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## Research Article

# The effects of Semen Parameters and age on Sperm Motility of Iranian men

## Abstract

**Objective:** Sperm motility is one of the most important factor in fertility of men. Because, it describes the ability of sperm to move properly through the female reproductive tract and reach the egg in order to fertilize it. Various factors, such as semen quality and other parameters are known to be effect on sperm motility. This study was designed to determine, how semen parameters and individuals age influence on sperm motility.

**Method:** Samples were collected from 273 men undergoing evaluation and treatment for infertility in Infertility Treatment Center, ACECR branch of Qazvin, Qazvin, Iran. Semen analysis was performed according to World Health Organization (WHO) criteria. In this procedure we considered on determination of sample volume, sperm concentration, motility, normal morphology and liquefaction time.

**Results:** In this analysis, our results showed, increasing in sperm count, sample volume and normal morphology increased sperm motility significantly. In contrast, increasing in liquefaction time and age decreased sperm motility significantly. Our statistical analysis demonstrated that sperm concentration and semen volume have the most and lowest effect on motility respectively.

**Conclusion:** In our study, semen parameters tend to have an influence on sperm motility.

## Introduction

Male infertility refers to male inability to cause pregnancy in a fertile female and it commonly due to deficiencies in the semen quality. Male infertility evaluation includes a physical exam, hormonal evaluation, semen analysis and genetic testing [1]. Semen analysis is the primary and the most important part of the infertility assessment, which is done by World Health Organization (WHO) manual for the examination of human semen parameters [2].

The most broadly used parameters are sperm concentration (count), motility, progressive motility, sperm morphology, semen liquefaction time and semen PH [3].

The normal count of sperm is more than or equivalent to 20 million per milliliter. In according to that normal volume of ejaculation is 2ml, at least 40 million sperm is necessary to fertilize an egg. The other important factors in male fertility are motility and normal morphology. These two factors influence sperm swimming to reach egg as well as the viability of the embryo which might be created [4].

Sperm cells are generated during spermatogenesis and

spermiogenesis. However, the generated spermatozoa are immature. They must undertake many modifications due to be able to participate in fertilization process. Spermiogenesis done in the testis but spermatozoa undergoes maturation process in the epididymis until ejaculation [5]. The prostate which is the largest sexual gland is crucial for male fertility. Prostate secretions partly supply seminal plasma, which protects and nourishes sperm after ejaculation [6].

Spermatozoa are not motile in male body and they obtain motility after ejaculation. Sperm motility is usually started by changes in special situation such as ionic concentrations or osmotic stimulation [5].

Sperm motility is a fundamental factor in the fertilization. There are still major gaps in the knowledge of how sperm motility is controlled or modulated [7]. In all our analysis on semen parameter, WHO standards are used as a reference [8]. It is well known that sperm motility affects male fertility but other semen parameter relation with sperm motility and how they have an effect on each other has not yet been evaluated. So, this study was designed to define the sperm motility in patients with infertility and find how sperm motility and semen other parameters may affect each other.

## Material and Methods

### Subjects

This study was approved by the Ethical Committee of the ACECR Telemedicine Infertility Center. The study was conducted for 25 months (June, 2013 – August 2015) on 473 patients who referred to ACECR Telemedicine Infertility Center for infertility problem. All subjects gave written informed consent. In this research, we excluded our samples with these criteria (had smoking, vasectomy, chemotherapy, radiotherapy, infertility). Our studied men were referred to infertility center for his wife fertility problem. Based on these exclusion criteria among the 473 men, we evaluated 273 of them.

### Semen collection and laboratory evaluation

The instructions, were to abstain for a minimum of 2 up to 7 days. The man masturbates and collects the ejaculated sample into a cup. Then, semen should be examined within a few hours, to achieve the most accurate results. The following parameters was evaluated:

#### Semen volume

The semen samples was evaluated by volume measurement of the seminal fluid by using a graduated measuring cylinder. 2 milliliters was a normal volume. A very low volume indicated that the seminal vesicles may not be making enough fluid or that these ducts may be blocked. It may also indicated a problem with the prostate gland.

#### PH measurement

In this regard, pH paper ranges from 0 to 14 was used (Merck, KGaA). Each types of pH paper be used for this analysis, its accuracy be checked against based on known standards before the use in routine semen analysis [9,10].

