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Review

MicroRNAs in Assisted Reproduction and their Potential Role in IVF Failure

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Abstract. *MicroRNAs (miRNAs) have recently emerged as important regulators of gene expression stability. In the endometrium, miRNAs are involved in the dynamic changes associated with the menstrual cycle, implicated in implantation and in reproductive disorders. We performed a review in an attempt to assess the potential biological pathways linking altered miRNAs profiles with in vitro fertilisation (IVF) failure. Crucially, as miRNAs appear to have a significant role in the course of reproduction, they are excellent research candidates with the potential to enable a better understanding over the underlying molecular activities that prevent implantation and further progression of the embryo. Further steps include in-depth pathway mapping of the implantation process and the characterization of the respective miRNAs and associated links. The efficiency of any intervention should determine whether miRNA profiling could possibly be adopted in routine practice to substantially improve the diagnostic accuracy and, in parallel, the directed treatment of the next-generation IVF.*

Although assisted human reproduction has evolved vastly over the past few decades marked by numerous milestone technological advances, some barriers are yet to be overcome. Among these is the control of the implantation process by fully understanding and successfully dealing with possible difficulties arising during this crucial step. Adequate endometrial receptivity and embryo development are essential for the pursued goal of increased pregnancy rates and reduced early pregnancy loss (1). Implantation failure is strongly related to the embryo transfer technique itself, embryo quality and endometrial receptivity and a synchronized dialogue between the maternal tissues and the blastocyst (2). In assisted reproductive techniques (ARTs), where the high-quality embryos are transferred, implantation remains the rate-limiting step for the success of treatment (3, 4). Therefore, a better understanding over the implantation process and the importance of the factors involved is warranted.

With the development of 'omics' technologies, numerous whole-genome expression analyses of the human endometrium have revealed hundreds of simultaneously up- and down-regulated genes that play a role in endometrial receptivity, embryo development and embryo-endometrial signaling (5, 6). Nevertheless, the molecular mechanisms regulating the expression of these genes are poorly understood.

In recent years, it has been demonstrated that small non-coding microRNAs (miRNAs) are important regulators of gene expression (7-9) and several miRNAs have been identified in the human endometrium (5). These small, single-stranded, non-protein-coding RNAs are a novel class of molecules with the ability to control gene expression at the post-transcriptional level through degradation, translational repression or silencing (10, 11). More than 2,000 different human miRNA species have so far been discovered in the human genome that may regulate the expression of one third of our genes and this number is steadily increasing (12). Every miRNA has a unique nucleotide sequence and expression

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pattern in a specific cell type (13-15). The biological transport of intercellular miRNAs and the current theories regarding the origin and the biological function of extracellular miRNAs are discussed in a recent review by Turcinovich *et al*. (15), while the fundamental approaches most commonly used for miRNA detection and target determination or regulation are discussed in recent reports (16-24). These studies point to their role, which is not yet fully elucidated, in key biological processes and physiological networks, including cell differentiation, proliferation, development and apoptosis. Consequently, and most importantly, their dysregulation is associated with the pathogenesis of various human disorders, such as infection, endometriosis, polycystic ovarian syndrome, recurrent miscarriage, subfertility and cancer.

Analyses of miRNAs in female human reproductive tissues have shown that both the uterus (endometrium, myometrium and cervix), as well as the ovaries, have high enrichment in individual miRNAs (22, 23, 25-27). The involvement of miRNAs in altered endometrial receptivity, abnormal pregnancies, endometriosis, gynaecological malignancies and fertility disorders has been reported (5, 28). Furthermore, in the ovary, events that reappear in a cyclic pattern, such as recruitment of growing follicles, atresia, ovulation and luteal tissue formation and regression, are believed to be regulated by genes controlled by miRNAs (26); similarly, miRNAs play a semantic role in the regulation of the oocyte and cumulus cells crosstalk (27).

Since their role in modifying gene expression is now well-established, an overview of the level of their involvement in human reproduction could possibly provide new insights into the management of implantation failure in *in vitro* fertilisation (IVF). The purpose of the present review was to assess the potential biological pathways linking altered miRNA profiles and IVF failure using the most up-to-the-minute available data through detailed investigation of the studies addressing this issue and the respective references.

