REVIEW

Assessment of sperm motility, and its relationship with sperm function and fertility

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Abstract
The 2010 World Health Organization laboratory manual for the examination and processing of human semen is provided and favored for assisted reproductive technology units in the investigation of male infertility. Conventional semen analysis is the primary measure for the evaluation of male fertility. This review critically examines conventional sperm motility assessment methods and reports on the lack of standardization and reproducibility associated with subjective measures and absence of a biological standard. The role of sperm motility in the development of more sophisticated fertility-related diagnostics and testing methods are discussed, with focus on the genetic causes of asthenozoospermia and the potential development of genetic biomarkers. Sperm preparation methods are closely examined to highlight factors which can negatively affect the reliability of sperm motility assessment. The association between sperm motility and clinical outcomes in IUI and ICSI cycles is also examined, with IUI and ICSI as comparable examples of a natural and non-natural sperm selection environment for which known sperm motility values can be assessed. Male accessory gland infection/inflammation is discussed regarding its role in impaired sperm motility, and in the case of Human Papilloma virus as a possible explanation for unknown male infertility. Finally, although this review concentrates specifically on sperm motility, by considering outcomes from total motile sperm counts, this review acknowledges the advantages of assessing multiple semen parameters.

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Introduction

Conventional semen analysis is the method of choice to assess male infertility in most assisted reproductive technology (ART) units. Sperm motility is the only measure of sperm function and is considered the most important measure of sperm quality, making assessment of sperm motility fundamental in implicating male infertility. The 2010 World Health Organisation laboratory manual for the examination and processing of human semen (Manual 5th Edition WHO, 2010) aims to provide standardization for sperm motility assessment although is limited by subjective measures, resulting in uncertainty regarding obtained sperm motility results and their clinical value (Tomlinson, 2016). Accordingly, there is a need for the development of more reputable and better-quality testing methods for sperm motility. Whilst all sperm parameters are considered to impact upon fertility and reproductive outcomes, this review will examine the implications of abnormal motility on clinical outcomes and focus specifically on the role of sperm motility in the development of new fertility-related diagnostics and testing methods for male infertility.

Sperm Motility Assessment In-Vivo and In-Vitro

Motility is the only measure of sperm function able to be assessed in clinical semen analysis and is plausibly the semen parameter most closely associated with sperm viability and fertilization capacity. In natural conception successful fertilization relies on the ability of sperm to travel within the female reproductive tract and reach the site of fertilization in the ampulla. During this journey, sperm are subject to a natural selection process by having to overcome many physiological hurdles, for which progressive motility is a crucial factor. Sperm possessing poor progressive motility are unable to penetrate through the cervical mucus and as such are filtered out of the natural selection process (Sakkas et al, 2015). Williams et al (1992), revealed just how selective this process is by recovering significantly reduced numbers of inseminated sperm from the uterine tubes of a patient who underwent a hysterectomy.

However, data is lacking from in-vivo studies on the overall characteristics of sperm which are successfully able to reach and fertilize the oocyte. Consequently, we can only speculate that this is a highly favored group, for which motility has been a major factor in their selection. Whether this highly selected group of sperm are more fertile than other motile sperm in the ejaculate, and whether all motile sperm are fertile, remains undetermined (Sakkas et al, 2015). Data from ART treatments have shown that in samples from subfertile men, the majority of motile sperm are incapable of fertilizing an oocyte (Sakkas et al, 2015). Leading us to question if this could be resolved by developing better techniques for selecting more fertile spermatozoa in the ejaculate.

The development of intracytoplasmic sperm injection (ICSI) has perhaps prevented the clinical need for this area to be explored in more depth than is presently, since manual insemination appears to override the importance of selecting the sperm most capable of fertilization. Kirkman-Brown and Smith (2001) suggested that reconsideration of the viscous effects of the female reproductive tract, and the role it plays in the selection and modulation of sperm behavior, could potentially assist in the development of improved ART selection techniques, diagnoses and treatments. Ola et al (2003), have already demonstrated a good predictor of fertility is the measurement of the concentration of sperm able to travel a set distance through mucus or viscous analogues.

The gap in knowledge regarding natural sperm selection mechanisms means that although only relatively few sperm are required to reach the oocyte for fertilization to occur, the actual threshold and motility characteristics remains unknown. Interestingly, in-vitro studies contradict the in-vivo concept that only low numbers of sperm are needed for successful fertilization, since increased numbers of sperm in ART treatments equate to better outcomes (Sakkas et al, 2015). Tournaye at el (2002) improved fertilization rates in IVF cycles by increasing the number of sperm to oocyte ratio from 5000:1 to 20000:1. Despite the absence of a biological standard for sperm motility, previous studies have concluded that at the lower ends of the motility scale the likelihood of subfertility is significantly increased (Macleod & Gold, 1951; Hargreave & Elton, 1986; Guzick et al, 2001; Cooper et al, 2010; Barratt et al, 2011). Regarding ART outcomes recent studies have implied a negative effect of low sperm motility. Harris et al (2019) found that both postprocessing sperm concentration and progressive sperm motility on the day of oocyte retrieval are predictive of low fertilization rates in conventional IVF cycles, and Bartolacci et al (2018) found a negative correlation between sperm motility and fertilization rates in ICSI cycles, with motility <5% having significantly lower fertilization rates than the control group with motility >32% (66.7 vs 75, $p<0.001$).

Asthenozoospermia is defined in the Manual 5th Edition (WHO, 2010) as a semen sample which contains less than the recommended lower reference limits of 40% for total motile sperm, and 32% for progressive motile sperm. Despite the publication of these guidelines, conventional semen analysis is not without error and is severely lacking standardization and reproducibility (Tomlinson, 2016). Regarding motility, assessment is greatly limited by subjective measures, absence of a biological standard, and a lack of attention given to how individual sperm swim, which are genetically capable of generating a healthy live birth outcome (Tomlinson, 2016). Tomlinson (2016) emphasized that overall, semen analysis is associated with poor consistency demonstrated by external quality assurance (EQA) schemes (Keel et al, 2000, 2002; Tomlinson, 2010;

Filimberti et al, 2013); variation in practice in relation to treatment decision-making based on sperm quality (Andersen et al, 2008; Tomlinson et al, 2013; Ombelet et al, 2014); continued perception of poor predictive values (Lefie vre et al, 2007; Chen et al, 2009; Kini et al, 2010, Wang & Swerdloff, 2014; Lemmens et al, 2016; Mali c Voncina et al, 2016); and general acceptance that the Manual 5th Edition (WHO, 2010) guidelines are the gold standard (Björndah, 2010). Evidently, there exists a high level of uncertainty associated with motility assessment in clinical andrology, highlighting the need for more innovation and improved testing.

