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World Congress on
Embryology and In vitro Fertilization
November 02-03, 2017 | Chicago, USA

Non-invasive mass spectrometric viability assessment of *in vitro* fertilized embryos using the alpha-1 chain of human haptoglobin

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Background: Viability assessment prior embryo transfer is a crucial question since the success rate of IVF experiments is below expectations (approx. 30%). The practice of multiple embryo transfer to overcome this limitation of IVF is sometimes accompanied by multiple gestation adding several new risk factors. The leading consensus is that the best possible option is the practice of single embryo transfer. The adoption of this policy however requires the best tools to predict the embryo's implantation potential during the first couple of days of development. In parallel with the use of the routinely used morphology based viability assessment assay known as the Istanbul Consensus Scoring System (ICCS), huge effort is made worldwide to find new markers of embryo viability, preferably in a non-invasive way due to ethical issues. The search for markers of embryo viability in the embryo culture medium seems to be an ideal approach. The aim of our work was to find any biomarker present in the embryo culture medium using mass spectrometry, which would qualitatively or quantitatively differ in the samples of viable and non-viable embryos, and help predicting implantation potential.

Methods: Spent embryo culture medium samples (n=201) were measured in a series of retrospective, blind experiments, all were suitable for transferation according to the ICCS. No sample preparation was made, 15 µl of sample was directly injected into the instrument after the addition of internal standard solution. A Dionex Ultimate 3000 (Dionex Corp., USA) analytical HPLC equipped with an autosampler and a column thermostat set at 30ºC was used. Separation was carried out on a Kinetex C18 2.6 µm, 2.1 x 100 mm analytical column (Phenomenex, USA) with a multi-step gradient elution at a flow rate of 200 µL/min. The mass spectrometer coupled was a Bruker micrOTOF accurate mass instrument (Bruker Daltonik, Germany) equipped with an electrospray ionization source (ESI) operated in the positive ion mode.

Results: A protein marker was found which significantly ($p<0.001$) differed in quantity between the samples of embryos which did (clinical pregnancy), or did not (no pregnancy) implant. Respective lots of unconditioned culture media were used as controls. Deconvolution of the obtained mass spectra revealed that this protein has a molecular mass of 9186.4 Da. The protein was identified using tandem mass spectrometry as the alpha-1 chain (HptA1) of the human haptoglobin (Hpt) molecule. It was observed that Hpt was present as a contaminant in the purified human serum albumin used to supplement the culture medium therefore it is not secreted by the embryo. A significant correlation ($p<0.001$) was found when comparing the clinical outcome (clinical pregnancy or no pregnancy) and the amount of HptA1. The positive predictive value (PPV) of the biochemical analysis was 51.2% while the negative predictive value (NPV) was 100%. On the current material the ICCS assay performed a PPV of 31.3%.

Discussion: The increased amount of HptA1 in the media of non-viable embryos compared viable embryos is caused by the increased reduction of the intramolecular disulphide bonds within Hpt. This reaction (leading to HptA1 liberation) is catalyzed in a higher extent by embryos with failed pregnancy outcome. The biochemical evaluation can select embryos having good morphological aspects but low implantation potential due to visually (microscopically) unnoticeable reasons. The increased PPV observed (51.2% vs. 31.3%) is due to the fact that the mass spectrometric analysis theoretically decreased the number of false positive cases of ICCS by 40% (n=78). Since the assay has an NPV of 100% these 40% were all true negative cases. The results suggest a possible contra selection tool, screening the embryos with good morphological aspects, but no implantation potential.

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