CALL FOR PAPERS | Comparative Genomics

Gene expression profiles of bovine caruncular and intercaruncular endometrium at implantation

Nadéra Mansouri-Attiia, 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

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 Bressovilliers, Leudeville, France; and 6Center for Regenerative Biology, Department of Animal Sciences, Storrs, Connecticut

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

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 Pttn, Plac8, and Cxcl12 as interferon-τ (IFNT) target genes and Mx1 and Cxcr7 as IFNT-regulated genes, whereas C11orf34 was not an IFNT-regulated gene. Our transcriptomic data provide new 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.

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.
This type I IFN is exclusively produced by the mononuclear trophoderm cells of the elongating conceptus during the peri-implantation period (27). In addition to its antiluteolytic 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 placentaion 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 kit; 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 and 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 transcriptomes 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 aminovalyl-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.i-p-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 [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,
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 μm) 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 2h 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 μg/ml of ovine recombinant IFNT (64). Control cells were left untreated and washed with cold PBS, recovered by scraping, 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 (radial S-adenosyl methionine domain containing 2: 10-fold), MXI (myxovirus “influenza virus” resistance 1, interferon-inducible protein p78: 4.6 10-fold), JSP.1 (MHC Class I JSP.1 similar to human HLA-A: 4 10-fold), FABP3 (fatty acid binding protein 3: 4.2 10-fold), or ISG15 (ISG15 ubiquitin-like modifier: 2.4 10-fold). The most pronounced downregulation of gene expression upon pregnancy was found for NPY (neuropeptide Y: 325-fold), COLIA2 (collagen, type I, alpha 2: 201-fold), LOC5534369 (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 COLIA2 (4.1-fold), OXTR (oxtocin 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 insights 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-
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 metallopeptidase 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 cyclic. 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 $\geq 5$ 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 $\geq 3$ 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 $\geq 5$ genes revealed significant association ($P < 0.01$; Supplementary Table S4) including cell cycle growth and proliferation (120 genes), cellular movement (69 genes), cell death (96 genes), and cellular development (88 genes). Eleven canonical pathways with $\geq 3$ 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 MX1 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

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Unigene ID</th>
<th>Expression</th>
<th>Microarray Fold Δ</th>
<th>Real-time Fold Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11ORF34</td>
<td>Hs.632097</td>
<td>AI</td>
<td>528</td>
<td>28,000‡</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Hs.522891</td>
<td>cycle</td>
<td>9</td>
<td>14*</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Hs.593413</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR7</td>
<td>Hs.471751</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFIT5</td>
<td>Hs.252839</td>
<td>AI</td>
<td>42</td>
<td>9*</td>
</tr>
<tr>
<td>MX1</td>
<td>Hs.517307</td>
<td>AI</td>
<td>4,660</td>
<td>68‡</td>
</tr>
<tr>
<td>MX2</td>
<td>Hs.8143</td>
<td>AI</td>
<td>659</td>
<td>336†</td>
</tr>
<tr>
<td>NPY</td>
<td>Hs.1832</td>
<td>cycle</td>
<td>334</td>
<td>15†</td>
</tr>
<tr>
<td>PLAC8</td>
<td>Hs.546392</td>
<td>AI</td>
<td>182</td>
<td>12‡</td>
</tr>
<tr>
<td>PTN</td>
<td>Hs.371249</td>
<td>AI</td>
<td>11,000</td>
<td>99‡</td>
</tr>
<tr>
<td>RSAD2</td>
<td>Hs.17518</td>
<td>AI</td>
<td>2.2</td>
<td>4*</td>
</tr>
<tr>
<td>SCARA5</td>
<td>Hs.591833</td>
<td>AI</td>
<td>2.2</td>
<td>4*</td>
</tr>
</tbody>
</table>

