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Differential expression profiles of mRNAs, miRNAs and proteins during embryo implantation

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1. ABSTRACT

Embryo implantation is a critical step in mammalian reproduction and includes apposition of the blastocyst, attachment to the uterine epithelium and decidualization of the uterine stroma. However, the key mechanism underlying embryo implantation remains to be elucidated. In recent years, high-throughput transcriptomic and proteomic approaches make it possible for studying the expression levels of thousands of genes and proteins simultaneously and thus accelerate the understanding of gene regulatory networks involved in embryo implantation. This review briefly summarizes the advance on mRNA, miRNA and protein expression profile analysis during embryo implantation in human and a variety of animal models.

2. INTRODUCTION

Embryo implantation is a critical step for mammalian reproduction, involving reciprocal interaction between a competent blastocyst and receptive uterus (1). Based on morphological studies, embryo implantation is characterized by three stages, apposition, attachment and invasion (2). Depending on the species, the invasion of trophoblast into the endometrium may remain superficial or continue deep into the endometrium. Using conventional gene-by-gene methods, such as gene knockout, Northern blot and *in situ* hybridization, many specific factors have been identified during embryo implantation. However, the key molecular mechanism underlying embryo implantation process is still unknown.

In recent years, high-throughput transcriptomic and proteomic approaches have been adopted in this field. These 'omics'-based methods are able to study the expression levels of thousands of genes and proteins simultaneously. To date, transcriptomic methods, including oligonucleotide microarrays, cDNA microarrays, and serial analysis of gene expression (SAGE), have been applied to study the implantation process of human, mouse, rat, monkey, cow and pig. The Affymetrix GeneChip® oligonucleotide microarrays (http://www.affymetrix.com) have been widely used and are considered as the gold standard for global gene expression analysis. Affymetrix microarrays are available for human, mouse, rat, rhesus monkey, cow and pig. Noticeably, microRNAs (miRNAs) have emerged as a novel part of the transcriptome. MiRNAs are a family of ~22-nucleotide non-coding RNAs discovered in most metazoan. In animals, they are involved in posttranscriptional gene regulation by mRNA decay and/or translation repression (3). Using specially designed oligonucleotide microarrays, global miRNA expression profiles have been investigated in human decidual cells (4) and mouse uterus (5, 6) during embryo implantation. However, the real functional molecules of the cell are proteins. Due to posttranscriptional and posttranslational regulation on protein expression, strong gene expression does not necessarily mean that the corresponding protein is also abundant or indeed active. Therefore, proteomic approaches that identify changes in proteins can be of key importance. A few proteomics-based studies have been undertaken to understand the embryo implantation process in human, mouse, monkey and cow. A variety of proteomic platforms are utilized, including two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), two-dimensional differential in-gel electrophoresis (2D-DIGE) and isotope-coded affinity tags (ICAT).

The advance on the global analysis of gene expression during embryo implantation has been reviewed previously (7-11). In this review, we will briefly summarize recent studies on mRNA, miRNA and protein expression profile in human and animal models that address the molecular mechanisms involved in embryo implantation.

3. TRANSCRIPTOMIC APPROACH TO UNDERSTAND IMPLANTATION

Transcriptomic approach analyzes all the RNA molecules transcribed from DNA in cells. Several high-throughput profiling technologies have been developed to detect thousands of transcripts simultaneously, such as oligonucleotide microarrays, cDNA microarray and serial analysis of gene expression (SAGE). Microarray analysis is the most common method applied in transcriptomic studies.

3.1. Humans

Human endometrium is highly dynamic throughout the menstrual cycle. Based on an ideal 28-day cycle, menstrual cycle is divided into menstruation phase, proliferative phase and secretory phase. The window of implantation in human endometrium is believed to reside in the mid-secretory phase (12). The proliferative phase is widely used as a pre-receptive control to find differentially expressed transcripts in the receptive endometrium.

Global gene expression changes during the window of implantation have been determined by five independent groups (13-17). The Affymetrix HG-U95A microarray, which contains 12686 human genes and expressed sequence tags, was used in these studies. However, a strong consistency is not observed. The expression trends of 8 genes (matrilysin, protocadherin 68, carbonic anhydrase precursor, SEC14L gene, receptor protein-tyrosine kinase, c-fos, HLA-D class II antigen DO beta chain, and NADP-dependent isocitrate dehydrogenase) are controversial among these studies. A set of 107 genes (72 up-regulated and 35 down-regulated) are consistently regulated in 2 or more than 2 studies. Only one gene, osteopontin, is consistently up-regulated in all the five studies (9).

Most recently, another transcriptomic screen was performed with Affymetrix HG-U133 plus 2.0 microarrays by Haouzi *et a*l (18). As a successor of the HG-U95A microarray, the HG-U133 plus 2.0 microarray (containing 54,675 probe sets or 21,083 genes) provide a better coverage of the entire human transcriptome. There are 746 genes up-regulated and 51 genes down-regulated in the window of implantation. Interestingly, the osteopontin gene was also identified as one of the up-regulated genes in this study.

