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Title:

NORMAL GROWTH VERSUS EARLY DEVELOPMENTAL ARREST OF THE HUMAN EMBRYO: UNDERSTANDING MOLECULAR NETWORK PERTURBATIONS

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Objective:

The development of a reproductively competent human embryo depends on a cascade of dynamic, cellular events during the preimplantation period. Embryo selection in the modern IVF laboratory is based on morphological criteria and chromosomal copy number assessment by preimplantation genetic testing (PGT-A). Despite our ability to enhance transfer selection and boost pregnancy outcomes using PGT-A, certain embryos do not develop to the blastocyst stage and up to a third of transferred euploid embryos fail to implant, suggesting there may be a variety of stresses and conditions, other than aneuploidy, that impede embryo development. Understanding key molecular pathways that mediate embryonic competence leveraging gene expression profiling could serve as one of the staples for the next generation of embryo quality testing. This study aimed to characterize variations in molecular networks associated with growth arrested and viable embryos.

Design:

Prospective cohort study on human embryos donated for research.

Materials and Methods:

The study included patients who donated fresh embryos at various stages of development during an IVF cycle between January, 2016 and June, 2016. Embryos were classified into 2 groups based on routine evaluation from day 3 to 7 of extended culture (Group 1: arrested; Group 2: viable/ongoing). Embryos were biopsied, and approximately 2-4 cells were removed for aneuploidy screening by next generation sequencing (NGS). The remaining cells of the embryo were designated for RNA sequencing. Read counts per gene were summed across embryo cohorts and normalized using the median of ratios. Differential gene expression between embryo cohorts was calculated using DESeq2. A likelihood ratio test was used to account for heterogeneity due to patient, batch, ploidy status, and developmental stage. Pathway analysis was performed and genes were grouped according to major cellular functions (listed in Table 1). The adjusted threshold for significance was $p<0.05$.

Results:

Of the 81 embryos that underwent RNA sequencing, 33 ongoing embryos (comprising of 32 blastocysts, 1 morula; 10 being euploid) were compared with 48 arrested embryos (11 blastocysts, 8 morula, 29 cleavage stage; 6 being euploid). Of the 20,055 protein coding transcripts were interrogated, 38 were found to have significant differential expression among ongoing vs. arrested embryos. Compared with those that arrested, ongoing embryos were characterized by massive downregulation of genes encompassing all cellular function categories, including cell death. The few genes that were significantly upregulated in ongoing embryos (n=5) are involved in metabolism and biosynthesis, immune function and cell division and proliferation. In a sub-analysis restricted to euploid embryos only (n=16), ongoing embryos were found to have 6 key differentially expressed genes compared with their arrested counterparts, only one of which was upregulated (SNRNP70, which codes for spliceosome proteins

required for alternative splicing and mRNA translation). Notable downregulated genes in ongoing, euploid embryos included: ZNF33A (encodes for a DNA binding transcription factor that promotes increased entry into cell cycle S phase); COMMD8 (via activation of NF-kappa-B, mediates immune and inflammatory response, cellular growth and apoptosis); EIF4EBP3 (encodes a repressor of translation initiation); and MMAB (encodes a protein which converts vitamin B into a vital co-enzyme for methylmalonyl-CoA mutase).

Conclusion:

The transcriptome of viable, ongoing human embryos is characterized by massive gene downregulation. As an adaptive mechanism to intrinsic and extrinsic stress, embryos in danger of developmental arrest may compensate for the loss of embryonic cells by upregulating gene expression that drives key cellular processes. While aneuploidy is heavily implicated in embryonic developmental arrest, our analysis revealed other possible causes. Arresting euploid embryos may suffer from cell cycle defects, for example entering S phase more rapidly than ongoing embryos, which could represent a regulatory mechanism that counteracts cellular apoptosis. Immune-mediated inflammatory cascades and defects in vitamin B12 pathways may also play causative roles in developmental arrest. While the blockage of vitamin B12 metabolism has been implicated in mouse embryonic arrest (Moreno et al., 2018), this is the first study to provide evidence that direct effects on cobalamin metabolism may drive human embryonic arrest. Elucidating gene expression pathways that mediate abnormal preimplantation embryo development and arrest may improve our ability to identify developmentally competent embryos based on their transcriptomic signature, optimize and personalize culture conditions, and pave the way for the development of accurate, non-invasive assessment.

