



HAL
open science

Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation

Nadera Mansouri-Atiia, Julie Aubert, Pierrette Reinaud, Corinne Giraud-Delville, Geraldine Taghouti, Laurent Galio, Robin, E. Everts, Severine Degrelle, Christophe Richard, Isabelle Hue, et al.

► **To cite this version:**

Nadera Mansouri-Atiia, Julie Aubert, Pierrette Reinaud, Corinne Giraud-Delville, Geraldine Taghouti, et al.. Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation. *Physiological Genomics*, 2009, 39 (1), pp.14-27. 10.1152/physiolgenomics.90404.2008 . hal-01197513

HAL Id: hal-01197513

<https://hal.science/hal-01197513v1>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Nadéra Mansouri-Attia, Julie Aubert, Pierrette Reinaud, Corinne Giraud-Delville, Géraldine Taghouti, Laurent Galio, Robin E. Everts, Séverine Degrelle, Christophe Richard, Isabelle Hue, Xiangzhong Yang, X. Cindy Tian, Harris A. Lewin, Jean-Paul Renard and Olivier Sandra
Physiol Genomics 39:14-27, 2009. First published Jul 21, 2009;
doi:10.1152/physiolgenomics.90404.2008

You might find this additional information useful...

Supplemental material for this article can be found at:

<http://physiolgenomics.physiology.org/cgi/content/full/90404.2008/DC1>

This article cites 89 articles, 46 of which you can access free at:

<http://physiolgenomics.physiology.org/cgi/content/full/39/1/14#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://physiolgenomics.physiology.org/cgi/content/full/39/1/14>

Additional material and information about *Physiological Genomics* can be found at:

<http://www.the-aps.org/publications/pg>

This information is current as of September 9, 2010 .

CALL FOR PAPERS | *Comparative Genomics*

Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation

Nadéra Mansouri-Attia,¹ Julie Aubert,² Pierrette Reinaud,¹ Corinne Giraud-Delville,¹ Géraldine Taghouti,¹ Laurent Galio,³ Robin E. Everts,⁴ Séverine Degrelle,¹ Christophe Richard,⁵ Isabelle Hue,¹ Xiangzhong Yang,⁶ X. Cindy Tian,⁶ Harris A. Lewin,⁴ Jean-Paul Renard,¹ and Olivier Sandra¹

¹Institut National de la Recherche Agronomique, Unité Mixte de Recherche (UMR) 1198 Biologie du Développement et Reproduction, Jouy-en-Josas; Ecole Nationale Vétérinaire d'Alfort, Maisons Alfort; ²AgroParisTech, UMR 518 Mathématiques et Informatiques Appliquées; Institut National de la Recherche Agronomique, UMR 518 Mathématiques et Informatiques Appliquées, Paris; ³Institut National de la Recherche Agronomique, UR1196, Unité Génomique et Physiologie de la Lactation, Jouy-en-Josas, France; ⁴Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois; ⁵Institut National de la Recherche Agronomique, UE1298 Unité Commune d'Expérimentation Animale de Bressonvilliers, Leudeville, France; and ⁶Center for Regenerative Biology, Department of Animal Sciences, Storrs, Connecticut

Submitted 19 December 2008; accepted in final form 16 July 2009

Mansouri-Attia N, Aubert J, Reinaud P, Giraud-Delville C, Taghouti G, Galio L, Everts RE, Degrelle S, Richard C, Hue I, Yang X, Tian XC, Lewin HA, Renard JP, Sandra O. Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation. *Physiol Genomics* 39: 14–27, 2009. First published July 21, 2009; doi:10.1152/physiolgenomics.90404.2008.—At implantation the endometrium undergoes modifications necessary for its physical interactions with the trophoblast as well as the development of the conceptus. We aim to identify endometrial factors and pathways essential for a successful implantation in the caruncular (C) and the intercaruncular (IC) areas in cattle. Using a 13,257-element bovine oligonucleotide array, we established expression profiles at *day 20* of the estrous cycle or pregnancy (implantation), revealing 446 and 1,295 differentially expressed genes (DEG) in C and IC areas, respectively (false discovery rate = 0.08). The impact of the conceptus was higher on the immune response function in C but more prominent on the regulation of metabolism function in IC. The C vs. IC direct comparison revealed 1,177 and 453 DEG in cyclic and pregnant animals respectively (false discovery rate = 0.05), with a major impact of the conceptus on metabolism and cell adhesion. We selected 15 genes including *C11ORF34*, *CXCL12*, *CXCR4*, *PLAC8*, *SCARA5*, and *NPY* and confirmed their differential expression by quantitative RT-PCR. The cellular localization was analyzed by in situ hybridization and, upon pregnancy, showed gene-specific patterns of cell distribution, including a high level of expression in the luminal epithelium for *C11ORF34* and *MX1*. Using primary cultures of bovine endometrial cells, we identified *PTN*, *PLAC8*, and *CXCL12* as interferon- τ (IFNT) target genes and *MSX1* and *CXCR7* as IFNT-regulated genes, whereas *C11ORF34* was not an IFNT-regulated gene. Our transcriptomic data provide novel molecular insights accounting for the biological functions related to the C or IC endometrial areas and may contribute to the identification of potential biomarkers for normal and perturbed early pregnancy.

transcriptome; interferon-tau; pregnancy; cattle

IN MAMMALS, the establishment and maintenance of pregnancy require a subtle and tightly regulated communication between

the conceptus (embryo and embryonic annexes) and the maternal environment (85). The success of implantation relies on several essential steps including the adjustment of the uterine environment to support the development of the conceptus and the profound remodeling of the endometrium structure necessary for the apposition, adhesion, and invasion phases (36). In contrast to human and rodents, the invasion of the maternal tissue by the fetal tissue is very limited in ruminants (71) and leads to a synepitheliochorial placentation (86). Since the trophoblast appears to be intrinsically invasive in mammals (11), apposition, adhesion, and invasion processes are thought to be controlled by the endometrium (83). In mammalian species presenting an invasive implantation, decidua restrains the invasion of the embryo in a spatiotemporal manner (20). The expression and the regulation of some factors involved in the apposition, adhesion, and invasion aspects of implantation have been reported in ruminants (62, 79), but, overall, the comparative cascade of molecular mechanisms remains largely unknown.

The sequence of events occurring during implantation involves a variety of maternal and embryonic factors (80). Uterine factors include enzymes, cytokines, growth factors, ions, hormones, glucose, transport proteins, and adhesion molecules (collectively termed histotroph) that have been shown to be mainly synthesized by the endometrial glands (65). In ruminants, these glands are specifically localized in large endometrial areas [intercaruncular zones (IC)] while small aglandular caruncular areas of stromal origin [caruncles (C)] are scattered over the endometrium surface. The endometrial glands of the IC areas have been shown to be crucial for the development of the conceptus (33). The C areas are present in the cyclic endometrium, and they fuse with the fetal cotyledons to give rise to placentomes in the pregnant animals (3). Therefore, given the structural differences and biological functions associated to the C and IC areas, these two distinct endometrial zones have to be analyzed separately for a more comprehensive understanding of the implantation process in ruminants.

Uterine receptivity to implantation has been shown to be dependent on P4, whereas interferon- τ (IFNT) has been identified as the major signal of pregnancy recognition in ruminants

Address for reprint requests and other correspondence: O. Sandra, INRA, UMR 1198 INRA-ENVA, Biologie du Développement et Reproduction, Domaine de Vilvert, F-78352 Jouy-en-Josas, France (e-mail: olivier.sandra@jouy.inra.fr).

(74). This type I IFN is exclusively produced by the mononuclear trophoblast cells of the elongating conceptus during the peri-implantation period (27). In addition to its antitumor actions, IFNT acts on endometrial genes (IFNT-stimulated genes or ISGs) in a specific spatial and temporal manner (8). In the bovine and ovine species, expression profiling analyses have recently been carried out in the endometrium to decipher the molecular processes taking place during the pre-implantation (6, 34, 51) as well as the postimplantation and early placentation periods (45, 82). Nevertheless, regarding the differentially expressed genes (DEG) identified from the bovine transcriptomic analyses, the contribution of IFNT in their regulation has not been investigated.

To gain new insights on the cellular and molecular mechanisms involved during the initial phase of the physical and permanent interactions between the trophoblast and the endometrium, we have undertaken a high-throughput analysis of the endometrial transcriptome at *day 20* postestrus of the cycle or pregnancy in cattle. The timing of sample collection was also based on earlier studies that detailed embryonic losses as mainly occurring before this stage in cattle (22, 43). Taking advantage of a 13,257-element bovine oligoarray (26, 61, 63), we established gene expression profiles in the C and IC areas separately; then, for a selection of genes, the differential expression was confirmed by quantitative RT-PCR, the cellular localization was analyzed by *in situ* hybridization, and the direct regulation by IFNT was established using primary cultures of bovine endometrial cells.

MATERIALS AND METHODS

Animals

The experiments were performed in accordance with the International Guiding Principles for Biomedical Research Involving Animals, as promulgated by the Society for the Study of Reproduction and with the European Convention on Animal Experimentation. Eighteen cyclic cows (eight Holstein-Normand breed heifers and 10 Charolais breed cows) were estrus synchronized by the Crestar method, then nine of them (four Holstein-Normand breed heifers and five Charolais breed cows) were artificially inseminated (AI) as published previously (14).

Tissue Collection and Preparation for RNA Extraction and *In Situ* Hybridization

Animals were slaughtered at *day 20* of estrous cycle or pregnancy. The uteri from the AI and cyclic animals were collected then flushed with PBS. Embryos recovered from the pregnant animals were observed by microscopy to determine the stage of the development (17). Only the ipsilateral horn to the corpus luteum carrying intact conceptuses with similar development age were used for gene expression analysis. The ipsilateral uterine horn was longitudinally opened by scissors. C areas were first carefully cut out then the intercaruncular endometrium was sampled (Supplementary Fig. S3¹). Dissection of the tissue was carried out by the same person for all the animals. Sections of the ipsilateral uterine horn were fixed in PAF 4%, then washed in PBS 1X followed by 15 and 18% sucrose solution. Total RNA from frozen tissue was isolated by homogenization using TRIzol Reagent (Invitrogen, Cergy-Pontoise, France) according to the manufacturer's recommendations. All RNA samples were purified on Qiagen columns according to the manufacturer's protocol (RNeasy

Mini kit; Qiagen, Courtaboeuf, France). Quality and integrity of total extracted and purified RNA were determined by Agilent 2100 bio analyzer (Massy, France). The RNA integrity number of each RNA sample ranged from 7.5 to 9.5 (69).

Experimental Design

Study 1. To determine the impact of pregnancy on the endometrial transcriptome in the C independently from the IC areas, we used a dye-switch loop design (66). Gene expression patterns were established to compare the transcriptome of cyclic (*day 20* of estrous cycle; $n = 9$) and AI pregnant animals (*day 20* of pregnancy; $n = 9$) for C and IC areas, respectively.

Study 2. Using a dye-swap loop design, gene expression profiles were established to analyze the differences in gene expression between the C and the IC areas of five cyclic (*day 20* of estrous cycle) and five AI pregnant animals used in *study 1* (*day 20* of pregnancy).

