miRNAs as potential spermatogenesis biomarkers in patients with non-obstructive azoospermia

Universitat Autònoma de Barcelona

Joan Roncero Carol | Biomedical Sciences | Autonomous University of Barcelona | Faculty of Biosciences

1. INTRODUCTION

miRNAs

microRNAs (miRNAs) are non-protein coding RNAs of about 22 nucleotides long, with an essential role in post-transcriptional regulation. They regulate various biological processes, including embryonic development, cell death and cell proliferation. It is predicted that more than 60% of our proteins are targets of them.

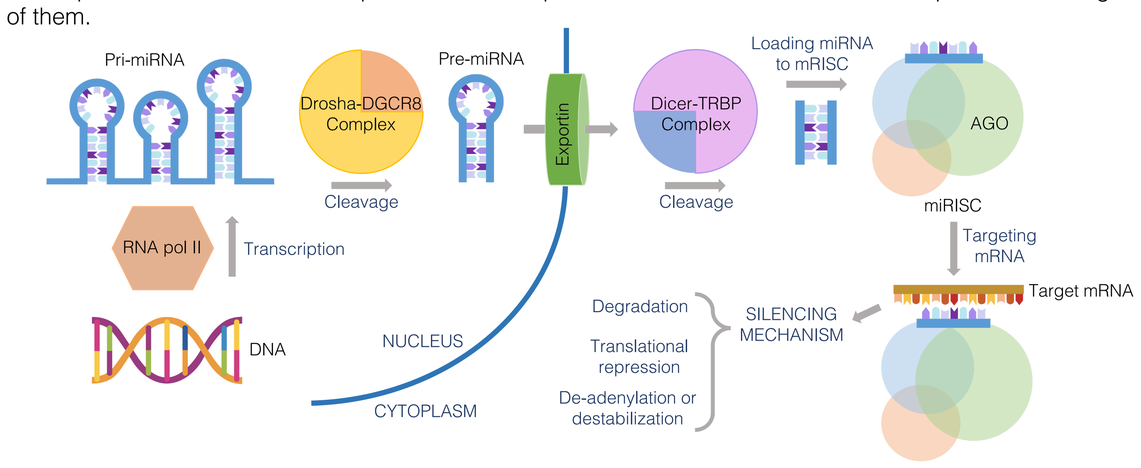

Figure 1. **miRNA biogenesis and mechanisms of action.** The precursor molecules of miRNAs (pri-miRNAs) are transcribed in the form of long hairpin structures by RNA polymerase II from intergenic or intragenic (introns) regions. The nuclear Drosha-DGCR8 complex processes the pri-miRNA into an intermediate called pre-miRNA. This pre-miRNA is transported to the cytoplasm where is processed by Dicer-TRBP resulting in a double-stranded miRNA of about 20 bp. This miRNA duplex is unwound, and only one strand associates with Argonaute (AGO) proteins to form the RNA-induced silencing complex (miRISC). miRNA, as a part of miRISC, binds to the 3'UTR of a target mRNA by complementarity and leads to its translational repression or degradation.

There are many evidences that miRNAs play an important role in spermatogenesis. They follow phase-specific expression patterns inside the testis and some of them are specifically expressed by each cell population.

Non-obstructive azoospermia

Non-obstructive azoospermia (NOA) is the most severe diagnosis in cases of male infertility, and it is caused by a severely impaired spermatogenic function of the testis. There are different histopathological patterns of NOA:

- Hypospermatogenesis: tubules with a very reduced population of germ cells, but they are present.
- Maturation arrest: arrest of the spermatogenic maturation sequence.
- Sertoli cell-only syndrome: tubules only populated by Sertoli cells, which are not germinal ones.

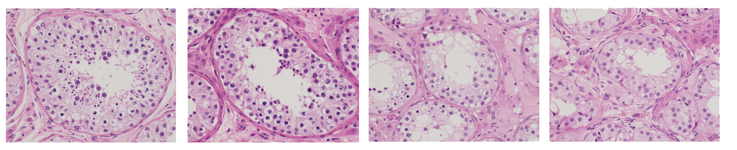

Figure 2. Seminiferous tubules of each histopathological pattern of NOA.

In some NOA cases, a sperm retrieval can be done by microdissection testicular sperm extraction (microTESE) in order to fertilize oocytes through intracytoplasmatic sperm injection (ICSI). However, there is a lack of possible biomarkers of spermatogenesis for a non-invasive diagnosis before the microTESE.

