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Effect of Time between Ejaculation and Analysis on Sperm Motility

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ABSTRACT

Objective To evaluate the effect of time between ejaculation and analysis on sperm motility.
Study design Observational study.
Setting The Andrology Laboratory, Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital.
Subjects Fifty-seven semen samples from patients attending the infertility clinic from August to October 2006.
Intervention Semen samples were kept in a 37°C incubator and were analyzed for sperm motility using the Computer-Aided Semen Analysis (CASA) at 30 minutes, 1, 2, 3 and 4 hours after ejaculation respectively.
Main outcome measure Sperm motility.
Results Mean (±SD) of sperm motility at 30 minutes, 1, 2, 3 and 4 hours were 53.1±50.5, 42.1±39.6, 29.1±28.4, 24.6±22.5 and 19.9±16.3 %, respectively. Motility significantly decreased at 60 minutes after ejaculation ($p < 0.017$).
Conclusion Sperm motility decreased when the time between ejaculation and analysis increased and significantly decreased at 60 minutes after ejaculation, approximately 20% changed.

Keywords: semen analysis, sperm motility, CASA

We now recognize that abnormalities in males are the sole cause of infertility in approximately 20 % of infertile couples and an important contributing factor in another 20-40% of couples with reproductive failure.[1] The list of known causes of male infertility is relatively long, but can be divided into three major categories: obstruction in the ductal system, abnormalities of sperm production and sperm function.[2]

Semen analysis is a key element in population-based studies of male reproductive health, dealing with infertility problems.[3,4] It can provide information on testicular output and functional properties of the spermatozoa also the

secretory function of accessory genital glands.[5,6] The aim of basic semen analysis is to evaluate descriptive parameters of ejaculation such as visual appearance, smell, liquefaction, viscosity, volume, concentration, total number of spermatozoa, sperm motility and vitality.[5,7]

Sperm motility is believed to be one of the most important parameters in evaluating the fertilizing ability of the ejaculated spermatozoa.[8-11] According to the manual of the World Health Organization (WHO), the assessment of sperm motility should begin either immediately or within 30-60 minutes to avoid deterioration caused by temperature or dehydration.[3,12] On the other hand, it is inconvenient for men who have difficulties in collecting a semen sample at a laboratory or men who collect samples at home and cannot deliver them to the laboratory within an hour. Although some studies show decreasing of sperm motility along with the time after ejaculation, but they do not known how rapidly changes in sperm motility.[13,14] The objective of this study was to evaluate the effect of time between ejaculation and analysis on sperm motility.

Materials and Methods

The study protocol was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Subjects were recruited from patients attending the Infertility clinic, Department of Obstetrics and Gynaecology, Ramathibodi Hospital from August to October 2006. Exclusion criteria included: (1) The volume of semen sample was less than 0.5 ml; (2) Complete liquefaction did not occur within 30 minutes. ; (3) Standard semen analysis revealed azoospermia. All subjects were informed and signed consent forms.

Semen collection

A two-to-seven days abstinence period was advised prior to semen collection. The semen was collected by masturbation and ejaculated into a clean plastic container. The ejaculation time was the time at 0 minute. All samples were collected in a room near the laboratory.

After liquefaction, fresh semen samples were immediately evaluated by conventional semen analysis according to the WHO guideline.[12] Then 0.5 ml of semen samples were kept in a 37°C incubator and evaluated again by Computer-Aided Semen Analysis (CASA) at 30 minutes, 1, 2, 3 and 4 hours after ejaculation, respectively.

Computer-Aided Semen Analysis (CASA)

Sperm count and motility were analyzed using the CASA (Hamilton Thorn Research version 10.7, Bevery, MA, USA) by one observer. A 5 μl drop of sample was loaded into a 10 μm depth Makl er chamber (Sefi-Mahidol Instruments, Haifa, Israel) and placed on the prewarmed stage (37°C) of the CA SA. Analysis was performed using x10 objective lens on 5 random fields from each sample. The CASA settings were followed according to the manufacture's standard setting : frames acquired 30; frame rate 60 Hz; minimum contrast 80; minimum cell size 3 pixels; static head size 1.00-2.90; static head intensity 0.60-1.40; and magnification 1.95. The motility defined as the sperm moving with rapid and slow progressive [rapid (class a): average path velocity (VAP) > 25 μm/s, slow (class b): VAP 5-25 μm/s].

