Zygote

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MicroRNA expression in infertile men: its alterations and effects

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Review

Cite this article: Kiani M *et al*. (2019) MicroRNA expression in infertile men: its alterations and effects. *Zygote* **27**: 263–271. doi: 10.1017/S0967199419000340

Received: 20 February 2019
Accepted: 20 May 2019
First published online: 15 August 2019

Keywords:
Biomarker; Male; MicroRNA; Non-invasive; Unexplained infertility

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Summary

Infertility is an important reproductive health problem, and male infertility is especially important in more than half of infertility cases. Due to the importance of genetic factors in this condition, analysis of semen alone is not enough to recognize men with idiopathic infertility. A molecular non-invasive investigation is necessary to gain valuable information. Currently, microRNAs (miRNAs) are being used as non-invasive diagnostic biomarkers. miRNAs, single-stranded non-coding RNA molecules, act as post-transcriptional gene silencing regulators either by inhibition or repression of translation. Changes in the regulation of miRNAs have been investigated in several different types of male infertility, therefore the biological role of miRNA and gene targets has been defined. The purpose of this study was to review recent research on the altered expression of miRNA in semen, sperm, and testicular biopsy samples in infertile males with different types of unexplained infertility. Changes in miRNA regulation were investigated using microarray and the miRNA levels were confirmed by real-time qRT-PCR. This review explains why creating a non-invasive diagnostic method for male infertility is necessary and how changes in miRNA expression can be used as new diagnostic biomarkers in patients with differing spermatogenic and histopathologic injury.

Introduction

Infertility in couples is defined as 12 months of unprotected intercourse without a resulting successful pregnancy. This worldwide reproductive health problem involves approximately 15% of couples. Male factors are the cause of half the cases of infertility (Okada *et al*., 2008). Reduced fertility in men has several causes including varicocele, immunological and genetic factors, endocrine disturbances and urogenital aberrations. For 60–75% of men, the clear cause of unexplained infertility could not be detected, resulting in the term 'idiopathic male infertility'. Results of physical examinations are usually normal without any previous history of fertility problems (Quaas and Dokras, 2008; Guzick *et al*., 2001). Genetic anomalies are involved in some men with idiopathic infertility, which is divided into two major categories of quantitative and qualitative abnormalities that include azoospermia, cryptozoospermia, oligoasthenozoospermia and asthenospermia, teratozoospermia and necrospermia, respectively (Hargreave, 2000).

Invasive and non-invasive diagnostic methods for male infertility can be applied. Identification of subsets of patients is based on testicular and prostate biopsy, but these invasive diagnostic approaches may cause some impairment. Similarly, a slightly less invasive procedure, fine-needle aspiration, is often used in gene expression studies to evaluate epigenetics, cytological and histologic diagnoses, provided that inadequate amounts of sample are collected. The use of non-invasive diagnostic methods, therefore, can play a major role in addressing the issue of male infertility (Hamada *et al*., 1998; Kline *et al*., 2006; Huang *et al*., 2009). The assessment of causes of male infertility requires a total history alongside semen examination, however this evaluation alone is insufficient to recognize men with unexplained infertility. Therefore, non-invasive molecular investigation is needed to gain valuable information when assessing and diagnosing these men (Khazaie and Nasr Esfahani, 2014). The study of miRNA is a major area of interest within the field of non-invasive diagnostic biomarkers of diseases (Baldassarre *et al*., 2017). In patients with various types of male infertility, a quantitative miRNA expression profile of testicular tissue, sperm, and original plasma can prove to be a helpful molecular assessment, complementing histopathologic diagnostics and governing the level of spermatogenic weakening (Wang *et al*., 2011; Barceló *et al*., 2018).

miRNAs are short (20–23 nucleotides), single-stranded non-coding RNA molecules that manage post-transcriptional gene silencing, and initiate translational hindrance or restraint (He and Hannon, 2004). miRNAs play a crucial role in different biological procedures including differentiation, growth, cell propagation and apoptosis (Bartel, 2004; He and Hannon, 2004; Pasquinelli *et al*., 2005). Recent research has demonstrated that miRNAs are produced in serum,

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plasma, semen, and other body fluids (Poon *et al.*, 2000; Li *et al.*, 2004). Therefore, changes in miRNA expression and in their recognized locations are likely to contribute to human illness, including sperm defects. There is some evidence to suggest that miRNAs concentrations in seminal plasma, sperm, and testicular biopsy may offer valuable data on gene expression in the male generative system. These data could also provide a new way to develop innovative non-invasive biomarkers of male infertility (Wang *et al.*, 2011).