The Manual 5th Edition (WHO, 2010) has attempted to simplify motility assessment by excluding the reporting of sperm velocity, choosing only to recommend the reporting of three grades of motility; progressive, non-progressive and immotile (WHO, 2010). Although this method has led to improved consistency in results, it fails to recognize the evidence on the importance of sperm velocity (Barratt et al, 1993; Larsen et al 2000; Garrett et al, 2003). This adds to the uncertainty of any result obtained for motility and arguably reduces its clinical value (Tomlinson, 2016).

The temperature at which sperm motility is assessed is another factor that can cause variation in results. Recommendations by Manual 5th Edition (WHO, 2010) regarding optimal temperature for assessing sperm motility have changed over time. Earlier editions recommended sperm motility be assessed at ambient laboratory room temperature, which was subsequently changed in the 1992 edition to include the option of making assessments at 37°C. Birks et al (1994) demonstrated how motility profiles obtained using conventional semen analysis methods, were markedly different between samples analyzed at either room temperature or 37°C, for both fresh and frozen-thawed samples. These findings led the authors to conclude that only one temperature of 37°C should be recommended for sperm motility assessment (Birks et al, 1994). Currently the Manual 5th Edition (WHO, 2010) recommends a temperature of 37°C. However, Ouitrakul et al (2018) acknowledged that it is difficult to store semen samples at 37°C, and thus studied the effects of time ranges post ejaculation on the motility and viability of sperm at laboratory room temperature (25°C to 26°C). The results showed sperm motility starts to decline after 60 minutes post ejaculation and sperm velocity and viability begin to be affected after 120 minutes. Therefore, the authors concluded that for effective and accurate motility assessments, samples should be analyzed within one-hour post ejaculation when stored at laboratory room temperature.

Additionally, there is a considerable overlap between the values exhibited by males who conceive and those from subfertile males (Ollero et al, 2001; Eliasson, 2010; De Jonge, 2012; Esteves et al, 2012). There is also wide variation among semen samples collected from the same individual (Sakkas et al, 2015). Overall, these problems with motility assessment support the need for the development of more sophisticated functional testing methods.

The aforementioned problems with conventional sperm motility assessment, have spurred interest in developing more reputable and better-quality tests to enhance or replace conventional methods. Highly developed measurements of sperm movement by computer assisted sperm analysis (CASA) have attempted to improve motility testing. CASA is able to incorporate measurements of sperm velocity which may add more clinical value to motility assessment (Tomlinson & Naeem, 2018). Although this automated approach provides more objective measurements, results can be impaired by a range of technical factors associated with the type of analyzer used and difficulties in validation due to interference of seminal debris and non-sperm cells affecting accuracy (Tomlinson & Naeem, 2018). Consequently, the acceptance and uptake of CASA systems into clinical practice has remained low (Tomlinson & Naeem, 2018).

DNA Fragmentation and Motility

Another area of development are tests that determine the quality of sperm DNA. There are several tests for sperm DNA fragmentation currently available, but no general agreement on the most appropriate one, the best test specimen (fresh or washed sperm), or what level of fragmentation is of clinical concern (Pacey, 2018). However, regarding sperm motility and asthenozoospermia, interesting associations have recently been made between decreased sperm motility and increased sperm DNA fragmentation (SDF) levels.

Belloc et al (2014) revealed in a large cohort of infertile men, both mean SDF level and the proportion of men with high levels of SDF, were significantly higher in men with isolated asthenozoospermia, compared to men with either isolated oligospermia or teratospermia. These findings indicate that abnormal motility is the sperm parameter most closely allied with DNA damage (Belloc, 2014). Although, the implications of this finding on fertility potential are unclear. Thus, the relationship between sperm motility and DNA fragmentation warrants further investigation, to compare outcomes between fertile and infertile men assessed for sperm motility and DNA damage.

Elbashir et al (2018) in a retrospective study aimed to define the relationship between SDF levels and progressive sperm motility, between infertile asthenozoospermic men and men of proven fertility. The findings showed that SDF levels were lesser in the fertile group of men than in the infertile asthenozoospermic group, and there was a significant negative correlation between SDF level and both motility and progressive motility (Elbashir et al, 2018). However, although these findings support the previous study by Belloc et al (2014), data is still lacking on pregnancy and healthy live birth outcomes, therefore as yet, no conclusions can be drawn on the implications of this research on fertility.

To better understand the emerging association between sperm DNA damage and sperm motility, it is of interest to investigate the underlying nature of this relationship, which is currently not fully distinguished (Belloc et al, 2014). It is possible that the explanation lies in developmental processes during spermatogenesis. Only 5-15% of histones are retained in the sperm nucleus (Schon et al, 2019), and this drastic loss aids in sperm motility, via optimizing the head shape, which subsequently maximizes sperm hydrodynamic efficiency (Gillies et al, 2018). The development of the flagellum also originates in spermatogenesis, and experimental studies have shown how targeted disruption of DNA compaction is associated with the development of an abnormal flagellum and defective motility (Cho et al, 2001; Yu Ye et al, 2000). Therefore, although speculative, there is evidence to suggest that disruptions during spermatogenesis processes provide a link between levels of DNA fragmentation and abnormal motility.

Sperm Preparation and Motility Assessment

When assessing sperm motility in ART procedures it is vital to avoid any factors that can negatively impact on results. Prior to commencing ART techniques, semen samples must be prepared, and sperm selection techniques utilized. Swim up and density gradient centrifugation techniques are commonly used for selection of good sperm features, such as progressive motility. Cryopreservation techniques are also used to store and preserve sperm ahead of potential treatment. However, survival yields from thawed sperm are low and sperm viability is severely affected as a result of cryodamage. Sperm mitochondria are also damaged during cryopreservation, which results in decreased sperm motility post thaw (O'connell et al, 2002; Satirapod et al, 2012; Oberoi at el, 2014).

