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Mini Review

Implication of Metabolomics in Reproductive Technology

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Abstract

Metabolomics is one of the emerging omics technologies of the postgenomic age vital to systems biology. By quantitatively characterising the dynamic variations of metabolites using analytical techniques, primarily mass spectrometry (MS) and nuclear magnetic resonance, it analyses the pathophysiological condition of the patient (NMR). Sperm, oocytes, and embryos are altered using a process called assisted reproductive technology (ART) to aid in conception. It has recently come to light that metabolomics methods may be used to measure metabolites in ART samples, which can be used to evaluate the quality of gametes and embryos. This chapter reviews the state of the art in metabolomics research and how this technology is used in ART to inform future research and development.

Keywords: Systems Biology; Metabolomics; Postgenomic; Nuclear Magnetic Resonance (NMR); Mass Spectrometry (MS) and Assisted Reproductive Technologies (ART)

Introduction

The fields of transcriptomics, proteomics, and genomes were developed before the 1970s and 1990s when metabolomics research began to take shape. The field is a crucial component of systems biology, which describes how different metabolites in organisms fluctuate dynamically and quantitatively to interpret the pathophysiological state of the study subject. Proteomics and transcriptomics-based genomic investigations shed light on the regulatory level in systems biology. At the same time, metabolomics is the preferred method for examining the metabolic phenotypes and phenotypic disorders of diseases influenced by dynamic environmental changes. To summarise, metabolomics is the final step in the biological process, while genomes, transcriptomics, and proteomics are the initial stages. Metabolomics offers several benefits [1]. First, since metabolites are easily observed, changes in them might immediately reflect changes in the body, magnifying changes in genes and proteins. Secondly, there is a finite number of metabolites overall. Therefore, there is no need to create an extensive database. Third, it is simple to analyse metabolomics data. Fourth, sample pretreatment and collection are easy and can be done using standard technology. These benefits have led to the application of metabolomics technology in assisted reproductive technologies (ART). ART involves modifying sperm, oocytes, and embryos to facilitate fertilisation. Artificial reproductive methods, including artificial insemination (AI), in vitro fertilisation and embryo transfer (IVF-ET), and its offshoots, are referred to as "test-tube babies".

However, the prevalence of infertility due to age and other causes has been steadily rising in recent years, making ART an essential part of infertility treatment. The clinical pregnancy rate using IVF-ET technology is rising consistently as science and technology advance and improve. An increasing number of infertile patients can fulfil their ambition of becoming parents because of ongoing research and the optimisation of new techniques and technology. The most notable aspects of reproduction include a high rate of multiple pregnancies and a low rate of implantations; yet, as a novel medical technology, ART and its associated technologies are not without problems. Although gametes and embryos are still evaluated and chosen using morphological assessment, this method cannot accurately represent the quality of gametes and embryos or identify genetic or epigenetic problems in embryos with normal morphology. To increase implantation rates and prevent multiple pregnancies, new techniques are required to assess the potential of gametes and embryos in clinical settings.

Numerous recent studies have effectively used metabolomics to quantify various metabolites, such as amino acids, proteins, reactive oxygen species, and specific small molecules, in the semen, follicular fluid, and embryo culture conditions. Although there is no evidence to support its ability to improve ART outcomes, metabolomics has been widely used in research to analyse the fluid components of the female reproductive system, which could lead to new technologies and approaches to treating infertility (such as clinical pregnancy and live birth rates). Metabolites associated with oocyte

quality may be used to predict ART outcomes and oocyte maturation. These small compounds can be used to evaluate the quality of gametes and embryos, raising pregnancy rates and decreasing the chance of multiple pregnancies. However, it's essential to consider each type of ART's therapeutic benefits and safety [2].

Technical platforms

To perform metabolomics research, established protocols gather samples, and metabolites are extracted using technical procedures to evaluate statistical differences in metabolites across groups and carry out additional studies. No analytical techniques are available to measure every metabolite in human samples. Researchers have employed various analytical methods to perform complementary coverage analysis of metabolites; nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the two most popular technology platforms in metabolomics. These technical platforms are introduced in the following sections, and Table 1 compares their sensitivity, specificity, and other attributes.

Basic Parameters	Nuclear Magnetic Resonance (NMR)	Mass Spectrometry (MS)
Selectivity and Sensitivity	Selectivity – Used for both Selective and Non-Selective Analysis, i.e., targeted and non-targeted analysis. Highly Sensitive – Max. Femto mole.	Utilised mainly for non-selective analysis with less Sensitive,
Sample Measurement and Recovery Rate	Sample measurement requires different chromatographic techniques, but the recovery rate is fast.	A little more sample is required, and a little more time is taken for recovery, but a single technique is enough because the samples can be stored and analysed for a little more extended time.
Sample Preparation, Target Analysis and Repeatability	Advanced Optimisation is required to perform a perfect targeted analysis.	Sample preparation is relatively easy, and optimisation is optional because targeted analysis can't be achieved.

