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Review Article

The Role of Circular RNAs in Male Infertility and Reproductive Cancers: A Narrative Review

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Abstract

Infertility is a global health problem affecting about 15% of all couples, of which 50% are due to male infertility. Although the etiology of infertility is known in most infertile men, idiopathic male infertility remains a challenge. Therefore, there is a need for novel diagnostic methods to detect the underlying mechanisms and develop appropriate therapies. Recent studies have focused on the role of non-coding RNAs (ncRNAs) in male infertility. Circular RNAs (CircRNAs), a type of ncRNAs, are found to play a key role in the development of some pathological conditions, including cardiovascular diseases, diabetes, cancers, autoimmune diseases, etc. Several studies have reported the presence of CircRNAs and their target genes in the human reproductive system. In addition, their expression in testicular tissues, sperm cells, and seminal fluid has been identified. Abnormal expression of CircRNAs has been associated with azoospermia and asthenozoospermia in infertile men. The present narrative review provides a brief description of the role of CircRNAs in spermatogenic cells, male infertility, and reproductive cancers. In addition, some CircRNAs have been identified as potential biomarkers for disease detection and treatment.

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What's Known

- Circular RNAs (CircRNAs) are highly expressed in human testes, especially in sperm cells, and affect sperm quality.
- CircRNAs are shown to play a role in male infertility-related diseases.

What's New

- An overview of the association of CircRNAs with problems related to the male reproductive system is presented.
- CircRNAs can be transferred from sperm to oocyte during fertilization, indicating their role in embryo development. They can be used as reliable biomarkers for disease detection and treatment.

Introduction

Infertility is one of the most common chronic diseases of the reproductive system affecting approximately 15% of all couples.[1] In recent decades, male infertility has increased worldwide and now accounts for up to 50% of infertile couples.[2,3] Factors affecting male infertility include genetic disorders, congenital anomalies, hormonal imbalance, infection, psychological distress, lifestyle, and environmental factors.[4] However, in 30% to 50% of men, the exact mechanism causing infertility is unknown.[5] Consequently, a proportion of infertile people will not receive proper medical treatment.[6,7] Therefore, there is a need to develop novel diagnostic methods to improve male fertility. Recently, several studies have addressed the potential of non-coding RNAs (ncRNAs) as diagnostic and therapeutic molecules.[8-10] ncRNAs regulate gene expression in different processes such as transcription, translation, RNA splicing, and editing, gene imprinting, and chromosome separation and maintenance. These RNAs include rRNA, tRNA, small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), small interfering RNA (siRNA), and others.[11]

Circular RNA (CircRNA) has attracted much attention in recent years.[12] In the past, CircRNAs were thought to be abnormal molecules resulting from a splicing error. However, today, we know that their expression is highly regulated.[13, 14] CircRNA molecules do not have a 3' poly (A) tail and 5' cap and may build up in certain tissues or cells due to their specific stability compared to linear RNAs.[15, 16] Some studies have reported a possible association between CircRNAs and some pathological conditions, e.g., cardiovascular diseases,[17, 18] diabetes,[19] autoimmune diseases (multiple sclerosis),[20] cancers,[21-23] Alzheimer's,[24] and age-related diseases.[25] Recently, the expression of CircRNAs in reproductive tissues such as the testes,[12] placenta,[26], and ovarian cells[27] have been reported. It is also shown that CircRNA expression is stage-specific in pre-implantation embryos and may be involved in embryo development.[28]

Given the role of CircRNA in various reproductive organs and cells, as well as associated diseases, there is a need for further studies on this RNA molecule. In the present study, we provide an overview of the association between CircRNAs and problems related to the male reproductive system (table 1). The information will help other researchers to develop potential diagnostic and therapeutic methods.

Types of CircRNAs

Three main types of CircRNAs have been identified, namely exonic CircRNA (eCircRNA),[36] circular intronic RNA (ciRNA),[37] and exon-intron CircRNA (EIciRNA).[38] The eCircRNA is located primarily in the cytoplasm and comprises more than 80% of all CircRNAs in the cell.ciRNA and EIciRNA are also found in the nuclei.[39]

Regulation and Biogenesis of CircRNA

The exact mechanism of CircRNA biogenesis remains poorly understood. However, spliceosomal machinery has been suggested as the modulator of the process. Generally, a mature linear RNA transcript with a polarity of 5' to 3' is generated when the spliceosome cleaves out the introns of a pre-mRNA. This process is initiated when a 2'-OH in an intronic conserved adenine nucleotide attacks the 5'-splice site, resulting in the formation of a lariat-3'-exon and a 3'-OH available at the end of 5'-exon. It is followed by a nucleophilic attack on the 3' splice site by the generated 3'-OH leading to the separation of the lariat structure and joining of exons in the next phase.[40-42] Through back-splicing, the binding of the 3′ to 5′ end of an exon may occur or a 3' end of an exon connects to the 5′ end of an upstream exon that forms CircRNAs.[38, 43-45] It was shown that CircRNAs generation requires splicing signals in the back-splicing mechanism.

