Development of an Uteroplacental Microarray and Analysis of the Expression Profile of Placental Genes during Bovine Gestation

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Abstract: Microarray technology provides new insights into the field of reproductive biology as well as other fields of medicine and biology. We fabricated a bovine uteroplacental cDNA microarray and investigated the key factors involved in the establishment and maintenance of gestation. Microarray-based global gene expression analyses on bovine placenta and trophoblast cells suggest that the expression profiles of specific genes depend on the cells and tissues in which they are expressed as well as the time of the gestation period. This custom-made microarray revealed that trophoblast-specific genes such as placental lactogen, pregnancy-associated glycoproteins, prolactin-related proteins, and those of the sulfotransferase family were mainly expressed in the trophoblast giant cells and that their expression increased as gestation progressed. The expression of these genes was extremely temporal and spatial. Further, the expression of the transcription factor AP-2 increased in the trophoblast giant cells as gestation progressed. Thus, the AP-2 gene family may play a major role in regulating the functions of bovine trophoblast giant cells. Microarray technology provides information not only on thousands of genes simultaneously but also on their regulatory mechanisms in cells. Bioinformatic tools could greatly aid biological and biomedical research; therefore, active efforts must be undertaken in this field.

Key words: Custom-made cDNA microarray, Bovine, Gestation, Bioinformatics

Introduction

Viviparity is an evolutionary feature of mammals. An essential feature of mammalian pregnancy is the presence of a fetomaternal interface, i.e., the placenta. The placenta connects the mother and the fetus and plays a crucial role in fetal growth and the maintenance of pregnancy. However, despite a large body of research that has been conducted to elucidate the mechanisms underlying implantation, placentation, fetogenesis, and delivery, the detailed mechanisms remain unclear. The complex cell-to-cell communication occurring during gestation, which extends for approximately 280 days in cattle, is modulated by hormones, cytokines, and growth factors. Placentomes in cattle are composed of the fetal cotyledary and maternal caruncular tissues [1]. Establishment of the placenta involves successive processes, namely, implantation and placentation. During these processes, fetal trophoblast cells invade the maternal endometrium. This is a complex process, and successful implantation requires appropriate communication between the embryo and the maternal endometrium. The onset of gestation is orchestrated by various molecules that are synthesized and secreted by the trophoblast cells [2–4]. In cattle, the maternal and fetal tissues are distinguished as the cotyledon, which is
the fetal component, and the caruncle, which is the maternal component [1]. The main reason for reproductive wastage in farm animals is early embryonic loss [2, 4]. Placental dysfunction leads to premature termination of pregnancy. These conditions eventually decrease animal productivity. To eliminate or reduce the effects of these factors, more detailed information is required regarding the mechanisms and molecular cascade involved in the establishment and maintenance of pregnancy.

Innovating technologies provide new insights into various fields of scientific; however, they also lead to misconceptions and unreasonably high expectations with regard to resolving complex issues. Since the last decade, molecular technologies have improved our understanding of genomic information. Molecular biology techniques such as northern and Southern blotting have been used on a large scale; however, the major limitations of these techniques are that they permit simultaneous analysis of only a limited number of genes and that they do not provide insights into differential gene expression [5]. An interesting molecular tool is microarray analysis, by which information on several thousands of genes can be obtained simultaneously. It provides not only genomic but also functional, proteomic, and metabolic information [6, 7]. It is a useful tool for research in the field of reproduction since numerous intricate issues pertaining to gestation, the functioning of the feto-maternal interface, immunological regulation in the fetal semi-allograft, production of placenta-specific molecules, expression and functions of retroviruses in placenta, etc., are yet to be elucidated. During the last decade, cDNA and/or oligonucleotide array techniques have been developed to examine gene expression in various species of organisms and in various physiopathological conditions [8–12]. Moreover, microarray techniques have been used to study the multigenic regulation of implantation in humans and mice [13–17].

We have previously developed a bovine-specific microarray system to analyze placental functions, namely, implantation, placentation, and the maintenance of gestation, in cattle [12, 18]. In this review, we mainly introduce the fabrication of cDNA microarray and practical application of this microarray to bovine uteroplacental tissues. Many reviews regarding the applications of microarray technology, both practical and theoretical, have been reported; these have provided detail information on the subject [19–23].

