Acta Scientiae Veterinariae, 2011. 39(Suppl1): s263 - s272.

ISSN 1679-9216 (Online)

Application of gene expression profiling to the study of placental and fetal function

Jorge A. Piedrahita

ABSTRACT

***Background*:** One of the key biological principles that differentiate mammals from other phyla is the presence of the placenta. This is a unique and complex tissue that plays an essential role in the development of the fetus and has both short terms effects in terms of reproductive efficiency, and long term effects in terms of adult onset diseases exacerbated by an inadequate fetal environment. Yet in spite of decades of intense study there is still a large number of unanswered questions regarding the function of this organ. This is true for both humans and domestic animals species.

***Review*:** In the past, studies focused on placental function and placental/fetal interactions have examined at most a few genes/proteins at a time; what is commonly referred to as the candidate gene approach. While this approach has been quite successful, and has helped develop the knowledge base that we have at present, it is also limiting in scope. New genomic technologies allow simultaneous analysis of most, if not all, of the genes expressed in placental and fetal tissues. However these technologies also present significant challenges due to the massive amount of information that is generated. This entails not just how to handle the generated data, but also how to properly analyze it and interpret it. Fortunately, a large number of computer algorithms have been, and continued to be, developed that permit not just the identification of which genes are disregulated in the system being studied, but perhaps more importantly, permits the identification of which biological pathways are affected. This in effect convert a list of genes that in most cases defy interpretation, into a more biologically-oriented set of results that provide a better understanding of the system. This knowledge can then be used to design direct biochemical and physiological experiments that, in essence, allow the investigator to move from the gene level to the system level. This review will cover some of the fundamental aspects of gene expression profile data capture, and the different approaches that are used to analyze the data generated, including brief descriptions of some of the most commonly used web-based gene expression profiling analysis programs. In addition, methods for searching and downloading dataset of interest that can help complement your own data will be reviewed. In particular, focus will be placed on how this technology can be utilized to study placental and fetal growth abnormalities in humans, with an emphasis on fetal growth restriction and preeclampsia. Additionally, similar approaches will be described that helped to elucidate the conservation and possible function of imprinted genes in swine.

***Conclusion*:** This manuscript describes methods for capturing and analyzing gene expression profiles with an emphasis of how this technology was applied to the study of placental function in humans and swine. In addition, it provides a description of web-based systems that can be used to analyze data generated by your own studies as well as method for searching and downloading data generated by others.

Keywords: Epigenetics, placenta, transcriptome, imprinting.

Center for Comparative Medicine and Translational Research and Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, U.S.A. CORRESPONDENCE: J.A. Piedrahita [jorge_piedrahita@ncsu.edu]. Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., ZIP CODE: 27606, Raleigh, NC, U.S.A.

I. INTRODUCTION

"Functional genomics" can be defined as the application of genomic technologies to the understanding of biological function both at the cellular and organismal level. It can cover areas such as the application of gene expression profiling as a tool to understand biological function, and the generation of genetically modified animals as a way of understanding the function of a gene in the context of the whole animal. For the purpose of this review functional genetics refers to the application of gene expression profiling to enhance our knowledge of the function of the placenta in both humans and mammals. In the most basic sense there are three areas that are key to this technology: a) how to collect the data; b) how to analyze the data and; c) how to apply the results generated.

II. GENE EXPRESSION PROFILES; HOW ARE THE DATA GENERATED

Scientists have been doing gene profiling for decades by low output technologies such as Northern's and RT-PCR (Reverse transcription-polymerase chain reaction). These techniques can be highly accurate but are limiting in scope as only a few genes can be analyzed simultaneously. As a result, most scientists relied on developing a hypothesis, selecting a few candidate genes thought to be involved in the process being studied, and generating the required information about those few genes; what is commonly referred to as the candidate gene approach. The candidate gene approach is still in use today and while it remains highly valid, and was the basis for most of the knowledge on gene expression generated to date, it is limiting in scope. In the last fifteen years a new alternative was developed. This approach has been referred to as the RNA microarray system, gene expression profiling, or transcriptome analysis. The initial microarray versions consisted of partial or complete sDNA to the gene of interest being "printed" into small glass slides. The capacity of these initial slides were in the hundreds of genes and most, if not all, were custom made by different groups interested in addressing specific systems - see [58] for comprehensive review in this area. While useful, this approach was limiting due to the time and effort required to build and test these arrays, as well as by technical issues related to early glass slide printing methods. A significant advance in this technology was the development of the oligonucleaotide-based arrays. Now instead of whole or partial cDNAs, genes could be measured by using carefully designed oligonucleotides ranging is size from 21 to approximately 60 base pairs [58]. The lower costs of oligonucleotide synthesis, combined with the completion of a wide range of genomic sequencing projects, including humans, pigs, horses, cattle, and dogs to name a few, allowed the development of species-specific commercial microarrays targeting most if not all of the transcripts being made by a particular species. In addition, printing variability issues were reduced by either avoiding the use of the glass slide completely or by printing the oligonucleotides directly into a solid matrix. Many groups including us have examined the utility of these commercial arrays. My group, specifically compared the utility of glass arrays versus commercial oligonucleotide arrays (Affymetrix) to determine which system was better adapted to address questions related to gene expression changes in swine [53]. While both methods were capable of detecting changes between two samples tissues, the commercial platform was found to be considerably better at detecting significant differentially expressed genes. In a side by side comparison using the same test cDNA samples, glass spotted arrays representing 12,000 genes were able to identify 3 differentially expressed genes while commercial microarrays, representing approximately 24,000 genes, identified 210 differentially expressed genes [53]. Since then, arrays continue to increase in coverage and in technical reproducibility further increasing their usefulness. However, while still the main option available for many laboratories worldwide there is new technology that has an even greater capacity for capturing transcriptome data than the microarrays. This is the so called deep sequencing,