Table 1: An overview of biological functions, expression level, and diseases associated with CircRNA in the reproductive system

Article	Biological functions	Expression level	Disease	CircRNAs
Kong Z et al.[29]	Oncogenic activity	Significantly increased in blood serum and prostate tissue	Prostate cancer	CircFOXO3
Liu L et al.[30]	Spermatogenesis	Downregulated in a seminal plasma sample and upregulated in a blood sample	Idiopathic non-obstructive azoospermia	hsa_circ_0049356
Mo-Qi Lv et al.[31]	1. Negatively correlated with the Johnsen score. 2. Associated with a low rate of successful testicular sperm retrieval. 3. Inhibitor of spermatogenesis by suppressing miR-449 activity.	Upregulated in testicular tissue specimens	Non-obstructive azoospermia	hsa_circ_0000116
Ji C et al.[32]	Predictive biomarkers for successful sperm retrieval	Higher expression level in both seminal plasma and testes of patients after successful sperm retrieval	Idiopathic non-obstructive azoospermia	hsa_circ_0007773 hsa_circ_0060394 hsa_circ_0000277
Manfrevola F et al.[33]	Regulation of gene expression (*CRISP2*, *PATE1* and *CATSPER1*)	Downregulated in poor-quality sperm	Asthenozoospermia	CircEPS15, CircRERE CircTRIM2
Chen D et al.[34]	Aggressiveness of cancer	Upregulated in prostate cancer tissue	Prostate cancer	CircHIPK3
Kong Z et al.[35]	Oncogenic activity	Upregulated in plasma samples and cancer tissues	Prostate cancer	CircNFIA
	Tumor suppressor	Downregulated expression level	Prostate cancer	CircZNF561

Moreover, splice site mutation results in decreased circulation efficiency. Furthermore, inhibition of conventional spliceosome decreases both linear and circular RNAs formation.[46] However, a previous study showed that blocking spliceosome resulted in a higher CircRNA formation while lowering the amount of related linear RNAs.[47] Exon skipping and direct back-splicing mechanisms have been shown to generate eCircRNA and EIciRNA.[36, 48] Using an exon skipping model, CircRNA is generated by alternative splicing of pre-mRNA followed by back-splicing.[49] In a direct back-splicing model, the process occurs by pairing flanking introns or RNA-binding proteins, which brings the upstream and downstream splice sites closer together.[49] CircRNA is then directly generated in addition to an exon-intron-exon structure that can be processed or probably degraded (figures 1a and 1b).[49]

The biogenesis of CiRNA requires a known motif consisting of Seven nucleotides (GU-rich) at the 5' junction and 11 nucleotides (C-rich) at the side of the branchpoint (figure 1c). These motifs have not been observed in other types of CircRNAs nor regular introns.[37] After splicing, they may develop when lariats escape exonucleolytic degradation leading to the 2' 5' circle formation.[37]

CircRNAs synthesis can be regulated by trans-factors (e.g., RNA binding proteins [RBPs]) and cis-elements (e.g., intronic sequences [inverted *Alu* repeats]) (figure 2).[38, 44, 50] Some RBPs (NF90/NF110,[51] FUS,[52] Muscleblind Like Splicing Regulator 1,[44] Quaking,[53] DHX9,[54] NOVA2,[55] and others[56]) were shown to interact with flanking intron sequences to regulate CircRNAs generation. Exon circularization is aided by inverted repeating Alu pairs in flanking introns, whereas pairings in the same intron facilitate linear splicing.[57]

Biological Functions of CircRNAs

The main function of CircRNA is still unknown, however, it has been shown that CircRNAs participate in various cellular processes including a potential role in regulating gene expression. They can act as miRNA sponges that inhibit miRNA function and consequently regulate miRNA target gene expression at the post-transcriptional level.[58] Some CircRNAs could interact with RBPs and influence biological processes including gene transcription, cell cycle, proliferation, apoptosis, and cell survival.[29, 59] Furthermore, some studies reported that some CircRNAs are involved in the regulation of their parental gene transcription by interacting with the transcriptional machinery.[37, 38] Besides, it was shown that some CircRNA molecules could be translated into proteins[60, 61] and played a role in the regulation of splicing.[44]