It has been debated whether sperm preparation before or after cryopreservation yields better quality sperm samples. Sperm preparation post thaw may be considered if an ART patient has a poor sperm baseline. However, this protocol is not accepted as a general approach, despite some studies suggesting that the antioxidants, polyunsaturated fatty acid and heparin-binding proteins within seminal plasma, provide a level of protection against the detrimental effects of cryoprotectants and temperature change (Martínez-Soto et al, 2013; Patel et al, 2016; Barrios et al, 2001). Donnelly et al (2001) revealed that progressive motility was higher when sperm preparation was performed post thaw. However, these results are somewhat contested by Petyim et al (2014) and Esteves et al (2000), who found improved motility percentages when sperm were prepared prior to cryopreservation despite preparation post thaw yielding better results when analyzed in terms of total motile sperm count (TMSC).

Rios et al (2018), examined the effects of cryopreservation before and after sperm preparation on TSMC, sperm motility and sperm viability; and also sought to verify whether sperm viability could be assessed based on sperm motility. The results of this study revealed that all evaluated parameters showed significant

decreases when samples were prepared via swim up technique before cryopreservation compared to when samples were prepared post thaw. Additionally, an exploratory test was included in this study to determine whether hypo-osmotic swelling testing (HOS-test) for sperm viability, could be substituted for motility evaluation to assess sperm viability.

The rationale behind this experiment was that the HOS-test is prone to false positives, due to spontaneously developed tail swellings (Martini et al, 2016), and second there is a strong association between sperm motility and viability, since motile sperm are effectively viable (Rios et al, 2018). The results for progressive and total motility were close to the those obtained for viability via HOS-test, revealing a relationship between sperm motility and sperm viability, found in both fresh and prepared sperm samples (Rios et al, 2018). Therefore, the findings of this study suggest that in order to achieve better outcomes for ART techniques, sperm preparation post thaw should be considered. Although, whether this is a practical protocol for all ART labs is questionable. The Study also suggests that the use of sperm motility outcomes to predict HOS-test viability results is a reliable, objective and less laborious alternative. Additionally, it also emphasizes that sperm motility is the parameter most closely related to viability and thus fertilization capacity.

Another factor to consider regarding both sperm preparation and motility assessment is abstinence time. The Manual 5th Edition (WHO, 2010) recommends an abstinence time for semen analysis of a minimum of 2 and maximum of 7days. However, studies have inferred that even though volume decreases with more frequent ejaculations, the same might not be so for sperm count, motility and viability (Mayorga-Torres, 2015), which could have implications for clinical outcomes (Agarwal, 2016).

Hanson et al (2018), conducted a systematic review of 28 publications regarding the impact of abstinence time on semen parameters and fertility outcomes since the year 2000. The effects of abstinence time on motility rates were assessed in 23 of the publications, with ten showing no significant differences between motility and abstinence times (Agarwa et al, 2016; Mayorga-Torres et al, 2015; Gonzalo et al 2013; DeJong et al, 2004; Welliver et al, 2016; Lehavi et al, 2014; Sanchez-Martin et al, 2013; Li et al, 2003; Raziel et al, 2001; Carlsen et al, 2004). Whilst the other 13 studies did reveal a significant effect of abstinence time on motility (Levitas et al, 2005; Sobreiro et al, 2005; Makkar et al, 2001; Wang, 2007; Marshburn et al, 2010; Sugiyam 2008; Zhang, 2009, Pasqualotto et al, 2006, Rivaroli et al, 2009; Jurema et al, 2005; AlAwlaq & Hammadeh, 2017; Bahadur et al, 2016; Elzanaty et al, 2005). Of the 13 studies that found a difference, ten found motility peaked following 3 days or less of abstinence, whilst the over 3 studies (Sobreiro et al, 2005; Rivaroli et al, 2009; Elzanaty et al, 2005), found that motility peaked following 4 or 5 days of abstinence. None of the studies found peak motility at an abstinence time of more than 5 days.

The results of Hanson et al (2018) are ambiguous and contradictory, although there does appear to be a trend towards a shorter abstinence time to improve sperm motility amongst those studies that did find an effect. However, shorter abstinence time to improve motility may oppose improvements in volume and sperm count. It could be speculated that in regard to ART assessments, more than one ejaculate should be obtained to accommodate the differing optimal abstinence times between the different parameters. Although, the practicalities and implications of this in an ART setting are questionable, and perhaps this method would be better suited to more research-based assessments. However, Barratt et al (2017) have addressed the question of whether an evaluation of a single ejaculate versus two is sufficient for referral to infertility investigation and treatment. This review suggests that there perhaps is a requirement for a repeat semen analysis for patients with scores in an intermediate range, but on the whole a single ejaculate is sufficient to determine the most appropriate investigation and treatment pathway (Barratt et al, 2017).

Sperm Motility and ART Treatment Outcomes

IUI Outcomes

Intra uterine insemination (IUI) for ART treatment more closely resembles natural conception given that if successful fertilization occurs, it does so in the natural environment of the female reproductive tract under natural sperm selection mechanisms. In terms of

assessing the clinical outcomes associated with sperm motility, IUI is unique in that it allows for prior assessment of sperm, before insemination back into the natural environment of the female reproductive tract. Therefore, in IUI cycles outcomes of sperm parameters can be assessed within a natural environment. However, data for single sperm parameters in IUI cycles are limited, with many showing no significant correlations between any single sperm parameter and pregnancy rate (PR).

Kuriya et al (2018), conducted a retrospective study to investigate the effects of abnormal semen parameters on PR in IUI cycles. The study included 981 patients couples with a total of 2231 IUI cycles. The included abnormal semen parameters of volume, concentration and motility were based on the Manual 5th Edition (WHO, 2010), however when either one, two or all three were abnormal, PR was seemingly unaffected. Interestingly, when total sperm count was accounted for, PR did decrease with counts below 39 million ($p= 0.04$). The authors of this study therefore suggest that more attention should be given to the total amount of sperm present (Kuriya et al, 2018). However, although this study found no significance between sperm motility and PR, other studies have suggested that both sperm motility and total sperm count be considered by assessing the total motile sperm count (TMSC).

