

Embrió életképesség vizsgálata a tápoldatban található peptid biomarkerek segítségével

Montskó Gergely^{1,2}, Zrínyi Zita^{1,2}, Janáky Tamás³, Szabó Zoltán³,
Várnagy Ákos⁴, Kovács L. Gábor^{1,2}, Bódis József⁴

¹ Pécsi Tudományegyetem, Laboratóriumi Medicina Intézet

² Pécsi Tudományegyetem, Szentágothai János Kutatóközpont

³ Szegedi Tudományegyetem, Orvosi Vegytani Intézet

⁴ Pécsi Tudományegyetem, Szülészeti és Nőgyógyászati Klinika

Összefoglalás

A fejlett országokban végbemenő negatív demográfiai változások, amelyek a társadalmak elöregedéséhez vezetnek, valamint a születések számának csökkenése az asszisztált reprodukciós technikák nagyobb hatékonyságát követelnék meg. Azonban az ezen módszerek segítségével elért születések aránya nem több, mint az embrióbeültetések egyharmada. Alternatív megoldásként merül fel az embriót körülvevő tápoldatkörnyezet molekuláris szintű vizsgálata, mely során olyan markereket keresünk, melyek lehetővé teszik az embriók új szempontú, nem invazív minőségi értékelését. Folyadékkromatográfiával kapcsolt tömegspektrometria segítségével vizsgáltunk meg kilencven tápoldat mintát (életképes és nem életképes embriók), mely során azonosítottunk egy 9 kDa tömegű fehérjét, amely mint mennyiségi marker szignifikáns eltérést mutatott az életképes és a nem életképes embriók mintái között. Módszerünk újdonsága, hogy méréseinket vakon, valós körülmények között végeztük 100%-os biztonsággal kiszűrve az életképtelen embriókat.

Kulcsszavak: in vitro fertilizáció, vak klinikai vizsgálat, embrió életképesség, biomarker, tömegspektrometria

Embryo viability assessment using biomarkers of the culture medium

Summary

Negative demographic changes and the decreasing number of births in developed countries require the improvement of embryo viability assessment techniques. The success rate of *in vitro* fertilization however is not more than 30%. As an alternative, the search for biomarkers in the culture medium might serve to determine embryo viability using an approach hopefully more successful than the routinely used microscopic assay. In our research liquid chromatography coupled mass spectrometry was used in the search for possible biomarkers. We have found a 9kDa polypeptide which significantly differed in quantity between the samples of the viable and non-viable embryos. The novelty of our work is that our measurements were done in a blind way under real clinical circumstances. Using our assay the identification of non-viable embryos is possible with 100% confidence.

Keywords: in vitro fertilization, blind assay, embryo viability, biomarker, mass spectrometry

Irodalom:

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* **1978**;8085:366.
2. Seli E, Sakkas D, Scott R, Kwok SC, Rosendahl SM, Burns DH. Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization. *Fertil Steril* **2007**;88:1350-7.
3. de Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V et. al. European IVF-Monitoring (EIM); Consortium for the European Society on Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE. *Hum Reprod* **2012**;27:954-66.
4. Ferraretti AP, Goossens V, de Mouzon J, Bhattacharya S, Castilla JA, Korsak V et. al. European IVF-monitoring (EIM); Consortium for European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2008: results generated from European registers by ESHRE. *Hum Reprod* **2012**;27:2571-84.
5. Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA et. al. European IVF-monitoring (EIM); Consortium, for The European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. *Hum Reprod* **2013**;28:2318-31.
6. Corcoran D, Fair T, Lonergan P. Predicting embryo quality: mRNA expression and the preimplantation embryo. *Reprod Biomed Online* **2005**;11:340-8.
7. Lane M, Gardner DK. Embryo culture medium: which is the best? *Best Pract Res Clin Obstet Gynaecol* **2007**;21:83-100.
8. Jones GM, Trounson AO, Vella PJ, Thouas GA, Lolatgis N, Wood C. Glucose metabolism of human morula and blastocyst-stage embryos and its relationship to viability after transfer. *Reprod Biomed Online* **2001**;3:124-32.
9. Hardy K, Stark J, Winston RM. Maintenance of the inner cell mass in human blastocysts from fragmented embryos. *Biol Reprod* **2003**;68:1165-9.
10. Fancsovits P, Toth L, Takacs ZF, Murber A, Papp Z, Urbancsek J. Early pronuclear breakdown is a good indicator of embryo quality and viability. *Fertil Steril* **2005**;84:881-7.
11. Dawson KJ, Conaghan J, Ostera GR, Winston RM, Hardy K. Delaying transfer to the third day post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. *Hum Reprod* **1995**;10:177-82.
12. Scott R, Seli E, Miller K, Sakkas D, Scott K, Burns DH. Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. *Fertil Steril* **2008**;90:77-83.
13. Kovalevsky G, Patrizio P. High rates of embryo wastage with use of assisted reproductive technology: a look at the trends between 1995 and 2001 in the United States. *Fertil Steril* **2005**;84:325-30.
14. Nel-Themaat L, Nagy ZP. A review of the promises and pitfalls of oocyte and embryo metabolomics. *Placenta* **2011**;32(Suppl 3):257S-63S.
15. Botros L, Sakkas D, Seli E. Metabolomics and its application for non-invasive embryo assessment in IVF. *Mol Hum Reprod* **2008**;14:679-90.