Fold change (Δ) is expressed as arbitrary units. RPL19 was used as a housekeeping gene. Data are means ± SD; *P < 0.05, †P < 0.01, ‡P < 0.001.
and various genes whose expression is affected by pregnancy, glandular epithelial origin

Table 3. Overview of IFNT-dependent gene regulation in trial expression of two IFNT-induced genes, Situ Hybridization

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Unigene ID</th>
<th>Estrous Cycle</th>
<th>Area Expression</th>
<th>Microarray Fold Δ</th>
<th>Real-time Fold Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C11ORF34</td>
<td>Hs.632097</td>
<td>C</td>
<td>1.9</td>
<td>6*</td>
<td></td>
</tr>
<tr>
<td>CXCL12</td>
<td>Hs.522891</td>
<td>C</td>
<td>3.1</td>
<td>4+</td>
<td></td>
</tr>
<tr>
<td>GJA1</td>
<td>Hs.74471</td>
<td>C</td>
<td>6.1</td>
<td>5†</td>
<td></td>
</tr>
<tr>
<td>LGALS1</td>
<td>Hs.445351</td>
<td>C</td>
<td>3.7</td>
<td>3.7†</td>
<td></td>
</tr>
<tr>
<td>MX1</td>
<td>Hs.621772</td>
<td>IC</td>
<td>4.1</td>
<td>4†</td>
<td></td>
</tr>
<tr>
<td>NPY</td>
<td>Hs.1832</td>
<td>IC</td>
<td>2.1</td>
<td>5†</td>
<td></td>
</tr>
<tr>
<td>PLAC8</td>
<td>Hs.546392</td>
<td>IC</td>
<td>1.7</td>
<td>1.7†</td>
<td></td>
</tr>
<tr>
<td>PTN</td>
<td>Hs.371249</td>
<td>C</td>
<td>7.7</td>
<td>2.8</td>
<td></td>
</tr>
</tbody>
</table>

Fold change (Δ) is expressed as arbitrary units. RPL19 was used as a housekeeping gene. Data are means ± SD; C, carnuncles; IC, intercarnuncular 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, MX1, 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 MX1 in the luminal epithelium of pregnant cows. C11ORF34 showed a clear shift from a 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. 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, RASD2, 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 (osteonecin) 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, RASD2, and IFITM3. Overall, the comparison between former studies with our work has high-
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 FN1. 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. 2. Regulation of gene expression by interferon-γ (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 ± SD; ns, nonsignificant. *P < 0.05, **P < 0.01, ***P < 0.001.
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-GPLD1, 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
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 cultured 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 placenta- tion 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 placenta- tion in human (23, 38). CXCR4 has been reported to

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Physiological Status</th>
<th>Luminal Epithelium</th>
<th>Subluminal Stroma</th>
<th>Stroma</th>
<th>Glands</th>
<th>Endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSAD2</td>
<td>estrous cycle</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MX1</td>
<td>pregnancy</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LGALS1</td>
<td>estrous cycle</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>SCARA5</td>
<td>estrous cycle</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>C11orf34</td>
<td>estrous cycle</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PTN</td>
<td>estrous cycle</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>MSX1</td>
<td>estrous cycle</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NPY</td>
<td>estrous cycle</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

- No staining; +, weak staining; ++, pronounced staining; ++++, intense staining; ++++, very intense staining.
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 feto-maternal cross talk.

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 trophoderm 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, UBEIL, 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 adaptative 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, IFR2 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 genes 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 ureterine 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; C, 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 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 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. AgroParisTech-INRA, Paris, France) for help in the experimental designs and for the management of the GEO data. We are grateful to J. J. Daudin, M. L. Court, San Diego, CA 92121.

Author contributions: N. Mansouri-Attia, J. Aubert, and O. Sandra designed the research; N. Mansouri-Attia, P. Reinaud, C. Giraud-Delville, G. Taghouti, and O. Sandra 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.


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


Physiol Genomics • VOL 39 • www.physiolgenomics.org


