Noncoding RNA Profiling as an Indicator of Male Gamete Reproductive Potential

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ABSTRACT

Objective: In men with unexplained infertility, supplementary tests may be pivotal to gain insight into the paternal contribution to the zygotic genome. Noncoding RNAs (ncRNA) are functional and regulatory RNA molecules that are transcribed from DNA but not translated into proteins. We aimed to determine if sperm ncRNA have an influential role in profiling the infertile male partner and predict his ability to achieve a viable pregnancy.

Design: In the course of a 15month period, we assessed the RNA profile of men undergoing infertility screening with specific focus on ncRNA via next generation RNA-Sequencing (RNA-Seq). RNA extraction was carried out on semen samples provided by 25 consenting men undergoing infertility screening. The analysis was performed by measuring the abundance of small noncoding RNA (sncRNA) and long noncoding RNA (lncRNA) in relation to ICSI reproductive outcome. A cohort of couples unable to sustain a pregnancy were compared to a men able to conceive naturally.

Materials and Methods: Human semen specimens ranging in concentration from 7 to 25x106/mL were utilized to isolate total RNA using a spin column commercial kit. Their spermatozoal nucleic acid quality and concentration were measured. Expression values were calculated in fragments per kilobase of transcript per million mapped reads (FPKM).

Results: Of the 25 men screened, 8 were selected for sperm RNA-Seq with an average female partner age of 34.8±3 years and male age of 26±5 years that presented with a sperm concentration of 27.3±27x10⁶/mL, motility of 46.6±24%, and normal morphology. From the 23,260 genes assessed, statistical analysis evidenced 28 (0.12%) ncRNAs that were differently expressed (P<0.0005) between the control cohort (n=3) and men (n=5) treated by ICSI (female partner age 34.8 ± 3) achieving a fertilization of 71.4% (30/42) but not capable of sustaining a pregnancy. Additionally, these RNA genes (n=16) were completely unexpressed in the cohort of men unable to conceive. Of these transcripts, 21/28 (75.0%) were long noncoding RNA and 7/21 (25.0%) were small noncoding RNA. In relation to their function, most of these (11/16, 68.8%) nonproteincoding RNA are considered to guide chemical modification of other RNAs, influence methylation, and modulate stability and translation of messenger RNA. Interestingly, 13/16 (81.3%) of these ncRNA were located on autosomes, while only three genes were located on the sex chromosomes; following similar distribution of the spermatogenesis related genes.

Conclusions: The ncRNAs contributed by the spermatozoon at the time of fertilization are chiefly regulatory molecules that can affect embryo development. Profiling men via RNA-seq to supplement standard semen analysis may aid in the diagnosis and management of these couples with unexplained infertility. Screening men for an epigenetic imbalance of sncRNA and lncRNA provides crucial information on the etiology of unexplained infertility and overall reproductive capacity of the infertile male.

INTRODUCTION

In men with unexplained infertility, supplementary tests may be pivotal to gain insight into the paternal contribution to the zygotic genome. Noncoding RNAs (ncRNA) are functional and regulatory RNA molecules that are transcribed from DNA but not translated into proteins. We aimed to determine if sperm ncRNA have an influential role in profiling the infertile male partner and predict his ability to achieve a viable pregnancy.

METHODS

In the course of a 15month period, we assessed the RNA profile of men undergoing infertility screening with specific focus on ncRNA via next generation RNA-Sequencing (RNA-Seq). RNA extraction was carried out on semen samples provided by 25 consenting men undergoing infertility screening (Figure 1). The analysis was performed by measuring the abundance of small noncoding RNA (sncRNA) and long noncoding RNA (lncRNA) in relation to ICSI reproductive outcome. A cohort of couples unable to sustain a pregnancy were compared to a men able to conceive naturally.

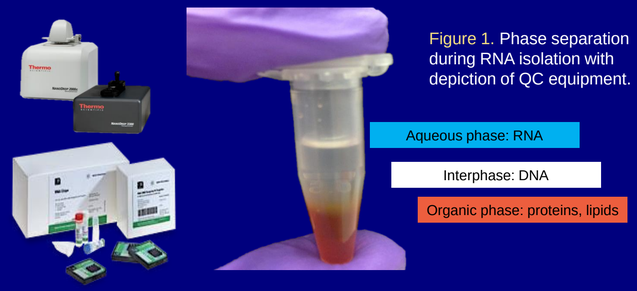

Figure 1. Phase separation during RNA isolation with depiction of QC equipment.

Human semen specimens ranging in concentration from 7 to 25x106/mL were utilized to isolate total RNA using a spin column commercial kit. Their spermatozoal nucleic acid quality and concentration were measured. Expression values were calculated in fragments per kilobase of transcript per million mapped reads (FPKM).

RESULTS

A total of 25 consenting men provided semen samples and we were able to get adequate nucleic acid that was processed for RNA-Seq. Participants had an average age of 26±5 years and presented with a sperm concentration of 27.3±27x10⁶/mL, a motility of 46.6±24%, and normal morphology (Table 1).

Participants	
Infertile Men	25
Male age (M yrs ± SD)	39.6 ± 5
Concentration (M x10⁶/ml ± SD)	46.3 ± 19
Motility (M% ± SD)	44.8 ± 14

Table 1. Patient characteristics and semen parameters.

	NC	ART
Couples	3	5
Female Age (M ± SD)	38.3 ± 5	34.8 ± 1
Male Age (M ± SD)	33.0 ± 7	37.6 ± 3
Cycles	-	5
Fertilization (%)	-	30/42 (71.4)
Clinical Pregnancy (+FHB)	3	0
Delivered	3	0

Table 2. Clinical outcome of fertile couples compared to couples attempting ART.