Statistical Analysis

The data were analyzed using Stata version 9.0 (StataCorp, Texas, USA). The data were presented as the mean $\pm$ SD in continuous data and the percentage in grouping data. One factor repeated measure analysis of variance (ANOVA) and two factors variance (ANOVA) with repeated measures on one factor were used to compare the mean of sperm motility at 30 minutes, 1, 2, 3 and 4 hours after ejaculation, respectively. A p-value < 0.05 was considered statistically significant. A sign test was used to compare among sperm motility at 30 minutes and other times after ejaculation with adjustment of the significance level to take account of type I error.

Results

A total of 57 men were included in this study. The mean (± SD) age was 34.2 (± 4.8) years. General semen characteristics measured at 30 minutes after ejaculation are shown in Table 1.

Table 2 shows the mean (±SD) of sperm motility at each time after ejaculation. Sperm motility at 30 minutes, 1, 2, 3 and 4 hours were 53.1±50.5, 42.1±39.6, 29.1±28.4, 24.6±22.5 and 19.9±16.3 %, respectively. We found that motility significantly decreased since the time of ejaculation to analysis was 1 hour or more ($p < 0.05$).

Table 3 shows the percentages changed of the means (±SD) of sperm motility at 30 minutes compared to others. The motility at 1, 2, 3 and 4 hours decreased from at 30 minutes after ejaculation 20.0±32.1%, 35.1±44.8%, 48.2±39.1% and 65.4±22.9%, respectively.

The semen samples were divided into 2 groups by the percentage of sperm motility at 30 minutes: 36 samples were in the normal group (motility ≥ 50%) and 21 samples were in the abnormal group (motility < 50%).[12] The mean of sperm motility in each group is shown in Table 4. When sperm motility at 30 minutes was compared with each other time after ejaculation it was found that there were significant decreases in sperm motility (p-value < 0.017) which appeared in 2 comparisons. One was the comparison between the mean of sperm motility of the normal group at 30 minutes and 60 minutes after ejaculation (p-value = 0.0001). The other was a comparison between the mean of sperm motility of the abnormal group at 30 minutes and 120 minutes after ejaculation (p-value = 0.0007).

Table 1. General semen characteristics at 30 minutes after ejaculation (N = 57 samples)

Semen parameters	Mean ± SD
Liquefaction (minutes)	13.35 ± 7.65
pH	6.90 ± 7.65
Volume (ml)	2.59 ± 1.13
Concentration (M/ml)	48.60 ± 30.71
Percent of motility	57.88 ± 13.78
Morphology	
- Normal (%)	15.91 ± 2.02
- Abnormal (%)	84.10 ± 2.05

Table 2. Sperm motility at each time after ejaculation (N = 57 samples)

Time (minutes)	Percent of motility (mean ± SD)
30	53.1 ± 50.5
60	42.1 ± 39.6
120	29.1 ± 28.4
180	24.6 ± 22.5
240	19.9 ± 16.3

* Statistical analysis by repeated measure ANOVA (repeated on time), p value < 0.05

Table 3. Mean of percent change of sperm motility at 60, 120, 180 and 240 minutes compared with motility at 30 minutes after ejaculation. (N = 57)

Time (minutes)	Percent change of motility from 30 minutes (mean ± SD)
60	20.1 ± 32.1
120	35.1 ± 44.8
180	48.2 ± 39.1
240	65.4 ± 22.9

* Statistical analysis by repeated measure ANOVA (repeated on time), p value < 0.05

Table 4. Sperm motility at each time after ejaculation was divided into 2 groups. (N = 57)

Time (minutes)	Percent of motility (mean ± SD)	
	Normal group (N=36)	Abnormal group (N=21)
30	70.5 ± 57.1	16.0 ± 14.9
60	54.5 ± 45.1[a]	14.2 ± 18.3[b]
120	38.7 ± 31.1	10.8 ± 12.5[c]
180	34.1 ± 31.4	8.4 ± 10.4
240	22.6 ± 22.4	5.5 ± 5.5

