Review Article

J Genet Med 2021;18(1):1-7
https://doi.org/10.5734/JGM.2021.18.1.1
ISSN 1226-1769 (Print) 2383-8442 (Online)

JGM Journal of Genetic Medicine

Cumulus and granulosa cell biomarkers: a good predictor for successful oocyte and embryo developmental competence in human *in vitro* fertilization

Eun Jeong Yu[1] and Sang Woo Lyu[2,*]

[1]Department of Obstetrics and Gynecology, CHA Fertility Center Seoul Station, CHA University School of Medicine, Seoul, Korea
[2]Department of Obstetrics and Gynecology, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul, Korea

The oocyte quality is of great importance in infertility as it reflects the follicle developmental potential and further affects the embryo development, clinical pregnancy outcomes. The analysis of gene expression in somatic cells is an important study to better clinical *in vitro* fertilization (IVF) outcomes in embryo selection reflecting the appropriate communication between the oocyte and somatic cells. Specifically, somatic cell transcriptomic technology can help assess biomarkers of oocyte and embryo ability. The present article aims to overview the basic aspect of folliculogenesis and review studies involving changes in candidate gene expression of cumulus or granulosa cell related to clinical outcomes in human IVF.

Key words: Cumulus cells, Granulosa cells, Biomarkers, *In vitro* fertilization, Human.

Introduction

Since the successful birth in 1978 of Louise Brown, pregnancies after *in vitro* fertilization (IVF) have increased much more rapidly and have improved pregnancy rates, obstetric and neonatal outcomes [1]. Oocyte developmental capacity or oocyte quality greatly limit in female fertility because it affects fertilization, embryo development, pregnancy rate, and fetal development. Therefore, a central step in IVF cycle is to assess the oocyte developmental capacity to establish embryos viability for transfer. At the present time, embryo assessment mainly relies on embryo morphology alone or are used in combination with morphologic characteristics and invasive method such as preimplantation genetic testing [2].

However, some embryos with good morphology have implantation failure and only 25% of all IVF treatments are successful [3]. Moreover, even euploid embryos transferred fail to implant 33%-45% of the time [4]. The limitations of these evaluations for embryos have led clinicians to seek adjunctive methods for the assessment of the embryonic reproductive potential before transfer. These methods include the evaluation of the genome, transcriptome profiling analysis of somatic cells such as granulosa and cumulus cells, metabolic and proteomic analysis of embryo [2]. Among them, transcriptomic technology provides

Received: 24 May 2021, Accepted: 15 June 2021, Published: 30 June 2021
*Corresponding author: Sang Woo Lyu, M.D. https://orcid.org/0000-0001-5572-0898
Department of Obstetrics and Gynecology, CHA Gangnam Medical Center, CHA University School of Medicine, 566 Nonhyeon-ro, Gangnam-gu, Seoul 06135, Korea.
Tel: +82-2-3468-3000, Fax: +82-2-3468-0320, E-mail: dung5038@cha.ac.kr
Conflict of interest: The authors declare that they do not have any conflicts of interest.

quantitative real-time polymerase chain reaction (RT-PCR) for genes expression assessment, as well as microarrays and RNA sequencing technology for whole genome transcriptome profiling. Analysis of cumulus/granulosa cells transcriptome profiling related to oocyte developmental competence can help identify new diagnostic markers as an addition or alternative to existing embryo assessment criteria. Identifying key genes in the oocyte–somatic cells will provide deeper understanding of the complex mechanisms on oocyte developmental capacity and discover new regulators and significant predictors of oocyte quality. Ultimately, these efforts will improve the effectiveness and clinical outcomes of IVF.

In the present article, we review the basic aspect of folliculogenesis and summarize potential candidate genes to aid oocyte developmental competence for better clinical outcomes in IVF.

Folliculogenesis

Folliculogenesis is the physiological process including the primordial structures activation, granulosa or theca cell development, oocyte maturation, follicle growth and ovulation. This is a markedly complex process that regulate carefully orchestrated expression of multiple factors for synchronization between oocyte maturation and development of the surrounding somatic cells. Folliculogenesis begins from migration of the primordial germ cells, that is undifferentiated cells that have developing potential into spermatozoa or oocyte [5], to made up the primordial structures in the fetal ovary [6]. Primordial (non-growing) follicles are recruited from the primary follicles to begin to growth and differentiation [7]. The first sign of this recruitment of primordial follicles is that granulosa cells begin to turn into a cuboidal shape and vigorous cell division begins [8].

