Journal of Andrology, Vol. 29, No. 5, September/October 2008

Temporal Decreases in Sperm Motility: Which Patients Should Have Motility Checked at Both 1 and 2 Hours After Collection?

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ABSTRACT: A decrease in sperm motility, and thus total motile sperm count (TMSC), over a period of hours might have clinical implications in counseling couples considering intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). The objective of this study was to identify patients with decreases in sperm motility from 1 to 2 hours after collection and examine predictive relationships with semen analysis parameters. Between 2001 and 2005, 2313 semen samples were analyzed. Sperm motility was evaluated at both 1 and 2 hours after time of collection. Relevant seminal parameters were compared between patients, with a decrease in 1-hour to 2-hour motility (n = 384) compared with those that showed no change (n = 1929). The same analysis was performed in a subset of patients with a TMSC between 10 and 40 million. In the total patient population, only 16% (384/2313) demonstrated a decrease in 1-hour to 2-hour motility. In patients displaying a decrease in the 1–2-hour motility, sperm concentration (33.5 vs 79 million/mL, $P < .0001$) and percent normal morphology (7% vs 8%, $P < .0001$) were significantly lower. Additionally, a significantly higher incidence of 1–2-hour motility decrease was seen in patients with midpiece anomalies (33.3% vs 15.9%, $P = .01$). Within the subpopulation of 10–40 million TMSC, the only statistically significant difference was in patients with midpiece anomalies (80.0% vs 28.2%, $P = .02$) who demonstrated a higher incidence of the 1–2-hour motility decrease. Overall, patients with a TMSC between 10 and 40 million showed a significantly higher incidence of 1–2-hour motility decrease compared with the rest of the patient population (29.0% vs 14.6%, $P < .0001$). Because decreases in 1–2-hour sperm motility affect only a small portion of patients, it is not necessary to check 2-hour motility on all patients. However, because patients with a TMSC between 10 and 40 million were significantly more likely to show a decrease in sperm motility—a decrease that could have possible clinical implications in couples deciding between IUI, IVF, or ICSI—checking 2-hour sperm motility should be considered in this population.

Key words: Assisted reproduction, infertility, semen, semen analysis.

J Androl 2008;29:558–563

Male factor infertility is the sole cause of infertility in approximately 20% of infertile couples and is a contributing factor in another 30%–40% (Mosher and Pratt, 1991; Thonneau et al, 1991). Thus, semen analyses remain an essential component of infertility evaluation. Normal semen quality is usually based on World Health Organization (WHO, 1999) criteria for multiple seminal parameters, including seminal volume, pH, sperm concentration, motility, and morphology. The WHO criteria (1999) for normality of sperm motility is 50% or more, with forward progression within 60 minutes of ejaculation. Given these criteria, many laboratories might not check sperm motility after >1 hour.

Sperm motility is an important factor to consider in couples pursuing assisted reproductive technologies (ARTs). Several studies have demonstrated the influence of sperm motility on the outcomes of various ARTs, including intrauterine insemination (IUI; Lee et al, 2002; Yalti et al, 2004; Zhao et al, 2004), conventional in vitro fertilization (IVF; Donnelly et al, 1998; Repping et al, 2002), and IVF with intracytoplasmic sperm injection (ICSI; Stalf et al, 2005). Although the exact total motile sperm count (TMSC) cutoff for defining severe male factor infertility varies somewhat among studies, a TMSC of 10 million in the ejaculate has been reported to be a useful threshold for decisions about treating a couple with IUI or IVF (Van Voorhis et al, 2001). This is also many times a practical threshold for conventional insemination or ICSI. Therefore, a decrease in sperm motility, and thus TMSC over a period of a few hours, could have relevant clinical implications in counseling these couples as to whether to pursue IUI, IVF, or ICSI. This decrease could have an even greater effect in oligospermic patients with already borderline low TMSC around this threshold value of 10 million.

The objective of this study was to determine the incidence of decreases in sperm motility seen from 1 to

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Received for publication August 16, 2007; accepted for publication May 23, 2008.

DOI: 10.2164/jandrol.107.004002

2 hours after collection in a large population of patients presenting to an infertility clinic and to identify related seminal parameters that help predict these decreases. In this retrospective study over a 4-year period, all patients who demonstrated a decrease in sperm motility from 1 to 2 hours after collection were identified, and multiple relevant seminal parameters were analyzed to identify any predictive parameters. A similar subset analysis was also performed on patients with a baseline TMSC between 10 and 40 million, in that decreases in this population are more likely to cause the TMSC to drop below the aforementioned threshold value of 10 million and, thus, be useful for decision making in couples contemplating IUI, IVF, or ICSI.