Table 1:

Differentially expressed genes when comparing the transcriptomic profile of ongoing (n=33) vs. arrested embryos (n=48). Asterisk (*) indicates significant differentially expressed genes among the subset of euploid embryos when comparing ongoing (n=10) vs. arrested (n=6) embryos. The adjusted threshold for significance was p<0.05 for both the full and sub-analysis.

	Downregulated			Upregulated		
Pathways	**Gene**	**Log2 fold Δ**	**Adjusted p value**	**Gene**	**Log2 fold Δ**	**Adjusted p value**
Cell death	**MDM4**	-5.42	0.02			
	MECOM	-8.05	0.04			
	MIR4454	-3.82	0.01			
	RP11-727A23.10	-4.63	0.02			
Metabolism & biosynthesis	**PDK1**	-7.97	0.03	**CBR1**	7.01	0.04
	FAM46C	-7.32	0.03	**GLO1**	5.41	0.02
	MMAB*	-6.84	0.007			
Immune-mediated activity	**C1QTNF6**	-6.48	0.02	**IL10RB**	5.51	0.04
	RASGRP3	-6.35	0.03			
	REL	-5.79	0.02			
	SCGB3A2	-4.49	0.03			
	SYTL2	-8.13	0.01			
	YPEL5	-4.40	0.02			
	COMMD8*	-10.6	0.007			
	NPB*	-9.77	0.04			
Cell division and proliferation	**KIF3A**	-6.90	0.01	**IL10RB**	5.51	0.04
	ZNF280B	-6.23	0.03	**OAZ2**	5.51	0.04
	MECOM	-8.05	0.04			
Cell cycle regulation	**FAM46C**	-7.32	0.03			
	RGS2	-5.65	0.01			
	USP2	-7.56	0.03			
	YPEL5	-4.40	0.02			
Cell adhesion	**CDH3**	-4.09	0.02			
	FUT3	-3.47	0.04			
Cell signaling and transport	**ANKRD36C**	-6.70	0.03			
	BAALC	-15.7	0.03			
	CNNM2	-5.00	0.03			
	GABRP	-4.72	0.02			
	KCNE4	-4.12	0.03			
	ZP3	-5.47	0.02			
	ZP4	-10.2	0.007			
Protein/nucleic acid binding	**REL**	-5.79	0.02	**SNRNP70***	4.71	0.047
	RPH3A	-9.05	0.047			
	SCGB3A2	-4.49	0.03			

	TPRXL	-5.04	0.04			
	ZNF333	-3.92	0.0497			
	ZNF788	-4.52	0.04			
	EIF4EBP3*	-7.99	0.007			
	ZNF33A*	-7.81	0.04			
Protein folding	**ZNF280C**	-6.85	0.03			
	ZNF333	-3.92	0.0497			
	ZNF788	-4.52	0.04			
Uncategorized	**CTC-210G5.1**	-2.97	0.03	**AC005523.2**	8.61	0.04
	KIAA1210	-7.09	0.3			
	RP11-556O5.5	-5.82	0.02			

References:

1. Moreno-Garcia MA, Rosenblatt DS, Jerome-Majewska LA. The methylmalonic aciduria related genes, Mmaa, Mmab, and Mut, are broadly expressed in placental and embryonic tissues during mouse organogenesis. Molecular Genetics and Metabolism 2012; 107(3):368-74.
2. Yang JJ. A novel zing finger protein, ZZaPK, interacts with ZAK and stimulates ZAK-expressing cells re-entering the cell cycle. Biochem Biophys Res Commun 2003; 301(1): 71-7.