3.2. Mice

Although there are substantial mechanistic differences between rodent and human implantation, mouse model has been a powerful tool in elucidating gene function during embryo implantation (1).

3.2.1. Implantation sites versus inter-implantation sites

The first transcriptomic study on mouse implantation was performed by Reese *et al* (19). Whole uterus from day 4 evening (2300~2400h) was used for analysis by Affymetrix microarrays. Comparing with inter-implantation sites, there are 36 up-regulated genes and 27 down-regulated genes at the implantation site. According to functional assignments, these genes fall into the following categories: growth factors/cytokines and their receptors, transcription factors, structural proteins, genes associated with cell proliferation and calcium-related genes.

The comparison on expression profiles between the implantation sites and inter-implantation sites (total uterus) on day 5 of pregnancy have also been examined using serial analysis of gene expression (SAGE) technology. A total of 16,677 and 16,696 unique transcripts were detected at implantation sites and inter-implantation sites using 11-bp tag profiling, respectively. Compared with inter-implantation sites, there are 100 genes up-regulated and 127 genes down-regulated at the implantation sites (20). Among these up-regulated genes, Fst, Hsp105 and Igfbp3 are also reported by Reese *et al* (19).

The embryo attachment occurs on the luminal epithelium. However, the luminal epithelium represents

only 5~10% of the major uterine cell types (21). Because the whole uterus was used in the above-mentioned studies, it is difficult to characterize the specific gene expression in the luminal epithelium because of the interference from large amounts of uterine stroma and myometrium. Chen *et al* (22) used enzymatically isolated luminal epithelium for microarray analysis. There are a total of 136 genes up-regulated and 223 genes down-regulated by at least 2 folds at implantation sites compared with inter-implantation sites, and some of the significantly changed genes were validated by semi-quantitative RT-PCR and in situ hybridization. They also found that Cyr61 is specifically expressed in the luminal epithelium at implantation sites and its expression is dependent upon the presence of an active blastocyst, suggesting that Cyr61 may play a role in embryo attachment. In another study, the luminal epithelium was isolated from mouse uterus of implantation sites and inter-implantation sites by laser-capture micro-dissection (LCM). Microarray analysis showed that 73 genes are highly expressed at implantation sites, while 13 genes are highly expressed at inter-implantation sites (23). There are 3 genes detected in these two independent studies: gamma-glutamyl hydrolase, inhibitor of DNA-binding 3, and secreted acidic cysteine-rich glycoprotein.

3.2.2. Delayed implantation and activation

In mice, mating taking place at the postpartum estrus leads to a lactational delay of embryo implantation. Similar to natural delay of implantation, an experimentally induced model can be achieved through ovariectomized pregnant females on day 4 of pregnancy followed by progesterone treatment to maintain the delayed implantation. A single dose of estrodiol can re-initiate the implantation process. Using microarrays, Reese *et al* (19) found that 128 genes are up-regulated and 101 genes are down-regulated after activation of the delayed implantation. Among the up-regulated genes, there are more genes involved in DNA processing, cell cycle and enzyme activity.

Based on a combination of laser-capture micro-dissection (LCM) and cDNA arrays, the gene expression profiles of luminal and glandular epithelium 8 h after activation were compared with delayed implantation. Among the significantly changed genes, 153 genes are significantly up-regulated in the luminal epithelium and enriched in lipid, metal-ion binding, and carbohydrate-metabolizing enzymes, while 118 genes are significantly up-regulated in the glandular epithelium, of which 5 genes are involved in immune response (24).

Embryo implantation is a reciprocal interaction between the blastocyst and receptive uterus. A competent embryo is also essential for embryo implantation. Several groups performed the gene expression profile analysis in embryonic development during peri-implantation period (25-31). However, it is hard to dissect embryonic factors that are important for embryo implantation from these studies. Blastocysts from delayed implantation and activation provided an opportunity for characterizing genes that are crucial for embryo implantation. Hamatani *et al* (32) identified 229 genes that are differentially expressed between dormant and activated blastocysts using microarrays. The major functional categories of these differentially expressed genes include cell cycle, cell signaling, and energy metabolic pathways. This work provided important information about the embryo side of uterine receptivity.

3.2.3. Pseudo-pregnancy

When a fertile female mouse is mated with a vasectomized male mouse, the series of changes in pseudo-pregnant uterus resemble those during the first four days of natural pregnancy. Pseudo-pregnant mice are widely used to determine receptive phases of mouse uterus. Compared to natural early pregnancy, pseudo-pregnant uterus is free from embryo contamination.