TMSC is obtained by multiplying the volume of the ejaculate by the sperm concentration and proportion of progressively motile sperm divided by 100% and has been implicated as an important predictive factor for PR in ART treatments (Speyer et al, 2013; van Weert et al, 2004; Khalil et al, 2001; Berker et al, 2012; Demir et al, 2011; Dorjpurev et al, 2011; Merviel et al, 2010; Tomlinson, 2013). Other studies have found TMSC to be a good predictor of pregnancy because it incorporates multiple semen parameters (Dickey et al, 2002; Dorjpurev et al, 2011; Ombelet et al, 2014; Soria et al, 2012). Additionally, Hamilton et al (2015) showed that TMSC analysis before sperm preparation has a better correlation with ongoing PR than the WHO 2010 classification system.

Nevertheless, literature on the value of TMSC for predicting IUI outcomes has been inconsistent. Ombelet et al (2014) consider TMSC with a cut-off value of 5-10 million to be a substantial discriminative parameter for success rates in IUI. Additionally, total motility was found to be a good predictor of success with a cut-off value of 30% (Omberlet et al, 2014). Xiao et al (2016), also make claim that TMSC is a good predictor of pregnancy rate following IUI, but that clinical significance of poor TMSC on the day of IUI from patient samples with previously normal semen analysis is undetermined. The purpose of their study was therefore to assess whether patients with poor TMSC, on the day of IUI, compared to outcomes from patients who had previously been diagnosed with male factor infertility.

The conclusion from the findings was that patients who had a normal semen analysis within 6months prior to IUI, but had low TMSC on the day of insemination, did not perform as poorly as those with diagnosed male factor infertility. Ultimately, this adds value to pre-treatment semen analysis but somewhat devalues TMSC. Furthermore, a retrospective observational study by Lemmens et al (2016) found that in 1166 patient couples with a total of 4251 IUI cycles, total progressive motility sperm count (TPMSC) had no predictive value for pregnancy rate after the first IUI cycle, or first finished IUI episode. Evidently, the value of assessing sperm motility in IUI cycles remains uncertain, and although success rates are likely associated with progressive sperm motility, other factors including, patient age, cause of infertility, ovulation induction method and number of mature follicles may also be involved. However, Siccteri et al (2018) evaluated the relationship between the aforementioned factors and pregnancy rates in 237 IUI cycles performed from 2011 to 2015 and found that only patient age was a contributing factor to rates of pregnancy.

ICSI Outcomes

In contrast to IUI, ICSI depends on the unnatural selection of sperm. The major advantage of ICSI lies in the treatment of asthenozoospermic men, who before ICSI had in effect minimal chances of having children. However, the routine use of ICSI treatment is controversial. Although ICSI appears to evade the sperm functions necessary for normal fertilization (Liu & Baker, 2002), outcomes regarding abnormal sperm motility and ICSI are not well understood (Lehti et al, 2012). The safety of ICSI with abnormal sperm warrants additional investigation, considering much of the

literature is inconsistent regarding adverse long-term outcomes (Harris et al, 2019).

When ICSI is the treatment of choice, there is a greater need for selecting a good quality sperm cell (Simopoulou et al, 2016). In-vitro sperm selection should consider markers of viability such as motility and morphology (Irez et al, 2012). Polyvinylpyrrolidone (PVP) media is often used for sperm selection in ICSI cycles, its high viscosity results in a deceleration of sperm motility, helping with selection, immobilization, and handling of spermatozoa before insemination (Simopoulou et al, 2016).

Interestingly, the presence of sperm motility within PVP, independent of other sperm parameters, has demonstrated to be a valuable predictor of ICSI success rates. Irez et al (2013) separated ICSI cycles into two groups based on only the presence or absence of sperm motility during PVP incubation prior to insemination. There were significant differences between the PVP motility positive and negative groups, with the positive group showing higher pregnancy rates and a higher rate of grade 1 embryo transfers. The study therefore concluded that sperm motility in PVP is associated with higher pregnancy and percentage of good-quality embryological development.

However, there are many disadvantages with PVP, including the inevitability that traces of it will be present in resulting embryos (Van Steirteghem et al, 1993) and its effects are not well investigated. Suggestions have been made that PVP may cause defects in sperm structure (Strehler et al, 1998), and may damage sperm mitochondria resulting in lower fertilization rates (Kato & Nagao, 2012).

The disadvantages associated with PVP led to the use of hyaluronic acid (HA) (Fraser et al, 1997), which confers good control due to its viscous nature coupled by the assurance of selecting good quality mature sperm for ICSI. In natural conception mature sperm, unlike immature sperm, have a high density of HA receptors and are able to bind to HA surrounding the oocyte (Cayli et al, 2003). Sperm that express HA receptors are found to have normal shape, minimal DNA fragmentation and low frequency of chromosomal aneuploidies (Beck-Fruchter et al, 2016). ICSI utilizes HA as a check-point for natural sperm fecundity (Parmegiani et al, 2012), and the literature on HA supports that it is less harmful and less toxic than PVP (Simopoulou et al, 2016). Additionally, Parmegiani et al (2010) found significantly better implantation rates and embryological development outcomes with the use of HA and Huszar et al (2007) reported significantly lower miscarriage rates within ICSI cycles utilizing HA.

In the past, successful outcomes of ICSI were thought to be unrelated to the principal parameters of sperm (Kupker et al, 1995; Nagy et al, 1995). In recent times, further research has since inferred the contrary, with reports suggesting that failed outcomes of ICSI may arise from sperm-derived factors (Tesarik, 2005; Tesarik et al, 2006). A thought-provoking study by Borge et al (2016), compared different ranges of TMSC in ICSI outcomes to examine the predictive efficacy of TMSC compared with the 2010 WHO semen analysis cut-off values. The study included 518 patients undergoing an ICSI cycle who all had male factor infertility in accordance with the WHO criteria; 106 were classed as asthenozoospermic, 148 as oligozoospermia and 361 as teratozoospermia. Alternatively, when TMSC count was considered, only 190 patients (36.7%) were classed as having abnormal sperm. The findings showed that a normal TMSC of >20 million, received higher fertilization rates and lower miscarriage rates compared to abnormal TMSC, and in contrast to the WHO cut-off values TMSC was the only predictor of good-quality embryological outcomes on days 2, 3 and 5 as well as the odds of miscarriage. This study therefore concluded that TMSC had a greater predictive value in ICSI cycles than the 2010 WHO cut-off values.

