New Approach for Chemometric Analysis of Mass Spectrometry Data

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ABSTRACT OF SUPPORT INFORMATION (SI). We included in the SI an expanded description of experimental procedures. We described in detail the in vitro fertilization (IVF) techniques and the HPLC-MS analysis with the experimental conditions. We showed the figures perform with the results of chemometric analysis using robust PCA (Figure S-1) and interval PLS (Figure S-2).
EXPANDED EXPERIMENTAL

The protocol includes pituitary desensitization with an agonist and ovarian stimulation with a combination of recombinant human FSH and hMG. Ovarian response was monitored by transvaginal ultrasound (TVUS) and E2 concentration. Ovulation was induced with 250 mg of recombinant hCG. Under sedation, oocytes were aspirated by a TVUS-guided needle 36 hours after hCG administration. On day 1, embryos fertilized with two pronuclei were placed into individual 50-μL drops of CM (three days culture medium) culture medium (Cook Medical), under standard incubation conditions to the cleavage stage and then transferred into the patients on day 3 or transferred into 50-μL drops of CCM (five days culture medium) culture medium (Vitrolife, Goteborg, Sweden). After the embryos were transferred, the culture medium was obtained from the clinic and used in the analysis.

The LC-ESI-MS-MS analysis was performed with the Agilent (Santa Clara, CA) 1100 series HPLC instrument. The LC system was coupled with the Agilent 1100 Series LC/MSD Trap SL, with the possibility of carrying out MS/MS analysis. The mass spectrometer was operated in the positive ESI mode, and the ion spray voltage was set at 4 kV. Nitrogen was used as the sheath gas (30 psi), and the ion transfer capillary was heated to 350 C. Injections were carried out using an HTC Pal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 20 μL sample loop. The culture medium solutions were infused into the flow of the HPLC system (10 μL) through a T connection, under the following conditions: flow rate of the HPLC system, 0.3 mL/ min (30 mM ammonium acetate and 5% acetic acid at a ratio of 12.5:87.5, pH 2.5, solvent A). The LC separations were carried out with Phenomenex (Torrance, CA) Luna 5μ SCX 100 Å column, 150 mm X 2.0 mm internal diameter, at 25 °C. For the elution of
the metabolites, an isocratic step was programmed with solvent A for 15 minutes. The overall flow rate was adjusted to 0.3 mL/min. Before use, the new SCX column was flushed overnight with 150 mM of ammonium acetate solution.
**Figure S-1.** Figure caption. Scores (figure top) and loadings of PC1 (figure bottom) plots for robPCA of human embryo culture media. In loadings plot (black line) it also shows the 2D pseudo-spectra from CM (red line) and CCM medium sample (blue line).
Figure S-2. Figure caption. The selected interval 55 in the iPLS model (figure top) and the predicted vs. measured data for this model (figure bottom). The insert in the figure top was the interval 55 zoom.