As miRNAs have a pivotal role in spermatogenesis and also fertilization of the oocyte, offspring phenotype may also be affected (Kotaja, 2014; Reilly *et al.*, 2016). The issue of altered miRNAs has received considerable critical attention in the field of male infertility in recent years. Aberrant expression of certain miRNAs is linked to certain male infertility dysfunctions. For this reason, miRNA expression might act as a suitable molecular biomarker for diagnosis of male infertility disorders and provide non-invasive biomarkers to diagnose patients more effectively (Lian *et al.*, 2009; Wang *et al.*, 2011; Salas-Huetos *et al.*, 2015). This study was undertaken to review recent research on altered expression of microRNA in semen, sperm and testicular biopsy samples from males with different types of unexplained infertility.

Mechanisms of the function of microRNA

MicroRNAs are usually synthesized in the nucleus using RNA polymerase II to produce a primary miRNA transcript (pri-miRNA). Drosha, an RNase III endonuclease, together with DGCR8, form part of the Microprocessor complex and recognize the double-stranded RNA (dsRNA) hairpin structure in pri-miRNA (Han *et al.*, 2004; Landthaler *et al.*, 2004). Two strands of pri-miRNA are cut and, accordingly, produce a precursor miRNA (pre-miRNA) (Lee *et al*, 2003). Pre-miRNAs are exported into the cytoplasm and the RNase III enzyme Dicer cleaves the hairpin structure of pre-miRNA. This endoribonuclease interacts with 5′ and 3′ modifications of the hairpin and removes the loop joining the 3′ and 5′ arms. Therefore, an incomplete miRNA of around 22 nucleotides in length is created. The subsequent miRNA duplex is then untwisted by the action of a helicase and, although the miRNA strand (the developed strand) is cleaved, it is incorporated into the RNA-induced silencing complex (RISC) (Yi *et al.*, 2003; Lund *et al.*, 2004; Wilson and Doudna, 2013). RISC incorporates the mature miRNA strand to form a complex, which acts to cleave an mRNA target. Resulting miRNA/mRNA complementarity success leads to both translational suppression and mRNA neutralization. MicroRNAs can act by diminishing target mRNA levels or by hindering their translation (Wahid *et al.*, 2010; Kotaja, 2014).

MicroRNAs in spermatogenesis

Self-reestablishment and propagation of spermatogonia, the meiotic division of spermatocytes, and postmeiotic separation of spermatids into spermatozoa are three important phases in the complex differentiation process of spermatogenesis. This procedure, at both the transcriptional and the post-transcriptional levels, is a firmly regulated process that allows continuous production of a gamete throughout adulthood (Bouhallier *et al.*, 2010; Ni *et al.*, 2011). An important regulator of spermatogenesis is the innovative application of post-transcriptional control that is controlled by microRNAs. Some examinations have evaluated the key role of non-coding RNAs as post-transcriptional controllers of spermatogenesis. Long non-coding RNAs basically are used as transcriptional repressors and chromatin alteration factors, piwi-interacting RNAs, which are known to control transposon silencing of microRNAs, and managing the expression of specific target messenger RNAs (McLachlan, 2013; Kotaja, 2014).

miRNAs are an important component of spermatogenesis and play vital roles in the progress of spermatozoa, principally in germ cells and somatic cells. However, it is widely accepted that any deregulation in miRNA expression can have significant effects on spermatogenesis pathways and lead to reproduction aberrations.

Alteration in microRNA expression in different varieties of male infertility

Variation in miRNA expression patterns has been allied to dissimilar human testicular histopathologic patterns (Sertoli cell only, mixed atrophy, and germ-cell arrest), and spermatogenic impairments (asthenozoospermia, oligozoospermia, oligoasthenozoospermia). Evaluation of the seminal plasma of patients with azoospermia or with sperm with abnormal morphology has indicated that miRNAs could be used as special biomarkers to appraise human male fertility (Abu-Halima *et al.*, 2013; Kotaja, 2014).

The following section describes changes in the regulation of miRNA in some types of male infertility assessed by microarray; miRNA levels were confirmed by real-time qRT-PCR.