Table 1: Comparison based on basic parameters in Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS).

Mass spectrometry

Gas chromatography

Gas Chromatography–Mass Spectrometry (GC–MS) is an analytical tool used to measure the ion-charge mass ratio, also known as the charge-mass ratio. Depending on the ionisation source, two types of GC-MS are used in metabolomics studies: chemical ionisation mass spectrometry and electron bombardment mass spectrometry. However, the application of GC-MS has been somewhat limited in recent years due to the requirement that processed samples meet specific standards (i.e., they must be volatile and stable after volatilisation). The recent development of whole two-dimensional gas chromatography (GC×GC), with its short processing time, excellent resolution, and other advantages over GC–MS, is considered to make it more suitable for metabolomics investigation of complicated samples.

Liquid chromatography

In recent years, metabolomics research has extensively used liquid chromatography–mass spectrometry (LC–MS) because of its superior sensitivity and separation degree, quicker separation time, and less reagent dosage than GC–MS [2-4]. Based on the column efficiency of front-end liquid chromatography, two types of LC-MS are available: ultra-high-performance liquid chromatography (UHPLC) and high-performance liquid chromatography (HPLC). These forms of LC-MS address different analytical requirements. Furthermore, based on the ionisation method of the back-end mass spectrometry, LC-MS techniques can be divided into atmospheric pressure chemical ionisation source and electrospray ion source approaches. This makes it possible to examine metabolites thoroughly. Thanks to its numerous front-end and back-end configurations, the different LC-MS combinations combined with ultra-performance liquid can be used with various measuring techniques.

NMR technology

Using nuclear magnetic resonance spectroscopy on biological body fluids, NMR technology may extract rich details about every tiny molecule metabolite in an organism. The biological significance of these changes has been revealed at the molecular level through multivariate statistical analysis and pattern recognition processing of the data. These have clarified the status and dynamic changes in functional genomics, pathophysiology, and other aspects of related organisms. NMR causes no damage; its test conditions can be selected within a range of temperatures and buffers, enabling the study of chemical exchange, diffusion, and internal motion. In addition, various editing techniques with flexible and diverse test methods can be designed. NMR technology allows relatively simple sample analysis; the pretreatment step can be omitted, and the sample can be injected directly.

Metabolomics – Sample collection and pretreatment

Metabolomics is used to examine the metabolites of biological materials because traditional omics cannot adequately explain complicated physiological and pathological responses. In contrast, metabolomics aims to describe all the metabolites within cells or biological systems.

Numerous materials, such as blood, urine, cerebrospinal fluid, saliva, biopsy tissue, or cell extracts, can be used for metabolomics investigation. The study objectives, sample availability, and analytic platform are considered while selecting sample sources. For human metabolomics studies, blood and urine are the most often used samples; however, other tissue samples, including saliva, tonsil, adipose, and exhaled breath condensate, can also be used to identify metabolites. Furthermore, specimens associated

with systemic disorders may be chosen for metabolite analysis to increase detection sensitivity and specificity. For instance, it is possible to select respiratory system diseases using exhaled breath condensates, reproductive system disorders using follicular fluid, digestive system diseases using faeces and gastric juice, and salivary diseases [3].

During the sample-collecting procedure, appropriate collection tubes with anticoagulants are needed for blood samples. Separating the serum or plasma and removing the proteins at four degrees centigrade is a recommended procedure before analysis, and it may be the primary cause of pre-analysis errors in blood metabolomics investigations. It is generally accepted that there should be no more than 35 minutes between the collection of blood samples and the separation of the cells, as this could allow for glucose metabolism in the blood cells and raise the level of lactic acid. Furthermore, it is best to avoid repeating freeze-thaw processes in studies. Compared to blood samples, urine samples have a lower protein content and a more straightforward biological makeup [4]. Therefore, they usually don't need further metabolite extraction procedures; they are still high.

Nevertheless, it is challenging to regulate the factors of nutrition, sex, age, weight, circadian rhythms, and metabolomics samples. This restricts metabolomics research using human samples from an ethical and financial standpoint.

Metabolomics – Data processing

Metabolomics data processing frequently makes use of pattern recognition techniques, including principal component analysis (PCA), partial least squares (PLS), orthogonal signal correction (OSC), and orthogonal partial least squares (OPLS), which combine PLS and OSC.

The most straightforward and least supervised approach is PCA, which may efficiently identify and remove aberrant samples while reflecting the initial state of the data. High-dimensional datasets are reduced in dimension using this approach to capture as many varieties in the data as feasible. PCA is frequently only used as a starting step to assist in constructing models with better-supervised classification methods because directed approaches can increase PCA's accuracy [5]. It is challenging to derive valid findings when there are little differences between groups and significant disparities within groups. Upon identification of prospective biomarkers, the most dependable biomarkers can be found using supervised techniques (like PLS) to maximise regional categorisation. Group variables can be artificially added to compensate for the inadequacies.