Search Strategy and Results

This review was conducted according to an initial protocol agreed by all authors. Studies in English language from 1976 to July 2014 were compiled with no study design restrictions using the following search algorithms in two major scientific/medical databases (Medline/PubMed, ScienceDirect): ("miRNA" OR "microRNA") AND ("IVF" OR "*in vitro* fertilization" OR "*in vitro* fertilisation" OR "assisted reproduction" OR "Reproductive Techniques, Assisted" OR "ART" OR "reproduction" OR "implantation failure" OR "IVF failure"). Search results were confirmed through the SCIRUS database. Reference lists of relevant articles were hand-searched for potentially eligible studies.

Data were retrieved from 80 out of the 248 eligible titles found at the initial search. The main text of the review was divided in 3 parts; data from experimental models, the alterations of miRNAs during controlled ovarian hyperstimulation (COH) for IVF and the association of these alterations with the procedure of human implantation. The potential pathways retrieved from the sum of the studies are presented in Figure 1.

Animal Studies

miRNA-mediated regulation of uterine gene expression in the context of implantation has been demonstrated in animal models with findings that confirm and underline the necessity of the dialogue between maternal and embryonic tissues (29, 30). Nothnick *et al*. (22), in a comprehensive review, included studies demonstrating that miRNAs are essential for normal development and function of the female mice reproductive tract, for example through the conditional deletion of the miRNA processing enzyme Dicer1 and an miRNA expression profile linked with mice reproductive pathology. Paradigms on the involvement of differentiated miRNAs in implantation in animals are numerous.

Cui *et al*. (31) and Murchison *et al*. (32) showed that Dicer1 was highly expressed in oocytes, with the highest-level expression being in the transcriptionally repressed germinal vesicle oocyte. Dicer1$^{-/-}$ mice were infertile, although their ovaries were histologically-normal and responsive to gonadotropins due to the association of the changes in the spindles, which were misaligned and disorganized, resulting in a loss of meiotic maturation and polar body production. Microarray analysis of these Dicer1$^{-/-}$ oocytes showed that 861 mRNAs were up-regulated >2-fold, with 173 of these having previously been shown to be degraded during meiotic maturation (32). Just recently, Zhang *et al*. reported the first microRNA-target mRNA pair (mmu-miR-376a modulating the expression of Pcna) that regulates the primordial follicle assembly in mice (33).

Moreover, dre-miR-430 was found to bind to over 300 maternal transcripts in zebrafish promoting their down-regulation directly before the beginning of the embryonic transcription (34), while mmu-miR-101a and mmu-miR-199a* were reported to be spatiotemporally expressed in the mouse uterus during implantation and post-transcriptionally regulate the expression of cyclooxygenase-2 (29). bta-miR-122a and * bta-miR-199a* were specifically expressed in day 30 bovine embryos (35) targeting cyclin G1 and extracellular signal-regulated kinase 2, respectively (36, 37). Accordingly, ssc-miR-181a and ssc-miR-181c play significant roles in the regulation of genes and pathways involved in embryo implantation and placentation in pigs (38).

Also, up-regulation of rno-miR-98 in ratus uterus during the receptive phase is linked to the decrease of endometrial stromal cell (ESC) proliferation *via* targeting B-cell lymphoma–extra large gene (*Bcl-xl*) (39).

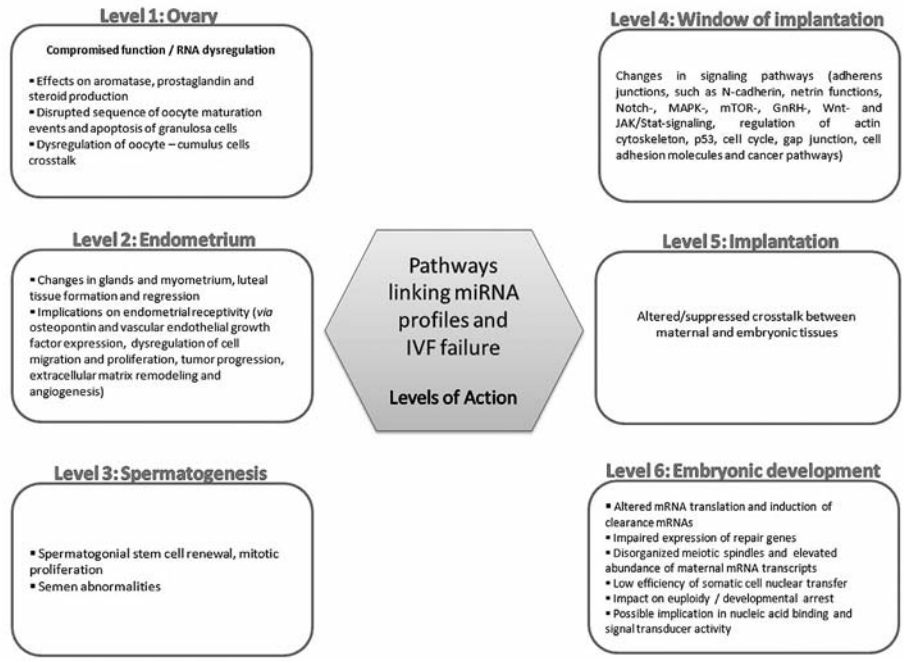

Figure 1. Pathways linking altered miRNAs profiles and IVF failure: Levels of action in the process of gametogenesis, endometrial receptivity, implantation and embryonic development.

