SPERM MOTILITY AND VIABILITY: OVERVIEW OF THE CELLULAR AND PHYSIOLOGICAL ASPECTS THAT SUPPORT THESE FUNCTIONS

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ABSTRACT

This review briefly summarises the cellular and physiological aspects of sperm motility (SM) and viability from the point of view of male fertility/infertility. We discuss the SM patterns and maturation processes during the epididymal transit, including the effects of seminal plasma proteins, and while moving through the female reproductive tract. In connection with SM and viability, the oxidative stress, the mitochondrial markers of SM and related predictive value of the proportion of motile sperm, and the effect of male age on sperm function are reviewed within the current literature. Furthermore, some of the potential techniques to determine molecules involved in sperm motion are presented. Other key points are sperm maturation and the markers of sperm maturity, including sperm-hyaluronic acid binding and DNA integrity, as well as the proportion of hyaluronic acid-bound sperm with respect to sperm morphology and tyrosine phosphorylation. Finally, proteins regulating SM and assessment approaches of sperm viability are pointed out in this review.

Keywords: Human sperm, motility, viability, fertilisation potential.

INTRODUCTION

Infertility is a troubling medical condition with social and economical influences for couples who desire pregnancy. In fact, the data collected from all Society for Assisted Reproductive Technologies member clinics indicated that 17% of assisted reproductive technology patients were diagnosed with only male factor infertility in 2012.[1] However, the aetiology for male factor infertility is multifactorial. Most studies focus on sperm motility (SM), viability (SV), and DNA integrity since these parameters are important characteristics of sperm function. Therefore, the purpose of this article is to provide an overview of human SM and SV in the concept of sperm function, including fertilising potential.

SPERM MOTILITY AND MATURATION

Normal development of sperm plays an essential role in enabling reproductive capacity. Spermatozoa acquire motility and fertility in the epididymal lumen which is a complex microenvironment. Phosphorylation, glycosylation, and further processing are several of the posttranslational modifications that sperm proteins undergo during epididymal transit, resulting in changes in protein function, ultimately leading to mature spermatozoa.[2]

The comparison of seminal plasma proteome in fertile and infertile men showed that ten seminal proteins are significantly up-regulated in the infertile group, in line with statistically significant differences in motility and sperm count between fertile and infertile men.[3] Thus, it has been proposed that a variety of peptides in seminal

fluid appear to have a role in SM.[4] In fact, there is increasing recognition of the role that peptides present in seminal plasma, such as seminal plasma-neutral endopeptidase and aminopeptidase N, have in determining SM (for a review[4]). Nonetheless, there is not much known about their specific roles in male fertility/infertility.

The activation of sperm is not entirely completed upon release from the male genital tract and is further modified while moving through the female reproductive tract. Once deposited inside the female reproductive tract, spermatozoa seek to reach the oocyte first and acquire hyperactivated progressive motility, defined as moving actively, either linearly or in a large circle, regardless of linear speed.[5] Spermatozoa gain hyperactive motility upon arrival to the oviduct.

Sperm movement is generated by the flagellum which constitutes more than 90% of the length of the mammalian spermatozoon.[6] Paoli et al.[7] described SM as the result of the propagation of transverse waves along the flagellum in a proximal-to-distal direction producing a hydrodynamic impulse that pushes the spermatozoon through the female genital tract to penetrate the cumulus and zona pellucida of the oocyte. Most mammalian sperm display two types of physiological motility: activated motility, as is seen in freshly ejaculated sperm, and hyperactivated motility, as is seen in most sperm recovered from the site of fertilisation.[8] However, these motility patterns are temporary and may vary in a time-related fashion. Turner et al.[9] defined that the flagellum of an activated sperm generates a symmetrical, lower amplitude waveform that drives the sperm in a relatively straight line. Once sperm from most species becomes hyperactivated, the flagellar beat becomes asymmetrical and of higher amplitude, which results in circular or figure-eight trajectories.[10] The propagation of a calcium-induced wave produced from the opening of calcium channels along the flagella is a necessary milestone in sperm maturation and makes hyperactivated motility possible.[11]