#### Sperm motility

A fixed volume of semen (not more than 10  $\mu$ l) was delivered onto a clean glass slide and covered with a 22 $\times$ 22 mm cover slip. The freshly made wet preparation was left to stabilize for one minute then examined with a microscope using 40 $\times$  magnification, then the percentage and grade of spermatozoa motility was recorded. Motility estimation can conveniently be carried out at a room temperature between 18 and 24 $^{\circ}$ C. At temperatures outside this range, some alteration in sperm motility will occur and this must be standardized in the laboratory. The microscopic field be scanned and the motility of each spermatozoa on encountered is graded a, b, c or d according to whether it shows [10]: (a) rapid progressive motility, (b) slow or sluggish progressive motility (c) non-progressive motility, (d) Immobility.

#### Sperm count

Sperm concentration of gently mixed samples was determined for three independent loadings on a Neuberhemocytometer.

### Sperm morphology

Determination of sperm morphology comprises the following steps (which are described in detail in subsequent sections). 1: Preparing a smear of semen on a slide. 2: Smears were made on the glass slides and the slides were fixed in 95% ethyl alcohol for about 30 minutes. Sperm morphology was classified according to the WHO criteria [10]. The air-dried smears, were fixed in ethanol and ethere, then stained by a modified Papanicolaou technique [10]. 3: Mounting the slide with a coverslip if the slide was to be kept for a long time. 4: Examining the slide with bright field optics at  $\times$ 1000 magnification with oil immersion. 5: Assessing approximately 200 spermatozoa per replicate for the percentage of normal forms and abnormal forms. 6: Comparing replicate values to see if they were close acceptably: if so, proceeding with calculations; if not, re-reading the slides. The slides were then examined microscopically for morphology. Normal results are when 4% or more of the sperm have normal shaped heads. Men with less than 4% of normal shaped sperm may have a significant infertility problem.

### Statistical analysis

Statistical evaluation was made with IBM SPSS Statistics version 22.0.0.0 64 bit edition. Data (presented as means $\pm$ SEM) were analyzed using repeated-measures Pearson Correlation, Kendall's tau, Regression, Bar chart, scatter/dot followed for significant differences. The significance level was fixed at 0.05. Spearman Correlations were calculated between parameters of semen.

### Results

The average age of participants in this research was 33.65 $\pm$ 1.78. Among the 273 individuals that we evaluated, 77.1% had no smoking history and 22.4% had smoking history. Evaluation of different sperm parameters showed, there was no significant difference in sperm concentration, motility, morphology and semen liquefaction between these groups ( $P > 0.05$ ). Other criteria which we assessed was varicocelesurgery history, 24.3% of men had surgery and 7.16% of them had no history of surgery. There was a significant decreasing in sperm concentration in men with varicocele surgery in compared to men with any surgery, 81 $\pm$ 1.97 versus 104 $\pm$ 1.85 ( $P < 0.05$ ). 35.7% of individuals had drug use and 63.8% of them had no history of drug using. We didn't observe significant difference in sperm parameters between these groups ( $P > 0.05$ ). In as much as smoking, surgery and drug use interfere in our work, we excluded 200 persons from the study and our investigation (the correlation between semen parameters and sperm motility) was conducted on 273 men. Results showed, the sperm count, morphology and semen volume had significant effects on motility ( $P < 0.000$ ) which it means, they cause an increase in sperm motility significantly ( $P$ -value for trend, 0.000) (Figures 1-3). Figure 4 illustrates that age of patients significantly affects motility ( $P < 0.001$ ) which an increase in age cause a significant decrease in sperm motility. Moreover, 273 men examined for the relationship between semen liquefaction time and sperm motility. There

was good evidence that confirm the correlation between sperm motility and liquefaction time which it means with liquefaction time increasing, motility of sperm significantly decreased ( $P < 0.001$ ) (Figure 5).

In other section of this research, we assessed the predictor importance of different semen parameters on sperm motility, in this regard our results showed, among the different parameters concentration had high predictor importance (0.43) and volume the lowest predictor importance (0.06) (Figure 6) (Tables 1,2).

### Discussion

Fertilization ability of human sperm has been shown to correlate intimately with sperm motility [11,12]. In this study, we have attempted to determine if there is any relationship between other semen parameters and motility. Our results

