

Original Article

Asian Pacific Journal of Reproduction

Journal homepage: <https://journals.lww.com/apjr/>

doi: 10.4103/apjr.apjr_39_25

Seminal exosomes and miRNA-mediated regulation of SRD5A2 in male infertility: An observational study

Adnan F. AL-Azaawie^{1✉}, Mousa Jasim Mohammed¹, Ahmed AbdulJabbar Suleiman²¹Department of Biology, College of Science, Tikrit University, Salah-aladin, Iraq²Department of Biotechnology, College of Science, University of Anbar, Anbar, Iraq

ABSTRACT

Objective: To investigate the levels of seminal plasma exosomes and the expression of the *SRD5A2* gene in Iraqi men with different types of male infertility (asthenozoospermia, oligozoospermia, and azoospermia), and to explore the regulatory role of selected microRNAs (miRNAs) in the modulation of *SRD5A2* gene expression.

Methods: A total of 90 male participants were categorized into four groups: asthenozoospermia ($n=24$), oligozoospermia ($n=24$), azoospermia ($n=18$), and normozoospermia ($n=24$). Seminal plasma exosome levels were quantified using ELISA. RNA, including miRNAs, was extracted, followed by cDNA synthesis. The expression of *SRD5A2* mRNA and five selected miRNAs (*miR-6090*, *hsa-miR-5189-5p*, *miR-23a-5p*, *miR-1914-3p*, and *miR-4540*) was evaluated using qRT-PCR. Correlation analyses were conducted between exosome levels, miRNA expression, and *SRD5A2* expression.

Results: Infertile men exhibited significantly lower seminal exosome levels than normozoospermic men ($P<0.01$). The expression of *SRD5A2* mRNA was decreased in asthenozoospermic and oligozoospermic men but elevated in azoospermic men. Elevated levels of *hsa-miR-5189-5p*, *miR-6090*, and *miR-23a-5p* were observed in infertile groups. Correlation analysis revealed a significant negative relationship between these miRNAs and *SRD5A2* expression in asthenozoospermic and oligozoospermic men, but a positive correlation in azoospermic men. Exosome levels were also significantly correlated with the expression of these miRNAs, suggesting their role as molecular carriers in regulating gene expression.

Conclusions: This study highlights the potential role of seminal exosomes and specific miRNAs in regulating *SRD5A2* gene expression among infertile men. The altered levels of exosomes and dysregulated miRNA expression, particularly *hsa-miR-5189-5p* and *miR-6090*, may serve as novel biomarkers for the diagnosis and management of male infertility. Further research is warranted to validate these findings and explore their therapeutic implications.

KEYWORDS: miRNA regulation; 5 α -reductase type 2; Exosomal biomarkers; Male infertility; Non-coding RNAs in infertility

1. Introduction

Male infertility is defined as the failure to conceive after at least 12 months of regular, unprotected sexual intercourse. It is a

Key Points

Question: What is the association between seminal exosomes, miRNA expression, and *SRD5A2* gene regulation in different types of male infertility?

Findings: In this case-control study of 90 men, exosome levels were significantly lower in infertile men, with altered *SRD5A2* mRNA expression—reduced in asthenozoospermia and oligozoospermia, but elevated in azoospermia. Three miRNAs (*miR-5189-5p*, *miR-6090*, and *miR-23a-5p*) showed differential expression and correlated with *SRD5A2* levels.

Meaning: Exosomal miRNAs and *SRD5A2* expression may serve as novel biomarkers for diagnosing and understanding male infertility pathogenesis.

✉To whom correspondence may be addressed. E-mail: ahmed.suleiman@uoanbar.edu.iq

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2025 Asian Pacific Journal of Reproduction Produced by Wolters Kluwer- Medknow.

How to cite this article: AL-Azaawie AF, Mohammed MJ, Suleiman AA. Seminal exosomes and miRNA-mediated regulation of *SRD5A2* in male infertility: An observational study. *Asian Pac J Reprod* 2025; 14(4): 161-170.

Article history: Received: 10 February 2025; Revision: 8 April 2025; Accepted: 16 June 2025; Available online: 31 July 2025

significant health concern worldwide for 7% of men, contributing to 40%-50% of all infertility cases[1,2]. Infertility can be classified into two subtypes as either primary, where a woman is unable to conceive at all, or secondary, where conception occurs once or a child is born, but subsequent pregnancies do not occur. Research shows that 10% to 15% of couples globally experience infertility, with male factors contributing as a primary cause in approximately half of these instances. Infertility is a complex condition, presenting in various forms such as endocrine disorders, congenital or acquired urogenital abnormalities, immune-related issues, and both quantitative and qualitative sperm defects[3]. Furthermore, it can be broadly classified based on underlying causes, including genetic, idiopathic, and obstructive factors, into several types such as azoospermia, oligozoospermia, teratozoospermia, and asthenozoospermia[4].

Of note, human semen is a complex fluid containing spermatozoa and seminal plasma, which is a mixture of secretions from the prostate, epididymis, and seminal vesicles. Extracellular vesicles (EVs) in seminal plasma are lipid bilayer nanovesicles that carry various molecules and facilitate cell-to-cell communication. These EVs are diverse and originate from various parts of the male reproductive system. Furthermore, these EVs are categorized into exosomes, microvesicles, and apoptotic bodies based on their size and origin[5].

Studies show that vesicles, like exosomes and microparticles, deliver information to the cellular microenvironment, potentially influencing target cell behavior[5]. Exosomes are saucer-shaped microvesicles derived from multivesicular bodies, ranging from 30–150 nm in diameter[6]. Secreted through exocytosis, they are released into the extracellular space to interact with target cells, facilitating cell-to-cell communication by transferring intracellular cargoes like proteins, lipids, and nucleic acids, including mRNA, microRNA, long non-coding RNA, and circular RNA[7]. Initially considered cellular waste, exosomes are now recognized for their role in regulating intercellular communication. Once taken up by recipient cells, they release bioactive cargo that influences cell behavior, spreading information throughout the body[8]. Given their role in communication, exosomes have attracted interest for their diagnostic and therapeutic potential in various diseases. While exosome biomarkers are under clinical trial, they are not yet in routine use. Ongoing research explores their potential as diagnostic tools and therapeutic delivery systems[9].