Microarray, Probe Labeling, and Array Hybridization

A 13,257-element bovine oligoarray (National Center for Biotechnology Information Gene Expression Omnibus: GPL2853) was used to determine the transcript profiles (26, 61, 63). We converted 20 μg of each RNA sample into aminoallyl-labeled cDNA with oligo-dT primers, amino-allyl-dUTP (Sigma, Saint-Quentin-Fallavier, France), and superscript II Reverse Transcriptase (Invitrogen). The cDNA was purified on a Microcon PCR column according to the manufacturer's protocol (Millipore, St-Quentin-en-Yvelines, France) and added to an aliquot of fluorescent dye (Cy3 or Cy5; GE HealthCare, Vélizy, France). Unused reactive sites were blocked by 4 M hydroxylamine, and fluorescent probes were purified on QiaQuick columns according to the manufacturer's recommendations (Qiagen). Every probe was quantified using a Nanodrop spectrophotometer and quality was assessed using an agarose gel electrophoresis 1X 1% TAE. The mean lengths of the fluorescent probes were analyzed using the Typhoon Imaging system scanner (GE HealthCare). Samples were then combined according to their fluorescence intensity (in picomoles), denatured 2 min at 95°C, and cohybridized on the 13,257 oligoarray at 42°C for 16 to 18 h using the Corning Pronto! Universal Microarray Kits according to the manufacturer's recommendations (Fischer Scientific Bioblock, Illkirch, France). After stringent washes to remove unbound cDNA, slides were scanned using a GenePix 4000B scanner (Axon instrument, France) and features were analyzed with GenePix Pro Version 4.0 software (Axon Instrument).

Analyses of Microarray Data

Data were normalized by a global loess regression (88) followed by a subtraction of the median by block and analyzed using a mixture model that identifies clusters of genes with equal variance (18) using the "anapuce" R package (<http://www.r-project.org/>). Statistical significant raw P values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure that controls the false discovery rate (FDR) (9). The FDR was set at 0.08 for the [AI vs. estrous cycle] and at 0.05 for [C vs. IC] comparisons respectively. The DEG were analyzed with Metacore (GeneGo, <http://trials.genego.com>; version 4.5, build 11165) and Ingenuity Pathway Analysis (IPA: <http://www.ingenuity.com>; release 5.5) software, to classify the DEG according to their Gene Ontology biological process, to identify significant differences in biological functions, and to distribute the DEG into canonical pathways or networks.

Validation of Gene Expression Data by Real-time RT-PCR

The purified RNA samples analyzed by array hybridizations were used for the quantitative RT-PCR (qRT-PCR). For each sample, cDNA was synthesized from 1 μg of total RNA then qPCR were performed with the Master Mix SYBR Green and the ABI Prism 7000 sequence detection system (Applied Biosystems, Cergy-Pontoise,

¹ The online version of this article contains supplemental material.

France). Primers were designed (Primer Express Software v2.0, Applied Biosystems) to specifically amplified 15 selected DEG and the housekeeping gene RPL19 (Supplementary Table S5). Every amplified PCR fragment was sequenced to assess whether the correct gene was amplified. According to the relative standard curve method (52), the relative quantification of mRNA amount of the selected DEG against RPL19 was calculated. To assess differential expression, data from duplicates were pooled, and a mixed model was run on the pooled qRT-PCR values. Genes were classified as differentially expressed if the mixed model *P* value was <0.05.

In Situ Hybridization

The pT7T3Pac or pGEMZf11 (+) plasmids containing T7 or T3/SP6 RNA polymerase promoters were used. The digoxigenin (DIG)-labeled uridine triphosphate cRNA probes were synthesized by *in vitro* transcription using T7 or T3/SP6 polymerase according to the manufacturer's protocol (Roche Diagnostics, Meylan, France). Three cyclic and three pregnant animals were used from the pools of animals analyzed by microarray and qRT-PCR, and three frozen sections (12 μ m) were cut for each animal. The *in situ* hybridization procedure was performed as described previously (12). Negative controls were made by hybridization with sense probes. Images were captured by photomicrography using an Olympus-DP50 microscope coupled to a digital camera system and software (Olympus, Rungis, France).

Primary Cultures of Bovine Endometrial Cells and IFNT Treatment

The primary endometrial cell cultures (fibroblasts or glandular epithelial cells) were derived from two individual cyclic cows sampled during the luteal phase. Briefly, using the uterine horn ipsilateral to the corpus luteum, we carefully dissected the endometrium from the myometrium and then washed it in Hanks solution. Endometrium was minced and dispersed with collagenase and hyaluronidase (Sigma) in an Earle buffer saline solution containing 15 mM HEPES and 2% BSA for 2 h at 37°C. Clamps of uterine glands cells were isolated with a strainer. Glandular epithelial cell suspensions were seeded into dishes. Fibroblast cells were resuspended in culture medium, seeded in dishes (PRIMARIA dishes; VWR, Fontenay-sous-Bois, France), and then incubated for 2 h in 20% CO₂. At the end of the preculture step, fibroblasts adhered and floating cells were carefully eliminated by washes. Twenty-four hours later, fibroblasts were treated with trypsin (TrypLE Express; GIBCO-Invitrogen, Cergy-Pontoise, France) and seeded. DMEM-F12 medium supplemented with 10% (vol/vol) fetal calf serum (FCS, GIBCO-Invitrogen), insulin (Actrapid-Novo, Novo-Nordisk, France), penicillin, and streptomycin (Sigma) was used for culturing glandular epithelial cells and fibroblasts at 37°C and 5% CO₂. Culture medium was changed every 2 days until the preconfluence stage was reached.

At the preconfluence stage, cells were starved for 24 h in the culture medium devoid of serum. Glandular epithelial cells and fibroblast cells prepared from each cow were treated for 30 min, 2 h, or 24 h with 100 ng/ml or 1 μ g/ml of ovine recombinant IFNT (64). Control cells were left untreated and washed with cold PBS, recovered by scrapping, and then centrifuged at 4°C for 2 min at 10,000 rpm to eliminate supernatant. Cell pellets were stocked at -80°C until further use for RNA extraction. Gene expression levels were analyzed by qRT-PCR as described above.

RESULTS

Microarray Hybridization Analyses

Study 1. Gene expression profiling of C and IC areas in the pregnant vs. estrous cycle comparison. The transcriptome analyses were carried out by hybridizing C or IC cDNAs to bovine 13,257-oligonucleotide microarrays using a dye-switch loop design. Nine cyclic animals were analyzed at *day 20* of

the estrous cycle, and nine pregnant animals were analyzed at *day 20* of pregnancy. In the C and IC areas, an FDR of 0.08 was used to increase the sensitivity to detect DEG between cyclic and pregnant females.

ANALYSES OF THE EXPRESSION PROFILES IN THE C AREAS. By comparing the expression profiles of the C areas between cyclic and pregnant animals, 446 genes were differentially expressed including 431 genes showing a twofold or greater differential expression (Fig. 1A). Among the 446 DEG (Supplementary Table S1), 202 genes were upregulated in cyclic cows, whereas 244 were upregulated in AI pregnant cows. Upon pregnancy the highest increase in gene expression was detected for *RSAD2* (radical S-adenosyl methionine domain containing 2: 10⁴-fold), *MXI* (myxovirus "influenza virus" resistance 1, interferon-inducible protein p78: 4.6 10³-fold), *JSP.1* (MHC Class I JSP.1 similar to human HLA-A: 4.4 10³-fold), *FABP3* (fatty acid binding protein 3: 4.2 10³-fold), or *ISG15* (ISG15 ubiquitin-like modifier: 2.4 10³-fold). The most pronounced downregulation of gene expression upon pregnancy was found for *NPY* (neuropeptide Y: 325-fold), *COL1A2* (collagen, type I, alpha 2: 201-fold), *LOC534369* (similar to Probable cation-transporting ATPase 13A1: 74-fold), *COL3A1* [Collagen alpha 1(III) chain precursor: 70-fold], or *RARRES* (similar to retinoic acid receptor responder 1: 66-fold). To assess biological meaning, the 446 DEG between cyclic and pregnant C areas were analyzed using the IPA software (Supplementary Table S1). The analysis identified 39 IPA Bio function terms with ≥ 5 genes and significant association (*P* < 0.01), including cellular growth and proliferation (118 genes), gene expression (53 genes), DNA replication, recombination and repair (32 genes), cellular movement (58 genes), and cell death (96 genes). Eight canonical pathways were affected in the AI vs. estrous cycle comparison with ≥ 3 genes and significant association (*P* < 0.05), including interferon signaling (7 genes), hepatic fibrosis/hepatic stellate cell activation (12 genes), antigen presentation pathway (5 genes), leukocyte extravasation signaling (11 genes), and tight junction signaling (9 genes).

ANALYSES OF THE EXPRESSION PROFILES IN THE IC AREAS. Using the same animals as those used for determining the C area transcriptome, 1,295 genes were found to be differentially expressed between cyclic and pregnant IC areas, including 66 genes with twofold or more differential expression (Supplementary Table S2). Among the 1,295 genes, 762 DEG and 533 DEG displayed a higher expression in cyclic or pregnant animals, respectively. The highest increase in gene expression in the pregnant IC areas was detected for *RSAD2* (5.4-fold), *IF16* (interferon, α -inducible protein 6: 4.5-fold), *ISG15* (ISG15 ubiquitin-like modifier: 4-fold), *MXI* (3.9-fold), or *JSP.1* (3.15-fold). In the cyclic IC areas, the highest upregulation was found for *COL1A2* (4.1-fold), *OXTTR* (oxytocin receptor: 3.7-fold), *SNAI2* (snail homolog 2: 3.5-fold), *C10ORF58* (chromosome 10 open reading frame 58: 3.4-fold), and *NPY* (3.2-fold).

To gain insight into the biological processes involved at implantation in the IC areas, the 1,295 DEG were analyzed using the IPA software (Supplementary Table S2). Forty-one IPA Bio function terms with ≥ 5 genes revealed significant association (*P* < 0.01), including cellular growth and proliferation (286 genes), posttranslational modification (101 genes), protein folding (17 genes), RNA posttranscriptional modifica-