massive parallel sequencing, next generation sequencing, or whole genome sequencing technology [36]. This technology can generate sequence over 55 billion (55 Gb) bases in a single day [62]. To put that number in perspective, the complete human genome is 3.4 billion bp, thus, in a single day this technology has the potential to sequence with a 10-15 fold coverage the genome of a single individual; A task that required several years and hundreds of millions of dollars just a few years ago. In essence, this new sequencing technology allows one to capture and sequence every transcript that is being made in a cell. It is highly accurate and quantitative, as it will calculate the relative proportion of each transcript within your population. It measures both mRNA and microRNAs and will examine all transcripts, even those that have not previously been described. Thus, in terms of data collection, this approach is comprehensive and unbiased and thus has many advantages over oligonucleotide-based arrays, even those arrays designed to cover the complete transcriptome. At present the application of this new approach is limited due to costs, which at presents can be 2-5x greater than microarrays (on a per-sample costs basis), and equally important by the computing and bioinformatic resources required to compile and analyze the massive amounts of data that are generated. Moreover, while implementation of deep sequencing to commonly studied species such as humans and mice is relatively straightforward due to the high quality of the whole genome sequencing information available, the same cannot be stated for species such as swine. While some groups have been able to implement this technology in swine successfully [1], it is not a project that most laboratories can manage on their own. Until cost of generating the data are reduced, and methods for rapid extraction of the expression data from the massive amounts of sequence information are developed, the broad applicability of this new approach will remain limited in scope. However, it is likely that this method will eventually become the method of choice for most, if not all, investigators interested in gene expression profiling.

Finally, an often overlooked source for data collection is data repositories. At present most journals require that the original raw data used to generate the results included in any manuscript has to be deposited in free-access databases such as the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) [4]. In May 2011, GEO contained information on 2,800 experiments covering over 563,000 samples. In domestic species, GEO had information from 158 pig projects representing over 3332 samples, 169 cattle projects representing over 3309 samples, 29 sheep projects representing 444 samples, and 9 goat projects representing 118 samples. When examining data from GEO it is important, however, to determine whether the data was generated using cross-species hybridization approaches. That is, in species such as goats where no goat-specific arrays are available investigators have used cattle specific arrays. While cross-species hybridization approaches can generate some valuable information it has drawbacks that need to be taken into consideration [52]. Even with this caveat, GEO and similar databases are a highly underutilized resource that could be of tremendous use to investigators and students to generate unique hypotheses to be tested and to become familiar with data analysis programs before generating their own datasets.

III. HOW TO ANALYZE GENE EXPRESSION PROFILING/ MICROARAY DATA

This section is not intended as a complete review of this field that combines complex statistical approaches with computing problems related to the large amounts of data that need to be processed. There is in addition to issues of quality control and data normalization that are beyond the scope of this review. And lastly, there are dozens if not hundreds of programs available for transcriptome analysis some with significant overlaps and others with highly specialized goals. The Gene Ontology consortium alone has over fifty different optional programs listed in their website (http://www.geneontology.org/GO.tools.microarray.shtml). What is described here is a single method that illustrates how one can go from a large microarray dataset to a more defined and biologically relevant set of results- See [43] for a more detailed review of this topic.

In general, when comparing two or more treatments, the following broad questions are asked:

1. Which genes are differentially expressed between the different treatments? This is a simple gene list that should contain fold change as well as si-

gnificance (p values). It is usually generated by either commercially available software or by using free web-based statistical programs.

2. What does the data collectively tell you about what biological pathways or processes are affected by the treatment(s)? This area, usually referred to as functional analysis, is where gene lists are converted into more biologically relevant information that can lead you to identify the biologically-relevant process that is (are) affected. Without this type of analysis, moving forward from a gene list it fraught with difficulties as, in some cases, several hundred gene are found to be statistically different.

