REVIEW

Markers of oocyte quality

Chiang Mee Voon[1], Savni Sawant[2]

[1]Valencian Institute of Infertility, University of Valencia, Valencia, Spain

[2]Wayne State University, Detroit, Michigan, United States of America

Abstract

Selection of the best oocyte for fertilization and embryo development is shown to be an essential step in human assisted reproduction treatment. Recent studies using OMICs-based approaches and morphological selection criteria have allowed the identification of key molecular markers that non-invasively predict oocyte quality that is anticipated to provide better clinical outcomes and efficient infertility treatment. This review will investigate and discuss the different types of markers of oocyte quality and their relationship to assisted reproductive outcomes. A search of the literature on oocyte markers was performed. The review on oocyte morphology was quite contradictory, and gave no clear markers of predictive value. The only exception was the meiotic spindle, which was found to have prognostic value for oocyte developmental competence. Studies found that there were significant differences in average oxygen consumption rates for fertilization, embryo quality, maternal age, and type of stimulation. The depletion or appearance of key amino acids was linked to the maturation potential of human oocytes and the patient's age. For telomere length studies, some research confirmed that the calculated telomere length decreased from oocyte to cleavage rates, but significantly increased between the cleavage stages to the blastocyst stage. Besides these, the expression levels of GDF9 and BMP15 were closely related to oocyte maturation, fertilization, embryo quality, and pregnancy outcomes. In conclusion despite the intensive research and some promising results, these contradictory data underline the importance of more intensive and fully coordinated research to reach a consensus in the analysis of the key markers of oocyte quality. Oxygen consumption and amino acid turnover as well as the expression of GDF9 and BMP15 factors appear promising as markers of oocyte quality. It appears obvious that a single marker cannot properly indicate the potential of the oocyte and that further research is definitely needed.

Disclaimer: The authors have no conflicts of interest.
J Reprod Biotechnol Fertil 11:96-103
Correspondence: Jessy MV Chiang; email: mvchiang77@gmail.com
Compliance acknowledgement: This article was edited by the Australian Editorial Services (www.nativeenglisheditor.com)
Keywords: Bone Morphogenetic Protein 15, Growth Differentiation Factor 9, oocyte marker, oxygen consumption, telomere length

Introduction

In vitro fertilization technology involves the selection and fertilization of oocytes, and then the development and transplantation of embryos or blastocysts to patients. The quality of the oocytes is crucial and has a direct impact on their fertilization and development competence. The criteria for the quality of oocytes can be categorized as morphological, cellular, and molecular. The overall efficiency of human assisted reproduction may increase if the non-invasive selection of developmentally competent human oocytes is achieved. It is especially crucial in countries where legal, religious, and social factors restrict the production of multiple embryos. Presently, the practice in clinical embryology is to fertilize all available oocytes that are in the MII-stage. With the selection based only on the morphological aspects after fertilization, seemingly suitable but intrinsically defective or abnormal embryos may be cultured,

resulting in compromised in vitro development and when transferred, poor clinical outcomes.

Oxygen consumption has been considered a valuable marker for evaluating oocyte and embryo metabolism, and it seems to provide an alternative method for assessing their development competence before transfer (Leese et al., 2012). Oxygen is important for the generation of cellular energy called ATP in the mitochondria via oxidative phosphorylation.

Despite intensively developed research in searching for the best and most appropriate markers of oocyte quality, the knowledge about the proteomics and metabolomics of an oocyte is still in its infant stage. Alternative approaches for non-invasive assessments have focused on profiling selective classes of metabolites where amino acids have featured heavily. A study on amino acid turnover was found to be largely independent of other known determinants of clinical pregnancy outcomes such as morphological grade (Sinclair et al., 2008).

Telomere length is known to affect senescence, cell division, and fertility. With each cell division, a small amount of the telomere is lost due to the "end replication problem". After many cell divisions, significantly short telomere length results in cell aging associated with chromosome instability, unless other mechanisms actively restore telomere length. The most important roles of the telomeres are to protect chromosome ends from being recognized as DNA breaks by the nucleases, and properly attach the chromosome ends to the nuclear matrix or membrane. They also play important roles in the formation of correct pairing, chiasmata, and synapses between homologous chromosomes (Aubert et al., 2012). Direct investigation of whether the length of telomere DNA in human oocytes is associated with maturation and quality has few limitations. These include low available quantities of DNA, difficulty in obtaining access to normal human oocytes, and the need to develop an approach to simultaneously quantify telomere DNA and diagnose aneuploidy from the same oocyte. Alternatively, abnormalities in the telomere length and telomerase activity in human granulosa cells may serve as molecular markers of occult ovarian insufficiency (Butts et al., 2009), as compromised telomerase maintenance of telomeres in granulosa cells may harm women's fertility.