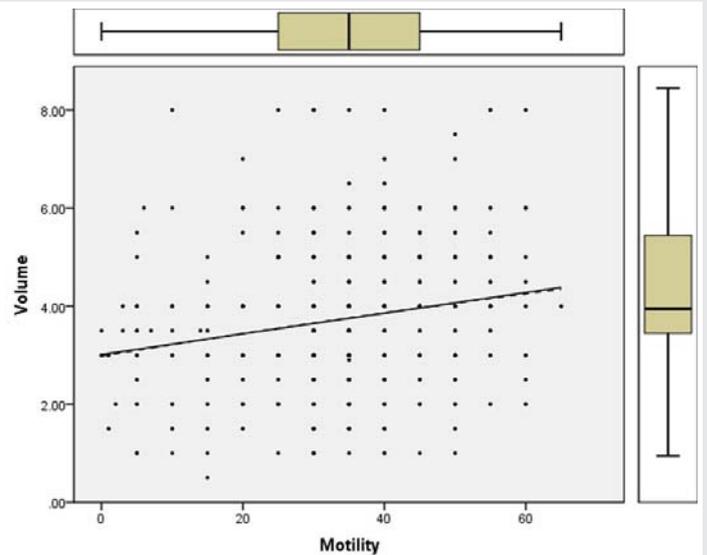


Figure 3: The influence of volume on sperm motility. Significant differences:  $P < 0.000$ .

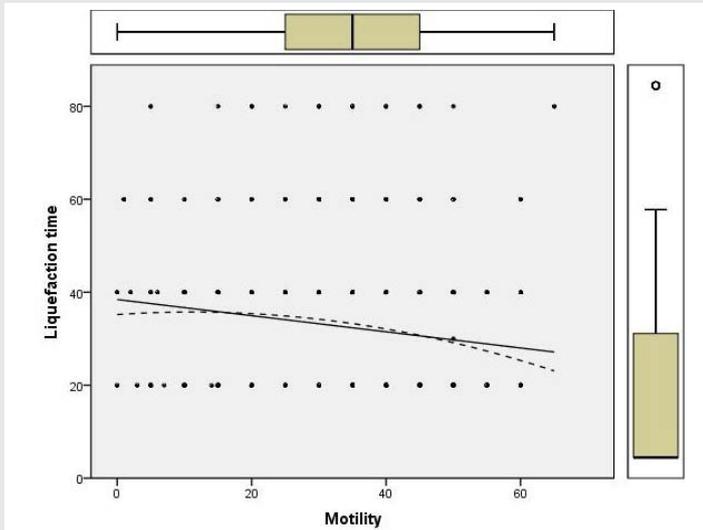


Figure 1: The influence of sperm count on sperm motility. Significant differences:  $P < 0.000$ .

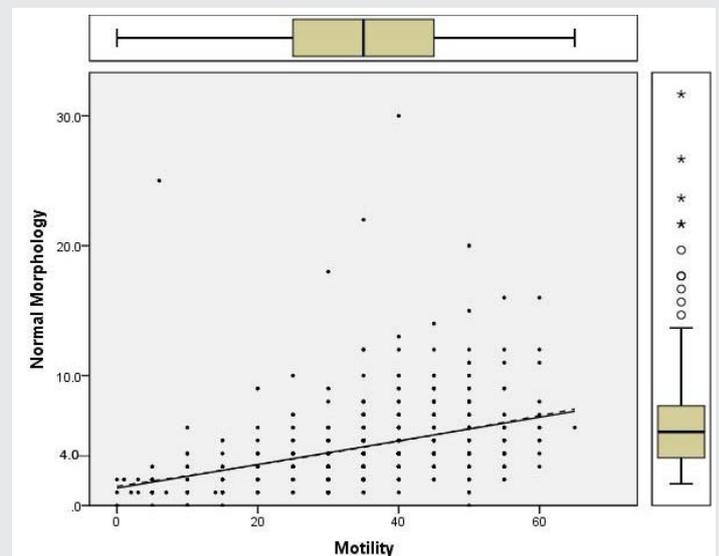


Figure 4: The influence of age on sperm motility. Significant differences:  $P < 0.001$ .

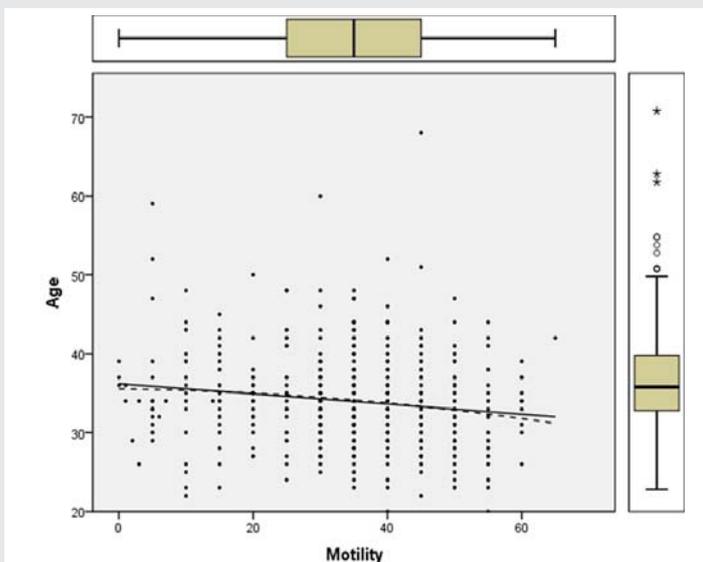


Figure 2: The influence of sperm normal morphology on sperm motility. Significant differences:  $P < 0.000$ .

