The use of proteomics in the assessment of health status of offspring born after intracytoplasmic sperm injection (ICSI)

Ioanna Kosteria[1], Alexandra Gkourogianni[1], Dimitrios Loutradis[2], Ioannis Papassotiriou[3], George P. Chrousos[1], George Tsagaris[4], Christina Kanaka-Gantenbein[1]

[1]Division of Endocrinology, Diabetes and Metabolism, First Department of Pediatrics, National and Kapodistrian University of Athens Medical School, Aghia Sophia Children's Hospital, Athens, Greece, [2]Division of Assisted Reproduction, First Department of Obstetrics & Gynaecology, National and Kapodistrian University of Athens Medical School, Alexandra Hospital, Athens, Greece, [3]Division of Clinical Biochemistry, Aghia Sophia Children's Hospital, Athens, Greece, [4]Institute of Biomedical Research, Foundation of the Academy of Science, Athens, Greece.

Introduction & Objectives: Several studies have correlated Assisted Reproduction Technologies (ART) including classic IVF and Intacytoplasmic Sperm Injection (ICSI) with epigenetic alterations in the offspring that could have long lasting unfavorable metabolic effects. Proteomics, a state-of-the-art technology used for the identification of early biomarkers of disease, has already been implemented in the search of success in ART but not yet for such markers evaluation in offspring of ART.

Our aim was to investigate the metabolic status of children born after ICSI with the use of proteomics.

Methods: Demographic, auxological, biochemical and hormonal parameters of 42 ICSI-conceived children and 42 age-matched naturally conceived (NC) children were assessed (mean age: 6.8±2.1 years). All children were prepubertal in order to avoid the confounding effect of pubertal insulin resistance. Amongst them, 10 couples of children (5♀ and 5♂) further matched for birthweight (SGA/AGA/LGA) and parity (twins/singles) were selected for comparative plasma proteomic analysis [2-D Electrophoresis- Matrix-Assisted Laser Desorption Tandem Time Of Flight Mass Spectrometry (MALDI-TOF-MS)]. The derived proteomic profiles were compared in pairs. Simultaneously, we have created two plasma pools from the ICSI and the NC group respectively, using 1 μL from each sample. The two pools were subsequently processed following the same methodology. Proteins were classified according to their biological characteristics, using their gene symbol (http://www.uniprot.org/) and were subsequently used for pathway analysis by submitting protein entry names to the STRING database.

Results & Conclusions: The ICSI group was characterized by a shorter duration of gestation, increased percentage of caesarean sections, smaller birthweight and birth length and advanced maternal age. No differences were observed regarding auxological and initial laboratory data, apart from decreased systolic blood pressure and increased T3 in the ICSI group. Of the spots that were found to be differentially expressed in one-to-one comparisons, 98 were identified and subsequently corresponded to 24 different proteins (Table 1A). In order to validate and further strengthen our results we proceeded to the comparison of the proteomic profiles of the pooled samples. Of the 20 selected spots, 15 were identified, corresponding to six proteins (Table 1B). By co-evaluating the results of the comparative analysis of both individual and pooled samples, we compiled a list of 22 differentially expressed proteins, of which 19 were overexpressed and 3 were underexpressed in the ICSI group (Table 2).

Our study has identified a panel of proteins that were consistently either over- or underexpressed in the ICSI group. The majority of the overexpressed proteins are implicated in the acute phase reaction, blood coagulation, activation of the complement pathway and iron and lipid metabolism, suggesting an unfavorable cardiometabolic profile of these children, at a subclinical level (Figure1). The main functional interactions identified were between Apo A1, A4 and E, between gelsolin and ApoA1, gelsolin and transthyretin, A2 macroglobulin and ApoE, A1 antitrypsin and A2 macroglobulin, fibrinogen γ-chain and prothrombin, as well as between transferrin and ApoA1 (Figure 2).

Conclusions: The results of this study highlight the importance of close, long term follow-up of children born after ICSI, especially regarding cardiometabolic risk factors, and underpin the role of proteomics in the early identification of markers of metabolic disturbance in children born after ICSI, long before any derangements become evident at the biochemical level.