Gene expression profiles in purified luminal epithelium of pseudo-pregnant mouse uterus have been examined by microarray analysis (33). Gene expression levels were determined at 5 time points with 12h intervals from pre-receptivity, the implantation window to refractoriness. Among the genes whose abundance changes significantly over the measurement period, some were previously found to be implicated in the Lif-Stat3 signaling pathway (e.g. Lifr, Osmr, Il1r1, Stat3, Jak, Areg, A130010J15Rik, Calb3, Coch, Isgf3g, Slfn3, and Igfbp3), and the prostaglandin pathway (e.g. Edg7, Pla2g4a, Pla2g10, Ptgds, Ptgs1, Ptger2, Pla2g7, Pafah1b3, Anxa3/8, Alox5ap, Alox15, and Alox12e) during embryo implantation.

3.3. Non-human primates

Primates are the closest extant relative to humans. A regular menstrual cycle also occurs in the higher primates. Non-human primates are ideal models for studying embryo implantation in humans.

Endometrial gene expression profiles of rhesus monkey on both day 13 and days 21~23 during artificial menstrual cycles have been compared using Affymetrix HG-U95A microarrays which are designed according to the human genome (34). Using an arbitrary 2-fold cut-off for every gene, 39 genes are up-regulated and 69 genes are down-regulated. The up-regulated genes include secretoglobin (uteroglobin), histone 2A, polo-like kinase (PLK), spermidine/spermine acetyltransferase 2 (SAT2), secretory leukocyte protease inhibitor (SLPI) and metallothionein 1G (MT1G). Meanwhile, transforming growth factor beta-induced (TGFBI or BIGH3), matrix metalloproteinase 11 (stromelysin 3), proenkephalin (PENK), cysteine/glycine-rich protein 2 (CSRP2), collagen type VIIalpha 1 (COL7A1), secreted frizzled-related protein 4 (SFRP4), progesterone receptor membrane component 1 (PGRMC1), chemokine (C-X-C) ligand 12 (CXCL12) and biglycan (BGN) are among the down-regulated genes.

3.4. Cows

In adult cows, the uteri are comprised of large caruncles and inter-caruncular areas. The caruncluar areas are the sites of implantation (35). The embryo implantation in cows is epitheliochorial and has a prolonged apposition

and attachment phase. Cows are good models for studying the early phase of embryo implantation.

In cows, Ishiwata *et al* (36) compared gene expression levels of non-pregnant uteri with pregnant ones using cDNA microarrays. They have identified 77 genes up-regulated and 12 genes down-regulated in the pregnant uteri. Among them, pregnancy-associated glycoprotein-1(PAG-1), placental lactogen (PL) and prolactin-related protein-1 (PRP-1) were previously defined as pregnancy-specific genes (37-39).

3.5. Pigs

A lengthy pre-attachment period is observed in pig, during which the spherical blastocyst elongates to form a filamentous conceptus of about 10cm in length. Like cows, the prolonged apposition and attachment phase makes the pig a good candidate model to study the early phase of embryo implantation.

In pigs, genome-wide comparison of gene expression of pregnant and non-pregnant uteri on day 14 after insemination was performed by Ostrup *et al* (40) using Affymetrix microarrays. Of 20,201 genes represented on the microarray, 146 genes are up-regulated and 117 genes are down-regulated in the pregnant animals. Among these genes, IL6R, LIFR, IL11RA, MUC4, ERBB3, FGF9, and FGFR3 are shown to be crucial in the gene interaction network associated with embryo implantation.

4. MIRNAS AND IMPLANTATION

MiRNAs are ~22 nucleotides endogenous non-coding RNAs which function as post-transcriptional regulators of gene expression. MiRNAs bind to the 3' untranslated regions (UTRs) of their target mRNAs, leading to mRNA degradation or translational repression (41). Gene ablation experiments indicate that miRNAs play a role in the postnatal development of mouse uterus and oviducts (42-44).

4.1. Humans

Recently, Kuokkanen *et al* (45) comparatively analyzed the miRNA and mRNA expression profiles in isolated endometrial epithelial cells during late proliferative phase and mid-secretory phase. A total of 12 miRNAs (miR-29b, miR-29c, Mir-30b, miR-30d, miR-31, miR-193a-3p, miR-203, miR-204, miR-200c, miR-210, miR-582-5p, and miR345) were significantly up-regulated and 12 miRNAs (miR-105, miR-127, miR-134, miR-214, miR-222, miR-369-5p, miR-370, miR-376a, miR-382, miR-450, miR-503, and miR-542-3p) were significantly down-regulated in the mid-secretory phase compared to the late proliferative phase. Importantly, the up-regulated miRNAs in the mid-secretory phase were predicted to target many genes involved in DNA replication licensing and in the cell cycle. These miRNAs are likely to play an important role in helping endometrial epithelial cells to exit cell proliferation and enter differentiation during the window of implantation.

4.2. Mice

The miRNA profiles of mouse uterus on days 1 and 4 of natural pregnancy was analyzed by Chakrabarty *et el* (5). Microarray results showed that 32 miRNAs are significantly up-regulated on day 4 and only 5 miRNAs are expressed at higher levels on day 1. Using in situ hybridization, the spatiotemporal expression of miR-101a and miR-199a* were found to be co-localized with Cox-2 mRNA. Furthermore, the Cox-2 protein is inversely correlated with miR-101a expression during artificial decidualization process after oil infusion. Luciferase reporter assay confirmed that Cox-2 is a direct target of miR-101a and miR-199a*. These findings demonstrate that miRNAs are linked to embryo implantation through regulating the expression of important implantation factors, like Cox-2.

