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Proteomics in human reproduction

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Abstract:

Assisted reproduction techniques (ART) have significantly advanced since the first successful *in vitro* fertilization (IVF). However, most *in vitro*–produced embryos fail to implant. Key steps in ART are the correct infertility diagnosis, in order to manage the individual treatments, and the assessment of gamete and embryo viability, to identify the embryo with the best implantation potential. Non-invasive approaches to correctly diagnose infertility and to access embryo development potential have the advantages of increasing the knowledge of embryo physiology, therefore allowing the development of methods to predict developmental competence and viability. These approaches include proteomic profiling. This article presents a brief review of proteomics in ART and raises the question of whether proteomics is a good alternative for the future of ART.

Keywords: *Proteomics, oocyte, embryo quality, male infertility.*

1. INTRODUCTION

Proteomics is a new frontier of the research in the field of human reproduction that is evolving very fast and provides the opportunity to elucidate complex biological systems, including fertilization, embryo implantation, and pregnancy [1].

Indeed, the large-scale identification of proteins and the study of their interactions may lead to the complete characterization of biological mechanisms [2] and may allow the identification of valuable biomarkers of gamete quality and embryo development [3].

Mass spectrometry–based proteomics has become the method of choice for the analysis of complex protein samples in which the identification of peptides is based on the ionization process. Each mass/ionic charge (m/z) peak is compared to a database with all known proteins. The proteomic screening must be preceded by analytical protein separation, whose resolution can simplify the identification of specific proteins [4].

2. PROTEOMIC AND OOCYTE

Follicular fluid is an ideal substrate in which to perform a proteomic analysis because it consists of a huge amount of proteins with significant roles in oocyte growth and development. Besides that, in ART procedures, it is possible to collect a high amount of this biological fluid [3].

The elucidation of its protein composition can identify reliable, valid biomarkers of ART outcomes. The comparison of plasma and follicular fluid in samples of patients undergoing IVF cycles may point to proteins related to follicle permeability during the oocyte maturation process [5].

Angelucci et al. [6] identified in follicular fluid a high concentration of proteins involved in the inflammatory process and antioxidant enzymes expressed in the follicle only after its complete maturation.

Other authors have analyzed follicular fluid's proteomic profile. Twigt et al. [7] have described the proteomic profiles of 246 patients with coagulation disorders and anti-inflammatory reactions associated with a follicle maturation deficiency. Jarkovska et al. [8] detected 43 proteins exclusively expressed in patients who presented hyper-stimulation. In another study, eight proteins were identified as biomarkers of hyper-stimulation [9].

Finally, 11 proteins were differentially expressed in patients with positive responses to IVF when compared with those that did not achieve pregnancy [10].

3. PROTEOMICS AND MALE INFERTILITY

The investigation of male infertility is mainly based on the analysis of seminal parameters (sperm concentration, motility and morphology) [11], hormonal profile (Testosterone, FSH, LH, etc.), testicular ultrasound analysis, genetic testing

(karyotype, Y chromosome microdeletion), and other sperm function tests (DNA fragmentation, acrosomal integrity, mitochondrial activity) [12-14]. However, this approach is not completely informative about the cause of male infertility. Therefore, new tests are needed to complete male infertility diagnosis and lead to conceptions. In this matter, the proteomic analysis of male fertility has become increasingly important [15].

More than 6,198 proteins present in human semen have been identified to be involved in the most diverse functional processes of spermatozoa [16]. These proteins are basically divided into two groups: regulatory proteins and structural proteins [17]. Proteins such as semenogelin 1 and 2, olfactory receptor 5R1, lactoferrin, hCAP18, spindling, and clusterin appear to be linked to male fertility; therefore, they are potential biological markers [18].

Studies on testicular tissue's proteomic profile have found a wide range of proteins; however, fewer than 200 of these proteins are common among these studies. This indicates that experimental variation can generate different sets of data, which impairs the use of these findings [19-21]. In 2011, Li et al. [20] showed that heterogeneous nuclear ribonucleoprotein L (HnRNPL) was under-expressed in the testes of patients with Sertoli Cell Only Syndrome, revealing the probable importance of this protein as a regulator of the growth and apoptosis of the spermatogonia. There is still great difficulty in analyzing testicular material, since many proteins are only expressed or activated at more advanced stages of spermatogenesis, besides the difficulty of obtaining testicular material and processing it [22]. Thus, most studies have used seminal plasma or the supernatant resulting from a centrifugation to perform the analysis [23].