1. From primary follicle to the preantral follicle

Then the granulosa cells made up multiple layers of somatic cells, forming secondary follicle. After further growth of these secondary follicles, they reach the preantral stage. One of the most critical changes is the creation of a theca layer in the development of a secondary follicles [9]. Thecal layer acquires a vascular cover consisting of networks of capillary, possibly through angiogenesis. This is an important event because ensuring greater vascular supply is an essential course in the maturation and selection of the dominant follicle, providing nutrients and hormones from the secondary follicle to waste products and secretion [10].

2. From antral follicles to preovulatory follicles

Following the formation of secondary follicles, small and fluid-filled cavities is formed with the follicle and merges to form the tertiary (antral) follicle [11]. The first sign of the onset of the tertiary follicle is the development of a fluid-filled cavitation in granulosa cells. Antral follicles are divided into two granulosa cell subgroups: 1) the mural granulosa cells lying the basement wall of follicle; 2) cumulus cells surrounding oocyte [12].

Without gonadotropin stimulation, the antral follicles become atretic. However, the antrum continues to enlarge, forming of a preovulatory follicle in the stimulation of follicle stimulating hormone (FSH). The oocyte is enveloped by a unique type of granulosa cell, cumulus cells, which are distinguished from the granulosa cells in the preovulatory stage [13].

Interactions of Oocyte and the Stromal Cells for Oocyte Developmental Competence

Ovarian follicles made up of oocyte and somatic follicle cells. Bidirectional communication between somatic cell and oocyte ensures the maturation and growth of oocytes and the potential to maintain fertilization and produce embryos of high viability [14]. Fine crosstalk between somatic cell and oocyte begins during the preantral follicle phase of folliculogenesis [15,16]. The changes in oocyte gene expression do not act in isolation but interact in various ways forming complex information networks [17,18]. Oocytes can promote follicle development and differ-

Table 1. Cumulus cell genes and function affected by oocyte secreted factors for oocyte development

Role	Gene documented/ studied	Up/down	Material	Individual versus pooled sampling	Reference
Oocyte maturation	HAS2	Up	Cumulus cell	Individual COC	[39]
	PTGS2		Cumulus cell	Individual COC	[40] [41]
Cumulus cell expansion	BDNF	Up	Cumulus cell	Pooled COC	[40]
	TNFAIP6				
	PTX3				

COC, cumulus-oocyte complex.

entiation via growth factors that act in paracrine factors that affect surrounding somatic cells. These factors regulate oocyte development and maturation [19]. There are three major factors for supporting interaction between oocyte and somatic cells in follicular maturation and development.

1. Oocyte-secreted factors

Oocyte-secreted factors (OSFs) play a vital role in determining the oocyte quality or the fertilization capacity of oocytes to sustain embryonic viability [20]. Growth-differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15) are representative OSFs and have a significant impact on proliferation of cumulus cell and the development of Cumulus Oocyte Complexes (COCs) [21,22]. Table 1 summarizes cumulus cell gene and function regulated by OSFs for oocyte development. GDF-9 promotes the cumulus cells expansion via recruitment of expression of the main components of extracellular matrix such as hyaluronic acid synthase 2 (HAS2), PTX3, TNFAIP6, and prostaglandin-endoperoxide synthase 2 (PTGS2) [23,24]. GDF-9 maintains the consistency of the COC in preovulatory follicles stage and even after ovulation [25]. BMP-15 promotes ovarian and follicle maturation under the growth phase that is independent of gonadotropins. They also regulate FSH-sensitive follicular granulosa cells and prevent the granulosa cell apoptosis [26]. As the relevant members of the transforming growth factor β superfamily, GDF-9 and BMP-15 are activated by the *Sma*- and *Mad*-related (SMAD) family of transcription factors [27]. While GDF-9 signals through SMAD 2 and 3, while the BMP-15 signals via SMADs 1, 5, and 8 [28,29].

2. KIT ligand

KIT ligand (KITL) is derived from granulosa cells and is a positive modulator that simulates somatic cell proliferation and oocyte growth [30]. FSH mediate granulosa cell production of KITL to promote activation of KIT receptor in oocytes and down-regulation the phosphatidylinositol 3-kinase (*PI3K*)/protein kinase B (*AKT*) signaling to stimulate oocyte growth [31]. Several components of the PI3K signaling cascades have an important role as inhibitors of follicular activation in the early follicular developmental stage [32]. PTEN (tumor suppressor phosphatase with tensin homology) and FOXO3A are essential negative effectors of KIT expression in oocytes [33].

