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A fatty acid profiling method using liquid chromatography–high resolution mass spectrometry for improvement of assisted reproductive technology

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ABSTRACT

Background: Liquid chromatography–high resolution mass spectrometry (LC–HRMS) can be useful to improve *in vitro* fertilization (IVF). This study aims to find out an association between embryonic growth and embryonic uptake of free fatty acid (FFA) from culture media by using LC–HRMS.
Methods: Embryos (n = 55) from 15 couples undergoing IVF were studied. An embryo was cultivated for up to 6 days in a 20 μl-medium drop under mineral oil, and classified by a morphological grading system into the good-growth group (n = 32; good quality blastocysts) and the poor-growth group (n = 23; non-blastocysts). The control study was set up without embryo. Extracted ion chromatogram of FFAs was collected in negative-ion mode for each medium sample obtained after use.
Results: The percent change from control to sample in mass area for docosahexaenoic acid showed a decrease in the good-growth group than that in the poor-growth group ($p < 0.05$). Decrease in %change of docosahexaenoic acid might indicate proper embryonic growth. Similar but insignificant change was observed for other essential FFAs, but not for non-essential FFAs.
Conclusion: The proposed metabolomic approach using LC–HRMS might be a powerful tool for non-invasive evaluation of embryonic growth.

1. Introduction

For separation of biological samples, gas chromatography (GC) and high-performance liquid chromatography (HPLC or LC) have been used widely. GC needs vaporization of analytes, and therefore, is applicable to only thermally stable compounds. Hence, time-consuming and laborious derivatization such as methylation is required before GC. Contrastingly, HPLC does not need derivatization in most cases. The combination of HPLC and mass spectrometry (MS), or LC/MS, is getting popular in clinical chemistry along with advance in equipment and applications of LC/MS. High-resolution mass spectrometry (HRMS) has the excellent capacity to determine molecular formulas from accurate mass measurements in complexed matrices such as serum, cell, and culture medium. The combination of LC and HRMS, or LC–HRMS, can provide highly sensitive, qualitative, comprehensive, and semi-quantitative analysis in one run of a wide range of analytes.

LC–HRMS can be a powerful tool to improve assisted reproductive technology, where a sensitive method for accurate and comprehensive information of embryonic growth is needed. This study aims to find out an association between the growth of human embryo and the change in free fatty acid (FFA) in the embryonic culture medium obtained in *in vitro* fertilization (IVF). Since the birth of the world's first "test tube" baby in Britain in 1978 [1], assisted reproductive technology has brought great benefits to the patient with infertility. Currently, morphological embryo grading methods are used commonly in IVF, whereas the outcomes of these methods are not satisfactory [2]. The poor accuracy and precision were pointed out for these methods by experienced embryologists [3,4].

Embryologists' selection of a single embryo of the best quality will meet a difficulty when several embryos show similar scores in a morphological scoring system. In that case, embryologists need a biomarker that can provide complementary biochemical information relevant to embryonic growth. The sample used for the measurement of biomarker must be obtained without a risk for injury to the embryo. Hence, the medium after use in the embryonic culture and otherwise to be discarded can be the best specimen. To date, several potential biomarkers in the used culture medium have been reported. They include pyruvate, glucose, lactate metabolism by microfluorometry or ^{1}H-nuclear magnetic resonance (^{1}H-NMR) [5–10], amino acid analysis by

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fluorescence labeling HPLC or ^{1}H-NMR [11,12], metabolomics by Raman spectroscopy or near-infrared spectroscopy (NIRS) [13–16]. Several other potential biomarkers and techniques have been reported [17]. However, no report adopting LC–HRMS can be found in the literature to the best of our knowledge. In this study, we applied LC-HRMS to a metabolomic approach on FFAs in the culture medium after use in cultivation of human embryo in IVF.

2. Materials and methods

2.1. Ethical consideration

Informed consents were obtained from the couples for participating in the research on the analysis of used culture media that is otherwise to be disposed. The ethical committees of Kamiya Ladies Clinic (approval number 10) and the Faculty of Health Sciences, Hokkaido University (approval number 14–4) approved this study.

2.2. Reagents

Methanol, acetonitrile, and distilled water (LC/MS grade) were from Kanto Chemical Co., Inc. Ammonium acetate (LC/MS grade) was from Wako Pure Chemical Industry. Palmitic acid (hexadecanoic acid, FFA 16:0) and stearic acid (octadecanoic acid, FFA 18:0) were from Tokyo Chemical Industry Co., Ltd. Oleic acid ((*Z*)-octadec-9-enoic acid, FFA 18:1), linoleic acid ((9*Z*,12*Z*)-octadeca-9,12-dienoic acid, FFA 18:2), α-linolenic acid ((9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid, FFA 18:3), arachidonic acid ((5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoic acid, FFA 20:4), eicosapentaenoic acid ((5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoic acid, FFA 20:5) or EPA, and docosahexaenoic acid ((4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid, FFA 22:6) or DHA were from Sigma-Aldrich.