Importantly, exosomes present in various body fluids, such as semen, amniotic fluid, blood, and breast milk, enable non-invasive detection methods[10]. Moreover, it has been reported that semen contains a greater concentration of exosomes compared to other fluids. Seminal plasma exosomes mainly produced by the epididymis and prostate contain proteins, nucleic acids, and a high content of cholesterol and sphingomyelin[11]. In male fertility, semen exosomes are believed to play a role in sperm capacitation

and the acrosome reaction. They selectively transport and deliver regulatory molecules to the female reproductive system, contributing to fertilization[12]. Notably, exosomes from oligoasthenozoospermic donors exhibited different miRNA and protein profiles compared to those from normozoospermic donors. Additionally, exosomes from normal human semen promoted sperm motility and capacitation, while those from asthenozoospermic donors showed impaired effects. These findings indicate that seminal plasma exosomes are functional regulators of male fertility and suggest that exosomal dysfunction may contribute to male infertility[13].

Gene expression during spermatogenesis is dynamically regulated post-transcriptionally by microRNAs (miRNAs), short noncoding RNAs of 22 to 24 nucleotides that inhibit the translation of target mRNAs by forming semicomplementary structures in their 3' untranslated regions (3'UTR)[14]. The exosomal miRNAs have been shown to be involved in processes such as the acrosome reaction and sperm motility, which are essential for successful fertilization[15]. Furthermore, exosomal miRNAs have been identified as potential biomarkers for diagnosing reproductive disorders, offering insights into the etiology of infertility and aiding in the development of therapeutic strategies[16]. MiRNAs are key regulators of gene expression in male infertility, affecting sperm count, motility, and morphology. Studies have identified several miRNAs associated with male infertility, such as *miR-34*, *miR-122*, *miR-146b-5p*, *miR-181a*, *miR-374b*, *miR-509-5p*, and *miR-513a-5p*, which are linked to idiopathic male infertility and could serve as diagnostic biomarkers[17].

The miRNA profile in semen varies with the type of genital tract obstruction, such as congenital obstruction versus vasectomy, suggesting that exosomal miRNAs in seminal plasma may serve as biomarkers to differentiate obstructive azoospermia from spermatogenic failure[18]. For instance, *miR-31-5p*, *miR-539-5p*, and *miR-941* have shown promise in predicting residual spermatogenesis[19]. Additionally, *hsa-miR-141*, *hsa-miR-34b*, and *hsa-miR-122* are linked to various sperm defects, highlighting their potential as molecular markers for male infertility. Their involvement in pathways like mismatch repair emphasizes their importance in spermatogenesis and potential for new therapies[20]. Furthermore, *miR-31-5p* demonstrates high diagnostic efficiency for azoospermia, serving as a non-invasive biomarker for assessing spermatogenic failure[21]. Seminal plasma exosomes containing miRNAs regulate sperm maturation, motility, and capacitation, influencing fertilization outcomes by interacting with cell receptors or being internalized to release miRNA cargo[22]. Studying miRNAs in male infertility reveals molecular mechanisms and offers potential biomarkers for diagnosis and therapeutic targets, guiding new treatments while reducing invasive procedures[23].

Given that spermatogenesis and sperm maturation are androgen-dependent processes, primarily regulated by dihydrotestosterone

(DHT), which is produced from testosterone through the action of the enzyme 5 α -reductase (SRD5A)[24]. The *SRD5A2* gene plays a vital role in the metabolism of steroid hormones and regulates testosterone biosynthesis. Impaired SRD5A2 (specific to the epididymis, seminal vesicles, and prostate) activity can lead to 5 α -reductase type 2 deficiency, impacting DHT synthesis[25]. The SRD5A2, found on chromosome 2, encodes a 254 amino acid protein[26]. Genetic variations in the *SRD5A2* gene, which can cause different enzyme activities among ethnic groups, are associated with male reproductive disorders[27]. Notably, mutations such as R227Q result in nearly absent enzymatic activity, while polymorphisms like V89L reduce DHT levels[28].

The specific role of exosomes and miRNAs in male infertility is an area that requires further research, their involvement in cell-to-cell communication suggests they may have implications in understanding and potentially addressing male reproductive health issues[6]. Hence, given the importance of SRD5A2, exosomes and miRNAs in male reproductive disorders, this study aims to investigate the correlation between exosomal levels and SRD5A2 expression and to explore the role of miRNAs and exosomes in regulating SRD5A2 in Iraqi men with asthenozoospermia, oligozoospermia, and azoospermia.

2. Methods

2.1. Study population and exclusion criteria

This was a cross-sectional observational study conducted to investigate the role of seminal exosomes and miRNA in the regulation of SRD5A2 among infertile Iraqi men. The study was conducted at the male private infertility clinic in Tikrit, Iraq, between April 2023 and April 2024. All participants were enrolled and examined during this period. A total of 90 male subjects, ranging in age from 20 to 40 years, were incorporated into this investigation, comprising 24 individuals classified as infertile due to asthenozoospermia, 18 individuals categorized as having oligozoospermia, and an additional 24 individuals diagnosed with azoospermia, alongside 24 men exhibiting normal semen parameters as normozoospermia, demonstrating proven fertility and having fathered children. A formal sample size calculation was not performed. The sample size was based on available cases within the study period.

Male participants were included if they sought assistance at the male infertility clinic for infertility intervention and underwent a comprehensive semen analysis. An abnormal spermiogram indicated subfertility, while normal results indicated fertility. The criteria for exclusion were specified as follows: tobacco usage, substance abuse, exposure to environmental or occupational toxic agents, presence

of sexually transmitted infections, cryptorchidism, anomalies of the genitourinary system, and surgical interventions related to infertility management.

2.2. Sample collection

Seminal samples were collected from each participant through masturbation following three days of sexual abstinence, each sample was divided into two parts, the first was 250 μ L, mixed well with 750 μ L of Trizol and kept frozen for the RNA extraction, and the second part permitted liquefaction for 30 min at 37 $^{\circ}$ C followed by immediate processing in accordance with the World Health Organization's 2010 guidelines[29]. Centrifugation of the seminal samples were carried out for 10 min at 500 $\times$ g. The supernatant (seminal plasma) was transferred to the Eppendorf tube for determination of exosomes levels.

2.3. Exosomes levels

The levels of seminal plasma exosomes were determined using an enzyme-linked immunosorbent assay (ELISA) kit supplied by (Sunlong company, China), following the manufacturer's instructions.

2.4. Total RNA, including miRNAs isolation

Total RNA, including miRNAs, was extracted by combination of Trizol (phenol/guanidine-based lysis of samples) and silica membrane-based purification for total RNA and miRNAs. The concentration and purity were measured using a Nanodrop ND-2000 spectrophotometer, and all RNA samples exhibited an integrity value of less than 2.3.