3. Gap-junctional communication

At the end of transzonal projections, heterologous gap junctions are formed between oocyte and companion somatic cells. These supply nutrients, amino acid precursors, and signaling molecules via gap junctional communication [34]. The natriuretic peptide C/natriuretic peptide receptor 2 system that produces cGMP in cumulus cells is critical for the maintaining meiotic arrest of oocytes [35]. The inflow of cyclic guanosine monophosphate (cGMP) via gap junctions into the oocyte prevents cyclic nucleotide phosphodiesterase 3A (PDE3A) activation to maintain high concentrated cyclic adenosine monophosphate (cAMP) [36].

Table 2. Summary of published literature of human cumulus or granulosa cell markers for embryo development

Role	Gene documented/studied	Up/down	Material	Individual versus pooled sampling	Reference
Day 3 embryo morphology	COX2, GREM1, HAS2, TRPM7, ITPKA	Up	Cumulus cell	Individual COC	[39,41,42] [43]
	BDNF	Down	Cumulus cell	Pooled COCs	[40]
Early cleavage status	CCND2, CXCR4, DVL3, CTNND1, DHCR7, HSPB1, GPX3, TRIM28	Down	Cumulus cell	Individual COC	[38]
Day 5 embryo morphology	STAR, AREG, SCD1/4/5, ITPKA, CYP11A1	Up/down	Cumulus cell	Individual COC	[41,43,47]
Pregnancy	SDC4, VCAN, EFNB2, CAMK1D, STC1, BCL2L11, PCK1, NF1B, DPP8, HIST1H4C, UBQLN1, CALM1, NPR1, PSMD6	Up	Cumulus cell	Individual COC	[41,43,48,50]
	CYP19A1, CDC42, SERPINE2, 3BHSD, UGP2, PHLDA1	Up	Granulosa cell	Pooled COCs	[51]
Live birth	VCAN, PTGS2, EFNB2, CAMK1D, STC2	Up	Cumulus cell	Individual COC	[43,52]

COC, cumulus-oocyte complex.

After the luteinizing hormone-triggered signaling, PDE3A becomes activated, decreasing the cAMP expression, which trigger a subordinate signaling pathways for meiotic resumption during ovulation [37].

Candidate Gene for Embryo Development in Cumulus Granulosa Cells

The biomarker for embryo development would improve oocyte and embryo selection, increasing the likelihood of a successful pregnancy with IVF and allowing embryos to be transferred.

The cumulus cells may provide a more manageable biomarker than the oocyte. Previous studies have indicated several transcripts expressed in the cumulus cells, providing a developmental capacity model that represent a non-invasive means to predict oocyte quality [38]. Table 2 provides an overview of published literature to date on human cumulus or granulosa cell markers that are regulated up or down in the somatic cells of capable oocytes [39-41].

The investigation of gene expression profiles in oocyte and somatic cells that reflect the embryo developmental potential was first introduced in 2004. McKenzie et al. [39] reported that cumulus cell gene expression of HAS2, PTGS2, commonly known as cyclooxygenase-2 (COX-2) and gremlin (GREM1) was higher on oocytes that resulted in high-quality cleavage compared with the lower quality embryos by RT-PCR assay in intracytoplasmic sperm injection (ICSI) patients. The higher expression HAS2 and GREM1 in cumulus cell is in agreement with RT-PCR findings by Cillo et al. [42] in gonadotropin-releasing hormone agonist and recombinant FSH (rFSH)-stimulation cycle. Inositol-trisphosphate 3-kinase A (ITPKA) and transient receptor potential cation channel subfamily M member 7 (TRPM7) expression was markedly upregulated and brain-derived neurotrophic factor was significantly downregulated in cells from oocytes that did not develop with low quality cleavage embryo [40,43].