Wang *et al*. (40) extracted total RNA from 2-cell and 4-cell mice embryos and observed that 192 miRNAs were differentially expressed between these two (122 up-regulated and 70 down-regulated), thus providing a developmental map for a large number of miRNAs; the authors' results obtained through microarray analysis were further confirmed by real-time quantitative RT-PCR for six miRNAs (mmu-miR-467h, mmu-miR-466d-3p, mmu-miR-292-5p, mmu-miR-154, mmu-miR-2145 and mmu-miR-706), all appearing to exert a special, but not yet elucidated role, in reproduction. Interestingly, a differentiation of miRNAs has been proven between implantation and inter-implantation sites (41).

miRNA levels were found to be different in IVF porcine embryos and *in vivo* and were related to the stage of embryonic development and to culture conditions (42). Tang *et al*. (43) found that between the 1-cell and 2-cell stage there was a 60% decrease in miRNA expression levels indicating that miRNA may be degraded similarly to maternal mRNA. However, between the 2-cell and 4-cell stage, as embryonic transcription proceeds, miRNA expression increases ~2.2-fold. Those from the miR-290 cluster have previously been shown to play a role in DNA methylation and telomere-length homeostasis (43, 44).

Alterations of miRNAs During COH

Several studies have been published on the physiological endometrium in an attempt to determine its gene profile (45-48). In the endometria of sub-fertile women, genetic analysis has demonstrated dysregulation of the respective pathways linked with leukocyte extravasation signalling, lipid metabolism and detoxification (49). Gene expression of endometrial cells is mainly under the influence of ovarian steroids and in COH, during IVF, ovarian steroid secretion is elevated (50), with estradiol levels increasing by 5-fold (51) or higher.

The basic concept of miRNAs' involvement in implantation lies on the modulation of endometrial and function / receptivity (52). In IVF, COH itself alters the gene expression profile through alteration of the mRNAs profile, when compared with natural cycles (53). In contrast, during the menstrual cycle of fertile women, there is no association between the changes of the female body with the expression of the circulating miRNAs (54).

Receptivity is negatively affected by the COH itself (28, 55, 56). This is accomplished through structural and functional alterations, which include promotion of histological functions and of pinopode maturation, as well as down-regulation of steroid receptors (53). During COH, along with a "genomic delay' of the endometrium, there is a localized expression of a unique set of miRNAs, in both the endometrium and the ovary, which is found to be altered, especially during the implantation period in the former (57, 58). This set of miRNAs is diverse when compared to that of natural cycles as shown by the deviant numbers and species found to be down-regulated or, more rarely, up-

regulated. As a result, the necessary function, and especially regression of specific target gene expression, is altered at a cellular level. It is well-accepted that if critical genes are not expressed during the window of implantation (WOI), implantation will not happen (59). It seems that during COH, an atypical pathway is followed further involving genes responsible for anti-inflammatory and anti-apoptotic roles and those implicated in angiogenesis (60, 61).

In addition, most reports conclude that the alterations during COH can consequently alter the WOI due to the abnormal hormonal environment to which the endometrium is exposed (50, 62-64). Interestingly, a recent report has provided a link between the type of ovulation trigger and luteal phase support during COH for IVF and endometrial receptivity and early implantation of the embryo (57). Of note, at the site of implantation, endometrial gene expression differs between fertile women and women with repeated implantation failure during IVF or recurrent miscarriages. Lédée *et al*. (65) performed a large-scale microarray analysis to provide evidence of an extensive endometrial dysregulation in these patients, which seems to reflect the impaired ability of the endometrium to co-act with the embryo at the time of implantation. Moreover, high levels of steroids themselves promote variations in the concentration of cytokines, adhesion molecules, growth factors and enzymes, such as leukemia inhibitory- and heparin-binding epidermal growth factor (EGF)-like growth factors, interleukins 4, 6, 10, 11, 13, 15 and tumor necrosis factor-α (TNF-α) (66) and in the physiological angiogenesis in the placental site (67).