2.5. The polyadenylation and complementary DNA (cDNA)

The polyadenylation of mature miRNAs was done by poly (A) polymerase according to the kit protocol supplied by the Tinzyme company, China. The cDNA synthesis was carried out using the 5 $\times$ RT kit from the Tinzyme company, China.

2.6. Quantitative real time PCR (qRT-PCR) of RNA and miRNA

The expression level for RNA and five selected miRNAs (*miR-6090*, *hsa-miR-5189-5p*, *miR-23a-5p*, *miR-1914-3p* and *miR-4540*) was determined using Luna SYBR Green Kit (Biolabs). Briefly, 10 μ L of Luna master mix was mixed with 0.5 μ L of forward and reverse primer and 2 μ L of cDNA and completed to 20 μ L with DNase-RNase free water. All qRT-PCR reactions were set up using

a by qRT-PCR applied biosystem 7500 at 95 °C for 3 min for 40 cycles for 15 s and 60 °C for 45 s. Melting curve analysis was used to control for the specificity of qRT-PCR products. Additionally, the analysis was conducted with SDS Relative Quantification Software version 2.3 (Life Technologies).

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism Software version 10.3 (GraphPad Software, USA). Prior to applying statistical tests, the normality of data distribution was assessed using the Shapiro–Wilk test. Data that conformed to a normal distribution were analyzed using parametric tests, while data that did not follow a normal distribution were evaluated using nonparametric methods. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test was used for normally distributed data. For non-normally distributed data, the Kruskal–Wallis test followed by Dunn's multiple comparisons test was employed. Receiver operating characteristic (ROC) curve analysis was also conducted to assess the diagnostic performance of seminal exosome levels, and the area under the curve (AUC) was calculated to determine sensitivity and specificity. Results are presented as mean±standard deviation (mean±SD). Statistical significance was defined as $P<0.05$, and highly significant results were considered at $P<0.01$. Calculating the a Log2 fold change ($2^{-\Delta\Delta CT}$) by using Livak method[30].

2.8. Ethics statement

The research was sanctioned by the institutional review board (No. 195/11) issued at 13 March 2023 affiliated with Tikrit University, in the Salah-alidin Governorate of Iraq. Informed consent, based on a thorough understanding, was obtained from each participant in the study.

3. Results

3.1. Characteristics of study subjects

We analyzed 90 seminal plasma samples from men with asthenozoospermia ($n=24$), oligozoospermia ($n=24$), azoospermic men ($n=18$), and normozoospermic men ($n=24$). The characteristics of the men are summarized in Table 1. Nonparametric analysis revealed no significant differences in mean age, semen volume, or semen pH between normozoospermic men and oligoasthenozoospermic subfertile men. However, there were significant differences between normozoospermic men and oligoasthenozoospermic men concerning the count, motility, and morphology of the spermatozoa.

3.2. Basic parameters of the infertile men and normozoospermic controls

Infertile men were significantly different compared to normozoospermic men in terms of seminal volume, morphology, count, motility and immotile ($P<0.01$). Whereas, other parameters, such as age, weight, and long, were not significantly different as shown in Table 1.

3.3. Exosomes levels

Highly significant differences were observed in seminal plasma exosomes levels among all infertility groups compared to normozoospermic men, as shown in Figure 1A ($P<0.01$). Importantly, a gradual decrease in exosome levels was noted among the infertility groups, with exosome concentrations recorded as follows: oligozoospermia at 141.987 pg/mL, azoospermia at 140.847 pg/mL, and asthenozoospermia at 130.923 pg/mL, as illustrated in Figure 1A. The receiver operating characteristic (ROC) curve visualizes the trade-off between sensitivity (true positive

Table 1. Characteristics of patients and seminal fluid of the study groups.

Characteristic	Asthenozoospermia ($n=24$)	Oligozoospermia ($n=24$)	Azoospermia ($n=18$)	Normozoospermia ($n=24$)	<i>P</i> value
Age, years	31.79±4.19 ^a	31.25±5.77 ^a	31.33±5.89 ^a	31.12±4.56 ^a	0.970
Weight, kg	81.50±10.68 ^a	81.29±11.52 ^a	83.44±14.94 ^a	79.37±9.08 ^a	0.720
Length, cm	173.00±6.93 ^a	173.20±7.13 ^a	171.38±8.03 ^a	172.95±5.90 ^a	0.830
Seminal volume, mL	2.80±0.55 ^b	2.93±0.83 ^b	2.73±0.59 ^b	3.66±0.99 ^a	<0.001
Rate of anomol morphology, %	53.72±6.45 ^b	63.61±10.42 ^a	-	47.78±2.49 ^c	<0.001
Count, 10 ⁶ /mL	47.80±12.79 ^b	7.55±2.13 ^c	-	64.83±10.19 ^a	<0.001
Motile sperm, %	48.37±8.31 ^b	57.10±8.18 ^b	-	77.24±5.47 ^a	<0.001
Motility, %					
Fast progressive	7.23±1.27 ^b	6.05±1.42 ^b	-	33.18±7.89 ^a	<0.001
Slow progressive	22.18±5.07 ^b	22.03±2.54 ^b	-	30.41±6.40 ^a	<0.001
Local motile	18.95±4.32 ^b	29.00±6.72 ^a	-	13.63±2.64 ^c	<0.001
Immotile, %	51.62±10.58 ^a	42.90±9.83 ^b	-	22.76±5.64 ^c	<0.001

Data are expressed as mean±SD. Fast + Slow + Local = Motility; Motility + Immotile =100%. Letters (a, b, c) indicate statistical significance.

