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Metabolomic profiling as a predictor of oocyte quality in assisted reproductive technology

Abstract

Human follicular fluid provides a key environment for follicular development and oocyte maturation and contributes to oocyte quality and in vitro fertilization (IVF) outcome. This study aims to understand better the role of metabolomic profiling in assessing oocyte quality in IVF program. Many biochemical materials can be isolated from follicular fluid. The concentration of each material varies. The concentration of intra-follicular material has a positive or negative effect on the oocytes quality. Many study reported that biochemical materials in follicular fluid were correlated with oocyte quality. Measurement of intrafollicular biomarker levels is more accurate and better for assessing oocyte quality. Compared with the morphological parameters, the cellular and molecular predictors of oocyte quality have been proven to be more precise and objective, further studies and refinement of techniques are still needed.

Keywords: *metabolomic, follicular fluid, oocyte quality, in vitro fertilization*

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Introduction

In many cultures and civilizations, marriage is a way to continue family line or to produce offspring. Thus, marriage is a physical, social and cultural phenomenon as well. Although not entirely, most of married couples are expected to get offspring. Not all couples who plan their pregnancy immediately get pregnant spontaneously. Some couples fail to conceive despite having unprotected sexual intercourse for one year. Infertility is a condition characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner[1].

The development of medical science and technology allows couples who are facing difficulties to have children to get pregnant and give birth by assisted reproductive technology (ART). ART is an option for couples who are difficult to get pregnant. ART is programmed through several stages. The first stage is the selection stage aimed to prepare the couple. In this stage, interviews and basic examinations are conducted to explore possible causes and to determine their requirements. This will be followed by ovarian stimulation stage. The purpose of this stage is to trigger the growth and development of follicles, thus, obtaining sufficient quantities of follicles with good quality[2].

Many factors are associated with the low success rate of ART, one of which is poor oocyte quality[2]. Assessment of oocyte quality is the main goal of an embryologist in an ART program. Traditionally, the method used to assess oocyte quality is based on the classification of follicular morphology, cumulus-oocyte complex, polar body and/or meiotic spindles. Many studies have reported that examining and studying metabolites and metabolomic of follicular fluids have shown better prediction[2-4].

Assessing Oocyte Quality

One of the early stages in ART is ovum pickup (OPU). During this stage, it is expected to get a sufficient number of oocytes with good quality. Thus, assessing the quality of oocytes is one of the embryological goals in ART. Many methods have been developed to assess oocyte quality, such as evaluation of positive or negative criteria, polarizing microscopy analysis, genetic expression assessment of granulosa cells as well as the oocyte itself, and also observation and measurement of specific molecular markers of the oocyte. Polar body biopsy and analysis can also be performed to detect possible defects on chromosomes[3,4].

Analysis of Human Follicular Fluids

Human follicular fluid (HFF) provides a very important microenvironment in oocyte development. Follicular fluid is a product derived from materials in the plasma fluid which crossed the blood-follicular barrier and also from secretion of granulosa cells and theca cells. HFF assessment can be used not only to assess oocyte quality, but also to predict the success of fertilization and embryonic development. Analysis of the follicular fluid component also reflects the changes in blood serum metabolism. This suggests that there is a link between the intra-follicular microalocyte environment

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and the biochemical environment in the general circulatory system[4,5].

Follicular fluid can be collected simultaneously during OPU. Aspiration of follicular fluid may be performed either on a single follicle or in conjunction with other follicles. Aspiration of HFF is done by using flush needle with culture medium or double needle technique, to ensure the oocyte retrieval from the targeted follicle. However, in this procedure, many punctures may increase the likelihood of infection and bleeding. Another problem is assessing the maturity of the oocyte nuclei (nuclear maturity). Nuclear maturity can be fully assessed after oocyte recovery in intra-cytoplasmic sperm injection cases, which may lead to bias. Recent researches over the last few years have involved complex molecular analysis (metabolomics) by analyzing all the biological fluid content[3-7].

It is important to know the content of biochemical predictors in HFF to assess oocyte quality. Biomarkers in follicular fluid can be grouped into several categories, such as hormones, transforming growth factor-beta, other growth factors and interleukins (ILs), reactive oxygen species (ROS), anti-apoptotic factors, proteins, peptides, amino acids, sugars, and prostanoids[3,4].