Oocyte-secreted factors (OSFs) such as growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), play a crucial role in the process of follicular development and oocyte maturation. Since OSFs are expressed in oocytes and cumulus granulosa cells, many recent studies are trying to explore whether the expression levels of GDF9 and BMP15 mRNAs in cumulus granulosa cells can be used as molecular markers for predicting an oocyte's developmental potential. The purpose of this bibliography review is to evaluate the availability of different oocyte quality markers in recent research and their relation to the oocyte's development competence, predictive value as a marker, and the clinical outcome achieved with the oocyte.

Oocyte morphology

Cumulus-oocyte complex (COC)

The study of oocyte morphology can be very subjective and produce conflicting results. Morphological criteria obtained by non-invasive methods in association with further development potential were investigated in a few studies. Ebner et al. (2008a) found a correlation between a very dense corona radiate layer and lower maturity of oocytes. Also, Lin et al (2003) found an association between the in vitro developmental potential and blastocyst quality based on the morphology of COCs.

Perivitelline space

Rienzi et al. (2008) found that large perivitelline space was associated with lower clinical outcomes. There is an inverse relationship between their oocyte morphological scoring system and pregnancy rates (Rienzi et al., 2008).

Zona pellucida

Birefringence elevation in the inner layer of the zona pellucida was found to be correlated with increased in vitro efficiency, including fertilization rates and embryo quality (Montag et al., 2008). This was contradictory to the recent publication of Madaschi et al. (2009), as there was no association between high or low zona

birefringence and fertilization rates or embryo development in their studies.

First polar body (PB1)

According to Verlinsky et al. (2003), irregular shape or fragmented first polar body (PB1) was not associated with embryo quality, blastocyst development, aneuploidy, or implantation rate. De Santis et al. (2005) also did not find any correlation between polar body fragmentation and fertilization rate, embryo quality, and blastocyst formation, although there was insufficient data to evaluate the effect of the shape of PB1 on further development. In contrast, Navarro et al. (2009) found a relationship between having a large PB1 and lower fertilization plus cleavage rates, as well as poor embryo quality.

Vacuoles and cytoplasmic inclusions

The existence of vacuoles in oocytes was negatively associated with cryo survival, and the developmental potential of embryos after fertilization (Balaban et al., 2008), although fertilization rates and embryo quality were not affected. Increased biochemical pregnancy rates as a consequence of embryos transferred that were derived from oocytes with vacuoles. According to some researchers, inclusions did not appear to harm fertilization, embryo quality, and implantation rates (De Sutter et al., 1996). Others however reported decreased fertilization and poorer embryo development with inclusions (Otsuki et al., 2007).

Centrally located cytoplasmic granularity (CLCG)

Oocytes with CLCG can cause a low survival rate and impair in vitro development after cryopreservation (Balaban et al., 2008). Ongoing clinical pregnancy rates were compromised when embryos derived from centrally granulated oocytes were transferred. However, Kahraman et al. (2000) found that CLCG oocytes were not associated with fertilization rates, embryo development, and pregnancy rates.

Meiotic spindle

It is well known that more than 80% of detected aneuploidies in embryos derive from meiotic errors in the oocyte. Conflicting results have been reported regarding the predictive value of the spindle in human oocytes by polarized microscopy. Some studies showed higher fertilization rates and higher pregnancy rates (Madaschi et al., 2008) whereas others found no difference in all or some of these outcomes (Chamayou et al., 2006).

There was a significant difference in spindle normality and spindle density in the oocytes of pregnant patients compared with the oocytes of non-pregnant patients (Kilani et al., 2009).

Oxygen consumption

Oxygen consumption was not affected by the morphology of oocytes. Morphologically normal oocytes had similar oxygen uptake compared to those having other phenotypes such as large perivitelline space, refractile bodies, granular oocytes, or oocytes with fragmented first polar bodies. However, there is a study that showed that average oxygen consumption rates were significantly different between successfully fertilized oocytes and those that failed to fertilize (Aberto Tejera et al., 2011). Oocytes that gave rise to good quality embryos displayed a higher tendency of average oxygen consumption rate on Day 2 or Day 3 compared to poor quality embryos but there was no significant difference (Aberto Tejera et al., 2011).