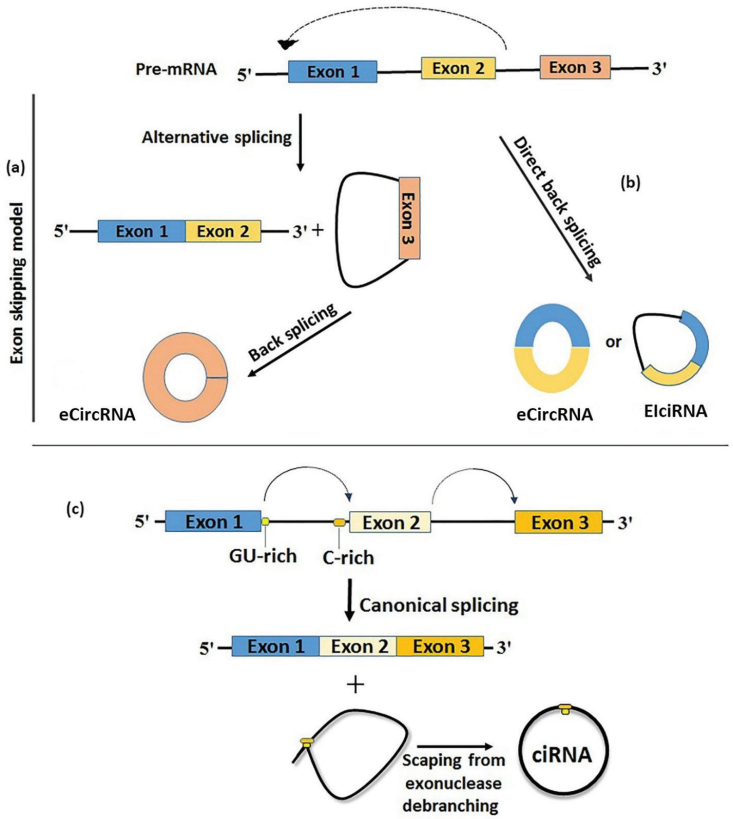

Figure 1: A schematic representation of CircRNA biogenesis in (a) an exon-skipping model, (b) direct back-splicing, and (c) ciRNA biogenesis.

