Decoding Idiopathic Male Infertility: A Molecular Perspective on Spermatogenesis and Fertilization

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Abstract- Male infertility significantly impacts approximately half of all couples struggling with infertility. A notable proportion of male infertility cases are classified as idiopathic, where the cause remains unidentified. Traditionally, sperm DNA has been the primary focus in understanding the male's contribution to fertilization. However, recent studies emphasize the critical roles of various sperm transcripts and proteins in processes like the acrosome reaction, sperm-oocyte fusion, and subsequent embryo development. This review offers a thorough and current examination of the molecular biology underlying spermatogenesis. It highlights the evidence for spermatogenetic failure, with a particular focus on the molecular factors carried by sperm that are vital for oocyte fertilization and embryonic growth. This information serves as a foundational step toward discovering new diagnostic markers and potential therapeutic targets for what is currently classified as idiopathic male infertility.

I. Introduction

Infertility, defined as the inability to achieve pregnancy after 1-2 years of regular, unprotected intercourse, is a prevalent issue in industrialized nations, affecting up to 15% of couples of reproductive age. Various factors can cause infertility, including issues with gamete production in either partner, difficulties in gamete interaction or fusion, and challenges with embryo development. Although several etiological factors, such as disorders of gametogenesis, gamete quality issues, and genital tract dysfunctions, have been identified, the underlying cause remains unknown in many cases.

Male factor infertility contributes significantly, accounting for half of all instances of couple infertility and being the sole cause in 30% of these cases. Despite this, the male partner's role in infertility and recurrent pregnancy loss (RPL) is sometimes underemphasized. Historically, research has focused on sperm DNA, which led to the development of assisted reproductive technologies (ART) designed to inject sperm DNA directly into the oocyte. However, the success rates of ART suggest that factors beyond DNA fragmentation are crucial.

Alarmingly, in about 70% of infertile couples where the male partner is affected, the cause of infertility remains unidentified, underscoring the need for more in- depth research into molecular targets.

Spermatogenesis is a highly intricate process, where spermatogonial stem cells (SSCs) proliferate and differentiate into spermatozoa. Advances in "omic" technologies have uncovered the complexity of spermatozoa, which carry a diverse array of RNAs and proteins. Investigating the contributions of the sperm genome, transcriptome, proteome, and epigenetic regulation to embryo development is essential for identifying novel molecular targets associated with male infertility.

This review seeks to provide a comprehensive and up-to-date understanding of the molecular biology involved in spermatogenesis. It emphasizes the role of molecular factors carried by sperm, produced during spermatogenesis, that are critical for the fertilization of the oocyte and the development of the embryo.

II. Physiology of Spermatogenesis

Spermatogenesis is an intricate, 74-day process that transforms spermatogonial stem cells (SSCs) into fully mature spermatozoa. This process occurs within the seminiferous tubules of the testes and involves three major stages: mitosis, meiosis, and spermiogenesis. The success of spermatogenesis depends on complex interactions between various cell types, including Sertoli cells, germ cells, and epithelial tubular cells, as well as the integrity of the blood-testis barrier (BTB).

SSCs possess the unique ability to both self-renew, thereby maintaining their population, and to differentiate into A-paired (Ap) spermatogonia. These Ap spermatogonia then undergo further development into A-aligned (Aal) spermatogonia through repeated cell divisions. Specific genes regulate the activities of SSCs, Ap, and Aal spermatogonia, controlling processes such as self-renewal, proliferation, and differentiation. Examples of these regulatory genes include Grfα1, Ret, Nanos, and Plzf. Sertoli cells, which are stimulated by follicle-stimulating hormone (FSH), secrete growth factors such as glial cell line-derived neurotrophic factor (GDNF). GDNF prompts Aal spermatogonia to differentiate further into A1 spermatogonia.

Through a series of mitotic divisions, these cells then become A2, A3, A4, and eventually B spermatogonia. The transition from undifferentiated to differentiated spermatogonia (A1-4 and B) requires the downregulation of self- renewal genes and the upregulation of genes associated with differentiation, such as Sohlh1, Sohlh2, and Dnmt1. B spermatogonia subsequently give rise to preleptoten spermatocytes, which must maintain close contact with Sertoli cells and rely on a functional BTB to progress into the adluminal compartment and develop into leptotene, zygotene, and pachytene spermatocytes.

Preleptotene spermatocytes enter a 16-day meiotic division, ultimately producing secondary spermatocytes. The first stage of meiosis, prophase I, is particularly critical, as it involves the formation of double-strand breaks (DSBs), pairing of homologous chromosomes, and crossing over (CO). A variety of proteins with enzymatic activity, such as PLK-4, SPO11β, and RPA1, play key roles in ensuring accurate chromosome segregation, CO recombination, and DSB repair.

Following the completion of crossing over, homologous chromosomes separate, a process dependent on intact intercellular bridges. These bridges, which are essential for communication and synchronization between germ cells, involve proteins like TEX14, a critical component linked to spermatogenetic failure (SPGF).