The miRNA expression profiles of implantation sites and inter-implantation sites of pregnant mice on day 5 were also examined (6). Of 249 mouse miRNAs designed on locked nucleic acid (LNA) microarrays, 13 miRNAs are significantly up-regulated and 2 miRNAs are significantly down-regulated at implantation sites compared with inter-implantation sites. MiR-21, being one of the up-regulated miRNAs, is predicted to target Reck. The interaction between miR-21 and Reck was experimentally confirmed using luciferase assay. Moreover, Reck suppresses MMP-9 activity in cultured uterine stromal cells. Thus, miR-21 may play an important role in the process of embryo invasion by regulating the activity of MMP-9 through its target gene Reck.

Recently, deep sequencing technology has been used to profile miRNAs. This approach has the advantage not only to detect low abundance sequences, but also to provide absolute quantitative data (46, 47). This technology was used to identify miRNA changes in mouse uterus under delayed implantation and activation (48). There are 20 miRNAs up-regulated and 42 miRNAs down-regulated during activation. Interestingly, the percentage of let-7 RNA editing at positions 4 and 5 was significantly higher under delayed implantation than that under activation. As perfect pairing to the miRNA seed (nucleotides 2 to 7) is important for target recognition, miRNA editing at positions 4 and 5 may interrupt the miRNA-mRNA binding. As a result, when G to C editing occurs at position 5 of let-7a, it loses the ability to regulate its target genes, for example, Klf9, Gatm and Dnajb9; meanwhile, the edited form of let-7a was shown to target a novel set of genes by seed sequence paring, Tmem55a, Timp3 and Smad7. The target shifts were confirmed by luciferase reporter assay. This study adds valuable insights to the complexity of the miRNAome in uterus.

5. PROTEOMIC ANALYSIS OF EMBRYO IMPLANTATION

Proteins are key players in cells. The correlation between mRNA and protein expression is typically modest due to substantial posttranscriptional regulation (49). Moreover, posttranslational modifications and subcellular locations largely affect the function and activity of proteins.

These diverse features are not predictable from gene expression levels. Thus, proteomics may provide a better understanding of the molecular event involved in embryo implantation at the protein expression level.

5.1. Humans

Protein expression profile during the window of implantation was studied by several groups using a variety of proteomic methods, including two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), two dimensional differential in-gel electrophoresis (2D-DIGE) and isotope-coded affinity tags (ICAT).

Using 2D-PAGE coupled with MALDI-TOF-TOF, Parmar *et al* (50) found that calreticulin, beta chain of fibrinogen, adenylate kinase isoenzyme 5, and transferrin are down-regulated, while annexin V, alpha 1- antitrypsin, creatine kinase, and peroxidoxin 6 are up-regulated in mid-secretory phase endometrium in comparison with proliferative phase. This investigation also revealed the presence of heat-shock protein 27, transferrin, and alpha precursor in both endometrial tissues and uterine fluids during proliferative phase.

Through 2D-DIGE, Chen *et al* (51) identified 76 differentially expressed proteins, representing 41 unique gene products in the endometrium between the proliferative and secretory phases. The observed changes in 3 proteins (Rho-GDIR, CLIC1 and PGRMC1) were confirmed by immunohistochemistry. Similarly, Dominguez *et al* (52) studied the proteome of endometrial biopsies obtained at LH+2 and LH+7 from the same women in two separate experiments. In this study, 32 differentially expressed proteins were indentified but only annexin A2 and stathmin 1 were consistently regulated in the two experiments performed. These 2 proteins were validated by Western blot and immunohistochemistry.

Proteomic analysis of human endometrium during the proliferative and secretory phases was also carried out with isotope-coded affinity tags (ICAT) technologies (53). In total, 119 proteins were identified by ProICAT software followed by manual inspection procedure, of which 5 proteins showed consistent differential expression in all three sample pairs, including glutamate NMDA receptor subunit zeta 1 precursor and FRAT1.

Besides, secreted proteins from endometrial tissues are also under investigation. Using a combination of 3 proteomic strategies, Casado-Vela *et al* (54) have shown the evidence for presence of 803 different protein species in the endometrial fluid during secretory phase of menstrual cycle. In another study, the secretome changes between the pre-receptive (LH+4) and the receptive (LH+9) phases have been compared (55). In all, 152 proteins have been identified, of which 82 are differentially expressed. These differentially expressed proteins are involved in host defense, coagulation, apoptosis regulation, and stress response.

