Spermatozoa from patients with seminal alterations exhibit a differential micro-ribonucleic acid profile

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Objective: To compare the microRNA (miRNA) expression profile in spermatozoa from three infertile populations vs. a group of fertile men.
Design: Evaluation of the expression level of 736 miRNAs in human spermatozoa using TaqMan quantitative reverse transcription–polymerase chain reaction.
Setting: University research facility.
Patient(s): Semen samples with a single seminal alteration were collected from infertile individuals: asthenozoospermic (n = 10), teratozoospermic (n = 10), and oligozoospermic (n = 10).
Intervention(s): None.
Main Outcome Measure(s): Correlation of the expression level of each miRNA with seminal parameters, age, and chromosome instability; clustering of the individuals according to their miRNA expression profiles and influence of the seminogram, age, chromosome instability, and assisted reproductive technology outcome in the clustering; analysis of the differentially expressed miRNAs (DE-miRNAs) in each infertile population; genome annotation of these DE-miRNAs; and ontological analysis of their predicted targets.
Result(s): The hsa-miR-34b-3p correlated with age, the hsa-miR-629-3p with sperm motility, and the hsa-miR-335-5p, hsa-miR-885-5p, and hsa-miR-152-3p with sperm concentration. The individuals clustered into two groups, and only the seminogram was differentially distributed. We identified 32 DE-miRNAs in the asthenozoospermic group, 19 in the teratozoospermic group, and 18 in the oligozoospermic group. The up-regulated miRNAs presented an enriched localization in introns, affecting relevant genes for spermatogenesis. The predicted targets of the DE-miRNAs contained critical genes associated to infertility, and their ontological analysis revealed significantly associated functions related to the seminal alterations of each group.
Conclusion(s): Spermatozoa from patients with seminal alterations exhibit a differential miRNA profile. This provides new evidence that miRNAs have an essential role in spermatogenesis, contributing to the mechanisms involved in human fertility. (Fertil Steril® 2015;104: 591–601. ©2015 by American Society for Reproductive Medicine.)
Key Words: Infertility, microRNA, spermatozoa, seminal alterations, sperm biomarkers

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Received March 26, 2015; revised May 22, 2015; accepted June 15, 2015; published online July 2, 2015.
A.S.-H. has nothing to disclose. J.B. has nothing to disclose. F.V. has nothing to disclose. A.G. has nothing to disclose. M.G. has nothing to disclose. M.C.P. has nothing to disclose. S.F.-F. has nothing to disclose. N.G. has nothing to disclose. E.A. has nothing to disclose.
Supported by the projects 2011FE16 (Ayudas Merck Serono 2011; Investigación Clínica en Fertilidad, Spain), FIS/PS09-00330 (Ministerio de Ciencia e Innovación, Gobierno de España, Spain), and 2014/SGR00524 (Agència de Gestió d'Ajuts Universitaris i de Recerca, Generalitat de Catalunya, Catalonia, Spain). A.S.-H. is the recipient of Personal Investigador en Formació grant 456-01-1/E2010 (Universitat Autònoma de Barcelona).
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Fertility and Sterility® Vol. 104, No. 3, September 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.06.015

Infertility is a disease defined by the failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (1). Fertility problems are estimated to affect 15% of couples worldwide, and a male factor contributes in approximately 50% of cases (2). Reduced male fertility can result from several reasons (e.g., urogenital abnormalities, infections of male accessory glands,

varicocele, endocrine disturbances, or immunologic factors), but the only abnormality detected in 40%–60% of patients is the presence of abnormal seminal parameters (3). Although in some cases the origin of these abnormalities has been associated with specific gene alterations (4), most of the time the cause remains idiopathic. In fact, the classification of infertile individuals mostly relies on the raw results derived from this conventional seminal analysis. The seminogram categorizes a semen sample only according the sperm physical status in relation to predefined universal thresholds, which in addition have been subjected to changes over time (1, 5). Nevertheless, this classification does not always match with the fertile potential of the individual, and several authors have claimed the need for new, additional male fertility indicators (6, 7). Because the ultimate factors that would determine the fertility potential of spermatozoa would depend on their genetic and epigenetic load, studies focused on defining and understanding infertility at a molecular level would be of great interest.

