

LONG-READ TRANSCRIPTOMICS OF ARRESTED HUMAN EMBRYOS IMPLICATES SPLICING FAILURE, RETROTRANSPOSON DEREPRESSION AND FAS-ASSOCIATED STRESS SIGNALLING

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Abstract

Preimplantation developmental arrest remains a major cause of embryo loss during in vitro culture, including in embryos for which morphology alone does not explain developmental failure. Because short-read RNA sequencing has limited ability to resolve transcript isoforms, intron retention, and repetitive-element-derived transcripts, we applied Oxford Nanopore long-read sequencing to 16 post-ICSI samples from seven patients, including four 0PN oocytes and 12 developmentally arrested embryos. The arrested samples showed marked heterogeneity in mitochondrial transcript fraction and mitochondrial variant profiles. In addition, arrested embryos displayed a recurrent long-read signature characterized by increased chimeric alignments, elevated intron retention, impaired developmental isoform switching, and enhanced LINE-1-associated signal. The most disorganized embryos also showed reduced expression of subcortical maternal complex components, particularly NLRP7, together with weaker BRCA1-related transcript output and a FAS-associated stress/apoptosis profile. These findings support a model in which a subset of

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arrested human embryos undergo coordinated failure of RNA processing, repeat control, and maternal genome-stability-associated programs. Long-read transcriptomics may provide a useful framework for studying embryo arrest at the level of transcript structure and repetitive-element activity, features that are not readily captured by gene-level short-read approaches.

Key words: nanopore sequencing, embryo arrest, NLRP7, retrotransposons, FAS, alternative splicing

Introduction. Preimplantation development depends on a tightly coordinated transition from the maternal oocyte program to embryonic genome activity. Developmental arrest remains a frequent outcome in assisted reproduction, and chromosomal abnormalities do not explain all cases. Increasing evidence suggests that arrest may also involve defects in the maternal-to-zygotic transition, genome maintenance, metabolic adaptation, and regulated cell-death responses [1–4]. Long-read sequencing is particularly relevant to this problem because it can resolve transcript features that are poorly captured by standard gene-level approaches, including isoform usage, intron retention, chimeric transcripts, and repeat-associated signal [5]. This is important in early embryos, where transcript remodelling is a central developmental process and where transposable element activity must remain developmentally controlled [6–9]. In parallel, maternal-effect factors such as NLRP7 have emerged as potential regulators of genome stability and alternative splicing during early human embryogenesis [10–12]. In the present study, we used Oxford Nanopore (ONT) long-read transcriptomics to profile 0PN oocytes and developmentally arrested human embryos. We asked whether arrest in this cohort is associated with coordinated changes in mitochondrial transcript balance, transcript-structure integrity, intron retention, repeat-associated signal, and expression of maternal genome-stability factors, particularly NLRP7.

Materials and methods. Patient cohort and ethics. A total of 16 surplus post-ICSI samples from seven patients were analyzed, including four oocytes without visible pronuclei (0PN) and 12 embryos arrested between days 2 and 4, from cleavage to pre-morula stages. The study was approved by the Ethics Committees of Medical Center Neovitro LLC and IBIR-BAS. Written informed consent was obtained, and all analyses were performed on de-personalized material. Total RNA was extracted from single blastomeres or whole arrested samples (1–10 cells) and amplified using the Qiagen Single Cell RNA Kit.

Nanopore sequencing and primary processing. Full-length cDNA libraries were prepared with the ONT Native Barcoding Kit (SQK-NBD114-24) and sequenced on a PromethION P2 Solo using R10.4.1 flow cells. Raw POD5 files were basecalled with Dorado v1.3 (sup model), then demultiplexed, trimmed, quality filtered, and aligned to GRCh38 with Minimap2.