So what exactly is functional analysis In essence, investigators have compiled lists of genes that are involved in different biologically processes, or that work in certain cellular compartments. For instance, there is a curated list of genes that are known to be involved in apoptosis. What functional analysis does, is compare your microarray data to that curated list of apoptosis-related genes and ask whether there is any evidence that the genes that are affected by your treatment are enriched for genes belonging to the apoptosis group. It does this through statistical methods and gives you a probability associated with the likelihood that your data indicates that genes in the apoptosis pathway are being affected. The end result being that you have just gone from a gene list to a biological function that can be tested experimentally *in vitro* or *in vivo*.

Thus, the value of this analysis is that it moves from single genes to a biological function that can be tested *in vitro* or *in vivo* depending on your system. And as the field matures, more and more of these groupings or categories of genes are available, thus increasing the value and usefulness of the information generated. One caveat for those working in domestic species is that the groupings or categories are developed using data generated in species such as Drosophila, Saccharomyces, and mice. While this is not likely to affect results in a highly conserved category such as apoptosis, it could have significant drawbacks if the phenotype of interested is more species-specific.

One of the better known system used to categorize gene function is known as the Gene Ontology or GO (http://www.geneontology.org) [2]. This system assigns each gene properties at three levels; cellular compartment (i.e. nucleus, membrane, mitochondria); molecular function (i.e. kinase, transcription factor, phosphatase); and biological function (i.e. apoptosis, signal transduction, cholesterol transport). This system is hierarchical in structure and you can go from a very broad to a highly specific category. A more comprehensive system that encompasses GO but also adds additional gene datasets is the Molecular Signatures Database or MSigDB (http://www.broadinstitute.org/gsea/msigdb/collections.jsp#C2) [45]. This database contains close to 7,000 annotated gene sets divided onto five major collections; each collection targeted to a particular goal. The different collections are:

1) C1 or positional gene set which can be used to determine if there are certain chromosomal locations that are affected into the system under study. In other words, does the data suggest that a particular chromosomal region is showing either a hotspot of gene activation, or conversely, gene down-regulation?

2) C2 a collection of gene sets compiled from online pathway databases such as Biocarta and KEGG, published literature, and experts in the field. In compares the experimental data to these known groups and determine whether there is a probability that the data is enriched for genes in one of these groupings. This collection can tell whether the dataset suggests that a particular biochemical pathway is affected (i.e. cholesterol biosynthesis) or whether the gene expression profile it is similar to that found in specific systems such as in cancer or stem cells.

3) C3 a motif gene collection that can help identify microRNA and transcription factors that are involved in the system under study. It analyses the data and determines whether the genes that are affected in the system are enriched for genes containing certain transcription factor binding sites; suggesting that that transcription factors are co-regulating all those genes. Similarly, it determines whether genes affected in the dataset are enriched for targets of one of more microRNA suggesting that those microRNAs may be behind the effect being seen.

4) C4 is a cancer related collection that groups known cancer-related molecular signatures and compares the data to those signatures to see if there are commonalities.

5) C5 is the Gene Ontology (GO) compilation that can be used to examine the data for enrichment into any of the tree GO categories mentioned above; cellular location, molecular function, and biological function.

The MsigDB forms the backbone of a web-based analysis program known as Gene Set Enrichment Analysis (GSEA). GSEA will not give a list of genes per se but will give processes, pathways, and a host of additional information about the data. It is an excellent way to mine the data for biologically relevant information and is very comprehensive as described above. A detailed set of instructions on how to use this program can be found at http://www.broadinstitute.org/gsea/index.jsp. As mentioned previously, this is not intended as a comprehensive review of this field but as a brief description of the type of analysis that lead to the information that will be presented in the following section.

IV. APPLICATION OF FUNCTIONAL GENOMIC APPROACHES TO THE STUDY OF PLACENTAL FUNCTION

One of the most fascinating and poorly understood organs in the mammalian system is the placenta. It is critical for normal fetal development and plays a crucial role in the flow of nutrients from the mother to the fetus. In humans, it plays a central role in diseases such as preeclampsia and in domestic species it has been postulated to affect litter size in species such as swine. While the candidate gene approach has been useful for elucidating some of the basic mechanisms behind these phenomena there are still may questions remaining to be answered. As a result, we applied a functional genomics approach to this question in order to determine of this approach was useful in helping unravel the complexity of each of these two phenotypes.

4.1 Application to human placental disorders; preeclampsia

Up to 8% of human pregnancies results in maternal high blood pressure [38] most due to preeclampsia. Preeclampsia is a pregnancy-associated disorder triggered by placental dysfunction and characterized by maternal hypertension and protein in the urine [37]. It also increases the incidence of preterm labor and about 15% of preterm births are due to preeclampsia [15]. Risk factors for preeclampsia include: first pregnancy, family history of preeclampsia, diabetes, and multiple pregnancies [32]. redmanPreeclampsia is usually separated into two distinct stages: 1) An initial stage characterized by abnormalities in placentation due to inadequate uterine remodeling and placental invasion and 2) a later stage where the maternal symptoms such as high blood pressure, associated with factors secreted by the affected placenta [35], are observed. Some of these factors include Activin-A and Inhibin A (INHA) [30, 21], Leptin (LEP) [28], soluble Endoglin (sENG) [55], soluble fms-like tyrosine kinase-1 (sFlt-1) [26], and placental growth factor (PGF) [51].

