Clin. Lab. 2024;70:XXX-XXX

ORIGINAL ARTICLE

Identification of Potential Biomarkers Associated with Spermatogenesis in Azoospermia

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SUMMARY

Background: **Azoospermia, characterized by the absence of spermatozoa in the ejaculate, affects approximately 1% of all men and 10 - 15% of infertile males, representing the most severe form of male infertility. It is classified into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA), with the latter often resulting from unexplained failures in spermatogenesis. This study endeavored to clarify the molecular underpinnings of spermatogenesis in NOA and to identify viable therapeutic targets.**

Methods: **We analyzed expression data from NOA and normal spermatogenesis samples obtained from the GEO database. Differential expression analysis was performed to identify differentially expressed genes (DEGs). We then intersected these DEGs with genes known to be related to spermatogenesis to pinpoint spermatogenesis-related DEGs specific to NOA. Subsequent analyses, including gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichments, aimed to elucidate potential signaling pathways involved. A protein-protein interaction (PPI) network was constructed to highlight hub genes, whose diagnostic potential was assessed by using ROC curve analysis. Additionally, miRNA and transcription factor (TF) regulatory network for hub genes were analyzed. The efficacy of identified hub genes as biomarkers was validated through RT-qPCR and Western blotting in a mouse model of NOA.**

Results: **This study identified 68 NOV-specific spermatogenesis-related genes. Enrichment analyses in GO and KEGG pathways highlighted their involvement in cellular processes related to reproduction in multicellular organism and endocrine and other factor-regulated calcium reabsorption. Seven hub genes were identified, with ROC curve analysis affirming their significant diagnostic value. Constructed networks revealed intricate interactions among miRNAs, hub genes, and TFs.**

Conclusions: **We identified seven hub genes (CATSPER1, CATSPER3, CATSPER4, CATSPERG, OAZ3, ODF1, and SUN5) significantly associated with spermatogenesis in NOA, demonstrating their potential as biomarkers for diagnosing and monitoring the disease.**

(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240541)

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Manuscript accepted July 3, 2024

Supplementary Data

Table S1. Primers for Real Time PCR target genes.

Genes	Sequences
GAPDH	5'-TCAGTTCGGAGCCCACACGC-3'
	5'-ACCAGGGAGGGCTGCAGTCC-3'
CATSPER1	CACGCTGATCACCATCATGAGGGCC
	GCAACGGCACTATCACGGAGATC
CATSPER3	AAAATGTCCCAACATTTTCACC
	GGCAAGACTGAGCAAGATTA
CATSPER4	TACAGGAATTCATCACTCAAAT
	CTTTCTGCTGCTGTCCAACG
CATSPERG	TTTTGAGGAACGGTCTGGAGGA
	GTGGGACAGGGTTGATGGAGC
OAZ3	AAACGAGAGAGTCGATCTTACAAAC
	CACCGAACTGCTACCTTGATTAGAC
ODF1	CACCGAAAACCCTCCAAAGAAATAG
	AAACCCTATTTCTTTGGAGGGTTTTC
SUN5	CACCGCTTGCTGTGAAAGTAAATGC
	AAACGCATTTACTTTCACAGCAAGC

GAPDH - Glyceraldehyde-3-phosphate dehydrogenase, CATSPER - cation channel of sperm, OAZ3 - ornithine decarboxylase antizyme 3, ODF1 - outer dense fiber gene, SUN5 - SUN domain-containing protein 5.

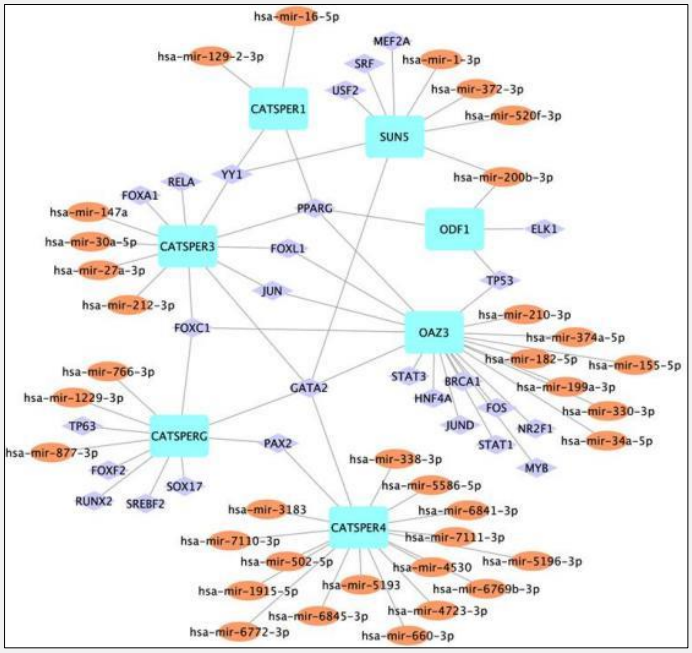

Figure S1. The miRNAs-genes-TFs network.