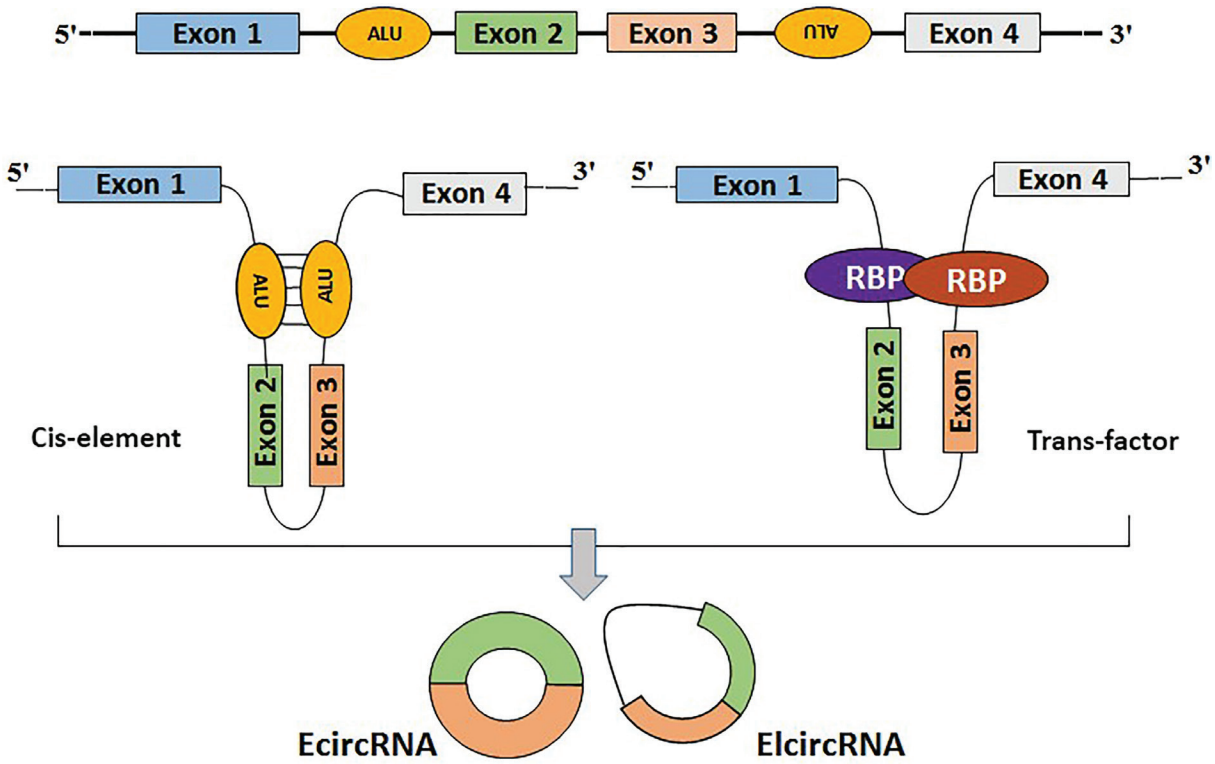

Figure 2: A schematic representation of CircRNA biogenesis regulation by cis-elements (inverted *Alu* repeats) and trans-factors (RNA binding proteins).

CircRNA and the Male Reproductive System

Testes: The testes are the most important male reproductive organs producing sperm and testosterone.[62] Spermatogenesis is the process by which sperm cells are produced. The process is divided into three steps, namely mitotic renewal (spermatogonia proliferation/differentiation), spermatocyte meiosis, and spermatid differentiation (spermiogenesis).[63] Each step requires highly precise temporal and spatial gene expression, which is strictly regulated by transcriptional gene expression, post-transcriptional changes, and epigenetic modifications.[42, 64] Testicular cells actively generate RNAs, including various non-coding and protein-coding mRNAs.[3] Recently, the presence of CircRNAs in the testes has been reported.[12, 62] The testicular CircRNAs were initially identified from gene Sry gene (Sex-determining Region Y) in the mouse.[65] Sry CircRNA (circSry) regulates gene expression by acting as a miR-138 sponge.[66] For the first time, Dong and colleagues reported the presence of CircRNAs in human testes and seminal plasma. Using next-generation deep sequencing, they identified over 15,000 CircRNAs of which 10,792 were novel. They showed that testis-derived CircRNAs can bind to proteins in seminal plasma and are very stable. They concluded that these CircRNAs can be used as non-invasive biomarkers for male infertility.[12] Gao and colleagues compared CircRNA expression in the testes of neonatal calves and adult cattle. They found that 4,248 CircRNAs were differentially expressed between the two groups. In adult cattle, 2,225 (10.2%) of the CircRNAs were upregulated and 2,023 (9.3%) were downregulated. In addition, they stated that the source gene of CircRNAs was involved in transforming growth factor β (TGFβ) signaling pathway, cell junction, and reproduction.[62] Li and colleagues used high-throughput RNA sequencing and demonstrated CircRNA expression at different reproductive stages in Tibetan sheep testes.[67] Furthermore, CircRNA expression in different tissues and developmental stages in rats was investigated. It was shown that CircRNAs were overexpressed in the testes and brain compared to other organs.[68] In another study, You and colleagues found that, after the brain, testicular tissue was most enriched with CircRNAs, implying that CircRNAs may play an essential role in its functioning.[69] Another study found a dynamic and age-dependent pattern of CircRNA expression in testes, such that it significantly increased with sexual maturity and decreased with aging.[68] Age dependency is strongly associated with spermatogenesis, indicating CircRNAs have specific physiological activities rather than simply being a by-product of RNAs and can act as a biomarker of reproductive aging.[68] Zhang and colleagues compared the

number of CircRNAs in the testes of adult boar and piglets and identified 2,326 differentially expressed CircRNAs. Some of these CircRNAs, associated with spermatogenesis (CircRNA 10979) and germ cell development (CircRNA 18456), were upregulated in the testes of adult boar and some others (CircRNAs 6682, 10187, 18456, 10979) and could be used as indicators of sexual maturation in pigs. They concluded that CircRNA is dynamically expressed during testicular development.[70]

Given the high expression of CircRNAs in the testes as well as their high stability, it is likely that these RNAs are the key factor involved in spermatogenesis or the pathogenesis of infertility-related diseases.