Hyperactivated motility and the associated protein tyrosine phosphorylation in the flagellum are initiated during *in vitro* capacitation, and their maintenance in zona pellucida-bound spermatozoa is of importance. Previously, we showed that the levels of capacitation-related tyrosine phosphorylation in the sperm neck and principal piece, a pattern that is a marker of sperm activation, increases in a time-related manner.[12] Progesterone hormone was previously described to initiate an immediate increase in intracellular calcium within human sperm cells through potentiation of CatSper channel[13] and also rapidly triggers hyperactivated motility and initiation of the acrosome reaction. Sperm intracellular pH (pHi) is a key regulator for the initiation of motility and hyperactivation. Even the basal SM is pH-sensitive since dynein's ability to hydrolyse adenosine triphosphate (ATP) and provide axonemal bending greatly increase with the rise of pHi.[11]

There have been numerous attempts to identify pharmacological agents such as theophylline that might improve SM, particularly in testicular sperm extraction cases where the isolating of a clean preparation of usable sperm is difficult due to the declined tendency of the sperm to detach from the testicular tissue owing to its limited motility.[14] The study indicated that 64 out of 65 patients (98.5%) showed a significant improvement in thawed testicular SM when theophylline is used, but this result has to be confirmed by independent laboratories. In addition, hyaluronic acid in the medium increased the velocity and retention of motility and viability in freshly ejaculated as well as in cryopreserved–thawed human spermatozoa.[15,16] The effects of hyaluronic acid upon sperm are likely to be receptor mediated due to the presence of the hyaluronic acid receptor in human sperm. We also found that the tyrosine phosphorylation patterns of sperm bound either to zona pellucida or to hyaluronic acid were similar.[12] Therefore, we think that there is a common regulatory pathway of tyrosine phosphorylation related to sperm ability. It is possible that such a regulatory pathway originated in the synchronous formation of the zona pellucida and hyaluronic acid receptors in the sperm plasma membrane, following the remodelling process related to the progress of spermiogenesis.

SPERM MOTILITY AS AN IMPORTANT FACTOR IN MALE FACTOR INFERTILITY

Studies demonstrated that SM correlates well with fertilisation and pregnancy rates after intrauterine insemination or *in vitro* fertilisation.[17,18] Based on 358 semen samples from a group of men reflecting the general male population, Larsen et al.[19] reported that the concentration of motile spermatozoa, defined as spermatozoa with curvilinear velocity

>25 μm/s, was the most significant and independent computer-assisted semen analysis parameter in predicting the chance of natural conception.

SM is also an important parameter when investigating men for potential environmental exposures or male infertility. Exposure to environmental and occupational toxicants may adversely affect SM and motion characteristics, and thus male reproductive potential is affected by environmental factors during sperm development or epididymal storage. Thus, there have been previous attempts to develop methods for preservation of SM by various semen extenders for semen analysis or assisted reproduction.[20] We have previously demonstrated that the use of phenylmethylsulfonyl fluoride and 4°C conditions during shipping for next-day semen analysis methods can preserve various sperm attributes, including sperm concentration, heat shock chaperone protein levels, chromatin maturity, DNA integrity, and sperm shape.[21-23] In a recent publication we reported that loading sperm with the MitoTracker® reagent allows the delayed assessment of SM based on the analysis of mitochondrial activity.[24]

OXIDATIVE STRESS AND SPERM MOTILITY

Oxidative stress can be initiated by a variety of factors in the male germ line including infection, age, obesity, and exposure to a variety of adverse environmental influences. It is caused by an excess of reactive oxygen species that damages proteins, lipids, and DNA in human spermatozoa and is considered a major cause of impaired sperm function.[25,26] One of the first functions affected by oxidative stress and lipid peroxidation is SM. It is known that sperm is responsible for reactive oxygen species production.[27] It was reported that regulation of reactive oxygen species levels is involved in sperm capacitation, motility acquisition, and acrosome reaction.[28,29] Agarwal et al.[30] showed that infertile men have reduced semen parameters and elevated reactive oxygen species levels compared to proven fertile men who have established a pregnancy recently or in the past, suggesting that measurement of reactive oxygen species levels in the seminal ejaculates might provide clinically-relevant information to clinicians.

