ROLE OF METABOLOMICS IN MALE INFERTILITY AND ASSISTED REPRODUCTIVE TECHNIQUES

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Abstract

Metabolomics is the systematic study of metabolites as small molecule biomarkers that represent the functional phenotype in a cell, tissue or organism. The ultimate goal of metabolomic analysis is to identify the global metabolite profiles of a given biological system. Detection of crucial disturbances in the concentration of metabolites by metabolomic profiling of key biomarkers can be beneficial in the management of various medical conditions including the male factor infertility. Recent studies have demonstrated the potential role of this rapid, non-invasive analysis in the investigation of male infertility. In addition, metabolomic analysis has been used to assist with gamete and embryo selection in assisted reproduction and has shown promise in predicting the pregnancy outcomes. Metabolomics can provide us with better, more useful information with higher throughput at a lower cost than genomics, transcriptomics or proteomics. Further research is however needed to confirm these preliminary observations.

Keywords

Metabolomics; biomarkers; infertility; semen analysis; ART

Introduction

Approximately 15%-20 % of all couples who attempt to conceive face the problem of infertility. Male factor infertility is the sole or contributing factor in nearly half of these cases [1]. Despite improvements in both diagnostic assessment and treatment of infertile couples, management of male factor infertility still remains a challenge, largely because of two factors: the lack of a rapid, non-invasive test to evaluate semen quality and investigate the cause of male infertility; and the inability to predict gamete quality and embryo viability, which in turn lead to low success rates and a high incidence of multiple births after in-vitro fertilization (IVF).

To overcome these limitations, a new science known as metabolomics has been conceived with an expectation that body fluid analysis can be optimized to create a low-cost, informative and medically relevant means of measuring metabolic changes, even when standard clinical chemistry markers are within normal limits [2]. Metabolomics is the systematic study of the inventory of metabolites, as small molecule biomarkers that represent the functional phenotype in a cell, tissue or organism [3, 4]. The metabolome constitutes the dynamic, quantitative complement of all low-molecular weight molecules (typically < 3000 m/z) present in cells in a particular physiological or pathological state. Ultimately, metabolomics is the mining of the global population of biomarkers, which unveils the phenotype of the system (cell, tissue or organism).

Rationale Behind Metabolomics

The central dogma of molecular biology suggests that there is a unidirectional flow of

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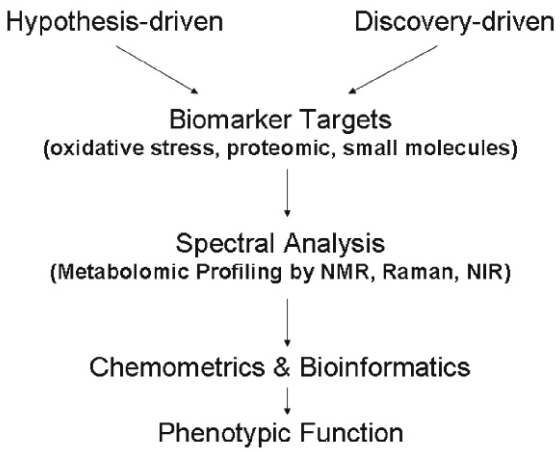
information from gene to transcript to protein. However, it is now known that in addition to gene expression, post-transcriptional and post-translational events regulate metabolic fluxes. Hence, the metabolome is considered to be closer to the phenotype rather than the transcriptome or proteome [5]. Although, metabolomics is complimentary to transcriptomics and proteomics, it provides us with a real-time snapshot of the downstream events that characterize gene expression [6]. While changes in the levels of individual enzymes have little impact on metabolic fluxes, they do have a significant effect on the concentration of a variety of individual metabolites [7]. Furthermore, with the downstream results of gene expression, changes in the metabolome are amplified relative to changes in the transcriptome and the proteome, which allows for increased sensitivity. Thus, metabolomics can provide us with better, more useful information with higher throughput at a lower cost than genomics, transcriptomics or proteomics and is therefore well suited for widespread investigations.