Construction of the Uteroplacental cDNA Microarray

Microarray techniques are based on the fundamental principle of hybridization of 2 complementary DNA strands, similar to northern blotting and Southern blotting. It enables simultaneous comparison of the expression levels of thousands of genes. Microarrays are generally produced by spotting cDNA or oligo-DNA onto glass or silicon chips at a high density [9, 13, 21, 24]. We first fabricated cDNA microarrays in 1999 by using bovine uterine and placental tissues and subsequently generated a bovine liver microarray comprising approximately 8,000 spotted clones [12, 18, 25]. Recently, Takahashi et al. of the National Institute of Agrobiological Sciences (Tsukuba, Japan) constructed an 11-k oligoarray comprising uterine and placental genes from the abovementioned microarray and other bovine gene ESTs obtained from public genome databases such as DDBJ [12, 25, Takahashi et al., unpublished data]. In this review, we provide an overview of cDNA microarray construction.

Tissue collection for constructing cDNA libraries

We collected endometrial and placental tissues from Japanese black cows in order to establish cDNA libraries. Tissue collection is one of most crucial factors involved in constructing microarrays. Further, the condition of the animals used for tissue collection is also a significant factor. Therefore, we collected samples from different parts of the endometrium throughout the estrous cycle and during gestation; further, we also separately collected the cotyledonary and intercotyledonary fetal membranes, as described previously [26, 27].

Library construction

Total RNA was isolated from the tissues by using Isogen (Nippon Gene, Toyama, Japan), according to the manufacturer’s instructions. After preparing poly(A)+ RNA from the total RNA, a phage cDNA library was constructed using the ZAP Express Vector kit (Stratagene, San Diego, CA). The cDNA fragments used to construct the library were approximately 500–2,500 bp in length. A phagemid cDNA library was excised in vivo from the phage library by using the ExAssist helper phage (Stratagene).

Clone selection and construction of a normalized cDNA library

We constructed a phagemid cDNA library containing
approximately 1,200,000 clones and randomly selected approximately 5,000 of these by the hit-picking method [12, 25, 28]. In brief, cDNA fragments were amplified by performing PCR, and bacterial colonies were mechanically inoculated and cultured. Further, macroarray filters were generated and hybridized with DIG-labeled probes. Following color development, the macroarray membranes were scanned, and numerous clones were mechanically separated. This was used as a normalized cDNA library for the spotting and sequence analysis (Figs. 1 and 2).

**cDNA microarray analysis**

Approximately 4,000 cDNA clones selected by the hit-picking method were amplified by PCR and mechanically spotted onto glass slides to obtain a uteroplacental microarray. These clones were simultaneously sequenced using the MegaBACE 1000 DNA sequencing system (Amersham Pharmacia Biotech, Piscataway, NJ) and annotated using the BLAST program. Further, 2 µg poly(A)+ RNA was extracted from the tissues and transcribed using Cy3- or Cy5-conjugated dUTP (Amersham Pharmacia Biotech).
and Superscript II reverse transcriptase (Life Technologies, Rockville, MD). The hybridization probes were applied to the microarray, and the system was incubated overnight. The slides were washed with various concentrations of SSC via several steps and subsequently dried by low-speed centrifugation. The hybridized slides were scanned on the GenePix 4000B system (Axon Instruments, Union City, CA), and the images were analyzed using the GenePix Pro3.0 software (Fig. 3).

Microarray data normalization

Images of the gene expression intensities were obtained; the data were then normalized, and cluster analysis was performed. Normalization compensates for nonspecific hybridization, technical variation, noise, etc. [21, 29–33]. We used the Lowess normalization method, which is commonly used to eliminate artifactual signals and smooth of data. In brief, the background intensity was smoothed using a locally weighted regression smoother (loess) in each spot, and this data was subtracted from the feature intensity data. The subtracted data were subjected to nonparametric regression and local variance normalization; the former can reduce intensity-dependent bias. This improves the accuracy of the data, provided the points in the Cy3 vs. Cy5 scatter plot are not distributed along a straight line. The variance method performed using the bovine uteroplacental array data produced highly reliable normalized ratios. All the data were deposited in the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo) and the GEO accession numbers are shown in Platform: GPL1221. Detail information regarding to tissues was shown in our previous report [32]. The minimum information about a microarray experiment (MIAME; http://www.mged.org/Workgroups/MIAME/miame.html) guidelines were used for unambiguous interpretation of the results and to potentially reproduce the experiment.

