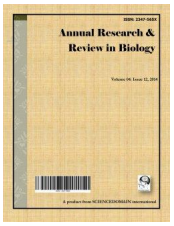

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Seminal Cell-Free DNA Test for the Management of Male Infertility

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of extracellular or circulating nucleic acids (Cfs), as a diagnostic or prognostic tool in oncology, has been broadly documented. However, their use in gynecology-obstetrics as non-invasive biomarkers in the management of infertility has become a recurring fact. The circulating nucleic acids are constituted by: free DNA which can be long or short DNA strands resulting from the apoptotic or necrotic processes, the free RNA containing: micro-RNAs (miRNAs) which are short single-stranded ribonucleic acids (RNA) that are able to deter the production of protein from a gene, Piwi-interacting RNAs (PiRNAs) that are small RNAs expressed in germ cells or even early embryos and small interfering RNAs (siRNAs) that are small RNAs that can bind specifically to a messenger RNA sequence and prevent gene expression by cleaving that RNA. The presence of circulating nucleic acids in many biological fluids such as: urine, seminal plasma and serum, the fact that they

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are easy to detect, the variation of their level according to the physiopathological conditions of the body and their implication in many biological processes such as folliculogenesis, steroidogenesis and spermatogenesis make nucleic acids circulating important biomarkers of interest in the management of male infertility. They compose a real complementary help for practitioners of medically assisted procreation. As a result, circulating nucleic acids are a promising avenue in the prevention of implantation failures. In this article, we will seek to affirm further, their importance in the management of male infertility, by highlighting their different uses.

Keywords: Circulating nucleic acids; male infertility; spermatogenesis; teratozoospermia; asthenozoospermia and oligozoospermia.

ABBREVIATIONS

Cf : Circulating nucleic acid
DNA : Deoxyribonucleic acid
ART : Assisted reproductive technology
RNA : Ribonucleic acid
mRNA : Messenger RNA
miRNA : MicroRNA
PiRNAs: Interaction of RNA with piwi
SiRNA : Small interfering RNA
OA : Obstructive Azoospermia
NOA : Non Obstructive Azoospermia
ROS : Reactive oxygen species
DFI : Fragmentation of DNA
MEST : Mesoderm-Specific Transcriptase
PTGDS: Prostaglandin-D-Synthase

1. INTRODUCTION

Interest in extracellular or circulating nucleic acids as biomarkers of interest in obstetric gynecology is well established. Circulating nucleic acids are used as potential biomarkers in the management of human infertility. Because they are easily detectable in many biological fluids [1], their rate varies depending on the physiopathological conditions of the body [2] and their involvement in many biological processes: steroidogenesis [3], spermatogenesis [4]. They are commonly used in the management of female infertility as a non-invasive biomarker in the detection and / or monitoring of pathologies of pregnancy [5], fetal and / or embryonic abnormalities [6] and finally in the evaluation of the functional state of the ovary [7]. Thus nucleic acids offer new perspectives, both from an innovative diagnostic and prognostic point of view in the management of human infertility. In this article we will not only explore the different components of circulating nucleic acids but also show the different uses of these in medically assisted procreation (PMA).

2. THE CIRCULATION OF NUCLEIC ACIDS OR EXTRACELLULAR IN BIOLOGICAL FLUIDS

Circulation of nucleic acids plays an important role in human pathophysiology. New data in the essay suggests that circulating nucleic acids plays a vital role in the management of male infertility [8].

2.1 Circulation of Nucleic Acids (CfDNAs and CfRNAs)

Circulation of nucleic acids consists of free DNA and free RNA which consists of: messenger ribonucleic acid (mRNA) and three large non-coding RNAs: microRNA (miRNA), Piwi-interacting RNA (PiRNA), small interfering RNAs (siRNA) [9]. The rate of nucleic acids varies according to pathophysiological conditions [10]. This variation reflects the physiopathological conditions and explains the use of nucleic acids as non-invasive biomarkers in different medical disciplines, particularly in human reproduction [11].