High levels of nitric oxide are associated with alterations in sperm function, particularly with decreased motility.[5,19,31,32] Studies indicated that hydrogen peroxide was the most cytotoxic oxygen metabolite; that superoxide and hydroxide probably also played a role in the immobilisation of spermatozoa by reactive oxygen species in preventing SM loss under such circumstances.[33] Even though the axonemes are reported to be affected, mostly as a result of ATP depletion,[28,29] the molecular mechanism underlying this loss of motility in spermatozoa under oxidative stress is not clearly known.

Uribe et al.[34] have shown that *in vitro* peroxynitrite causes decreased motility and mitochondrial membrane potential in human spermatozoa, compromising vital functions of the male gamete without affecting viability.[34] Oxidative stress results in redox-dependent protein modifications, such as tyrosine nitration and S-glutathionylation. Thus, in a very recent paper, Morielli et al.[35] showed that oxidative stress promotes a dose dependent increase in tyrosine nitration and S-glutathionylation, and alters motility and the ability of spermatozoa to undergo capacitation in normozoospermic sperm samples from healthy individuals. Furthermore, Talevi et al.[36] demonstrated that *in vitro* treatment of human spermatozoa with zinc, D-aspartate, and coenzyme Q10 exerts a direct protective effect on SM, kinetics, lipid peroxidation, and DNA fragmentation during handling, extended culture, and cryopreservation, particularly during assisted reproductive procedures.

Ebner et al.[37] presented a sperm-selection chamber called the Zech-selector that exclusively enables the accumulation of spermatozoa with fast progressive motility without exposure to centrifugation stress. Since the spatial separation of spermatozoa of highest motility happened in a time-dependent manner, the idea is that spermatozoa of fast progressive motility are those most likely to be DNA intact.[38] Spermatozoa showing numeric (17.4% of patients without aneuploidy) or structural chromosomal abnormalities (90% of patients without strand-breaks) are reported to be separated most effectively with this sperm processing technique.[38] However, the efficiency of this technique in oligoasthenoteratozoospermic patients is not yet known.

MITOCHONDRIAL DEFECTS AND SPERM MOTILITY

During spermatogenesis there is a significant reduction of mitochondria number per cell due to

mitochondrial DNA (mtDNA) replication arrest. Mitochondria may supply sperm with energy for several purposes, including motility.[32] Experimental evidence suggesting an association between mitochondrial functionality and sperm quality was presented in previous papers.[6,39] Indeed, the structural and functional defects in sperm mitochondria, and the presence of mutant mtDNAs are associated with decreased SM in men.[7,40]

Mutations in the mitochondria have been implicated in infertility[41] via new candidate genes such as human GALNTL5 and are suggested to result in male infertility with the reduction of SM.[42,43] Tian et al.[44] suggest that mtDNA copy number and epigenetic factors including LINE-1 element may be linked to semen quality via SM. This aspect also brings the idea of the assessment of the genetic and epigenetic modifications as diagnostic information in addition to the information gained by the routine semen analysis in the evaluation of male infertility, especially in patients with potential risks.[44]

MALE AGE

There is an increasing trend for older men to have children. The advanced age-associated increases in sperm defects are a big concern, in particular for older men who are attending assisted reproductive clinics in order to father children. Using data from 90 studies (93,839 subjects), a systematic review and meta-analysis to quantify the effect of male age on motility revealed that male age is associated with a decrease in the percentage of motility and the percentage of progressive motility.[45] Thus, it has been suggested that DNA fragmentation and progressive motility would be better diagnostic parameters during fertility treatments of ageing couples.

Schmid et al.[46] identified that older men (≥65 years) had significantly higher levels of zinc, copper, and calcium in their sperm and higher levels of sulphur in their seminal fluid than younger men (≤28 years). Both higher sperm calcium and copper were reported to be associated with lower SM and increased frequency of DNA fragmentation. In addition, seminal plasma sulphur was found to be negatively associated with SM and structural aberrations, and positively associated with DNA fragmentation. These findings demonstrated that there are differences even in the elemental composition of whole sperm and seminal plasma between younger and older men

and that these elements are quantitatively associated with increased risks for poorer semen quality and genomic defects in the sperm of older men.[46]

