The Analysis of Sperm Small Non-coding RNA Expression Profiles Based on PANDORA-seq Technology and Their Association with Sperm Quality

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Abstract

As male gametes, sperm contain vital genetic material for the development of the embryo and the wellbeing of subsequent generations. Numerous small non-coding RNAs (SncRNAs) in sperm have been discovered recently; these RNAs are essential for spermatogenesis, fertilization, and the early stages of embryonic development. However, the presence of RNA modifications makes it difficult for traditional RNA sequencing technology to detect SncRNAs in sperm with accuracy. Consequently, PANDORA-seq technology was created as a potent instrument to thoroughly map the range of sperm SncRNAs. The sensitivity of SncRNA detection is increased by the efficient removal of RNA modifications with the use of T4PNK and ALKB treatments. The EpiTM Mini Long RNA-seq Kit is then used to create RNA libraries using cDNA synthesis and reverse transcription procedures. A thorough profile of SncRNAs is obtained by high-throughput sequencing after library fragments are enriched by PCR amplification. The findings show that PANDORA-seq allows identification beyond miRNAs (20 nt) and reveals a significant presence of tsRNAs and rsRNAs when compared to conventional short RNA library preparation methods. Significantly, this approach effectively detects a number of difficult-to-detect piRNAs (18–31 nt) that are essential for male infertility. Infertile males and fertile control groups exhibit significantly different levels of piRNA expression, according to specific analysis; patients with asthenozoospermia exhibit downregulated levels of piR-1207 and piR-2107, while their expression levels are significantly correlated with sperm motility ($p < 0.01$). More thorough detection capabilities for evaluating sperm quality and locating biomarkers linked to high-quality sperm are offered by PANDORA-seq technology.

Keywords

Sperm, SncRNA, PANDORA-seq, miRNA, piRNA