Hormones

Gonadotropin

The levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in follicular fluid are influenced by its concentration in plasma. In the IVF, their concentration in plasma is determined by the administration of external gonadotropin. Its high level promotes oocyte maturation and determines the success of the IVF. Higher follicular LH level in follicles containing oocytes is associated with embryonic development and higher success rate of ART. It is clear that gonadotropin plays a crucial role in secreting the substances produced by granulosa cells (e.g., hyaluronic acid), which impact the development and maturation of oocytes. These hormones also synergize with estradiol (E2) in enhancing oocyte cytoplasm maturation through cyclic adenosine monophosphate (cAMP) secretion, and control of meiosis oocytes. High level of gonadotropin can improve these processes, resulting in higher number of quality oocytes and embryos, also increasing pregnancy rates[3-6].

Growth hormone

Growth hormone improves the performance of FSH on granulosa cells to produce E2. This initially involves the formulation of FSH and LH receptors on granulosa layer. Growth hormone is synthesized in the follicle[3].

Prolactin

High prolactin level and low cAMP level in follicular fluid are more commonly found in follicles whose oocytes can be fertilized compared to unfertilized oocytes. Many studies have sought to find an association between high prolactin levels and low cAMP levels in follicular fluids with successful fertilization and pregnancy. However, the results are still conflicting. Thus, the presence of prolactin in follicular fluid is not a reliable bio-marker for assessing oocyte quality[3,4].

Estrogen, Progesterone, and Androgen

It has been well-established that intrafollicular environment is estrogenic. This suggests that good follicular development and growth are estrogen-dependent. Estrogen (E2) is also found to improve oocyte cytoplasmic maturation through various direct non-genomic actions in cellular membrane level, which induces extracellular calcium ion transport into intracellular compartment, mediated by specific pattern of calcium ion oscillation. Higher E2/progesterone (P) ratio is associated with better oocyte maturation and higher pregnancy rates[3,6,7].

There are also disagreements about the level of progesterone in follicular fluid. Some researchers suggest that high level of progesterone or low E2/P ratio are negatively correlated with implantation and pregnancy because they reflected progressive luteinization and decreased aromatic activity which is needed in the final stage of oocyte maturation. On the contrary, high progesterone level may indicate the oocyte postmaturity that causes fertilization abnormalities and even multipronuclear embryos occurrence. It appears that optimal progesterone exposure has a positive effect on oocyte characteristics, whereas excessive level will cause cellular damage. It is essential to determine the threshold value of progesterone[3,6,7].

Increased level of androgens (e.g. testosterone) is associated with poor oocyte quality. Although fertilization occurs, there may be poor cell division. A higher E2/T (estradiol/testosterone) ratio is associated with better follicular quality and a higher likelihood of pregnancy. Existing data indicate that low E2/T ratio in follicular fluid is associated with early follicular atresia. This results in a progestogenic effect on oocyte viability and minimizes the possibility of fertilization and pregnancy. The androgenic atmosphere in the follicle triggers early follicular atresia, but on the other hand, it is also agreed that a certain amount of testosterone in the follicle is required for optimal follicular development. In fact, in an ART program where the response to FSH is unsatisfactory, the addition of LH (which triggers androgen synthesis by theca cells) promotes follicular growth and oocyte maturation[3,5].

Corticoids

The corticoids in the follicular fluid are thought to play an important role in the final stage of oocyte maturation. Some researchers also suggest that corticoids are also involved in the implantation of the embryo. Thus, high cortisol/cortisone ratio in follicular fluid is associated with higher pregnancy rate in ART programs[3,5].

Growth factors of the Transforming growth factor-beta superfamily

Inhibin and Activin

Inhibin level produced by granulosa cells and its presence in follicular fluid reflect the amount and activity of granulosa cells in each follicle. Inhibin A is increased while inhibin B is decreased in the follicular

phase. Inhibin A level is significantly higher in patients with endometriosis. Recent prospective studies have shown that higher levels of Inhibin A and B are related to the number of oocytes present at OPU, but are not associated with oocyte quality and fertilization rates. Thus, inhibin B may also be a useful marker about the quality of the embryo. Other studies have also made the same conclusion. Others research found that high levels of inhibin A and B in follicular fluid are associated with increased fertilization and pregnancy rates. In others study found a positive correlation between oocyte quality and inhibin A levels, but not with inhibin B. He suggested that the role of inhibin A as a marker of oocyte quality should be investigated further[5-8].