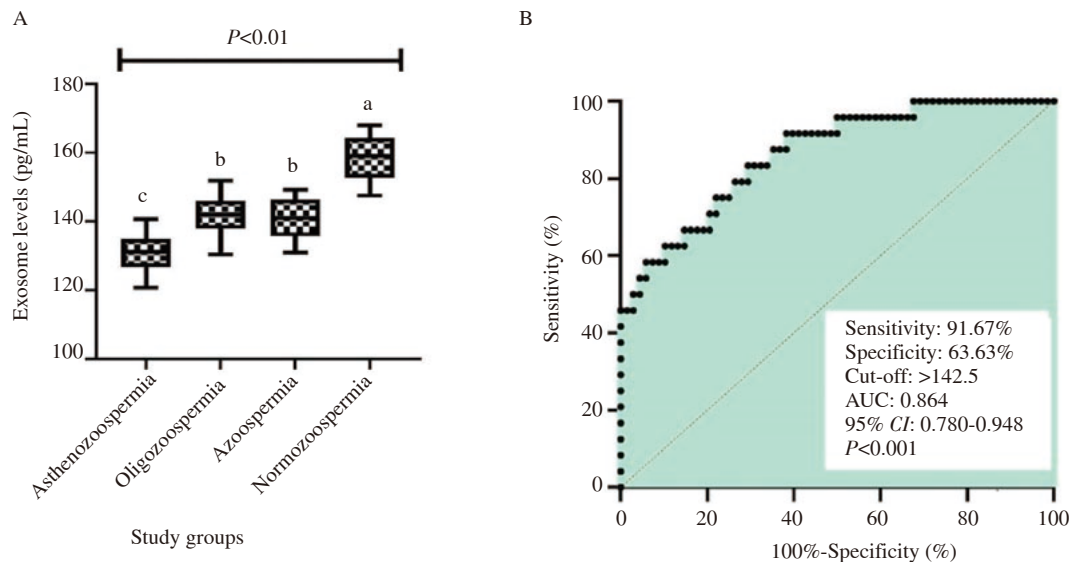


Figure 1. Seminal plasma exosomes levels. A. Exosomes levels of the study groups; Letters (a, b, c) indicate statistical significance. B. Receiver operating characteristic of the exosome's levels. AUC: area under the curve. CI: confident interval.

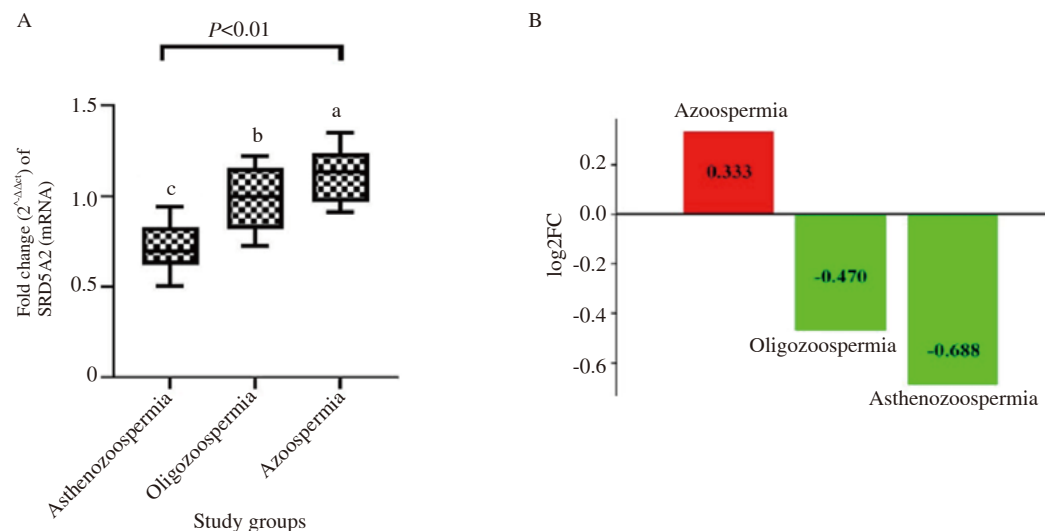


Figure 2. Differential gene expression analysis of the SRD5A2 enzyme of the study groups. A. Fold change (2^{-ΔΔCT}) of the SRD5A2 enzyme of the infertile men compared with normozoospermic men; Letters (a, b, c) indicate statistical significance. B. Bar blot. The X-axis indicates the mRNA and the Y-axis indicates Log2 fold change (FC).

rate) and specificity (1-false positive rate) across various threshold settings of a diagnostic test. The sensitivity of the seminal plasma exosome levels was 91.67%, while the specificity was 63.63%. The area under the curve (AUC) value was 0.864, as shown in Figure 1B.

3.4. SRD5A2 mRNA expression levels

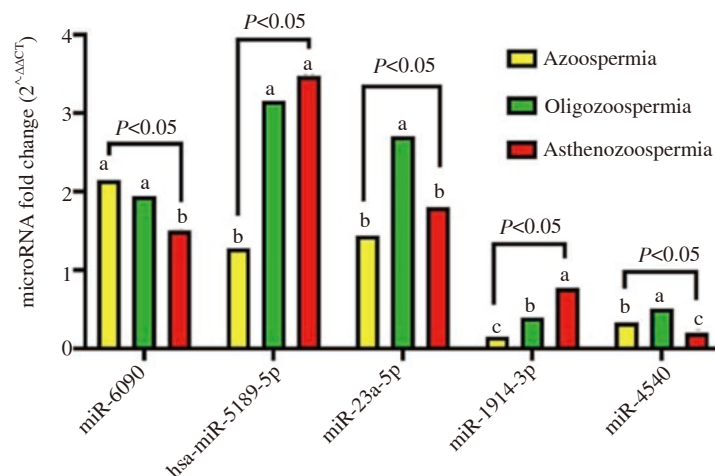
We compared the mRNA expression levels of SRD5A2 between infertile men and normozoospermic individuals and found significant differences between them. Notably, azoospermic men showed an increase in mRNA expression, with a fold change (2^{-ΔΔCT}) of 1.111 and a Log2 fold change of 0.33 compared to age-matched individuals. In contrast, oligozoospermic men demonstrated a fold change of 0.985 and a Log2 fold change of -0.470, whereas

asthenozoospermic men had a fold change of 0.725 and a Log2 fold change of -0.688 ($P < 0.01$). These findings are illustrated in Figure 2A and B.

3.5. Correlation between exosomes levels and SRD5A2 expression

Exosome levels were significantly positively correlated (correlation coefficient 0.634 with P -value 0.004) with the SRD5A2 expression in azoospermic men. In contrast, a significant negative correlation (correlation coefficient -0.505 with P -value 0.011) in asthenozoospermic and (correlation coefficient -0.419 with P -value 0.038) oligozoospermic men were observed between exosome levels and SRD5A2 expression.