Spermatogenic Cells: Spermatogenesis is the biological process of sperm production in mammals, driven by a pool of self-renewing cells known as spermatogonial stem cells (SSCs). SSCs undergo mitotic divisions and differentiation, resulting in the production of meiotic spermatocytes. Round spermatids are formed after two meiotic reduction divisions and undergo a major morphological transformation during spermiogenesis to become sperm cells.[71] This complex process involves an accurate balance between differentiation and SSCs self-renewal, in which CircRNAs play a key role.[72] Zhang and colleagues reported that CircRNAs show a different expression pattern in the testes of adult boar and piglets. Some of these differentially expressed CircRNAs play a role in signaling pathways such as fibroblast growth factor receptor 1 *(FGFR1)*, AKT serine/threonine kinase 3 *(AKT3)*, SMAD family member 4 *(SMAD4)*, Frizzled Class Receptor 3 *(FZD3)*, and Activin A receptor type 1 *(ACVR1)* that regulate pluripotency in stem cells.[70] In addition, CircRNAs are abundantly expressed in spermatogenic cells. A previous study reported that 15,101 CircRNAs were identified in mouse spermatogenic cells, of which 7,220 were expressed in round spermatids and the rest in SSCs (5,573), spermatogonia type A (5,596), preleptotene (6,686), and pachytene spermatocytes (4,677).[73] Similar results in rat studies showed that most expressed CircRNAs are involved in spermatid development and acquisition of sperm motility (morphogenesis of flagellum).[68] Tang and colleagues reported that during spermatogenesis in mice, the generation of CircRNAs increases. Spermatocytes evolve into round spermatids at late pachytene and then show elongated morphologies (figure 3a) due to association with linear mRNA degradation in

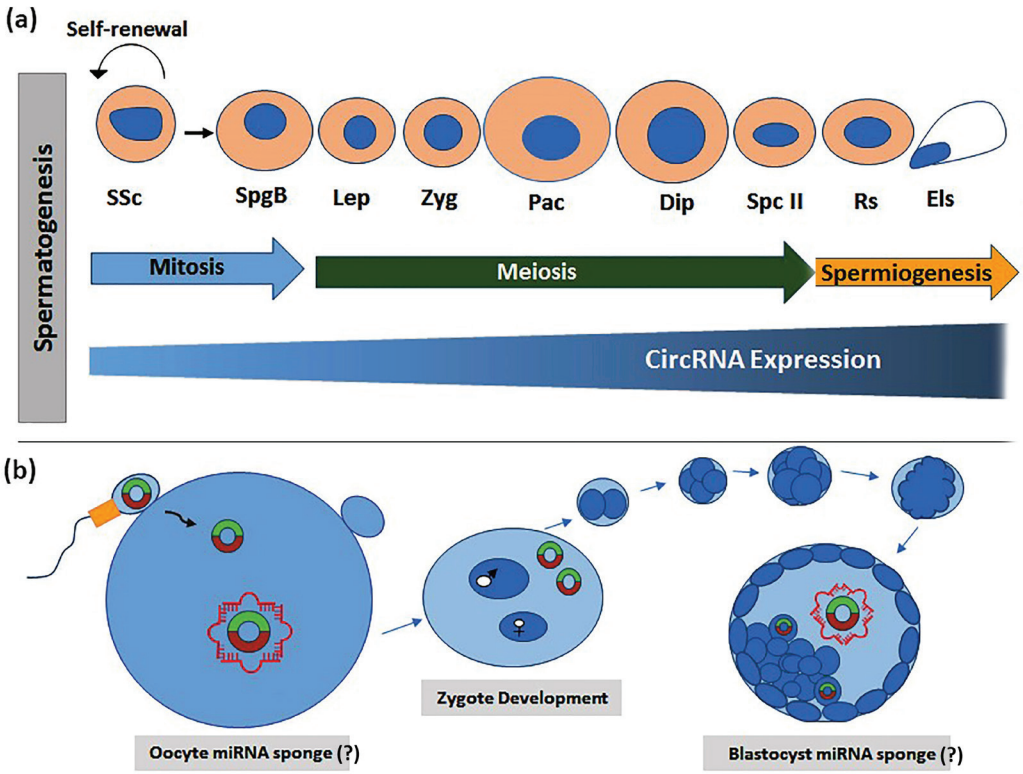

Figure 3: A schematic representation of (a) spermatogenesis and expression level of CircRNA and (b) possible sperm CircRNAs role as miRNA sponges in oocyte, embryonic cell, blastocoel fluid, and zygote development. SSC: Spermatogonia stem cell; SpgB: Spermatogonia B; Lep: leptotene; Zyg: Zygotene; Pac: Pachytene; Dip: Diplotene; Spc II: Spermatocyte II; Rs: Round spermatid; Els: Elongated spermatid; Spz: Spermatozoa

spermatids. Since most of these CircRNAs are exonic, they could be translated into proteins.[74] They also reported that sperms with high fertilization ability (IVF>25%) contained higher levels of CircRNAs, indicating an association between CircRNA function and sperm quality. Chioccarelli and colleagues used a microarray technique to characterize CircRNAs in human sperm and identified 10,726 CircRNAs of which 28% were novel. They also identified 148 differentially expressed CircRNAs correlating to good- or bad-quality sperm cells based on motility and morphological parameters. The results of subcellular localization of differentially expressed CircRNAs showed that most of these molecules were localized in the sperm head. It has been hypothesized that they may play a functional role in regulating the early stages of embryonic development by transferring sperm to oocyte during fertilization.[75]