Anti-mullerian Hormone

Data on the relationship between anti mullerian hormone (AMH) level and oocyte quality are still under debate. Serum AMH level ranging from 1.66-4.56 ng/mL were associated with good oocyte quality and good embryo morphology. Follicular AMH level is independently correlated with oocyte maturity and development. In contrast, other study found that the oocyte would be more likely to be fertilized if the follicles that produced higher AMH. AMH level in follicular fluid of successfully fertilized oocytes is three times higher than non-fertilized oocytes[6,7].

Bone Morphogenetic Protein-15

Bone morphogenetic protein-15 (BMP-15) was found to reflect oocyte quality and maturity. BMP-15 level was higher in follicular fluid whose oocytes were fertilized compared to unfertilized oocytes with no cleavage. In the same study, BMP-15 level was correlated with E2 levels in certain follicle. But, this still requires more extensive research[3].

Other Growth factors and Interleukin

Insulin-like Growth Factors

Insulin-like growth factors (IGF-I) and -II are polypeptides that trigger cell proliferation and differentiation in some tissues. Biological availability of IGFs is regulated by IGF-binding proteins (from IGFBP-1 to IGFBP-6). Intrafollicular IGF-II, IGFBP-3 and IGFBP-4 levels were correlated with higher fertilization rate, cell division, progression and embryo morphology score on day 3. In the same study, a combination of IGFBP-3 and IGFBP-4, and pregnancy-associated plasma protein-A was significantly correlated with fertilization and embryonic development[3].

In another study, IGF-I and IGFBP-1 levels were both positively correlated with oocyte quality and maturation. IGF-1/IGFBP-1 ratio was significantly higher in both serum and follicular fluid of patients who successfully conceived in an ART program. However, there are also studies that reported no correlation between IGF-I level and the quality of embryos and the success of ART program. Hence, research on the role of IGFs and IGFBPs as bio-marker of oocyte quality in clinical practice is still needed[3].

Interleukins

Proinflammatory cytokines can be found locally produced in follicular fluids and are removed when the follicles are maturing and ovulating. One of these cytokines is IL-1 beta which is derived from plasma ultrafiltrate and the synthesis of luteinized granulosa cells. There is a positive relationship between IL-1 beta concentration and E2 level on the day of human chorionic gonadotropin injection. High concentrations of IL-1 beta in follicular fluid are associated with normal fertilization. But the concentration in follicular fluid is low on oocytes that finally become better embryos and undergo successful ART program. It is possible that IL-1 beta affects cytoplasm maturation and fertilization but does not play a role in post-fertilization embryo development[3].

In some studies, IL-2 and IL-10 levels are correlated with specific hormonal atmosphere within the follicle, but not with the success of ART. IL-2 and interferon concentrations in follicular fluid were significantly higher in oocytes that undergo cleavage early in embryonic formation. In the same study, the high concentration of IL-12 in follicular fluid was associated with highly fragmented embryos, and granulocyte colony-stimulating factor. This is also specifically related to the high implantation potential. Other study reported no significant association between IL-1alpha, IL-2, tumor necrosis factor alpha (TNF-α) and leukotriene B4 (LTB4) levels with oocyte maturity, fertilization and pregnancy occurrence. On the other hand, IL-1alpha/TNF-alpha, IL-1 alpha/LTB4 ratio, TNF-alpha/LTB4 levels are significantly different in the follicular fluid of females that successfully conceive compared with non-successful females. He believes that IL-1alpha, TNF-alpha, and LTB4 may play an important role in the follicular degradation process and are associated with a better intrafollicular environment, thus optimizing oocyte development and maturation[3].

Reactive Oxygen Species

Follicular vascularity, intra-follicular oxygen content and mitochondrial activity are factors that maintain optimum and sustained oocyte development. Research on vascularization and oxygenation of the follicle may be able to provide information about the potential development of each oocyte in the follicle[3,5,6].

Oxidative stress is likely to induce deoxyribonucleic acid damage which leads to apoptosis. There is evidence to support that hypoxic follicles contain high-frequency oocyte abnormalities of the myotical spindle which may decrease the likelihood of embryonic development. Cytoplasmic defects of oocytes and multinucleated blastomeres are commonly found in hypoxic follicles. The impact of oxidative stress on oocyte maturity appears to be very destructive, although its mechanism is unclear. However, many studies have found an association between ROS and oocyte maturity parameters. Other study reported that women who were pregnant after ART showed significantly lower ROS content in their follicular fluid compared to those who failed. On the other hand, ROS level may be a sign of poorer embryonic

development. Thus, the presence of low ROS in the follicular fluid is a predictor of ART success[3-8].