Bioinformatic analysis. Transcript quantification and mitochondrial analysis. Transcript quantification was performed with *featureCounts* using Gencode

v47 annotation and a transposable-element-aware counting strategy. Raw counts were normalized to transcripts per million (TPM). Mitochondrial transcript burden was assessed as the proportion of mitochondrial-mapped reads relative to nuclear-mapped reads. A mitochondrial-to-nuclear transcript ratio was calculated from TPM values of mitochondrial-encoded transcripts and the nuclear-encoded mitochondrial regulators *TFAM* and *POLG*. Mitochondrial heteroplasmy was evaluated from mitochondrial single-nucleotide variants with allele frequency above 20%. Filtered variants were used for hierarchical clustering and genomic-coordinate visualization of mitochondrial profiles.

Transcript-structure and repeat-associated analyses. Transcript-structure disruption was estimated as the rate of inter-chromosomal chimeric reads per million mapped reads. Intron retention was evaluated by an intron retention index (IRI), defined as the ratio of intronic to corresponding mature exonic reads. Isoform switching was assessed per sample for selected genes associated with the normal oocyte-to-embryo transcript transition. Repeat-associated transcription, including *LINE-1*-related signal, was quantified from the aligned datasets using the TE-aware workflow.

Results and discussion. Mitochondrial transcriptomic heterogeneity and mt-variant remodelling in arrested samples. The cohort included 16 post-ICSI samples from seven patients, comprising four 0PN oocytes and 12 embryos arrested between the cleavage and pre-blastocyst stages (Table 1). Global mapping profiles showed marked variation in mitochondrial read contribution across the cohort (Fig. 1A, G). The mitochondrial-to-nuclear expression ratio was

T a b l e 1
Patient, Sample Type, ID, Morphological Arrest Type

Patient ID	Object	Sample ID	Sample type	Morphological category of Day 5
1	1o.1	sample 09	Oocyte	0PN / infertile, 0PN Oocyte
2	2e.1	sample 10	Embryo	Cleavage-stage arrest ($\approx$ D3-like), D3 arrest
4	4e.1	sample 01	Embryo	Cleavage-stage arrest, D2-D3 arrest
4	4e.2	sample 02	Embryo	Cleavage-stage arrest, D2-D3 arrest
4	4e.3	sample 11	Embryo	Arrest near compactization / early morula, D4 arrest
5	5e.1	sample 12	Embryo	Arrest in Early Embryonic Division (D2 arrest)
5	5e.2	sample 13	Embryo	Arrest near compactization / early morula, D4 arrest
6	6e.1	sample 15	Embryo	Cleavage-stage arrest (D3 arrest)
6	6e.2	sample 16	Embryo	Arrest near compactization / early morula
7	7e.1	sample 17	Embryo	Cleavage-stage arrest (D2–D3 arrest)
8	8o.1	sample 06	Oocyte	0PN / infertile, 0PN Oocyte
8	8o.2	sample 07	Oocyte	0PN / infertile, 0PN Oocyte
8	8o.3	sample 08	Oocyte	0PN / infertile, 0PN Oocyte
8	8e.1	sample 03	Embryo	Pre-blastocyst arrest (cleavage $\rightarrow$ compactization), D4 arrest
8	8e.2	sample 04	Embryo	Pre-blastocyst arrest (cleavage $\rightarrow$ compactization), D4 arrest
8	8e.3	sample 05	Embryo	Pre-blastocyst arrest (cleavage $\rightarrow$ compactization), D4 arrest