These events appear to lead to the observed asynchrony between the endometrium and the embryo stage, observed in repeated IVF implantation failures following COH (68, 69). According to recent data, the alteration of specific subsets of miRNAs results in the differentiated expression of, for example, cytokines, such as osteopontin (OPN) and vascular endothelial growth factor (VEGF) and the inability to stabilize the endometrial expression of specific genes, which, in turn, lead to the decreased endometrial receptivity and subsequently poor pregnancy rates in IVF patients (60, 70).

In conclusion, although there is no direct evidence indicating that altered miRNAs' expression results in the differentiated expression of these genes and abnormal expression of miRNAs is just one of potential reasons for altering genes expression, it seems that continuous research will eventually firmly link the pieces of the puzzle.

Embryo Implantation

During the normal procedure of implantation and maintenance of pregnancy, a precise series of molecular interactions between the blastocyst and endometrium is necessary. Both biological processes are based essentially on adequate gene expression that, in turn, depends on miRNAs function. The conditions under which this molecular network functions determine the fate of the blastocyst itself. Unfortunately, it is not easy to provide an exemplified pathway to assist our understanding over the underlying functional networks.

In subfertility, there is a marked difference in the miRNA profiles when compared to fertile population. This difference seems to be age-related as described through the detection of miRNAs in membrane-enclosed vesicles of human follicular fluid (71). There also has been observed a significant decrease in the expression of six miRNAs in comparison with fertile donor control blastocysts derived from patients with polycystic ovaries or male factor sub-fertility while being morphologically similar (72). Analysis of the target genes demonstrated significant altered expression consistent with aberrant miRNA profiles (72). As a result, there is an etiological derivation in major biological processes, such as in cell growth and maintenance, transcription and molecular functions implicated in nucleic acid binding and signal transducer activity.

In the same context, differential miRNA expression between euploid and aneuploid embryos has also been reported to be a potentially early indicator of their prognosis or a mechanism behind their eventual fate (73). Similarly, Rosenbluth *et al*. found that miRNAs (miR-645, -191 and -372) into culture media of embryos at day 5 positively correlated with embryonic aneuploidy and pregnancy outcomes (74).

Assou *et al*. (27) using a deep-sequencing approach studied the identification and quantification of miRNAs in human MII oocytes and cumulus cells (CCs). The authors report that *BCL2* and *CYP19A1* mRNA levels were decreased upon MIR23a over-expression concluding that miRNA could play a role in the regulation of the oocyte and CC crosstalk (27). Li *et al*. (70) performed a gene pathway analysis of miRNA and mRNAs examining the effects of a differential expression between normal and elevated progesterone (P) in women undergoing IVF: the analysis identified OPN and angiogenin and reduced VEGF, as playing an important part in impaired endometrial receptivity in cases of elevated P, leading to poor pregnancy rates. The authors demonstrated that several of the differentially-expressed genes have been identified as target genes for miRNA, which are believed to play a crucial role in reducing endometrial receptivity. Furthermore, they identified four miRNAs and 22 mRNAs that were differentially expressed between normal and elevated P endometrium. Of note, the studies assessing endometrial gene expression have included only a small number of patients due to the cost, on one hand, and the plethora of the involving genes, on the other. In addition, the results reported are quite different through possibly different assays and methods used; accordingly, the conclusions that can be drawn are limited (28).

In the special group of cases with repeated implantation failures, a reduced action of both miRNAs and the targeted

molecular pathways has been demonstrated as compared to those of fertile women (21, 75). Possible causes were attributed to inappropriate timing, the lack of expression or the asynchronization between miRNAs and the gene target expression itself. As a consequence, the differentiated miRNA-regulated molecular pathways lead to defects in endometrial receptivity and, hence, to unsuccessful embryo implantation.

Conclusions and Next Targets

MicroRNAs are involved in numerous processes affecting the function of the human reproductive system and, moreover, conception, implantation process and embryonic development. Crucially, as they play a significant role in the course of reproduction, they represent excellent research candidates with the potential to enable a better understanding over the underlying molecular activities that prevent implantation and embryo progression. During an IVF cycle, COH triggers differential miRNA expression and, since these are responsible for the suppression of target gene expression, this has an adverse effect on the implantation process. Recent research allowed for the characterization of specific genes whose expression is associated with implantation and their functional interplay with miRNAs. A schematic summary of the potential pathways linking altered miRNAs profiles and IVF failure is presented in Figure 1.