5.2. Mice

Low molecular weight proteins expressed on days 5 and 6 of pregnancy were screened using in situ imaging Matrix-assisted laser desorption/ionization (MALDI) (56). A total of 230 distinct peaks were found in wild type mouse uterus, of which 50 peaks were differentially expressed at implantation sites compared to inter-implantation sites. There are 4 peaks identified as ubiquitin (m/z 8565), calgizzarin (m/z 10952), calcyclin (m/z 9962), and transthyretin (m/z 13641). The spatial localization of these proteins was validated by in situ hybridization at the mRNA level.

5.3. Non-human primates

The receptive and non-receptive endometria obtained from vehicle-treated control and onapristone-treated bonnet monkeys were compared using two-dimensional proteomic technologies (57). Through mass spectrometric analysis, 2 differentially expressed proteins are identified. One is calreticulin expressed in the glandular epithelium. The other one is protein disulfide-isomerase (PDI) which is located in the stroma. They are both highly expressed in non-receptive endometrium.

5.4. Cows

The protein expression profiles of pregnant and non-pregnant endometrium in the pre-attachment period (day 18 of pregnancy) were analyzed by 2-D DIGE (58). Statistical analysis revealed that the expression levels of 4 proteins are significantly affected: Rho GDP dissociation inhibitor beta, 20 alpha-hydroxysteroid dehydrogenase, soluble NADP1-dependent isocitrate dehydrogenase 1 and acyl-CoA-binding protein. All of them are increased in pregnant animals.

6. CONCLUSIONS AND PERSPECTIVES

Transcriptomic and proteomic methods have shown great success and promise in implantation biology over the last decade. A large repository of mRNA, miRNA and protein expression profiles has been built during embryo implantation, covering a variety of model organisms, including human, monkey, mouse, rat, sheep, cow and pig. Collectively, these high throughput studies indicate that cell cycle, cell proliferation, intracellular signaling, transcription and translation, and cellular metabolism are the main biological processes controlling the window of embryo implantation.

However, due to the differences of animal models and sample collection protocols, it is difficult to make an integrative or comparative analysis of all the heterogenic data that are now available. Furthermore, the choice of platforms and statistical criteria makes it even more difficult to compare between different studies because of a lack of benchmarking frame. In addition, microarrays are limited by the number and nature of the spots on each array and suffer from non-specific background levels of hybridization. In human endometrium, even if the same microarray was used in five studies, only one gene, osteopontin, is consistently up-regulated in all the 5 studies (9). The mRNA-sequencing, which utilizes the latest massively parallel sequencing, has provided an alternative way to obtain mRNA expression profiles at unprecedented sensitivity (59, 60). Besides transcriptome quantification,

mRNA-sequencing is suitable to reveal more details of transcriptome, including RNA editing, alternative splicing and gene fusion. The gene fusion of JAZF1-JJAZ1 was observed in normal endometrium (61). Little is known if this is a rare or common event until we have endometrial transcriptome sequenced. Similarly, small RNA-sequencing (deep sequencing) has shown to have the potential to replace miRNA microarrays in miRNA profiling studies. As for proteomic methods, current techniques are confronted with problems of resolution, precision and accuracy. One of the major advances in proteomics in recent years is shotgun proteomics. It is a label-free proteomic method utilizing high performance liquid chromatography combined with mass spectrometry (62). It is a promising next generation proteomic method, yet there are relatively few descriptions of its use in implantation biology. The only case was reported on the study of glycomics and proteomics of mouse uterine luminal fluid (63).

Furthermore, analytical tools in the genomic field are evolving fast. Beyond transcriptomic, miRNAomic and proteomic technologies, there is a need to expand our repertoire to include epigenomic, metabolomic, glycomic, and other 'omic' approaches. The application of these powerful high throughput methods will enable us to look closer than ever before at the molecular mechanism of embryo implantation.

7. ACKNOWLEDGMENT

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8. REFERENCES

1. Wang, H. & S. K. Dey: Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*, 7, 185-99 (2006)

2. Enders, A. C. & S. Schlafke: Cytological aspects of trophoblast-uterine interaction in early implantation. *Am J Anat*, 125, 1-29 (1969)

3. Nilsen, T. W.: Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet*, 23, 243-9 (2007)

4. Qian, K., L. Hu, H. Chen, H. Li, N. Liu, Y. Li, J. Ai, G. Zhu, Z. Tang & H. Zhang: Hsa-miR-222 is involved in differentiation of endometrial stromal cells in vitro. *Endocrinology*, 150, 4734-43 (2009)

5. Chakrabarty, A., S. Tranguch, T. Daikoku, K. Jensen, H. Furneaux & S. K. Dey: MicroRNA regulation of cyclooxygenase-2 during embryo implantation. *Proc Natl Acad Sci U S A*, 104, 15144-9 (2007)

6. Hu, S. J., G. Ren, J. L. Liu, Z. A. Zhao, Y. S. Yu, R. W. Su, X. H. Ma, H. Ni, W. Lei & Z. M. Yang: MicroRNA expression and regulation in mouse uterus during embryo implantation. *J Biol Chem*, 283, 23473-84 (2008)

7. White, C. A. & L. A. Salamonsen: A guide to issues in microarray analysis: application to endometrial biology. *Reproduction*, 130, 1-13 (2005)