Azoospermia

A large percentage of male infertility is due to azoospermia (the complete lack of spermatozoa upon semen assessment) instigated by blockage of the excurrent ductal system (obstructive azoospermia, OA) or by abnormal/absent testes (non-obstructive azoospermia, NOA), with the latter found in 1% of all mature men (Lian *et al.*, 2010; Wosnitzer *et al.*, 2014). Tables 1 and 2 show a brief description of changes in miRNA regulation in azoospermia and non-obstructive azoospermia patients.

Asthenozoospermia

The term asthenozoospermia or asthenospermia refers to a biological event characterized by decline in sperm motility. It is diagnosed when the percentage of motile sperm is less than 50% or when the percentage of motile sperm moving in a straight line and with a speed of 25 μm/s is less than 25% (Youn *et al.*, 2011). Table 3 presents the results from assessment of changes in miRNA expression in asthenozoospermia patients.

Oligoasthenoteratozoospermia

Previous studies have mostly defined oligospermia and oligozoospermia as male infertility due to low concentrations of sperm in semen. Often semen with a reduced sperm concentration may also show significant abnormalities in sperm morphology and motility (McLachlan, 2013). Microarray and quantitative real-time qRT-PCR results for oligoasthenoteratozoospermia patients related to changes in miRNA regulation are summarized in Table 4.

Teratozoospermia

The broad use of the term teratospermia or teratozoospermia is characterized by the presence of sperm with irregular morphology that affect male fertility. According to the criteria in a World

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

Table 1. Changes in the regulation of miRNAs in the human testicular biopsy, semen, and sperm samples from azoospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation) by microarray	MicroRNA name (upregulation) microarray	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	Gene target	Reference
Azoospermic	Testicular samples	hsa-mir-19b-1, hsa-mir-19b-2, hsa-mir-20a, hsa-mir-302a, hsa-mir-371a, hsa-mir-372, hsa-mir-373, hsa-mir -383, hsa-mir-491, hsa-mir-520d, hsa-mir-92a-1, hsa-mir-92a-2						Lian *et al.*, 2009
Azoospermic	Seminal plasma	hsa-miR-1, hsa-miR-122, hsa-miR-124, hsa-miR-128, hsa-miR-146b-5p, hsa-miR-181a, hsa-miR-219-2-3p, hsa-miR-34c-5p, hsa-miR-374b, hsa-miR-495, hsa-miR-509-5p, hsa-miR-513a-5p, hsa-miR-890, hsa-miR-9	hsa-miR-106b, hsa-miR-193a-3p, hsa-miR-19b, hsa-miR-21, hsa-miR-532-5p	miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p				Wang *et al.*, 2011
Azoospermic	Testicular tissues		hsa-mir-141, hsa-mir-429, hsa-mir-7-1, hsa-mir-7-2, hsa-mir-7-3					Abu-Halima *et al.*, 2013
Azoospermic	Germ-cell arrest miRNAs compared with normal	hsa-miR-449a, hsa-miR-34b, hsa-miR-34c-5p, hsa-miR-34b, hsa-miR-449b	hsa-miR-135a, hsa-miR-3137, hsa-miR-99b, hsa-miR-3692	hsa-miR-34b, hsa-miR-34b, hsa-miR-34c-5p, and hsa-miR-449a		Bcl-2 plays a serious role in male germ-cell development by interceding spermatogonial apoptosis	Caspase-2, bcl2 (mir-449a)	Abu-Halima *et al.*, 2013
Azoospermic	Testicular biopsy from males with pathologic alterations of spermatogenesis; Sertoli cell only miRNAs compared with normal Sertoli cell only (SCO)	hsa-miR-34b, hsa-miR-34c-5p, hsa-miR-449a, hsa-miR-574-5p, hsa-miR-15b, hsa-miR-125b, hsa-miR-125a-5p, hsa-miR-16, hsa-miR-204, hsa-miR-1260, hsa-miR-23a, hsa-miR-145, hsa-miR-1260b, hsa-miR-30b, hsa-miR-25, hsa-miR-1274a, hsa-miR-22, hsa-miR-34b, hsa-miR-19a, hsa-miR-574-3p, hsa-miR-92a	hsa-miR-3925, hsa-miR-135a, hsa-miR-1471, hsa-miR-642b, hsa-miR-617, hsa-miR-3180-3p, hsa-miR-718, hsa-miR-3200-5p, hsa-miR-99b, hsa-miR-3945, hsa-miR-3648, hsa-miR-575, hsa-miR-936, hsa-miR-3137, hsa-miR-548q, hsa-miR-4322, hsa-miR-1181, hsa-miR-125a-3p, hsa-miR-371-5p, hsa-miR-373*, hsa-miR-3197, hsa-miR-3656, hsa-miR-3194	hsa-miR-34b, hsa-miR-34b, hsa-miR-34c-5p, and hsa-miR-449a	hsa-miR-10b	Apoptosis, cell proliferation and cell cycle, Bcl-2 plays a critical role in male germ-cell development by mediating spermatogonial apoptosis	RNA polymerase II, TATA box binding protein-associated factor (TAF5), Insulin-like growth factor-binding protein 5 (IGFBP5), caspase-2, bcl2 (mir-449a)	Abu-Halima *et al.*, 2013
Azoospermic	Mixed atrophy (MA)	hsa-miR-34c-5p, hsa-miR-34b, hsa-miR-449a, hsa-miR-509-5p, hsa-miR-514, hsa-miR-34b, hsa-miR-517a, hsa-miR-506, hsa-miR-514b-5p, hsa-miR-129-3p	hsa-miR-127-3p, hsa-miR-410, hsa-miR-199a-5p, hsa-miR-379	hsa-miR-34b, hsa-miR-34b, hsa-miR-34c-5p, and hsa-miR-449a	hsa-miR-10b	Bcl-2 plays a critical role in male germ-cell development by mediating spermatogonial apoptosis	Caspase-2, bcl2 (mir-449a)	Abu-Halima *et al.*, 2013
Azoospermic	Testicular biopsies	miR-122, miR-31, miR-34b, miR-, 34c-5p, miR-375, miR-449a, miR-449b, miR-449b*, miR-515-5p, miR-517b, miR-517c, miR-518e, miR-518e*,miR-518f, miR-519a, miR-519b-3p, miR-519d, miR-520b, miR-52°c-3p, miR-520f, miR-520g, miR-520h and miR-, 526b*		miR-34b/c and miR-449 (consisting of three members: miR-449a, miR-449b, and miR-449c)	miR-10b*, the Sertoli cell expressed miR-202*10, miR-210, miR-504, miR-551a	Cell cycle regulation, spermatogenesis	Targeting the E2F-pRb pathway TGIF2 and NOTCH2 are targets of miR-34c and NOTCH1, regulated by both miR-34b and miR-34c, is acute for germ-cell differentiation and survival during spermatogenesis	Muñoz *et al.*, 2015