Early cleavage, defined as cell division event completing the first mitotic division within 25-27 hour after insemination [44], is a significant marker in determining the developmental competence of embryo [45,46]. van Montfoort et al. [38] identified embryo selection parameters based on the study using elective single embryo transfer. The investigators compared the gene expression pattern in cumulus cells from individual oocyte that exhibited early cleavage status to those from oocytes that exhibited non-early cleavage embryos. They found that cyclin D2 (CCND2), chemokine receptor 4 (CXCR4), glutathione peroxidase (GPX3), catenin delta 1 (CTNND1), 7-dehydrocholesterol reductase (DHCR7), heat shock 27 kDa protein 1 (HSPB1), disheveled homolog 3 (DVL3), and tripartite motif containing 28 (TRIM28) had negative correlation with early cleavage embryos. Cumulus cells from oocyte that have become non-early cleavage embryo had hypoxia or a delayed oocyte maturation.

Blastocyst morphology was associated with enhanced gene expression of Amphiregulin (AREG), steroidogenic acute regulatory protein (STAR), and stearoyl-Coenzyme A Desaturase 1 and 5 (SCD1 and 5) by cumulus cells [47]. Cytochrome P450 family 11 subfamily A polypeptide 1 (CYP11A1) is also responsible for the biosynthesis of the steroid hormones and improved the blastocyst quality [43].

Despite syndecan-4 (SCD4) and versican (VCAN) were reliable biomarkers for predicting pregnancy, they had not been studied about embryo morphology [43]. Assou et al. [48] reported a relationship between pregnancy outcomes and embryo developmental capacity in the transcriptome of cumulus cells. They analyzed the differential expression of predominantly up-regulated gene for Bcl-2-like protein 11 (BCL2L11), Phosphoenolpyruvate Carboxykinase 1 (PCK1) and on down-regulated gene for Nuclear Factor 1B (NFIB) between cumulus cell from top-quality embryos with negative pregnancy outcomes and cumulus cells with top-quality embryos associated with successful pregnancy outcomes. BCL2L11 and PCK1 play roles in controlling apoptosis and the regulation of gluconeogenesis, respectively [49].

Previous studies identified significant indicators for selecting the embryo with good oocyte quality and successful pregnancy outcomes. Assidi et al. [50] individually selected cumulus cells based on both good morphology and sub-cellular oocyte structure, such as high zona pellucida birefringence and found important components: histone cluster 1, H4c (HIST1H4C), neuropilin 1 (NRP1), ubiquilin 1 (UBQLN1), calmodulin 1 (CALM1), and proteasome (prosome, macropain) 26S subunit, non-ATPases 6 (PSMD6). Wathlet et al. [43] found significant different expression pattern of calcium/calmodulin-dependent protein kinase 1D (CAMK1D) and EphrinB2 (EFNB2) among the pregnancy versus nonpregnancy groups in the cumulus cell.

Hamel et al. [51] identified potential follicular markers from the transcriptome of granulosa cells from oocyte resulting in a successful pregnancy. A tendency toward up-regulation in CC from competent oocyte for cytochrome P450 aromatase (CYP19A1), cell division cycle 42 (CDC42), and serin proteinase inhibitor clade E member 2 (SERPINE2) were significantly associated with pregnancy outcomes. They also found that 3-beta-hydrozystereoid dehydrogenase (HSD3b1), UPD-glucose pyrophosphorylase 2 (UGP2), and pleckstrin homology-like domain

family A member 1 (PHLDA1) were correlated with embryo quality with a positive pregnancy.

More recently, some investigators compared the cumulus cell gene expression profiling of patients with live birth outcomes to patients who were not born. Gebhardt et al. [52] identified significant expression of VCAN, PTGS2, and EFNB2 in the cumulus cells of oocytes with healthy live birth outcomes. CAMK1D, stanniocalcin-2 (STC2) were up-regulated in the pregnant groups [43].

Conclusion

Suitable interaction between the oocyte and the companion somatic cells are important to the oocyte development and embryos viability. It is a little clear that the oocyte has a vital role in indicating the follicle growth and differentiation by the secretion of paracrine growth factors. However, this is still a relatively new field and much research remains on identifying OSFs and their related genes. The overview of the results published in studies evaluating somatic transcriptomes show limited consensus on the identification of markers. The information about these markers will aid improvement in pregnancy outcome in the setting of IVF once the mechanism is fully understood.

Authors' Contributions

Conception and design: SWL. Acquisition of data: EJY. Analysis and interpretation of data: EJY, SWL. Drafting the article: EJY, SWL. Critical revision of the article: SWL. Final approval of the version to be published: EJY, SWL.