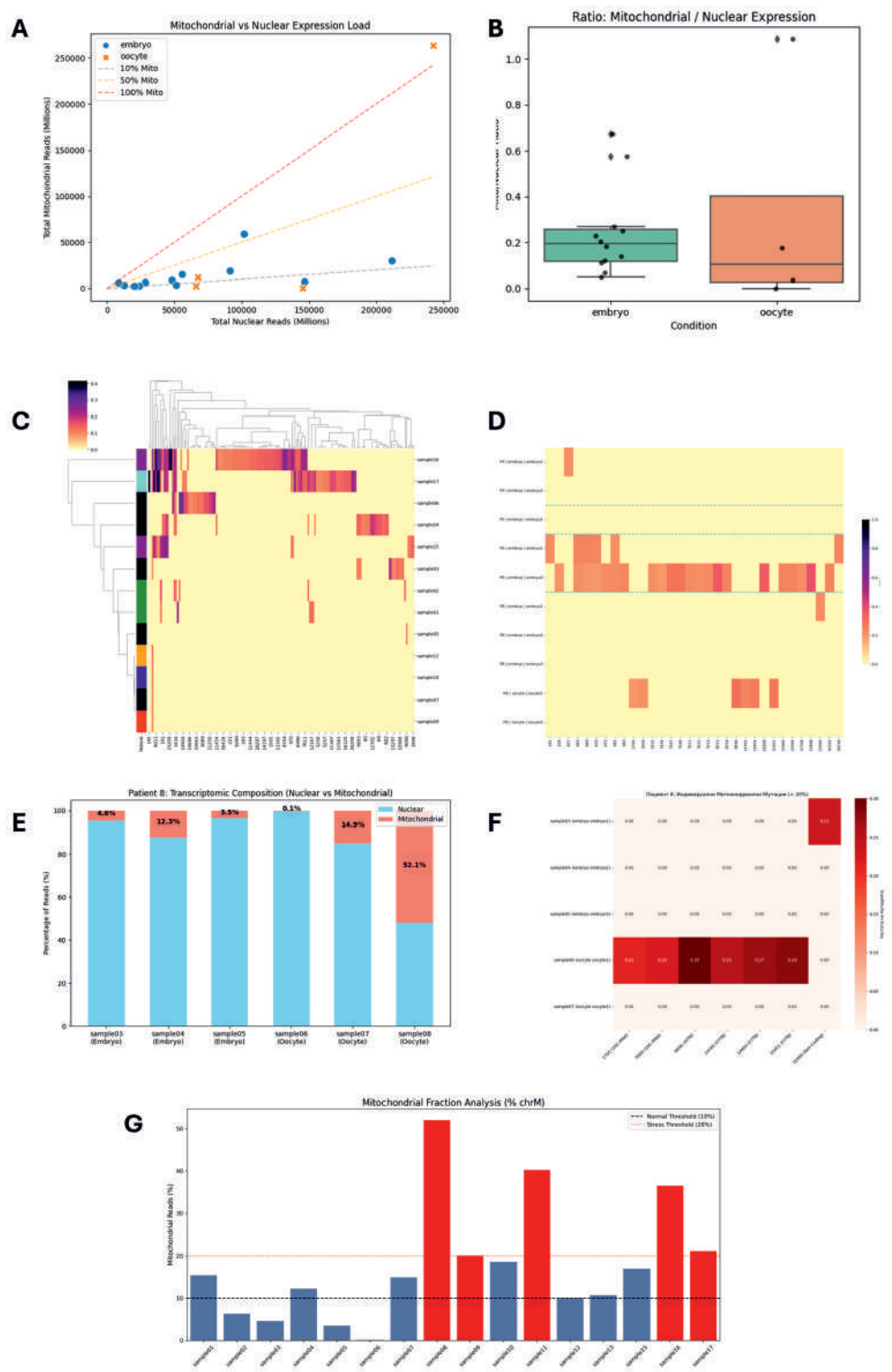


Fig. 1. Mitochondrial transcriptomic and variant profiles in OPN oocytes and arrested embryos. (A) Distribution of sequencing reads mapped to the mitochondrial genome (ChrM) and nuclear genome across the analysed samples. (B) Mitochondrial-to-nuclear transcript ratio across OPN oocytes and arrested embryos. (C) Unsupervised hierarchical clustering of samples based on mitochondrial single-nucleotide variant profiles. (D) Patient-grouped alignment of mitochondrial variants with allele frequency above 20% plotted along mitochondrial genome coordinates. (E) Relative contribution of mitochondrial and nuclear transcript fractions in the Patient 8 sample series. (F) Heatmap of variant allele frequencies for selected mitochondrial loci in the Patient 8 sub-cohort. (G) Proportion of mitochondrial-mapped reads per sample across the cohort