Note: Statistical analysis by two factor ANOVA with repeated measure on one factor (time) ,adjusting significant level p < 0.017

a; Motility of normal group at 60 minutes compare with 30 minutes, p-value < 0.017

b; Motility of abnormal group at 60 minutes compare with 30 minutes, p-value > 0.017

c; Motility of abnormal group at 120 minutes compare with 30 minutes, p-value < 0.017

Discussion

Semen analysis is important in the diagnosis of male infertility.[8-11] Some studies showed that sperm motility decreases along with the time after ejaculation, but it was not known how rapidly sperm motility changes.[15-17] From the previous study, sperm motility and viability appeared to decline when the ejaculate-to-analysis delay exceeded two hours, although the only statistically significant change in mean values was for viability between 2 and 3 hours. Due to the very small numbers of samples analyzed after delay more than 3 hours and considering the great variability of parameters of sperm quality, no conclusion of real biological significance was feasible.[13]

In this study, sperm motility revealed a significantly decreased since at 60 minutes after ejaculation. It was superior to the previous study because of repetitive measurements, larger sample size and more accurate sperm motility evaluation method: CASA to reduce the subjective bias. [3,18-21] The decrease of motility should be due to the detrimental effect of reactive oxygen species (ROS)[22-24], secondary to bacterial overgrowth[15-17] or metabolic activity of spermatozoa leading to depletion of nutrient.[25-26]

We further analyzed by dividing into two groups using the percentage of motility; normal group

(motility > 50%) and abnormal group (motility < 50%).[14] In the normal motility group, the result was still the same. However, in the abnormal group, sperm motility statistically significantly decreased at 120 minutes and was nearly significant at 60 minutes after ejaculation. The reason of this difference may be explained by the small numbers of each subgroup (samples analyzed after division into two groups), or the very low beginning of mean of sperm motility in the abnormal group.

The limitation of this study was the great variability of semen sample including both normal and abnormal semen groups. We suggested that semen analysis should be performed within an hour and preferably within 30 minutes after ejaculation. The suitable place for semen collection near the laboratory should be prepared. Further studies, comparing between normal and abnormal groups with final outcomes such as pregnancy should be measured.

Conclusion

Sperm motility decreased when the time between ejaculation and analysis increased and significantly decreased at 60 minutes after ejaculation, approximately 20% changed. Physicians should be cautious in the interpretation of semen analysis at different time from ejaculation to analysis.

References

1. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three regions (1988-1989). Hum Reprod 1991;6:811-6.
2. Leon S, Marc A F. Clinical gynecologic endocrinology and infertility. 7th ed. USA: Lippincott William & Wilkins, 2005:1135-72.
3. Shanis BS, Check JH, Bollendorf A. Interpretation and misinterpretation of semen parameters. Arch Androl 1989;23:213-27.
4. David SG, James WO, Pam F, Charlene K. Sperm morphology, motility and concentration in fertile and infertile men. N Eng J Med 2001;345:1388-93.
5. Eliasson R. Analysis of semen. In:Burger H, de Kretser D, editors. The testis. New York: Raven Press, 1981:381-99.
6. Atiken RJ. Sperm function tests and fertility. Int J Androl 2006;29:69-75.
7. NAFA and ESHRE-SIGA. (2002). Manual on basic semen analysis 2002. [Online] Available: http://www.ki.se/org/nafa [2006, August].
8. Bongso TA, Ng SC, Mok H, Lim MN, Teo HL, Wong PC, et al. Effect of sperm motility on human in vitro fertilization. Arch Androl 1989;22:185-90.
9. Brasch JG, Rawlins R, Tarchala S, Radwanska E. The relationship between total motile sperm count and success of intrauterine insemination. Fertil Steril 1994;62:150-4.
10. Bollenndorf JG, Check JH, Lurie D. Evaluation of the effect of the absence of sperm with rapid and linear progressive motility on subsequent pregnancy rates following intrauterine insemination or in vitro fertilization. J Androl 1996;17:550-7.
11. Zaini A, Jenning MG, Baker HW. Are conventional sperm morphology and motility assessments of predictive value in subfertile men ?. Int J Androl 1985;8:427-35.
12. World Health Organization. WHO laboratory manual for the examination of human semen of sperm-cervical mucous interaction.4th ed. Cambridge University Press, 1999.
13. Mortimer D, Templeton AA, Lenton EA, Coleman RA. Influence of abstinence and ejaculation to analysis delay on semen analysis parameter of suspected infertile men. Arch Androl 1982;8:251-6.
14. Brackelt NL, Santa-Cruz C, Lynne CM. Sperm from spinal cord injured men lose motility faster than sperm from normal men. J Urol 1997;157:2150-3.
15. Appell RA, Evans PR, Blandy JP. The effect of temperature on the motility and viability of sperm. Br J Urol 1977;49:751-6.
16. Appell RA, Evans PR. The effect of temperature on sperm motility. II. Is bacterial growth a factor ?. Fertil Steril 1978;30:436-8.
17. Makler A, Urbach V, Lefler E, Merzbach D. Factors affecting sperm motility . VI. Sperm viability under the influence of bacterial growth in human ejaculates. Fertil Steril 1981;35:666-70.
18. Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology 2002;57:149-79.
19. Pusch H, Wiesspeiner G, Hausl A. Evaluation of semen by electronic spermcounter. Andrologia 1982;14:113-7.
20. Spiropoulas J. Computerized semen analysis (CASA): effect of semen concentration and chamber depth on measurements. Arch Androl 2001;46:37-42.
21. Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, et al. Intra- and Inter-individual variability in human sperm concentration, motility and viability assessment during a workshop involving ten laboratories. Human Reprod 2000;15:2360-8.
22. Esfandiari N, Saleh RA, Blaut AP, Sharma RK, Nelson DR, Thomas AJ Jr, et al. Effect of temperature on sperm motion characteristics and reactive oxygen species. Int J Fertil Womens Med 2002;47:227-33.