A



B

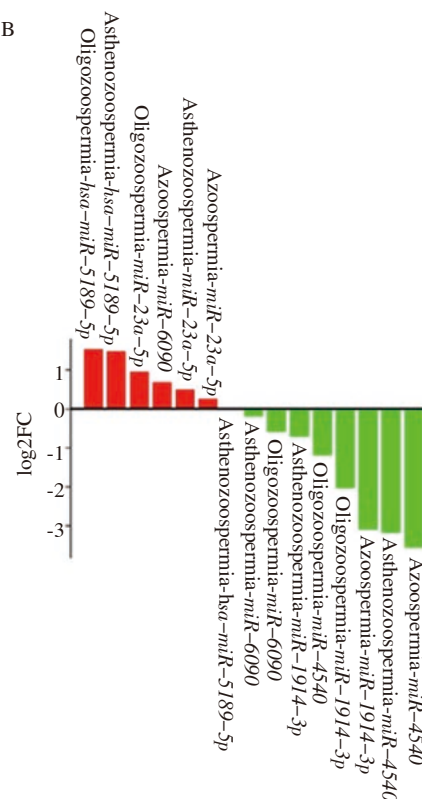


Figure 3. Expression profiles of selected miRNAs (*miR-5189-5p*, *miR-6090*, *miR-23a-5p*, *miR-1914-3p*, *miR-4540*) in infertile subgroups. A. Fold change ($2^{-\Delta\Delta CT}$) of the microRNA types of the infertile men compared with normozoospermic men; Letters (a, b, c) indicate statistical significance. B. Bar blot. The X-axis indicates the mRNA and the Y-axis indicates Log2 fold change (FC) of the microRNA types of the infertile men compared with normozoospermic men.

3.6. Differential expression of miRNAs associated with *SRD5A2* regulation in male infertility

Moreover, to predict the potential miRNAs that may potentially regulate *SRD5A2* mRNA expression in infertility, bioinformatics analysis was performed. Among multiple potential miRNAs, five miRNAs (namely: *miR-6090*, *hsa-miR-5189-5p*, *miR-23a-5p*, *miR-1914-3p* and *miR-4540*) were selected for further analysis. Therefore, these miRNAs were further experimentally validated for their relative expression levels in asthenozoospermia, oligozoospermia and azoospermia compared with normozoospermia as shown in Figure 3A. The miRNAs *hsa-miR-5189-5p*, *miR-6090*, and *miR-23a-5p* exhibited higher expression than *miR-1914-3p* and *miR-4540* across all three infertile groups. Specifically, *hsa-miR-5189-5p* showed the expression (log2FC) of 3.474, 3.157, and 2.145 in asthenozoospermia, oligozoospermia, and azoospermia, respectively, with a mean log2FC of 2.636, significantly higher than *miR-4540* log2FC expression of 0.351. In the upregulated group, *hsa-miR-5189-5p* had log2FC of 1.521 and 1.473 in oligozoospermia and asthenozoospermia, respectively. However, *miR-23a-5p* showed log2FC of 0.945, 0.491, and 0.258 in oligozoospermia, asthenozoospermia, and azoospermia, respectively. Moreover, *miR-6090* was upregulated only in azoospermia, with a log2FC of 0.677. In contrast, *miR-4540* had lower log2FC values of -3.552 and -3.162 in azoospermia and asthenozoospermia, respectively as shown in Figure 3B.

3.7. Correlation between exosomes levels and miRNA expression

To clarify the role of exosomes in the transfer of miRNAs, we analyzed the correlations between exosome levels and the log2FC of miRNAs. As summarized in Figure 4A, exosome levels showed a significant positive correlation with *hsa-miR-5189-5p* and *miR-6090* in men with asthenozoospermia, oligozoospermia, and azoospermia. This suggests that exosomes transfer these specific miRNAs from one cell to another. Conversely, a significant negative correlation was observed between exosome levels and *miR-23a-5p* in oligozoospermia and *miR-1914-3p* in azoospermia. Other miRNAs exhibited weak positive and negative correlations with exosome levels across the three infertile groups.

3.8. Correlation between miRNA and *SRD5A2* expression

Our results revealed significant positive correlation between the miRNA types (*miR-6090*, *hsa-miR-5189-5p* and *miR-23a-5p*) and *SRD5A2* expression as shown in Figure 4B. Notably, the expression of *miR-1914-3p* in asthenozoospermia and *miR-4540* in oligozoospermia exhibited a significant positive correlation with *SRD5A2* expression. While miRNAs *miR-6090*, *miR-23a-5p*, and *hsa-miR-5189-5p* in men with asthenozoospermia showed a significant negative correlation with *SRD5A2* expression. In azoospermia, *hsa-miR-5189-5p*, *miR-1914-3p*, and *miR-4540*

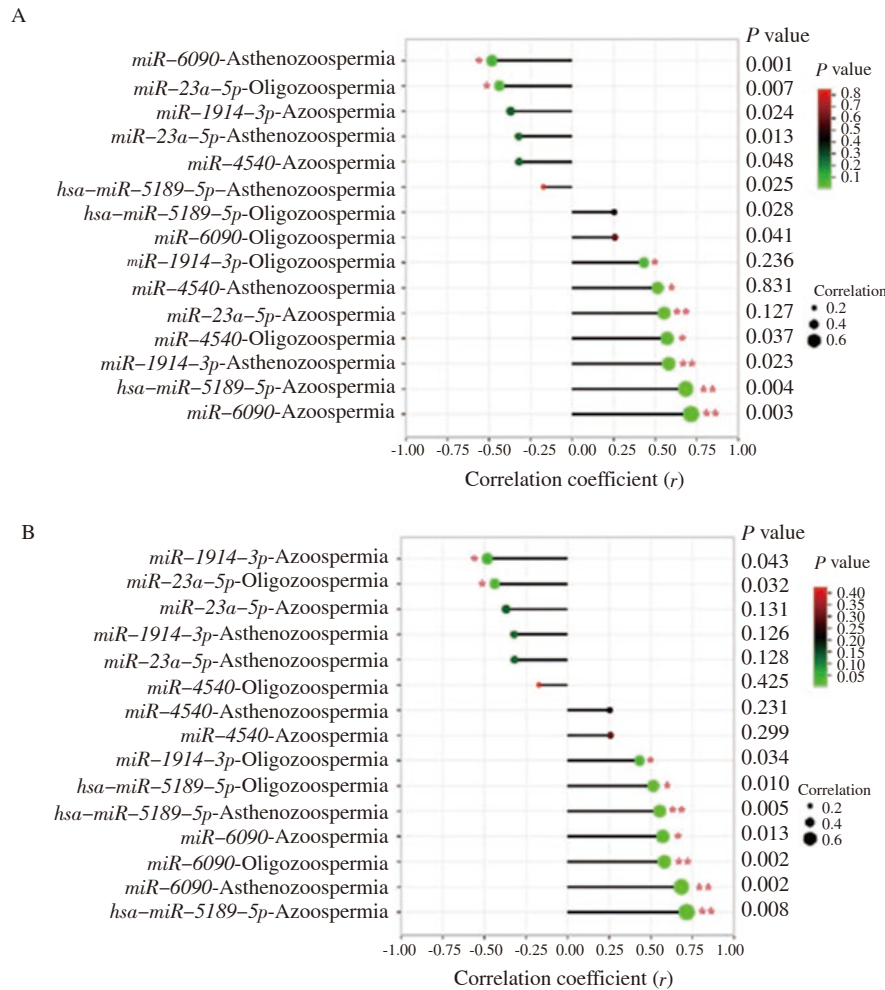


Figure 4. Correlations of exosomal levels with miRNAs and *SRD5A2* mRNA expression across different infertility types. A. Correlation coefficient between exosomal levels and mean fold change ($2^{\Delta\Delta CT}$) of microRNA (*SRD5A2*) of the infertile men. B. Correlation coefficient between mean fold change ($2^{\Delta\Delta CT}$) of *SRD5A2* and microRNA of the infertile men.