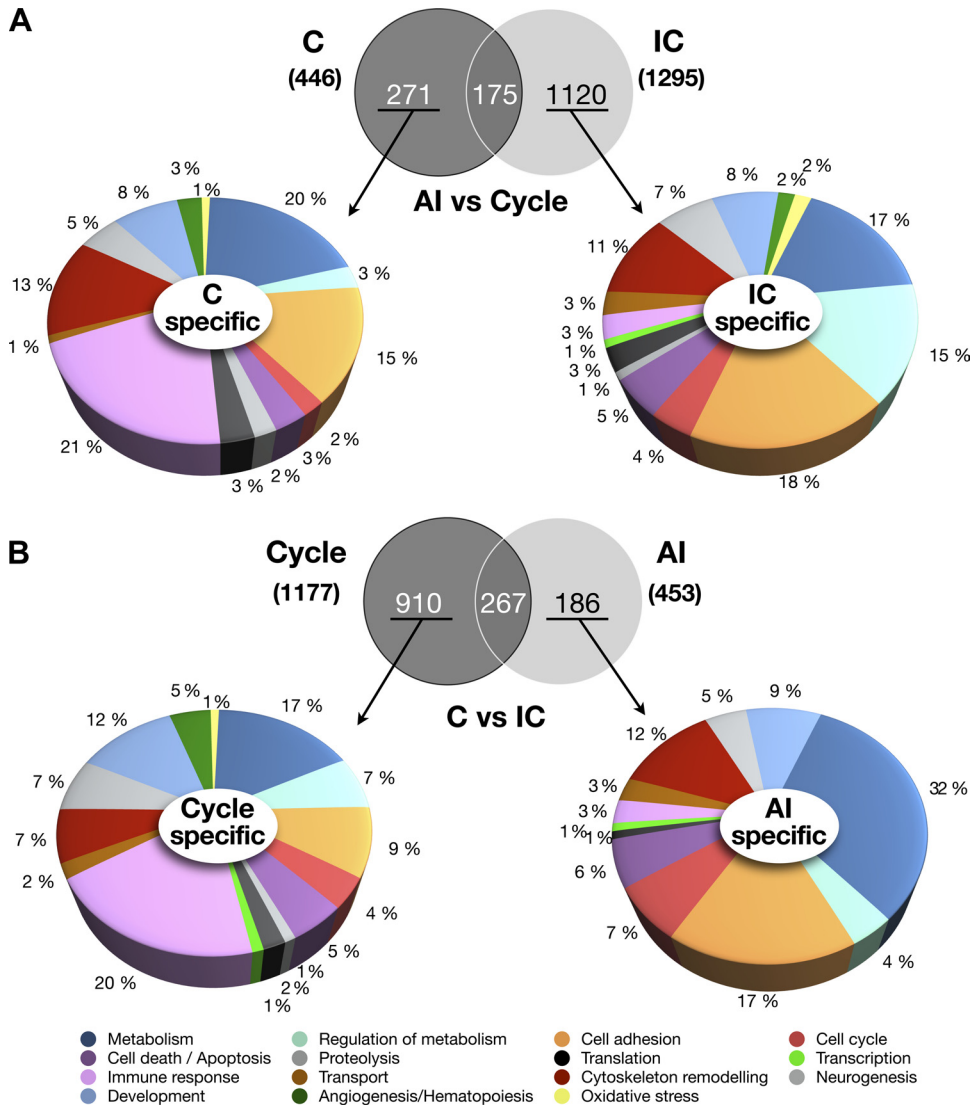


Fig. 1. Analysis of the bovine microarray data in endometrial caruncles (C) or intercaruncular (IC) areas in estrous cycle and pregnancy at implantation. *A, top:* Venn diagram showing the artificial insemination (AI) vs. cycle differentially expressed genes (DEG) common or specific to C or IC areas. *Bottom:* Gene Ontology classification of the AI vs. cycle DEG specifically expressed in C or in IC areas. *B, top:* Venn diagram showing the C vs. IC DEG common or specific to estrous cycle or pregnancy. *Bottom:* Gene Ontology classification of the C vs. IC DEG specifically expressed in cyclic or in AI animals.

tion (31 genes), and cell death (245 genes). Twenty canonical pathways with ≥ 3 genes were significant ($P < 0.05$) such as protein ubiquitination pathway (37 genes), oxidative phosphorylation (25 genes), antigen presentation pathway (11 genes), ubiquitin biosynthesis (13 genes), and VEGF signaling (15 genes).

ANALYSES OF THE C- AND IC-SPECIFIC GENE EXPRESSION PROFILES IN CYCLIC AND PREGNANT ANIMALS. In the AI vs. estrous cycle comparison, the Venn diagram showed 271 DEG and 1,120 DEG to be specifically regulated in the C and the IC areas respectively (Fig. 1A). The C-specific DEG that displayed the highest expression in cyclic samples were *LOC534369* (Probable cation-transporting ATPase 13A1: 74-fold), *COL12A1* (collagen, type XII, alpha 1: 36-fold), *MMP2* (matrix metalloproteinase 2: 31-fold), *PRSS11* (protease, serine, 11: 28-fold), and *MGC140440* (similar to limitrin and human matrix-remodeling associated 8: 25-fold). The DEG that showed the highest C-specific expression in pregnant samples were *MX2* (myxovirus resistance 2: 659-fold), *C11ORF34* (placenta expressed transcript protein 1; *PLET1*: 531-fold), *DKK1* (Dickkopf-1: 504-fold), *CA2* (carbonic anhydrase II: 300-fold), and *IFITM3* [interferon-induced transmembrane

protein 3 (1–8U): 292-fold]. Using Genego Metacore software, we classified the DEG according to their biological functions in the C and IC areas (Fig. 1A). The most striking differences appeared for two biological functions whose percentage differs between the C and the IC area, namely “immune response” (22% in C, 15% in IC) and “metabolism and regulation of metabolism” (15 and 2% in C, 19 and 7% in IC, respectively). Using the IPA software, we found many biological functions implicated in cell regulation, such as cell adhesion, cellular growth and proliferation, cell signaling, cell morphology, or cell death affected by pregnancy in both C and IC areas (Supplementary Fig. S1).

Study 2. gene expression profiling of the C vs. IC comparison in cyclic and pregnant bovine females. We investigated the differences in gene expression between the C and IC areas in five cyclic animals and five pregnant animals at day 20 post-estrus. Using a dye-swap loop design, we compared the C areas with the IC areas from the same animal. An FDR of 0.05 was applied.

In cyclic animals, 1,177 C vs. IC DEG were found (129 genes displaying a twofold or higher differential expression), and 453 C vs. IC DEG were found for pregnant animals (84

genes displaying a twofold or higher differential expression). The top DEG are listed in the Supplementary Tables S3 and S4 for cyclic and pregnant cows, respectively. The Venn diagram comparing the C vs. IC DEG between cyclic and AI pregnant animals (Fig. 1B) showed 267 DEG (59%) in AI to be common with cycle. The DEG were classified according to their biological functions using the GeneGo Metacore software. Many biological functions were represented, such as metabolism, cell adhesion, immune response, cytoskeleton remodeling, cell cycle control, and angiogenesis/hematopoiesis (Fig. 1B). Using the IPA software, we found the comparison of the 1,177 DEG in the C vs. IC cyclic profile to show significant association ($P < 0.01$) for 39 Bio function terms having ≥ 5 genes (Supplementary Table S3) including cellular growth and proliferation (251 genes), cell death (231 genes), cell morphology (156 genes), and cell-to-cell signaling and interaction (133 genes). Eighteen canonical pathways with ≥ 3 genes were significantly regulated ($P < 0.05$, Supplementary Table S3) including IGF-I signaling (13 genes), hepatic fibrosis/hepatic stellate cell activation (16 genes), glutamate metabolism (7 genes), propanoate metabolism (9 genes), and complement system (6 genes). For the C vs. IC AI profiles, 36 IPA Bio function terms with ≥ 5 genes revealed significant association ($P < 0.01$; Supplementary Table S4) including cell growth and proliferation (120 genes), cellular movement (69 genes), cell death (96 genes), and cellular development (88 genes). Eleven canonical pathways with ≥ 3 genes were significantly affected ($P < 0.05$; Supplementary Table S4) including dopamine receptor signaling (8 genes), complement system (5 genes), IGF-I signaling (7 genes), Wnt/ β -catenin signaling (10 genes), and chemokine signaling (5 genes).

By classifying the C vs. IC pregnant-specific and cycle-specific DEG, we found that metabolism, cell adhesion, and cytoskeleton remodeling were predominantly affected among the pregnant-specific DEG, whereas immune response was more affected among the cycle-specific DEG (Fig. 1B). Using the IPA software, we found that many biological functions implicated in cell regulation, such as cellular growth and proliferation, cellular development, cellular movement, cellular assembly and organization, and cell cycle, were affected in both C vs. IC cyclic and pregnant profiles (Supplementary Fig. S2).

Analysis of Selected Genes by qRT-PCR

To validate the results obtained from the microarray analyses, we selected 15 genes and analyzed their expression by qRT-PCR (Table 1). We selected 12 genes on the basis of their differential expression between pregnant and cyclic animals (study 1). The abundance of nine transcripts was higher in the pregnant animals. The *CXCL12*, *CXCR7*, and *NPY* transcripts were downregulated by pregnancy. For the C vs. IC expression profiles (study 2), eight genes were analyzed by qRT-PCR. *NPY* and *PLAC8* were found as DEG in cyclic cows only, whereas *C11ORF34* and *LGALS1* were found as DEG in AI pregnant cows (Table 2). All the results obtained by array hybridizations were confirmed by qRT-PCR except for pleiotrophin (*PTN*) whose expression in the C vs. IC comparison exhibited a trend towards the increase in the C areas (p value 0.057).

In Vitro Regulation of Pregnancy-associated DEG by IFNT

To determine if the genes elicited from the studies 1 and 2 were regulated by IFNT, primary cultures of endometrial fibroblasts or glandular epithelial cells were derived and then treated by IFNT. When the regulation occurred within 2 h or at 24 h, the gene was considered as an "IFNT induced" gene or an "IFNT regulated" gene, respectively. The regulation of 15 selected DEG is summarized according to the cell type and the time of incubation (Table 3), including nine DEG whose regulation is detailed in Fig. 2. Our findings showed that *RSAD2* and *MX1* were strongly induced by IFNT from 2 h onward in both fibroblast and glandular epithelial cells, with a stronger and sustained effect in fibroblast cells for *RSAD2*. The regulation of *IFIT5* was similar to *MX1* with a lower fold induction. *CXCL12*, *PLAC8*, and *PTN* were identified as new IFNT-induced genes. *CXCL12* was significantly induced by IFNT from 2 h onward in fibroblasts. Very interestingly, the induction and the regulation of *PTN* transcriptional expression by IFNT clearly differed between fibroblasts and glandular epithelial cells (Fig. 2). *CXCR7* and *MSX1* were shown to be new IFNT-regulated genes in fibroblasts, whereas *C11ORF34*, *LGALS1*, and *SCARA5* were not regulated by IFNT in either type of endometrial cell cultures (Table 3).

Table 1. Quantification of mRNA levels for selected genes in the AI vs. estrous cycle comparison by real-time RT-PCR

Gene Symbol	Unigene ID	AI vs. Estrous Cycle					
		Caruncles			Intercaruncular Areas		
		Expression	Microarray Fold Δ	Real-time Fold Δ	Expression	Microarray Fold Δ	Real-time Fold Δ
<i>C11ORF34</i>	Hs.632097	AI	528	28,000‡			
<i>CXCL12</i>	Hs.522891	cycle	9	14*	cycle	1.3	21*
<i>CXCR4</i>	Hs.593413				AI	1.7	4†
<i>CXCR7</i>	Hs.471751				cycle	1.5	3.5*
<i>IFIT5</i>	Hs.252839	AI	42	9*			
<i>MX1</i>	Hs.517307	AI	4,660	68‡	AI	3.9	36‡
<i>MX2</i>	Bt.8143	AI	659	336†			
<i>NPY</i>	Hs.1832	cycle	334	15†	cycle	3.2	3*
<i>PLAC8</i>	Hs.546392	AI	182	12‡			
<i>PTN</i>	Hs.371249				AI	2.2	4*
<i>RSAD2</i>	Hs.17518	AI	11,000	99‡	AI	5.4	199.5‡
<i>SCARA5</i>	Hs.591833				AI	3.1	5†

Fold change (Δ) is expressed as arbitrary units. RPL19 was used as a housekeeping gene. Data are means \pm SD; * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Table 2. Quantification of mRNA levels for selected genes in the C vs. IC areas, comparison by real time RT-PCR

Gene Symbol	Unigene ID	Estrous Cycle			AI		
		Area Expression	Microarray Fold Δ	Real-time Fold Δ	Area Expression	Microarray Fold Δ	Real-time Fold Δ
<i>C11ORF34</i>	Hs.632097				C	3.0	2.5*
<i>CXCL12</i>	Hs.522891	C	1.9	6*	C	1.5	9*
<i>GJA1</i>	Hs.74471	C	3.1	4†	C	3.3	6 *
<i>LGALS1</i>	Hs.445351				C	1.6	1.5*
<i>MSX1</i>	Hs.621772	IC	4.1	4†	IC	2.3	2.5*
<i>NPY</i>	Hs.1832	C	6.1	5†			
<i>PLAC8</i>	Hs.546392	IC	1.5	3.7*			
<i>PTN</i>	Hs.371249	C	7.7	2.8	C	3.8	2†

Fold change (Δ) is expressed as arbitrary units. RPL19 was used as a housekeeping gene. Data are means \pm SD; C, carnuncles; IC, intercaruncular area. * $P < 0.05$, † $P < 0.01$.