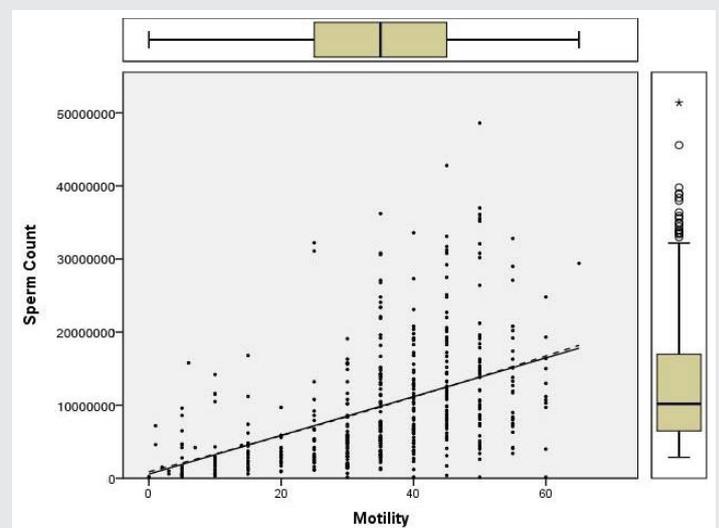


Figure 5: The influence of liquefaction time on sperm motility. Significant differences:  $P < 0.001$ .

showed the significant correlation between several semen parameters and sperm motility of men referred to Telemedicine Infertility Treatment Center, ACECR branch of Qazvin. This investigation demonstrated a direct correlation between sperm motility and concentration, morphology and volume whereas a reverse correlation was observed between sperm motility with age and liquefaction time.

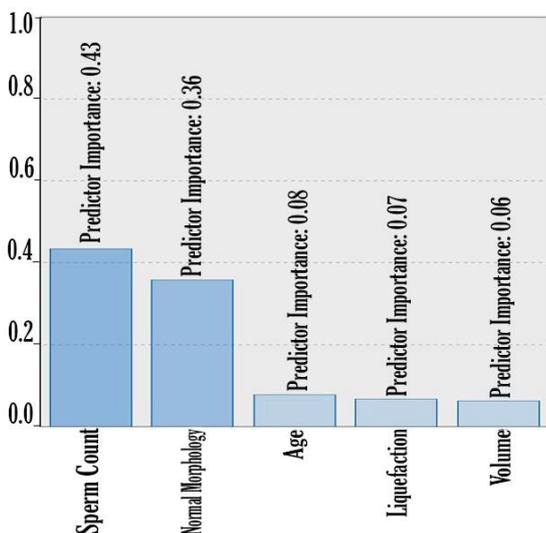
Sperm concentration and morphology significantly affect motility [13]. In according to our results, the most important semen parameter which had the largest effect on motility was concentration. In this line was shown, linear velocity was affected by the concentration of cells present in the counting chamber [14]. More significant linear velocity in semen were observed in samples with sperm concentration less than  $40 \times 10^6$  cells/ml [14]. Moreover, in Gundogana *et al.*, 2010 study, it was observed that concentration profoundly had effects on some sperm characteristic such as motility and morphology

[13]. Sing *et al.*, 2010 showed that sperm motility has progressive linear correlation with sperm concentration. They have been done a cross sectional study of sperm motility index (SMI) in two-hundred subject consists of normozoospermic and oligoasthenoteratozoospermic males which showed that a positive correlation between SMI and sperm concentration [15].

Motility, was found to be affected by sperm morphology which an increase in normal morphology result in an increment in motility. Although sperm tail morphology is the most important factor in motility but head morphology is also important for motility [14]. This fact was supported in one study that the cervical mucus chooses the spermatozoa based on their morphology: spermatozoa with head abnormalities or abnormalities in the principal or middle pieces of the tail, are stopped by the mucus [16].