A vital step is to support the continuity of current research for in-depth pathway mapping of the implantation process and the characterization of the respective miRNAs and associated links so as to unveil possible new roles of both previously and newly discovered molecules. More specifically, there is an urgent need for the identification, validation and functional characterization of specific miRNAs involved in implantation along with parallel assessment of their targets in order to clarify their role in fertility and implantation. To this end, various novel miRNA-associated molecules in the form of agonists / mimics or antagonists and synthetic decoy targets have been put forward. Advanced techniques and methodologies have already been proposed for combining miRNAs by integrating different co-expression networks in order to be accurately utilized in biological models. Since a single miRNA can potentially affect multiple targets, an indisputable point here is that, before a proposed protocol is applied in humans, any potential intervention in these pathways for therapeutic purposes should first be monitored in variant models to weigh its positive against any adverse effects.

In addition, relevant data from gene expression studies should be integrated to find markers of clinical value. Thus, targeting specific miRNAs can potentially represent a valuable approach in the directed treatment of specific cases where multiple factors affect fertility and result in IVF failure. Moreover, precise characterization could possibly uncover an important marker of fertility status in both sexes or even markers for monitoring, analyzing and potentially improving gamete quality and endometrial receptivity.

Cost effectiveness should be explored and, furthermore, trials on the efficiency of any intervention should determine whether miRNA profiling could possibly be adopted in routine practice to substantially improve the diagnostic accuracy and, in parallel, the directed treatment of the next generation IVF. Cost reduction to a minimum of any potential future application should be seriously considered so that such services may be accessible to all groups of patients. Up-to-date published data and ongoing trials are limited, probably attributable to the fact that cost could place a series of obstacles in performing large-scale studies for the purpose of establishing robust conclusions on the effectiveness of this practice.

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References

1 Aplin J: Embryo implantation: the molecular mechanism remains elusive. Reprod Biomed Online *13*: 833-839, 2006.
2 National Institute for Clinical Excellence (NICE): Fertility: Assessment and Treatment for People with Fertility Problems. NICE Clinical Guidelines, No. 11. National Collaborating Centre for Women's and Children's Health (UK). London (UK): RCOG Press; February 2004. ISBN-10: 1-900364-97-2.
3 Edwards RG: Clinical approaches to increasing uterine receptivity during human implantation. Hum Reprod *10*: 60-66, 1995.
4 Macklon NS, Stouffer RL, Giudice LC and Fauser BC: The science behind 25 years of ovarian stimulation for *in vitro* fertilization. Endocr Rev *27*: 170-207, 2006.
5 Altmäe S, Esteban FJ, Stavreus-Evers A, Simón C, Giudice L, Lessey BA, Horcajadas JA, Macklon NS, D'Hooghe T, Campoy C, Fauser BC, Salamonsen LA and Salumets A: Guidelines for the design, analysis and interpretation of 'omics' data: focus on human endometrium. Hum Reprod Update *20*: 12-28, 2014.
6 Altmäe S, Reimand J, Hovatta O, Zhang P, Kere J, Laisk T, Saare M, Peters M, Vilo J, Stavreus-Evers A and Salumets A: Research resource: interactome of human embryo implantation: identification of gene expression pathways, regulation, and integrated regulatory networks. Mol Endocrinol *26*: 203-217, 2012.
7 Ambros V and Lee RC: Identification of microRNAs and other tiny noncoding RNAs by cDNA cloning. Methods Mol Biol *265*: 131-158, 2004.
8 Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell *116(2)*: 281-297, 2004.
9 Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS and Johnson JM: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature *7027*: 769-773, 2005.
10 Lim LP, Glasner ME, Yekta S, Burge CB and Bartel DP: Vertebrate microRNA genes. Science *299*: 1540, 2003.

11 Engels BM and Hutvagner G: Principles and effects of microRNA-mediated post-transcriptional gene regulation. Oncogene *25*: 6163-6169, 2006.

12 miRBase: the microRNA database (University of Manchester) [http://www.mirbase.org/]

13 Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foà R, Schliwka J, Fuchs U, Novosel A, Müller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M and Tuschl T: A mammalian microRNA expression atlas based on small RNA library sequencing. Cell *129*: 1401-1414, 2007.

14 Turchinovich A, Weiz L and Burwinkel B: Extracellular miRNAs: the mystery of their origin and function. Trends Biochem Sci *37(11)*: 460-465, 2012.

15 Turchinovich A, Samatov TR, Tonevitsky AG and Burwinkel B: Circulating miRNAs: cell-cell communication function? Front Genet *4*: 119, 2013.