Declaration statement: No conflict of interest to declare for all authors.

Title	Accession	Mascot Score	MS Coverage	Protein MW	pI
A. Individual Samples					
Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=	A1AG1_HUMAN	85	44	23725	4.80
Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	A1AT_HUMAN	168	51	46878	5.30
Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1	FETUA_HUMAN	72	23	40098	5.40
Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3	A2MG_HUMAN	283	34	164613	6.00
Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1	APOA1_HUMAN	274	68	30759	5.50
Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3	APOA4_HUMAN	73	14	45371	5.20
Apolipoprotein E OS=Homo sapiens GN=APOE PE=1 SV=1	APOE_HUMAN	172	61	36246	5.50
Complement C1s subcomponent OS=Homo sapiens GN=C1S PE=1 SV=1	C1S_HUMAN	80	18	78174	4.70
Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	CO3_HUMAN	256	28	188569	6.00
Complement factor B OS=Homo sapiens GN=CFB PE=1 SV=2	CFAB_HUMAN	80	18	86847	6.70
DAN domain family member 5 OS=Homo sapiens GN=DAND5 PE=2 SV=	DAND5_HUMAN	53	53	20737	12.30
Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2	FIBA_HUMAN	225	35	95656	5.60
Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3	FIBG_HUMAN	216	62	52106	5.30
Gelsolin OS=Homo sapiens GN=GSN PE=1 SV=1	GELS_HUMAN	50	8	86043	5.90
Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	HPT_HUMAN	74	16	45861	6.10
Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	IGKC_HUMAN	73	75	11773	5.50
Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=	K1C10_HUMAN	68	22	59020	5.00
Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1	K1C9_HUMAN	147	41	62255	5.00
Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1	K2C1_HUMAN	77	24	66170	8.82
Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2	PLMN_HUMAN	321	43	93247	7.30
Prothrombin OS=Homo sapiens GN=F2 PE=1 SV=2	THRB_HUMAN	118	21	71475	5.60
Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2	TRFE_HUMAN	366	57	79280	7.00
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	ALBU_HUMAN	334	59	71317	5.90
Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1	VTDB_HUMAN	276	56	54526	5.30
B. Pooled Samples					
Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	A1AT_HUMAN	170	52	46878	5.3
Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1	APOA1_HUMAN	75	36	30759	5.5
Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3	APOA4_HUMAN	101	30	45371	5.2
Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3	FIBG_HUMAN	201	58	52106	5.3
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	ALBU_HUMAN	184	42	71317.00	5.9
Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1	TTHY_HUMAN	64	55	15991	5.4

Tables 1A & 1B. List of the proteins identified by MALDI-ToF-Mass Spectrometry from the spots that were selected as differentially expressed between groups (ICSI & Natural Conception) after 2D Electrophoresis

ICSI	
Overexpressed proteins	
Alpha-1-acid glycoprotein	
Alpha-1-antitrypsin	Gelsolin
Alpha-2-HS-glycoprotein	Haptoglobin
Alpha-2-macroglobulin	Ig kappa chain C region
Apolipoprotein A-I	Keratin, type I cytoskeletal 10
Apolipoprotein A-IV	Keratin, type I cytoskeletal 9
Apolipoprotein E	Keratin, type II cytoskeletal 1
Complement C1s subcomponent	Prothrombin
Complement C3	Serotransferrin
Complement factor B	Serum albumin
Transthyretin	Vitamin D-binding protein
Underexpressed proteins	
DAN domain family member 5	
Fibrinogen alpha chain	
Plasminogen	

Table 2. List of proteins that were found to be differentially expressed between ICSI and Natural Conception groups