While the pathophysiology of the placenta and its downstream effects are well understood, it has been more difficult to identify potential triggers that lead to the defective placenta. Recently, we utilized a microarray-based approach and compared gene expression profiles of preeclamptic and normal term human placentas [52]. The data was analyzed for differentially expressed gene as well as pathways affected. Our data confirmed overexpression of the ENG, FLT1, and INHA as had been previously reported. In addition, pathway analysis identified several immune-regulated pathways as being affected including Fc Receptor mediated phagocytocis in macrophages and monocytes, CXCR4 signaling, and leukocyte extravasation signaling. CXCR4 is a chemokine receptor specific for stromal-derived factor-1 (SDF-1) and ubiquitin and has been implicated in inflammation as well as the process of implantation [39,23,40,13]. Thus, the pathway analysis was supportive of a role for immune system involvement in preeclampsia. This has been previously reported [13] but the cause of the immune activation was not clear. What our results suggest is that the sialic acid pathways, a pathway central to self-recognition by the immune system [48] is disregulated in preeclamptic placentas. This allowed us to propose a model whereby preeclampsia is initiated by an abnormal sialic acid pathway resulting in a form of autoimmune disease where the placenta is not recognized as self. This results in abnormal branching and shallow trophoblast invasion with the end result being preeclampsia. While there is still considerable work to be done to fully understand the disease, the gene profiling approach allowed us to uncover the previously unknown role of sialic acid

in the process of autoimmune-related placentas abnormalities. It is highly unlikely that we could have reached this point, this rapidly, using a candidate gene approach, as there are hundreds if not thousands of molecules that could play a role in immune responses. Thus, the combination of the large amount of data generated and the ability to examine that data in multiple ways led us to make a crucial observation regarding this disorder.

4.2 Application to domestic species; pig placenta.

As mentioned earlier, there are commercial arrays available for use in species such as swine, cattle, and chicken. Additionally, with the completion of the genomic sequencing projects for these species it is now also possible to utilize the deep sequencing technology. In cattle, gene expression profiling has been utilized as a method for increasing our understanding of early embryonic development under a variety of experimental system [48, 8], milk production [7], oocyte quality [24], adipose tissue in dairy cattle during lactation [46], spermatozoa [9], IVF embryos [14], endometrial changes during the estrus cycle [10], muscle marbling [17], and meat tenderness [61], among others. In swine, this approach has been used to examine muscle growth, and regulation of muscle growth [50,34,22,49] regulation of the immune system and response to infections [54,16,12,11], factors controlling ovulation [56], and gene expression in embryos [59] and placentas [44, 6].

We have utilized this technology as a way of understanding the family of imprinted genes in swine [5]. The imprinted gene family is particularly relevant as it play a major role in placental and fetal development and function. While 99% of genes in mammalian species are expressed from both the paternal and maternal allele (biallelic expression), imprinted genes are mono-allelically expressed. That is, either the maternal or paternal allele is expressed, but not both. This results in a form of dosage compensation and is controlled by epigenetic modifications including DNA methylation and histone modifications [19, 18].

Of particular interest to my laboratory is the concept of parental conflict put forward by Moore and Haig [29]. This hypothesis states that imprinted genes expressed from the maternal allele evolved as a mechanism of resource conservation for the mother (at the expense of the fetus), while imprinted genes expressed from the paternal alleles increase the extraction of resources from the mother (favoring the fetus). In a normal pregnancy these two competing forces balance each other resulting in normal fetal growth. However, if the system is unbalanced it can result in either intra uterine growth restriction or large for gestational age fetuses (too small or too large). One experimental approach to study imprinted genes and their role in fetal and placental development is the use of uniparental embryos. Uniparental embryos are composed only of maternal DNA (two haploid maternal genomes; known as gynogenotes or parthenotes) or of paternal DNA (two haploid paternal genomes; known as androgenotes) [25, 47]. Litters from gynogenotes or parthenotes yield intrauterine growth-restricted (IUGR) conceptuses, and a small hypo-vascular placenta [25, 47]. Androgenotes, in contrast, develop into a very large abnormal placenta but lack a fetus proper [27]. Both phenotypes support the main idea behind the parental conflict hypothesis.

Further evidence for the conflict theory between maternal and paternally imprinted genes comes from transgenic mouse studies. In the mouse, the insulin-like growth factor *Igf2* is paternally expressed and increases placental and fetal weights as well as nutrient flow, while its receptor is expressed maternally and sequesters the function of *Igf2* by binding and, subsequently, trafficking to the lysosome [3]. Also, inactivation of *Peg10* or *Peg3*, two paternally expressed genes, result in smaller fetuses and placentas [33, 20]. Thus, the evidence for the opposing roles of maternally and paternally imprinted genes is strong.