Asthenozoospermic men appear to have reduced expression of the heat shock protein (HSPs) when compared to normozoospermic men, suggesting that this protein plays an important role in egg-sperm interaction [24, 25]. A total of 101 proteins were differentially expressed in asthenozoospermic patients when compared to healthy donors. Overexpressed proteins were fructose-bisphosphate-aldolase A, glyceraldehyde-3-phosphate dehydrogenase, legumain precursor, and epididymal-secretory protein E4, while DJ-1, which seems to be related to the reduction of oxidative stress, was under-expressed [25, 26]. Sperm motility was also correlated with the expression of proteasome alpha 3 [27].

Proteomics may aid the diagnosis of azoospermias (obstructive – OA – vs. non-obstructive – NOA). Studies have identified different protein expression when fertile patients have been compared with OA and NOA patients [28, 29]. The ECM1 expressed in the epididymis and the TEX101 expressed in the testis appear to be highly sensitive and specific markers to differentiate OA patients from NOA patients [30].

Azoospermic patients show an overexpression of prostatic acid phosphatase when compared to normozoospermic, asthenozoospermic, and oligozoospermic patient groups; in addition, other proteins are differently expressed in such patients,

including fibronectin, proteasome subunit alpha type-3, beta-2-microglobulin, galectin -3-binding protein, prolactin-inducible protein, and cytosolic nonspecific dipeptidase [31].

Concerning the use of proteomics as a predictive tool for pregnancy outcome among normozoospermic patients, previous studies have presented different findings. The A2LD1, ATP1B3, and FBXO2 proteins were differentially expressed when patients who achieved fatherwood were compared with those who did not [32]. Azpiazu et al. found 66 other proteins differently expressed among these groups, and the relationship of these proteins with lipoprotein metabolism and chromatin assembly may explain failure to conceive [33].

Proteomic analysis has also been associated with other seminal parameters, such as mitochondrial activity (annexin A7, endoplasmic reticulum resident protein 44, and glutathione S-transferase Mu3), acrosomal integrity (phospholipid transfer protein), and DNA fragmentation (cysteine-rich secretory proteins, retinoic acid receptor responder protein1, proteasome subunit alpha type-5) [34].

Not only the expression level of the proteins but also the post-translational modifications are important for the fertilization mechanism. One example is phosphorylation, which changes proteins' structures and appears to be strongly linked to the process of sperm capacitation [35-37]. Acetylation of lysines also appears to play a key role in sperm capacitation and sperm motility [38].

There is still lack of consensus concerning which biomarkers may be used as a diagnosis toll in male infertility, but studies are advancing, and in the next future, proteomics will be part of the clinical routine of male infertility.

4. PROTEOMICS AND EMBRYO QUALITY

Previous reports on proteomics and embryo quality have correlated the concentration of amino acids in the culture media with embryo developmental potential. Patients with positive result regarding ongoing pregnancy presented a different profile of amino acids than did those with negative results, regardless of maternal age [39].

More recently, studies have focused on proteomics profiles and been able to identify biomarkers of embryo quality, blastocyst formation, and implantation [40-43]. Other authors have correlated the presence of a specific protein responsible for inflammatory stress with the incidence of embryo aneuploidy [44].

5. CONCLUSION

Although there are still obstacles to overcome concerning the use of proteomics technology in assisted reproduction, studies have suggested that this technology is a highly sensitive method with quantitative efficiency. Its use in clinical practice goes beyond the identification of the oocytes and embryos with the best developmental potentials or prediction of a pregnancy's outcome. It may add to the diagnosis of both male and female infertility, adding to our understanding of cell biology, and in

the future, it also may be a laboratory tool that will contributes to the birth of a healthy child.

