



in-Vitro Fertilization Growth Media Proteins in Competent Embryos

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Background and Significance

In the most recent Society of Assisted Reproductive Technology 2005 review (https://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?ClinicPKID=0), 37%, 29%, 20%, and 11% of IVF cycles in women <35, 35-37, 38-40, and 41-42 years, respectively, result in a live birth.¹ At the NYU Fertility Center, 48%, 38%, 28%, and 12% of cycles in women <35, 35-37, 38-40, and 41-42 years, respectively, result in a live birth.² Furthermore, 40% of these births in women <35 years at the NYU Fertility Center are twin deliveries. One of the greatest criticisms of assisted reproductive technologies (ART) is the high incidence of multiple and high order multiple gestations. Since 1980, there has been an over 80% increase in twins and 500% increase in triplet and higher order births. It is well established that fetuses and mothers of multiple and high-order multiple pregnancies are faced with increased morbidity and mortality.

Grifo et al recently described their clinic's progression to blastocyst transfer as a means to reduce the high-order multiple rate.¹ The ART community has addressed the need for more single embryo transfers (SET) but also recognizes the lowered pregnancy rates that may ensue. The ability to identify additional markers associated with embryo viability and competence has been the greatest challenge towards promoting SET. In a recent study of 3 and 5 day growth media, we have identified gi|223976 haptoglobin Hp2, mass 41717. ², Two more proteins were identified in our latest report, gi|90108928 1 Chain H, Orally Available Factor7a Inhibitor, mass 28582, and gi|119573737 hCG1793647 [Homo sapiens]Mass: 6112.3

Materials and Methods

The IVF growth media protein from 3 and 5 day embryos was extracted with organic solvent and high pressure using ProteoSolve and the Barycycler respectively (Pressure BioSciences, West Bridgewater, MA). The protein fraction was trypsinized, and the peptides studied with LCMS (Hitachi NanoFrontier nLC, Dallas, TX).

Results

The combination of high pressure protein extraction with an organic solvent and LCMS significantly increased the yield of peptides obtained from the growth media digest and identification of heretofore unidentified proteins in competent embryos. Table 1

Discussion

The combination of high pressure protein extraction with an organic solvent and LCMS increased the yield of peptides, and improved subsequent NCBI nr data base identification of proteins. These proteins almost certainly represent biomarkers of competent embryos

Table 1 Both competent embryos have the exact same proteins identified from the growth media fluid of 5 day embryos. Both embryos represent a single live birth from a single transferred embryo. The only protein common to all four to all four embryos was the albumin in the growth media. The other two proteins in sample 27 may represent apoptotic proteins in a degenerating embryo

Sample 17 Competent embryo

Serum Albumin Precursor
Gamma-aminobutyric-acid receptor subunit
Tetratricopeptide repeat protein 9

Sample 21 Competent embryo

HSA
Gamma-aminobutyric-acid receptor subunit
Tetratricopeptide repeat protein 9

Sample 27 Non-Competent embryo

HSA
Grainyhead-like protein 2 homolog
Myosin-6

Sample 29 Non-Competent embryo

HSA

Conclusion

These identified proteins probably represent new biomarkers of competent embryos. This should enhance embryo selection and result in more live births from single embryo transfers.

REFERENCES

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