MALDI-TOF MS FINGERPRINTING OF BLOOD SERUM USED FOR RUMINANT EMBRYO *IN VITRO* PRODUCTION

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Overview

Blood serum (BS) is used as a culture medium supplement for ruminant embryo *in vitro* production (IVP). Its use must be avoided because of many reasons, including its effects on embryo lipid composition, which affect cryosensivity. This work evaluated MALDI-TOF as a simple and fast method to study the lipid composition of blood serum from domestic animal species of interest for IVP. This information may contribute with the design of synthetic culture media.

Introduction

Blood serum, commonly used for ruminant embryo *in vitro* production (IVP) presents many negative characteristics such as sanitary problems, influence on embryo development velocity, gene expression and lipid composition. The aim of this work was to use MALDI-TOF MS as a simple and fast method to study the lipid composition of bovine, sheep and goat BS, and compare the MALDI-TOF MS fingerprints with the profile of one commercially available blood serum replacement (SR) supplement for cell culture. Information of lipid composition profile of BS could be useful to observe species differences and to design synthetic serum replacements more suitable for the metabolic needs during *in vitro* embryo development.

Methods

Samples of BS from bovine fetuses (fetal calf serum –FCS; n=9), sheep BS (n=2), goat BS (n=1) used for embryo *in vitro* production and one sample of commercial SR were submitted to lipid extraction (Bligh & Dyer, 1959). Shortly, 300μL of sample, 375μL chloroform and 750μL methanol were vortexed. Next, 375μL chloroform and 300μL ultrapure water were added and the bottom phase of the solution was dried and diluted in 100μL methanol. DHB 1.0M was used as matrix. A MALDI-Q-TOF Premier mass spectrometer (Waters, Manchester, UK) equipped with a 200-Hz solid-state laser was used in the reflectron mode. Operating conditions used were in the positive ion mode, 250 a.u. (laser energy) and 10V (sample plate).

Results

Bovine FCS, sheep and goat BS and the SR presented characteristic MALDI-TOF MS fingerprints in the *m/z* range of 600-1200 (Figure 1). Ions of *m/z* 647.5 and 685.5 were detected for all 13 samples. Ions of *m/z* 652.6, 761.7, 774.6, 797.6, 807.7 were detected only for the bovine FCS samples. Ions of *m/z* 747.6 and 785.7 were detected exclusively for goat samples. Samples of SR presented a distinct MS profile characterized by a series of ions separated by 44 *m/z* units, which is indicative of polyethoxylated (C_2H_4O) compounds. Polyethoxylate compounds in SR could have been added as surfactants. Ions of *m/z* 666.5, 723.5, 725.6, 750.6, 782.6, 784.6, 786.7, 788.7, 806.6, 810.7, 812.7, 830.6, 832.7, 834.7, 836.7 were present in all samples but not in the SR. Principal component analysis (PCA; Figure 2) clearly grouped bovine samples and also separated them from sheep, goat and the SR samples. Therefore, our preliminary data on lipid profile acquired under these conditions indicates that the MALDI-TOF MS profiles are characteristic for each species. Most ions detected in the fingerprints have the same *m/z* values reported for phospholipids such as phosphocholines and phosphoethanolamines species, which are normally detected with the use of DHB as matrix in the positive ion mode. MS/MS analyses are being performed to confirm PL identities.

Conclusions

MALDI-TOF MS lipid fingerprinting is shown to provide a practical approach to study composition of BS used for embryo IVP. Knowledge of BS lipidomics will contribute to comprehend BS influence on ruminant embryo *in vitro* development, as well as with the formulation of definite synthetic replacements which could appropriately meet embryo lipid metabolic needs during *in vitro* culture and avoid BS use.

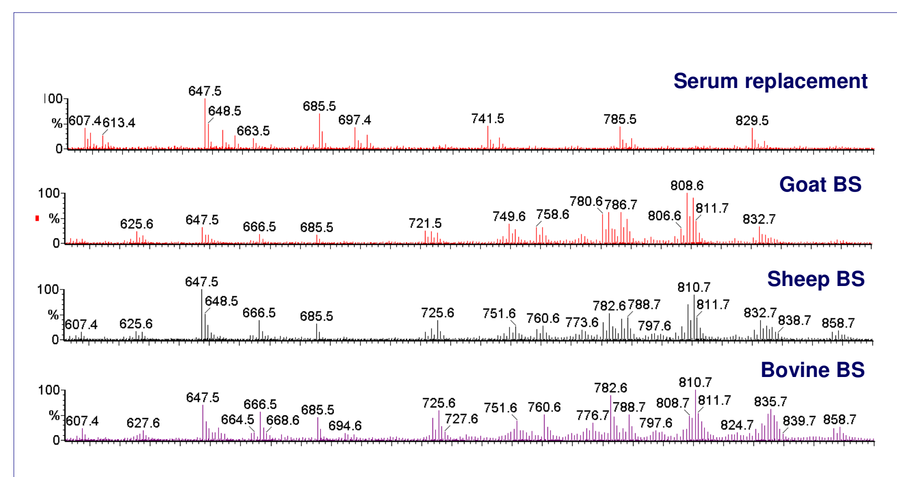

Figure 1. MALDI-TOF Spectra showing the lipid profile of serum replacement and blood serum (BS) samples from goat, sheep and bovine.

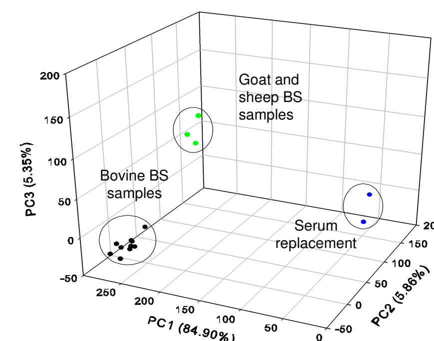

Figure 2. Principal Component Analysis showing distinct grouping of bovine BS samples, goat and sheep BS samples, and serum replacement.

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BLIGH, E. C. & DYER, W.J. 1959. A rapid method of total lipid. Extraction and Purification. **Can. J. Biochem. Physiol., 37:** 911-917.