REFERENCES

1. Kolialexi A.,Mavrou A.,Spyrou G., Tsangaris G.T. Mass spectrometry-based proteomics in reproductive medicine. *Mass Spectrom Rev*, **2008**; 27(6): p. 624-34.
2. Tsangaris G.T. From proteomics research to clinical practice. *Expert Rev Proteomics*, **2009**; 6(3): p. 235-8.
3. Jauniaux E.,Greenwold N.,Hempstock J., Burton G.J. Comparison of ultrasonographic and Doppler mapping of the intervillous circulation in normal and abnormal early pregnancies. *Fertil Steril*, **2003**; 79(1): p. 100-6.
4. Kosteria I.,Anagnostopoulos A.K.,Kanaka-Gantenbein C.,Chrousos G.P., Tsangaris G.T. The Use of Proteomics in Assisted Reproduction. *In Vivo*, **2017**; 31(3): p. 267-283.
5. Schweigert F.J.,Gericke B.,Wolfram W.,Kaisers U., Dudenhausen J.W. Peptide and protein profiles in serum and follicular fluid of women undergoing IVF. *Hum Reprod*, **2006**; 21(11): p. 2960-8.
6. Angelucci S.,Ciavardelli D.,Di Giuseppe F.,Eleuterio E.,Sulpizio M.,Tiboni G.M.,Giampietro F.,Palumbo P., Di Ilio C. Proteome analysis of human follicular fluid. *Biochim Biophys Acta*, **2006**; 1764(11): p. 1775-85.
7. Twigt J.,Steegers-Theunissen R.P.,Bezstarosti K., Demmers J.A. Proteomic analysis of the microenvironment of developing oocytes. *Proteomics*, **2012**; 12(9): p. 1463-71.
8. Jarkovska K.,Martinkova J.,Liskova L.,Halada P.,Moos J.,Rezabek K.,Gadher S.J., Kovarova H. Proteome mining of human follicular fluid reveals a crucial role of complement cascade and key biological pathways in women undergoing in vitro fertilization. *J Proteome Res*, **2010**; 9(3): p. 1289-301.
9. Wu Y.T.,Wu Y.,Zhang J.Y.,Hou N.N.,Liu A.X.,Pan J.X.,Lu J.Y.,Sheng J.Z., Huang H.F. Preliminary proteomic analysis on the alterations in follicular fluid proteins from women undergoing natural cycles or controlled ovarian hyperstimulation. *J Assist Reprod Genet*, **2015**; 32(3): p. 417-27.
10. Estes S.J.,Ye B.,Qiu W.,Cramer D.,Hornstein M.D., Missmer S.A. A proteomic analysis of IVF follicular fluid in women <or=32 years old. *Fertil Steril*, **2009**; 92(5): p. 1569-78.
11. Cooper T.G.,Noonan E.,von Eckardstein S.,Auger J.,Baker H.W.,Behre H.M.,Haugen T.B.,Kruger T.,Wang C.,Mbizvo M.T., Vogelsong K.M. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*, **2010**; 16(3): p. 231-45.
12. Agarwal A.,Sharma R.K.,Sharma R.,Assidi M.,Abuzenadah A.M.,Alshahrani S.,Durairajanayagam D., Sabanegh E. Characterizing semen parameters and their association with reactive oxygen species in infertile men. *Reprod Biol Endocrinol*, **2014**; 12: p. 33.