also exhibited a significant negative correlation with *SRD5A2*. Additionally, in men with oligozoospermia, *miR-23a-5p*, *hsa-miR-5189-5p*, and *miR-6090* demonstrated a significant negative correlation with *SRD5A2* expression. Moreover, *hsa-miR-5189-5p* was negatively correlated with asthenozoospermia and oligozoospermia, while showing a positive correlation in azoospermia.

4. Discussion

Numerous efforts have been made in the last decade in order to develop specific diagnostic and prognostic biomarkers for male infertility ultimately enhancing reproductive system treatments[8]. Exosomes as biomarker have been previously reported due to high stability, specificity and sensitivity in comparison with other biomarkers[31]. Exosomes derived from seminal plasma may play a crucial role in enhancing sperm motility and facilitating the maturation of spermatozoa[14]. Exosomes are essential for

spermatogenesis, facilitating communication between Sertoli, Leydig, and germ cells by transferring regulatory molecules critical for testicular development and sperm production, thus highlighting their potential in addressing male infertility[32].

To the best of our knowledge, previously no research has been carried out to determine the levels of exosomes in human male infertility. Therefore, our study is aimed to evaluate the correlation between exosomal levels and *SRD5A2* expression, in addition to explore the role of miRNAs in the regulation *SRD5A2* expression in three of Iraqi infertile men as asthenozoospermia, oligozoospermia, azoospermia. A few studies included determination of exosome levels such as Elsharkawi and colleagues[31] which estimated the exosome concentration in urine and serum as novel biomarkers.

In our study, seminal plasma levels in normozoospermic individuals were significantly higher than in other infertile men. This may be linked to decreased exosome levels in male infertility, influenced by factors like inflammatory conditions, such as orchitis caused by infections like uropathogenic *Escherichia coli*. Inflamed testes produce exosomes that carry pro-inflammatory signals, like *miR-*

155–5p, disrupting the immunosuppressive environment needed for normal testicular function. This inflammatory response can reduce exosome levels and contribute to infertility[33].

A similar study found that unilateral varicocele, a condition affecting male fertility, leads to dysregulation of exosome-associated proteins where significant changes in the expression of proteins like ANXA2 and KIF5B in seminal plasma are noted, which are essential for exosome function. This dysregulation can impair exosome machinery, resulting in reduced exosome levels and compromised sperm function. Exosome levels can be influenced by environmental factors like pH, temperature, and storage conditions. Research shows that extreme pH and repeated freeze-thaw cycles significantly reduce exosome concentration, potentially affecting their role in fertility[34].

The *SRD5A2* gene regulates testosterone biosynthesis by encoding the 5- α -reductase enzyme, which converts testosterone into DHT. This enzyme is crucial for spermatogenesis and sperm maturation due to its androgen dependence. However, no studies have examined *SRD5A2* mRNA or miRNA in male infertility.

Our study revealed significantly lower *SRD5A2* mRNA expression in men with asthenozoospermia and oligozoospermia compared to normozoospermic men, while azoospermic men exhibited higher expression. The decreased *SRD5A2* levels can be attributed to factors such as small prostate size and female pseudohermaphroditism during fetal development, which leads to reduced *SRD5A2* expression. Additionally, epigenetic modifications, including DNA methylation, histone modifications, and miRNA changes, likely contribute to the diminished *SRD5A2* expression in some infertile men by altering gene expression without changing the DNA sequence[25].

Genetic variants in the *SRD5A2* gene can significantly reduce enzyme activity and testosterone conversion, contributing to infertility, with mutations linked to 46, XY disorders of sex development disrupting DHT biosynthesis, and conditions like varicocele associated with lower *SRD5A2* content in sperm further exacerbating infertility issues[35,36].

Through bioinformatic analysis, we identified five miRNAs associated with *SRD5A2* expression to investigate their regulatory roles in three groups of fertile and infertile sperm samples. We then conducted qRT-PCR to measure the expression levels of *miR-6090*, *hsa-miR-5189-5p*, *miR-23a-5p*, *miR-1914-3p*, and *miR-4540* in seminal fluid. Our results confirmed that the levels of *hsa-miR-5189-5p*, *miR-6090*, and *miR-23a-5p* were significantly higher in infertile men compared to *miR-1914-3p* and *miR-4540*. Among these, *hsa-miR-5189-5p* showed the highest expression in infertile men, suggesting that these three miRNAs play a crucial role in regulating *SRD5A2* gene expression in male infertility.

The correlation analysis revealed that *miR-5189-5p* and *miR-6090* have a significant negative correlation with men suffering from

asthenozoospermia and oligozoospermia, while showing a positive correlation with azoospermic men. These findings align with the *SRD5A2* expression levels, which decrease in asthenozoospermia and oligozoospermia but increase in azoospermia. Notably, our validation confirms that *miR-5189-5p* and *miR-6090* regulate *SRD5A2* expression in male infertility. Our results highlight their potential as sperm quality biomarkers and as promising candidates for further investigation into their roles in spermatogenesis.

Exosome levels were high in azoospermic men and showed a significant positive correlation with *SRD5A2* expression, while levels were low and negatively correlated with *SRD5A2* in asthenozoospermic men. The mean expression values of *hsa-miR-5189-5p* and *miR-6090* were also elevated, with a significant positive correlation between exosome levels and these miRNAs in all three groups: asthenozoospermia, oligozoospermia, and azoospermia. This highlights the importance of transferring these microRNAs within seminal fluid. Exosomes serve as a novel delivery medium that transmits genetic information over long distances for diagnostic purposes[20].

This transfer of miRNAs *via* exosomes is a critical aspect of male reproductive biology, offering insights into the molecular mechanisms underlying fertility and the potential for developing novel diagnostic and therapeutic strategies for male infertility[23,37]. The intricate network of proteins and miRNAs within seminal exosomes suggests a complex regulatory mechanism that impacts gene expression, contributing to the maintenance of sperm quality and fertility potential. This regulatory capacity of exosomes underscores their potential as biomarkers for diagnosing male infertility and as therapeutic targets for enhancing reproductive outcomes[38,39].