8. Giudice, L. C.: Application of functional genomics to primate endometrium: insights into biological processes. *Reprod Biol Endocrinol*, 4 Suppl 1, S4 (2006)

9. Horcajadas, J. A., A. Pellicer & C. Simon: Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Hum Reprod Update*, 13, 77-86 (2007)

10. Groothuis, P. G., H. H. Dassen, A. Romano & C. Punyadeera: Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. *Hum Reprod Update*, 13, 405-17 (2007)

11. Evans, A. C., N. Forde, G. M. O'Gorman, A. E. Zielak, P. Lonergan & T. Fair: Use of microarray technology to profile gene expression patterns important for reproduction in cattle. *Reprod Domest Anim*, 43 Suppl 2, 359-67 (2008)

12. Wilcox, A. J., D. D. Baird & C. R. Weinberg: Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med*, 340, 1796-9 (1999)

13. Carson, D. D., E. Lagow, A. Thathiah, R. Al-Shami, M. C. Farach-Carson, M. Vernon, L. Yuan, M. A. Fritz & B. Lessey: Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod*, 8, 871-9 (2002)

14. Kao, L. C., S. Tulac, S. Lobo, B. Imani, J. P. Yang, A. Germeyer, K. Osteen, R. N. Taylor, B. A. Lessey & L. C. Giudice: Global gene profiling in human endometrium during the window of implantation. *Endocrinology*, 143, 2119-38 (2002)

15. Borthwick, J. M., D. S. Charnock-Jones, B. D. Tom, M. L. Hull, R. Teirney, S. C. Phillips & S. K. Smith: Determination of the transcript profile of human endometrium. *Mol Hum Reprod*, 9, 19-33 (2003)

16. Riesewijk, A., J. Martin, R. van Os, J. A. Horcajadas, J. Polman, A. Pellicer, S. Mosselman & C. Simon: Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. *Mol Hum Reprod*, 9, 253-64 (2003)

17. Mirkin, S., M. Arslan, D. Churikov, A. Corica, J. I. Diaz, S. Williams, S. Bocca & S. Oehninger: In search of candidate genes critically expressed in the human endometrium during the window of implantation. *Hum Reprod*, 20, 2104-17 (2005)

18. Haouzi, D., K. Mahmoud, M. Fourar, K. Bendhaou, H. Dechaud, J. De Vos, T. Reme, D. Dewailly & S. Hamamah: Identification of new biomarkers of human endometrial receptivity in the natural cycle. *Hum Reprod*, 24, 198-205 (2009)

19. Reese, J., S. K. Das, B. C. Paria, H. Lim, H. Song, H. Matsumoto, K. L. Knudtson, R. N. DuBois & S. K. Dey: Global gene expression analysis to identify molecular markers of uterine receptivity and embryo implantation. *J Biol Chem*, 276, 44137-45 (2001)

20. Ma, X. H., S. J. Hu, H. Ni, Y. C. Zhao, Z. Tian, J. L. Liu, G. Ren, X. H. Liang, H. Yu, P. Wan & Z. M. Yang: Serial analysis of gene expression in mouse uterus at the implantation site. *J Biol Chem*, 281, 9351-60 (2006)

21. Finn, C. A. & D. G. Porter: The Uterus: Cells and Tissues of the Endometrium. Publishing Sciences Group, Inc.(1975).

22. Chen, Y., H. Ni, X. H. Ma, S. J. Hu, L. M. Luan, G. Ren, Y. C. Zhao, S. J. Li, H. L. Diao, X. Xu, Z. A. Zhao & Z. M. Yang: Global analysis of differential luminal epithelial gene expression at mouse implantation sites. *J Mol Endocrinol*, 37, 147-61 (2006)

23. Yoon, S. J., D. H. Choi, W. S. Lee, K. Y. Cha, S. N. Kim & K. A. Lee: A molecular basis for embryo apposition at the luminal epithelium. *Mol Cell Endocrinol*, 219, 95-104 (2004)

24. Niklaus, A. L. & J. W. Pollard: Mining the mouse transcriptome of receptive endometrium reveals distinct molecular signatures for the luminal and glandular epithelium. *Endocrinology*, 147, 3375-90 (2006)

25. Zhang, P., M. Zucchelli, S. Bruce, F. Hambiliki, A. Stavreus-Evers, L. Levkov, H. Skottman, E. Kerkela, J. Kere & O. Hovatta: Transcriptome profiling of human pre-implantation development. *PLoS One*, 4, e7844 (2009)

26. Chen, H. W., J. J. Chen, S. L. Yu, H. N. Li, P. C. Yang, C. M. Su, H. K. Au, C. W. Chang, L. W. Chien, C. S. Chen & C. R. Tzeng: Transcriptome analysis in blastocyst hatching by cDNA microarray. *Hum Reprod*, 20, 2492-501 (2005)