In another study, CircRNAs expression in boar sperm was investigated and 1,598 CircRNAs were identified, 80% of which had not been reported previously. Sperms with low and high motility showed 148 differentially expressed CircRNAs, indicating their association with sperm quality. Of these, two CircRNAs (ssc_circ_1321 and ssc_circ_1458) were suggested as biomarkers for sperm motility.[76] Recently, the presence of some sperm RNAs in the oocyte has been confirmed as a result of fertilization and their function in zygote and embryo development.[77] It is shown that injection of sperms treated with RNase into MII oocytes resulted in decreased blastocyst development and live birth outcome, while the rate of embryo development increased after injecting total sperm RNAs into these oocytes.[78]

As mentioned earlier, the majority of CircRNAs are found in the sperm head, where they can be transferred to the oocyte during fertilization and influence gene expression at the early stages of development. Ragusa and colleagues investigated the presence of CircRNAs in human and mouse spermatozoa and showed that circNAPEPLDiso1 and circNAPEPLDiso2 were highly expressed. In murine-unfertilized oocytes, the expression level of circNAPEPLDiso1 and circNAPEPLDiso2 was low and high, respectively. However, after fertilization, circNAPEPLDiso1 expression increased significantly, whereas circNAPEPLDiso2 expression remained constant. However, in human spermatozoa, circNAPEPLDiso1 could interact with a variety of miRNAs expressed in the oocyte, blastocyst cells, and blastocoel fluid. In this regard, it is suggested that circNAPEPLDiso1 serves as a paternal-derived miRNA sponge after fertilization and regulates early embryonic development by suppressing the anti-proliferative functions of some oocyte miRNAs.[79] Chioccarelli and colleagues also reported that circCNOT6L is transferred from sperm to oocyte during fertilization where it may support zygote development (figure 3b).[77]

Effect of CircRNAs on Male Infertility

Azoospermia: Azoospermia refers to the absence of sperm in the ejaculate and is the most severe form of male infertility, accounting for 10-15% of infertile men.[80] Azoospermia is divided into two types, namely obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). In OA, seminal tract closure obstructs the transport of sperm despite normal spermatogenesis. NOA is the most problematic type of azoospermia (about 60% of patients) caused by the failure of spermatogenesis in the testes.[81] To date, the etiology of more than 80% of NOA cases is unknown, and genetic abnormalities have been identified in only 20% of NOA patients.[56] Micro-dissection testicular sperm extraction (micro-TESE) is the commonly used surgical method for sperm retrieval in NOA patients.[82] However, the success rate of sperm retrieval is around 50%, implying that testicular biopsy is unsuccessful in about half of the patients.[83, 84] Therefore, a biomarker that could predict the outcome of micro-TESE would play an important role in reducing unnecessary invasive procedures.

Several studies showed the potential of CircRNAs as a biomarker for disease detection and treatment.[85, 86] For the first time, Ge and colleagues reported that the expression pattern of CircRNA is different in the testes of NOA compared to OA patients.[87] This indicates that CircRNAs may play an important role in regulating sperm production and can be used in NOA diagnosis and treatment. In another study, Bo and colleagues showed similar results by comparing the expression pattern of CircRNA in the testicular tissue of NOA and OA patients. They reported that some of the differentially expressed CircRNAs are associated with several signaling pathways that may play a role in NOA pathogenesis. For example, hsa_CircRNA_402130 inhibits the function of the let7_miRNA family involved in the regulation of stemness.[88] Liu and colleagues compared hsa_circ_0049356 expression levels in the seminal plasma and blood samples of idiopathic NOA patients and healthy individuals. Hsa_circ_0049356 is associated with the *CARM1* gene (co-activator-associated arginine methyltransferase), which has an important