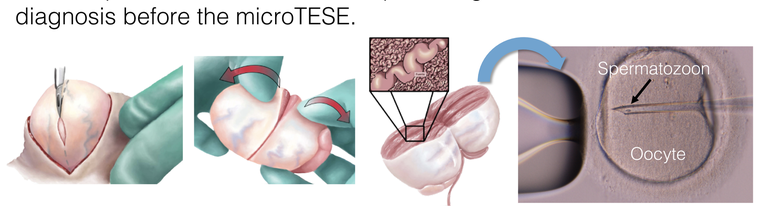

Figure 3. microTESE and ICSI techniques.

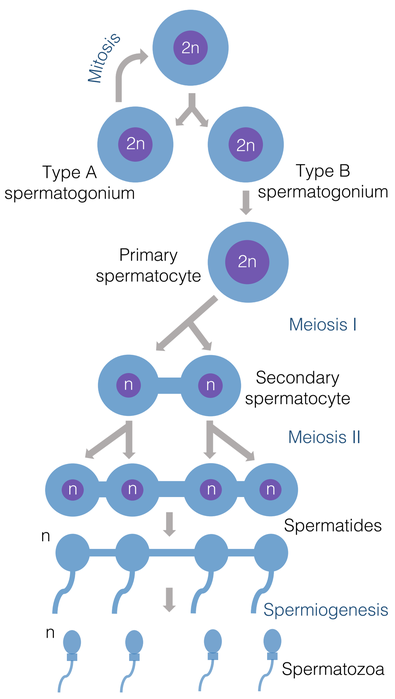

Figure 4. **Spermatogenesis.** Spermatogenesis is the process in which diploid spermatogonial cells become haploid spermatozoa cells. It can be divided into 3 steps: mitosis, meiosis and spermiogenesis.

2. OBJECTIVES

The main objectives of this review are:

- To provide a broad vision of the pathology (NOA) and to describe miRNAs, their generation and their implications in spermatogenesis.
- To describe a miRNA or a set of miRNAs present in seminal plasma that may predict the possibility of obtaining sperm from the testes of a patient with NOA.
- To relate the alteration of the miRNA selected in the context of infertility.

3. METHODOLOGY

The methodology consisted on a bibliographic search.

- The most used databases were Pubmed and Google Scholar. The articles selected were published from 2010 onwards.
- Some keywords used were: *non-obstructive azoospermia*, *miRNAs*, *successful sperm retrieval*, *microTESE*.
- The most important reviews and articles were read and summarized.

4. RESULTS

There is a correlation between the probability of having a successful sperm retrieval in each histology and their number of deregulated miRNAs: the more deregulated miRNAs, the more difficult to obtain sperm. Considering this, miRNAs could evaluate the possibility of having a successful sperm extraction.

Histopathological pattern	Sperm retrieval rates (%)	Deregulated miRNAs (n)
Sertoli cell-only syndrome	22,5 – 41	46
Maturation arrest	36,4 – 75	27
Hypospermatogenesis	81 – 100	0

Table 1. Sperm retrieval rates after performing microTESE and number of miRNAs deregulated according to testicular histology of patients with NOA.

Two studies have focused on the ability of some miRNAs to predict a successful sperm retrieval. The expression of some miRNAs was compared between NOA patients with a successful obtaining of sperm and NOA patients without it.

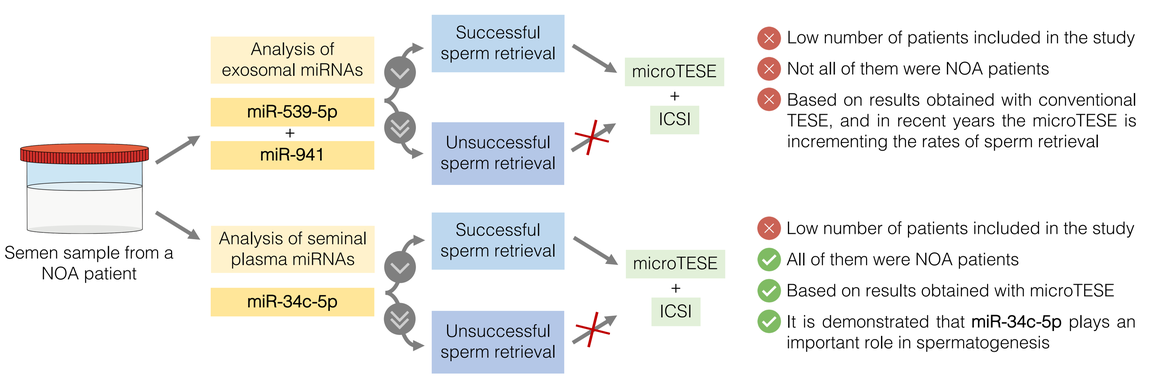

Figure 5. Possible diagnostic procedures, according to the studies, for patients with NOA interested in undergoing assisted reproduction techniques. The analysis of the exosomal miR-539-5p and miR-941 or the seminal plasma miR-34c-5p could predict their sperm retrieval and, therefore, the utility of performing the microTESE. Advantages and disadvantages of each study are commented on the right. ⌄: downregulated, ⌄⌄: very downregulated