Cluster analysis of the microarray data

Cluster analysis is a fundamental strategy used to analyze gene expression and function. The theory underlying the cluster analysis performed in this study was based on a previous report by D’haeseleer and is shown in Fig. 4 [31]. We used the TIGR MultiExperiment Viewer (MeV) 3.0 program (http://www.tigr.org/software/tm4/) for this analysis [33]. Data for individual genes was estimated based on the average value obtained for the corresponding spots on the microarray. The transformed log2 values were considered in the cluster analysis. A total of 1,446 unique genes, except those that exhibited unreliable low expression, were applied to the K-means algorithm, and the data were represented by using an eight-dimensional vector. The K-means clusters were divided into 10 centroid centers, and the distance between the gene vectors was calculated using the cosine coefficient (vector angle).

Analysis of Gene Expression Profiles

Features of the bovine uteroplacental cDNA microarray

The custom-made cDNA microarray in this study...
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contained approximately 4,800 spotted clones. However, after using the hit-picking normalization method to eliminate redundant clones, approximately 1,780 clones were confirmed to carry single genes, including functionally unknown genes. The efficiency of the hit-picking normalization was estimated at approximately 17% [12, 28]. Although this value may indicate a rather lower efficiency, we emphasize the potential of this DNA microarray system since it included 17 of 22 pregnancy-associated glycoproteins (PAGs), which are specific genes in bovine placenta. To our knowledge, no previously developed microarray has effectively included such placenta-specific genes [11, 12, 18, 34–37]. Functional classification of the microarray revealed at least 10 categories of genes: those encoding enzymes and coenzymes (11%), cytokines (including growth factors) and hormones (9%), DNA/RNA-binding proteins (9%), membrane proteins (3%), chaperones (3%), and cell-adhesion molecules (2%) and ribosomal (10%), ECM and MMP-related (5%), cytoskeletal (5%), apoptosis- and cell cycle-related (3%), functionally unknown (10%), and other (30%) genes [8]. These data indicate one of the most specific features of cDNA microarrays. Annotated genes are important for the functional analysis of tissues and/or organs; however, a group of functionally unknown and other genes imply new genes or new sites of placental function. We analyzed certain functionally unknown and other genes and identified several new and/or functionally novel genes such as prolactin-related protein (PRP) VII-IX and BCL2 related protein A1 (BCL2A1) [38–40]. Another aspect that suggests the specificity of this microarray is that it contained at least 40 genes that are highly variable in the placenta/endometrium as compared to those in the endometrium during the estrous cycle [12, 41]. The abovementioned oligomicroarray developed by Takahashi et al. was classified using the GeneSpring software (Agilent Technologies) and contained genes functioning in at least 11 categories of biological processes, as shown in Fig. 5.

As mentioned earlier, the technical variation in the hybridization conditions and the array procedure and the variability in sample material should be carefully

Fig. 4. Examples of different types of cluster analysis. We randomly selected 20 sample genes from the entire data set as a model for the analysis. A: Genes were compared under 2 different conditions. B: Hierarchical cluster analysis. C: K-means cluster analysis. D: Self-organizing map (SOM) cluster analysis. These classification categories and graphic concepts were described in the report by D’haseleer (2005).
compensated for in microarray data analysis. The accuracy and reproducibility of the array can be confirmed by reverse labeling using Cy3 and Cy5; the correlation coefficients between samples are generally estimated by performing reverse labeling [32]. The data obtained by performing reverse labeling in duplicate using poly(A)+ RNA samples obtained from the endometrium during the estrous cycle were in the range of 50–200% when compared with the theoretical value (100%). The placental and endometrial tissues exhibited high and reproducible correlation coefficients ($r = 0.9003$). These data indicate that gene expression levels that were either less than 50% or more than 200% exhibited significant difference.

