Original Article

Are Immotile Spermatozoa, Immotile or are they Immotile but Resting Spermatozoa? – A Prospective Study

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Abstract

Objective: To observe the immotile spermatozoa over a period of time in semen samples to find out if they are immotile or resting.

Type of study: Prospective Study

Study place: Reproductive Medicine Department, Chettinad Super Speciality Hospital.

Materials and Methods: Data collected during November 2011 to October 2012. After routine semen analysis according to WHO 2010 criteria, the immotile spermatozoa in each semen sample were observed for about 5minutes totally and recordings noted down at 2 minutes and at 5 minutes.

Results: In 304 patients, 612 immotile spermatozoa were observed continuously for 5 minutes and the following was observed:

1. At 2 minutes (12.7% became progressively motile and 6.6% became non-progressively motile).
2. At 5 minutes (10.1% became progressively motile and 5.7% became non progressively motile).

Conclusion: We observed that some spermatozoa noted to be immotile at some point, might pick up motility at a later point of time. These spermatozoa may be resting spermatozoa, which may resume motility later on. This is a pilot study and we are continuing to study more spermatozoa for the same observation.

Key Words: Immotile Spermatozoa, Resting Spermatozoa, Spermatozoa Motility.

Introduction

Semen analysis is currently the gold standard for evaluating male infertility, despite all its limitations. There are several parameters analyzed during routine semen analysis of which motility of spermatozoa is one of the important parameters. Several factors are known to influence motility of spermatozoa, for example: calcium, kinases, phosphatase, cell volume and osmolarity, reactive oxygen species[1-7]. In fact, during the passage of spermatozoa from the testis to the epididymis, the osmolarity of the luminal fluid increases and normal spermatozoa counteract shrinkage by increasing the uptake of organic osmolytes such as L-carnitine and aminoacids secreted by the epithelium in the epididymis[4].

Knowledge about the fundamental structure of the spermatozoon is necessary to understand the motility mechanism at the cellular level. Basic structure "flagellum" is responsible for the motility, which consist of four regions, which are the connecting piece, the mid-piece, the principal piece and the terminal or end piece[8].

The flagellum also consists of the axoneme and outer dense fibres(fig 1). Axoneme is composed of two central microtubules, connected by linkages[9], surrounded by nine microtubule doublets[8] (the '9 + 2' pattern). Each doublet has an A subunit forming a complete microtubule, and a B subunit which is C-shaped with its ends attached to the A subunit. A central sheath composed of a spiral of two fibres surrounds the two central microtubules[9]. Dynein arms are the microtubule doublets of A subunit[5,6,10]. Dynein arm bends by translating the chemical energy into kinetic energy and cause flagellar movement[6]. This occurs in an attachment–detachment cycle between the dynein arms and the adjacent doublet[7].

Each microtubule doublets are connected by nexin links[6,11] between the A and B subunits[11]. Role of nexin ring is elastic recoil which helps in regulating the amplitude of flagellar bending[12] and give adequate spacing of the microtubules to optimize tubulin – dynein interaction[13] .

Adjacent microtubule doublets are connected by nexin links[7,11] between the A and B subunits. It has been suggested that these are elastic elements which allow regulation of the shear forces during doublet sliding, or

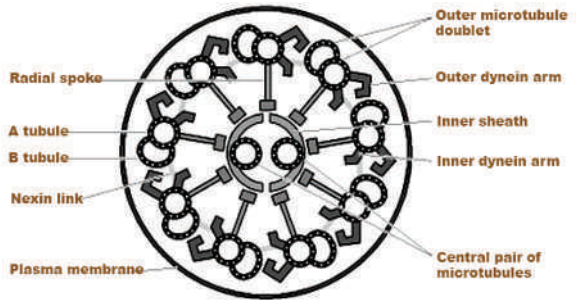

Fig 1 - Cross section of the flagellum

that they assist in the retention of axonemal symmetry during sliding[14]. It has been shown that nexin can be digested by elastase, causing an increase in the flagellar bend angle, and a concomitant decrease in flagellar beat frequency, suggesting that the nexin links are elastic elements involved in the regulation of the amplitude of flagellar bending[12]. However, it has also been observed that the nexin links undergo cycles of displacement to permit microtubule sliding which would suggest that their role is not purely for the provision of elastic recoil. It has also been proposed that the links regulate the spacing of the microtubules to optimize dynein–tubulin interaction[13]. The precise interaction between dynein and tubulin is still not clear, although it is known that the dynein-tubulin binding which allows active sliding involves the B subunit of the neighboring microtubule doublet, and is influenced by ATP[15]. A subunit have a projection called radial spokes[13,14,15] which is composed of 17 proteins. Its function is to attach and detach during the microtubule doublet sliding cycle which helps in movement as shown in Fig1.

The flagellar movement takes place with the coordination of all the ultrastructures. Calcium is an important regulatory molecule in flagellar beating and cyclic adenosine monophosphate is needed for the initiation of flagellar movement and hence spermatozoa movement[16,17,18,19].