Furthermore, the lifestyle and environmental factors may adversely affect human health and reproductive performance. Kumar et al.[47] reported that there is a non-significant lowering of sperm count and total progressive motility between tobacco smokers and non-smokers among the oligozoospermic patients. Besides deterioration in sperm count, total progressive motility and normal sperm morphology is observed among alcohol consumers who had oligozoospermia. However, no statistical significance is observed, possibly due to low frequency of alcohol consumers in the study population. In another study, cigarette smoking and alcohol consumption separately and combined were found to have a deleterious effect on sperm parameters and sperm DNA fragmentation.[48]

This also brings up the possibility that the increased use of certain new technologies such as portable computers may decrease male fertility. Avendaño et al.[49] examined the effect of portable computers on human spermatozoa *in vitro.* They have demonstrated that laptop computers connected wirelessly to the internet decrease the sperm progressive motility and increase the proportion of sperm with DNA fragmentation. However, the mechanisms involved in mediating the decrease in SM and DNA integrity require further investigation.

DETERMINATION OF PROTEINS INVOLVED IN HUMAN SPERM MOTILITY

SM can be affected by several molecules. Lin et al.[50] showed that nerve growth factor could promote human SM *in vitro* by increasing the movement distance and the number of A-Grade spermatozoa in a dose-dependent manner. Although the number of such related publications has risen dramatically in the past few years, unfortunately there is as yet no comprehensive model of the myriad molecular mechanisms controlling SM. Two clear signalling pathways are known to be involved in SM regulation: cyclic monophosphate/protein kinase A pathway and calcium signalling. There is an increasing number of papers reporting several proteins to be necessary for sperm flagellum movements. This also brings on different approaches to study SM and determine the proteins that support human SM. Currently, the

use of patch clamp technique and mathematical analysis of calcium dynamics,[51,52] the use of imaging and fluid mechanics simulation of sperm swimming to reveal the influence of media viscosity in SM,[53] and the analysis of protein phosphatase 1 complexes[54] can be counted as some of these potential techniques. More recently, Amaral et al.[55] have suggested a high-throughput differential proteomic strategy to identify proteins involved in SM (de)regulation.

SPERM VIABILITY ASSESSMENT

SV can be described with multiple criteria that collectively contribute to sperm-oocyte activation and fertilisation, and also further developmental steps during embryogenesis. There are several proteins to be potentially used as markers for SV. Most of them rely on the sperm membrane proteins since the fusion of the sperm and oocyte membranes is one of the critical steps for *in vivo* fertilisation. While this mechanism provides many proteins that may be studied as markers of SV, it is important to note that this fusion step is bypassed in intracytoplasmic sperm injection (ICSI) by the embryologist. Even if the ICSI method is used, the mature and viable sperm has to be selected accurately and predictably for injection into the ooplasm. Thus, it is necessary to identify markers that are vital to sperm function downstream of sperm-oocyte fusion. In addition, proteins involved in the sperm DNA fragmentation have been considered as potential markers for poor viability.[56] There is still an increasing effort to identify markers that possess a functional role during sperm maturation and also following delivery to the oocyte, including sperm-derived mRNA and micro-RNA transcripts.[57]

We have previously shown that sperm that are able to bind to hyaluronic acid are mature and have completed the spermiogenetic processes of sperm plasma membrane remodelling, cytoplasmic extrusion, and nuclear histone-protamine replacement, as well as demonstrating high DNA integrity. In addition, we determined an increase of the Tygerberg normal spermatozoa in the hyaluronic acid bound sperm fractions.[58] The increase in hyaluronic acid-selected spermatozoa with normal morphology attributes was also associated with concomitant improvements in the sperm maturity biochemical markers. Clearly, only viable sperm exhibited hyaluronic acid binding.[59,60] Thus, hyaluronic acid binding by human sperm indicates cellular maturity and also viability. This sperm-hyaluronic acid binding assay conforms to the basic idea that sperm DNA integrity should always be studied in motile sperm.

CONCLUSION

In conclusion, the physiology of SM and SV are very complex functions, and there are several molecules reported to regulate these sperm functions. In fact, special care has to be taken in the advent of assisted reproductive technologies such as ICSI that require the bypass of SM. However, this might also increase the genetic defects passed to subsequent generations. Thus, further studies are required to highlight the molecular mechanisms underlying these processes, to decrease possible negative outcomes and concerns in assisted reproductive technologies.

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