The results of our study demonstrated that an increase in age cause a significant decrease in sperm motility. In parallel, this fact has been demonstrated by some other studies [17-19]. This decrease may be partly related to the ageing of the male reproductive tract and sexual dysfunctions [20]. It has been reported that testosterone is required for the production of sperm with normal mobility and preserve activity of testis and sex glands [21]. The progressive decrease in testosterone levels along ageing in men is undeniable but this decline shows a large variations between men, with possible related factors, such as obesity, drugs, alcohol, genetic factors and chronic diseases [20]. Studies of sperm morphology and motility revealed that there is a reduction in sperm motility in older men whereas the percent of abnormal shape sperm is increased [21]. Recently, it was described that prostate secretions -specially secretions of the prostate dorsal lobe - are essential for progressive sperm motility in uterus and it is also known that old men show prostate alterations too [21].

In the present study we showed that high semen volume significantly causes an increase in sperm motility. The probable reason can be the protective effects of seminal plasma and its contents of spermatozoa. It is recommended that the



**Figure 6:** The predictor importance of semen parameters on sperm motility. This chart shows the effect of semen parameters on sperm motility. Concentration with the predictor importance of 0.43 and volume with the predictor importance of 0.06 had the most and the least effect on motility, respectively.

**Table 1:** Age and semen parameters of the studied samples by selected characteristics.

Variable	Number	Age	volume	Concentration	Normal morph (%)	Motility	Liquefaction time
				( $10^6$ /ml) (Mean)		(%)	(Minute)
<b>Abstain</b>							
<5 days =	311(65.7%)	33.54±1.2	3±0.48	99±3.1	4±0.01	34±2.3	33±2.1
Days5>	162(34.2%)	34.73±1.1	4±0.49	97±2.95	4±0.01	34±2.1	30±1.9
<b>Tobacco use</b>							
Never	365 (77.1%)	33±1.45	3±0.23	99±2.98	4±0.012	35±1.98	31±2.4
Ever	106(22.4%)	34±1.7	3±0.22	94±2.65	4±0.011	34±1.4	33±2.21
<b>Surgery Varicocele</b>							
Yes	115(24.3%)	34±1.85	3±0.32	81±1.97*	4±0.014	30±2.1	33±1.25
No	356(77.16%)	33±1.65	3±0.31	104±1.85	4±0.013	36±2.4	31±1.44
<b>Drug use</b>							
Yes	169(35.7%)	34±2.21	3±0.21	93±1.89	3±0.012	31±1.2	33±2.1
no	302(63.8%)	33±2.12	3±0.32	101±1.95	4±0.014	36±1.1	31±2.2

\*Shows significant difference in sperm concentration between men with and without surgery. Regard the other criteria we did not see significant difference in semen parameters.

**Table 2:** Semen parameters of the studied samples based on different ranges of motility.

Motility (%)	Number	Age (Mean)	Concentration (10 <sup>6</sup> /ml) (Mean)	Normal Morph (%)	Liquefaction Time (Minute)	Volume (Mean)
≥40	224	33±1.25	132±1.95*	5±0.049	29±1.1	4±0.055
<40	247	34±1.2	67±1.85	3±0.045	34±1.2	3±0.045

Shows significant difference in sperm concentration between men with sperm motility ≥ and < 40%.

seminal plasma which is covering each spermatozoon protect them from premature aging and improve other criteria such as motility. It means, the higher volume of seminal plasma and its components may be one of the reasons for better conservation of functional and structural parameters in semen fluid [22]. The change in semen volume is possibly is affected by the dietary habit [23]. Some other studies show a positive correlation between seminal volume and the length of short-term abstinence. In one hand, there is an association between serum testosterone level and seminal fructose concentration and in the other hand, testosterone decreased within a decline in Zinc level. Moreover, one of the first evidence of zinc depletion may be reduced seminal volume, induced by an alteration in Leydig cell output [23]. So, it is suggested that the higher volume of seminal plasma may be one of the reasons for better motility of sperm which it can prevent spermatozoa premature aging during storage. The possible physiological reasons may be the effects of seminal plasma volume and its components on suppressing of endogenous free radical production [23].

This study showed that by liquefaction time increasing, motility of sperm significantly decreased. There are some studies which have shown a relationship between the coagulation-liquefaction property of human ejaculates and their semen quality [24]. Chomsrimek *et al.* 2008, reported that sperm motility reduced when the time between semen ejaculation and semen analysis increased. This reduction has been seen profoundly at 60 minutes after ejaculation [25].

## Conclusion

This study demonstrated that sperm motility also appeared to be affected by other semen parameters. In according to our results, there is a direct correlation between sperm motility with concentration, morphology and volume whereas a reverse correlation was observed between sperm motility compare to age and liquefaction time. Since Sperm motility is a fundamental factor in the fertilization, according to factors effect to this parameter is important clinically, specifically in IVF procedures.

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