In a retrospective study of 1219 patient couples by Bartolacci et al (2018), fertilization rates and embryological outcomes of ICSI were examined to assess whether abnormal sperm concentration and sperm motility had any negative influence on reproductive outcomes. Interestingly, abnormal sperm motility of <5% significantly affected fertilization rates but no other analyzed outcomes. Abnormal sperm concentration however, affected both fertilization rates and blastulation rate per oocyte retrieve. Neither abnormal concentration or motility affected pregnancy rates or good-quality blastocyst formation rates. Resultingly, this study only provides support for abnormal motility as a prognostic marker for low ICSI fertilization rates, which has previously been proposed by (Borges et al, 2016; Zheng et al, 2016) but leads to speculation on whether TMSC would be a

better predictor of outcomes then either motility or concentration alone.

Interestingly, Yang et al (2018), present a case study of a man with multiple morphological abnormalities of the sperm flagella (MMAF) who underwent ICSI treatment. Although the volume and sperm concentration of his semen were within normal ranges, all sperm in his analyzed ejaculates were immotile with morphologically abnormal flagellum structure as assessed via transmission electron microscopy assays. The study detailed how testicular sperm were obtained for use, with most being tailless and totally immotile. Crucially, for this method to work, live sperm must be selected (Yang et al, 2018), hence HOS-testing was used to test for viability. Two ICSI cycles were undertaken with fertilization rates of 5/11 and 4/10 in cycles one and two respectively. The second cycle, after one blastocyst transfer, resulted in a successful pregnancy and the birth of a healthy 3150g female infant delivered at term.

Overall, this study demonstrates that in cases of MMAF the use of testicular sperm in ICSI can result in successful outcomes regardless of flagellar defects. However, this is an isolated case and more studies would need to be undertaken to compare a pooled set of results for MMAF cases. Additional support for the use of immotile testicular sperm in ICSI cycles is provided by Lee et al (2018). In this study all clinical outcomes were comparable between 141 cycles using ejaculated sperm and 37 cycles using testicular sperm. The outcomes included rates of fertilization, blastocyst formation and quality, implantation, clinical pregnancy, miscarriage and live delivery. In conclusion, the role of sperm motility in ICSI outcomes warrants further investigation.

Genetic Causes of Asthenozoospermia and Development of Diagnostics

Conventional semen analysis is limited by the fact it is unable to test for suspected sperm motility dysfunctions (Hashemitabar et al, 2015). This has been acknowledged by recent proteomic research aimed at identifying potential protein biomarkers of sperm motility that could foresee the fertilizing capacity of sperm in ART patients. It is speculated that defective proteins involved in sperm motility may cause fertilization failure (Pixton et al, 2004), and Amaral et al (2013) have demonstrated that certain proteins of the sperm flagella could potentially influence the quality of embryo development. Hashemitabar et al (2015) conducted a proteomic analysis study on sperm flagellum in low motility sperm from asthenozoospermia patients, to identify potential biomarkers in sperm dysfunction diagnoses and embryo quality. Fourteen proteins with altered expression were identified in asthenozoospermic patients, ten of which had previously been associated with asthenozoospermia and four of which were identified for the first time, HSPA9, TUBB2B, SPANX B, and ASRGL1 (Hashemitabar et al, 2015).

Understanding the role of genes encoding the proteins involved in defective sperm motility in genetically determined asthenozoospermia, has much potential for the development of fertility-related diagnostics and therapeutics, via the detection of specific biomarkers (Lehti & Sironen, 2017). Asthenozoospermia is commonly caused by anomalies in the ultrastructure of the flagellum's axoneme. The dynein arms within the axoneme provide the major force for motility, while the fibrous sheath (FS) and outer dense fibers (OFDs) mechanically reinforce the axonemes and support movement of the flagellum (Lehti & Sironen, 2017).

Primary ciliary dyskinesia (PCD), a heterogenous autosomal recessive disease distinguished by immotile cilia resulting in chronic respiratory tract infections, is often indicated in cases of asthenozoospermia (Pereira et al, 2015). PCD causes defects in both cilia and sperm flagella because the axonemes of each are highly conserved through eukaryotic evolution (Inaba & Mizuno, 2016). It is thought that 70-80% of PCD cases result from mutations in genes encoding proteins for the outer dynein arms (ODAs) of the axoneme (Castleman et al, 2009; Shoemark et al, 2012). Mutations in the DNAI1 and DNAH5 genes are the most widespread and result in defective cilia and flagellum formation (Bragina et al, 2016). Additionally, deletion of the dynein axonemal assembly factor 2 DNAAF2 gene results in a partial or complete absence of both ODAs and inner dynein arms (IDAs) (Inaba & Mizuno, 2016).

Another genetic condition associated with asthenozoospermia is dysplasia of the FS (DFS) (Elinati et al, 2016). DFS is characterized by hypertrophy and hyperplasia of the FS, with

deficiency or absence of the annulus and mitochondria (Chemes et al, 1998). DFS has been reported in infertile patients who lack AKAP3 and AKAP4 genes, which encode A-Kinase anchoring proteins (AKAP) making up the cytoskeletal structure of the FS (Eddy et al, 2003). However, some of the literature does not support these genes being the genetic cause of DFS (Turner et al, 2001; Moretti et al, 2007), and this highlights that the role of these genes is unclear. Pereira et al (2015) speculate that DFS is most likely a multigenic disease. The FS also functions to provide metabolic pathways for energy production and consists of cation channels of sperm (CatSper) ion channel proteins involved in calcium signaling for the hyperactivation of sperm motility in the female reproductive tract (Qi et al, 2007). Mutations in the CATSPER1 and CATSPER 2 genes encoding CatSper subunits cause infertility by resulting in the inability of sperm to hyperactivate (Miller et al, 2015; Singh et al; 2015; Hidebrand et al, 2010; Ray et al, 2007).