16. Nagy ZP, Sakkas D, Behr B. Symposium: innovative techniques in human embryo viability assessment. Non-invasive assessment of embryo viability by metabolomic profiling of culture media ('metabolomics'). *Reprod Biomed Online* **2008**;17:502-7.
17. Nagy ZP, Jones-Colon S, Roos P, Botros L, Greco E, Dasig J et. al. Metabolomic assessment of oocyte viability. *Reprod Biomed Online* **2009**;18:219-25.
18. Sela R, Samuelov L, Almog B, Schwartz T, Cohen T, Amit A et. al. An embryo cleavage pattern based on the relative blastomere size as a function of cell number for predicting implantation outcome. *Fertil Steril* **2012**;98:650-56.
19. Ng ST, Chang TH, Wu TC. Prediction of the rates of fertilization, cleavage, and pregnancy success by cumulus-coronal morphology in an in vitro fertilization program. *Fertil Steril* **1999**;72:412-7.
20. Boiso I, Veiga A, Edwards RG. Fundamentals of human embryonic growth in vitro and the selection of high-quality embryos for transfer. *Reprod Biomed Online* **2002**;5:328-50.
21. Magli MC, Gianaroli L, Ferraretti AP, Lappi M, Ruberti A, Farfalli V. Embryo morphology and development are dependent on the chromosomal complement. *Fertil Steril* **2007**;87:534-41.
22. Cortezzi SS, Cabral EC, Trevisan MG, Ferreira CR, Setti AS, Braga DP et. al. Prediction of embryo implantation potential by mass spectrometry fingerprinting of the culture medium. *Reproduction* **2013**;145:453-62.
23. Wrenzycki C, Herrmann D, Niemann H. Messenger RNA in oocytes and embryos in relation to embryo viability. *Theriogenology* **2007**;68(Suppl 1):77S-83S.
24. Biase FH, Martelli L, Puga R, Giuliatti S, Santos-Biase WK, Fonseca Merighe GK et. al. Messenger RNA expression of Pabpn1 and Mbd3l2 genes in oocytes and cleavage embryos. *Fertil Steril* **2010**;93:2507-12.
25. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril* **2012**;97:870-5.
26. Singh R, Sinclair KD. Metabolomics: approaches to assessing oocyte and embryo quality. *Theriogenology* **2007**;68(Suppl 1):56S-62S.
27. Bromer JG, Seli E. Assessment of embryo viability in assisted reproductive technology: shortcomings of current approaches and the emerging role of metabolomics. *Curr Opin Obstet Gynecol* **2008**;20:234-41.
28. Devreker F, Hardy K, Van den Bergh M, Winston J, Biramane J, Englert Y. Noninvasive assessment of glucose and pyruvate uptake by human embryos after intracytoplasmic sperm injection and during the formation of pronuclei. *Fertil Steril* **2000**;73:947-54.
29. Gardner DK, Lane M, Stevens J, Schoolcraft WB. Noninvasive assessment of human embryo nutrient consumption as a measure of developmental potential. *Fertil Steril* **2001**;76:1175-80.
30. Sturmey RG, Brison DR, Leese HJ. Symposium: innovative techniques in human embryo viability assessment. Assessing embryo viability by measurement of amino acid turnover. *Reprod Biomed Online* **2008**;17:486-96.
31. Gada RP, Daftary GS, Walker DL, Lacey JM, Matern D, Morbeck DE. Potential of inner cell mass outgrowth and amino acid turnover as markers of quality in the in vitro fertilization laboratory. *Fertil Steril* **2012**;98:863-9.
32. Gardner DK, Wale PL. Analysis of metabolism to select viable human embryos for transfer. *Fertil Steril* **2013**;99:1062-72.

33. Shi CZ, Collins HW, Garside WT, Buettger CW, Matschinsky FM, Heyner S. Protein databases for compacted eight-cell and blastocyst-stage mouse embryos. *Mol Reprod Dev* **1994**;37:34-47.
34. Vergouw CG, Botros LL, Judge K, Henson M, Roos P, Kosteljik EH et. al. Non-invasive viability assessment of day-4 frozen-thawed human embryos using near infrared spectroscopy. *Reprod Biomed Online* **2011**;23:769-76.
35. Katz-Jaffe MG, Linck DW, Schoolcraft WB, Gardner DK. A proteomic analysis of mammalian preimplantation embryonic development. *Reproduction* **2005**;130:899-905.
36. Katz-Jaffe MG, Gardner DK, Schoolcraft WB. Proteomic analysis of individual human embryos to identify novel biomarkers of development and viability. *Fertil Steril* **2006**;85:101-7.
37. Hachey DL, Chaurand P. Proteomics in reproductive medicine: The technology for separation and identification of proteins. *J Reprod Immunol* **2004**;63:61-73.
38. Katz-Jaffe MG, McReynolds S. Embryology in the era of proteomics. *Fertil Steril* **2013**;99:1073-7.
39. Upadhyay RD, Balasinor NH, Kumar AV, Sachdeva G, Parte P, Dumasia K. Proteomics in reproductive biology: Beacon for unraveling the molecular complexities. *Biochim Biophys Acta* **2013**;1834:8-15.
40. Wright PC, Noirel J, Ow SY, Fazeli A. A review of current proteomics technologies with a survey on their widespread use in reproductive biology investigations. *Theriogenology*. **2012**;77:738-65.
41. Brill A, Torchinsky A, Carp H, Toder V. The role of apoptosis in normal and abnormal embryonic development. *J Assist Reprod Genet* **1999**;16:512-9.
42. Darcel CL, Kaldy MS. Further evidence for the heterogeneity of serum albumin. *Comp Biochem Physiol B* **1986**;85:15-22.