It seems that a balance between oxygen and anti-oxidant availability is essential for normal spindle formation and chromosomal normality in meiosis process. In mice, normal chromosomes are found in follicles with high oxygen and anti-oxidant contents. Overall, high level of ROS reflects pathological conditions associated with infertility. The destructive capabilities demonstrated by the presence of ROS can be neutralized in the presence of free radicals or endogenous anti-oxidants, such as enzyme super-oxide dismutase (SOD), and selenium-dependent glutathione peroxidase (SeGPx). The presence of SOD in human follicular fluids has been reported. High SOD concentrations in follicular fluid are associated with failure of fertilization. In contrast, high SeGPx was found in successful fertilization, and low SeGPx levels are associated with failure of fertilization[3-8].

The hormone melatonin produced by the pituitary gland utilizes antioxidant to protect the oocyte from oxidative stress. Its concentration in follicular fluid is inversely proportional to the 8OH-deoxyguanosine level. Administration of melatonin 3 mg/day orally increases fertilization rates. It has been reported that total anti-oxidant capacity (TAC) is significantly greater in follicles with fertilized oocytes compared to follicles with non-fertilizable oocytes. TAC reflects anti-oxidant activity, and is associated with the quality of the embryo and pregnancy rate. Higher TAC levels of follicular fluid signify anti-oxidant activity in granulosa cells. The increased TAC was evident in more mature follicles with high-quality oocytes[3-8].

Nitric Oxide

The granulosa cells contain endothelial cells that synthesize nitric oxide (NO) or eNOS. NO is a highly unstable gas molecule with very short half-life, making it very difficult to measure directly. This gas is very easily transformed into nitrite and nitrate that can be measured in biological fluids[3,6].

Nitrite and nitrate levels are significantly lower in follicles containing matured oocytes, fertilized oocytes, and embryo (>6 cells). Conversely, their high levels are correlated with more fragmented embryonic cells and reduced likelihood for implantation. Moreover, NO level in follicular fluid was also higher in patients with endometriosis or hydrosalpinges. Both of these conditions are associated with poorer oocyte quality and lower fertility potential. Interestingly, the up-regulation of serum NO level was associated with failure of implantation in patients with tubal and peritoneal factors. The above data states that excessive production of NO in the environment around the oocyte may trigger apoptosis before fertilization occurs and affect the development of oocytes. The concentration of nitrite and nitrate in follicle fluid can be used as predictors of oocyte quality[3,7].

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) plays an important role in perifolic angiogenesis and regulates intrafollicular oxygen. VEGF level was significantly correlated with gradation of perifolic vessels. A prospective study noted that VGEF concentrations were higher in oocytes that failed to fertilize. Also, high levels of VEGF were also correlated with poor embryonic morphology and lower conception rates in the ART program[3,4].

The synthesis of VEGF is a response to the hypoxia of oocyte-cumulus complex which explains why VEGF level is a useful biomarker in assessing oocyte quality. The state of hypoxia is responsible for the intrafollicular increase of this peptide. In the ART program, measurement of VEGF level in follicular fluid can be used as an index to exclude hypoxic oocytes. But until now this examination is still very expensive, making it difficult to be applied in clinical setting[3,4].

Anti-apoptotic Factors

Changes in the environment of apoptotic micro-follicles can affect the success of the ART program. Highly apoptotic granulosa cells will cause lower oocyte quality. Apoptosis of granulosa cells is significantly higher in older women, and is related to the quality of oocytes and embryos[3,7,8].

Activation of specific cellular pathways such as TNF and Fas-ligand (Fas-L) is essential in determining the occurrence of apoptosis of the follicle. Even in the oocyte itself, several receptors of specific cellular pathways such as TNF receptor and Fas are also expressed. Soluble FAS (sFas) and sFas-L are both detectable in amniotic fluid. It is likely that both regulate follicular and oocyte maturation during folliculogenesis. The sFas levels were significantly higher in follicular fluid of matured oocytes, whereas low sFas and sFas-L levels were associated with high apoptosis and poorer oocyte quality. The sFas and sFas-L levels in the follicular fluid were both significantly different in patients with polycystic ovarian syndrome who were either pregnant or not. Both are unfavorable indicators of oocyte and embryo quality[3,7,8].