more variable in the OPN group, with several high outliers, whereas arrested embryos were distributed at generally lower values (Fig. 1B). Mitochondrial variant-based clustering revealed both patient-related grouping and within-patient divergence, indicating heterogeneous mitochondrial variant composition among related samples (Fig. 1C, D). This heterogeneity was particularly evident in the Patient 8 series. In that subgroup, one oocyte showed pronounced mitochondrial transcript enrichment whereas another displayed minimal mitochondrial contribution, while the corresponding embryos showed altered variant patterns relative to the oocyte subgroup (Fig. 1E, F). Together, these data indicate that arrested samples are not defined by a single uniform mitochondrial state, but by pronounced mitochondrial transcriptomic and heteroplasmic heterogeneity.

This pattern suggests that mitochondrial imbalance in arrested embryos is heterogeneous rather than stereotyped. Instead of supporting one fixed mitochondrial arrest signature, the data are more consistent with patient- and sample-specific remodelling of mitochondrial contribution and variant composition, potentially reflecting different trajectories of developmental failure.

Arrested embryos show disrupted transcript structure, intron retention, and LINE-1-associated signal. To evaluate transcriptome integrity beyond gene-level abundance, we examined chimeric alignments, repeat-associated signal, intron retention, and isoform usage. Chimeric-read burden was prominent across the cohort and was especially evident in many arrested embryos (Fig. 2A). LINE-1-associated signal also varied substantially, with the strongest enrichment confined to a subset of samples rather than uniformly distributed across all arrested embryos (Fig. 2B). Intron retention was broadly elevated in arrested embryos (Fig. 2C), consistent with impaired RNA processing. Developmental isoform switching was also disrupted, as shown by the altered switching pattern in the arrested group (Fig. 2D). A representative severely affected embryo displayed a genome-wide map of transcriptional disorganization accompanied by dense TE-associated signal (Fig. 2E). Taken together, these findings support a model in which a subset of arrested embryos accumulates coupled defects in RNA processing, transcript structure, and repeat control.

These observations extend previous studies of arrested embryos by highlighting transcript-level disorganization. Because long-read sequencing preserves tran-