Despite the novel findings of this study regarding the roles of seminal exosomes, miRNAs, and *SRD5A2* gene expression in male infertility, several limitations must be acknowledged. First, the sample size was relatively small and restricted to a single geographical region (Iraq), which may limit the generalizability of the findings to broader populations with varying genetic and environmental backgrounds. Second, while bioinformatics tools were employed to predict miRNA targets, *in vitro* functional validation of the miRNA–*SRD5A2* interactions was not conducted, which may limit the mechanistic insights drawn from expression correlations alone. Third, although potential confounding variables such as smoking and genitourinary disorders were excluded, other factors like hormonal profiles, lifestyle influences, and environmental exposures were not systematically assessed. Additionally, the use of ELISA for exosome quantification provides total levels but lacks detailed profiling of exosomal subpopulations or surface markers, which could provide more nuanced insight into their origin and function. Lastly, the cross-sectional design limits causal inferences;

thus, longitudinal studies and functional assays are recommended for future investigations.

In conclusion, this study indicates the significant roles of exosomes, miRNAs, and the *SRD5A2* gene in male infertility among Iraqi men with asthenozoospermia, oligozoospermia, and azoospermia. Our results indicate that lower exosome levels in seminal plasma correlate with infertility, potentially linked to testicular inflammatory conditions. Additionally, the altered expression of *SRD5A2* mRNA and specific miRNAs, particularly *hsa-miR-5189-5p*, *miR-6090*, and *miR-23a-5p*, highlights their potential as biomarkers for male infertility. This study provides a foundation for further research into the molecular mechanisms of male infertility and the development of targeted diagnostic and therapeutic strategies.

Conflict of interest statement

The authors declare that they have no competing interests.

Funding

The study receives no extramural funding.

Data availability statement

All data in this manuscript are available.

Authors' contributions

Adnan F. AL-Azaawie performed conceptualization, methodology design, sample collection, experimental analysis, data interpretation, and manuscript drafting. Mousa Jasim Mohammed performed laboratory work including RNA extraction, qRT-PCR assays, statistical analysis, and result visualization. Ahmed AbdulJabbar Suleiman contributed to supervision, bioinformatics analysis of miRNA targets, critical revision of the manuscript, correspondence with the journal, and final approval of the version to be published. All authors have read and approved the final manuscript.

Publisher's Note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- [1] Junahar D, Dwiputri R, Adikusuma W, Darmawi D, Afdal A, Irham LM, et al. Potential biomarker signatures in male infertility: Integrative genomic analysis. *Egypt J Med Hum Genetics* 2024; **25**(1): 1-8.
- [2] Wang Z, Wang R, Wang X, Zheng S, Li M, Yu Y, et al. Infertility risk assessment with ultrasound in congenital adrenal hyperplasia male patients. *Sci Rep* 2024; **14**(1): 1-9.
- [3] Sohail A, Arshad A, Tariq T, Bibi A, Aslam S, Irfan M. Role of *MDR1* gene polymorphisms in human male infertility: A meta-analysis. *Am J Mens Health* 2023; **17**(2): 15579883231166645. doi: 10.1177/15579883231166645.
- [4] He X, Yang S, Lv M. Editorial: Genetic factors in male infertility. *Front Genet* 2023; **14**: 1187445. doi: 10.3389/fgene.2023.1187445.
- [5] Farrokhyar S, Baharara J, Eidi A, Roodbari NH. Effect of exosomes derived from bone marrow mesenchymal stem cells on ovarian granulosa cells of immature NMRI mice. *Cell J (Yakhteh)* 2024; **26**(1): 28-38.
- [6] Mobarak H, Rahbarghazi R, Lolicato F, Heidarpour M, Pashazadeh F, Nouri M, et al. Evaluation of the association between exosomal levels and female reproductive system and fertility outcome during aging: A systematic review protocol. *Syst Rev* 2019; **8**(1): 1-6.
- [7] Jeon H, Seo SM, Kim TW, Ryu J, Kong H, Jang SH, et al. Circulating exosomal miR-1290 for diagnosis of epithelial ovarian cancer. *Curr Issues Mol Biol* 2022; **44**(1): 288-300.
- [8] Gaffo E, Garbin A, Galligani I, Tosato A, Damanti CC, Lovisa F, et al. MiR-26a-5p as a reference to normalize microRNA qRT-PCR levels in plasma exosomes of pediatric hematological malignancies. *Cells* 2021; **10**(1): 101. doi: 10.3390/cells10010101.
- [9] Grunt M, Failla AV, Stevic I, Hillebrand T, Schwarzenbach H. A novel assay for exosomal and cell-free miRNA isolation and quantification. *RNA Biol* 2020; **17**(4): 425-440.
- [10] Yue D, Yang R, Xiong C, Yang R. Functional prediction and profiling of exosomal circRNAs derived from seminal plasma for the diagnosis and treatment of oligoasthenospermia. *Exp Ther Med* 2022; **24**(5): 649. doi: 10.3892/etm.2022.11586.
- [11] Gholipour H, Amjadi FS, Zandieh Z, Mehdizadeh M, Ajdary M, Delbandi AA, et al. Investigation of the effect of seminal plasma exosomes from the normal and oligoasthenoteratospermic males in the implantation process. *Rep Biochem Mol Biol* 2023; **12**(2): 294-305.
- [12] Xiao D, Ohlendorf J, Chen Y, Taylor DD, Rai SN, Waigel S, et al. Identifying mRNA, microRNA and protein profiles of melanoma exosomes. *PLoS One* 2012; **7**(10): e46874. doi: 10.1371/journal.pone.0046874.
- [13] Barceló M, Mata A, Bassas L, Larriba S. Exosomal microRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue. *Hum Reprod* 2018; **33**(6): 1087-1098.
- [14] Zhang X, Song D, Kang H, Zhou W, Chen H, Zeng X. Seminal plasma exosomes evoke calcium signals via the CatSper channel to regulate human sperm function. *BioRxiv* 2020. doi: 10.1101/2020.05.21.094433.