2.1.1 Free DNA (CfDNAs)

The free DNA or CfDNAs are double-stranded DNA molecules moreover [12]. They are present in the bloodstream in the form of fragments of the order of 180 to 360 base pairs. The interest of the study of CfDNA is no longer debated in certain medical disciplines, particularly in oncology [13]. They are used as non-invasive biological markers for cancer pathologies [14] detection or monitoring, the diagnosis of pathologies related to pregnancy [15,16]. In the treatment of male infertility, CfDNAs offer innovative new perspectives both from the point of view of diagnosis and prognosis [17].

2.1.2 Free RNA (CfRNAs)

CfRNAs like CfDNAs also vary according to pathophysiological conditions. They are also used as blood biomarkers for the detection or diagnosis of certain diseases as well as in treatments monitoring. They are detectable in many biological fluids of patients with breast cancer [18], nosopharyngeal carcinoma [19], malignant melanoma [20] and colorectal cancer [21]. The cfRNAs are also used as non-invasive biomarkers in the management of infertility [22].

2.1.3 MicroRNAs (miRNA)

MicroRNAs are short strands of non-coding RNA of the order of 19 to 25 nucleotides [23]. MicroRNAs do not code for proteins [24]. Their main and basic role is to block the translation of proteins into the mRNA to which they are attached [25]; they are involved also in various pathologies such as: foamy virus [26], HIV [27], vesicular stomatitis [28], hepatitis C [29], cancer [30].

However, MiRNAs are also important non-invasive biomarkers in human fertility especially for men, diagnosis as well as in the prevention of human abnormalities in general [31].

2.1.4 Piwi-interacting RNA (PiRNAs)

The use of circulating nucleic acids as non-invasive biomarkers has been extended to piwi interacting RNA (PiRNA). PiRNAs are a class of small RNAs of the order of 24 to 31 nucleotides [32]. The function of PiRNAs is to block the activity of the mobile elements present in the DNA. They are used as biomarkers because they have been shown to be unregulated in certain cancerous conditions [33], such as: Colorectal cancer [34], prostate cancer [35], and pancreas cancer [36]. They also take part in maintaining the integrity of germinal DNA. Consequently, they constitute important non-invasive biomarkers in infertility management.

*2.*1.5 Small interfering RNAs (siRNA)

The small RNAs are interfering RNAs exact same way as the miRNAs. SiRNAs are able to bind specifically to an mRNA sequence; therefore, they prevent expression by cleaving that RNA. They are present in many biological fluids such as: urine [37], seminal plasma [38] spermatozoa [39] and serum [38]. Several researchers have shown that the level of nucleic acids can be a tool for early diagnosis in certain pathologies.

2.2 In Human Plasma, Serum, Urine, Seminal Plasma and Sperm

The detect-ability of CfDNA in many biological fluids makes them real biomarkers of interest, not only for the detection of many diseases but also for the follow-up of certain treatments.

Schwarzenbach et al. have shown that the level of CfDNAs in serum is an early detection tool for colorectal cancer. Indeed, they found a high level of CfDNA (22, 3 to 9.22 ng / ml) in the serum of patients with colorectal cancer compared to healthy donors (5-16ng / ml) [40]. In the same vein, the team of Schaw has found that there was a difference in CfDNA levels in the plasma of patients with breast cancer and that of healthy donors [41]. In the same way Gormally et al. have shown the utility of CfDNAs in plasma and serum as non-invasive biomarkers for the detection or control of breast and prostate cancer, they found that patients with prostate cancer have a high level of serum CfDNAs compared to healthy donors [42].