References

1. Zhu L, Zhang Y, Liu Y, Zhang R, Wu Y, Huang Y, et al. Maternal and live-birth outcomes of pregnancies following assisted reproductive technology: a retrospective cohort study. Sci Rep 2016;6:35141.
2. Seli E, Robert C, Sirard MA. OMICS in assisted reproduction: possibilities and pitfalls. Mol Hum Reprod 2010;16:513-30.
3. Ferraretti AP, Goossens V, de Mouzon J, Bhattacharya S, Castilla JA, Korsak V, et al. Assisted reproductive technology in Europe, 2008: results generated from European registers by ESHRE. Hum Reprod 2012;27:2571-84.
4. Simon AL, Kiehl M, Fischer E, Proctor JG, Bush MR, Givens C, et al. Pregnancy outcomes from more than 1,800 in vitro fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism-based preimplantation genetic testing for aneuploidy. Fertil Steril 2018;110:113-21.
5. Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. Endocr Rev 2009;30:624-712.
6. Picton H, Briggs D, Gosden R. The molecular basis of oocyte growth and development. Mol Cell Endocrinol 1998;145:27-37.
7. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev 2000;21:200-14.
8. Gougeon A, Chainy GB. Morphometric studies of small follicles in ovaries of women at different ages. J Reprod Fertil 1987;81:433-42.
9. Magoffin DA. Ovarian theca cell. Int J Biochem Cell Biol 2005; 37:1344-9.
10. Yamoto M, Shima K, Nakano R. Gonadotropin receptors in human ovarian follicles and corpora lutea throughout the menstrual cycle. Horm Res 1992;37 Suppl 1:5-11.
11. Soyal SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. Development 2000;127:4645-54.
12. Clarke HJ. Regulation of germ cell development by intercellular signaling in the mammalian ovarian follicle. Wiley Interdiscip Rev Dev Biol 2018;7:e294.
13. Diaz FJ, Wigglesworth K, Eppig JJ. Oocytes determine cumulus cell lineage in mouse ovarian follicles. J Cell Sci 2007;120(Pt 8):1330-40.
14. Eppig JJ, Wigglesworth K, Pendola FL. The mammalian oocyte orchestrates the rate of ovarian follicular development. Proc Natl Acad Sci U S A 2002;99:2890-4.
15. Reynaud K, Driancourt MA. Oocyte attrition. Mol Cell Endocrinol 2000;163:101-8.
16. Choi Y, Rajkovic A. Genetics of early mammalian folliculogenesis. Cell Mol Life Sci 2006;63:579-90.
17. Skinner MK. Regulation of primordial follicle assembly and development. Hum Reprod Update 2005;11:461-71.
18. Hutt KJ, McLaughlin EA, Holland MK. Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. Mol Hum Reprod 2006;12:61-9.
19. Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. Reproduction. 2001;122:829-38.
20. Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update 2008;14:159-77.
21. Huang Z, Wells D. The human oocyte and cumulus cells relationship: new insights from the cumulus cell transcriptome. Mol Hum Reprod 2010;16:715-25.
22. Kathirvel M, Soundian E, Kumanan V. Differential expression dynamics of Growth differentiation factor9 (GDF9) and Bone morphogenetic factor15 (BMP15) mRNA transcripts during in vitro maturation of buffalo (Bubalus bubalis) cumulus-oocyte complexes. Springerplus 2013;2:206.
23. Elvin JA, Yan C, Wang P, Nishimori K, Matzuk MM. Molecular charac-

