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COMMENTARY

Spermatozoal RNA profiling towards a clinical evaluation of sperm quality

Toshio Hamatani

Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan
E-mail address: toshiohamatani@z3.keio.jp

Abstract Human spermatozoal RNAs were recently profiled using microarrays and explored as clinical markers of male infertility. An appropriate study design with a considerable number of biological replicates (sperm samples) is necessary to validate the accuracy and reproducibility of these microarray data. If the genes identified as sperm quality markers by microarray studies are successfully attributed to the pathogenesis of male infertility, then the microarray strategy may be used as a clinical diagnostic tool for male infertility. On the other hand, spermatozoal RNAs may contain not only remnant RNAs after spermatogenesis, but also RNAs that may contribute extragenomically to early embryonic development. Therefore, spermatozoal RNA profiling may enable a better understanding of what is contributed to the oocyte by sperm, in addition to their genome, to facilitate early embryonic development.

KEYWORDS: ICSI, male fertility, RNA profiling, sperm, spermatozoa, transcriptome

Assessments of male reproductive fitness have typically relied upon microscopic evaluation using semen parameters including morphology, motility, sperm concentration, presence of any cell types other than mature spermatozoa, and semen volume. The observation of normal semen features using these parameters does not necessarily guarantee male fertility. Recently, morphological real-time observation at high magnification ($>\times$6000) has been used to select sperm for intracytoplasmic sperm injection (ICSI). The morphological normalcy of the sperm nucleus is defined by both its shape (smooth, symmetric and oval) and its chromatin content (homogeneity of the chromatin mass containing no extrusion or invagination and no more than one vacuole involving less than 4% of the nuclear area) (Bartoov et al., 2002). Most publications have reported better rates of implantation and clinical pregnancy as well as a reduction in the rate of abortion where sperm cells were strictly morphologically selected at high magnification (Souza Setti et al., 2010). Prospective randomized clinical studies are still needed to confirm the preliminary findings on the efficacy of intracytoplasmic morphologically selected sperm injection (IMSI) over conventional ICSI. The further improvement of diagnosis and treatment of male infertility will need a new method to evaluate sperm quality based on molecular analysis, rather than on morphological observation.

Mature spermatozoa have little cytoplasm and a highly condensed chromatin architecture that is enriched in protamines. These structural features led to the long held view that mature spermatozoa are inert cells but both transcription and translation occur in the mitochondria, and not in the cytoplasm, of mature spermatozoa (Miller and Ostermeier, 2006). Spermatozoal nuclei, containing RNA polymerase and abundant transcription factors, are capable of transcribing RNA from endogenous templates (Hecht and Williams, 1978). Although mature spermatozoa do not contain some of the essential components of the 80S cytoplasmic ribosomes such as 28S and 18S rRNAs; 55S mitochondrial ribosomes are present in spermatozoal polysomal fractions (Gur and Breitbart, 2006). The incorporation of labeled amino acids into polypeptides occurs during sperm

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capacitation, and is completely inhibited by mitochondrial translation inhibitors but not by a cytoplasmic translation inhibitor (Gur and Breitbart, 2006). Therefore, it is apparent that mitochondrial ribosomes are actively involved in protein translation in spermatozoa.

The first mRNA that was identified in human mature spermatozoa was the c-Myc mRNA (Kumar et al., 1993). The existence of a complex population of mRNAs in ejaculated human mature spermatozoa was shown by expression profiling using oligo DNA microarrays (Ostermeier et al., 2002). Although these mRNAs were previously thought to be non-functional remnants of stored mRNAs that are synthesized at earlier stages of spermatogenesis, Ostermeier et al. proposed that a specific set of functional RNAs may be delivered into oocytes and support early embryonic development (Ostermeier et al., 2004). Although the specific functional significance of these mRNAs in mature ejaculate spermatozoa remains poorly investigated; they have been demonstrated to influence the phenotypic traits of offspring (Miller and Ostermeier, 2006). The poor developmental ratios relative to normal of both parthenogenetic embryos and cloned embryos obtained from somatic-cell nuclear transfer, are consistent with a developmental role for spermatozoal mRNAs.