16 Pan Q, Luo X, Toloubeydokhti T and Chegini N: The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol Hum Reprod *13*: 797-806, 2007.

17 van Rooij E: The art of microRNA research. Circ Res *108(2)*: 219-234, 2011.

18 Tang G, Tang X, Mendu V, Tang X, Jia X, Chen QJ and He L: The art of microRNA: various strategies leading to gene silencing *via* an ancient pathway. Biochim Biophys Acta *1779(11)*: 655-662, 2008.

19 Bueno MJ, Pérez de Castro I and Malumbres M: Control of cell proliferation pathways by microRNAs. Cell Cycle *7(20)*: 3143-3148, 2008.

20 Lessey B: Fine tuning of endometrial function by estrogen and progesterone through microRNAs. Biol Reprod *82(4)*: 653-655, 2010.

21 Revel A, Achache H, Stevens J, Smith Y and Reich R: MicroRNAs are associated with human embryo implantation defects. Hum Reprod *26(10)*: 2830-2840, 2011.

22 Nothnick WB: The role of micro-RNAs in the female reproductive tract. Reproduction 143(5): 559-576, 2012.

23 Baley J and Li J: MicroRNAs and ovarian function. J Ovarian Res *5*: 8, 2012.

24 Moreno-Moya JM, Vilella F and Simón C: MicroRNA: key gene expression regulators. Fertil Steril *101(6)*: 1516-1523, 2014.

25 Shingara J, Keiger K, Shelton J, Laosinchai-Wolf W, Powers P, Conrad R, Brown D and Labourier E: An optimized isolation and labeling platform for accurate microRNA expression profiling. RNA *11*: 1461-1470, 2005.

26 Carletti MZ and Christenson LK: MicroRNA in the ovary and female reproductive tract. J Anim Sci *87(14)*: E29-38, 2009.

27 Assou S, Al-Edani T, Haouzi D, Philippe N, Lecellier CH, Piquemal D, Commes T, Aït-Ahmed O, Dechaud H and Hamamah S: MicroRNAs: new candidates for the regulation of the human cumulus-oocyte complex. Hum Reprod *28(11)*: 3038-3049, 2013.

28 Galiano D and Pellicer A: MicroRNA and implantation: Fertil Steril *101*: 1531-1544, 2014.

29 Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H and Dey SK: MicroRNA regulation of cyclooxygenase-2 during embryo implantation. PNAS *104(38)*: 15144-15149, 2007.

30 Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, Ma XH, Ni H, Lei W and Yang ZM: MicroRNA expression and regulation in mouse uterus during embryo implantation. J Biol Chem *34*: 23473-23484, 2008.

31 Cui XS, Shen XH and Kim NH: Dicer1 expression in preimplantation mouse embryos: Involvement of Oct3/4 transcription at the blastocyst stage. Biochem Biophys Res Commun *352(1)*: 231-236, 2007.

32 Murchison EP, Stein P, Xuan Z, Pan H, Zhang MQ, Schultz RM and Hannon GJ: Critical roles for Dicer in the female germline. Genes Dev *21(6)*: 682-693, 2007.

33 Zhang H, Jiang X, Zhang Y, Xu B, Hua J, Ma T, Zheng W, Sun R, Shen W, Cooke HJ, Hao Q, Jie Q and Shi Q: microRNA 376a Regulates Follicle Assembly by Targeting Pcna in Fetal and Neonatal Mouse Ovaries. Reproduction *148(1)*: 43-54, 2014.

34 Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ and Schier AF: Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science *312(5770)*: 75-79, 2006.

35 Coutinho LL, Matukumali LK, Sonstegard TS, Van Tassell CP, Gasbarre LC, Capuco AV and Smith TP: Discovery and profiling of bovine microRNAs from immune-related and embryonic tissue. Physiological Genomics *29*: 35-43, 2007.

36 Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, Calin GA, Giovannini C, Ferrazzi E, Grazi GL, Croce CM, Bolondi L and Negrini M: Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res *67(13)*: 6092-6099, 2007.

37 Kim S, Lee UJ, Kim MN, Lee EJ, Kim JY, Lee MY, Choung S, Kim YJ and Choi YC: MicroRNA miR-199a* regulates the MET proto-oncogene and the downstream extracellular signal-regulated kinase 2 (ERK2). J Biol Chem *283(26)*: 18158-18166, 2008.