function in establishing sperm epigenome. They showed that the expression of this CircRNA was highly upregulated in whole blood samples of NOA patients, but significantly downregulated in seminal plasma samples, which could be attributed to the presence of a blood-testis barrier. These findings indicated the important role of hsa_circ_0049356 in spermatogenesis. In this regard, it is recommended to analyze CircRNA expression levels in the blood or seminal specimens from NOA patients as a diagnostic approach. Moreover, the bioinformatic analysis revealed that the expression patterns of hsa_circ_0049356 and the network of hsa_circ_0049356-miRNA-mRNA differ in NOA patients compared to healthy individuals. Most target mRNAs with sponges are important factors affecting cytoskeleton re-arrangement of germ cells during spermatogenesis, which is needed for their differentiation.[30] Another study reported that hsa_circ_0000116 was highly expressed in testicular tissue specimens of NOA compared to OA patients.[31] It has also been shown that expressions of hsa_circ_0000116 were negatively associated with the Johnsen score in NOA patients. In the study by Liu and colleagues, patients in the NOA group were divided into three subgroups, namely hypospermatogenesis (reduced sperm production in the presence of all germ cells), maturation arrest of premature spermatogenesis, and Sertoli cell-only syndrome (SCOS) characterized by the total absence of germ cells. They showed that the expression of hsa_circ_0000116 was different between the subgroups, with significant upregulation in SCOS compared to the hypospermatogenetic subgroup. Furthermore, overexpression of hsa_circ_0000116 was shown to be correlated with the reduced success of testicular sperm retrieval. In addition, they showed that miR-449 could be a target of hsa_circ_0000116 sponges. Since high miR-449 levels are expressed during testicular development and spermatogenesis in adults, spermatogenesis can be suppressed by blocking hsa_circ_0000116, leading to NOA. On the other hand, autophagy-related genes (*ATG4B, Bcl2*) can be regulated by miR-449. Probably, as a result of hsa_circ_0000116 upregulation in NOA patients, autophagy dysfunction causes damage to the spermatogenesis.[31]

In a recent study, Zhu and colleagues investigated CircRNA expression patterns in SCOS patients. They found that 1,594 CircRNAs were differentially expressed in SCOS compared to OA patients with normal spermatogenesis. Bioinformatic analysis revealed that differentially expressed CircRNAs in SCOS have a role in regulating spermatogenic cell proliferation and intercellular communication. In addition, abnormal expression of these vital CircRNAs is related to pathogenic changes in SCOS through spermatogonial dysplasia or interfering with normal spermatogenesis.[89] In another recent study, Ji and colleagues investigated the potential application of CircRNAs in seminal plasma to predict the success rate of micro-TESE in patients with idiopathic NOA. They found that hsa_circ_0007773, hsa_circ_0060394, and hsa_circ_0000277 have higher expression levels in both seminal plasma and testes of patients after successful sperm retrieval. Furthermore, samples from healthy individuals showed constant CircRNAs expression levels after 48 hours at room temperature. They suggested that these CircRNAs could be used as reliable diagnostic biomarkers for seminal plasma samples in patients with idiopathic NOA.[32]

Asthenozoospermia: Sperm motility is a key factor for successful fertilization.[90] Asthenozoospermia, a common infertility problem in men, is defined as progressive motility of less than 32% or total motility of less than 40% in at least two separate semen analyses.[91] Some of the causes of asthenozoospermia include varicocele, sperm dysfunction, partial blockage of the seminal tract, infection, and genetic factors. Nonetheless, asthenozoospermia could be idiopathic.[92]

The molecular mechanism that impairs sperm motility is not known, however, evidence has shown the association of sperm quality with sperm RNAs.[93, 94] Recently, some studies have investigated the role of CircRNAs in asthenozoospermia. Manfrevola and colleagues stated that 22% of CircRNAs in asthenozoospermic patients have not been previously reported. They compared the expression pattern of CircRNAs in high- and low-quality sperm samples, based on morphology and motility, between the patients and control groups. It was shown that 1,432 CircRNAs were differentially expressed and associated with mitochondrial functions and sperm motility. A mixture of the oral amino acid (L-arginine, citrulline, and ornithine) was administered to treat asthenozoospermic patients. The results showed that the expression of five upregulated CircRNA decreased significantly post-treatment, whereas the expression of five downregulated CircRNA increased to approximately the level of healthy patients. It was concluded that CircRNA molecules could be used as a biomarker for the diagnosis of asthenozoospermia. In addition, it was found that the modulation of CircRNAs with oral amino acid supplements increased

the viability and motility of sperms, indicating their potential in therapeutic approaches. Manfrevola and colleagues also investigated the putative molecular mechanisms of infertility in asthenozoospermic patients. They evaluated the mRNA expression of Cysteine-Rich Secretory Protein 2 *(CRISP2*), Prostate and Testis Expressed 1 (*PATE1*), and Cation Channel Sperm Associated 1*(CATSPER1*) in the semen of asthenozoospermic individuals. The results showed that low-quality sperms exhibited a significant decrease in the expression of these three mRNAs. Bioinformatic analysis revealed that three miRNAs (hsa-miR-138-5p, hsa-miR-27b, and hsa-miR-6721-5p) showed inhibitory effect on *CRISP2, PATE1,* and *CATSPER1* mRNAs, which were associated with differentially expressed CircRNA in the sperm cells of asthenozoospermic individuals. It was shown that the expression levels of circRERE, circEPS15, and circTRIM2 (upstream of *CRISP2*, *PATE1,* and *CATSPER1* mRNAs, respectively) were significantly downregulated in low-quality sperms. In addition, after administrating a mixture of oral amino acid supplements, the expression levels of these CircRNAs increased, indicating dysregulation of CircRNAs expression in asthenozoospermia and its potential as a targeting molecule for treatment.[33] Another study reported that reduced expression of circBoule RNAs, circEx2-7, and circEx3-6 is associated with asthenozoospermia in humans.[95]