Biomarkers of Disease

About 3000 small molecule metabolites make up the human metabolome. The metabolites arising from the post-transcriptional and post-translational events that can be assessed systematically in the study of metabolomics serve as biomarkers [3]. Steroids, amino-acids and various markers of oxidative stress (OS) have all been used as biomarkers by different researchers in the field of reproduction [8-11]. The current trend is to use multivariate biomarkers rather than a single biomarker [12]. The Metabolomics Study Group for Assisted Reproductive Techniques, which is credited with pioneering research in this field, is especially focused on the biomarkers of OS [13-15]. Oxidative stress arises as a consequence of excessive production of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms [16]. -CH, -NH, -SH, C=C and -OH serve as biomarkers of OS. These biomarkers have been found in both the male and female reproductive tracts and are known to affect sperm quality and function, oocyte quality and embryo viability [17, 18]. Recent reports have found high levels of ROS in 25% to 40% of semen samples from infertile men [19]. Furthermore, enough evidence is available to suggest that ROS originating from embryo metabolism and the surrounding environment act on the cellular molecules of the embryo and block early embryo development [20-22].

Analysis of Biomarkers

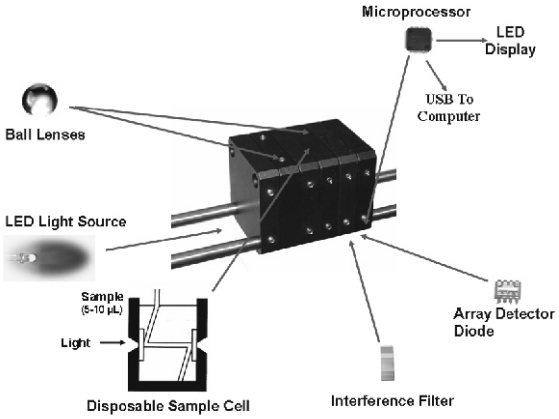
The identification of metabolites or biomarkers, which are indicative of a disease, is an active area of research. These biomarkers are quantified by various forms of analytical, biochemical and spectral analysis to establish the quantitative lists or "signatures" of the metabolites for healthy control population and test subjects with specific illnesses [3]. Unique metabolomic profiles describing differences in the concentration of specific biomarkers can be used to differentiate and grade test subjects from controls. To make the analysis as predictive as possible, stringent statistical validation is carried out using multivariate mathematical methods collectively known as bioinformatics or computational biology [23-25] (**Figure 1**).



The biomarker analysis typically involves the use of following analytical technologies [26]: 1) Gas chromatography (GC), High performance liquid chromatography (HPLC) or Capillary electrophoresis (CE) for separation of the biomarkers. 2) Mass spectroscopy (MS), Nuclear magnetic resonance (NMR), Fourier transfer infrared, Raman or Near infrared spectroscopy (NIR) for identification and quantification of the biomarkers.

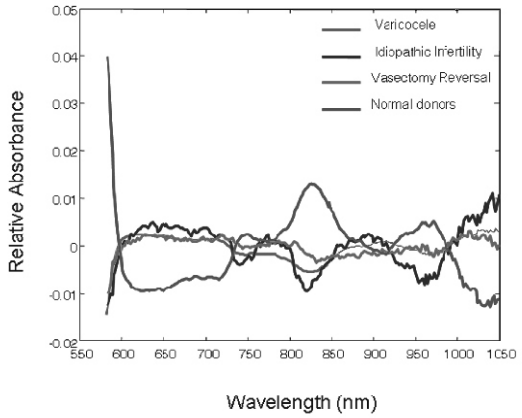
Gas Chromatography combined with Mass spectroscopy is the most widely used method in metabolomics research; it is often referred to as GCMS [27]. However, the Metabolomics Study Group for Assisted Reproductive Techniques has focused on "biospectroscopy based metabolomics," which is the application of different forms of spectral analysis in human biology to identify, quantify and validate proteomic and metabolomic biomarkers [13]. Raman and NIR spectroscopy has been utilized because they have several added advantages: a) sample preparation is not necessary, and analysis does not destroy specimens; b) they are less time consuming because they analyze multiple biomarkers simultaneously; c) lesser chemical bias; and d) they are less expensive [28].

Instrument Prototype : A disposable sample cell containing 5-10 μL of specimen is inserted into a fixed position in the light path within the instrument. The stable light source passes light into the sample, which then travels through a wavelength filter to select a specified wavelength for the analysis. A light-detecting diode captures the photons of energy emerging from the filter and delivers them to the bioinformatics microprocessor chip. Here, biomarker profiles are calculated using proprietary and non-proprietary chemometrics and bioinformatics to determine the probability of normal cellular function vs. the presence of a pathological state of cellular activity. The total analysis time is approximately 1 minute. The instrument can be interfaced with any computer system to download data to patient files (**Figure 2**).