38 Su L, Liu R, Cheng W, Zhu M, Li X, Zhao S and Yu M: Expression Patterns of MicroRNAs in Porcine Endometrium and Their Potential Roles in Embryo Implantation and Placentation. PLoS One *9(2)*: e87867, 2014.

39 Hong-Fei Xia, Xiao-Hua Jin, Zong-Fu Cao, Tian Shi and Xu Ma: MiR-98 is involved in rat embryo implantation by targeting Bcl-xl. FEBS Lett *588(4)*: 574-583, 2014.

40 Wang P, Cui J, Zhao C, Zhou L, Guo X, Shen R, Zhang J and Ling X: Differential expression of microRNAs in 2-cell and 4-cell mouse embryos. Zygote *26*: 1-7, 2013.

41 Geng Y, He J, Ding Y, Chen X, Zhou Y, Liu S, Liu X and Wang Y: The Differential Expression of MicroRNAs Between Implantation Sites and Interimplantation Sites in Early Pregnancy in Mice and Their Potential Functions. Reprod Sci *21(10)*: 1296-1306, 2014.

42 Stowe HM, Curry E, Calcatera SM, Krisher RL, Paczkowski M and Pratt SL: Cloning and expression of porcine Dicer and the impact of developmental stage and culture conditions on MicroRNA expression in porcine embryos. Gene *501(2)*: 198-205, 2012.

43 Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K and Surani MA: Maternal microRNAs are essential for mouse zygotic development. Genes Dev *21(6)*: 644-648, 2007.

44 Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, Schoeftner S, Murchison E, Andl T, Chen T, Klatt P, Li E, Serrano M, Millar S, Hannon G and Blasco MA: A mammalian microRNA cluster