2.3. Equipment

Chromatographic analyses were performed with an HPLC Prominence, equipped with a binary LC-20 AD pumping system, SIL-20 A auto-sampler, and CTO-20 A column oven (Shimadzu Corpp.). High-resolution mass spectrometric analysis was performed using LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Inc.) combined with Surveyor MS pump and an auto-sampler. Electrospray ionization tandem mass spectrometry analysis was performed in negative-ion mode, and mass spectra were obtained in Fourier-transform mode.

2.4. LC/MS analysis

Eight molecular species, that is, FFAs 16:0 (palmitic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (linoleic acid), 18:3 (α-linolenic acid), 20:4 (arachidonic acid), 20:5 (EPA), and 22:6 (DHA), were studied.

Ten microliters of the prepared sample was injected into Hypersil Gold C8 column (2.1 μm × 50 mm i.d., particle size 1.9 μm; Thermo Fisher Scientific, Inc.) maintained at 40 °C. Gradient elution was performed with a mobile phase composed of 10 mmol/L aqueous ammonium acetate (Solvent A) and acetonitrile (Solvent B), at a flow rate of 0.2 mL/min. The LC gradient elution program were as follows: 0.00–2.00 min

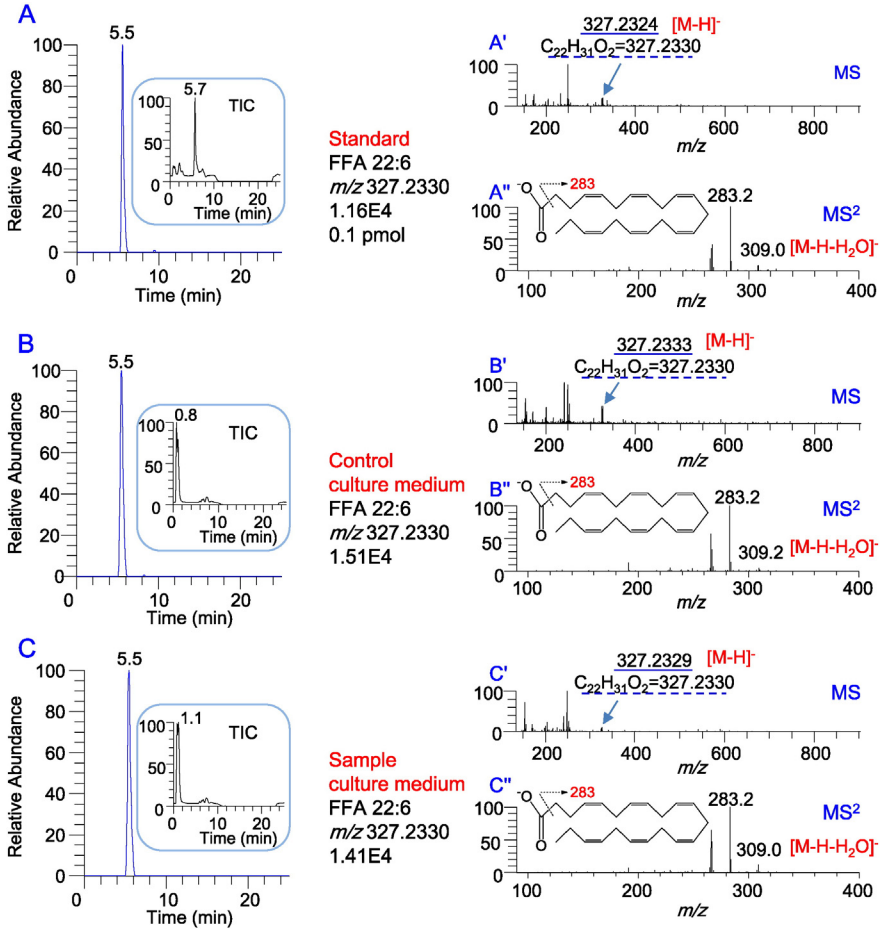

Fig. 1. LC–HRMS profiles of FFA 22:6 (DHA) in negative-ion mode. A typical TIC is shown in the inset in Fig. 1A, B, and C for the standard, a control culture medium, and a sample culture medium, respectively. Each of the EIC for *m/z* 327.2330, $[M–H]^-$, showed a single peak at RT of 5.5 min in Fig. 1A, B, and C, respectively. The direct introduction mass spectra are shown in Fig. 1A', B', and C', respectively. The MS^2 spectra are shown in Fig. 1A", B", and C", respectively.