Scott et al. (2008) worked with human oocytes and they observed that there was a difference in the respiration rates depending on maternal age and FSH concentration. They found that oocytes with respiration rates of between 0.48 and 0.55 nl O_2/h were viable, whereas lower respiration rates were consistent with a lack of continued development or atresia.

Amino acid turnover

The "Quiet embryo" hypothesis which was proposed by Leese (2001, 2012) stated that embryos with lower metabolism operate more efficiently, and have better development potential compared to those with higher metabolism as reflected in higher amino acid turnover (Houghton et al., 2002). There is an association between the degree of DNA damage and amino acid turnover that has been confirmed in porcine, bovine, and human embryos. Glutamic acid, glutamine, valine, arginine, and isoleucine appearance or depletion was all significantly different between degenerated and morphologically normal MII oocytes (Hemmings et al., 2013).

Moreover, their studies showed that MII oocytes from patients aged under 35 years depleted more glutamine, methionine, phenylalanine, and arginine significantly and had a greater reduction of total amino acids as well as a higher amino acid turnover than oocytes from older patients (≥35 years old). However, there were no significant differences in depletion, appearance, and turnover of amino acids between oocytes from women with PCOS compared to oocytes from women without PCOS according to Hemmings et al. (2013). For gonadotrophin stimulation, there were no significant differences in the turnover, depletion, and balance of amino acids from all mature oocytes retrieved from rFSH versus hMG-stimulated cycles (Hemmings et al., 2013).

Telomere length

Turner et al. (2010) reported that telomere length changes according to the stage of embryo development. He confirmed that telomere length decreased from the oocyte to cleavage stage embryos and increased between cleavage embryos and blastocysts. Cleavage-stage embryos have significantly shorter telomere lengths than GV or blastocyst nuclei ($P< 0.001$; Turner et al., 2010).

Cheng et al. (2013) showed that there was a significantly higher relative telomere length (T/S ratio) in cumulus cells surrounding oocytes that were fertilized in comparison to unfertilized oocytes. They also found that the relative telomere length was longer in cumulus cells from mature oocytes compared to cumulus cells from immature oocytes, and in cumulus cells from good-quality embryos compared to poor-quality embryos on Day 3.

Oocyte secreted factors (GDF9 & BMP 15)

Previous studies had indicated that higher GDF9 and BMP15 levels in follicular fluid are significantly related to oocyte maturation and embryo quality (Gode et al., 2011). These two factors may stimulate mitogen-activated protein kinase (MAPK) and M-phase-promoting factor (MPF) activity in oocytes and improve their quality and development potential. According to Yi Li et al. (2014), the expression levels of GDF9 and BMP 15 mRNA were significantly associated with age, BMI, oocyte maturation, and normal fertilization and cleavage rates ($p<0.05$). The GDF9 and BMP15 mRNAs expression levels in the group with good-quality embryos were significantly higher than those in the group with poor-quality embryos ($P < 0.05$). The expression levels of GDF9 and BMP15 mRNAs in the pregnancy group were also significantly higher than those in the no-prgnancy group ($P < 0.05$).

Recent advances in finding non-invasive biomarkers for oocyte quality

The last couple of years have seen tremendous progress in assaying and testing non-invasive biomarkers to predict the quality of an oocyte. This section shall review some of these upcoming markers and discuss their clinical relevance. MicroRNAs have emerged as promising small-non coding RNAs that can be extracted from bodily fluids and their upregulation or downregulation is correlated with diseased states. A 2020 study (Zhang et al., 2020) evaluated follicular fluid from women with poor oocytes and superior oocytes and it was observed that the group with poor oocytes had 7 miRNAs upregulated in their follicular fluid, all of which are associated with oocyte development.

Another marker of oocyte competence is the presence of Vitamin D (Ciepiela et al., 2018). Ciepiela et al. (2019) showed that the presence of 25-Hydroxy-vitamin D [25(OH)(D)] in follicular fluid negatively correlates with oocyte quality, i.e. lower concentration leads to higher fertilization rates, better quality embryos, and higher pregnancy rates. Taking a turn towards trends in the genome of the oocyte itself, Li and coworkers (Li et al.,2022) established that oocytes that had hypermethylation of CpG island in GNAS clusters were better in quality than those that were hypomethylated. This could be a potential epigenetic marker for oocytes derived from in-vitro maturation.