27. Xie, D., C. C. Chen, L. M. Ptaszek, S. Xiao, X. Cao, F. Fang, H. H. Ng, H. A. Lewin, C. Cowan & S. Zhong: Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. *Genome Res*, 20, 804-15 (2010)

28. Maekawa, M., T. Yamamoto, M. Kohno, M. Takeichi & E. Nishida: Requirement for ERK MAP kinase in mouse preimplantation development. *Development*, 134, 2751-9 (2007)

29. Jeong, H. J., H. J. Kim, S. H. Lee, K. Kwack, S. Y. Ahn, Y. J. Choi, H. G. Kim, K. W. Lee, C. N. Lee & K. Y. Cha: Gene expression profiling of the pre-implantation mouse embryo by microarray analysis: comparison of the two-cell stage and two-cell block. *Theriogenology*, 66, 785-96 (2006)

30. Hamatani, T., M. G. Carter, A. A. Sharov & M. S. Ko: Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell*, 6, 117-31 (2004)

31. Ushizawa, K., C. B. Herath, K. Kaneyama, S. Shiojima, A. Hirasawa, T. Takahashi, K. Imai, K. Ochiai, T. Tokunaga, Y. Tsunoda, G. Tsujimoto & K. Hashizume: cDNA microarray analysis of bovine embryo gene expression profiles during the pre-implantation period. *Reprod Biol Endocrinol*, 2, 77 (2004)

32. Hamatani, T., T. Daikoku, H. Wang, H. Matsumoto, M. G. Carter, M. S. Ko & S. K. Dey: Global gene expression analysis identifies molecular pathways distinguishing blastocyst dormancy and activation. *Proc Natl Acad Sci U S A*, 101, 10326-31 (2004)

33. Campbell, E. A., L. O'Hara, R. D. Catalano, A. M. Sharkey, T. C. Freeman & M. H. Johnson: Temporal expression profiling of the uterine luminal epithelium of the pseudo-pregnant mouse suggests receptivity to the fertilized egg is associated with complex transcriptional changes. *Hum Reprod*, 21, 2495-513 (2006)

34. Ace, C. I. & W. C. Okulicz: Microarray profiling of progesterone-regulated endometrial genes during the rhesus monkey secretory phase. *Reprod Biol Endocrinol*, 2, 54 (2004)

35. Gray, C. A., F. F. Bartol, B. J. Tarleton, A. A. Wiley, G. A. Johnson, F. W. Bazer & T. E. Spencer: Developmental biology of uterine glands. *Biol Reprod*, 65, 1311-23 (2001)

36. Ishiwata, H., S. Katsuma, K. Kizaki, O. V. Patel, H. Nakano, T. Takahashi, K. Imai, A. Hirasawa, S. Shiojima, H. Ikawa, Y. Suzuki, G. Tsujimoto, Y. Izaike, J. Todoroki & K. Hashizume: Characterization of gene expression profiles in early bovine pregnancy using a custom cDNA microarray. *Mol Reprod Dev*, 65, 9-18 (2003)

37. Green, J. A., S. Xie, X. Quan, B. Bao, X. Gan, N. Mathialagan, J. F. Beckers & R. M. Roberts: Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. *Biol Reprod*, 62, 1624-31 (2000)

38. Nakano, H., T. Takahashi, K. Imai & K. Hashizume: Expression of placental lactogen and cytokeratin in bovine placental binucleate cells in culture. *Cell Tissue Res*, 303, 263-70 (2001)

39. Schuler, L. A., M. A. Kessler, M. Tanaka & K. Nakashima: Nomenclature clarification for the bovine placental prolactin-related hormones. *Endocrinology*, 129, 2057 (1991)

40. Ostrup, E., S. Bauersachs, H. Blum, E. Wolf & P. Hyttel: Differential endometrial gene expression in pregnant and nonpregnant sows. *Biol Reprod*, 83, 277-85 (2010)

41. Bartel, D. P.: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116, 281-97 (2004)

42. Gonzalez, G. & R. R. Behringer: Dicer is required for female reproductive tract development and fertility in the mouse. *Mol Reprod Dev*, 76, 678-88 (2009)

43. Nagaraja, A. K., C. Andreu-Vieyra, H. L. Franco, L. Ma, R. Chen, D. Y. Han, H. Zhu, J. E. Agno, P. H. Gunaratne, F. J. DeMayo & M. M. Matzuk: Deletion of Dicer in somatic cells of the female reproductive tract causes sterility. *Mol Endocrinol*, 22, 2336-52 (2008)

44. Hong, X., L. J. Luense, L. K. McGinnis, W. B. Nothnick & L. K. Christenson: Dicer1 is essential for female fertility and normal development of the female reproductive system. *Endocrinology*, 149, 6207-12 (2008)

45. Kuokkanen, S., B. Chen, L. Ojalvo, L. Benard, N. Santoro & J. W. Pollard: Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. *Biol Reprod*, 82, 791-801 (2010)

46. Creighton, C. J., J. G. Reid & P. H. Gunaratne: Expression profiling of microRNAs by deep sequencing. *Brief Bioinform*, 10, 490-7 (2009)