Unfortunately, while there are a limited number of imprinted genes with known functions in placental development in mice and humans, there are a limited number of studies of their role in domestic species (see [60]for a more comprehensive review in this area). In order the gain a greater understanding of this gene family in an agriculturally relevant species we utilized a microarray based approach, combined with uniparental pregnancies, to a) determine the degree of conservation of imprinted genes among different mammalian species and b) to explore the feasibility of using this approach to understand the physiological role of imprinted genes.

To accomplish this we developed porcine parthenotes. In pigs, the parthenogenetic fetus develops as a small fetus with a small placenta and dies at approximately day 32 of gestation. Parthenogenetic and control fetuses were allowed to develop to days 28-30 and samples collected for microarray analysis. Data was analyzed to indentify individually affected genes as well as biological processes/pathways affected. The expectation was that uniparental embryos containing 2 doses of maternal DNA and no paternal DNA would have a double dose of maternally expressed imprinted genes and lack expression of paternally expressed genes. Comparison of this profile with the control fetuses carrying one dose of each, the maternally and the paternally imprinted genes, would allow the identification of which genes were imprinted in swine. In mice, this approach had been used successfully by others [42, 31]. As expected, we were able to identify a large number of imprinted genes using this approach, and found a high conservation of imprinting with the mouse and humans [5]. Equally important we were able to identify the following pathways affected in the parthenotes 1) phosphatidylinositol binding, 2) microtubule associated complex, 3) lipid transporter activity, 4) prothoracicotrophic hormone activity, 5) transmembrane receptor protein serine/threonine kinase signaling pathway and 6) double-stranded DNA binding. Overall, there were differences in proliferation, biogenesis and biosynthesis pathways. This is consistent with the role of imprinted genes as regulators of energy/nutrient flow between the mother and the fetus.

Additionally, using GSEA, we were able to identify several microRNAs affected in the partenote samples. Two of these microRNAs have been implicated as a target of angiogenesis receptor II type 1 AGTR1, a gene responsible for angiogenesis [41] vasoconstriction and increased pregnancy complication by preeclampsia [57]. Gross morphological examination of swine parthenote placentas shows reduced number of blood vessels, and this observation is also supported by differential expression of AGTR1 in placental tissues ($p<0.0009$).

In short, as for the preeclamptic work in humans, the gene expression profiling approach allowed us to rapidly test for imprinting conservation between pigs and other species as well as to understand, in the context of the whole organism, how these genes function to regulate fetal and placental growth. And as for the preeclampsia work, the microarray analysis serves as starting point for a large number of more focused experiments.

V. CONCLUSION

The development of novel technologies to collect and analyze the transcriptome of domestic species provides a unique opportunity to move from a single gene approach to a whole organism level. As the technology for capturing and analyzing the data continues to evolve and improve, it is critical that the new generation of scientists conducting basic and applied research on domestic species become familiar with these powerful and valuable technologies.

Acknowledgements. Portions of the work described here were supported by a National Research Initiative Grant (2005-35604-15343) from the USDA Cooperative State Research, Education, and Extension Service and grant HD 048510 from the National Institutes of Health.

REFERENCES

1 **Amaral A.J., Megens H.J., Kerstens H.H., Heuven H.C., Dibbits B., Crooijmans R.P., Den Dunnen J.T. & Groenen M.A. 2009.** Application of massive parallel sequencing to whole genome SNP discovery in the porcine genome. *BMC Genomics*. 10: 374.

2 **Ashburner M., Ball C.A., Blake J.A., Botstein D., Butler H., Cherry J.M., Davis A.P., Dolinski K., Dwight S.S., Eppig J.T., Harris M.A., Hill D.P., Issel-Tarver L., Kasarskis A., Lewis S., Matese J.C., Richardson J.E., Ringwald M., Rubin G.M. & Sherlock G. 2000.** Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics*. 25(1): 25-29.

3 **Barlow D.P., Stoger R., Herrmann B.G., Saito K. & Schweifer N. 1991.** The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*. 349(6304): 84-87.

4 Barrett T., Troup D.B., Wilhite S.E., Ledoux P., Evangelista C., Kim I.F., Tomashevsky M., Marshall K.A., Phillippy K.H., Sherman P.M., Muertter R.N., Holko M., Ayanbule O., Yefanov A. & Soboleva A. 2011. NCBI GEO: archive for functional genomics data sets—10 years on. *Nucleic Acids Research*. 39 (Database issue): D1005-1010.

5 Bischoff S.R., Tsai S., Hardison N., Motsinger-Reif A.A., Freking B.A., Nonneman D., Rohrer G. & Piedrahita J.A. 2009. Characterization of conserved and nonconserved imprinted genes in swine. *Biology of Reproduction*. 81(5): 906-920.

