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A Comprehensive Characterization of Cell-free RNA in Spent Blastocyst Medium and Quality Prediction for Blastocyst

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Abstract : Background: The biopsy of the preimplantation embryo may increase the potential risk and concern of embryo viability. Clinically discarded spent embryo medium (SEM) has entered the view of researchers, sparking an interest in noninvasive embryo screening. However, one of the major restrictions is the extremelty low quantity of cf-RNA, which is difficult to efficiently and unbiased amplify cf-RNA using traditional methods. Hence, there is urgently need to an efficient and low bias amplification method which can comprehensively and accurately obtain cf-RNA information to truly reveal the state of SEM cf-RNA. Result: In this present study, we established an agarose PCR amplification system, and has significantly improved the amplification sensitivity and efficiency by ~90 fold and 9.29 %, respectively. We applied agarose to sequencing library preparation (named AG-seq) to quantify and characterize cf-RNA in SEM. The number of detected cf-RNAs (3533 vs 598) and coverage of 3' end were significantly increased, and the noise of low abundance gene detection was reduced. The increasing percentage 5' end adenine and alternative splicing (AS) events of short fragments (< 400 bp) were discovered by AG-seq. Further, the profiles and characterizations of cf-RNA in spent cleavage medium (SCM) and spent blastocyst medium (SBM) indicated that 4-mer end motifs of cf-RNA fragments could remarkably differentiate different embryo development stages. Significance: This study established an efficient and low-cost SEM amplification and library preparation method. Not only that, we successfully described the characterizations of SEM cf-RNA of preimplantation embryo by using AG-seq, including abundance features fragment lengths. AG-seq facilitates the study of cf-RNA as a noninvasive embryo screening biomarker and opens up potential clinical utilities of trace samples.

Keywords : cell-free RNA, agarose, spent embryo medium, RNA sequencing, non-invasive detection
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