Interestingly, axonemal protein encoding genes with expression seemingly exclusive to the testes, have now been identified in infertile men presenting with severe asthenozoospermia (Lores et al, 2018). Mutations in these genes cause multiple morphological abnormalities of the sperm flagella (MMFA), independent of PCD symptoms. Li et al (2019) report that previous studies reveal that only approximately 50% of MMFA cases are caused by the so far identified genes involved in MMAF, providing scope to identify other causative genes or pathogenic processes (Khelifa et al, 2013; Tang et al, 2017; Coutton et al, 2018; Dong et al, 2018). With this as their rationale Li et al (2019) aimed to identify gene mutations for MMFA using human whole-exome sequencing (WES), in 65 infertile male patients diagnosed with MMFA who did not present with symptoms of PCD. The study successfully identified a pathogenic mutation in the cilia and flagella associated protein 251 gene (CFAP251) and suggested that biallelic loss of function mutations of this gene can cause defective sperm flagella and infertility (Li et al, 2019). However, this mutation was only found in 3 of the 65 patients (5%) and therefore may not be common amongst infertile men. Regardless, this study is a good example of how future research can advance knowledge for the genetic analyses of MMFA and assist in the development of better diagnostic methods for male infertility. Pereira et al (2015), support the use of WES as an effective approach for future studies on the genetic causes of astheno-zoospermia and infertility. In their investigation on the genetics of asthenozoospermia, for the identification of promising genetic biomarkers and treatments for male infertility, WES was compared against the gold standard of genetic testing, the Sanger sequencing method. The advantage of WES is that is has a higher throughput, suitable for the analysis of heterogenetic genetic diseases such as PCD and DFS and can be used as a robust discovery tool for new genetic causes (Knowles et al, 2013; Onoufriadia et al, 2014). However, limitations exist with WES including a marked rate of false-positive variants (Bamshad et al, 2011). Consequently, Sanger sequencing is still needed to confirm results (Pereira et al, 2015). In total this study identified nine new gene variants with two being likely candidates for genetic markers of asthenozoospermia (Pereira et al, 2015).

The molecular and genetic pathways of asthenozoospermia need to be defined for the development of diagnostic methods and better management of infertility treatment. Khosronezhad et al (2015), investigated the genetic mutations in the Nsun7 gene, implicated as a causative gene for sperm motility defects. The main finding from this study was that a deletion mutation in exon 4 of the Nsun7 gene, involved in mitochondrial rRNA processing in post-meiotic sperm, was significantly higher in asthenozoospermic men than in men with normal sperm motility. More recently, Pelloni et al (2018), investigated the co-expression of ROPN1 and CABYR genes in asthenozoospermic patients and found that in comparison to patients with normal sperm motility, ROPN1 and CABYR mRNA were downregulated and positively correlated to total progressive motility and total motile sperm count (TMSC). Both of these genes encode for proteins that interact with AKAPs in the FS (Carr et al, 2001; Fiedler et al, 2007; Lea et al, 2004; Hanlon et al, 2008; Li et al, 2011), and therefore support the notion that DFS is a multigenic disease. Interestingly, the authors suggest that although the cause of ROPN1 and CABYR downregulation is unknown, it may be the result of epigenetic regulation mechanisms (Pelloni et al, 2018).

Despite the interest in the genetic causes of asthenozoospermia is it clear that more investigation is needed in this area. Recently, the role of paternal epigenetics has been indicated as a key factor in the etiology and reproductive effects of asthenozoospermia (Schon et al, 2019). It is already well established that epigenetic mechanisms are involved in the regulation of spermatogenesis via post-translational modifications (PTMs), involving acetylation and methylation mechanisms and retention of histones, which result in either activation or suppression of specific genes (Zhao & Garcia, 2015). Interestingly, between 85 to 95 percent of histones are removed during sperm maturation to aid in condensing DNA within the sperm nucleus (Schon et al, 2019).

Schon et al (2019), investigated whether there were significant differences in the histones H3 and H4 PTM profiles between normal sperm and sperm with anomalies in either total motility, progressive motility, morphology or all three. Sperm samples abnormal for all three parameters revealed evident differences in H4 acetylation and H4K20 and H3K9 methylation, and decreased H4 acetylation was also evident in sperm abnormal for total and progressive motility. The authors conclude that these distinct variations in histone PTM profiles of abnormal sperm may be significant for normal sperm function and fertility (Schon et al, 2019). Additionally, Cheng et al (2019) investigated the role of a newly identified PTM named lysince glutarylation (Kglu), in human sperm. Kglu is found in several proteins located in the sperm tail and the results from this study revealed that its presence was significantly reduced in asthenozoospermic men, and was positively correlated with progressive motility, however the underlying mechanism requires further investigation.

Male Accessory Gland Infection/ Inflammation and Sperm Motility

The European Association of Urology includes male accessory gland infection/inflammation (MAGI) among the causes of male infertility (Jungwirth et al, 2015). MAGI comprises a set of inflammatory diseases of the male accessory sexual glands and is diagnosed in 2 to 18 percent of infertile patients (La Vignera et al, 2011). Clinical criteria for the diagnosis of MAGI includes oligospermia, asthenozoospermia and/or teratozoospermia with at least, a history or physical signs of a genitourinary infection in combination with either abnormal prostate fluid expression and/or abnormal urine after prostatic massage or leukocytospermia, bacteriospermia, or alteration in seminal biochemistry (Rowe et al, 1993). Didymo-epididymal and prostate-vesicular ultrasound scans, capable of locating the site and extent of the inflammatory process, provide a more precise classification of MAGI of either uncomplicated (prostatitis) or complicated (prostate-vesiculitis and prostate-vesciculo-epididymitis) (Calogero et al, 2017). Additionally, these scans can determine whether the infection/inflammation is unilateral or bilateral (Calogero et al, 2017), with complicated and bilateral forms of MAGI having the most impact on semen parameters (La Vignera et al, 2012). Alterations in semen parameters associated with MAGI include decreased semen volume, sperm concentration and total sperm count; percentage of progressive motility and normal forms; and increased concentrations of seminal leukocytes (La Vignera et al, 2012). Mechanisms involving MAGI in the impairment of semen parameters include production of reactive oxygen species (ROS) and/or inflammatory cytokines, impaired secretory capacity of the accessory glands, anatomical obstruction or sub-obstruction of the seminal tract and direct effects of microorganisms on sperm (La Vignera et al, 2011).