Cellular Localization of mRNA Expression by In Situ Hybridization

In situ hybridization was performed to localize the endometrial expression of two IFNT-induced genes, *RSAD2* and *MX1*, and various genes whose expression is affected by pregnancy, namely *LGALS1*, *SCARA5*, *C11ORF34*, *PTN*, *MSX1*, and *NPY*. No specific signal was observed in sections hybridized with sense oligonucleotide probes. Photomicrographs are presented in Fig. 3, and the results are summarized in Table 4.

In the luminal epithelium, the expression of *MX1* was strong in pregnant cows but very weak in cyclic cows. In addition, higher signal intensity was detected for *MX1* and *RASD2* in deep and subluminal stoma cells, glandular epithelium, and myometrium of pregnant cows compared with the cyclic ones. Regarding *LGALS1* the expression was prominent in the stroma of cyclic and pregnant cows, but the staining of the endometrial glands appeared dimmer upon pregnancy. For *SCARA5*, the staining was mainly localized in the stroma with a clear impact of pregnancy on the expression level in the subluminal stroma layer. A very intense staining was detected for *C11ORF34* (*PLET1*) and *MSX1* in the luminal epithelium of pregnant cows. *C11ORF34* showed a clear shift from a

Table 3. Overview of IFNT-dependent gene regulation in bovine endometrial cell cultures from fibroblast and glandular epithelial origin

Gene Symbol	Unigene ID	IFNT-induced Gene	IFNT-regulated Gene	No Regulation by IFNT
<i>C11ORF34</i>	Hs.632097			F/GE
<i>GJA1</i>	Hs.74471			F/GE
<i>CXCL12</i>	Hs.522891	F		GE
<i>CXCR4</i>	Hs.593413			F/GE
<i>CXCR7</i>	Hs.471751		F	GE
<i>IFIT5</i>	Hs.252839	F/GE		
<i>LGALS1</i>	Hs.445351			F/GE
<i>MSX1</i>	Hs.621772		F	GE
<i>MX1</i>	Hs.517307	F/GE		
<i>MX2</i>	Bt.8143	F/GE		
<i>NPY</i>	Hs.1832			F/GE
<i>PLAC8</i>	Hs.546392	F/GE		
<i>PTN</i>	Hs.371249	F/GE		
<i>RSAD2</i>	Hs.17518	F/GE		
<i>SCARA5</i>	Hs.591833			F/GE

Genes regulated at 30 min or 2 h were considered as early interferon- τ (IFNT)-induced genes. Genes regulated at 24 h were considered as IFNT-regulated genes. The regulation in each endometrial cell type is indicated. F, fibroblast; GE, glandular epithelium.

stromal expression in cyclic cows to a luminal and glandular epithelium expression in pregnant cows. *PTN* was undetectable in endometrial glands and mainly localized in stroma cells. *NPY* was the only gene whose transcript localized in stroma cells appeared to be strongly downregulated by pregnancy.

DISCUSSION

To better understand the biological processes leading to a successful or aborted pregnancy, the molecular and cellular events regulating early conceptus-endometrium interactions need to be investigated. Ruminants have recently been described as animal models relevant for the study of apposition and attachment at implantation (55). Gene expression profiles of the bovine C and IC areas from pregnant or cyclic animals were compared at *day 20* of the estrous cycle to unravel the biological processes, pathways, and networks contributing to this crucial step of pregnancy. Furthermore, additional studies were carried out on a selection of genes to establish the cellular localization of their expression in the endometrium as well as their direct regulation by IFNT, the major signal of pregnancy recognition in ruminants.

Comparison to Related Studies in Cattle and Sheep

In the last few years, several studies focusing on the preimplantation (6, 34, 51) have been carried out in ruminants. Using monozygotic twin cows and in vitro produced embryos at *day 18* of pregnancy, Klein et al. (51) investigated the genes upregulated in the pregnant endometrium (87 genes). A similar study using artificially inseminated cows was carried out by Bauersachs et al. (6). Both studies focused on the IC area, therefore eliminating the possibility of analyzing the biological events taking place in the C area. By comparing our results with these studies, we found a partial overlap of the DEG displaying a similar up- or downregulation of expression, including *B2M*, *IFIT3*, *ISG15*, *MX1*, *OAS1*, *OXTR*, *PTGS2*, *RSAD2*, *STAT1*, and *UBE1L*. Gray et al. (34) analyzed the effect of IFNT, progesterone, and pregnancy at *day 14* in the ovine endometrium, without separating the C and IC areas. The overlap of DEG with our study was 11 genes in C and in IC areas. The expression of two genes, *SPARC* (osteonectin) and *VIM*, showed inverse expressions, namely upregulated in sheep but strongly downregulated in cattle. Four genes were identified across the four studies, all known to be IFN-regulated genes: *B2M*, *MX1*, *RSAD2*, and *IFITM3*. Overall, the comparison between former studies with our work has high-

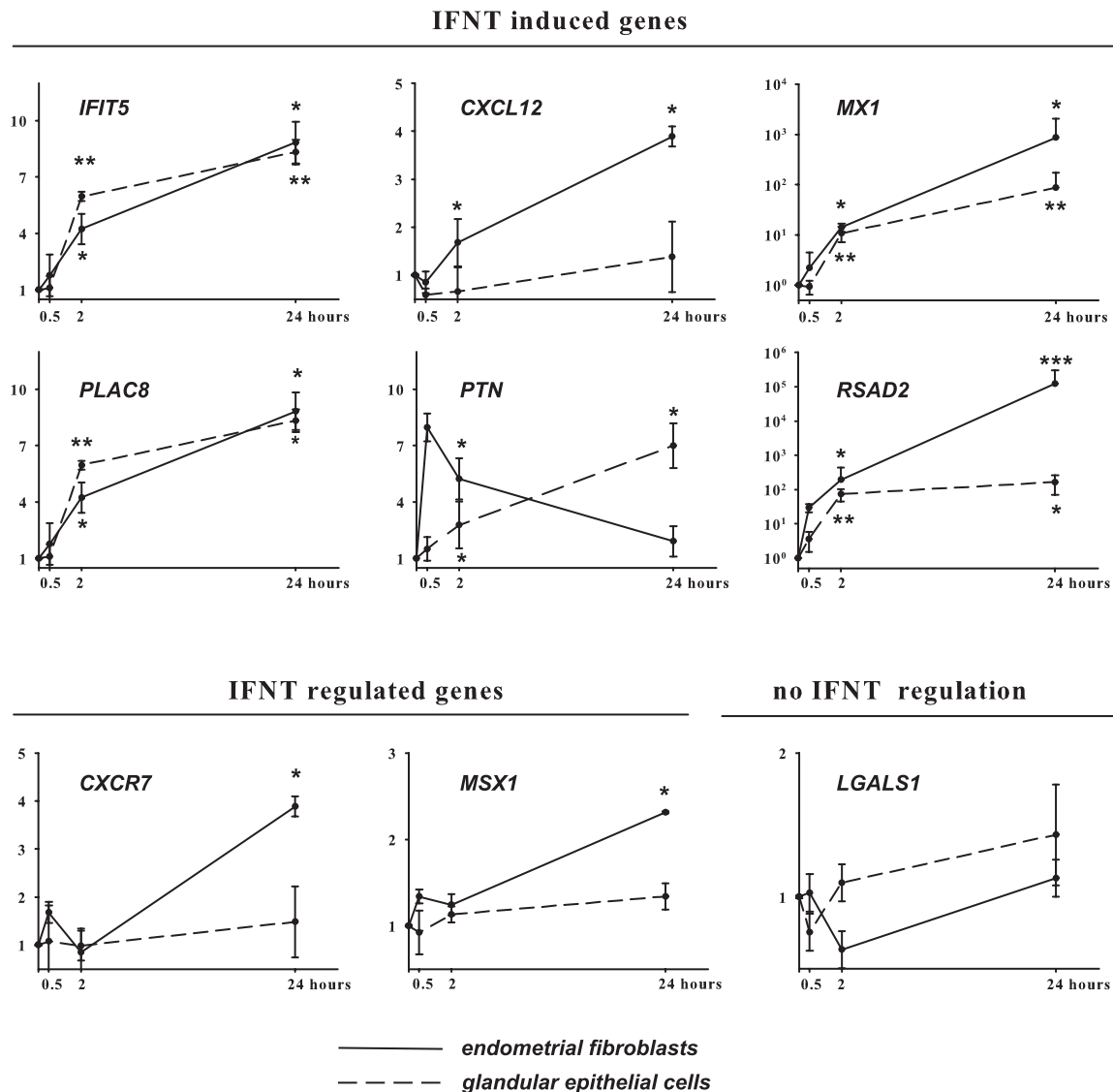


Fig. 2. Regulation of gene expression by interferon- τ (IFNT) in bovine endometrial primary cell cultures. Genes were selected on the basis of their differential expression (AI vs. estrous cycle). Gene expression was quantified by real-time RT-PCR using RPL19 as the housekeeping gene. Bovine endometrial fibroblasts (solid line) or glandular epithelial cells (dashed line) were incubated with IFNT for 30 min, 2 h, or 24 h. Control cells were left untreated and harvested at each incubation time. Fold increase was calculated as [IFNT-treated cells/untreated cells] ratio at 30 min, 2 h, or 24 h. Data are means \pm SD; ns, nonsignificant. * P < 0.05, ** P < 0.01, *** P < 0.001.

lighted a common set of endometrial genes whose expression is affected during the peri-implantation process, especially in cattle. Very interestingly, some of the DEG we identified at implantation (e.g., *GPX3*, *HSPA1A*, *MSX1*, *STC1*) were previously reported to be differently regulated in the C and the IC areas collected throughout bovine pregnancy (45, 82). Those genes may represent the base for future and deeper investigation during the placentation process. However, numerous additional factors related to various biological functions were found to be regulated in our study, thanks to the use of a bovine oligonucleotide array that included many genes associated with early growth and development (26, 61, 63).