Spermatogenesis is a complex differentiation process commonly divided into three main phases: self-renewal and proliferation of spermatogonia, meiotic division of spermatocytes, and postmeiotic differentiation of spermatids into spermatozoa. These events are controlled by well-coordinated transcriptional and posttranscriptional regulators. Several studies have analyzed the role of noncoding RNAs as posttranscriptional regulators of spermatogenesis (8–10), including [1] long noncoding RNAs, which mainly participate as transcription repressors and chromatin modification factors, [2] piwi-interacting RNAs, which are known to be responsible for transposon silencing, and [3] microRNAs (miRNAs), which regulate the expression of specific target messenger RNAs (mRNAs) (11).

Focusing on this last group of regulators, miRNAs are small molecules of 22–24 nucleotides that form partially complementary structures to their mRNA targets. MicroRNAs are transcribed into primary miRNA transcripts that are further processed in the nucleus to form the hairpin-shaped miRNA precursor (pre-miRNA). These pre-miRNAs can be exported to the cytoplasm, where they will be processed again, leading to the formation of the mature miRNAs. Their assembly with Argonaute proteins will form the ribonucleoprotein miRNA-induced silencing complex that will be responsible for executing the functional translational repression associated with these molecules (12).

It has been estimated that miRNAs can regulate up to 60% of protein-coding genes (13), and some studies have described their implication in the gene expression regulation of many biological processes, including spermatogenesis (14, 15). Recently some authors have observed a dysregulated expression of several miRNAs related to the particular fertility conditions of the individuals analyzed (16–20). Although all of these articles suggest a relationship between miRNA sperm cargo and male fertility, there is great variability among studies, either due to the types of biological material analyzed (spermatozoa, testicular cells, and seminal plasma) or regarding the origin of the reference values used to identify the differentially expressed miRNA, and also due to the way of interpreting the biological consequences of the results (from simple revision of previous published data to ontological analysis).

Additionally, the sperm miRNA profile has also been described to affect both early embryo development and assisted reproductive technology (ART) outcome (15). McCallie et al. (21) detected differential miRNA profiles in embryos from fertile and infertile individuals. In this study a significant decrease in the expression of six miRNAs in transferable-quality blastocysts was described in couples with male factor infertility. Besides the association between altered miRNA profiles and fertility, other authors have described a differential miRNA profile in young and old individuals, pointing to a potential regulatory role of miRNAs in the aging process (22). Furthermore, a differential expression of specific miRNAs has been associated with chromosome instability in tumoral processes (23–26). Because age (27) and sperm chromosome instability (28) are two of the factors related to male fertility, it would be interesting to consider the influence of these parameters over the sperm miRNA cargo in infertile patients.

In a recent study, we performed a comprehensive characterization of the miRNA expression profile in spermatozoa from 10 fertile individuals (29). Our results provided control reference values for 736 miRNAs that can be useful for determining the contribution of miRNAs as a possible underlying cause of idiopathic male infertility. We demonstrated that human sperm contain a stable population of miRNAs potentially related to embryogenesis and spermatogenesis.

The present study aimed to characterize the miRNA expression profile in spermatozoa from three different human infertile populations with a sole seminal parameter altered: a group of individuals with reduced sperm motility (asthenozoospermia), another group with abnormal sperm morphology (teratozoozpermia), and a third group with low sperm count (oligozoospermia). The analysis has been addressed to [1] evaluate a possible correlation between the expression level of every evaluated miRNA and the seminal parameters, age, and chromosome instability of the individuals analyzed; [2] cluster the individuals according to their miRNA expression profiles and determine the influence of the seminogram, age, chromosome instability, and ART outcome in the classification obtained; [3] identify differentially expressed miRNAs (DE-miRNAs) in each infertile population when compared with a previously described fertile control population (29); [4] evaluate the characteristics of the hosting regions encoding the DE-miRNAs; and [5] define the biological functions significantly associated with the predicted targets of these DE-miRNAs.

MATERIALS AND METHODS

Study Population and Sample Collection

Inclusion criteria for patient recruitment were directed toward the selection of semen samples from infertile individuals of unknown etiology showing the alteration of a single seminal parameter (i.e., reduced sperm motility, or abnormal sperm morphology, or lower sperm count) (World Health Organization 2010) (1, 30). Individuals presenting more than one alteration were discarded. According to