CfDNAs were also detected in the urine. Their presence is explained by the transfer of renal blood or directly from cells that have been in contact with this biological fluid [43]. An increase in the amount of CfDNAs was noted in the urine of patients with serious pancreatitis compared to healthy donors [44]. The team of Brygunova showed that smokers' urine had more CfDNAs than non-smokers (9.46 and 9.04 ng / ml for women, respectively 4.96 and 2.93 ng / ml) for men [45].

Salvi et al. have found an increase in the amount of CfDNAs in the urine of patients with acute pancreatitis compared with healthy controls [44].

3. SEMINAL CFDNAS AS SIGNALING MOLECULES IN CELLULAR COMMUNICATION

3.1 Seminal CfDNAs Resulting from the Apoptotic or Necrotic Process

The exact mechanism by which CfDNAs are released into the bloodstream is not always clear. However, many researchers believe that CfDNAs are produced by apoptotic cells [46]. Others argue that CfDNAs result from the

necrotic process or even phagocytosis [47]. Thus, it should be remembered that apoptosis remains the main contributor of CfDNAs even if the origin is still uncertain [48]. In view of the fact that Stroun's team has said that living cells can release CfDNAs [49].

3.2 Free DNA (CfDNAs) and Exosomes

Exosomes are small particles of the order of 30 to 140 nm. They are membrane-bound and result from multivariate fusion (MVB) with the plasma membrane [50]. Exosomes can be released into various biological fluids such as plasma, saliva and urine [51]. Exosomes are an important source of biomarker for early detection of tumors, monitoring and planning of cancer treatments. Moreover Grange noted some difference in the protein profiles of RNAs and MicroRNAs from cancer cell exosomes [52]. Zhang's team showed that in the case of breast cancer, exosomes derived from stroma contribute to the quiescence of these, cells via the CXCL-12 screening of miRNAs [53].

3.3 Free DNA (CfDNAs) Detection Information of Genetic and Epigenetic

The sperm is a mixture of liquids resulting from various secretions from the two testicles, the epididymis, the seminal vesicles, the Cowper glands and the prostate [54]. The epigenetic information contained in seminal CfDNAs which plays an important role in spermatogenesis. It is involved in the reorganization and condensation of the genome of germ cells during maturation. Indeed [40], it reflects the epigenetic aberrations of the testes [55], the problems of male infertility.

4. SEMINAL FREE DNA (CFDNAS) FOR THE DIAGNOSIS OF MALE INFERTILITY

The abundance of CfDNAs in sperm is due to significant apoptosis during spermatogenesis. According to the Costa's team, the presence of CfDNAs is associated with sperm parameters [56]. That explains why CfDNAs can be a biomarker for the diagnosis and evaluation of secretory sperm organs.

4.1 Free DNA (CfDNAs) CfDNA

CfDNAs are detectable in human sperm. Its concentration in sperm is much higher than in other biological fluids [40]. Its presence and concentration is directly correlated to sperm parameters such as speed, morphology or even mobility [55]. According to Li et al. the level of CfDNA is higher in the seminal plasma of patients with defective sperm parameters [57]. These observations explain the use of CfDNAs in the search for biological markers of sperm quality.

4.2 Free RNA (CfRNAs)

CfRNAs is an excellent biomarker for sperm quality. The team of Steger have detected high levels of Prm1 and Prm2 and mRNA in sperm obtained in patients whose in vitro fertilization (IVF) had failed compared to those whose in vitro fertilization was successful [58]. Bansal et al. have also established a probable correlation between the profile of sperm DNA and male infertility [59].

4.3 MicroRNAs (miRNA)

The expression of microRNAs, that are present in sperm, can be a very interesting approach and a valuable aid in routine practice of predicting sperm quality. According to the Salas-Huetos team, human sperm contains a stable population of miRNAs linked to embryogenesis and spermatogenesis [60]. Still Salas-Huetos et al. have shown that MIR-34-P, MIR-132-3P, mir30C-5P and miR-375 play a role in cell cycle progression and sperm differentiation [61].