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

Table 2. Changes in the regulation of miRNA in the human testicular biopsy, semen, and sperm samples from non-obstructive azoospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation)	MicroRNA name (upregulation)	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	Gene target	Reference
Non-obstructive azoospermia	Testicular tissues	154 differentially downregulated (ref: Lian *et al.*, 2009)	hsa-miR-129-5p, hsa-miR-193a-3p, hsa-miR-193a-5p, hsa-miR-210, hsa-miR-23a, hsa-miR-302a, hsa-miR-371-5p, hsa-miR-374a, hsa-miR-423-3p, hsa-miR-491-3p, hsa-miR-554, hsa-miR-557, hsa-miR-565, hsa-miR-572, hsa-miR-638, hsa-miR-654-5p, hsa-miR-663, hsa-miR-744, hsa-miR-801	miR-520d-3p and miR-383	miR-302a and miR-491-3p	miR-1, miR-181a, miR-221 and miR-9: TIMP-3 (active role in testicular development and differentiation), SOX9: miR-145, is required for Sertoli cell maturation and normal spermatogenesis, MiR-383, GADD45G can induce apoptosis and inhibit cell development in reply to stress shock	mir-520:PRMT5, mir-383: Late cornified envelope protein 2C (LCE2C) mir-302a: RPL13A, mir-491: SLCO4A1, miR-302a and miR-383 is MLH1, miR-491-3p: SCP1, miR-520d-3p: SCP3.	Lian *et al.*, 2009
Non-obstructive azoospermia	Testes			miR-383		Reduced the levels of the targets of IRF1, namely cyclin D1, CDK2 and p21	Interferon regulatory factor-1 (IRF1)	Lian *et al.*, 2010
Non-obstructive azoospermia	Seminal plasma				miR-141, miR-429 and miR-7-1-3p	Cell cycle and apoptosis in spermatogenesis	Cbl and Tgfb2 were downregulated by miR-141, while Rb1 and Pik3r3 were downregulated by miR-7-1-3p.	Wu *et al.*, 2013

