroviruses of sheep: teaching new lessons in retroviral interference and adaptation. J. Gen. Virol., 85, 1–13.

87) Rai, S.K., Duh, F.M., Vigdorovich, V., Danilkovitch-Miagkova, A., Lerman, M.I. and Miller, A.D. (2001): Candidate tumor suppressor HYAL2 is a glycosylphophatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelop protein of which mediates oncogenic transformation. Proc. Natl. Acad. Sci. USA, 98, 4443–4448.

88) Dunlap, K.A., Palmarini, M., Adelson, D.L. and Spencer, T.E. (2005): Sheep endogenous betaretroviruses (enJSRVs) and the hyaluronidase 2 (HYAL2) receptor in the ovine uterus and conceptus. Biol. Reprod., 73, 271–279.

89) Baba, K., Nakaya, Y., Shojima, T., Muroi, Y., Kizaki, K., Hashizume, K., Imakawa, K. and Miyazawa, T. (2011): Identification of novel endogenous betaretroviruses which are transcribed in the bovine placenta. J. Virol., 85, 1237–1245.

90) Wooding, F.B. and Beckers, J.F. (1987): Trinucleate cells and the ultrastructural localization of bovine placental lactogen. Cell Tissue Res., 247, 667–673.

91) Wooding, F.B. (1992): Current topic: the synepitheliochorial placenta of ruminants: Binucleate cell fusions and hormone production. Placenta, 13, 101–113.