Methods

Semen Analysis

Use of semen analysis outcome data for investigative purposes was approved after Investigational Review Board (IRB) application and review. Semen samples were collected from men presenting to the University of Michigan Center for Reproductive Medicine. All men were instructed to refrain from any sexual activity, including masturbation, for approximately 3–5 days before sample collection. Semen was collected by masturbation in a lab-approved sterile plastic container. Semen specimens were allowed to liquefy at 37°C before analysis approximately 1 hour after ejaculation. Semen volume was measured with a graduated pipette to the nearest 0.1 mL. A drop of semen was placed on pHydrion paper (Micro Essential Laboratory Inc, Brooklyn, New York), and pH was measured and recorded. Sperm concentration was determined for duplicate loadings on a Neubauer hemocytometer. Sperm motility was assessed at both 1 and 2 hours after collection. For assessment of motility, a minimum of 200 sperm from multiple fields was counted with the 37°C warming stage and ×20 magnification. Percentage of motile sperm with forward progression and speed of progression were also analyzed at this time. For assessment of morphology, 10 μL of semen was smeared, air dried, then stained with Papanicolau stain. Morphologic assessment was then carried out under the ×100 objective with immersion oil until 200 sperm were counted. Sperm morphology was determined according to the strict criteria described by Kruger et al (1986) at ×100 magnification. Gross head defects, midpiece defects, and tail defects also were scored. Presence of anti-sperm antibodies was evaluated with the use of Sperm-Mar (Conception Technologies, San Diego, California).

Statistical Analysis

Information was collected retrospectively on 2313 semen analyses collected from 2003 to 2006. Patients were divided into 2 groups: 1) those that showed a decrease in motility from 1 hour to 2 hours after collection (n = 384) and 2) those that showed no change in motility from 1 hour to 2 hours after collection (n = 1929). Decrease in motility was defined as those samples that had a ≥10% decline in 2-hour motility when compared with 1-hour motility. Statistical analysis with the use of Fisher's exact test was performed to evaluate the bivariate relationship between a decrease in motility from 1 hour to 2 hours after collection and other categorical semen analysis variables. The Wilcoxon 2-sample test was used to compare the bivariate relationship between a decrease in motility from 1 hour to 2 hours after collection and continuous semen analysis variables. $P < .05$ was considered statistically significant.

Additionally, a similar subset analysis was performed on patients with a baseline TMSC between 10 and 40 million (n = 321). Among this subset, patients were again divided into 2 groups: 1) those that showed a decrease in motility from 1 hour to 2 hours after collection (n = 93) and 2) those that showed no change in motility from 1 hour to 2 hours after collection (n = 228). Fisher's exact test and Wilcoxon 2-sample test were again used to evaluate the bivariate relationship between a decrease in motility from 1 to 2 hours after collection with categorical and continuous semen analysis variables, respectively.

Results

In the total patient population, only 16% of samples (384/2313) demonstrated a decrease in 1-hour to 2-hour motility. Table 1 shows the comparison of relevant seminal parameters between the group that demonstrated a decrease in 1-hour to 2-hour motility compared with those with no change. The range of sperm concentrations in the entire data set was 0 to 2432 × 10^6. Sperm concentration ($P < .0001$), percent normal morphology ($P < .0001$), and percentage of motile sperm with forward progression ($P = .0001$) were all lower in patients that had a decrease in 1-hour to 2-hour motility when compared with patients with no change in motility. Additionally, the percentage of abnormal sperm forms with head defects ($P = .001$) and midpiece defects, as well as the percentage of sperm bound with IgG anti-sperm antibodies ($P = .01$), were higher in patients that had a decrease in 1-hour to 2-hour motility. Table 2 displays several other categorical seminal parameters that were evaluated between the 2 groups. A greater incidence of 1-hour to 2-hour motility decrease was seen in patients whose semen analysis had morphologic midpiece defects ($P = .01$), $>5 \times 10^6$ round cells ($P = .01$), incomplete liquefaction ($P = .04$), and a sluggish speed of progression ($P < .0001$).

