OPTIMIZATION OF A METHOD TO ISOLATE AND PURIFY HUMAN SPERM TRANSCRIPTOME INCLUDING miRNAs

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Introduction:

MicroRNAs (miRNAs) are molecules of 22-24nt involved in the regulation of gene expression (1, 2). They play an important role in different biological proceses: cell cycle regulation (3, 4), embryo development (5) and gametogenesis (6, 7). Some authors have recently identified altered expression profiles of miRNAs in germinal cells in cases of idiopathic male infertility suggesting its fundamental role in fertility regulation (6, 8).

Integrated into an overall project with the main objective to assess the participation of alterations in the sperm transcriptome as the underlying cause of idiopathic male infertility, the aim of this study was to optimize a method for purifying the total human sperm transcriptome.

Material and methods:

1.Sample collection and sperm purification

Thirty ejaculated samples from fifteen volunteers were obtained. Somatic cells present in the ejaculate were disrupted by Somatic Cell Lysis (SCL) method (9), that consists with a mixture of 0.1% SDS and 0.5% Triton X-100. The maximum ratio accepted was 1 somatic cell per 1,000 spermatozoa.
Jurkatt cell-line was used as control of RNA integrity.

2.RNA isolation

1. Total RNA was isolated using *TRIzol* method (10) followed by DNase treatment.
2. To confirm the absence of DNA, RT-PCR followed by PCR for *Protamine 1 (PRM1)* was performed. Exon-exon primers were used to distinguish between the amplification products resulting from RNA amplification from those resulting from potential DNA contamination.

3.RNA quantification

Total RNA concentration and purity were quantified using a *Nanodrop-2000*.

4.Transcriptome analysis

Transcriptome was analyzed by *Agilent-2100 Bioanalyzer* platform, using two chips:

1. *Small-RNA*: to assess the presence of miRNAs.
2. *Nano-RNA*: to discard the existence of somatic RNA in sperm samples whereas confirming the RNA integrity.

Results:

The SCL method removed the majority of somatic cells with a loss of 28.41% ±22.13 (range=6.2-67.6%) spermatozoa. This method allowed to obtain highly purified sperm fractions thus minimizing the presence of somatic RNA in the samples analyzed (fig.1).

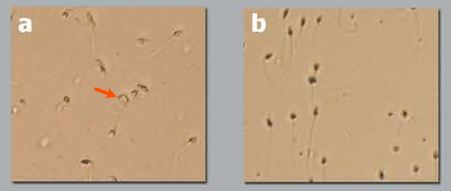

Figure 1:
Semen fraction before (a) and after (b) SCL method treatment. The arrow points a somatic cell. Note the absence of somatic cells following the treatment.

PCR results confirmed the absence of DNA in all the samples. Only one cDNA product with a length corresponding to *PRM1-mRNA* was amplified (fig.2).

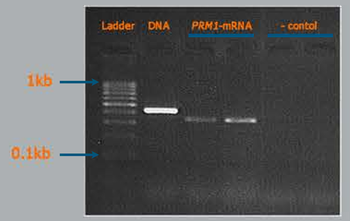

Figure 2:
Electrophoretic analysis (2% agarose) of spermatozoal RNA PCR products. Lane 2: positive control with DNA (422b). Lanes 3 and 4: spermatozoal cDNAs showing the 91b intronless cDNA product obtained from the reverse transcribed spermatozoal RNA (331b).

Sperm samples:
A260/A280 ratios ranged from 1.5-2.0 (average 1.70). The concentrations obtained described an average content of 20fg RNA/Spermatozoa which agreed with previously published data (11, 12).

Jurkatt samples:
A260/A280 ratios ranged from 1.9-2.0 (average 1.94). The concentrations obtained described an average content of 3900fg RNA/Jurkatt cell.

Small-RNA chips confirmed the suitability of the *TRIzol* method to isolate small RNAs (fig.3). As a mean, 58.23%±21.57 of the small RNA fraction correspond to miRNAs. *Nano-RNA* chips corroborated the proper isolation of the sperm fraction (absence of somatic RNA) and confirmed the optimal RNA integrity (RIN=9.45±0.37). RIN values were obtained from Jurkatt cells RNA (rRNA is not present in spermatozoa) and extrapolated to sperm samples (fig.4).

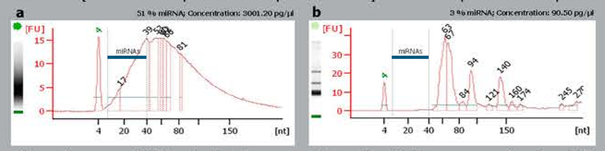

Figure 3: a. Elecropherogram of RNA extracted from spermatozoa. The fraction corresponding to miRNAs has been indicated. b. Electopherogram of RNA extracted from Jurkatt Cells. The three peaks displayed correspond to tRNAs (65nt), pre-miRNAs (90nt) and 5S-5.8S rRNAs (140nt).

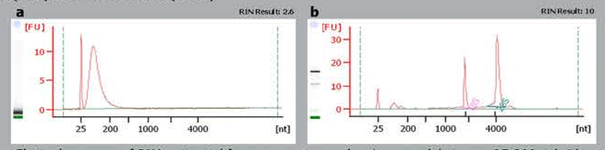

Figure 4: a. Electopherogram of RNA extracted from spermatozoa showing a peak between 25-200nt (without rRNAs). b. Electopherogram of RNA extracted from Jurkatt Cells. The two peaks displayed correspond to 18S rRNA (1869nt) and 28S rRNA (5025nt).

Conclusions:

The methodology described allows the obtainment of the total human sperm transcriptome, including miRNAs, of high quality and purity.

References: **1.** Ambros (2001) *Cell* 107, 823-826; **2.** Boyd (2008) *Lab Invest* 88, 569-578; **3.** Chan et al. (2005) *Cancer Res.* 65, 6029-6033; **4.** Lu et al. (2005) *Nature* 435, 834-838; **5.** Boerke et al. (2007) *Theriogenology* 68 Suppl 1, S147-155; **6.** Lian et al. (2009) *Reprod Biol Endocrinol* 7, 13-23; **7.** He et al. (2009) *Reproduction* 137, 901-911; **8.** B. McCallie et al. (2010) *Fertil Steril* 93:2374–2382; **9.** Goodrich et al. (2007) *Arch. Androl.* 53, 161-167; **10.** Chomczynski and N. Sacchi (2006) *Nat. Protoc.* 1, 581-585; **11.** Pessot et al. (1989) *Biochem. Biophys. Res. Commun.* 158, 272-278; **12.** Dadoune (2009) *Microsc Res Tech* 72, 536-551.

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