Microorganisms implicated in the cause of microbial and viral forms of MAGI include Escherichia coli, Chlamydia trachomatis, Ureaplasma urealyticum, Trichomonas vaginalis, Candida albicans, Human immunodeficiency virus type 1 (HIV) and Human Papilloma virus (HPV) (Calogero et al, 2017). There are two main mechanisms by which microorganisms directly alter sperm function independent of ROS and inflammatory cytokines. The first is by direct adhesion, effective by E. coli, T. vaginalis, C. albicans and HPV. Adhesions result in ultrastructural alterations and damage to the plasma membrane, acrosome, mid-piece and tail, consequently resulting in severely diminished motility and impaired fertilizing capacity of affected sperm (Diemer et al, 2000). The second mechanism is via the release of factors capable of altering sperm motility and/or inducing apoptosis, such as sperm immobilization factor (SIF) produced by E. coli, farnesol produced by C. albicans and C.

trachomatis lipopolysaccharide (La Vignera et al, 2011). SIF is a 56kDa molecule produced by E. coli and was identified by Prabha et al (2010). At concentrations of 0.8mg/mL and 2mg/mL isolated and purified SIF was found to cause instant immobilization and death of human sperm, respectively (Prabha et al, 2010).

Diemer et al (2003) reviewed the direct effects on sperm motility from bacteria, seminal leucocytes and pro-inflammatory cytokines and highlighted how most experimental studies regarding the effects of bacteria on sperm motility, have focused on the enterobacterium E. coli. E. coli is the most significant pathogen isolated from MAGI patients (Weidner et al, 1991; Diemer et al, 2000). However, Diemer et al (2003) concluded that although outcomes of experimental studies involving E. coli have shown significant decreases in sperm motility as well as complete immobilization (Huwe et al, 1998), these results were found under experimental conditions utilizing extreme concentrations of E. coli. Consequently, the in-vitro concentrations of E.coli were disproportionate to those found naturally in physiological concentrations under in-vivo conditions, and therefore do not provide substantial evidence that biological concentrations of E. coli impact on sperm motility in MAGI patients.

There appears to be inconsistencies in the literature regarding microbial forms of MAGI and their effects of sperm motility. C. trachomatis infection was shown to have no effect on sperm motility in asymptomatic men (Hosseinzadeh et al (2004), which contrasts with Gallegos et al (2008) who reported slight decreases in sperm motility in men with C. trachomatis infection. Interestingly, Kokab et al (2010) reported significantly lower percentage progressive motile sperm in infertile patients compared with fertile men with C. trachomatis infection. The most common microorganism found in infertile men is U. urealyticum, with a frequency of 10 to 40 percent (Dieterle et al, 2008). In men identified with genital tract infection, associations have been made between U. urealyticum and decreased sperm concentration without an effect being found on sperm motility (Upadhyaya et al, 1984). A later study by Wang et al (2006) confirmed U. urealyticum had no effect on sperm motility in men with isolated U. urealyticum infection, despite finding effects on viscosity, pH value, and sperm concentration. Gdoura et al (2007) also found no effect on sperm motility in men with diagnosed with U. urealyticum infection, however this study also found no significant effects on sperm concentration which contradicts the previous studies. Nevertheless, one study has reported significantly lower sperm motility in men with U. urealyticum infection compared to men with no infection (Zheng et al, 2008). Additionally, in-vitro studies have confirmed that T. vaginalis, a protozoan capable of binding to the sperm head and flagella resulting in agglutination, does have adverse effects on sperm motility (Jarecki-Black et al, 1988; Han et al, 2004; Kranjcic´-Zec et al, 2004; Benchimol et al, 2008).

Candidiasis caused by C. albicans and C. glabrata is the only sexually transmitted yeast infection (Achkar et al, 2010). Although this infection is more common in women (Alsterholm et al, 2008; Achkar et al, 2010) men can act as an infection reservoir (Ximena et al, 2018). The effects of C. glabrata on semen parameters are yet to be identified (Ximena et al, 2018). However, C. albicans is known to effect sperm quality in both the male and female reproductive tract, with in-vitro studies reporting decreases in sperm motility and viability in addition to increased DNA fragmentation and damage of mitochondrial membranes (Burrello et al, 2009; Tian et al, 2007; Tuttle et al 1977). An experimental study by Burrello et al (2009), found that co-incubation of C. albicans with sperm isolated from normozoospermic healthy men significantly reduced sperm motility. The association between C. albcians and reduced sperm motility may be a direct result of farnersol, a sesquiterpene alcohol virulence factor of C. albicans, which is found to reduce sperm motility as well as result in sperm apoptosis and necrosis (Rennemeier et al, 2009).

HIV and Antiretroviral Therapy Effects on Sperm Motility

Decreases in semen volume and sperm motility are associated with Human immunodeficiency virus type 1 (HIV) infection (Dulioust et al, 2002; Nicopoullos et al, 2004; Bujan et al, 2007). Bujan et al, (2007) investigated semen parameters in a large number of HIV-infected men and revealed that semen volume, percentages of progressive motile sperm, as well as total sperm counts were all lower in HIV-infected men compared to non-infected men

(Bujan et al, 2007). Additionally, Van Leeuwen et al (2008) found that the percentage of progressively motile sperm was significantly reduced in HIV-infected patients receiving combination antiretroviral therapy treatment. Combination antiretroviral therapy (cART) used in the treatment of HIV significantly reduces the risk of virus transmission (Cohen et al, 2011; Anglemyer et al, 2013). Treatment with cART in HIV-infected patients enables serodiscordant couples to safely undergo medical assistance to procreate (MAP). However, despite the association between cART and altered sperm motility it remains unclear whether the use of antiretroviral treatments in HIV-infected men significantly reduces their chances of fathering a child, and therefore subsequently increases the likelihood that they would need to access ART treatment.

Frapsauce et al (2015) found sperm motility was the only semen parameter which significantly varied according to the type of cART regime utilized by HIV-infected patients attempting to undergo MAP. The regimes assessed included nucleosidic reverse transcriptase inhibitors alone or in association with a protease inhibitor or a non-nucleosidic reverse transcriptase inhibitor of either efavirenz or nevirapine. Regimes that included efavirenz were associated with a significant impairment of sperm motility, whereas regimens without efavirenz were not associated with significant semen changes (Frapsauce et al, 2015). Additionally, the median percentage of rapid spermatozoa was 5% in the group of patients receiving efavirenz versus 20% in the other groups ($P = 0.0001$). Lambert-Niclot et al, (2011) previously reported similar results which show better sperm motility and vitality in HIV-infected men receiving nevirapine compared to men receiving efavirenz. However, and as acknowledged by Frapsauce et al (2015), a link between impaired sperm motility and efavirenz cannot be certain, as the possibility that patients receiving efavirenz were more exposed to other factors impairing sperm motility cannot be ruled out.