The TMSC range for the total population was 0–967 million. However, in our subset analysis, patients with a TMSC between 10 and 40 million were almost twice as likely to have a drop in motility from 1 to 2 hours after collection compared with the rest of the population

Table 1. *Seminal parameters in total patient population and subpopulation of a total motile sperm count (TMSC) of 10–40 × 10^6*

Semen Parameter	Total Patient Population			Subpopulation (TMSC 10–40 × 10^6)		
	1–2-h Motility Decrease	No Motility Change	*P* Value	1–2-h Motility Decrease	No Motility Change	*P* Value
Days of abstinence	4.8 ± 5.4	4.7 ± 6.2	.35	5.0 ± 9.1	5.4 ± 15.3	.56
Volume, mL	3.3 ± 1.9	3.3 ± 1.6	.51	3.0 ± 1.9	2.6 ± 1.6	.10
pH	7.53 ± 0.4	7.55 ± 0.2	.82	7.49 ± 0.6	7.57 ± 0.2	.21
Concentration, $\times 10^6$/mL	64.4 ± 109.1	102.5 ± 109.9	<.0001[a]	38.8 ± 51.2	35.3 ± 37.1	.77
Forward progression, %[b]	69.6 ± 15.0	72.5 ± 13.2	.0001[a]	67.7 ± 14.9	71.3 ± 6.3	.44
Morphology, % normal forms	7.4 ± 4.0	8.6 ± 4.8	<.0001[a]	7.5 ± 3.4	7.1 ± 2.9	.29
Head anomalies, % of abnormal forms	90.6 ± 4.7	89.6 ± 5.7	.001[a]	89.8 ± 4.1	90.2 ± 7.1	.06
Midpiece defects, % of abnormal forms	0.08 ± 0.5	0.02 ± 0.2	.005[a]	0.05 ± 0.2	0.004 ± 0.06	.01[a]
Tail anomalies, % of abnormal forms	1.75 ± 3.1	1.87 ± 2.4	.22	2.47 ± 2.6	2.63 ± 6.3	.64
IgA anti-sperm Ab, % sperm-bound	5.8 ± 19.2	2.6 ± 9.2	.52	8.0 ± 25.4	3.3 ± 11.1	.25
IgG anti-sperm Ab, % sperm-bound	8.4 ± 25.6	3.9 ± 17.3	.01[a]	12.0 ± 30.5	6.2 ± 22.4	.24

Abbreviations: Ab, antibody; Ig, immunoglobulin.

[a] Statistically significant.

[b] Percentage of motile sperm with forward progression.

Table 2. *Categorical seminal parameters in total patient population and subpopulation with a total motile sperm count (TMSC) of 10–40 × 10^6*

Semen Parameter	Total Patient Population		Subpopulation (TMSC 10–40 × 10^6)	
	Incidence of 1–2-h Motility Decrease, %	*P* Value	Incidence of 1–2-h Motility Decrease, %	*P* Value
Place of Collection				
Home	16.6	.90	30.1	.88
Lab	16.8		28.6	
Liquefaction				
Complete	16.5	.04[a]	28.8	.29
Incomplete	38.5		100.0	
Agglutination				
None	22.5	.13	31.1	.14
Slight	12.4		25.8	
Moderate	16.7		100.0	
Heavy	40.0		100.0	
Speed of rogression				
None–sluggish	24.6	<.0001[a]	33.3	.24
Good–excellent	14.3		26.8	
Round cells				
<5 × 10^6/mL	16.2	.01[a]	28.5	.29
≥5 × 10^6/mL	28.8		44.4	
Midpiece anomaly				
Absent	15.9	.01[a]	28.2	.01[a]
Present	33.3		80.0	

[a] Statistically significant.

(29.0% vs 14.6%, $P < .0001$; Figure 1). The results of the subset analysis of patients with a TMSC between 10 and 40 million are shown in Tables 1 and 2. The only statistically significant difference among the multiple seminal parameters examined was seen in patients with gross morphologic midpiece defects who subsequently had a higher incidence of 1-hour to 2-hour motility decrease compared with those without midpiece defects ($P = .03$). Among both the total patient population and the subpopulation of TMSC between 10 and 40 million, the presence of midpiece defects was the only seminal parameter that showed a statistically significant relationship to the incidence of 1-hour to 2-hour motility decrease in both populations (Figure 2).

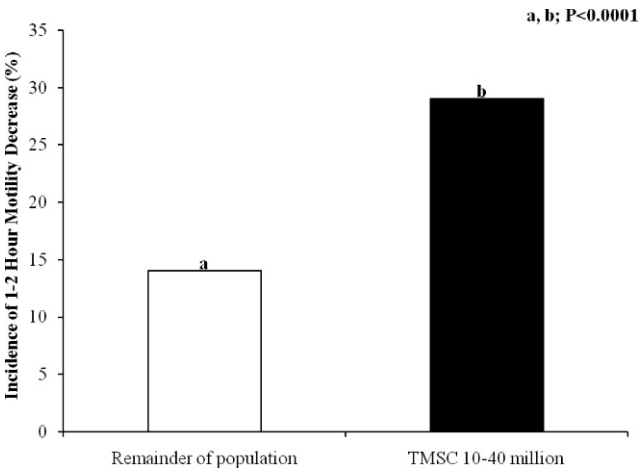

Figure 1. Incidence of decreases in 1-hour to 2-hour sperm motility in patients with total motile sperm count (TMSC) of 10–40 × 10^6 compared with the remainder of the patient population.