Metabolomics applications – Reproductive technologies Progress

The inability of a couple of reproductive age to conceive naturally following a year of consistent sexual activity without the use of contraception is known as infertility. According to studies, reduced fertility affects about 15% of couples; the underlying reasons may be attributed to either the male or the female, or they may remain unknown. To ensure a live birth, artificial reproductive technology (ART) processes human germ cells outside of the uterus in a manner consistent with exogenous ovarian stimulation and endometrial preparation. Regretfully, the effectiveness of ART is restricted because only 10–30% of embryos can be implanted successfully; only 30% of ART-produced embryos are transported to the uterus, and the majority do not result in live babies.

Factors associated with pregnancy rate

The quality of gametes, embryos, and endometrial receptivity are three crucial aspects that affect the outcome of assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI) and in vitro fertilisation (IVF). In clinical settings, the morphological score is used to evaluate the quality of the gametes. A more sophisticated evaluation approach than only morphology is needed when there is a low oocyte abundance. In clinical practice, poor morphological grade oocytes can lead to embryo development without a live birth. It is possible for high-quality oocytes to either not fertilise at all with ICSI or to generate embryos of inferior quality or without the ability to implant into the endometrium. The endometrium is usually assessed by ultrasound assessment of the endometrial thickness to determine endometrial receptivity.

Primary application

Traditional morphological evaluation is the most popular technique for screening embryos, which cannot adequately represent gametes or embryos' quality and developmental potential. A non-invasive, dependable, and precise embryo selection strategy would enhance assisted reproduction results for selective single embryo transfer. The end products of several physiological regulating mechanisms during egg and embryo development are known as low-molecular-weight metabolites. Measuring metabolites in follicular fluid or an embryo culture medium allows for the evaluation of oocyte and embryo development and facilitates embryo optimisation. The total shift in the organism's metabolic status is revealed by metabolomics, the qualitative and quantitative study of small molecule metabolites in living things. The potential applications of metabolomics in ART have received increased attention.

Secondary applications

By using nuclear magnetic resonance (NMR) to detect the quantities of lactate, choline, citric acid, alanine, glycerophosphocholine (GPC), glutamine, and other components in semen, Ashish., *et al*. discovered that sperm quality could be assessed using GPC, citric acid, tyrosine, and phenylalanine. This metabolomics method is a quick and non-invasive way to investigate infertility. Wallace., *et al*. removed the oocytes and associated follicular fluid from 58 IVF patients. They then analysed the follicular fluid that could be fertilised but not cleaved and the follicular fluid that could be both

fertilised and cleaved using 1H-NMR analytical techniques. They discovered notable variations in the metabolite profiles of the two types of follicular fluid, including those in glucose, lactate, protein, and choline/choline phosphate profiles [6].

Thirty women who underwent ART treatment had their oocyte metabolites examined by Yun., *et al*. The women were split into two groups: one for advanced maternal age and the other for young control. The scientists evaluated three hundred and eleven metabolites in the follicular fluid, and seventy revealed substantial variations between the groups. The age of the mother was positively and strongly correlated with eight metabolites. Among them, there was a negative correlation between three metabolites and the number of recovered oocytes and a negative correlation between five metabolites and cleaved embryos [7].

Verloes., *et al*. examined the follicular fluid and an in vitro culture medium at the cleavage and blastocyst phases for human leucocyte Ag-G (HLA-G) presence or absence. They discovered that oocytes, cleavage-stage embryos, and blastocyst-stage embryos were secreted. In another investigation, HLA-G was positively linked with the likelihood of embryo implantation and the pregnancy rate. Patients with polycystic ovary syndrome (PCOS) had downregulated metabolites of cholesterol, α-tocopherol, and high-density lipoprotein phosphorylcholine when compared to healthy women of childbearing age. However, linoleic acid metabolites were upregulated, fatty egg white, palmitic acid, unsaturated fatty acids, and low-density fatty egg white—the primary membrane lipid composition of plasma samples from PCOS patients [8].

Timothy., *et al*. compared 21 metabolomic studies of different biofluids in the female reproductive system. Little data suggests that standard embryo morphology predicts individual embryo viability and implantation rate better than embryo culture media. There needs to be more consistent data from various studies, some of which require more randomised controlled trials, and there needs to be proof that metabolomics can enhance ART results. However, metabolomics may offer a more thorough comprehension of developing oocytes and embryos [9].

Conclusion

There is a plethora of potential applications for metabolomics technology in disease research. Metabolomics, as opposed to conventional clinical chemistry and other research techniques, is a "holistic perspective" research idea that methodically and thoroughly captures an organism's metabolic properties. By employing high-throughput, high-resolution technology analysis in conjunction with pattern recognition analysis techniques, scientists can investigate the features and regularities of life activities at the metabolic level and establish a causal link between metabolite content and changes in biological phenotype. These methods are susceptible, quick, effective, non-invasive, and focused. The rapid advancement of metabolomics technology has also given rise to fresh approaches to ART bottleneck issues; nevertheless, additional study is required to refine these approaches.

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