13. Hamada A.,Esteves S.C.,Nizza M., Agarwal A. Unexplained male infertility: diagnosis and management. *Int Braz J Urol*, **2012**; 38(5): p. 576-94.
14. Sikka S.C., Hellstrom W.J. Current updates on laboratory techniques for the diagnosis of male reproductive failure. *Asian J Androl*, **2016**; 18(3): p. 392-401.
15. Kovac J.R.,Pastuszak A.W., Lamb D.J. The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. *Fertil Steril*, **2013**; 99(4): p. 998-1007.
16. Amaral A.,Castillo J.,Ramalho-Santos J., Oliva R. The combined human sperm proteome: cellular pathways and implications for basic and clinical science. *Hum Reprod Update*, **2014**; 20(1): p. 40-62.
17. Castillo J.,Amaral A.,Azpiazu R.,Vavouri T.,Estanyol J.M.,Ballesca J.L., Oliva R. Genomic and proteomic dissection and characterization of the human sperm chromatin. *Mol Hum Reprod*, **2014**; 20(11): p. 1041-53.
18. Milardi D.,Grande G.,Vincenzoni F.,Messana I.,Pontecorvi A.,De Marinis L.,Castagnola M., Marana R. Proteomic approach in the identification of fertility pattern in seminal plasma of fertile men. *Fertil Steril*, **2012**; 97(1): p. 67-73 e1.
19. Guo X.,Zhao C.,Wang F.,Zhu Y.,Cui Y.,Zhou Z.,Huo R., Sha J. Investigation of human testis protein heterogeneity using 2-dimensional electrophoresis. *J Androl*, **2010**; 31(4): p. 419-29.
20. Li J.,Liu F.,Liu X.,Liu J.,Zhu P.,Wan F.,Jin S.,Wang W.,Li N.,Liu J., Wang H. Mapping of the human testicular proteome and its relationship with that of the epididymis and spermatozoa. *Mol Cell Proteomics*, **2011**; 10(3): p. M110 004630.
21. Guo X.,Zhang P.,Huo R.,Zhou Z., Sha J. Analysis of the human testis proteome by mass spectrometry and bioinformatics. *Proteomics Clin Appl*, **2008**; 2(12): p. 1651-7.
22. Macleod G., Varmuza S. The application of proteomic approaches to the study of mammalian spermatogenesis and sperm function. *FEBS J*, **2013**; 280(22): p. 5635-51.
23. Duncan M.W., Thompson H.S. Proteomics of semen and its constituents. *Proteomics Clin Appl*, **2007**; 1(8): p. 861-75.
24. de Mateo S.,Martinez-Heredia J.,Estanyol J.M.,Dominguez-Fandos D.,Vidal-Taboada J.M.,Ballesca J.L., Oliva R. Marked correlations in protein expression identified by proteomic analysis of human spermatozoa. *Proteomics*, **2007**; 7(23): p. 4264-77.
25. Martinez-Heredia J.,de Mateo S.,Vidal-Taboada J.M.,Ballesca J.L., Oliva R. Identification of proteomic differences in asthenozoospermic sperm samples. *Hum Reprod*, **2008**; 23(4): p. 783-91.
26. Wang J.,Wang J.,Zhang H.R.,Shi H.J.,Ma D.,Zhao H.X.,Lin B., Li R.S. Proteomic analysis of seminal