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

Table 3. Changes in the regulation of miRNAs in human testicular biopsy, semen, and sperm samples from asthenozoospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation) by microarray	MicroRNA name (upregulation) microarray	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	Gene target	Reference
Asthenospermia	Seminal plasma	hsa-miR-1, hsa-miR-124, hsa-miR-128, hsa-miR-219-2-3p, hsa-miR-495, hsa-miR-513a-5p, hsa-miR-9	hsa-miR-106b, hsa-miR-122, hsa-miR-146b-5p, hsa-miR-181a, hsa-miR-193a-3p, hsa-miR-19b, hsa-miR-21, hsa-miR-34c-5p, hsa-miR-374b, hsa-miR-509-5p, hsa-miR-532-5p, hsa-miR-890		miR-34c-5p, 122, miR-146b-5p, miR-181a, miR-374b, miR-509 -5p, and miR-513a-5p	Spermatogenesis, germ-cell differentiation, miR-122, a liver-specific miRNA, is also overproduced in all yolk sac tumours, miR-181a has been implicated in the regulation of B-cell differentiation and T-cell receptor signalling, miR-146b, however, is associated with inflammation and innate immune responses, miR-146b to be involved in the modulation of mRNAs related to apoptosis processes, miR-513a-5p is another miRNA with a role in immunity		Wang *et al.*, 2011
Asthenozoospermia	Spermatozoa	hsa-miR-1973, hsa-miR-34b, hsa-miR-122	hsa-miR-30a, hsa-miR-363, hsa-miR-26a, hsa-miR-200a, hsa-miR-141, hsa-miR-429, hsa-miR-193b, hsa-miR-29a, hsa-miR-1274a, hsa-miR-24, hsa-miR-4286, hsa-miR-99a	miR-122 and miR-34b	miR-141 and miR-200a	miR-26a targets EH domain-containing 1 and oestrogen receptor 1, spermatogenesis, testis development or spermatogenesis (mir-122)	Downregulation of transition protein 2 (TNP2) through targeting the 3′ untranslated region of TNP2 mRNA, which is synthesized only in round spermatids (mir-122), miR-34b and miR-34c is Notch gene homologue 1 (NOTCH1)	Abu-Halima *et al.*, 2013
Asthenozoospermia		hsa-miR-342-3p, hsa-miR-520, hsa-miR-629-3p, hsa-miR-942, hsa-miR-184, hsa-miR-34b-3p	hsa-miR-615-5p, hsa-miR-548c-5p, hsa-miR-143-3p, hsa-miR-548d-5p, hsa-miR-616-3p, hsa-miR-548b-5p, hsa-miR-520d-3p, hsa-miR-591, hsa-miR-605, hsa-miR-27a-5p, hsa-miR-1303, hsa-miR-770-5p, hsa-miR-370, hsa-miR-604, hsa-miR-939-5p, hsa-miR-432-3p, hsa-miR-1275, hsa-miR-19b-1-5p, hsa-miR-1254, hsa-miR-212-3p, hsa-miR-636, hsa-miR-572, hsa-miR-1255b-5p, hsa-miR-324-3p, hsa-miR-638, hsa-miR-491-5p	hsa-miR-629-3p, hsa-miR-132-5p, hsa-miR-15b-5p, hsa-miR-335-5p, hsa-miR-34b-3p, hsa-miR-520h, and hsa-miR-9-3p	hsa-miR-27a-5p and, hsa-miR-34b-3p	Chromatin modification, embryonic development, embryonic development ending in birth or egg hatching, and cell motion, motility of spermatozoa		Salas-Huetos *et al.*, 2015