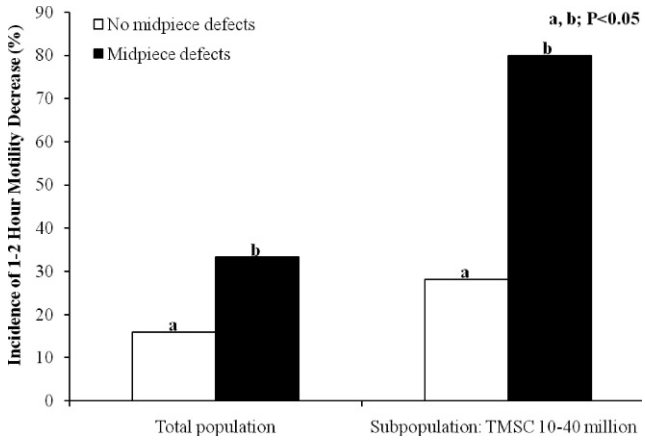

Figure 2. Relationship of sperm morphologic midpiece defects to the incidence of decreases in 1-hour to 2-hour sperm motility in the total population compared with the subpopulation of patients with total motile sperm count (TMSC) of 10–40 $\times$ 10^6.

Discussion

Semen analysis is an integral component of evaluation of infertility. Sperm motility is an important factor in the fertilizing potential of the sperm, and motility has been shown to correlate closely with fertilization rates of human oocytes in vitro (Bongso et al, 1989). Subsequently, sperm motility is also an important factor in the outcomes of various ARTs. Multiple studies have shown that IUI outcomes are affected by the percentage of motile sperm and total motile sperm count (Shulman et al, 1998; Pasqualotto et al, 1999; Lee et al, 2002; Miller et al, 2002; Yalti et al, 2004; Zhao et al, 2004). This effect on IUI outcomes has been demonstrated with both the percentage of motile sperm in the unprocessed ejaculate (Lee et al, 2002; Yalti et al, 2004; Zhao et al, 2004) and postprocessed sperm motility (Shulman et al, 1998; Pasqualotto et al, 1999). Both percentage of motile sperm and TMSC in unprocessed semen have been shown to influence IVF outcomes (Donnelly et al, 1998; Van Voorhis et al, 2001; Repping et al, 2002). Additionally, the fertilization potential of injected sperm during ICSI was found to be associated with motility in both ejaculated and testicular sperm (Stalf et al, 2005). Given this effect of sperm motility on ART outcomes, a decline in motility over a period of a few hours could have relevant clinical implications in couples pursuing ARTs.

Many laboratories do not routinely check repeated measures of sperm motility. Sperm motility can be time dependent. Under normal laboratory conditions, sperm motility will gradually decline, although significant signs of change are not usually apparent until 3 hours have elapsed (Glover et al, 1990). A decrease in motility of up to 20% in 3–4 hours is not unusual, but if the fall-off is greater or quicker, infection or some other abnormality should be suspected (Glover et al, 1990). Previous studies have looked at sperm motility longevity. Denil and coworkers (1992) reported that after 24 hours, samples from men with male factor infertility lost 48% of initial motility, whereas normal specimens lost only 34%. Makler and colleagues (1979) found 3 typical curves of motility change during the first 4 hours: increase in motility, moderate decline in motility, and rapid loss of motility. It was in this population of patients with rapid loss of motility over an hour that we sought to identify the incidence and evaluate their relationship with other relevant seminal parameters. In our retrospective study of 2313 semen analyses over a 4-year period, we found that decreases in sperm motility from 1 to 2 hours after collection affected only a relatively small proportion of patients, with an incidence of 16%. Singer and coworkers (1980) reported that percentile decreases in sperm motility after 5 hours were found to be higher in oligospermic specimens than in those with higher sperm densities. In this study, patients with decreases in motility from 1 to 2 hours after collection demonstrated lower mean sperm concentrations when compared with patients that showed no change in sperm motility. Although this difference was statistically significant, the mean sperm concentration in both groups was still high enough to be considered normospermic and thus would likely be of little clinical significance in such a way that would help guide which patients should have motility checked at both 1 and 2 hours postcollection. Percent normal morphology and percentage of motile sperm with forward progression were also lower in patients with decreases in 1-hour to 2-hour sperm motility. Similarly, although a significant observation from a statistical perspective, the actual differences are too small to affect clinical decision making in determining which patients should have sperm motility checked at both 1 and 2 hours.

