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Original Research Article

Raman spectroscopy analysis of differences in composition of spent culture media of in vitro cultured preimplantation embryos isolated from normal and fat mice dams

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
19 January 2016
Accepted 10 February 2016
Available online 24 February 2016

Keywords:
Preimplantation embryo
Obesity
In vitro culture
Raman spectroscopy

ABSTRACT

The aim of the present study was to compare overall patterns of metabolic activity of *in vitro* cultured preimplantation embryos isolated from normal and fat mice dams by means of non-invasive profiling of spent culture media using Raman spectroscopy.

To produce females with two different types of body condition (normal and fat), a previously established two-generation model was used, based on overfeeding of experimental mice during prenatal and early postnatal development. Embryos were isolated from spontaneously ovulating and naturally fertilized dams at the 2-cell stage of development and cultured to the blastocyst stage in synthetic oviductal medium KSOMaa. Embryos from fat mice (displaying significantly elevated body weight and fat) showed similar developmental capabilities *in vitro* as embryos isolated from normal control dams (displaying physiological body weight and fat).

The results show that alterations in the composition of culture medium caused by the presence of developing mouse preimplantation embryos can be detected using Raman spectroscopy. Metabolic activity of embryos was reflected in evident changes in numerous band intensities in the 1620–1690 cm^{-1} (amide I) region and in the 1020–1140 cm^{-1} region of the Raman spectrum for KSOMaa. Moreover, multivariate analysis of spectral data proved that the composition of proteins and other organic compounds in spent samples obtained after the culture of embryos isolated from fat dams was different from that in spent samples obtained after the culture of embryos from control dams.

This study demonstrates that metabolic activity of cultured preimplantation embryos might depend on the body condition of their donors.

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http://dx.doi.org/10.1016/j.repbio.2016.02.002

1. Introduction

The relationship between overweight in mammals and reproductive disorders is important, but controversial issue. Some human clinical studies have demonstrated that maternal obesity can contribute to anovulation, reduced conception rate, reduced response to fertility treatment, increased risk of spontaneous miscarriage, preeclampsia and congenital developmental anomalies [1,2]; however, other studies did not find any disorders in ovarian stimulation or any differences in either the oocyte fertilization rate or the numbers of successful pregnancies when comparing obese and non-obese patients [3,4]. In rodents with diet-induced obesity, defective ovarian function, poor oocyte quality, oocyte meiotic aneuploidy, significant delay in following *in vitro* cell cycle kinetics, reduced blastocyst survival rates, reduced blastocyst cell numbers and abnormal embryonic cellular differentiation have been recorded as well [5–9]. In our previous studies we have similarly demonstrated that female mice with obesity-like phenotype produced a higher number of immature oocytes; mature oocytes isolated from such dams showed significantly decreased deposits of neutral lipids in the cytoplasm; and naturally fertilized zygotes showed moderated reduction of DNA cytosine methylation signal in parental pronuclei. On the other side, higher fertilization index was recorded in fat dams [10]. We have shown that *in vivo* development of preimplantation embryos was slowed down as well, and blastocysts produced by overweight dams revealed a higher percentage of apoptotic cells. However, total cell numbers in such blastocysts remained unaffected [11]. Our results were obtained using a standardized two-generation animal model which simulates population heterogeneity in a manner analogous to naturally reproducing mammalian subjects, and allows the effect maternal body condition on reproductive parameters to be studied while minimizing the impact of the composition of actual nutrition (both control and overweight animals are fed standard diet only during the reproductive process), as well as the impact of maternal aging and that of hormonal treatment [12].

Based on these results we hypothesized that embryos produced by fat mothers might show different secretory and metabolomic patterns compared to embryos produced by mothers with physiological body weight and fat. The aim of the present study was to test this hypothesis by non-invasive profiling of media used for the culture of the 2-cell embryos isolated from mice dams with different body conditions. The profiling was performed using Raman spectroscopy.

Raman spectroscopy is a powerful technique ideal for studying the composition of biological samples. An important advantage of Raman spectra over infrared lies in the fact that water does not cause interference, indeed, Raman spectra can be obtained from aqueous solutions. Furthermore, since localized vibrations of multiple-bonded or electron-rich groups generally produce more intense Raman bands than do vibrations of single-bonded or electron-poor groups, the Raman spectrum of a protein (or a nucleic acid) is largely dominated by bands associated with the peptide main chain, aromatic side chains, and sulfur-containing side chains [13]. This technique has been increasingly used to study the biological changes in various cells or cellular components, even in oocytes [14–17] and 2- to 4-cell embryos [18]. Metabolomic profiling of embryo culture media using Raman spectroscopy had been recently proposed as a novel method of assessing human embryo quality [19] or a rapid, non-invasive, and clinically applicable technology for prediction of reproductive potential of human and bovine *in vitro* fertilized embryos [20–22]. In our study, Raman spectroscopic profiling of chemically-defined mouse embryo culture media was performed for the first time.

2. Materials and methods

2.1. *Animals and experimental design*

Experiments were performed on mice of the outbred ICR (CD-1 IGS) strain (Velaz, Prague, Czech Republic). To produce females with various types of body condition, a previously established two-generation model was used, based on overfeeding of experimental mice during prenatal and early postnatal development [10,11]. Briefly, fertilized mice of the parental generation were randomly divided into control and experimental groups. During the gestation period (21 days) and the lactation period (from birth to weaning: 21 days), dams in the control group were fed the standard pellet diet (M1, Ricmanice, Czech republic; 3.2 kcal/g) and dams in the experimental group were fed the standard diet with the addition of high-energy liquid product Ensure Plus (Abbot Laboratories, Netherlands, 1.5 kcal/mL) *ad libitum* [11]. After weaning, mice of the F1 generation in both groups were fed the standard diet only. On Day 34 of their age, F1 females were individually measured for their weight and scanned with EchoMRI (Whole Body Composition Analyser, Echo Medical System, Houston, TX) for the evaluation of exact amounts of body fat deposits. Females in control group were allocated into two groups: 1, normal controls with physiological body weight and body fat (7–8%) and 2, lean controls spontaneously displaying decreased body weight and body fat (<7%). Females in experimental group were classified as: 1, fat experimental mice with significantly elevated body weight and fat (>11%) and 2, experimental mice with physiological body weight and slightly elevated body fat (8–11%).

In the reproductive study, only normal controls with physiological body weight and body fat (CN) and fat experimental mice with significantly elevated body weight and fat (EXF) from the F1 generation were used. Starting at Day 35 of their age, spontaneously-ovulating female mice were mated with males of the same strain for one or more nights. Successful mating was confirmed by identification of a vaginal plug every morning. On Day 2 of pregnancy (approx. 32 h after supposed ovulation), the fertilized dams from both groups were killed by cervical dislocation and subjected to isolation of embryos at the 2-cell stage (number of donor dams: CN $n = 13$, EXF $n = 14$).

All animal experiments were reviewed and approved by the Ethical Committee for animal experimentation of the Institute of Animal Physiology, approved by the State Veterinary and Food Administration of the Slovak Republic (Ro 2296/13-221c), and were performed in accordance with Slovak legislation based on EU Directive 2010/63/EU on the