Cell Adhesion and Endometrial Remodeling at Implantation

In the context of cell adhesion, we found many integrins affected by pregnancy including *ITGAV*, *ITGA7*, *ITGA8*,

ITGB4, *ITGB1BP1*, and *ADAMDEC1* but also ligands such as *FNI*. As previously observed during the peri-implantation period in goat (30), bovine integrins appeared to be weakly up- or downregulated in either endometrial area. Integrins represent a family of cell surface glycoprotein receptors that mediate cellular adhesion (31). During implantation, integrins bind extracellular matrix (ECM) ligands, modifying the cytoskeleton organization and modulate cell growth and differentiation. *ITGB4* and *ITGAV* were found to be regulated by pregnancy in cattle (this study), but they were shown to be constitutively expressed in human endometrial luminal epithelium (2). An altered expression of integrins (especially a delayed *ITGB3* expression) was correlated with causes of infertility in human (56, 57). In sheep, *ITGAV* and some others integrins are constitutively expressed in both luminal epithelium and trophoblast (79). The expression of these integrins is constitutive

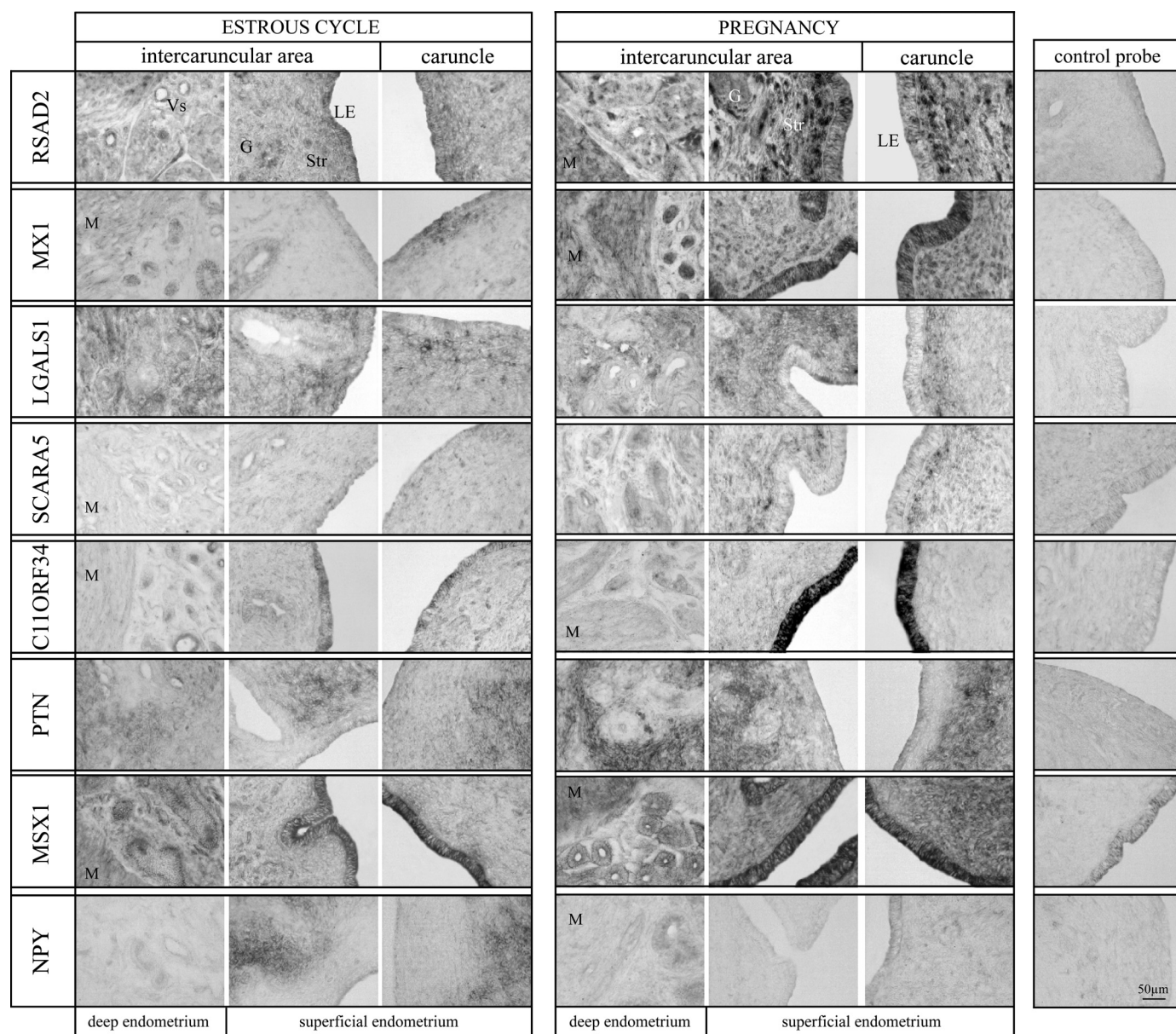


Fig. 3. Cellular localization of gene expression by in situ hybridization in cyclic or pregnant bovine endometrium. Endometrium was sampled at *day 20* of the estrous cycle or pregnancy. Endometrial sections were prepared and hybridized with labeled cRNA sense or antisense probes prepared from linearized plasmid cDNA clones. The corresponding negative controls (sense probes) are shown at *right*. G, glandular epithelium; LE, luminal epithelium; M, myometrium; Str, stroma; Vs, blood vessel. Bar scale: 50 μ m.

and is not influenced by the pregnancy or the conceptus. Likewise, in cattle *ITGAV* is not implicated in the trophoblast-endometrial cross talk (50). Consequently, it seems that implantation in ruminants is more associated to the regulation of integrin ligands or other ECM proteins (e.g., galectin, fibronectin) than to the regulation of integrins themselves.

In our analyses, other proteins of the glycocalyx were found to be regulated by pregnancy but not reported before, such as *PARP14* (also known as *BALB2*) and *PI-GLPDI*, both strongly upregulated at implantation in the C areas. The ADP-ribosylation process is involved in membrane trafficking and actin cytoskeleton (1) and could be part of the trophoblast-endometrial adhesion process. Interestingly, whereas *MUC1* was not significantly altered by pregnancy, *MUC13* was upregulated

(33-fold) in bovine C areas. As mucin proteins contain glycans that are recognized by the blastocyst (2), *MUC13* could be required for the adhesion between the trophoblast and the endometrial luminal epithelium in cattle.

ECM remodeling has been analyzed in human pathological pregnancy (4), in the murine developing neonatal uterus (42), and in ruminants (75, 87). Matrix metalloproteinases (MMP) induce ECM degradation and are regulated by tissue inhibitors of MMP (TIMP) that could also act as proliferation and differentiation factors in a paracrine/autocrine mechanism (15). In our data, the expression of *MMP2* was downregulated in the C areas at implantation, whereas *TIMP2* increased in both C and IC areas as previously reported (39). Other MMPs such as *MMP7* and *TIMP1* showed a minor decreased expression in the

Table 4. Distribution of selected genes for mRNA expression in bovine endometrium by *in situ* hybridization

Gene	Physiological Status	Luminal Epithelium	Subluminal Stroma	Stroma	Glands	Endothelium
RSAD2	estrous cycle	+	+	+	+	+
	pregnancy	++	+++	++	+	+
MX1	estrous cycle	-/+	—	—	+	—
	pregnancy	+++	++	++	++	—
LGALS1	estrous cycle	+	++	+	-/+	—
	pregnancy	-/+	++	+	—	—
SCARA5	estrous cycle	+	+	+	+	—
	pregnancy	-/+	+	++	+	—
C11ORF34	estrous cycle	+	+	+	+	++
	pregnancy	++++	+	+	+	+
PTN	estrous cycle	—	+	+	—	—
	pregnancy	—	-/+	++	—	—
MSX1	estrous cycle	+	+	+	+	—
	pregnancy	++	++	++	+	—
NPY	estrous cycle	—	—	+++	+	—
	pregnancy	—	—	+	+	—

—, No staining; +, weak staining; ++, pronounced staining; +++, intense staining; +++++, very intense staining.

IC areas. The decrease in *MMP7* expression was reported in women with recurrent implantation failure compared with women with normal fertility, implying a potential role of this factor in implantation (81). The regulation of *MMP7* during implantation has never been reported in the bovine endometrium, and this factor could constitute a new target factor in the analysis of endometrium remodeling and ECM degradation.

The C vs. IC expression profiles of pregnant cows showed genes whose products are involved in endometrial cell remodeling such as *GJA1*. In cattle, *GJA1* was localized in the caruncular connective tissue during pregnancy (72), and the present study indicated an IFNT-independent regulation in primary stroma or glandular cell cultures. Connexins were shown to be regulated by ovarian steroids hormones in rat (35), and the endometrial expression of *GJA1* was recently reported to be altered by somatic cell nuclear transfer (SCNT) embryos in recipient cows during implantation (7, 63). In mice, a *Gja1* knockout model demonstrated the essential function of the protein for decidual response and angiogenesis during implantation (54). Identifying the conceptus-related factors that regulate *GJA1* in the endometrium may help in better understanding the contribution of this connexin to the establishment of pregnancy in mammals.

In the C vs. IC profiles of pregnant cows, *LGALS1* appeared to be slightly upregulated in the C areas at implantation. The galectin family is known to be expressed in the mammalian endometrium and would be required for the correct conceptus-endometrial interactions by modulating cell growth, cell adhesion, and apoptosis as reported in cancer progression (53). Several galectins have been shown to take part to the conceptus attachment onto the endometrium, such as *LGALS15* in sheep and goats (58) or *LGALS9* in human (73). During the bovine estrous cycle, the expression of *LGALS1* decreased (68), and the present report indicated that *LGALS1* was not an IFNT-regulated gene. In pregnant animals, *LGALS1* was expressed in stroma cells, very weakly present in the luminal epithelium, and absent in the endometrial glands. In human (10, 46) as well as in mice (13), *LGALS1* was found to be upregulated during the window of implantation. Therefore, from the data published during the estrous cycle (68) or at implantation (this study), a discrepancy in *LGALS1* expression between bovine

and other species does exist and would be worth investigating, keeping the differences in implantation and placentation in mind.

In our data, the expression of a gene encoding the orphan protein *C11ORF34* (also known as *PLET1*; 91) was found to be dramatically increased in the C areas at implantation in keeping with a very intense staining displayed by the luminal epithelium but not by the endometrial glands of pregnant animals. The expression of *C11ORF34* was also detected in the stroma cells *in vivo*, but not affected by IFNT *in vitro*. Very interestingly, *C11ORF34* was recently shown to be affected in the endometrium of pregnant cows recipient for SCNT embryos compared with AI or IVF pregnant cows during the implantation phase (7, 63). We assume that this newly reported factor could be involved in the luminal epithelium-trophoblast interactions during the implantation process and could play a pivotal role in placental development.

Chemokines and Angiogenic and Immune Factors in the Bovine Endometrium

A successful pregnancy implies the establishment of a correct vascular supply to allow the growth and development of the conceptus but also to protect the fetal allograft from rejection by the maternal immune system. Several factors related to the immune response as well as vascular function were found to be differentially expressed in our microarray analyses.

Chemokine genes *CXCL2*, *CXCL12* (also known as *SDF1*), *CXCL14*, and *CX3CL1* and chemokine receptor genes *CXCR4*, *CXCR6*, and *CXCR7* were clearly affected by pregnancy. Chemokines constitute a super family of secreted factors related to cytokines that display chemotactic activity by targeting specific leukocyte populations (21). In the present study, *CXCR4* was upregulated by pregnancy in the IC areas, while its ligand *CXCL12* was downregulated in the C areas but still more expressed than in the IC areas. *CXCL12* and *CXCR7* were classified as new IFNT target genes in primary cultured stroma cells. The biological functions of chemokines have been analyzed at the maternal-fetal interface during implantation and placentation in human (23, 38). *CXCR4* has been reported to

be upregulated during the window of implantation in vivo (24) and by hypoxia in vitro (60, 77). In sheep, several chemokines such as CXCL9 and CXCL10 would be involved in the attachment of the conceptus to the endometrium during the peri-implantation period (44). Therefore, the contribution of the chemokine-related factors and their receptors to the implantation process in cattle is potentially as crucial as previously described in other mammalian species. Their biological functions would deserve to be analyzed in the context of angiogenesis regulation, hypoxia, and immune cells distribution, three major biological pathways found to be affected by pregnancy in the bovine endometrium.