23. de Lamirande E, Gognon C. Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. J Androl 1992;13:368-78.
24. Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, Sikka SC. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radic Biol Med 1999;26:869-90.
25. Davis ME, McCune WW. Fructolysis of human spermatozoa. Fertil Steril 1950;1:362-72.
26. Vaishwanar PS. Fructolysis of human spermatozoa in semen. Am J Obstet Gynecol 1958;75:139-43.

ผลของระยะเวลาหลังการหลั่งน้ำอสุจิต่อการเคลื่อนไหวของอสุจิ

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วัตถุประสงค์ *: เพื่อศึกษาระยะเวลาหลังการหลั่งน้ำอสุจิถึงการตรวจน้ำอสุจิ ที่จะมีผลต่อการเคลื่อนไหวของอสุจิ*
รูปแบบการวิจัย *: การวิจัยโดยการสังเกตุ*
สถานที่ทำวิจัย *: ห้องปฏิบัติการชีววิทยาการเจริญพันธุ์ หน่วยรักษาผู้มีบุตรยาก ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี*
กลุ่มตัวอย่าง *: น้ำอสุจิ 57 ตัวอย่าง จากผู้ป่วยที่เข้ารับการรักษาที่หน่วยรักษาผู้มีบุตรยาก ระหว่างสิงหาคม ถึงตุลาคม 2549*
การกระทำ *: นำน้ำอสุจิที่ได้เก็บไว้ที่อุณหภูมิ 37 องศาเซลเซียส และทำการตรวจการเคลื่อนไหวของอสุจิด้วยเครื่องคอมพิวเตอร์ ที่ระยะเวลา 30 นาที 1, 2, 3 และ 4 ชั่วโมง หลังการหลั่งน้ำอสุจิ*
ตัววัดที่สำคัญ *: การเคลื่อนไหวของอสุจิ*
ผลการวิจัย *: ค่าเฉลี่ยการเคลื่อนไหวของอสุจิที่ระยะเวลา 30 นาที 1, 2, 3 และ 4 ชั่วโมงหลังการหลั่งน้ำอสุจิคือ 53.1±50.5, 42.1±39.6, 29.1±28.4, 24.6±22.5 และ 19.9±16.3 % ตามลำดับ โดยมีการลดลงอย่างมีนัยสำคัญทางสถิติที่ระยะเวลา 60 นาทีหลังการหลั่งน้ำอสุจิ*
สรุป *: การเคลื่อนไหวของอสุจิมีการลดลงตามระยะเวลาหลังการหลั่งน้ำอสุจิที่เพิ่มขึ้น โดยลดลงอย่างมีนัยสำคัญ ที่ระยะเวลา 60 นาทีหลังการหลั่งน้ำอสุจิ ประมาณ 20%*