Table 4. Changes in the regulation of miRNAs in human testicular biopsy, semen, and sperm samples from oligoasthenoteratozoospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation)	MicroRNA name (upregulation)	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	Gene target	reference
Oligoasthenoteratozoospermia	Spermatozoa	hsa-miR-34b, hsa-miR-15b, hsa-miR-34c-5p, hsa-miR-34b, hsa-miR-449a, hsa-miR-1973, hsa-miR-122, hsa-miR-16, hsa-miR-19a	hsa-miR-141, hsa-miR-193b, hsa-miR-26a, hsa-miR-20°c, hsa-miR-29a, hsa-miR-200a, 429, hsa-miR-99a, hsa-miR-363	miR-122, miR-34c-5p, miR-34b, and miR-16	miR-141 and miR-200a	Spermatogenesis, testis development or spermatogenesis (mir-122)	Downregulation of transition protein 2 (TNP2) through targeting the 30 untranslated region of TNP2 mRNA, which is synthesized only in round spermatids (mir-122), miR-34b and miR-34c is Notch gene homologue 1 (NOTCH1), Bcl-2 (mir-34c), miR-15b is predicted to target the mRNA coding region for isocitrate dehydrogenase 3 (NADp) alpha (IDH3A). Low expression of IDH3A disrupts sperm motility by altering sperm energy metabolism	Abu-Halima *et al.*, 2013
Oligoasthenoteratozoospermia	Spermatozoa			hsa-miR-335-5p		Cell death, cell survival, and cell proliferation	BCL2, BIRC5, MAPK1, MERTK, PTPRN2, RASA1, and SOX4	Abu-Halima *et al.*, 2013

Health Organization manual (5th edition) (WHO, 2010) teratozoospermia is defined when the percentage of normal spermatozoa in the ejaculate is less than 4% (Herati *et al.*, 2015). As seen from the data in Table 5, teratospermia patients exhibit changes in miRNAs regulation.

Oligozoospermia

Another problem in male infertility is oligospermia. This is characterized by a low sperm count. The WHO classifies sperm counts at or above 15×10^6/ml sperm in semen as average. Anything below this number is considered low and is diagnosed as oligospermia (Kumar and Singh, 2015). From Table 6, we can see changes in the regulation of miRNAs in patients with oligospermia.

Discussion

miRNA analysis is a suitable option for diagnosing infertility

Grading of diagnostic markers is based on ease of measurement, harmlessness, rate efficiency, variability with use, and consistency across gender and ethnic groups. Currently, low specificity, affectability, and false-positive outcomes are the major limitations of available biomarkers. There are several reasons why miRNAs have become a suitable option for the diagnosis of infertility. These markers are evolutionarily conserved small non-coding RNAs that are also acknowledged as micro-coordinators of gene expression. They are effective tools as potential new diagnostic and predictive biomarkers with great precision and sensitivity. The role of miRNAs in the regulation of progressive and physiological processes is critical and their lack has been associated with several diseases. In addition, miRNAs are stable and can be easily isolated and quantified from body fluids and tissues (Faruq and Vecchione, 2015). Recently, the practicalities of using miRNAs or miRNA boards as biomarkers have been under consideration in the diagnosis of cancer. The expression patterns of these biomarkers in oncology have initiated new and widespread discussion. Recent experiments have shown that miRNAs are impaired in cancer and are assumed to have an indispensable role in the development and advancement of this disease. Accordingly, the exploration of such molecules is a goal for the cancer diagnosis, treatment and prediction, offering promising methodology (Hamam *et al.*, 2017).

There is no reliable molecular procedure to identify male infertility as a cohort diagnosis from semen analysis or to corroborate conservative histology of testes biopsies. Variations in miRNA expression profiles in patients with dissimilar spermatogenic and histopathologic deficiencies could potentially to be used as new biomarkers for diagnostic purposes. This literature review assesses the importance of miRNA applications in different types of male infertility as a non-invasive indicator of diagnosis. One or more miRNAs could be considered as indicators for diagnosis of some causes of male infertility.

Changes in miRNA profiles in patients with various spermatogenic and histopathologic impedances can possibly be used as new biomarkers related to symptoms. In total, 30% of protein-coding events are orchestrated by miRNAs either as a clear mRNA target or preceding it.