Role of Metabolomics In The Screening of Infertile Men

One of our recent studies that was presented at the 2006 American Society for Reproductive Medicine (ASRM) demonstrated a potential role of metabolomic profiling of biomarkers of OS as a diagnostic tool to evaluate semen function and quality [13]. In this study, we collected seminal plasma from a group of patients seeking infertility evaluation and a group of healthy donors. The subjects were divided into 4 different groups: idiopathic male infertility (n = 15); varicocele (n = 70); vasectomy reversal (n = 9); and healthy donors (n = 30). The semen specimens from all 4 groups showed unique spectral signatures that were statistically different from one another, indicating differences in the concentrations biomarkers of OS (–CH, -NH, –OH and ROH). The –CH to ROH content, which is reflective of the OS status, was also found out to be different on quantification of each metabolomic profile using direct exponential curve resolution algorithm and logistic regression analysis. The healthy donors and vasectomy reversal groups were well defined within the self organized map. However, the profile from the varicocele patients was found to be broadly distributed among all the groups and did not segregate as a separate population with uniquely identifiable biomarker characteristics. The idiopathic male infertility patients were represented as two regions in the map (**Figure 3**).

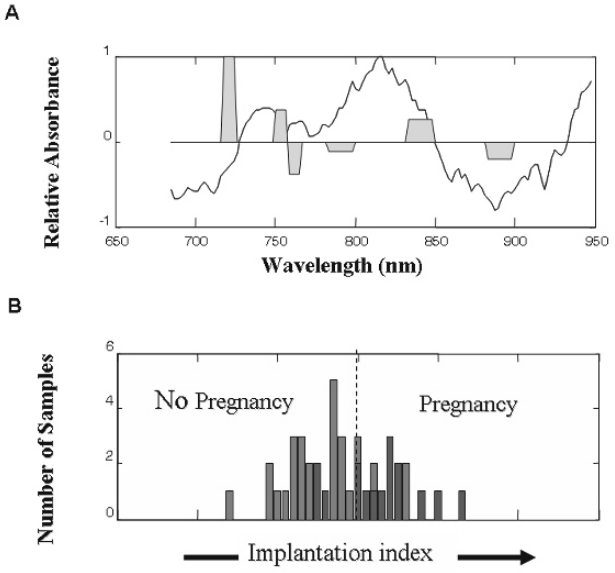


This study revealed that different levels of OS biomarkers are uniquely associated with semen plasma of normal men compared to different forms of male factor infertility**.** The total analysis time per sample was approximately 1 minute, and only10μL of seminal plasma was required to perform the analysis. The sensitivity and specificity of the different spectroscopic measurements reproducibly exceeded 80%. Hence, metabolomic profiling of semen, using NIR spectroscopy and proprietary chemometrics and bioinformatics, may provide a rapid, non-invasive and cost-effective diagnostic method of analyzing semen for abnormalities related to ROS damage and OS. The ability to quantify differences in the metabolomic profiles of various types of male infertility patients could prove as a diagnostic tool to evaluate semen quality and function.

Metabolomics and Assisted Reproduction

Assisted Reproductive Techniques (ART) is one of the most important treatment options available to infertile couples diagnosed with male factor infertility. A negative correlation has been observed between the ROS levels in follicular fluid and embryo culture media and ART outcomes [17]. The recent introduction of metabolomics in the field of ART might help predict pregnancy outcomes and improve its success rates. Studies have been conducted to correlate the levels of various biomarkers present in the follicular fluid and the embryo culture media with pregnancy outcomes using metabolomic profiling.

In a recent study, 83 follicular fluid samples were analyzed for biomarkers of OS at specific wavelengths using NMR, Raman and NIR spectroscopy. The metabolomic profiles were quantified using proprietary chemometrics, and bioinformatics and were correlated to the pregnancy outcome. The CH-to-ROH content in the follicular fluid was different between the pregnant and non-pregnant groups. Spectral regions obtained from all spectroscopic measurements revealed that an association existed between the OS biomarkers and pregnancy outcome with a sensitivity and specificity of 80 % [29] (**Figure 4**).



The study demonstrated for the first time the potential role of metabolomics in oocyte selection for ART procedures. Further studies are needed to confirm these observations.