controls DNA methylation and telomere recombination *via* Rbl2-dependent regulation of DNA methyltransferases. Nat Struct Mol Biol *15(9)*: 998, 2008.
45 Kuokkanen S, Chen B, Ojalvo L, Benard L, Santoro N and Pollard JW: Genomic Profiling of MicroRNAs and Messenger RNAs Reveals Hormonal Regulation in MicroRNA Expression in Human Endometrium. Biol Reprod *82(4)*: 791-801, 2010.
46 Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirney R, Phillips SC and Smith SK: Determination of the transcript profile of human endometrium. Mol Hum Reprod *9*: 19-33, 2003.
47 Horcajadas JA, Riesewijk A, Martín J, Cervero A, Mosselman S, Pellicer A and Simón C: Global gene expression profiling of human endometrial receptivity. J Reprod Immunol *63(1)*: 41-49, 2004.
48 Horcajadas JA, Pellicer A and Simon C: Wide genomic analysis of human endometrial receptivity: new times, new opportunities. Hum Reprod Update *13*: 77-86, 2007.
49 Altmäe S, Martinez-Conejero JA, Salumets A, Simon C, Horcajadas JA and Stavreus-Evers A: Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility. Mol Hum Reprod *3*: 178-187, 2010.
50 Rackow BW, Kliman HJ and Taylor HS: GnRH antagonists may affect endometrial receptivity. Fertil Steril *89*: 1234-1239, 2008.
51 MacLachlan V, Besanko M, O'Shea F, Wade H, Wood C, Trounson A and Healy DL: A controlled study of luteinizing hormone-releasing hormone agonist (buserelin) for the induction of folliculogenesis before *in vitro* fertilization. N Engl J Med *320*: 1233-1237, 1989.
52 Hull ML and Nisenblat V: Tissue and circulating microRNA influence reproductive function in endometrial disease. Reprod Biomed Online *27(5)*: 515-529, 2013.
53 Mirkin S, Nikas G, Hsiu JG, Díaz J and Oehninger S: Gene expression profiles and structural/functional features of the peri-implantation endometrium in natural and gonadotrophin-stimulated cycles. J Clin Endocrinol Metab *89*: 5742-5752, 2004.
54 Rekker K, Saare M, Roost AM, Salumets A and Peters M: Circulating microRNA Profile throughout the menstrual cycle. PLoS One *8(11)*: e81166, 2013.
55 Lass A, Peat D, Avery S and Brinsden P: Histological evaluation of endometrium on the day of oocyte retrieval after gonadotrophin-releasing hormone agonist-follicle stimulating hormone ovulation induction for *in vitro* fertilization. Hum Reprod *13(11)*: 3203-3205, 1998.
56 Ubaldi F, Bourgain C, Tournaye H, Smitz J, Van Steirteghem A and Devroey P: Endometrial evaluation by aspiration biopsy on the day of oocyte retrieval in the embryo transfer cycles in patients with serum progesterone rise during the follicular phase. Fertil Steril *67(3)*: 521-526, 1997.
57 Zhao Y, Zacur H, Cheadle C, Ning N, Fan J and Vlahos NF: Effect of luteal-phase support on endometrial microRNA expression following controlled ovarian stimulation. Reprod Biol Endocrinol *10*: 72, 2012.
58 Hawkins SM and Matzuk MM: Oocyte-somatic cell communication and microRNA function in the ovary. Ann Endocrinol *71(3)*: 144-148, 2010.
59 Nabilsi NH, Broaddus RR, McCampbell AS, Lu KH, Lynch HT, Chen LM and Loose DS: Sex hormone regulation of survivin gene expression. J Endocrinol *207*: 237-243, 2010.
60 Qun P and Nasser C: MicroRNA Signature and Regulatory Functions in the Endometrium during Normal and Disease States. Semin Reprod Med *26(6)*: 479-493, 2008.
61 Landskroner-Eiger S, Moneke I and Sessa WC: miRNAs as modulators of angiogenesis. Cold Spring Harb Perspect Med *3(2)*: a006643, 2013.
62 Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J and Zhu G: Serum progesterone level effectson the outcome of *in vitro* fertilization in patients with different ovarianresponse: an analysis of more than 10,000 cycles. Fertil Steril *97*: 1321-1327, 2012.
63 Zapantis G, Szmyga MJ, Rybak EA and Meier UT: Premature formation of nucleolar channel systems indicates advanced endometrial maturation following controlled ovarian hyperstimulation. Hum Reprod *28*: 3292-3300, 2013.
64 Van Vaerenbergh I, Van Lommel L, Ghislain V, In't Veld P, Schuit F, Fatemi HM, Devroey P and Bourgain C: GnRH antagonist/rec-FSH stimulated cycles, advanced endometrial maturation on the day of oocyte retrieval correlates with altered gene expression. Hum Reprod *24*: 1085-1091, 2009.
65 Lédée N, Munaut C, Aubert J, Sérazin V, Rahmati M, Chaouat G, Sandra O and Foidart JM: Specific and extensive endometrial deregulation is present before conception in IVF/ICSI repeated implantation failures (IF) or recurrent miscarriages. J Pathol *225(4)*: 554-564, 2011.
66 Achache H and Revel A: Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update *6*: 731-746, 2006.
67 Zhang B, Liu XX, He JR, Zhou CX, Guo M, He M, Li MF, Chen GQ and Zhao Q: Pathologically decreased miR-26a antagonizes apoptosis and facilitates carcinogenesis by targeting MTDH and EZH2 in breast cancer. Carcinogenesis *32*: 2-9, 2011.
68 Almog B, Shalom-Paz E, Dufort D and Tulandi T: Promoting implantation by local injury to the endometrium. Fertil Steril *94(6)*: 2026-2029, 2010.
69 Azpiroz A, Junovich G, Ferrer C, Pasqualini A, Pasqualini S and Gutierrez G: Is ovarian stimulation affecting endometrial NK cell response during implantation window? J Reprod Immunol *90*: 165-166, 2011.
70 Li R, Qiao J, Wang L, Zhen X, Liu P and Zheng X: MicroRNA array and microarray evaluation of endometrial receptivity in patients with high serum progesterone levels on the day of hCG administration. Reprod Biol Endocrinol *9*: 29, 2011.
71 Diez-Fraile A, Lammens T, Tilleman K, Witkowski W, Verhasselt B, De Sutter P, Benoit Y, Espeel M and D'Herde K: Age-associated differential microRNA levels in human follicular fluid reveal pathways potentially determining fertility and success of *in vitro* fertilization. Hum Fertil *17(2)*: 90-98, 2014.
72 McCallie B, Schoolcraft WB and Katz-Jaffe MG: Aberration of blastocyst microRNA expression is associated with human infertility. Fertil Steril *93(7)*: 2374-2382, 2010.
73 Rosenbluth EM, Shelton DN, Sparks AE, Devor E, Christenson L and Van Voorhis BJ: MicroRNA expression in the human blastocyst. Fertil Steril *99(3)*: 855-861, 2013.
74 Rosenbluth EM, Shelton DN, Wells LM, Sparks AE and Van Voorhis BJ: Human embryos secrete microRNAs into culture media – a potential biomarker for implantation. Fertil Steril *101*: 1493-1500, 2014.
75 Parveen F1 and Agrawal S. Recurrent Miscarriage and Micro-RNA Among North Indian Women. Reprod Sci 2014. [Epub ahead of print]

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