**Gene expression during gestation**

The bovine uterus has a characteristic morphology—one region termed the caruncle gives rise to the placenta, and another region termed the intercaruncle does not; however, these regions are not markedly differentiated during the estrous cycle. Once placentome development was initiated, the expression of various genes increased in the placentomal and intercaruncular regions, as shown in Fig. 6; the detailed expression patterns have been reported previously [32]. The intensity of gene expression was estimated based on global normalization, and the median value obtained for whole spots was considered as 1. By performing K-means cluster analyses, we identified 10 clusters; from each cluster, the 10 most upregulated and 50 most downregulated genes on day 25 of gestation were subjected to hierarchical cluster analysis, as shown in Fig. 6. Table 1 lists the 5 most upregulated genes in each K-means cluster. The expression intensity of many genes, particularly in the cotyledary tissues, increased immediately after implantation was initiated. In K-means cluster 2, an increasing number of genes, including CSH1 (placental lactogen), PRPs, PAGs, and sulfotransferase family 1E, estrogen-preferring, member 1 ($SULT1E1$), were concentrated in the embryonic membrane (cotyledary and intercotyledary tissues). As shown in Fig. 6 these genes were among the most upregulated ones during gestation; therefore, they may play a crucial role not only in implantation and placentation but also in placental function and the maintenance of the normal gestation period. The PAG gene family plays an important role in fetal growth [42-47]; these gene products are aspartic proteinases and hence may also play a role in the coordination of placental metabolism, immunomodulation, etc. [48]. Although their specific functions remain unclear, their expression levels are a good indicator of gestation in cattle [42, 49, 50]. CSH1 is a member of the prolactin gene family and is specifically expressed in bovine trophoblastic giant cells (TGCs) [51–56]. Another gene family largely expressed in trophoblast cells is the PRP family, also referred to as PRL/GH gene family; several PRP genes are expressed in the placenta throughout the gestation period [57–62]. These genes may play a specific role depending on the area of the endometrium where they are expressed temporally [18, 32, 38–41, 47, 51]. These specific cluster 2 genes are mainly localized to the TGCs and may participate in implantation, adaptation of the embryo to the
endometrial environment, and placentogenesis [55, 56, 60–62]. It is likely that many of these genes are regulated in a similar manner; however, the detailed mechanism regarding their regulation remains unknown. We recently reported that one particular transcription factor (belonging to the AP-2 family) may play a crucial role in the regulation of cluster 2 genes [32]. This hypothesis was based on the results of a microarray-based global gene expression analysis. Thus, microarray technology has provided a large amount of crucial information related to the intricate mechanisms underlying gestation [12, 18, 32].

In contrast to these trophoblast cell-specific genes, various principal endometrial genes such as uterine milk protein (UMP), gastrin-releasing peptide (GRP), selectin (SELL), and stanniocalcin (STC1) were classified under clusters 8 and 9. UMP, STC1, and GRP were mainly expressed in the intercaruncular region [56, 63–65]. UMP may play a significant role in implantation and immunomodulation in the feto-maternal interface [44, 48, 63]. STC1 is known to play some role(s) in endometrial receptivity; however, its precise role and functional mechanism in the endometrium have not been elucidated to date [66–68]. GRP expression has been confirmed in bovine placenta; however, its precise role should be investigated since it is homologous to bombesin, which is expressed as a type of neuroregulatory factor in the digestive duct [69, 70]. Cathepsins and their related genes are also classified under cluster 8 and appear to play a role in implantation and placentation [71–73]. These cluster analysis results strongly suggest that genes belonging to a single cluster operate with same factors and mechanism in regulating the feto-maternal relationship during gestation.

Our studies have focused on the gene expression profiles during bovine gestation. We fabricated a bovine cDNA microarray and determined the expression profiles of specific genes in the placenome and endometrium during placentation, i.e., around day 60 of gestation. We observed that CSH1, PRPs, and PAGs, play a major role during this time in the gestation period

Fig. 6. Hierarchical cluster analysis of the 100 most upregulated and 50 most downregulated genes on day 25 of gestation. The expression intensities of many trophoblast giant cell-specific genes increased as gestation progressed.
Further, we examined the embryonic and extraembryonic gene expression during the peri-implantation period, and several specific gene clumps were detected around the time of implantation [74]. Aberrations were detected in some trophoblast-specific genes in somatic cell nuclear-transferred (SNT) cloned cattle cells; no major variations were observed in the gene expression in the cloned trophoblast cells [41].