47. Creighton, C. J., A. L. Benham, H. Zhu, M. F. Khan, J. G. Reid, A. K. Nagaraja, M. D. Fountain, O. Dziadek, D. Han, L. Ma, J. Kim, S. M. Hawkins, M. L. Anderson, M. M. Matzuk & P. H. Gunaratne: Discovery of novel microRNAs in female reproductive tract using next generation sequencing. *PLoS One*, 5, e9637 (2010)

48. Su, R. W., W. Lei, J. L. Liu, Z. R. Zhang, B. Jia, X. H. Feng, G. Ren, S. J. Hu & Z. M. Yang: The integrative analysis of microRNA and mRNA expression in mouse uterus under delayed implantation and activation. *PLoS One*, 5, e15513 (2010)

49. Foth, B. J., N. Zhang, S. Mok, P. R. Preiser & Z. Bozdech: Quantitative protein expression profiling reveals extensive post-transcriptional regulation and post-translational modifications in schizont-stage malaria parasites. *Genome Biol*, 9, R177 (2008)

50. Parmar, T., S. Gadkar-Sable, L. Savardekar, R. Katkam, S. Dharma, P. Meherji, C. P. Puri & G. Sachdeva: Protein profiling of human endometrial tissues in the midsecretory and proliferative phases of the menstrual cycle. *Fertil Steril*, 92, 1091-103 (2009)

51. Chen, J., N. Hannan, Y. Mak, P. Nicholls, J. Zhang, A. Rainczuk, P. Stanton, D. Robertson, L. Salamonsen & A. Stephens: Proteomic Characterization of Mid-Proliferative and Mid-Secretory Human Endometrium. *J Proteome Res* (2009)

52. Dominguez, F., T. Garrido-Gomez, J. A. Lopez, E. Camafeita, A. Quinonero, A. Pellicer & C. Simon: Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated. *Hum Reprod*, 24, 2607-17 (2009)

53. DeSouza, L., G. Diehl, E. C. Yang, J. Guo, M. J. Rodrigues, A. D. Romaschin, T. J. Colgan & K. W. Siu: Proteomic analysis of the proliferative and secretory phases of the human endometrium: protein identification and differential protein expression. *Proteomics*, 5, 270-81 (2005)

54. Casado-Vela, J., E. Rodriguez-Suarez, I. Iloro, A. Ametzazurra, N. Alkorta, J. A. Garcia-Velasco, R. Matorras, B. Prieto, S. Gonzalez, D. Nagore, L. Simon & F. Elortza: Comprehensive proteomic analysis of human endometrial fluid aspirate. *J Proteome Res*, 8, 4622-32 (2009)

55. Scotchie, J. G., M. A. Fritz, M. Mocanu, B. A. Lessey & S. L. Young: Proteomic analysis of the luteal endometrial secretome. *Reprod Sci*, 16, 883-93 (2009)

56. Burnum, K. E., S. Tranguch, D. Mi, T. Daikoku, S. K. Dey & R. M. Caprioli: Imaging mass spectrometry reveals unique protein profiles during embryo implantation. *Endocrinology*, 149, 3274-8 (2008)

57. Parmar, T., S. Nimbkar-Joshi, R. R. Katkam, S. Gadkar-Sable, U. Chaudhari, D. D. Manjramkar, L. Savardekar, S. Jacob, C. P. Puri & G. Sachdeva: Differential expression of calreticulin, a reticuloplasmin in primate endometrium. *Hum Reprod*, 24, 2205-16 (2009)

58. Berendt, F. J., T. Frohlich, S. E. Schmidt, H. D. Reichenbach, E. Wolf & G. J. Arnold: Holistic differential analysis of embryo-induced alterations in the proteome of bovine endometrium in the preattachment period. *Proteomics*, 5, 2551-60 (2005)

59. Marioni, J. C., C. E. Mason, S. M. Mane, M. Stephens & Y. Gilad: RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res*, 18, 1509-17 (2008)

60. Wang, Z., M. Gerstein & M. Snyder: RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*, 10, 57-63 (2009)

61. Li, H., J. Wang, G. Mor & J. Sklar: A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells. *Science*, 321, 1357-61 (2008)

62. Swanson, S. K. & M. P. Washburn: The continuing evolution of shotgun proteomics. *Drug Discov Today*, 10, 719-25 (2005)

63. Kuo, C. W., C. M. Chen, Y. C. Lee, S. T. Chu & K. H. Khoo: Glycomics and proteomics analyses of mouse uterine luminal fluid revealed a predominance of Lewis Y and X epitopes on specific protein carriers. *Mol Cell Proteomics*, 8, 325-42 (2009)

Abbreviations: EST (expressed sequencing tags), SAGE (Serial Analysis of Gene Expression), 2D-PAGE (two-dimensional polyacrylamide gel electrophoresis), 2D-DIGE (two dimensional differential in-gel electrophoresis), ICAT (isotope-coded affinity tags), NGS (next generation sequencing).

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