Understanding the complex mechanism of motility is very unclear. It is believed that motility plays a major role in fertilization process. While the inherent motility appears to be so intricate, we were fascinated by the observation that some spermatozoa do not remain immotile for extended time of watching. Hence we decided to present our observations of the behavior of immotile spermatozoa in this paper. The objective of this study was to observe whether an immotile spermatozoon in a given semen sample remains immotile, or is it in resting state to regain motility later on.

Methodology

It is a prospective study conducted at the Department of Reproductive Medicine, Chettinad Super Speciality Hospital. Patients who came for treatment between November 2011 to October 2012, and whose semen samples were normozoospermic (304) were included in this study (age range: 26 to 35years).

Inclusion criteria: Normozoospermic patients who underwent routine semen analysis after which the samples that were to be discarded ,were included for this study.

Exclusion Criteria: Patients with history of vasectomy, increased round cells in semen, h/o smoking, alcohol use, high BMI, substance abuse, hypertension, diabetes, sample with liquefaction more than 1 hour, highly viscous sample, cryptozoospermic samples, total asthenozoospermic samples and samples collected after 7days of abstinence period were excluded from the study.

Semen Collection and analysis

The patients were instructed to abstain from ejaculation for 2-7 days before producing the semen sample. All men were instructed to collect semen by masturbation into a sterile, clean wide mouth collection container in the laboratory collection room. They were also advised to refrain from using any lubricants or drugs for facilitating ejaculation.

Semen analyses were performed in accordance with the guidelines of the "World Health Organization" 2010 for semen analysis by a single observer. Semen samples were kept for liquefaction, for not more than 60minutes at 37° C before analysis. Spermatozoa were counted in Makler Chamber (SEFI Medical Instruments Ltd). The sperm motility was assessed after liquefaction by grading the sperm cells as progressive, non-progressive and immotile. The methodology of assessing motility was done according to WHO 2010 manual. The Kruger "Strict Criteria" determined sperm morphology scores.

Method of standardization for analyzing the immotile spermatozoa

Semen analysis was performed according to WHO 2010 by focusing in one 40x field and the microscope was attached to monitor connected with the camera (Figure 2).

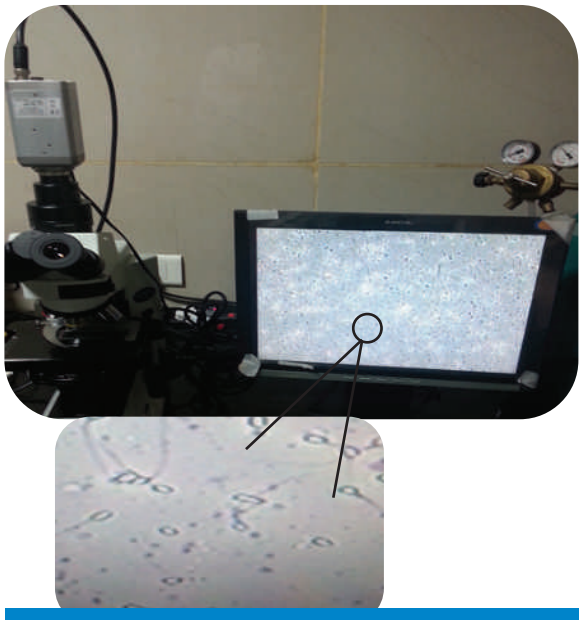

Fig 2 - Setup for observing immotile spermatozoa

The same immotile spermatozoa were observed for a total of 5minutes, readings were noted at 2minutes first and then at 5minutes.On an average of 2 to 3 immotile spermatozoa from single field were observed from each sample.

The values were noted and to rule out observer bias, other five embryologists confirmed observation at the same time, who also observed and noted the findings.

Results

In 304 patients, total of 612 immotile spermatozoa were observed continuously for 5 minutes and in which the total of 216(35.2%) spermatozoa regained motility when observed for 5 minutes.

The following was observed (Fig 3):

1. At 2 minutes (12.7% became progressively motile and 6.6% became non- progressively motile).
2. At 5 minutes (10.1% became progressively motile and 5.7% became non- progressively motile).

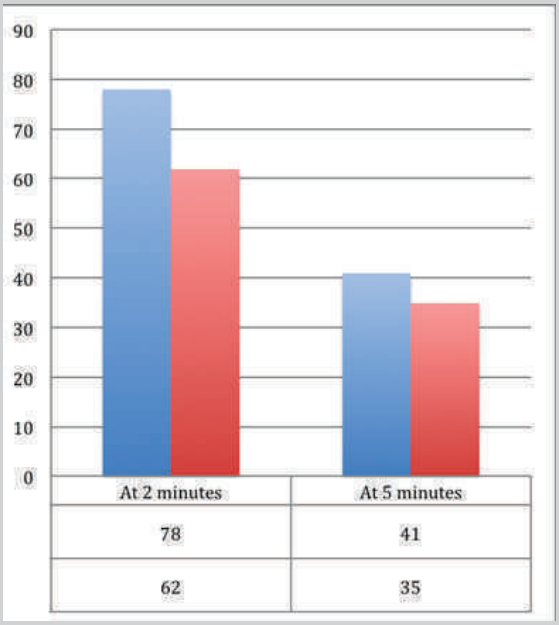

Fig 3 - Number of motile spermatozoon after 2 and 5 minutes observation

As observation of the immotile spermatozoa was interesting, we further decided to observe the motility behavior of the motile spermatozoa. So we further observed 25 motile spermatozoa in same set of patients. They were observed for a period of 30 minutes and we found that 12 motile spermatozoa stopped moving for 5 – 10 seconds after which they regained motility.