Apart from using follicular fluid or the oocyte, another non-invasive way to judge oocyte quality is by the granulosa cells that surround them. Liu et al., (2021) showed that when granulosa cells are divided into two categories, one from those that gave rise to good quality blastocysts, and the other that gave rise to poor blastocysts, RNA sequencing can be performed to show differing gene expression. In the group of granulosa cells whose oocytes gave good-quality blastocysts, it

was shown that 129 genes were differentially expressed, the most significant one being COL1A2 and renin. These results could potentially pave the way for non-invasive PGD to choose which embryo to transfer. Along with granulosa cells, cumulus cells also have the potential in predicting oocyte quality. A recent study by von Mengden and coworkers (von Mengden et al., 2022) showed that by using cumulus cell gene expression datasets and bioinformatic tools, two genes namely PTGS2 and CYPB1 affect oocyte quality positively and negatively respectively and this was correlated with the IVF cycle outcomes using oocytes with either gene elevated.

RNA sequencing has given rise to libraries of genes responsible for failed IVF cycles, and bioinformatics can be combined with this to predict oocyte or embryo potential. Oocyte maturation pathways require critical intercellular signaling and the fluctuation of calcium ions plays a crucial role. Since the calcium ion is stimulated by binding to proteins, it is of interest to look into calcium-calmodulin binding genes. Farshbaf and coworkers (Farshbaf et al., 2021) conducted bioinformatics analyses on several such genes and narrowed down Calcineurin, whose several catalytic subunits when present in cells surrounding oocytes gave rise to oocyte maturation and its absence caused oocytes to arrest in the GV stage. This makes Calcineurin a good predictor of oocyte fertilization.

As the number of IVF cycles increases worldwide, the number of IVF failures also comes to light. A large portion of these recurrent failures have an underlying genetic basis wherein there is a mutation in genes responsible for oocyte maturation, fertilization, and early embryonic development. Currently, 16 genes (PATL2, TUBB8, TRIP13, ZP1, ZP2, ZP3, PANX1, TLE6, WEE2, CDC20, BTG4, PADI6, NLRP2, NLRP5, KHDC3L, and REC114) have been reported as the cause of these processes going wrong (Sang et al, 2021). Most of these are inherited although they can be de novo. From this panel, NLRP2 is most strongly correlated with IVF outcomes and thus it could serve as a non-invasive biomarker for oocyte quality. Knowledge of which gene is mutated could serve as a target for gene therapy.

Discussion

Publications reporting on the morphological characteristics of oocytes are the result of an unavoidable compromise, as the use of terms to describe them is inconsistent between studies and is subjective among researchers. Experimental designs also vary considerably, with wide differences between papers regarding the outcome parameters. The only exception is the meiotic spindle study, where the data for the morphology features and the outcome parameters is reasonably homogenous and suitable for meta-analysis. Besides, there is no strict selection for the experimental designs, for publications investigating features of morphology.

Conventionally, the examination of spindle imaging was only dependent on fixing the oocyte on a slide. It was therefore difficult to evaluate the clinical IVF outcome by studying the spindle dynamics in live oocytes. The introduction of a novel polarized microscopic system has allowed for visualization of the meiotic spindle without destroying the oocytes. In a recent study, the spindle length is shorter in oocytes that gave pregnancy as compared to oocytes that did not. This suggests that there may be a cut-off limit for a spindle to be considered normal (Kilani et al., 2009) and there must be further experiments to confirm this suggestion.

Oocyte oxygen consumption values provided by an Embryoscope can be correlated to the mitochondrial load and the metabolomics activity of the oocyte. Mitochondria are energy-supplying organelles and their main functions are ATP synthesis and calcium supply (Krisher et al., 2004). As a consequence, mitochondrial functional integrity is pivotal for cellular survival and development. Recent studies found that oxygen consumption is associated with fertilization ability, which is consistent with previous data regarding higher ATP turnover in mature oocytes. Some research groups showed that a lower ATP production, reflected in oxygen consumption, could compromise not only fertilization but also embryo development (van Blerkom et al., 2009). Amino acid depletion/appearance in human oocytes is a feature of patient age, gonadotrophin preparation, and dose used for control ovarian stimulation.

The findings of Hemmings et al., (2013) have some limitations as the immature eggs collected at the time of ICSI were used as the model for maturation. These oocytes have failed to respond to hCG triggers in vivo and have limited capacity to support further embryo development in vitro. The lack of cumulus cells and in vitro stress may have influenced the turnover of amino acids and owing to small sample sizes, further studies are required to confirm these findings.