6 Chae J.I., Lee K.S., Kim D.J., Han Y.M., Lee D.S., Lee K.K. & Koo D.B. 2009. Abnormal gene expression in extraembryonic tissue from cloned porcine embryos. *Theriogenology*. 71(2): 323-333.

7 Connor E.E., Siferd S., Elsasser T.H., Evock-Clover C.M., Van Tassell C.P., Sonstegard T.S., Fernandes V.M. & Capuco A.V. 2008. Effects of increased milking frequency on gene expression in the bovine mammary gland. *BMC Genomics*. 9: 362.

8 Everts R.E., Chavatte-Palmer P., Razzak A., Hue I., Green C.A., Oliveira R., Vignon X., Rodriguez-Zas S.L., Tian X.C., Yang X., Renard J.P. & Lewin H.A. 2008. Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. *Physiological Genomics*. 33(1): 65-77.

9 Feugang J.M., Rodriguez-Osorio N., Kaya A., Wang H., Page G., Ostermeier G.C., Topper E.K. & Memili E. 2010. Transcriptome analysis of bull spermatozoa: implications for male fertility. *Reproductive Biomedicine Online*. 21(3): 312-324.

10 Forde N., Beltman M.E., Duffy G.B., Duffy P., Mehta J.P., O'gaora P., Roche J.F., Lonergan P. & Crowe M.A. 2011. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biology of Reproduction*. 84(2): 266-278.

11 Genini S., Delputte P.L., Malinverni R., Cecere M., Stella A., Nauwynck H.J. & Giuffra E. 2008. Genome-wide transcriptional response of primary alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus. *Journal of General Virology*. 89(Pt 10): 2550-2564.

12 Gladue D.P., Zhu J., Holinka L.G., Fernandez-Sainz I., Carrillo C., Prarat M.V., O'donnell V. & Borca M.V. 2010. Patterns of gene expression in swine macrophages infected with classical swine fever virus detected by microarray. *Virus Research*. 151(1): 10-18.

13 Gleicher N. 2007. Why much of the pathophysiology of preeclampsia-eclampsia must be of an autoimmune nature. *American Journal of Obstetrics and Gynecology*. 196(1): 5.e1-7.

14 Huang W., Yandell B.S. & Khatib H. 2010. Transcriptomic profiling of bovine IVF embryos revealed candidate genes and pathways involved in early embryonic development. *BMC Genomics*. 11: 23.

15 Kanasaki K. & Kalluri R. 2009. The biology of preeclampsia. *Kidney International*. 76(8): 831-837.

16 Lee G., Han D., Song J.Y., Lee Y.S., Kang K.S. & Yoon S. 2010. Genomic expression profiling in lymph nodes with lymphoid depletion from porcine circovirus 2-infected pigs. *Journal of General Virology*. 91(Pt 10): 2585-2591.

17 Lee S.H., Gondro C., Van Der Werf J., Kim N.K., Lim D.J., Park E.W., Oh S.J., Gibson J.P. & Thompson J.M. 2010. Use of a bovine genome array to identify new biological pathways for beef marbling in Hanwoo (Korean Cattle). *BMC Genomics*. 11: 623.

18 Lewis A. & Murrell A. 2004. Genomic imprinting: CTCF protects the boundaries. *Current Biology*. 14(7): R284-286.

19 Lewis A., Mitsuya K., Umlauf D., Smith P., Dean W., Walter J., Higgins M., Feil R. & Reik W. 2004. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. *Nature Genetics*. 36(12): 1291-1295.

20 Li L., Keverne E.B., Aparicio S.A., Ishino F., Barton S.C. & Surani M.A. 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. *Science*. 284(5412): 330-333.

21 Lindheimer M.D. & Woodruff T.K. 1997. Activin A, inhibin A, and pre-eclampsia. *Lancet*. 349(9061): 1266-1267.

22 Lobjois V., Liaubet L., Sancristobal M., Glenisson J., Feve K., Rallieres J., Le Roy P., Milan D., Cherel P. & Hatey F. 2008. A muscle transcriptome analysis identifies positional candidate genes for a complex trait in pig. *Animal Genetics*. 39(2): 147-162.

23 Majetschak M. 2011. Extracellular ubiquitin: immune modulator and endogenous opponent of damage-associated molecular pattern molecules. *Journal of Leukocyte Biology*. 89(2): 205-219.

24 Mamo S., Carter F., Lonergan P., Leal C.L., Al Naib A., Mcgettigan P., Mehta J.P., Evans A.C. & Fair T. 2011. Sequential analysis of global gene expression profiles in immature and *in vitro* matured bovine oocytes: potential molecular markers of oocyte maturation. *BMC Genomics*. 12: 151.

25 Mann J.R. & Lovell-Badge R.H. 1984. Inviability of parthenogenones is determined by pronuclei, not egg cytoplasm. *Nature*. 310(5972): 66-67.