Secondary spermatocytes proceed through meiosis II, where sister chromatids are separated, resulting in haploid round spermatids. During the final stage, spermiogenesis, the DNA of these spermatids is repackaged, and the acrosome and flagellum form, with excess cytoplasm being discarded. This 26-day process culminates in the formation of mature spermatozoa.

Mature spermatozoa are highly specialized cells, designed for the specific task of actively swimming through the female reproductive tract and successfully penetrating the oocyte. Due to their limited ability to neutralize toxins and their susceptibility to damage from reactive oxygen species (ROS), environmental factors that disturb the delicate balance between pro-oxidants and antioxidants can significantly impair sperm fertility.

Figure 1 likely illustrates various molecular factors that are involved in spermatogenesis, with mutations in the genes encoding these factors linked to spermatogenetic failure (SPGF). Beyond these proteins, RNA processing plays a complex role in mammalian spermatogenesis. Specifically, post-transcriptional modifications of mRNAs and long non-coding RNAs (lncRNAs), such as the reversible N6-methylation of adenosine residues (m6A), are crucial. This modification process involves a complex of proteins, including METTL3, METTL4, WTAP, RBM15, and KIAA1429, which add methyl groups, as well as proteins like FTO and ALKBH5 that remove them. Recent research indicates that these m6A RNA modifications are essential for spermatogenesis in mice.

III. Physiology of Fertilization and Embryo Development

Fertilization is a complex, multi-step process that begins when a sperm reaches an oocyte. It involves several critical stages:

- Capacitation: This stage involves various changes within the sperm, preparing it for fertilization. These changes only occur after the sperm has bound to the outer layer of the egg.
- Hyperactivation: At this point, the sperm's tail movement intensifies, enabling it to propel itself more effectively.
- Acrosome Reaction (AR): The acrosome, a cap-like structure at the head of the sperm, releases enzymes that help the sperm penetrate the protective outer layer of the egg, known as the zona pellucida (ZP).
- ZP Binding: The sperm's surface receptors recognize and bind to specific proteins within the zona pellucida, namely ZP1, ZP2, and ZP3.
- ZP Penetration: The sperm navigates through the zona pellucida with the assistance of enzymes and its hyperactive tail movement.
- Sperm-Oocyte Fusion: The membranes of the sperm and egg merge, allowing the sperm's contents, including DNA and the centriole, to enter the egg.
- Oocyte Activation: This triggers mechanisms within the egg that prevent additional sperm from entering. Once the sperm has entered the egg, the sperm and egg nuclei (pronuclei) undergo dramatic changes in preparation for fusion:
- Chromatin Remodeling: The tightly packed sperm DNA, which is initially organized with protamines, is reorganized with histones derived from the mother.
- DNA Methylation Changes: The sperm's DNA undergoes rapid demethylation, while the maternal DNA demethylates more slowly. Certain imprinted regions, which are controlled by the parent of origin, retain their methylation.
- Imprinted Genes: Genes that are imprinted and carried by the sperm, such as IGF2, may play a vital role in early embryonic development. Paternally imprinted genes are generally believed to promote fetal growth, whereas maternally imprinted genes may restrict it.

The sperm's DNA begins transcription even before the pronuclei fuse. Between the 4-cell and 8-cell stages in human development, the embryo genome becomes activated (EGA). Prior to this point, all developmental processes depend entirely on the molecules, such as RNA and proteins, that were present in the original sperm and egg.

After the pronuclei fuse, the zygote starts to undergo cell division, eventually forming a morula and then a blastocyst within approximately six days. The blastocyst sheds its zona pellucida and implants into the uterus, where further differentiation occurs, leading to the formation of the ectoderm, mesoderm, and endoderm—the essential layers that give rise to a fully functional organism.

IV. Discussion

Recent advancements in the understanding of sperm "omics" are reshaping our perception of the sperm's role in fertility. It is becoming increasingly evident that sperm are not merely DNA carriers; they also transport a wealth of molecules, including transcripts, proteins, and metabolites, which may be crucial for successful embryo development.

Particularly noteworthy are paternally-expressed imprinted genes, which may be transcribed within the sperm itself. These genes often exhibit low methylation patterns, a characteristic associated with active transcription. For example, IGF2 mRNA is present in human sperm, and the IGF2 protein is a known growth factor.

It is plausible that IGF2, carried by sperm, could play a role in early embryonic cell division, both before and after embryo genome activation (EGA). If this hypothesis is confirmed, IGF2 could become an invaluable target for diagnosing and potentially treating cases of idiopathic male infertility.

In addition to IGF2, "omic" analyses are uncovering numerous other factors carried by sperm. Future research must rigorously evaluate the levels of these molecules in both fertile and

infertile men, along with data from assisted reproductive technologies (ART), to determine their precise role in fertility. These insights may eventually lead to groundbreaking advancements in the clinical treatment of male infertility.

V. Conclusion

This review has highlighted the growing body of evidence suggesting that sperm play a more complex role in fertilization than merely delivering DNA. Sperm carry an array of transcripts and proteins that significantly influence embryo development. A deeper understanding of these molecules has the potential to revolutionize the way we diagnose and treat cases of male infertility that are currently considered idiopathic.

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