Proteins, Peptides and Amino Acids

The amniotic fluid contains some proteins that are derived from the blood plasma and secretions of granulosa cells and theca cells. The purpose of analyzing the protein composition in amniotic fluid is to identify molecules that can be used as biomarkers of follicular development to optimize the oocyte selection process. Research is done by detecting specific proteins in follicular fluid and linking them to oocyte quality. In recent years, many techniques have been introduced to study proteins and peptides in biological fluids that are included in terminology, 'proteomic'[3,9-12].

The main problem in analyzing these protein markers in biological fluids is their complex and dynamic composition. Thus, techniques that have high precision separation and identification capabilities are needed. The most popular approach to proteomic follicular fluid analysis is two-dimensional gel electrophoresis followed by protein digestion and spectrometry. Other techniques include protein pre-fractionation with isoelectric focusing and subsequent nano-liquid chromatography as well

as mass spectrometry. In general, knowledge of proteins and their role in the development and maturation of follicles and oocytes is limited[3,4,9-12].

Some proteins, peptides and amino acids that can be found in follicular fluids include Alpha-Fetoprotein, Carcinoembryonic Antigen and CA-125, CD44 (membrane-integrated protein) antigen, alpha1-antitrypsin, leptin, endothelin-2. But, their correlation with oocyte quality is still being investigated. There are also other proteins such as oocyte maturation inhibitor (OMI). OMI concentration in the follicular fluid decreases as soon as the oocytes are matured and ready for fertilization. Follicles with matured oocytes may undergo fertilization at lower OMI concentrations. Follicles with high OMI activity show atretic or immature oocytes, and pelucida zone damage. It was concluded that in cases with abnormal oocyte maturity and oocyte damage, OMI level in follicle fluid was high. Low level of homocysteine (HCY), a methionine metabolite with several functions not yet fully understood, was found in oocytes with better quality and maturity. Administration of folic acid leads to low HCY concentrations in both serum and follicular fluid. This suggests that pre-conception administration of folic acid may give a positive effect on ART outcomes[3,4,9-12].

Beta-endorphin

Data on the presence of beta-endodorphin (EP) in follicular fluid and oocyte quality are controversial and inconclusive. The content of beta-EP in follicle fluid is higher in follicles with fertilized oocytes. In contrast to other studies, beta-EP levels were found lower in follicles containing oocytes of good quality which ultimately progressed into embryos. Other proteins suggested are lactoferrin, angiotensin II, prorenin and other amino acids. Some amino acids like alanine and glycine in the follicular fluid show good predictive value on the success of ART program on cows. In humans, the concentration of D-aspartic acid in the follicular fluid is directly correlated with good oocyte quality, the occurrence of meiosis II, and higher fertilization rate. Lactoferrin is an iron-binding glycoprotein that originally can be isolated from milk. Its presence in follicular fluid can be detected and its levels are higher in the follicles with better oocyte quality. Angiotensin II (AT II) in the fluid is negatively correlated with progesterone concentrations. Yet, another study found no correlation between AT-II in individual follicular fluid and the ability of the oocyte to be fertilized. Some amino acids like alanine and glycine in the follicular fluid show good predictive value on the success of the assisted reproductive technology program on cows. In humans, the concentration of D-aspartic acid in the follicular fluid is positively correlated with the mentioned parameters[3,4,11,12].

Sugars

Some sugars are found in follicular fluids such as hyaluronan, myo-inositol, and prostanoid. Hyaluronan is a disaccharide composed of D-glucuronic acid and D-N-acetylglucosamine. Both are present in the extracellular matrix of the cumulus (cumulus-oocyte complex) which is then released in follicular fluid before ovulation. High myo-inositol level in follicular fluid is positively correlated with oocytes and embryos. However, myo-inositol is considered no better than E2 to assess oocyte quality. Prostaglandin F2-alpha (PGEF2), produced by granulosa cells upon stimulation of gonadotropin, is a useful biochemical marker for oocyte quality. Its high level in the follicular fluid reflects the number of oocytes that can be fertilized. Also, the concentrations of PGE2 and PGEF2 alpha in follicular fluid were higher in those with matured oocytes[3,4,9-11].

Metabolomic Profiling

Recent researches have shifted from the analysis of metabolite targets (limited to one metabolite) to the metabolomic profiling. Metabolomic profiling is a process of analyzing the combination of several metabolites or intermediate metabolites by using an analytical technique, as well as assessing the dynamics between them, which includes all molecules with low molecular weight[3,4,10-13].