26 Maynard S.E., Min J.Y., Merchan J., Lim K.H., Li J., Mondal S., Libermann T.A., Morgan J.P., Sellke F.W., Stillman I.E., Epstein F.H., Sukhatme V.P. & Karumanchi S.A. 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of Clinical Investigation*. 111(5): 649-658.

27 Mcgrath J. & Solter D. 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell*. 37(1): 179-183.

28 Mise H., Sagawa N., Matsumoto T., Yura S., Nanno H., Itoh H., Mori T., Masuzaki H., Hosoda K., Ogawa Y. & Nakao K. 1998. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. Journal of Clinical *Endocrinology and Metabolism*. 83(9): 3225-3229.

29 Moore T. & Haig D. 1991. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics*. 7(2): 45-49.

30 Muttukrishna S., Knight P.G., Groome N.P., Redman C.W. & Ledger W.L. 1997. Activin A and inhibin A as possible endocrine markers for pre-eclampsia. *Lancet*. 349(9061): 1285-1288.

31 Nikaido I., Saito C., Mizuno Y., Meguro M., Bono H., Kadomura M., Kono T., Morris G.A., Lyons P.A., Oshimura M., Hayashizaki Y. & Okazaki Y. 2003. Discovery of imprinted transcripts in the mouse transcriptome using large-scale expression profiling. *Genome Research*. 13(6B): 1402-1409.

32 Odegard R.A., Vatten L.J., Nilsen S.T., Salvesen K.A. & Austgulen R. 2000. Preeclampsia and fetal growth. *Obstetrics and Gynecology*. 96(6): 950-955.

33 Ono R., Nakamura K., Inoue K., Naruse M., Usami T., Wakisaka-Saito N., Hino T., Suzuki-Migishima R., Ogonuki N., Miki H., Kohda T., Ogura A., Yokoyama M., Kaneko-Ishino T. & Ishino F. 2006. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. *Nature Genetics*. 38(1): 101-106.

34 Ponsuksili S., Murani E., Phatsara C., Schwerin M., Schellander K. & Wimmers K. 2010. Expression quantitative trait loci analysis of genes in porcine muscle by quantitative real-time RT-PCR compared to microarray data. *Heredity*. 105(3): 309-317.

35 Redman C.W. & Sargent I.L. 2009. Placental stress and pre-eclampsia: a revised view. *Placenta*. 30 (Suppl A:S): 38-42.

36 Reinartz J., Bruyns E., Lin J.Z., Burcham T., Brenner S., Bowen B., Kramer M. & Woychik R. 2002. Massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms. *Briefs in Functional Genomics and Proteomics*. 1(1): 95-104.

37 Roberts J.M. & Redman C.W. 1993. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet*. 341(8858): 1447-1451.

38 Roberts J.M., Balk J.L., Bodnar L.M., Belizan J.M., Bergel E. & Martinez A. 2003. Nutrient involvement in preeclampsia. *Journal of Nutrition*. 133(5 Suppl 2): 1684S-1692S.

39 Saini V., Marchese A. & Majetschak M. 2010. CXC chemokine receptor 4 is a cell surface receptor for extracellular ubiquitin. *Journal of Biological Chemistry*. 285(20): 15566-15576.

40 Sales K.J., Grant V., Catalano R.D. & Jabbour H.N. 2011. Chorionic gonadotrophin regulates CXCR4 expression in human endometrium via E-series prostanoid receptor 2 signalling to PI3K-ERK1/2: implications for fetal-maternal crosstalk for embryo implantation. *Molecular Human Reproduction*. 17(1): 22-32.

41 Sasaki K., Murohara T., Ikeda H., Sugaya T., Shimada T., Shintani S. & Imaizumi T. 2002. Evidence for the importance of angiotensin II type 1 receptor in ischemia-induced angiogenesis. *Journal of Clinical Investigation*. 109(5): 603-611.

42 Schulz R., Menheniott T.R., Woodfine K., Wood A.J., Choi J.D. & Oakey R.J. 2006. Chromosome-wide identification of novel imprinted genes using microarrays and uniparental disomies. *Nucleic Acids Research*. 34(12): e88.

43 Slonim D.K. & Yanai I. 2009. Getting started in gene expression microarray analysis. *PLoS Computational Biology*. 5(10): e1000543.

44 Su L., Zhao S., Zhu M. & Yu M. 2010. Differential expression of microRNAs in porcine placentas on days 30 and 90 of gestation. *Reproduction Fertility and Development*. 22(8): 1175-1182.

45 Subramanian A., Tamayo P., Mootha V.K., Mukherjee S., Ebert B.L., Gillette M.A., Paulovich A., Pomeroy S.L., Golub T.R., Lander E.S. & Mesirov J.P. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of National Academy of Sciences*. USA, 102(43): 15545-15550.