From the 23,260 genes assessed, statistical analysis evidenced 28 (0.12%) ncRNAs that were differently expressed (P<0.0005) between the control cohort (n=3) and men (n=5) treated by ICSI (female partner age 34.8 ± 3) achieving a fertilization of 71.4% (30/42) but not capable of sustaining a pregnancy (Table 2) (Figure 2).

Additionally, these RNA genes (n=16) were completely unexpressed in the cohort of men unable to conceive. Of these transcripts, 21/28 (75.0%) were long noncoding RNA and 7/21 (25.0%) were small noncoding RNA (Table 3).

Gene	Chr	Description	Control	Study	*P*-Value
SNAR-C3	19	Non-coding RNA class	209.9	0	0.006
SNORD104	17	Small Nucleolar RNA, C/D Box 104, primarily guide chemical modifications of other RNAs, associated with methylation	1823.7	0	0.008
MIR3687	21	Involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs	8442.3	0	0.008
GNG8	19	Signal transducer activity and GTPase activity.	11.1	0	0.009
TTTY18	Y	Testis-Specific Transcript, Y-Linked	8.9	0	0.013
LOC644145	4	Exocyst Complex Component 1 Pseudogene	6.9	0	0.016
MIR181C	19	Involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.	397.3	0	0.017
LOC401164	4	Unannotated	5.1	0	0.018
TTTY22	Y	Testis-Specific Transcript, Y-Linked	10.6	0	0.024
TTTY16	Y	Testis-Specific Transcript, Y-Linked	11.6	0	0.035
AVP	20	Encodes a precursor protein consisting of arginine vasopressin and two associated proteins, neurophysin 2 and a glycopeptide, copeptin.	10.9	0	0.038
SNORD68	16	Small Nucleolar RNA, C/D Box 68, primarily guide chemical modifications of other RNAs, associated with methylation	3409.8	0	0.063
ALOX15P1	17	Arachidonate 15-Lipoxygenase Pseudogene 1	9.5	0	0.082
MIR3689B	19	Involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.	68.4	0	0.087
MIR636	17	Involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.	526.5	0	0.134
RNU5D-1	5	RNA, U5D Small Nuclear 1	18732.3	0	0.143

Table 3. List of ncRNA that are present in the control group but not expressed in the study cohort.

RESULTS cont'd

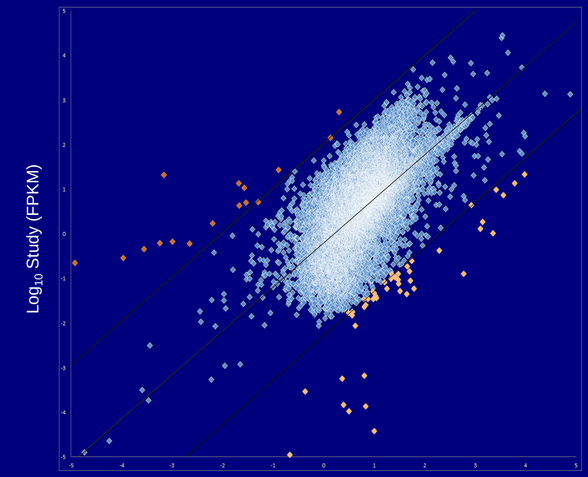

Figure 2. Log values of the study and control plotted to identify genes over or under expressed.

In relation to their function, most of these (11/16, 68.8%) non protein coding RNA are considered to guide chemical modification of other RNAs, influence methylation, and modulate stability and translation of messenger RNA. Interestingly, 13/16 (81.3%) of these ncRNA were located on autosomes, while only three genes were located on the sex chromosomes; following similar distribution of the spermatogenesis related genes (Figure 3).

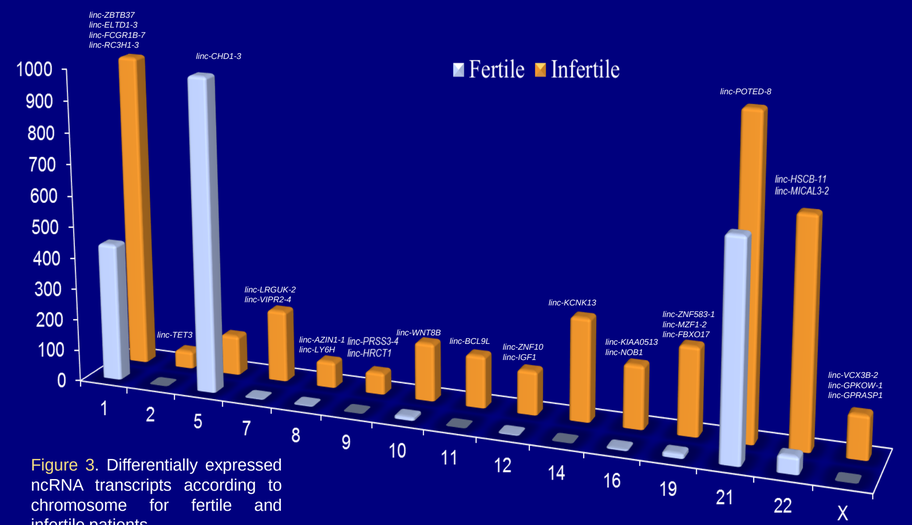

Figure 3. Differentially expressed ncRNA transcripts according to chromosome for fertile and infertile patients.

CONCLUSIONS

The ncRNAs contributed by the spermatozoon at the time of fertilization are chiefly regulatory molecules that can affect embryo development. Profiling men via RNA-seq to supplement standard semen analysis may aid in the diagnosis and management of these couples with unexplained infertility. Screening men for an epigenetic imbalance of sncRNA and lncRNA provides crucial information on the etiology of unexplained infertility and overall reproductive capacity of the infertile male.