- [15] Abu-Halima M, Belkacemi A, Ayesh BM, Simone Becker L, Sindiani AM, Fischer U, et al. MicroRNA-targeting in spermatogenesis: Over-expressions of microRNA-23a/b-3p and its affected targeting of the genes *ODF2* and *UBQLN3* in spermatozoa of patients with oligoasthenozoospermia. *Andrology* 2021; **9**(4): 1137-1144.
- [16] Salas-Huetos A, James ER, Aston KI, Carrell DT, Jenkins TG, Yeste M. The role of miRNAs in male human reproduction: A systematic review. *Andrology* 2020; **8**(1): 7-26.
- [17] Ghafourian M, Mahdavi R, Akbari Jonoush Z, Sadeghi M, Ghadiri N, Farzaneh M, et al. The implications of exosomes in pregnancy: Emerging as new diagnostic markers and therapeutics targets. *Cell Commun Signal* 2022; **20**(1): 1-19.
- [18] Han B, Cheng T, Zhu H, Yu J, Zhu WJ, Song HD, et al. Genetic analysis of 25 patients with 5 α -reductase deficiency in Chinese population. *Biomed Res Int* 2020; **2020**: 1789514. doi: 10.1155/2020/1789514.
- [19] Zeng XT, Su XJ, Li S, Weng H, Liu TZ, Wang XH. Association between *SRD5A2* rs523349 and rs9282858 polymorphisms and risk of benign prostatic hyperplasia: A meta-analysis. *Front Physiol* 2017; **8**: 688. doi: 10.3389/fphys.2017.00688.
- [20] Elsharkawi F, Elsabah M, Shabayek M, Khaled H. Urine and serum exosomes as novel biomarkers in detection of bladder cancer. *Asian Pac J Cancer Prevent* 2019; **20**(7): 2219-2224.
- [21] Mohammadi A, Shabani R, Bashiri Z, Rafiei S, Asgari H, Koruji M. Therapeutic potential of exosomes in spermatogenesis regulation and male infertility. *Biol Cell* 2024; **116**(6): e2300127. doi: 10.1111/boc.202300127.
- [22] Xu J, He C, Fang YW, Hu ZY, Peng ML, Chen YY, et al. Testicular exosomes disturb the immunosuppressive phenotype of testicular macrophages mediated by miR-155-5p in uropathogenic *Escherichia coli*-induced orchitis. *Asian J Androl* 2022; **25**(0): 389-397.
- [23] Panner Selvam MK, Agarwal A, Sharma R, Samanta L, Gupta S, Dias TR, et al. Protein fingerprinting of seminal plasma reveals dysregulation of exosome-associated proteins in infertile men with unilateral varicocele. *World J Mens Health* 2021; **39**(2): 324-337.
- [24] Cheng Y, Zeng Q, Han Q, Xia W. Effect of pH, temperature and freezing-thawing on quantity changes and cellular uptake of exosomes. *Protein Cell* 2019; **10**(4): 295-299.
- [25] Zhang W, Yu B, Luo W, Sun B, Zhang X, Wang X, et al. *In vitro* functional study of fifteen *SRD5A2* variants found in Chinese patients and the relation between the *SRD5A2* genotypes and phenotypes. *J Steroid Biochem Mol Biol* 2023; **235**: 106421. doi: 10.1016/j.jsbmb.2023.106421.
- [26] Li SP, Li LW, Sun MX, Chen XX, Wang XF, Li ZK, et al. Identification of a novel mutation in the *SRD5A2* gene of one patient with 46,XY disorder of sex development. *Asian J Androl* 2018; **20**(5): 518-519.
- [27] Aquila S, Montanaro D, Guido C, Santoro M, Perrotta I, Gervasi S, et al. Human sperm molecular anatomy: The enzyme 5 α -reductase (*SRD5A*) is present in the sperm and may be involved in the varicocele-related infertility. *Histochem Cell Biol* 2015; **144**(1): 67-76.
- [28] Dimik M, Abeyasinghe P, Logan J, Mitchell M. The exosome: A review of current therapeutic roles and capabilities in human reproduction. *Drug Deliv Transl Res* 2022; **13**(2): 473-502.
- [29] Gad YZ, Khairt R, Mazen I, Osman HG. Detection of the G34R mutation in the 5 alpha reductase 2 gene by allele specific PCR and its linkage to the 89L allele among egyptian cases. *Sexual Dev* 2007; **1**(5): 293-296.
- [30] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- $\Delta\Delta C_T$} method. *Methods* 2001; **25**(4): 402-408. doi: 10.1006/meth.2001.1262.
- [31] Elsharkawi F, Elsabah M, Shabayek M, Khaled H. Urine and serum exosomes as novel biomarkers in detection of bladder cancer. *Asian Pac J Cancer Prevent* 2019; **20** (7): 2219-2224.
- [32] Mohammadi A, Shabani R, Bashiri Z, Rafiei S, Asgari H, Koruji M. Therapeutic potential of exosomes in spermatogenesis regulation and male infertility. *Biol Cell* 2024; **116**(6): e2300127. doi: 10.1111/boc.202300127.
- [33] Xu J, He C, Fang YW, Hu ZY, Peng ML, Chen YY, et al. Testicular exosomes disturb the immunosuppressive phenotype of testicular macrophages mediated by miR-155-5p in uropathogenic *Escherichia coli*-induced orchitis. *Asian J Androl* 2022; **25**(3): 389-397. doi: 10.4103/aja202243.
- [34] Cheng Y, Zeng Q, Han Q, Xia W. Effect of pH, temperature and freezing-thawing on quantity changes and cellular uptake of exosomes. *Protein Cell* 2019; **10**(4): 295-299.
- [35] Li SP, Li LW, Sun MX, Chen XX, Wang XF, Li ZK, et al. Identification of a novel mutation in the *SRD5A2* gene of one patient with 46,XY disorder of sex development. *Asian J Androl* 2018; **20**(5): 518-519.
- [36] Aquila S, Montanaro D, Guido C, Santoro M, Perrotta I, Gervasi S, et al. Human sperm molecular anatomy: The enzyme 5 α -reductase (*SRD5A*) is present in the sperm and may be involved in the varicocele-related infertility. *Histochem Cell Biol* 2015; **144** (1): 67-76.
- [37] Sahoo B, Choudhary RK, Sharma P, Choudhary S, Gupta MK. Significance and relevance of spermatozoal RNAs to male fertility in livestock. *Front Genet* 2021; **12**. doi: 10.3389/fgene.2021.768196.
- [38] Guo XR, Ma Y, Ma ZM, Dai TS, Wei SH, Chu YK, et al. Exosomes: The role in mammalian reproductive regulation and pregnancy-related diseases. *Front Physiol* 2023; **14**: 1056905. doi: 10.3389/fphys.2023.1056905.
- [39] Baskaran S, Panner Selvam MK, Agarwal A. Exosomes of male reproduction. *Adv Clin Chem* 2020; **95**: 149-163.