Garcia-Herrero et al. used microarrays to investigate spermatozoal RNAs (this issue; Garcia-Herrero et al., 2011). They compared the profile gene of expression in spermatozoa that achieved pregnancy (group P) through an ICSI cycle in an oocyte donation program with the profile of those that did not achieve pregnancy (group NP) (Garcia-Herrero et al., 2011). In order to reduce female infertility as a bias factor, all of the oocytes originated from young female donors. Furthermore, the coupled pairs of women (pregnant and non pregnant) received the oocytes from the same donor. The total number of expressed transcripts detected in fresh sperm samples was 19,229. Of those transcripts, 16,035 (83.4%) were expressed in both groups, P and NP. Among these commonly expressed transcripts, only 44 sequences were overexpressed in group P versus NP and five in group NP versus P. Notably, the 44 differentially-expressed genes in group P included four cathepsins and six metallothioneins. Cathepsins are a family of cysteine proteases and are likely to prevent atrophy of seminiferous tubules and support spermatogenesis to pachytene spermatocytes (Gye and Kim, 2004; Wright et al., 2003). Metallothioneins function as detoxicants to prevent damage of the testes by heavy metals (Kusakabe et al., 2008). An ontology analysis by DAVID (http://david.abcc.ncifcrf.gov/home.jsp) of 1358 exclusively-expressed transcripts in group P, found the term 'embryo development ending in birth or egg hatching' as one of the highest-ranked gene ontology (GO) terms. The exclusively-expressed genes corresponding to this GO term include adducin 1 (*ADD1*), activin A receptor type-II like 1 (*ACVRL1*), androgen receptor (*AR*), and aryl-hydrocarbon receptor nuclear translocator (*ARNT*). All of those genes are potential pregnancy success markers rather than potential fertilization makers. In fact, because ICSI removes the physiological process of sperm entry, spermatozoal fertilization factors may not be necessary for pregnancy after ICSI. These highly expressed RNAs in group P may partially represent spermatozoal extra-genomic components that are required for successful pregnancies. In addition, DAVID also analyzed frozen spermatozoa used in ICSI in the same way, and demonstrated that the RNA profile of frozen spermatozoa was considerably changed by the sperm cryopreservation procedure.

Several other studies have profiled spermatozoal RNAs in clinical samples with the aim of finding a marker RNA or a distinctive expression pattern to represent sperm quality. A cross-platform microarray strategy was used to assess the profile of human spermatozoal transcripts from 13 fertile males who had fathered at least one child compared to those from eight teratozoospermic individuals (Platts et al., 2007). This analysis successfully distinguished between the normal and teratozoospermic groups using unsupervised hierarchical clustering. The teratozoospermic group lacked the RNAs of genes related to the ubiquitin-proteasome pathway and those genes transcribed at late stages of spermatogenesis including; an egg-activating sperm factor, *PLCZ1*; acrosomal proteins, *ACRV1* and *SPAM1*; and non-tubulin components of sperm tails, *ODF1-4*. These changes in gene expression are indicative of the failure of late-stage spermatogenesis in teratozoospermia. In a study by Lalancette et al., the spermatozoal RNAs of 24 healthy donors were expression profiled and a series of invariable transcripts were consistently present in all of the donor samples (Lalancette et al., 2009). Based on the expression of these consistently-expressed genes, only a single donor sample was not well correlated with the other 23 samples, suggesting that spermatozoal RNA profiling could be clinically applied to mark outliers. Furthermore, Garcia-Herrero et al. compared the transcriptomic profiles of sperm samples that achieved pregnancy after the first IUI to those that did not (Garcia-Herrero et al., 2009). They identified 756 genes that were significantly preferentially expressed in the pregnant group, and 194 genes that were significantly preferentially expressed in the non-pregnant group (Garcia-Herrero et al., 2009). Interestingly, these 756 genes include 20 of the 44 genes that were overexpressed in group P (pregnant after an ICSI in oocyte donation program) in the current study by the same group described in this volume of *Reproductive BioMedicine Online*. These 20 genes out of the 756 genes could be considered as potential pregnancy success markers rather than potential fertilization makers.

Thus far, microarray technologies have been used to assess the profiles of human spermatozoal RNAs and the utility of spermatozoal RNAs as clinical markers of male infertility. An appropriate study design with a considerable number of biological replicates (sperm samples) is necessary to validate the accuracy and reproducibility of these microarray data. If the genes identified as sperm quality markers by microarray studies are successfully attributed to the pathogenesis of male infertility, then the microarray strategy may be used as a clinical diagnostic tool for male infertility. On the other hand, spermatozoal RNAs may contain not only RNAs left over from failed or abnormal spermatogenesis, but also RNAs that may contribute extragenomically to early embryonic development. Therefore, spermatozoal RNA profiling may enable a better understanding of what is contributed to the oocyte by sperm, in addition to their genome, to facilitate early embryonic development.

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