**Deciphering the Role of DOT1L in Spermatogenesis: Insights from Single-Cell RNA Sequencing**

**Danial Hashemi Karoii (M.Sc.) 1, Zahra Hasani Mahforoozmahalleh (M.Sc.) 2 and Hossein Azizi (Ph.D.) 3\***

1. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran. Email: [d.hashemi.karoii@ut.ac.ir](mailto:d.hashemi.karoii@ut.ac.ir)

2. Department of Microbial Biotechnology, Amol University of Special Modern Technologies, Amol, Iran, Email: [Zahra.hasani@ausmt.ac.ir](mailto:Zahra.hasani@ausmt.ac.ir)

3. Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran, Email: [h.azizi@ausmt.ac.ir](mailto:h.azizi@ausmt.ac.ir)

**\*Corresponding author address: Hossein Azizi, Email: H.azizi@ausmt.ac.ir**

**Tel.: +981144153452, +989112206741; Fax: +981144154265**

**Abstract:**

Spermatozoa exhibit a distinct genomic organization characterized by chromatin that is largely devoid of histones and instead comprising protamines. This unique composition offers enhanced compaction and safeguards the paternal genome until fertilization. The critical transition from histones to protamines, essential for producing functional sperm, occurs in spermatids. Our study highlights the role of H3K79‐methyltransferase disruptor of telomeric silencing-1 (DOT1L) in this process. DOT1L facilitates spermatid chromatin remodeling, aiding in the reorganization and compaction of the spermatozoon genome. It also alters chromatin before histone removal, affecting genes linked to flagellum development and apoptosis during spermatid differentiation. Consequences of disrupted DOT1L activity include less compact sperm heads and reduced motility, leading to compromised fertility.

We utilized single-cell RNA sequencing (GEO access number: GSE216907 and their platform: Illumina HiSeq 2000) to analyze tissue and organ microenvironments at a molecular and single-cell level. For this purpose, 5 adult testicular samples with normal spermatogenesis and 7 samples from individuals with non-obstructive azoospermia (NOA) were examined to assess DOT1L's role in spermatogenesis. This technology revealed that DOT1L expression is lower in spermatogonia, spermatocytes, and sperm.

Furthermore, our analysis of the protein-protein interaction database indicated that DOT1L interacts with Histone H2B type 1-J (H2BC11), Histone H2B type 1-K (H2BC12), Histone H3.1 (H3C12), and Protein AF-10 (MLLT10). These interactions suggest that DOT1L and these proteins play a collective role in the differentiation of spermatocytes into sperm.

Our research indicates that the identified genes and their associated hub proteins are likely key factors in understanding the pathophysiology of germ cell abnormalities and genomic integrity in mitosis and meiosis. These genetic and protein interactions may provide critical insights into the underlying mechanisms driving these conditions, potentially leading to more targeted and effective treatments for infertility related to germ cell dysfunction.

**Keywords**: Spermatozoa, Protamines, Methyltransferase, DOT1L, Single-Cell RNA Sequencing, spermatogenesis