4.4 Piwi-interacting RNA (PiRNAs)

The presence in human spermatozoa, piRNAs represent 11% of CfRNAs [62]. PiRNAs are used in the evaluation of sperm quality and they offer new perspectives for diagnosis, prognosis and treatments in the management of male infertility [8].

5. NON-OBSTRUCTIVE AZOOSPERMIA

Due to a lack of adequate intrinsic gonadotropin stimulation or testicular insufficiency, non-obstructive azoospermia is diagnosed in approximately 10% of infertile men [63]. According to the Drabovich team, the concentration of CfDNA is much higher in sperm than in other human body fluids, with an average value of 1.34 pg / ml in normozoospermia and 2.56 pg / ml cases azoospermia cases [64]. The team of Li in a recent analysis of the different mRNA and microRNA profiles of patients with non-obstructive azoospermia and patients with

obstructive azoospermia (OA) found differences in profiles with control (normozoospermia) [65]. Meanwhile, Wang et al. demonstrated differences in seminal plasma microRNA expression patterns, performed studies in patients with non-obstructive azoospermia (NAO) versus fertile men and noted a sharp decrease in expression of seven microRNAs (miR-346-5p, miR-122, Mir 149 + -5p, miR181a, miR-374b, miR-509 and miR-513a-5p) in the seminal plasma of patients with NOA compared to the control [66]. However, in a study by Gunes's team in patients with azoospermia, they found the additional expression of miR-34c-5p, miR-122, miR-146b-5p, miR-181a, 374b Mir, miR-509- 5p and 513a-5P which is increased strongly in the case of asthenozoospermia [67]. In the same vein, the team of Wu analyzed the testicular tissues of NOA patients and found a significant increase in the expression of miR-141, miR-429 and miR-7-1-3p in plasma. Seminal NOA compared to fertile controls [68].

6. TERATOZOOSPERMIA, ASTHENO-ZOOSPERMIA AND OLIGOZOOS-PERMIA

Male fertility may be affected by abnormalities such as: reduced mobility (asthenozoospermia), abnormal morphology (Teratozoospermia), non-detectable sperm (azoospermia) or decreased sperm count (oligozoospermia).

6.1 Teratozoospermia

Teratozoospermia is characterized by the presence of spermatozoa with an abnormal morphology greater than 85% in spermatozoa. In the exploration of markers useful to better compensate for male infertility, Herati et al. made a comparison of sperm expression profiles of men teratozoospermia and found a decrease in the expression of SAH-miR-19b-3p, hsa-miR-28-5p, SAH-miR-148B and 106B-mir-5P [69]. This clarifies the interest of these miRNAs as biomarkers male infertility management.

6.2 Asthenozoospermia

Patients with asthenosozoospermal disease, Safarinejad et al. noted an increase in the expression of SAH-miR548c-5p, SAH-miR548c-5p, and SAH-miR-27a, SAH-mi-548b -5p -548d-5P [31]. While Abu-Halima and his collaborators noted a dysregulation of a-miR-34b-3p in patients with asthenoszoopermia [32].The same way, an increase in HSA-miR-27a expression has been observed in asthenozoospermic patients [70].

6.3 Oligozoospermia

In a study by Wang, in which he compared the expression patterns of miRNAs from normal and oligozoospermic patients, a significantly lower level of mir-34C-5p, Mir 122, miRNA expression was observed. 14BB-5P, miR -181A, miR-374b, miR-509-5p and miR-531a-5P noted by the controls [71]. Wu et al. have found a strong expression of miR-19b and miR -7bis in patients with oligospermia [68]. Bilge Özsait Selçuk found a significant increase in the expression level of miR-21 and miR-22. However, finding a threshold value for the diagnosis and prognosis of male infertility remains a problem.