plasma from asthenozoospermia patients reveals proteins that affect oxidative stress responses and semen quality. *Asian J Androl*, **2009**; 11(4): p. 484-91.

27. Siva A.B.,Kameshwari D.B.,Singh V.,Pavani K.,Sundaram C.S.,Rangaraj N.,Deenadayal M., Shivaji S. Proteomics-based study on asthenozoospermia: differential expression of proteasome alpha complex. *Mol Hum Reprod*, **2010**; 16(7): p. 452-62.
28. Batruch I.,Smith C.R.,Mullen B.J.,Grober E.,Lo K.C.,Diamandis E.P., Jarvi K.A. Analysis of seminal plasma from patients with non-obstructive azoospermia and identification of candidate biomarkers of male infertility. *J Proteome Res*, **2012**; 11(3): p. 1503-11.
29. Batruch I.,Lecker I.,Kagedan D.,Smith C.R.,Mullen B.J.,Grober E.,Lo K.C.,Diamandis E.P., Jarvi K.A. Proteomic analysis of seminal plasma from normal volunteers and post-vasectomy patients identifies over 2000 proteins and candidate biomarkers of the urogenital system. *J Proteome Res*, **2011**; 10(3): p. 941-53.
30. Drabovich A.P.,Dimitromanolakis A.,Saraon P.,Soosaipillai A.,Batruch I.,Mullen B.,Jarvi K., Diamandis E.P. Differential diagnosis of azoospermia with proteomic biomarkers ECM1 and TEX101 quantified in seminal plasma. *Sci Transl Med*, **2013**; 5(212): p. 212ra160.
31. Davalieva K.,Kiprijanovska S.,Noveski P.,Plaseski T.,Kocevska B.,Broussard C., Plaseska-Karanfilska D. Proteomic analysis of seminal plasma in men with different spermatogenic impairment. *Andrologia*, **2012**; 44(4): p. 256-64.
32. Zhu Y.,Wu Y.,Jin K.,Lu H.,Liu F.,Guo Y.,Yan F.,Shi W.,Liu Y.,Cao X.,Hu H.,Zhu H.,Guo X.,Sha J.,Li Z., Zhou Z. Differential proteomic profiling in human spermatozoa that did or did not result in pregnancy via IVF and AID. *Proteomics Clin Appl*, **2013**; 7(11-12): p. 850-8.
33. Azpiazu R.,Amaral A.,Castillo J.,Estanyol J.M.,Guimera M.,Ballesca J.L.,Balasch J., Oliva R. High-throughput sperm differential proteomics suggests that epigenetic alterations contribute to failed assisted reproduction. *Hum Reprod*, **2014**; 29(6): p. 1225-37.
34. Intasqui P.,Camargo M.,Antoniassi M.P.,Cedenho A.P.,Carvalho V.M.,Cardozo K.H.M.,Zylbersztejn D.S., Bertolla R.P. Association between the seminal plasma proteome and sperm functional traits. *Fertil Steril*, **2016**; 105(3): p. 617-628.
35. Porambo J.R.,Salicioni A.M.,Visconti P.E., Platt M.D. Sperm phosphoproteomics: historical perspectives and current methodologies. *Expert Rev Proteomics*, **2012**; 9(5): p. 533-48.
36. Ficarro S.,Chertihin O.,Westbrook V.A.,White F.,Jayes F.,Kalab P.,Marto J.A.,Shabanowitz J.,Herr J.C.,Hunt D.F., Visconti P.E. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. *J Biol Chem*, **2003**; 278(13): p. 11579-89.
37. Wang J.,Qi L.,Huang S.,Zhou T.,Guo Y.,Wang G.,Guo X.,Zhou Z., Sha J. Quantitative phosphoproteomics analysis reveals a key role of insulin growth factor 1 receptor (IGF1R) tyrosine kinase in human sperm capacitation. *Mol Cell Proteomics*, **2015**; 14(4): p. 1104-12.
38. Sun C.J.,Yan L.Y.,Wang W.,Yu S.,Wang X., Zhang W.Y. Proteomic analysis of the alteration of protein expression in the placenta of Down syndrome. *Chin Med J (Engl)*, **2011**; 124(22): p. 3738-45.
39. Brison D.R.,Houghton F.D.,Falconer D.,Roberts S.A.,Hawkhead J.,Humpherson P.G.,Lieberman B.A., Leese H.J. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. *Hum Reprod*, **2004**; 19(10): p. 2319-24.
40. Katz-Jaffe M.G.,Gardner D.K., Schoolcraft W.B. Proteomic analysis of individual human embryos to identify novel biomarkers of development and viability. *Fertil Steril*, **2006**; 85(1): p. 101-7.
41. Katz-Jaffe M.G.,Schoolcraft W.B., Gardner D.K. Analysis of protein expression (secretome) by human and mouse preimplantation embryos. *Fertil Steril*, **2006**; 86(3): p. 678-85.
42. Cortezzi S.S.,Garcia J.S.,Ferreira C.R.,Braga D.P.,Figueira R.C.,Iaconelli A., Jr.,Souza G.H.,Borges E., Jr., Eberlin M.N. Secretome of the preimplantation human embryo by bottom-up label-free proteomics. *Anal Bioanal Chem*, **2011**; 401(4): p. 1331-9.
43. Braga D.P.,Setti A.S.,Lo Turco E.G.,Cordeiro F.B.,Cabral E.C.,Cortezzi S.S.,Ono E.,Figueira R.C.,Eberlin M.N., Borges E., Jr. Protein expression in human cumulus cells as an indicator of blastocyst formation and pregnancy success. *J Assist Reprod Genet*, **2016**; 33(12): p. 1571-1583.
44. McReynolds S.,Vanderlinden L.,Stevens J.,Hansen K.,Schoolcraft W.B., Katz-Jaffe M.G. Lipocalin-1: a potential marker for noninvasive aneuploidy screening. *Fertil Steril*, **2011**; 95(8): p. 2631-3.