Indeed a characteristic of degenerating oocytes was a significant increase in the depletion of arginine, which may be utilized to generate nitric oxide (NO) by nitric oxide synthase. This is supported by a study that stated that high levels of NO have been associated with apoptosis in bovine embryos before implantation (Tranguch et al., 2003).

The results of telomere length are potentially valuable, but the oocytes were examined at different stages and further variation could also result from differences in the measurement techniques used. Moreover, the exact values of telomere length may be inaccurate as it is quite challenging to analyze cells that are in between the cell cycle.

Conclusion

Morphological features of MII human oocytes fail to predict fertilizing ability and developmental competence. Oxygen consumption and amino acid turnover indices could become useful markers of oocyte competence. Telomere length as marker needs further investigation. The expression of GDF9 and BMP15 factors were significantly associated with oocyte maturation, fertilization, embryo quality, and clinical outcome. It appears obvious that a single marker cannot properly indicate the developmental potential of the oocyte and that further research is definitely needed.

References

Aberto Tejera, Javier Herrero, M. J. de los Santos, Nicol_as Garrido, Niels Ramsing, and Marcos Meseguer. Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimens. Fertil Steril 2011;96:618-623.

Aubert G, Hills M, Lansdorp PM. Telomere length measurement-caveats and a critical assessment of the available technologies and tools. Mutat Res 2012;730:59–67.

Balaban B, Ata B, Isiklar A, Yakin K, Urman B. Severe cytoplasmic abnormalities of the oocyte decrease cryosurvival and subsequent embryonic development of cryopreserved embryos. Hum Reprod 2008;23:1778–1785.

Butts S, Riethman H, Ratcliffe S, Shaunik A, Coutifaris C, Barnhart K. Correlation of telomere length and telomerase activity with occult ovarian insufficiency. J Clin Endocrinol Metab 2009;94:4835–4843.

Chamayou S, Ragolia C, Alecci C, Storaci G, Maglia E, Russo E, Guglielmino A. Meiotic spindle presence and oocyte morphology do not predict clinical ICSI outcomes: a study of 967 transferred embryos. Reprod Biomed Online 2006;13:661–667.

Cheng EH, Chen SU, Lee TH, Pai YP, Huang LS, Chun-Chia Huang CC, Lee MS. Evaluation of telomere length in cumulus cells as a potential biomarker of oocyte and embryo quality. Hum Reprod 2013;28:929-936.

Ciepiela P, Dulęba AJ, Kowaleczko E, Chełstowski K, Kurzawa R. Vitamin D as a follicular marker of human oocyte quality and a serum marker of in vitro fertilization outcome. J Assist Reprod Genet. 2018;35(7):1265-1276. doi:10.1007/s10815-018-1179-4

Ciepiela P. Is it realistic to consider vitamin D as a follicular and serum marker of human oocyte quality?. *J Assist Reprod Genet*. 2019;36(1):175-176. doi:10.1007/s10815-018-1344-9

Daei-Farshbaf N, Aflatoonian R, Amjadi FS, Nikniyaz H, Taleahmad S, Bakhtiyari M. Identification of calcineurin as a predictor of oocyte quality and fertilization competence based on microarray data. Comput Biol Chem 2021;94:107561.

De Santis L, Cino I, Rabellotti E, Calzi F, Persico P, Borini A, Coticchio G. Polar body morphology and spindle imaging as predictors of oocyte quality. Reprod Biomed Online 2005;11:36–42.

De Sutter P, Dozortsev D, Qian C, Dhont M. Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum Reprod 1996;11:595–597.

Gode F, Gulekli B, Dogan E, Korhan P, Dogan S, Bige O, Cimrin D, Atabey N. Influence

of follicular fluid GDF9 and BMP15 on embryo quality. Fertil Steril 2011;95:2274–2278.

Hemmings KE, Maruthini D, Vyjayanthi S, Hogg JE, Balen AH, Campbell BK, Leese HJ, Picton HM. Amino acid turnover by human oocytes is influenced by gamete developmental competence, patient characteristics and gonadotrophin treatment. Hum Reprod 2013; 0:1-14.

Houghton FD, Hawkhead JA, Humpherson PG, Hogg JE, Balen AH, Rutherford AJ, Leese HJ. Non-invasive amino acid turnover predicts human embryo developmental capacity. Hum Reprod 2002;17:999–1005.

Kahraman S, Yakin K, Donmez E, Samli H, Bahce M, Cengiz G, Sertyel S, Samli M, Imirzalioglu N. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod 2000;15:2390–2393.