7. IDIOPATHIC INFERTILITY AND OTHER FUNCTIONAL SPERM DEFECTS (DNA FRAGMENTATION, ROS, METHYLA-TION, PROTEOMICS, mi-RNA)

Idiopathic male infertility is defined as the absence of a causal factor in sperm analysis when the sperm has abnormalities such as azoospermia, oligozoospermia, asthenozoospermia or teratozoospermia [31]. It can be affected by other factors such as: DNA fragmentation, oxidative stress, methylation or protein.

7.1 DNA Fragmentation

DNA fragmentation is an important factor in the etiology of male infertility [72] or even a good indicator of the potential of conventional sperm parameters [73]. Because sperms with normal sperm parameters can have DNA damage [74]. Das In a study of the etiology of age-related male infertility shows that DNA fragmentation can lead to reduced motility of spermatozoa [75]. Similarly, Twyma-Saint Victor's team in a study published and carried out on three patients suffering from male infertility reveals that the proportion of spermatozoa with fragmentation of the DNA varies between 4.4 and 28, compared to a percentage of DNA fragmentation of 1.20 ± 0.95 between controls [69]. Apart from DNA fragmentation, various conditions such as stress chromatin condensation, methylation can affect men [76].

7.2 Oxidative STRESS

Even if a low concentration of ERO is necessary for the critical stages (capacitation, acrosomal

reaction and fusion between the oocyte and sperm) of fertilization [77]. It turns out that, cellular stress in all its forms, can lead to DNA fragmentation and affect male fertility. According to Twigg seminal ERO levels can lead to sperm injury leading to male infertility. Only the lack of consensus on the physiopathological limits of ERO remains the crucial problem [78].

7.3 Methylation

The methylation of DNA modifies the genetic material. It is a causative factor of infertility [79]. The example of the modulating AMPC response element (PAC) gene, which has a high concentration, becomes a negative factor for normal motility and sperm morphology. Arifin's teams have shown that methylation contributes to the increase of mesoderm-specific transcriptase (MEST) in association with abnormal sperm parameters and male infertility [80].

7.4 Proteomics

In seminal fluid, sperm accounts for 10% of the total volume of ejaculation while 90% is a diverse molecular composition. The specific protein concentration provides a rich source of potential biomarkers in the assessment of male fertility [81]. The team of Mitsumoto observed in infertile men a decrease in the protein (DJ-1-A) involved in the regulation of oxidative stress [82]. Diamandis also found a positive correlation between the seminal concentration of Prostaglandin-D-Synthase (PTGDS) with the mobility and normal morphology of spermatozoa [83]. The proteomic study conducted in the search for biological markers of azoospermia conducted by Bieniek showed that proteins such as, PTGDS, ACRV1, LGALS3BP, ECM1 and TEX101 are seminal biomarkers for the evaluation of male infertility [84].

8. SEMINAL TECHNICAL APPROACH CFDNAS FROM THE ANIMAL MODEL TO HUMANS

The development of biomarkers for the diagnosis of male infertility, the provision of assistance for drug development and its application at the human level cannot be possible without having gone through the animal model, including the mouse [85]. The experts in reproductive biology use several animal models. But in this part, we will only talk about the proteomic technology of sperm. The mouse model led to the identification of 52 proteins at the spermatic level [86]. Bleil et al. from the sperm of the boar have identified the surface proteins of the spermatozoa responsible for connecting the spermatozoon to the oocyte [87]. The list of animals used as models is very long. The dog remains the best experimental model for comparative studies in humans because of the similarity of the prostate [88]. The main challenge according to Naughton is the translation of knowledge acquired from its animal models to the male infertility clinic for an improvement of existing treatment, the development of an accurate diagnosis and the formulation of male contraceptives with minimal side effects [46].

9. CONCLUSION

The use of nucleic acids as biomarkers of male fertility remains an innovative approach and is extremely promising because it offers new perspectives from a diagnostic as well as prognostic points of view. This is because of the relationship between the level of CfDNA and the presence or absence of spermatozoa. In addition it is a noninvasive procedure and therefore reduces the risks the patient is exposed to.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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