Because these decreases in sperm motility over a period of a couple hours affect only a small portion of patients, we propose that it is unnecessary to check both 1-hour and 2-hour motility on all patients. Even in some patients who display a decrease in motility, ART clinical decision making could be unaffected. For example, in a couple contemplating ART, if the man already has a high TMSC over 100 million, even after a 50% decrease in sperm motility, he would still have a relatively high TMSC, and IUI would still be the likely recommended first-line ART for the couple. On the other end of the spectrum, for a man with severely low TMSC of <1 million, a 50% decrease in sperm motility also would likely not affect ART decision making, in that ICSI would likely be recommended either way. However, the patients that could be most affected by a decrease in

sperm motility would be those with a TMSC between these 2 extremes. The exact TMSC threshold for defining severe male factor infertility has varied somewhat among studies, but threshold values of between 5 and 10 million in the ejaculate have been reported (Cohlen et al, 1998; Dickey et al, 1999; Van Voorhis et al, 2001). Van Voorhis and colleagues (2001) reported that the average TMSC in the ejaculate was a useful independent predictor of clinical pregnancy after IUI, with a threshold value of 10 million. Additionally, when the TMSC in the ejaculate was <10 million, IVF with ICSI was more cost-effective that IUI. A decrease in sperm motility and TMSC over a period of an hour would have the most relevant clinical implications in patients with a moderately low TMSC around this 10 million threshold and the subsequent decision whether to pursue IUI, IVF with conventional insemination, or ICSI. Thus, we performed a subset analysis in this population, identifying patients with a TMSC between 10 and 40 million. Patients in this subpopulation were twice as likely to show a decrease in 1-hour to 2-hour motility when compared with the rest of the population (29.0% vs 14.6%, $P < .0001$). Given the significantly increased incidence of motility decrease in patients with a TMSC between 10 and 40 million and its subsequent potential clinical implications for couples deciding between various ART interventions, checking both 1-hour and 2-hour sperm motility should be considered in this population.

Interestingly, the only common seminal parameter that showed a statistically significant relationship to the incidence of 1-hour to 2-hour motility decrease in both the total patient population and the subpopulation of TMSC between 10 and 40 million was presence of gross morphologic midpiece defects. The sperm midpiece is densely packed with mitochondria that provide energy in the form of ATP to the flagellum to propel the cell. Multiple studies have examined the relationship between the sperm midpiece and mitochondria with sperm motility. Motility depends largely on the mitochondrial volume within the sperm midpiece (Ruiz-Pesini et al, 1998) and morphologic defects of the midpiece can cause nonprogressive movement or immotility (Piasecka and Kawiak, 2003). Mundy and colleagues (1995) showed that sperm midpiece length was significantly shorter in asthenozoospermic subjects compared with controls. Additionally, Nakada and coworkers (2006) used a transmitochondrial mouse model to show that accumulation of mutant mitochondrial DNA induces asthenozoospermia, with the majority of sperm showing midpiece abnormalities. In our study, patients with morphologic defects of sperm midpiece on semen analysis were more than twice as likely to show a decrease in 1-hour to 2-hour sperm motility when compared with those with no midpiece defects. This relationship was seen consistently across our total patient population, as well as in our subpopulation analysis. This study is subject to the limitations of light microscopy to evaluate morphologic sperm defects. Further studies with electron microscopy would be necessary to fully evaluate the true morphologic structural abnormalities contributing to these temporal decreases in motility. However, given our current findings, in combination with the multiple previous studies, checking both 1-hour and 2-hour sperm motility should also be considered in patients with morphological midpiece defects on semen analysis.

As far as we know, this is the first study to determine the incidence and related seminal parameters of rapid declines in sperm motility longevity over a 1-hour period in a such a large sample of subjects from an infertility clinic population. The incidence of rapid declines in sperm motility from 1 to 2 hours after collection is relatively low, affecting only 16% of the population. Thus, it is likely unnecessary to make 2-hour sperm motility a routine part of semen analysis for all patients. However, patients with a TMSC between 10 and 40 million, as well as patients with morphologic midpiece defects of the sperm, had a significantly higher incidence of rapid declines in sperm motility over the 1-hour period. Because of the possible ART clinical implications of rapid declines in sperm motility in these patients, checking both 1-hour and 2-hour sperm motility should be considered in these populations.

Acknowledgments

The authors thank Dr Carrie Cosola-Smith for critical review of this manuscript and editorial comments. The authors also thank Rodney L. Dunn, MS, for his assistance with statistical analysis.

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