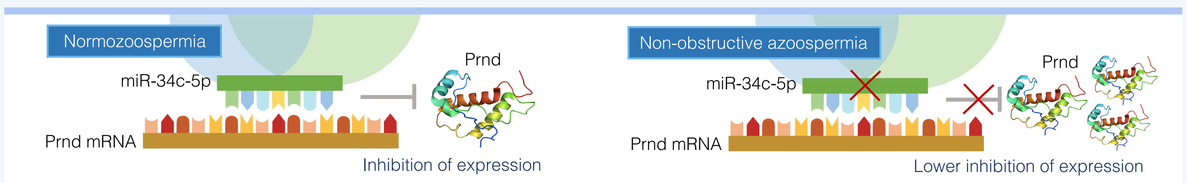

Figure 6. Effect of miR-34c-5p on Prion like protein doppel (Prnd), one of its targets. In NOA patients miR-34c-5p is downregulated, and consequently there is a lower inhibition of Prnd expression what causes a greater expression of the protein. Prnd is expressed in Sertoli cells, spermatozoa and seminal plasma. Therefore it may play a role in spermatogenesis, and a dysregulation of its expression could be another factor of the infertility of NOA patients.

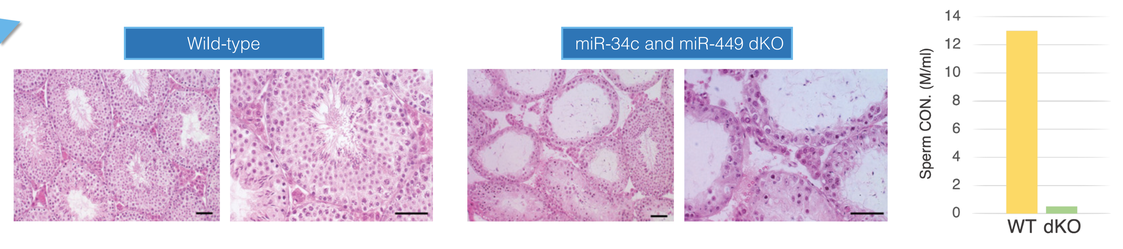

Figure 7. When a double knock-out was done for miR-34b/c and miR-449 clusters, mice presented a severely altered spermatogenesis. The epithelium of the seminiferous tubules was atrophic and disorganized, with only two layers of spermatogenic cells next to the basal membrane. Moreover, a very small number of spermatozoa was collected from dKO mice (about 5% of normal).

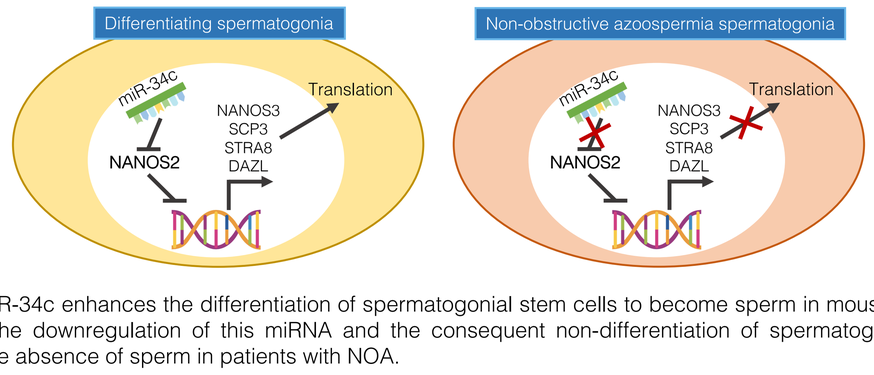

Figure 8. miR-34c enhances the differentiation of spermatogonial stem cells to become sperm in mouse by targeting NANOS2. The downregulation of this miRNA and the consequent non-differentiation of spermatogonia could be related to the absence of sperm in patients with NOA.

5. CONCLUSIONS

- There is a lack of biomarkers to predict a successful sperm retrieval in patients with NOA, and miRNAs are good candidates since they have been demonstrated to play an important role in spermatogenesis.
- One possible miRNA to predict a successful sperm retrieval before practicing the microTESE is miR-34c-5p, whose expressions in seminal plasma are significantly different between NOA patients with and without sperm retrieval.
- miR-34c-5p and its family are involved in spermatogenesis, but their redundant functions with other miRNAs make it necessary to focus not only on this single miRNA, but a profile of miRNAs.

6. BIBLIOGRAPHY

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