Among the DEG downregulated upon pregnancy, a marked reduction of *NPY* expression was detected in both C and IC areas, mainly occurring in the endometrial stroma cells. *NPY* is a neuronal factor involved in the control of feeding behavior, circadian rhythms, sexual function, anxiety responses, and blood flow (84). In the murine uterus, the expression of *Npy* increased in both luminal and glandular epithelia during the peri-implantation period (90). In the guinea pig, *NPY* was shown to induce contraction of uterine arteries (49), and pregnancy was associated with the reduction of *NPY* endometrial expression (28). In human, a significant decrease of *NPY* was also described in pregnant women but less pronounced in patients with pre-eclampsia (29). Thus, at implantation, the drop of *NPY* expression could promote the vasorelaxation of uterine arteries to increase the uterine blood flow of the pregnant uterus necessary for a correct fetomaternal crosstalk.

In the context of the innate immunity, *SCARA5*, a new member of the scavenger receptors family (47, 76) was found to be upregulated in the IC areas of pregnant cows. *Scara5* was shown to be expressed in the epithelial cells of various murine tissues but not in the uterus (47, 76). In situ hybridization showed a stromal and, to a lesser extent, glandular localization of *SCARA5* in cyclic endometrium. Upon pregnancy, a pronounced staining of the subluminal cell population appeared, likely related to immune cell populations. Our in vitro data showed that *SCARA5* is not regulated by IFNT. The regulation of *SCARA5* in the context of pregnancy is described for the first time in the present work, but the biological functions of this scavenger receptor will require additional studies.

ISG in the Bovine Endometrium

A set of genes strongly induced by pregnancy was identified, mainly in the C areas. Some of these genes are already known to be ISG, and their regulation reflects the endometrial response to IFNT, the major signal of maternal recognition secreted by the bovine trophoctoderm from day 12 to day 24 of pregnancy (27). The contribution of IFNT to the regulation of pregnancy-regulated DEG was determined in vitro in primary bovine endometrial cell lines.

New insights on previously known IFNT-stimulated genes. As previously reported in cattle (6, 63), *RSAD2*, *MX1*, *MX2*, *IFIT3*, *IFIT5*, *ISG15*, *DTX3L*, *UBE1L*, *IFITM3*, and *STAT1* were found in our analyses. Other known ISG (19, 32), such as interferon regulatory factor *IRF-1*, *-2*, and *-6*, were also affected by pregnancy. *RSAD2* is produced during a viral infection in response to interferons to limit viral replication and to modulate adaptive immunity (40). In our in situ hybridization data, *RSAD2* mRNA was expressed in the luminal epithelium,

the stroma, and to a lesser extent in the glandular epithelium, the myometrium, and the endothelium of blood vessels. *RSAD2* was strongly and rapidly induced by IFNT in both fibroblasts and glandular epithelial cells. As suggested for the ovine ortholog (78), *RSAD2* could act as an immunomodulatory factor preventing viral infection of the uterus during the critical stage of implantation.

Myxovirus resistance proteins are type I interferon-induced antiviral proteins and belong to the GTPase family (37). In keeping with the analysis of *MX* expression in cyclic and pregnant cows during preimplantation (41), *MX1* and *MX2* transcripts were strongly upregulated by pregnancy in the present study. In addition, we reported the induction of *MX1* and *MX2* by IFNT with a similar profile in bovine cultured fibroblast or glandular epithelial cells. *MX1* was localized in various endometrial cell types including an intense staining in the luminal epithelium and the endometrial glands at implantation. Interestingly, the expression of *RSAD2* and *MX1* IFNT-induced genes was not limited to the stroma or the glandular epithelium but was also detectable in the luminal epithelium at implantation. On the basis of the current working model for IFNT actions in sheep, *IRF2* expression in luminal epithelium appears to restrict IFNT induction of many ISG to stroma and glandular epithelium of the uterus (8). Our data raise the question of the appropriateness of the working model in cattle as well as the identity of the endometrial and/or other embryonic factors that could contribute to the regulation of these ISG.

Identification of new IFNT-induced or -regulated genes. In the present study, *PTN* (also known as *HBGF8*), a member of a family of secreted growth/differentiation cytokines (59), was found to be an IFNT-induced gene in cultured fibroblasts but an IFNT-regulated gene in the glandular epithelial cells. *PTN* was stimulated by pregnancy, displaying a higher expression in the C than in the IC areas, a similar result to the increase reported during decidualization in murine implantation sites (5). In addition to its angiogenic properties (89), it has been demonstrated that *PTN* controls the Wnt/ β catenin pathway by increasing the tyrosine phosphorylation of β -catenin (67). Considering the predominant caruncular expression for *PTN* in the bovine endometrium, this factor could participate to the proliferation of stroma cells, to the fetal-maternal cross talk by increasing angiogenesis and/or to the decidualization-like process described in ovine uterine cells (48).

Among the IFNT-regulated genes newly identified in the present study, very little is known about *PLAC8*. In cattle, this gene was first reported in a transcriptomic analysis of blastocysts where *PLAC8* was shown to be upregulated in biopsies from blastocysts resulting in calf delivery compared with resorption (25). In bovine endometrium, *PLAC8* was upregulated in pregnant cows compared with the cyclic ones (51). In our work, the upregulation of *PLAC8* was confirmed in the pregnant C areas. This factor appeared as a new IFNT-induced gene in both fibroblasts and glandular epithelial cells, but the biological functions of *PLAC8* are currently unknown.

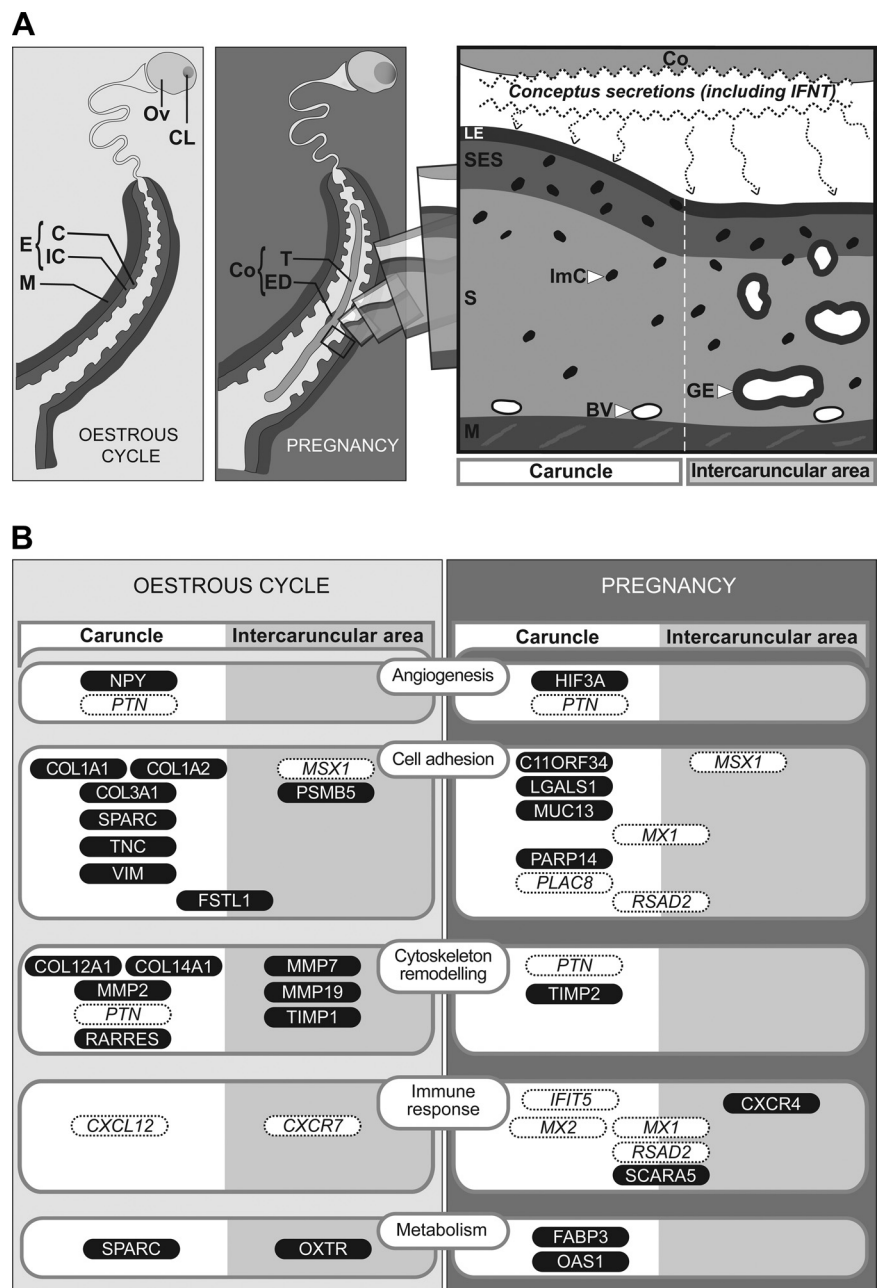
The transcription factor *MSX1* (also known as *HOX7-1*) was found to be an IFNT-regulated gene in endometrial stromal cells. As formerly described at days 56–64 of pregnancy (82), *MSX1* presented a higher expression level in the IC than in the C endometrial areas, and we detected an intense cellular staining for *MSX1* mRNA in the luminal epithelium and the

stroma at implantation. An opposite situation was described in the whole murine uterus including the luminal uterine epithelium, where the expression of *Msx1* was downregulated with the onset of implantation (70) independently from the presence of the implanting blastocyst (16). Collectively, the current data underline a species-specific regulation of *MSX1* at the cellular level at implantation whose biological meaning will require further investigation.