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

Table 5. Changes in the regulation of miRNA in human testicular biopsy, semen, and sperm samples from teratospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation) by microarray	MicroRNA name (upregulation) microarray	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	reference
Teratospermia	Sperm	hsa-miR-151-5p, hsa-miR-935, hsa-miR-125a-3p, hsa-miR-130b-5p, hsa-miR-191-3p, hsa-miR-132-5p, hsa-miR-320b, hsa-miR-195-5p	hsa-miR-101-5p, hsa-miR-1305, hsa-miR-32-3p, hsa-miR-16-1-3p, hsa-miR-198, hsa-miR-5095p, hsa-miR-6163p, hsa-miR-34a5p, hsa-miR-7705p, hsa-miR-605, hsa-miR-380-5p			Cell cycle, negative regulation of cell differentiation, cell morphogenesis, cell projection, morphogenesis, cellular component morphogenesis, cell morphogenesis involved in differentiation, cell part morphogenesis, and embryonic morphogenesis	Salas-Huetos *et al.*, 2015
Teratospermia	Sperm			hsa-miR-449a, hsa-miR-19b-3p, hsa-miR-28-5p, hsa-miR-148b-3p, hsa-miR-106b-5p			Herati *et al.*, 2015
Teratospermia	Sperm			miR-191, miR-425			Battista *et al.*, 2013

Table 6. Changes in the regulation of miRNA in human testicular biopsy, semen, and sperm samples from oligospermia patients compared with the normal controls determined by microarray and quantitative real-time qRT-PCR

Diseases	Sample	MicroRNA name (downregulation) by microarray	MicroRNA name (upregulation) microarray	MicroRNA (downregulation) by quantitative real-time qRT-PCR	MicroRNA name (upregulation) by quantitative real-time qRT-PCR	Function	Gene target	Year/ reference
Oligozoospermia	Sperm	hsa-miR-935, hsa-miR-30d-3p, hsa-miR-125a-3p, hsa-miR-9-3p 5, hsa-miR-151-5p, hsa-miR-195-5p, hsa-miR-132-5p, hsa-miR-335-5p, hsa-miR-34b-3p, hsa-miR-15b-5p, hsa-miR-320b, hsa-miR-139-5p, hsa-miR-517a-3p, hsa-miR-28-5p, hsa-miR-1180	hsa-miR-483-5p, hsa-miR-491-5p, hsa-miR-324-3p	hsa-miR-132-5p, hsa-miR-15b-5p, hsa-miR-335-5p, hsa-miR-34b-3p, hsa-miR-520h, and hsa-miR-9-3p		Chromatin modification, cell projection morphogenesis, cell part morphogenesis, and cell morphogenesis	The processes that were significantly enriched in this group included chromatin modification, cell projection morphogenesis, cell part morphogenesis, and cell morphogenesis, which can also ultimately be associated to a low sperm production	Salas-Huetos *et al.*, 2015
Oligozoospermia	Semen spermatozoa	miR-25, miR-34b, miR-122, miR-152, miR-192, miR-335	let-7b, let-7c, let-7g, miR-21, miR-22, miR-30a, miR-148a, miR-221, miR-320a, miR-375, miR-423-3p, miR-423-5p	miR-34b, miR-122				Abu-Halima *et al.*, 2013

Benefits and disadvantages of miRNA as a diagnostic marker

The many benefits of miRNAs as biomarkers have drawn attention. These benefits include:

1. In the human 3′ untranslated region (UTR), over 45,000 miRNAs target destinations are available and over 60% of human protein-coding genes are presumably organized by multiple miRNAs. It is predicted that more than 30% of protein-coding genes are arranged by miRNAs, either by linking target mRNA or by linkage to mutated regions in the 3′-UTR, resulting in regulation of gene expression at the translational level.
2. MicroRNAs are endogenous, naturally plentiful, relatively stable, evolutionarily conserved, small non-coding RNAs that operate in post-transcriptional gene regulation in important biological and pathological processes.
3. Stable miRNAs have been found in several biological fluids such as plasma, serum, saliva and milk, and the expression motifs of serum miRNAs are known to be related to several illnesses.
4. MicroRNAs have been identified in blood plasma, even under unfavourable conditions (long-term deposit at room temperature, high or low pH, or bubbling due to freeze–thaw cycles).
5. Using microarray, hybridization, deep-sequencing and qRT-PCR analysis, miRNA quantitation it is easy to perform.
6. MicroRNAs play the main role in an extensive range of developmental processes including cell proliferation, stem cell separation, muscle differentiation, metabolism, apoptosis, and brain morphogenesis (Friedman *et al.*, 2009; Felekkis *et al.*, 2010; Yi and Fuchs, 2011; Faruq and Vecchione, 2015).