**Table 1. Genes expression intensities on day 25 of gestation in bovine: five most higher genes in each cluster**

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Gene</th>
<th>Day 25 Value</th>
<th>K-means</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_175797</td>
<td>ARHGDIR: Rho GDP dissociation inhibitor beta</td>
<td>0.28</td>
<td>1</td>
</tr>
<tr>
<td>NM_174506</td>
<td>BCKDHA: Branched chain alpha-keto acid dehydrogenase</td>
<td>0.11</td>
<td>1</td>
</tr>
<tr>
<td>NM_173906</td>
<td>DCN: Decorin</td>
<td>0.24</td>
<td>1</td>
</tr>
<tr>
<td>NM_174464</td>
<td>SPARC: secreted protein acidic cysteine-rich</td>
<td>0.73</td>
<td>1</td>
</tr>
<tr>
<td>NM_174491</td>
<td>YWHAE: 14-3-3 epsilon</td>
<td>0.38</td>
<td>1</td>
</tr>
<tr>
<td>NM_181007</td>
<td>CSH1: Placental lactogen</td>
<td>0.49</td>
<td>2</td>
</tr>
<tr>
<td>NM_177488</td>
<td>SERPINB6: Serpin peptidase inhibitor clade B member 6</td>
<td>0.40</td>
<td>2</td>
</tr>
<tr>
<td>NM_174411</td>
<td>PAG1: Pregnancy-associated glycoprotein 1</td>
<td>0.69</td>
<td>2</td>
</tr>
<tr>
<td>NM_177488</td>
<td>SULT1E1: Sulphotransferase family 1E estrogen-preferring member 1</td>
<td>0.07</td>
<td>2</td>
</tr>
<tr>
<td>NM_174623</td>
<td>TMSB16: Thymosin beta 10</td>
<td>0.81</td>
<td>2</td>
</tr>
<tr>
<td>NM_181018</td>
<td>CLCA3: Chloride channel calcium activated family member 3</td>
<td>0.50</td>
<td>3</td>
</tr>
<tr>
<td>NM_174333</td>
<td>GRP58: Glucose regulated protein 58kdD</td>
<td>0.81</td>
<td>3</td>
</tr>
<tr>
<td>NM_001010995</td>
<td>TPM2: Tropomyosin 2</td>
<td>0.81</td>
<td>3</td>
</tr>
<tr>
<td>M62428</td>
<td>UBC: Polyubiquitin</td>
<td>0.68</td>
<td>3</td>
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<tr>
<td>NM_174217</td>
<td>VIL2: Villin 2</td>
<td>1.16</td>
<td>3</td>
</tr>
<tr>
<td>NM_001012519</td>
<td>AKR1B1: Aldose reductase</td>
<td>0.96</td>
<td>4</td>
</tr>
<tr>
<td>NM_174800</td>
<td>CFDP2: Craniofacial development protein 2</td>
<td>0.70</td>
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<tr>
<td>AF013213</td>
<td>EEF1A1: Eukaryotic translation elongation factor 1 alpha 1</td>
<td>1.33</td>
<td>4</td>
</tr>
<tr>
<td>NM_174550</td>
<td>HSPA1A: Heat shock 70 kD protein 1</td>
<td>0.91</td>
<td>4</td>
</tr>
<tr>
<td>NM_174087</td>
<td>IGF2: Insulin-like growth factor 2</td>
<td>0.53</td>
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</tr>
<tr>
<td>NM_181003</td>
<td>AQP4: Aquaporin 4</td>
<td>0.54</td>
<td>5</td>
</tr>
<tr>
<td>NM_174257</td>
<td>CALB3: Calbindin 3</td>
<td>0.55</td>
<td>5</td>
</tr>
<tr>
<td>NM_001034039</td>
<td>COL1A1: Collagen type I alpha 1</td>
<td>1.37</td>
<td>5</td>
</tr>
<tr>
<td>NM_174472</td>
<td>TIMP2: Tissue inhibitor of metalloproteinase 2</td>
<td>0.24</td>
<td>5</td>
</tr>
<tr>
<td>AF241780</td>
<td>TKDP5: Trophoblast Kunitz domain protein 5</td>
<td>0.67</td>
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<tr>
<td>NM_001046249</td>
<td>CALM1: Calmodulin 1</td>
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<tr>
<td>K00800</td>
<td>FN1: Fibronectin 1</td>
<td>0.48</td>
<td>6</td>
</tr>
<tr>
<td>NM_174556</td>
<td>IGFBP3: Insulin-like growth factor binding protein 3</td>
<td>0.13</td>
<td>6</td>
</tr>
<tr>
<td>NM_001033608</td>
<td>MIF: Macrophage migration inhibitory factor</td>
<td>0.20</td>
<td>6</td>
</tr>
<tr>
<td>NM_001015592</td>
<td>PFN1: Profilin 1</td>
<td>0.44</td>
<td>6</td>
</tr>
<tr>
<td>NM_174716</td>
<td>ANXA2: Annexin A2</td>
<td>0.29</td>
<td>7</td>
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<tr>
<td>NM_174798</td>
<td>MSX1: Msh homeo box 1</td>
<td>0.12</td>
<td>7</td>
</tr>
<tr>
<td>NM_174115</td>
<td>MUC1: Mucin 1</td>
<td>0.22</td>
<td>7</td>
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<tr>
<td>NM_205775</td>
<td>TKDP4: Trophoblast Kunitz domain protein 4</td>
<td>0.85</td>
<td>7</td>
</tr>
<tr>
<td>NM_174487</td>
<td>VEGFB: Vascular endothelial growth factor B</td>
<td>0.11</td>
<td>7</td>
</tr>
<tr>
<td>NM_173902</td>
<td>CLU: Clusterin</td>
<td>0.16</td>
<td>8</td>
</tr>
<tr>
<td>NM_174029</td>
<td>CST3: Cystatin C</td>