The present study has provided the first data showing the differential impact of pregnancy on the caruncular and intercaruncular endometrial transcriptome in cattle. The impact on the immune response function was more prominent in the C areas, whereas the regulation of metabolism function was more affected in the IC areas, likely in keeping with the secretory activities of the endometrial glands exclusively localized in this

endometrial zone. Therefore analyzing both C and IC areas helped in generating a comprehensive and integrative view of the molecular mechanisms taking place during the implantation phase (Fig. 4). New factors (e.g., *C11ORF34*, *SCARA5*) strongly affected by pregnancy were also identified that had never been investigated in the mammalian endometrium. The biological functions of these bovine orthologs in the regulation of implantation and the establishment of the very early steps of placentation clearly deserve investigation taking the sensor properties of the endometrium into account (63). Our results have also shed light on new IFNT target genes in nonimmune endometrial cells that may account for the diversity of IFNT actions in the bovine endometrium and represent targets for future studies in ruminants. Eventually, the list of endometrial genes provide new candidates for a comparative investigation

Fig. 4. Schematic representation of a selection of DEG in the bovine endometrial areas at *day 20* of the estrous cycle or pregnancy. **A**. Overview of the bovine uterine horn ipsilateral to the corpus luteum (CL) at *day 20* postestrous (*left*) or at *day 20* of pregnancy (implantation, *middle*). A detail of the pregnant uterine horn is magnified at *right*. Ov, ovary; E, endometrium; M: myometrium; Co, conceptus; T, trophoblast; ED, embryonic disc; LE, luminal epithelium; SES, subepithelial stroma; GE, glandular epithelium; S, stroma; IMC, immune cell; PV, blood vessel. **B**: the cyclic or pregnant endometrium is subdivided into C and IC areas. Each DEG is represented by a capsule-shaped box and placed in the major biological functions it was shown to belong to. The position of each DEG in the figure results from the compilation of the estrous cycle vs. pregnancy and caruncle vs. intercaruncular area transcriptomic analyses as well as qRT-PCR data when available. Each DEG was first placed according to the differential expression determined from the estrous cycle vs. pregnancy analyses (see DISCUSSION and Table 1). When no statistical difference between estrous cycle and pregnancy was detected, the DEG was placed in each physiological state. Then the position in each endometrial area was deduced from the caruncles vs. intercaruncular areas analyses (see Table 2). When no statistical difference between the C and the IC areas was detected, the DEG was placed at the boundary between the 2 endometrial areas. The names of the IFNT-induced or IFNT-regulated DEG (see Table 3) are written in italics and placed inside a white box with a dashed frame.



between cattle and other mammalian species to determine how the expression, the regulation, the cell localization, and the biological functions are associated with differences in the implantation process described in mammals.

ACKNOWLEDGMENTS

We warmly thank Philippe Bardou (INRA, SIGENAE, Toulouse, France) for the management of the GEO data. We are grateful to J. J. Daudin, M. L. Martin-Magniette, N. Coqué, A. Bar-Hen, and C. Bigorne (UMR 518 AgroParisTech-INRA, Paris, France) for help in the experimental designs and statistical analyses. We thank R. Oliveira (UIUC, US) for helping in the Metacore software use, D. Krauss for animal management, G. Chaouat and M. Guillomot for helpful discussions, as well as C. Poirier for helping in the preparation of the figures.

Author contributions: N. Mansouri-Attia, J. Aubert, and O. Sandra designed the research; N. Mansouri-Attia, P. Reinaud, C. Giraud-Delville, G. Taghouti, L. Galio, and O. Sandra performed the research; N. Mansouri-Attia, J. Aubert, P. Reinaud, and O. Sandra analyzed the data; R. E. Everts and H. A. Lewin provided tools and annotation, R. E. Everts, S. Degrelle, I. Hue, X. C. Tian, H. A. Lewin, J.-P. Renard, and O. Sandra contributed to the microarray development. N. Mansouri-Attia and O. Sandra wrote the paper. Every author read and annotated the paper.

Access links: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=1fabfeumeskkatg&acc=GSE13728> and <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=fjyzhgqwckyyarm&acc=GSE13664>.

Present address for R. E. Everts: SEQUENOM, Inc., 3595 John Hopkins Court, San Diego, CA 92121.

GRANTS

This research was partially supported by the European Network of Excellence on Embryo Implantation Control funded by the European Union Grant LSHN-CT-2004-512040. N. Mansouri-Attia is a Ministry of Education, Research and Technology fellow.

REFERENCES

- Ame JC, Spenlehauer C, de Murcia G. The PARP superfamily. *Bioessays* 26: 882–893, 2004.
- Aplin JD. Adhesion molecules in implantation. *Rev Reprod* 2: 84–93, 1997.
- Atkinson BA, King GJ, Amoroso EC. Development of the caruncular and intercaruncular regions in the bovine endometrium. *Biol Reprod* 30: 763–74, 1984.
- Bai SX, Wang YL, Qin L, Xiao ZJ, Herva R, Piao YS. Dynamic expression of matrix metalloproteinases (MMP-2, -9 and -14) and the tissue inhibitors of MMPs (TIMP-1, -2 and -3) at the implantation site during tubal pregnancy. *Reproduction* 129: 103–113, 2005.
- Bany BM, Schultz GA. Pleiotrophin messenger ribonucleic acid levels increase in mouse endometrial stromal cells during decidualization. *Endocrinology* 142: 955–958, 2001.
- Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Weni-gerkind H, Vermehren M, Sinowatz F, Blum H, Wolf E. Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. *Reproduction* 132: 319–331, 2006.
- Bauersachs S, Ulbrich SE, Zakhartchenko V, Minten M, Reichenbach M, Reichenbach HD, Blum H, Spencer TE, Wolf E. The endometrium responds differently to cloned versus fertilized embryos. *Proc Natl Acad Sci USA* 106: 5681–5686, 2009.
- Bazer FW, Spencer TE, Johnson GA. Interferons and uterine receptivity. *Semin Reprod Med* 27: 90–102, 2009.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc B* 57: 289–300, 1995.
- Blois SM, Ibarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 13: 1450–1457, 2007.
- Burton GJ. Human and animal models: limitations and comparisons. In: *The First Twelve Weeks of Gestation*, edited by Barnea ER, Husta J, Janiaux E. Berlin: Springer-Verlag, p. 469–485, 1992.
- Cammas L, Reinaud P, Dubois O, Bordas N, Germain G, Charpigny G. Identification of differentially regulated genes during elongation and early implantation in the ovine trophoblast using complementary DNA array screening. *Biol Reprod* 72: 960–967, 2005.
- Choe YS, Shim C, Choi D, Lee CS, Lee KK, Kim K. Expression of galectin-1 mRNA in the mouse uterus is under the control of ovarian steroids during blastocyst implantation. *Mol Reprod Dev* 48: 261–266, 1997.
- Constant F, Guillomot M, Heyman Y, Vignon X, Laigre P, Servely JL, Renard JP, Chavatte-Palmer P. Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. *Biol Reprod* 75: 122–130, 2006.
- Curry TE Jr, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev* 24: 428–465, 2003.
- Daikoku T, Song H, Guo Y, Riesewijk A, Mosselman S, Das SK, Dey SK. Uterine Msx-1 and Wnt4 signaling becomes aberrant in mice with the loss of leukemia inhibitory factor or Hoxa-10: evidence for a novel cytokine-homeobox-Wnt signaling in implantation. *Mol Endocrinol* 18: 1238–1250, 2004.
- Degrelle SA, Campion E, Cabau C, Piumi F, Reinaud P, Richard C, Renard JP, Hue I. Molecular evidence for a critical period in mural trophoblast development in bovine blastocysts. *Dev Biol* 288: 448–460, 2005.
- Delmar P, Robin S, Daudin JJ. VarMixt: efficient variance modelling for the differential analysis of replicated gene expression data. *Bioinformatics* 21: 502–508, 2005.
- De Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, Silverman RH, Williams BR. Functional classification of interferon-stimulated genes identified using microarrays. *J Leukoc Biol* 69: 912–920, 2001.
- Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. *Endocr Rev* 25: 341–373, 2004.
- Dimitriadis E, White CA, Jones RL, Salamonsen LA. Cytokines, chemokines and growth factors in endometrium related to implantation. *Hum Reprod* 11: 613–630, 2005.
- Diskin MG, Morris DG. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Domest Anim* 43, Suppl 2: 260–267, 2008.
- Dominguez F, Pellicer A, Simon C. The chemokine connection: hormonal and embryonic regulation at the human maternal-embryonic interface—a review. *Placenta* 24, Suppl B: S48–S55, 2003.
- Dominguez F, Galan A, Martin JJ, Remohi J, Pellicer A, Simon C. Hormonal and embryonic regulation of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in the human endometrium and the human blastocyst. *Mol Hum Reprod* 9: 189–198, 2003.
- El-Sayed A, Hoelker M, Rings F, Salliew D, Jennen D, Tholen E, Sirard MA, Schellander K, Tesfaye D. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. *Physiol Genomics* 28: 84–96, 2006.
- Everts RE, Chavatte-Palmer P, Razzak A, Hue I, Green CA, Oliveira R, Vignon X, Rodriguez-Zas SL, Tian XC, Yang X, Renard JP, Lewin HA. Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. *Physiol Genomics* 33: 65–77, 2008.
- Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW, Roberts RM. Expression of trophoblastic interferon genes in sheep and cattle. *Biol Reprod* 43: 210–218, 1990.
- Fried G, Hökfelt T, Terenius L, Goldstein M. Neuropeptide Y (NPY)-like immunoreactivity in guinea pig uterus is reduced during pregnancy in parallel with noradrenergic nerves. *Histochemistry* 83: 437–442, 1985.
- Fried G, Hökfelt T, Lundberg JM, Terenius L, Hamberger L. Neuropeptide Y and noradrenaline in human uterus and myometrium during normal and pre-eclamptic pregnancy. *Hum Reprod* 1: 359–364, 1986.
- García P, Nieto A, Sánchez MA, Pizarro M, Flores JM. Expression of alpha_v, alpha₄, alpha₅ and beta₃ integrin subunits, fibronectin and vitronectin in goat peri-implantation. *Anim Reprod Sci* 80: 91–100, 2004.
- Giancotti FG, Ruoslahti E. Integrin signalling. *Science* 285: 1028–1032, 1999.
- Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ, Ott TL. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *J Dairy Sci* 90: 274–280, 2007.

33. Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE. Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol Reprod* 64: 1608–1613, 2001.
34. Gray CA, Abbey CA, Beremand PD, Choi Y, Farmer JL, Adelson DL, Thomas TL, Bazer FW, Spencer TE. Identification of endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. *Biol Reprod* 74: 383–394, 2006.
35. Grümmer R, Chwalisz K, Mulholland J, Traub O, Winterhager E. Regulation of connexin26 and connexin43 expression in rat endometrium by ovarian steroid hormones. *Biol Reprod* 51: 1109–1116, 1994.
36. Guillomot M. Cellular interactions during implantation in domestic ruminants. *J Reprod Fertil Suppl* 49: 39–51, 1995.
37. Haller O, Stertz S, Kochs G. The Mx GTPase family of interferon-induced antiviral proteins. *Microbes Infect* 9: 1636–1643, 2007.
38. Hannan NJ, Salamonsen LA. Role of chemokines in the endometrium and in embryo implantation. *Curr Opin Obstet Gynecol* 19: 266–272, 2007.
39. Hashizume K. Analysis of uteroplacental-specific molecules and their functions during implantation and placentation in the bovine. *J Reprod Dev* 53: 1–11, 2007.
40. Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. *Hepatology* 42: 702–710, 2005.
41. Hicks BA, Etter SJ, Carnahan KG, Joyce MM, Assiri AA, Carling SJ, Kodali K, Johnson GA, Hansen TR, Miranda MA, Woods GL, Vanderwall DK, Ott TL. Expression of the uterine Mx protein in cyclic and pregnant cows, gilts, and mares. *J Anim Sci* 81: 1552–1561, 2003.
42. Hu J, Zhang X, Nothnick WB, Spencer TE. Matrix metalloproteinases and their tissue inhibitors in the developing neonatal mouse uterus. *Biol Reprod* 71: 1598–1604, 2004.
43. Humblot P. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. *Theriogenology* 56: 1417–1433, 2001.
44. Imakawa K, Imai M, Sakai A, Suzuki M, Nagaoka K, Sakai S, Lee SR, Chang KT, Echterkamp SE, Christenson RK. Regulation of conceptus adhesion by endometrial CXC chemokines during the implantation period in sheep. *Mol Reprod Dev* 73: 850–858, 2006.
45. Ishiwata H, Katsuma S, Kizaki K, Patel OV, Nakano H, Takahashi T, Imai K, Hirasawa A, Shiojima S, Ikawa H, Suzuki Y, Tsujimoto G, Izaike Y, Todoroki J, Hashizume K. Characterization of gene expression profiles in early bovine pregnancy using a custom cDNA microarray. *Mol Reprod Dev* 65: 9–18, 2003.
46. Jeschke U, Karsten U, Wiest I, Schulze S, Kuhn C, Friese K, Walzel H. Binding of galectin-1 (gal-1) to the Thomsen-Friedenreich (TF) antigen on trophoblast cells and inhibition of proliferation of trophoblast tumor cells in vitro by gal-1 or an anti-TF antibody. *Histochem Cell Biol* 126: 437–444, 2006.
47. Jiang Y, Oliver P, Davies KE, Platt N. Identification and characterization of murine SCARA5, a novel class A scavenger receptor that is expressed by populations of epithelial cells. *J Biol Chem* 281: 11834–11845, 2006.
48. Johnson GA, Burghardt RC, Joyce MM, Spencer TE, Bazer FW, Pfarrer C, Gray CA. Osteopontin expression in uterine stroma indicates a decidualization-like differentiation during ovine pregnancy. *Biol Reprod* 68: 1951–1958, 2002.
49. Jovanovic S, Grbovic L, Jovanovic A. Pregnancy is associated with altered response to neuropeptide Y in uterine artery. *Mol Hum Reprod* 6: 352–360, 2000.
50. Kimmins S, Lim HC, MacLaren LA. Immunohistochemical localization of integrin alpha V beta 3 and osteopontin suggests that they do not interact during embryo implantation in ruminants. *Reprod Biol Endocrinol* 2: 19, 2004.
51. Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H, Wolf E. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. *Biol Reprod* 74: 253–264, 2006.
52. Larionov A, Krause A, Miller W. A standard curve based method for relative real time PCR data processing. *BMC Bioinformatics* 6: 62, 2005.
53. Lau KS, Dennis JW. N-Glycans in cancer progression. *Glycobiology* 18: 750–60, 2008.
54. Laws MJ, Taylor RN, Sidell N, DeMayo FJ, Lydon JP, Gutstein DE, Bagchi MK, Bagchi IC. Gap junction communication between uterine stromal cells plays a critical role in pregnancy-associated neovascularization and embryo survival. *Development* 135: 2659–2668, 2008.
55. Lee KY, DeMayo FJ. Animal models of implantation. *Reproduction* 128: 679–695, 2004.
56. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *J Clin Invest* 90: 188–195, 1992.
57. Lessey BA, Castelbaum AJ. Integrins and endometriosis: fact or artefact? *Hum Reprod* 13: 3578–3580, 1998.
58. Lewis SK, Farmer JL, Burghardt RC, Newton GR, Johnson GA, Adelson DL, Bazer FW, Spencer TE. Galectin 15 (LGALS15): a gene uniquely expressed in the uteri of sheep and goats that functions in trophoblast attachment. *Biol Reprod* 77: 1027–1036, 2007.
59. Li YS, Milogor PG, Chauhan AK, Watson MA, Hoffman RM, Kodner CM, Milbrandt J, Deuel TF. Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. *Science* 250: 1690–1694, 1990.
60. Lima e Silva R, Shen J, Hackett SF, Kachi S, Akiyama H, Kiuchi K, Yokoi K, Hatare MC, Lauer T, Aslam S, Gong YY, Xiao WH, Khu NH, Thut C, Campochiaro PA. The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization. *FASEB J* 21: 3219–3230, 2007.
61. Loor JJ, Everts RE, Bionaz M, Dann HM, Morin DE, Oliveira R, Rodriguez-Zas SL, Drackley JK, Lewin HA. Nutrition-induced ketosis alters metabolic and signalling gene networks in liver of periparturient dairy cows. *Physiol Genomics* 32: 105–116, 2007.
62. MacIntyre DM, Lim HC, Ryan K, Kimmins S, Small JA, MacLaren LA. Implantation-associated changes in bovine uterine expression of integrins and extracellular matrix. *Biol Reprod* 66: 1430–1436, 2002.
63. Mansouri-Attia N, Sandra O, Aubert J, Degrelle S, Everts RE, Giraud-Delville C, Heyman Y, Galio L, Hue I, Yang X, Tian XC, Lewin HA, Renard JP. Endometrium as an early sensor of in vitro embryo manipulation technologies. *Proc Natl Acad Sci USA* 106: 5687–5692, 2009.
64. Martal J, Degryse E, Charpigny G, Assal N, Renaud P, Charlier M, Gaye P, Lecocq JP. Evidence for extended maintenance of the corpus luteum by uterine infusion of a recombinant trophoblast alpha-interferon (trophoblastin) in sheep. *J Endocrinol* 127: R5–R8, 1990.
65. Martal J, Chêne N, Camous S, Huynh L, Lantier F, Hermier P, L'Haridon R, Charpigny G, Charlier M, Chauat G. Recent developments and potentialities for reducing embryo mortality in ruminants: the role of IFN-tau and other cytokines in early pregnancy. *Reprod Fertil Dev* 9: 355–380, 1997.
66. Mary-Huard T, Aubert J, Mansouri-Attia N, Sandra O, Daudin JJ. Statistical methodology for the analysis of dye-switch microarray experiments. *BMC Bioinformatics* 9: 98, 2008.
67. Meng K, Rodriguez-Peña A, Dimitrov T, Chen W, Yamin M, Noda M, Deuel TF. Pleiotrophin signals increased tyrosine phosphorylation of beta catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. *Proc Natl Acad Sci USA* 97: 2603–2608, 2001.
68. Mitko K, Ulbrich SE, Wenigerkind H, Sinowatz F, Blum H, Wolf E, Bauersachs S. Dynamic changes in messenger RNA profiles of bovine endometrium during the estrous cycle. *Reproduction* 135: 225–240, 2008.
69. Mueller O, Lightfoot S, Schroeder A. RNA Integrity Number (RIN) - Standardization of RNA quality control. *Agilent Application Note*, publication number 5989-1165EN, 2004.
70. Pavlova A, Boutin E, Cunha G, Sassoon D. Msx1 (Hox-7.1) in the adult mouse uterus: cellular interactions underlying regulation of expression. *Development* 120: 335–345, 1994.
71. Pfarrer C, Hirsch P, Guillomot M, Leiser R. Interaction of integrin receptors with extracellular matrix is involved in trophoblast giant cell migration in bovine placentomes. *Placenta* 24: 588–597, 2003.
72. Pfarrer CD, Heeb C, Leiser R. Expression of gap junctional connexins 26, 32 and 43 in bovine placentomes during pregnancy. *Placenta* 27: 79–86, 2006.
73. Popovici RM, Krause MS, Germeyer A, Strowitzki T, von Wolff M. Galectin-9: a new endometrial epithelial marker for the mid- and late-secretory and decidual phases in humans. *J Clin Endocrinol Metab* 90: 6170–6176, 2005.
74. Roberts RM, Leaman DW, Cross JC. Role of interferons in maternal recognition of pregnancy in ruminants. *Proc Soc Exp Biol Med* 200: 7–18, 1992.

75. **Salamonsen LA, Nagase H, Woolley DE.** Matrix metalloproteinases and their tissue inhibitors at the ovine trophoblast-uterine interface. *J Reprod Fertil Suppl* 49: 29–37, 1995.
76. **Sarraj MA, McClive PJ, Wilmore HP, Loveland KL, Sinclair AH.** Novel scavenger receptor gene is differentially expressed in the embryonic and adult mouse testis. *Dev Dyn* 234: 1026–1033, 2005.
77. **Schutysse E, Su Y, Yu Y, Gouvy M, Zaja-Milatovic S, Van Damme J, Richmond A.** Hypoxia enhances CXCR4 expression in human microvascular endothelial cells and human melanoma cells. *Eur Cytokine Netw* 18: 59–70, 2007.
78. **Song G, Bazer FW, Spencer TE.** Pregnancy and interferon tau regulate RSAD2 and IFIH1 expression in the ovine uterus. *Reproduction* 133: 285–295, 2007.
79. **Spencer TE, Johnson GA, Bazer FW, Burghardt RC.** Implantation mechanisms: insights from the sheep. *Reproduction* 128: 657–668, 2004.
80. **Spencer TE, Sandra O, Wolf E.** Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. *Reproduction* 135: 165–179, 2008.
81. **Tapia A, Gangi LM, Zegers-Hochschild F, Balmaceda J, Pommer R, Trejo L, Pacheco IM, Salvatierra AM, Henríquez S, Quezada M, Vargas M, Ríos M, Munroe DJ, Croxatto HB, Velasquez L.** Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy. *Hum Reprod* 23: 340–351, 2008.
82. **Ushizawa K, Takahashi T, Hosoe M, Ishiwata H, Kaneyama K, Kizaki K, Hashizume K.** Global gene expression analysis and regulation of the principal genes expressed in bovine placenta in relation to the transcription factor AP-2 family. *Reprod Biol Endocrinol* 5: 17, 2007.
83. **Von Rango U.** Fetal tolerance in human pregnancy—a crucial balance between acceptance and limitation of trophoblast invasion. *Immunol Lett* 115: 21–32, 2008.
84. **Wójcik-Gładysz A, Polkowska J.** Neuropeptide Y-a neuromodulatory link between nutrition and reproduction at the central nervous system level. *Reprod Biol* 6, Suppl 2: 21–28, 2006.
85. **Wolf E, Arnold GJ, Bauersachs S, Beier HM, Blum H, Einspanier R, Fröhlich T, Herrler A, Hiendleder S, Kölle S, Prella K, Reichenbach HD, Stojkovic M, Wenigerkind H, Sinowatz F.** Embryo-maternal communication in bovine - strategies for deciphering a complex cross-talk. *Reprod Domest Anim* 38: 276–289, 2003.
86. **Wooding FB.** The role of the binucleate cell in ruminant placental structure. *J Reprod Fertil Suppl* 31: 31–39, 1982.
87. **Yamada O, Todoroki J, Takahashi T, Hashizume K.** The dynamic expression of extracellular matrix in the bovine endometrium at implantation. *J Vet Med Sci Mar* 64: 207–214, 2002.
88. **Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP.** Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* 30: e15, 2002.
89. **Yeh HJ, He YY, Xu J, Hsu CY, Deuel TF.** Up regulation of pleiotrophin gene expression in developing microvasculature, macrophages, and astrocytes after acute ischemic brain injury. *J Neurosci* 18: 3699–3707, 1998.
90. **Yonaha H, Minoura H, Yoshida T, Takeuchi S, Noda N, Tanaka K, Nishiura R, Kawato H, Toyoda N.** Expression of neuropeptide Y is increased in murine endometrial epithelium during the peri-implantation period under regulation by sex steroids. *Reprod Fertil Dev* 16: 355–361, 2004.
91. **Zhao SH, Simmons DG, Cross JC, Scheetz TE, Casavant TL, Soares MB, Tuggle CK.** PLET1 (C11orf34), a highly expressed and processed novel gene in pig and mouse placenta, is transcribed but poorly spliced in human. *Genomics* 84: 114–125, 2004.