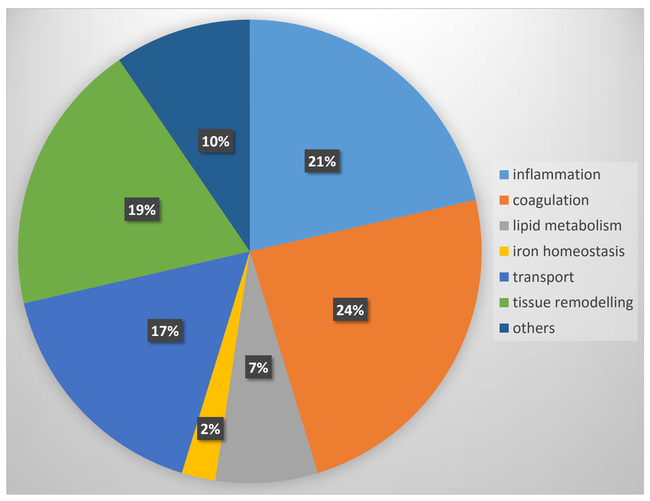
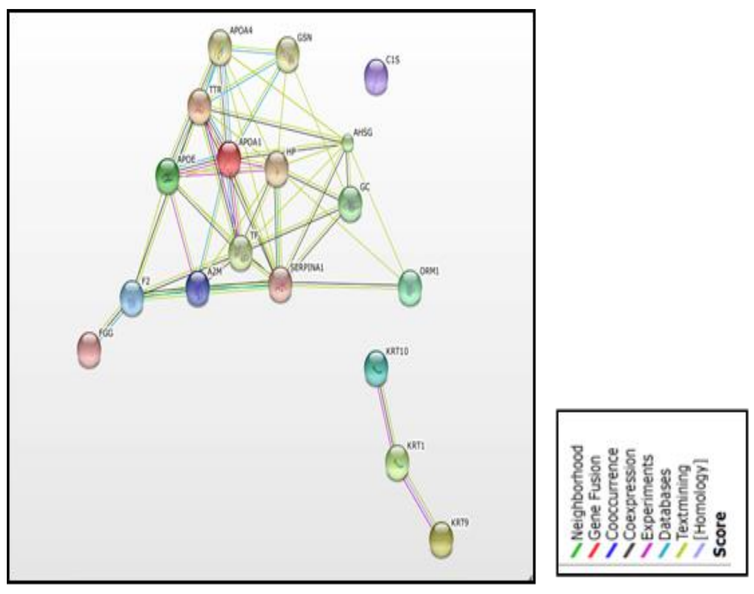


Figure 1.Representation of functional classification of identified, differentially expressed proteins (% total number of identified proteins)

Figure 2. Interaction networks and enriched functional annotations of proteins differentially expressed in examined samples. Thicker network lines demonstrate strong protein relation as well as neighbouring positions.

ORM1: α1 glycoprotein, **SERPINA1**: α1antithrypsin, **AHSG**: α2-HSglycoprotein, **A2M:** α2 macroglobulin **APOA1**: apolipoprotein A-I, **APOA4**: , apolipoprotein A-IV, **APOE**: apolipoprotein E, **C1S**: complement component 1, **FGG**: fibrinogen gamma chain;, **GSN**: gelsolin, **KRT 10**: keratin10, **KRT 9**: keratin 9, **KRT 1**: keratin 1 (keratin type II cytoskeletal 1), **F2**: prothrombin, **HP**: haptoglobin, **TF**: tranferrin, **GC**: vitamin D binding protein, **TTR**: transhyretin



References: 1. Anagnostopoulos AK; Tsangaris GT. et al. Proteomic analysis of amniotic fluid in pregnancies with Klinefelter syndrome foetuses. *Journal of proteomics* 2010;73(5):943–50. 2. Kassi E; Chrousos G. et al. Metabolic syndrome: definitions and controversies. *BMC Med* 2011;9:48. 3. Ceelen M; Delemarre-van de Waal HA et al. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J Clin Endocrinol Metab* 2008;93(5):1682–1688. 4. Kanaka-Gantenbein; G, Chrousos GP et al. Endocrine-related causes and consequences of intrauterine growth retardation. *Ann N Y Acad Sci* 2003;997:150–157.

55ESPE 13--2-RFC

Management of Obesity

Ioanna Kosteria

DOI: 10.3252/pso.eu.55ESPE.2016

Poster presented at: ESPE 2016 PARIS

PosterSessionOnline.com