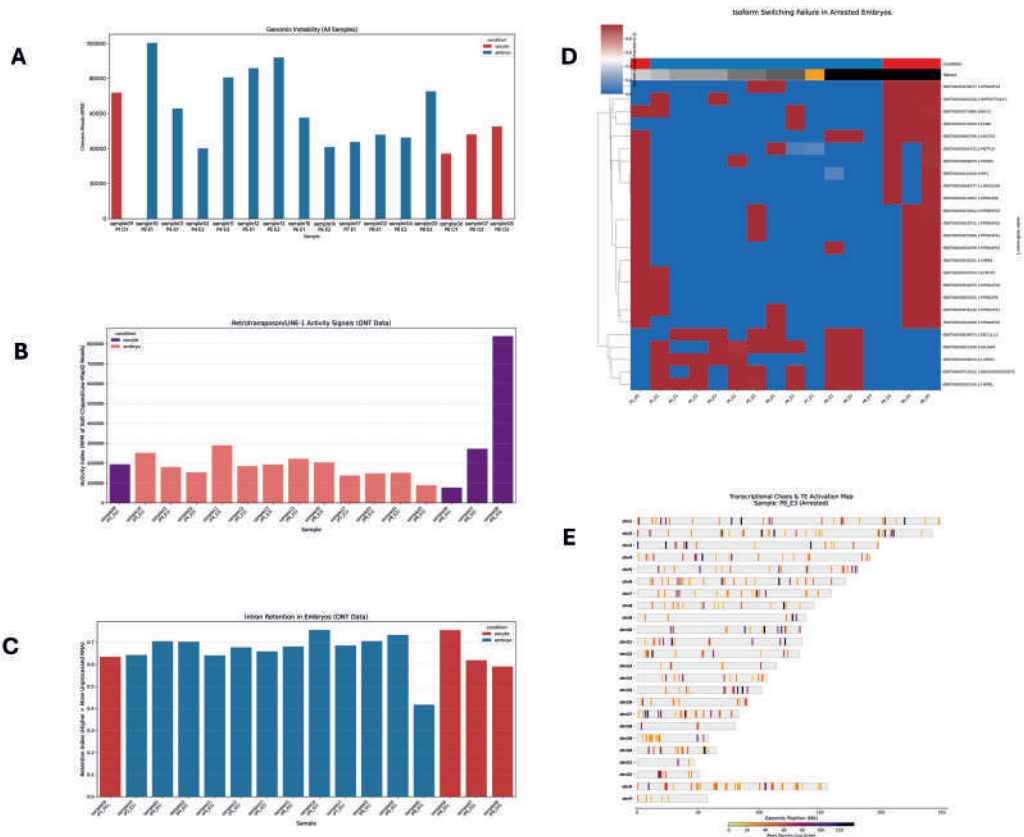


Fig. 2. Transcript-structure disruption, intron retention, isoform switching, and LINE-1-associated signal in OPN oocytes and arrested embryos. (A) Inter-chromosomal chimeric read index, expressed as reads per million mapped reads. (B) LINE-1-associated expression index across the analysed samples. (C) Intron retention index across the cohort. (D) Sample-level assessment of isoform usage for genes associated with the normal oocyte-to-embryo transcript transition. (E) Chromosomal distribution of assembled L1HS-associated transcripts in a representative arrested embryo (Patient 8, Embryo 3; P8_E3)

script structure, it is particularly useful for detecting abnormalities such as intron retention, altered isoform usage, and repeat-associated transcripts that may be underestimated by conventional gene-level analyses [5]. At the same time, the present data remain transcriptomic; they support repeat derepression or repeat-associated signal, but do not by themselves prove active retrotransposition in every arrested embryo [6–9].

NLRP7-low embryos show altered DDR-related transcripts and a FAS-associated stress signature. Immune- and stress-related transcripts detected in arrested embryos were dominated by chemokines, TNF superfamily members, interferon-related genes, and NLR/inflammasome-associated components (Fig. 3A). Expression of SCMC and DDR-associated genes was het-

linked apoptotic engagement, but transcript abundance alone cannot determine the dominant execution pathway [13, 14]. Thus, the present findings support a stress/apoptosis-associated state in the most disorganized embryos without establishing a single universal mechanism of death. Across the cohort, the most consistent pattern was not one isolated lesion, but convergence between mitochondrial heterogeneity, transcript-structure disruption, intron retention, repeat-associated signal, and altered expression of maternal and DNA-damage-related regulators. In this sense, the main contribution of the study is not to redefine embryo arrest as one mechanism, but to show that long-read transcriptomics can reveal a coordinated instability signature that is poorly captured by standard gene-level approaches.

Conclusions. Long-read profiling of 0PN oocytes and developmentally arrested embryos identifies a coordinated arrest signature characterized by mitochondrial heterogeneity, disrupted RNA processing, repeat-associated signal, reduced NLRP7/BRCA1-associated expression, and FAS-associated stress signalling. Embryonic arrest in this cohort does not appear to represent a single uniform molecular lesion; rather, the most disorganized embryos show combined failure of transcript processing and repeat control. Although this study is limited by its small and heterogeneous cohort, these findings support the use of long-read transcriptomics to refine the molecular classification of arrested human embryos.

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