Limitations of the study

It was a pilot study with a small sample size and also limited period of observation.

Discussion

Motility is one of the inherent biological characteristics of a spermatozoon. It is still unclear when motility is initiated for spermatozoa following their release into the lumen of seminiferous tubule. But motility of the spermatozoa becomes critical at the time of fertilization because it allows or at least facilitates passage of the sperm through the zona pellucida[4]. During our routine semen analysis, sperm motility gives an insight for the choice of treatment for the couples. From our study we have observed that, motile spermatozoa may go through a resting phase and they may resume motility later on. Single step observation parameters do not remain constant while assessing the motility; semen analysis may have to be repeated at varying periods of time at the same sitting to reconfirm the motility index of the spermatozoa. If we assess a sample as asthenozoospermia, time variable analysis may give us different motility and increase in motility percentage can happen. We need to observe single motile spermatozoon continuously to get more information regarding the assessing motility at different times. Since this is a pilot study and we are extending our study for more samples to reiterate our observation on the motility behavior of the spermatozoa. This may have a therapeutic implication on the selection of the motile spermatozoa for Intra Cytoplasmic Sperm Injection (ICSI), particularly when it is a severely asthe nozoospermic semen sample.

Conclusion

The immotile spermatozoa in the semen sample may be at rest for few seconds. Inherent motility of a spermatozoon is difficult to assess at a single point of time during the routine semen analysis.

The authors declare no conflict of interest

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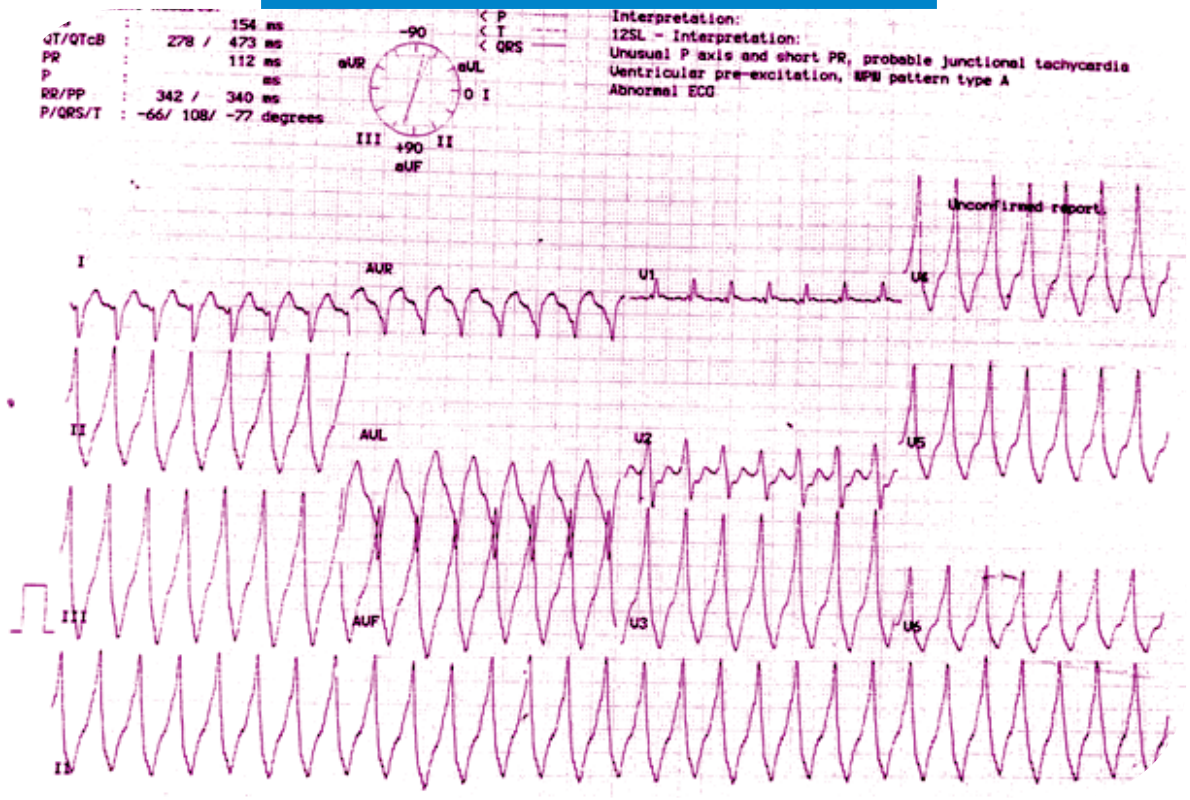
Diagnose the condition

37 year female came with complaints of palpitation



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