46 Sumner-Thomson J.M., Vierck J.L. & Mcnamara J.P. 2011. Differential expression of genes in adipose tissue of first-lactation dairy cattle. *Journal of Dairy Science*. 94(1): 361-369.

47 Surani M.A., Barton S.C. & Norris M.L. 1984. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature*. 308(5959): 548-550.

48 Surolia I., Pirnie S.P., Chellappa V., Taylor K.N., Cariappa A., Moya J., Liu H., Bell D.W., Driscoll D.R., Diederichs S., Haider K., Netravali I., Le S., Elia R., Dow E., Lee A., Freudenberg J., De Jager P.L., Chretien Y., Varki A., Macdonald M.E., Gillis T., Behrens T.W., Bloch D., Collier D., Korzenik J., Podolsky D.K., Hafler D., Murali M., Sands B., Stone J.H., Gregersen P.K. & Pillai S. 2010. Functionally defective germline variants of sialic acid acetylesterase in autoimmunity. *Nature*. 466(7303): 243-247.

49 Te Pas M.F., Hulsegge I., Coster A., Pool M.H., Heuven H.H. & Janss L.L. 2007. Biochemical pathways analysis of microarray results: regulation of myogenesis in pigs. *BMC Developmental Biology*. 7: 66.

50 Te Pas M.F., Keuning E., Hulsegge B., Hoving-Bolink A.H., Evans G. & Mulder H.A. 2010. Longissimus muscle transcriptome profiles related to carcass and meat quality traits in fresh meat Pietrain carcasses. *Journal of Animal Sciences*. 88(12): 4044-4055.

51 Torry D.S., Wang H.S., Wang T.H., Caudle M.R. & Torry R.J. 1998. Preeclampsia is associated with reduced serum levels of placenta growth factor. *American Journal of Obstetrics and Gynecology*. 179(6 Pt 1): 1539-1544.

52 Tsai S., Hardison N.E., James A.H., Motsinger-Reif A.A., Bischoff S.R., Thames B.H. & Piedrahita J.A. 2011. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. *Placenta*. 32(2): 175-182.

53 Tsai S., Mir B., Martin A.C., Estrada J.L., Bischoff S.R., Hsieh W.P., Cassady J.P., Freking B.A., Nonneman D.J., Rohrer G.A. & Piedrahita J.A. 2006. Detection of transcriptional difference of porcine imprinted genes using different microarray platforms. *BMC Genomics*. 7: 328.

54 Tuggle C.K., Bearson S.M., Uthe J.J., Huang T.H., Couture O.P., Wang Y.F., Kuhar D., Lunney J.K. & Honavar V. 2010. Methods for transcriptomic analyses of the porcine host immune response: application to Salmonella infection using microarrays. *Veterinary Immunology and Immunopathology*. 138(4): 280-291.

55 Venkatesha S., Toporsian M., Lam C., Hanai J., Mammoto T., Kim Y.M., Bdolah Y., Lim K.H., Yuan H.T., Libermann T.A., Stillman I.E., Roberts D., D'amore P.A., Epstein F.H., Sellke F.W., Romero R., Sukhatme V.P., Letarte M. & Karumanchi S.A. 2006. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nature Medicine*. 12(6): 642-649.

56 Verbraak E.J., Van 'T Veld E.M., Groot Koerkamp M., Roelen B.A., Van Haeften T., Stoorvogel W. & Zijlstra C. 2011. Identification of genes targeted by FSH and oocytes in porcine granulosa cells. *Theriogenology*. 75(2): 362-376.

57 Wallukat G., Homuth V., Fischer T., Lindschau C., Horstkamp B., Jupner A., Baur E., Nissen E., Vetter K., Neichel D., Dudenhausen J.W., Haller H. & Luft F.C. 1999. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *Journal of Clinical Investigation*. 103(7): 945-952.

58 Wheelan S.J., Martinez Murillo F. & Boeke J.D. 2008. The incredible shrinking world of DNA microarrays. *Molecular Biosystems*. 4(7): 726-732.

59 Whitworth K.M., Agca C., Kim J.G., Patel R.V., Springer G.K., Bivens N.J., Forrester L.J., Mathialagan N., Green J.A. & Prather R.S. 2005. Transcriptional profiling of pig embryogenesis by using a 15-K member unigene set specific for pig reproductive tissues and embryos. *Biology of Reproduction*. 72(6): 1437-1451.

60 Wildman D.E. 2011. Review: Toward an integrated evolutionary understanding of the mammalian placenta. *Placenta*. 32 (Suppl 2): 142-145.

61 Zhang Y., Zan L. & Wang H. 2011. Screening candidate genes related to tenderness trait in Qinchuan cattle by genome array. *Molecular Biology Reports*. 38(3): 2007-2014.

62 Zhao J. & Grant S.F. 2011. Advances in whole genome sequencing technology. *Current Pharmacology Biotechnology*. 12(2): 293-305.