<td>0.48</td>
<td>8</td>
</tr>
<tr>
<td>NM_174031</td>
<td>CTSL: Cathespin B</td>
<td>0.52</td>
<td>8</td>
</tr>
<tr>
<td>NM_174092</td>
<td>IL1A: Interleukin 1, alpha</td>
<td>0.29</td>
<td>8</td>
</tr>
<tr>
<td>NM_174707</td>
<td>MGP: Matrix Gla protein</td>
<td>0.10</td>
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<tr>
<td>NM_175784</td>
<td>ANXA1: Annexin I</td>
<td>0.30</td>
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<tr>
<td>AF515786</td>
<td>B4GALT1: Glycoprotein-4-beta-galactosyltransferase 2</td>
<td>0.30</td>
<td>9</td>
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<tr>
<td>NM_174032</td>
<td>CTSL: Cathespin L</td>
<td>0.24</td>
<td>9</td>
</tr>
<tr>
<td>NM_178319</td>
<td>GRP: Gastrin-releasing peptide</td>
<td>0.18</td>
<td>9</td>
</tr>
<tr>
<td>NM_176669</td>
<td>STC1: Stanniocalcin 1</td>
<td>0.30</td>
<td>9</td>
</tr>
<tr>
<td>NM_176648</td>
<td>CAPZB: Capping protein (actin filament) muscle Z-line, beta</td>
<td>0.21</td>
<td>10</td>
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<tr>
<td>NM_176644</td>
<td>CYP11A1: Cytochrome P450, family 11, subfamily A, polypeptide 1</td>
<td>0.11</td>
<td>10</td>
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<tr>
<td>NM_174100</td>
<td>LDHB: Lactate dehydrogenase B</td>
<td>0.13</td>
<td>10</td>
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<tr>
<td>NM_001034053</td>
<td>LMNA: Lamin A</td>
<td>0.30</td>
<td>10</td>
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<tr>
<td>NM_174745</td>
<td>MMP2: Matrix metalloproteinase 2</td>
<td>0.13</td>
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Specific gene expression was examined using a bovine trophoblast cell line (BT-1) as a model for in vitro analysis. Mononucleate trophoblast cells were observed to differentiate into placental lactogen-expressing TGCs [55, 62, 75]. By using microarray techniques and bioinformatics, recent studies have investigated global gene expression profiles throughout the gestation period and have explored a common regulatory factor for the expression of these genes. Many trophoblast- and/or endometrium-specific genes exhibited marked variations with temporal and spatial specificity [32]. We identified several features of bovine gene expression during gestation. (1) Of the genes that were classified by K-means cluster analysis, (i) trophoblast-specific genes such as CSH1, PAGs, PRP, and SULT1E1 were classified under cluster 2, and the expression levels for most of these genes increased as gestation progressed and (ii) endometrium-specific genes such as UMP, cathepsins, and SELL, were classified under clusters 8 and 9, and their expression was extremely temporal and spatial. (2) TGCs simultaneously expressed various molecules, namely, placental lactogen, PRPs, PAGs, heparanase, the antiapoptosis gene BCL2A1, SULT1E1, etc. [38–40, 43–47, 76]. (3) The transcription factor AP-2 may play a major role in regulating bovine TGC functions since this family of transcription factors was also expressed in TGCs and their expression increased as gestation progressed. These gene expression analyses emphasize the significance of the trophoblast cell lineage since many genes such as CSH1 and AP-2 are detected in TGCs in various rodent species as well as in humans and ruminants [32, 77–80].

During the last decade, microarray technology has developed rapidly and has easy applications. It has already been applied in various fields such as biology, toxicology, and medicine, and the data have been analyzed by bioinformatic methods [81–86]; however, the methods of analysis have not been standardized as yet [19, 20, 24, 87, 88]. Microarray technology can provide information regarding not only specific genes in tissues but also gene cascades and/or molecular interactions in cells and tissues. Currently, bioinformatics tools are available to analyze this information; however, there exists a communication gap between biologists and bioinformatics scientists. Bridging this gap is of paramount importance.

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