KD Sinclair, LA Lunn, WY Kwong, K Wonnacott, RST Linforth, J Craigon. Amino acid and fatty acid composition of follicular fluid as predictors of in-vitro embryo development. Reprod Biomed Online 2008;16:859-868.

Krisher RL. The effect of oocyte quality on development. J Anim Sci 2004;82(E-suppl):E14–23.

Leese HJ. Metabolism of the preimplantation embryo: 40 years on. Reproduction 2012;143:417–427.

Leese HJ. Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. Bioessays 2002;24:845–849.

Leese HJ. What does an embryo need? Hum Fertil (Camb) 2003;6:180–5.

Li Y, Li RQ, Ou SB, Zhang NF, Ren L, Wei LN, Zhang QX, Yang DZ. Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. Reprod Biol Endocrinol 2014;12:81.

Li Y, Sena Lopes J, Coy-Fuster P, Rivera RM. Spontaneous and ART-induced large offspring syndrome: similarities and differences in DNA methylome. Epigenetics. 2022;17(11):1477-1496. doi:10.1080/15592294.2022.2067938

Liu T, Huang Y, Lin H. Estrogen disorders: Interpreting the abnormal regulation of aromatase in granulosa cells (Review). *Int J Mol Med*. 2021;47(5):73 doi:10.3892/ijmm. 2021. 4906

Madaschi C, de Souza Bonetti T, de Almeida Ferreira Braga D, Pasqualotto F, Iaconelli AJ, Borges EJ. Spindle imaging: a marker for embryo development and implantation. Fertil Steril 2008;90:194–198.

Montag M, Schimming T, Köster M, Zhou C, Dorn C, Ro¨ sing B, van der Ven H, Ven der Ven K. Oocyte zona birefringence intensity is associated with embryonic implantation potential in ICSI cycles. Reprod Biomed Online 2008;16:239–244.

Navarro PA, de Araujo MM, de Araujo CM, Rocha M, dos RR, Martins W. Relationship between first polar body morphology before intracytoplasmic sperm injection and fertilization rate, cleavage rate, and embryo quality. Int J Gynaecol Obstet 2009;104:226–229.

Otsuki J, Nagai Y, Chiba K. Lipofuscin bodies in human oocytes as an indicator of oocyte quality. J Assist Reprod Genet 2007;24:263–270.

Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril. 2008;90:1692–700.

Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update 2011;17:34–45.

Sang Q, Zhou Z, Mu J, Wang L. Genetic factors as potential molecular markers of human oocyte and embryo quality. J Assist Reprod Genet. 2021;38(5):993-1002.

Scott L, Berntsen J, Davies D, Gundersen J, Hill J, Ramsing N. Symposium: innovative techniques in human embryo viability assessment. Human oocyte respiration-rate measurement—potential to improve oocyte and embryo selection? Reprod Biomed Online 2008;17:461–9.

Suha Kilani, Simon Cooke, Andrew Kan, Michael Chapman. Are there non-invasive markers in human oocytes that can predict pregnancy outcome? Reprod Biomed Online 2009;18: 674-680.

Tranguch S, Steuerwald N, Huet-Hudson YA. Nitric oxide synthase production and nitric oxide regulation of preimplantation embryo development. Biol Reprod 2003;68:1538–1544.

Turner S, Wong HP, Rai J, Hartshorne GM. Telomere lengths in human oocytes, cleavage stage embryos and blastocysts. Mol Hum Reprod 2010;16:685-694.

van Blerkom J. Mitochondria in early mammalian development. Semin Cell Dev Biol 2009;20:354–64.

Verlinsky Y, Lerner S, Illkevitch N, Kuznetsov V, Kuznetsov I, Cieslak J, Kuliev A. Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? Reprod Biomed Online 2003;7:336–341.

von Mengden L, De Bastiani MA, Grun LK, Barbé-Tuana F, Adriaenssens T, Smitz J, Arruda LS, Link CA, Klamt F. Bioinformatic Analysis of Human Cumulus Cells to Unravel Cellular's Processes that Could Be Used to Establish Oocyte Quality Biomarkers with Clinical Application. Reprod Sci. 2022; 26:1-4.

Zhang X, Wang T, Song J, Deng J, Sun Z. Study on follicular fluid metabolomics components at different ages based on lipid metabolism. Reprod Biol Endocrinol. 2020;18(1):42. Published 2020 May 12. doi:10.1186/s12958-020-00599-8