A study presented at the 2006 ASRM explored the role of metabolomics in embryo selection. A total of 35 embryos were evaluated. The spent media were collected on day 3 cleavage stage after 44 hours of embryo culture and evaluated using NMR and NIR. Changes in the hydroxyl modifications of various molecular constituents in the media were analyzed using the selective genetic algorithm; and a viability score was obtained for each sample based on its unique spectral profiles. A significant difference was found in the spectral profiles between the embryos that had the capability of implantation (and thus were reproductively competent) and the incompetent embryos [14]. A prospective multi-center trial was also presented at the ASRM 2006 involving two academic centers and a private ART center. In this study, the day 3 culture media of 108 embryos were collected and analyzed using NMR and NIR. The spectral profiles revealed that the concentrations of –CH, -NH, -OH, and ROH in the culture media of embryos that resulted in pregnancy were different than the concentrations of the culture media from the embryos that did not. The ratio of –CH to ROH was also different between the two groups. Results from the logistic regression analysis using Raman spectroscopy demonstrated a sensitivity of 95% and specificity of 80% whereas NIR resulted in a 73% sensitivity and 83% specificity [15]. Another study found unique metabolomic profiles of OS biomarkers in the discarded culture media (N=228) of day 3 and day 5 embryos resulting in pregnancy and those that did not. These observations were consistent with all the methods of spectroscopy used (NMR, NIR, and Raman), and the spectra from day 3 embryos were significantly different from that of the day 5 embryos (unpublished data).

Houghton et al investigated the amino acid turnover of embryo culture media by measuring the physiological mixture of 18 amino acids in the in-vitro culture media using high performance liquid chromatography. They observed that the amino acid patterns of the embryos developing into the blastocyst stage exhibited a pattern distinct from the growth-arrested embryos despite having similar morphological appearances [9]. Lopes et al observed oxygen consumption of bovine embryos and discussed the use of embryonic respiration rate for the assessment of embryo quality [10].

Thus far, these preliminary studies have demonstrated the usefulness of non-invasive metabolomic profiling of biomarkers to select viable embryos for ART.

Expert Opinion

The purpose of this article is to discuss the potential role of metabolomic profiling of biomarkers in the field of reproductive health. The tests available for analyzing semen quality are deficient and currently no consistent methodologies are available that can assist in gamete and embryo selection to ensure efficacy and safety of the IVF procedure. A number of recent studies have demonstrated the potential role of metabolomics in rapid, non-invasive testing of semen in infertile men; and oocyte and embryo selection for ART procedures.

Metabolomics testing in the field of reproductive health is still in its infancy. The technique has not yet been standardized and requires further research to validate the observations of the

preliminary studies. However it is likely to lead to a) more men seeking infertility evaluation; b) enhanced success rates in ART procedures; c) reduction in the incidence of multiple births due to feasibility of single embryo transfer technique; and d) reduced health care costs associated with providing medical care to multiple premature infants. Thus, this new technology paradigm is expected to fuel extensive research in near future.

FIVE-YEAR VIEW

Given the potential role of metabolomics in the management of infertility, several trials are underway to confirm the preliminary results achieved by the earlier investigators. Currently, a multicenter, multinational study is being conducted to demonstrate the relationship between an embryo's metabolomic profile and its implantation potential. Other potential applications of metabolomics in the field of reproduction are:

1. Gamete selection: Metabolomic profiling of biomarkers of OS can be developed as a routine method for assessing sperm function in ART.
2. Functional genomic testing: Aneuploidy screening and testing for other genetic conditions responsible for male infertility is a promising application of metabolomics.
3. Endometrial receptivity: The role of metabolomics in the non-invasive examination of the endometrial lining of the uterus just prior to embryo transfer is being investigated and it is expected to increase the success rates of IVF.
4. Fetal monitoring: Metabolomic analysis of biomarkers in amniotic fluid has a potential role in assessing fetal development.

The discovery of biomarkers is also gaining a great deal of interest worldwide as alterations of metabolites are involved in many reproductive disorders, and the identification of key metabolite markers will likely lead to greater diagnostic and therapeutic interventions. However, there are a number of challenges [3, 30] in developing the science of metabolomics:

1. Active metabolic pathways responsible for changes in the concentrations of various metabolites need to be identified.
2. More than half of the metabolites that are detected need identification of their chemical structures. A naming protocol has been suggested by Bino et al in order to name the unknown metabolites in which the chemical nature is unknown so that they can be recognized between different laboratories [31].
3. As metabolite data are multivariate and hence complex, it is essential that the data are validated prior to being uploaded. In order for metabolomics to develop, standards must be adopted that will allow the integration of large amounts of data generated in metabolomics experiments and enhance the reproducibility and credibility of the data.

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