Research on low molecular weight metabolites (amino acids, lipids, nucleotides, signaling molecules, etc.) in biological fluids generated through the activity of various proteins expressed from gene transcription and micro ribonucleic acid (mRNA) translation is captivating. Metabolites represent the relationship between genetic information and cellular function phenotypes. The metabolomic analysis is relatively faster compared with genomic or proteomic analysis[3,4,9].

Metabolomic analysis techniques are more consistent and informative to analyze the patterns of biological systems that can be applied in research on embryos and oocytes. The purpose of performing a metabolomic analysis is to identify and quantify all metabolites in biological fluids, such as follicular fluids, as functional information and physiological conditions at the same time. The main difficulties of this metabolomic are: many metabolites are naturally unstable, chemical structures and has extensive variations in their patterns of production, unavailability of methods to strengthen metabolites measurement sensitivity, as well as metabolomic analysis associated with several chemical molecules simultaneously[3,4].

Techniques used for metabolomic research should be sensitive and easy to use for screening molecules in large quantities simultaneously and in a short period. For example, mass spectrometry technique alone or combined with chromatography, gas chromatography, capillary electrophoresis or ultra performance liquid chromatography offers high efficiency[3,4].

Metabolomics in follicular fluid is very dynamic if measured quantitatively against low molecular weight material. As an end product in cellular metabolism, low-molecular-weight molecules can express follicle response to anything that affects their development. Analyzing these metabolites is more informative than a direct study of gene expression (genomics), mRNAs (transcriptomes), or proteins (proteomes). Increased gene activity

that results in increased synthesis of mRNA and protein synthesis should not affect or impair cell function and morphology. This is because the metabolomes reveal the actual function of the biological system and the cell itself[3,4,9-13].

The presence of Inhibin B in follicular fluid and blood serum is strongly correlated with the number of oocytes at OPU but not with the success of ART. Meanwhile, the level of inhibins may reflect ovarian response rather than oocyte or embryo quality. Inhibin B level in follicular fluid is an effective marker of follicular development. He also showed a significant correlation between follicular inhibin B level with embryonic score on the second and third days[14,15].

The development of the oocyte is controlled through the accumulation of transcriptase during its development phase. Gene expression in oocytes can be investigated through microarray and high-fidelity RNA amplification techniques that can identify thousands of genes simultaneously. However, this technique cannot be used to select oocytes because of cell lysis requirement. Many studies analyze RNA molecules in human oocytes using the technique of microarrays (transcriptomics). All mRNA molecules need to be translated into protein or metabolite after the specific post-translation process, so that they can be the actual cell function markers. Metabolites present the final product of the cell-setting process. It is now possible to profiling metabolites, which is secreted by oocytes to surrounding media including follicular fluids. Or with metabolomic fluid secreted by oocytes in culture media after oocyte excavation in TRB program ('exo-metabolomics' or 'ecretomics'). All profiling of follicular fluid or supernatant oocyte culture media can be used to assess oocyte quality. More than one metabolite used to evaluate the potential for oocyte development so that it can be used as a biomarker of oocyte quality and can be applied as a clinical diagnosis. This research is highly relevant to animals for profiling of some metabolites like fatty acids, amino acids, or sugar. Although this is still a preliminary study, it can be done immediately by profiling follicular fluid. The follicular fluid taken from large and small follicles reflects different biochemical profiles (different phases of oocyte maturity). This study found that oocytes can absorb glucose in large amount and convert them into lactates that indicate the potentiality of high fertilization. Other studies have also focused on evaluating oocyte metabolism by measuring its energy substrate or its oxygen consumption in a media culture[3,4,9-13].

Conclusions

Oocyte quality estimation during ART procedures is main issues so far, mainly based on morphological criteria that are considered largely unsatisfactory. Even several biomarkers have been identified, the outcomes of IVF procedures remain poor because of the limited clinical treatment strategy. In fact, despite fundamental progress of basic research, translational medicine still requires lots of effort to translate discoveries into clinical application. The scientific community is therefore focused on research of other approaches to this issue based on genomic, transcriptomic and proteomic molecular investigations about ovarian follicle components, including somatic cells and follicular fluid. HFF serve as a key for follicle development and oocyte maturation and contributes to oocyte quality and IVF outcome. ■

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