One possible explanation for the relationship between cART treatment and impaired sperm is nucleosidic reverse transcriptase inhibitor related mitochondrial toxicity (Brinkman et al, 1998; Coˆte´ et al, 2002; Lewis et al, 2003). Sperm contain an abundance of mitochondria which provide adenosine triphosphate (ATP), necessary to maintain progressive motility (Evenson et al, 1982; Graham et al, 1990). Therefore, damage to sperm mitochondria by nucleosidic reverse transcriptase inhibitors has been proposed to explain the association between compromised sperm motility in HIV-infected men undergoing cART treatment (White et al, 2001; Leandri et al, 2003; Pavili et al, 2010). However, Frapsauce et al (2015) emphasize how in their study this hypothesis alone does not explain how sperm motility was not reduced in patients receiving nucleosidic reverse transcriptase inhibitor alone or in combination with medications other than efavirenz.

HPV and Sperm Motility

Human Papilloma Virus (HPV) infected patients have a higher rate of complicated forms of MAGI (Calogero et al, 2017). HPV-infected men show significantly lower percentage of sperm with progressive motility (Calogero et al, 2017), which may negatively impact on their fertility. HPV is a common sexually transmitted virus found in both men and women (Bezold et al, 2007; Forman et al, 2012; Ventimiglia et al, 2016). High risk types of HPV are known to cause dysplasia leading to cancer, most commonly in the cervix but also in the vagina, vulva, anus, penis, mouth and throat (Dunne & Park, 2013; Ventimiglia et al, 2016). HPV infection in women has also been linked to adverse pregnancy outcomes (Souho et al, 2015). In men HPV is frequently detected in semen samples with a reported prevalence of 16% in infertile men (Laprise et al, 2014). However, HPV infection is asymptomatic, and testing is needed to confirm its presence. Due to the asymptotic nature of the infection and lack of testing for HPV infection in ART treatments, it has recently been claimed that an active HPV infection may impact on male fertility and provide an explanation for the large number of categorized cases of unexplained male infertility (Boeri et al, 2019).

Evidence for this claim comes from the many studies that have linked HPV infection to men with abnormal semen parameters. Foresta et al (2010) found a link specifically between sperm motility and HPV infection in sexually active males. These findings were confirmed by Garolla et al (2013), when it was found that the

motility in infected sperm was lower (29%) compared to non-infected sperm (48%).

Garolla et al (2016), investigated the impact of semen HPV infection in both natural and assisted conception in infertile patients. Within the timeframe of 6months prior to the commencement of ART treatment, no pregnancies had occurred naturally in the 54 patient couples with HPV positive semen. There were however 14 naturally conceived pregnancies reported in the 172 patient couples without HPV semen infection. Within 12 months of commencing ART treatment, 12 IUI and 40 ICSI pregnancies were achieved in the non-infected group, compared to 2 and 6 pregnancies respectively in the infected group. Furthermore, compared to the non-infected group the percentage of motility in the infected semen samples was significantly lower and the presence of anti-sperm antibodies was significantly higher.

The association between semen HPV infection and decreased motility reported in other previous studies (Foresta et al, 2010; Garolla et al, 2013; Yang et al, 2013) has yet to be clarified. The impact of HPV infection on semen parameters appears controversial, with some studies having found no association with HPV infection and semen quality (Golob et al, 2014; Luttmer et al, 2016).

Nevertheless, recent evidence for decreased motility in HPV infected sperm is strong and could implicate HPV infection in male infertility. In their cross-sectional real-life study, Boeri et al (2019), found HPV infection in 15.5% of their 729 male subjects, a figure that matches that previously claimed by (Laprise et al, 2014). Compared to the non-infected subjects, the HPV positive subjects, had significantly lower progressive sperm motility as well as higher sperm DNA fragmentation levels. This study also revealed that the most commonly found type of HPV in their infected subjects, was the high-risk type HPV16, which confirms findings from previous studies (Damke et al, 2017; Lyu et al, 2017).

HPV infection represents a pressing concern for fertility, as beyond reduced sperm motility the overall consequence of this infection could result in undesirable outcomes such as early miscarriage (Gizzo et al, 2014). This warrants greater attention to be given to assessing HPV infection in ART patients, particularly in men with unknown infertility. Recent pooled results from a study by Siristatidis et al (2018), have inferred that the effect of HPV infection in women is not significant for rates of live birth and ongoing pregnancy, but that contrarily pooled results for HPV infection in men, do show significant differences in rates of live birth and ongoing pregnancy as well as significant increases in the rates of miscarriage.

Therefore, HPV testing could in the future be implemented into ART regimes, and more understanding is needed on the relationship between HPV infection and reduced sperm motility. Furthermore, the increased presence of anti-sperm antibodies in HPV positive semen may also be associated with decreased motility (Garolla et al, 2013). Garolla et al (2013), demonstrated that infertile men with HPV positive semen had increased anti-sperm antibodies and lower sperm motility, and samples with anti-sperm antibodies had the lowest motility values.

Conclusion

This present review provides an insight into the role of sperm motility in male infertility and the implications of abnormal sperm motility on clinical outcomes. Natural sperm selection mechanisms have been discussed to investigate characteristics of fertile sperm, which can be evaluated with the use of in-vitro selection methods. Sperm motility has also been examined in IUI and ICSI outcomes, which provide examples of a natural sperm selection method and non-natural method and sperm preparation techniques have been analyzed with evidence presented for better outcomes with post thaw preparation. The potential underlying causes of asthenozoospermia have been examined to provide discussion about the potential development of more innovative diagnostics methods for male infertility. Whilst, inconsistencies have been highlighted regarding the role of MAGI in impaired sperm motility, HPV semen infection has been considered for its potential role in male infertility. Although this review focused specifically on sperm motility, the inclusion of TMSC outcomes highlights the importance of considering multiple semen parameters to evaluate fertility.

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