Effect of CircRNA on Male Reproductive Cancers

It has been suggested that CircRNAs can be a potential biomarker for cancer.[96] Aberrant CircRNA expression is associated with some malignancies and can serve as tumor inhibitors or activators.[97] In this regard, some studies have demonstrated the role of CircRNAs in reproductive cancers.[29, 98, 99] To the best of our knowledge, there are no reports on CircRNAs association with testicular cancer.

Prostate Cancer

The prostate gland produces an alkaline fluid and is an important part of the male reproductive system. Semen is a combination of sperm cells and secretions from the prostate gland and other glands such as the bulbourethral gland and seminal vesicle.[100] Prostatic diseases can affect sperm function and male fertility.[101] In 2020, prostate cancer (PCa) was reported as the second most common malignant cancer in men and the fifth leading cause of cancer-related deaths worldwide. According to GLOBOCAN 2020, a total of 1,414,259 new cases and 375,304 deaths due to prostate cancer has been reported globally.[102] Prostate-specific antigen (PSA), a known biomarker of prostate cancer, is routinely used to detect PCa. However, because of its low specificity, the PSA test often results in misdiagnosis and overtreatment. Currently, there is no consensus on the effectiveness of PSA screening methods in reducing the incidence of PCa-related death.[103] Therefore, there is a need to identify new biomarkers as a diagnostic tool.

Recently, some studies have shown the aberrant expression of CircRNAs in patients with PCa (table 1). Ge and colleagues identified 749 differentially expressed CircRNAs in PCa compared to healthy tissues, most of which were associated with tumor progression. They suggested that these CircRNAs could be potential biomarkers for the detection and treatment of PCa.[104] Similarly, Wu and colleagues identified 60 differentially expressed CircRNAs in PCa cells compared to normal tissues.[105] Chen and colleagues reported that circHIPK3 upregulation is associated with the aggressiveness of PCa, whereas its downregulation reduces the proliferation and invasion of cancerous cells.[34] It is also shown that CircRNAs can impact the cell cycle by enhancing the G2 to M transition that results in cell proliferation.[106] A previous study showed that the expression of another CircRNA, circFOXO3, was significantly increased in blood serum and PCa tissue, which may result in PCa development, indicating the potential of circFOXO3 as a biomarker for PCa.[29] Hansen and colleagues identified five new CircRNAs (circKMD1A, circTULP4, circZNF532, circSUMF1, and circMKLN1) with high cancer specificity and association with disease progression.[107] These were also recommended as predictive biomarkers for PCa. Several studies have reported the inhibitory effect of CircRNAs on PCa progression. For instance, a study showed that circDDX17 inhibits the transition, proliferation, and invasion of PCa cells.[108] Kong and colleagues identified dysregulated circZNF561 and circNFIA in tissue samples of PCa patients. The expression level of CircNFIA in plasma and tissue samples increased and had an oncogenic function, whereas circZNF561 showed a reduced expression level and tumor suppressive function.[35]

Conclusion

Disorders of the male reproductive system account for around 50% of infertility issues, indicating the need to identify the mechanisms involved. In the present review, a description of CircRNA and its function in different male reproduction mechanisms was presented.

Based on the findings of several studies, CircRNAs are highly expressed in the testes and have a dynamic and age-dependent pattern. These RNAs are involved in a variety of different processes such as germ cell self-renewal, spermatid cell development, sperm quality, and embryo development. Changes in CircRNA expression level are associated with different reproductive complications including asthenozoospermia, non-obstructive azoospermia, and prostate cancer. To date, there are no reports on the association of CircRNA with teratozoospermia or varicocele. Overall, CircRNAs can be a potential biomarker for the diagnosis and treatment of diseases related to the male reproductive system. Further *in vivo* studies, especially in humans, are recommended.

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Authors' Contribution

Z.D and SMB.T: Conception, design, acquisition of data, writing the manuscript. Z.D, SMB.T, S.B, S.A, J.F: Data interpretation, review of the manuscript. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

Conflict of Interest: None declared.

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