As with all diagnostic methods, this approach has some advantages and disadvantages, In reviewing the literature regarding the challenges facing miRNA use as diagnostic biomarkers, we concluded that all miRNA analysis need confirmatory steps, appropriate standardization of analytical procedures, and control of potential technical biases before being considered for use in clinical practices (Moldovan *et al.*, 2014). The capacity to take an extracellular miRNA signature from body fluids by non-invasive means exists, but ways to separate extracellular RNAs from plasma or serum must be extrapolated to other body liquids such as spittle or urine. Standardized and reliable methods need to be applied at many levels from the whole blood to plasma/serum preparation; handling and methods of RNA extraction and miRNA evaluation. To improve the efficiency of miRNA results, there should be minimal differences in procedures between laboratories and ways to limit inconsistencies among different operators. If methodological parameters are not considered in the design of these study, some individual and environmental factors in miRNA analysis could not be properly evaluated, and could plausibly influence miRNA application in clinical practices (Tiberio *et al.*, 2015).

Biological role of miRNA as a means for the diagnosis of infertility

miRNAs as biomarkers have increasingly gained a role in diagnosing abnormalities in spermatogenesis. The studies presented in Table 1 showed mir-499a in relation to caspase-2, bcl-2, RNA polymerase II, TATA box binding protein-associated factor (TAF5), and insulin-like growth factor-binding protein 5 (IGFBP5) gene expression, plus mir-449a playing an imperative role in apoptosis, cell proliferation and the cell cycle (Abu-Halima *et al.*, 2013). Bcl-2 plays a pivotal role in male germ-cell improvement by interceding in spermatogonial apoptosis. In azoospermia, miR-34b and miR-34c downregulate E2F-pRb, TGIF2 and NOTCH2, targets of miR-34c and miR-34b that are necessary for germ-cell differentiation and subsistence during spermatogenesis. In non-obstructive azoospermia (Table 2) miR-1, miR-181a, miR-221 and miR-9 affected the *TIMP-3* gene that has an intrinsic role in testicular progress and differentiation. miR-145 affects SOX9, which is essential for Sertoli cell maturation and normal spermatogenesis; and miR-383 regulates GADD45G, which can induce apoptosis and obstruct cell growth in answer to stress shocks. Cbl and Tgfb2 were downregulated by miR-141, and Rb1 and Pik3r3 were downregulated by miR-7-1-3p, which affects the cell cycle and apoptosis in spermatogenesis (Lian *et al.*, 2009, 2010; Wu *et al.*, 2013). In men with asthenospermia, miR-122 is important for spermatogenesis and germ-cell differentiation, miR-181a which is a liver-specific miRNA, and miR-146b which is related to the inflammation and the innate immune response, are involved in modulation of mRNAs linked to apoptosis. miR-513a-5p is another miRNA with a role in immunity that showed increase in expression (Wang *et al.*, 2011; Salas-Huetos *et al.*, 2015). Abu-Halima *et al.* (2013) showed that mir-122 was downregulated in infertile men, leading to the downregulation of transition protein 2 (TNP2), targeting the untranslated area of TNP2 mRNA, which is synthesized uniquely in round spermatids. This miRNA affects spermatogenesis, testis development and spermatogenesis. In teratospermia, miRNAs play a central role in cell cycle, negative control of cell differentiation, cell morphogenesis, cellular component morphogenesis and cell morphogenesis and were involved in differentiation, and embryonic morphogenesis. Salas-Huetos *et al.* (2015) showed that changes in miRNA expression in teratospermia coincided with a reduction in normal sperm, so changes in some miRNAs could be used as new biomarkers in diagnosing men with teratospermia.

A strong relationship has been reported between changes in miRNA expression in male infertility and gene expression. Based on evidence of miRNA changes in infertility, miRNA can be used as a diagnostic indicator for individual patients. Researchers have stated that they would focus on one miRNA for any infertile disease, so results could be used to provide a dedicated kit.

The following conclusions can be drawn from the present review: (1) Creating a non-invasive diagnostic method for male infertility is necessary. (2) miRNA is fast becoming a key instrument in the diagnosis of male infertility. (3) Changes in miRNA expression can be used as a new diagnostic biomarker in patients with different types of spermatogenic and histopathologic damage.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of interest. None.

Ethical standards. Not applicable.

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https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press

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https://doi.org/10.1017/S0967199419000340 Published online by Cambridge University Press