terization of the follicle defects in the growth differentiation factor 9-deficient ovary. Mol Endocrinol 1999;13:1018-34.
24. Gui LM, Joyce IM. RNA interference evidence that growth differentiation factor-9 mediates oocyte regulation of cumulus expansion in mice. Biol Reprod 2005;72:195-9.
25. Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Mol Endocrinol 2001;15:854-66.
26. Ebrahimi B, Valojerdi MR, Eftekhari-Yazdi P, Baharvand H, Farrokhi A. IVM and gene expression of sheep cumulus-oocyte complexes following different methods of vitrification. Reprod Biomed Online 2010;20:26-34.
27. Kaivo-oja N, Jeffery LA, Ritvos O, Mottershead DG. Smad signalling in the ovary. Reprod Biol Endocrinol 2006;4:21.
28. Mazerbourg S, Hsueh AJ. Genomic analyses facilitate identification of receptors and signalling pathways for growth differentiation factor 9 and related orphan bone morphogenetic protein/growth differentiation factor ligands. Hum Reprod Update 2006;12:373-83.
29. Reader KL, Heath DA, Lun S, McIntosh CJ, Western AH, Littlejohn RP, et al. Signalling pathways involved in the cooperative effects of ovine and murine GDF9+BMP15-stimulated thymidine uptake by rat granulosa cells. Reproduction 2011;142:123-31.
30. Horie K, Fujita J, Takakura K, Kanzaki H, Suginami H, Iwai M, et al. The expression of c-kit protein in human adult and fetal tissues. Hum Reprod 1993;8:1955-62.
31. Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002;296:1655-7.
32. Sobinoff AP, Nixon B, Roman SD, McLaughlin EA. Staying alive: PI3K pathway promotes primordial follicle activation and survival in response to 3MC-induced ovotoxicity. Toxicol Sci 2012;128:258-71.
33. Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, et al. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science 2008;319:611-3.
34. Eppig JJ. Intercommunication between mammalian oocytes and companion somatic cells. Bioessays 1991;13:569-74.
35. Wigglesworth K, Lee KB, O'Brien MJ, Peng J, Matzuk MM, Eppig JJ. Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. Proc Natl Acad Sci U S A 2013;110:E3723-9.
36. Norris RP, Ratzan WJ, Freudzon M, Mehlmann LM, Krall J, Movsesian MA, et al. Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development 2009;136:1869-78.
37. Sun QY, Miao YL, Schatten H. Towards a new understanding on the regulation of mammalian oocyte meiosis resumption. Cell Cycle 2009;8:2741-7.
38. van Montfoort AP, Geraedts JP, Dumoulin JC, Stassen AP, Evers JL, Ayoubi TA. Differential gene expression in cumulus cells as a prognostic indicator of embryo viability: a microarray analysis. Mol Hum Reprod 2008;14:157-68.
39. McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, et al. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum Reprod 2004;19:2869-74.
40. Anderson RA, Sciorio R, Kinnell H, Bayne RA, Thong KJ, de Sousa PA, et al. Cumulus gene expression as a predictor of human oocyte fertilisation, embryo development and competence to establish a pregnancy. Reproduction 2009;138:629-37.
41. Wathlet S, Adriaenssens T, Segers I, Verheyen G, Van de Velde H, Coucke W, et al. Cumulus cell gene expression predicts better cleavage-stage embryo or blastocyst development and pregnancy for ICSI patients. Hum Reprod 2011;26:1035-51.
42. Cillo F, Brevini TA, Antonini S, Paffoni A, Ragni G, Gandolfi F. Association between human oocyte developmental competence and expression levels of some cumulus genes. Reproduction 2007;134:645-50.
43. Wathlet S, Adriaenssens T, Segers I, Verheyen G, Janssens R, Coucke W, et al. New candidate genes to predict pregnancy outcome in single embryo transfer cycles when using cumulus cell gene expression. Fertil Steril 2012;98:432-9.e1-4.
44. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod 2001;16:2652-7.
45. Shoukir Y, Campana A, Farley T, Sakkas D. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Hum Reprod 1997;12:1531-6.
46. Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod 1998;13:182-7.
47. Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D. Gene expression in human cumulus cells: one approach to oocyte competence. Hum Reprod 2007;22:3069-77.
48. Assou S, Haouzi D, Mahmoud K, Aouacheria A, Guillemin Y, Pantesco V, et al. A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. Mol Hum Reprod 2008;14:711-9.
49. Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Cumulus cells apoptosis as an indicator to predict the quality of oocytes and the outcome of IVF-ET. J Assist Reprod Genet 2001;18:490-8.
50. Assidi M, Montag M, Van der Ven K, Sirard MA. Biomarkers of human oocyte developmental competence expressed in cumulus cells before ICSI: a preliminary study. J Assist Reprod Genet 2011;28:173-88.
51. Hamel M, Dufort I, Robert C, Léveillé MC, Leader A, Sirard MA. Identification of follicular marker genes as pregnancy predictors for human

IVF: new evidence for the involvement of luteinization process. Mol Hum Reprod 2010;16:548-56.
52. Gebhardt KM, Feil DK, Dunning KR, Lane M, Russell DL. Human cumulus cell gene expression as a biomarker of pregnancy outcome after single embryo transfer. Fertil Steril 2011;96:47-52.e2.