

# Characteristics of Semen Parameters among Iraqi Men at different ages presenting to ICSI Procedure

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## Abstract

**Purpose:** To evaluate a relationship between age and reduction in semen parameter used for Intra cytoplasmic sperm injection (ICSI) in Iraqi males population.

**Method:** Eighty four infertile men enrolled in this study; all of them were presented to the Reproduction and Infertility Unit in Kamal AL-Samarrai hospital / Baghdad from September 2020 through April 2021. In this study, all patient charts were reviewed for age, infertility etiology, smoking and semen characteristics. At day of ICSI, semen specimen was taken and analyzed. Semen analysis parameters were compared between different age groups (n=19, 25-31 yr), (n= 40, 32-40yr), (n= 18, 41-50yr) and (n= 7, 51-60yr) years using least significant difference –LSD and Pearson’s correlation coefficient (r) between parameters.

**Results:** Our patient had seminal volume within normal range and we notice a noticeable decrease with age but there was no significant differences amongst different ages group (P>0.05). PH were significantly increase in 1st group of age (P<0.05) in comparison with 3rd group (7.63 ±0.05 vs 7.48 ±0.03 respectively). Asthenozoospermia (having an overall reduced sperm motility below the 40%) was shown in all men at different ages, in addition, we noted the non-progressive motility of sperm (Grade C) in group of age 41-50 years were significantly lower differences in comparison to the group of age 25-31 years (12.50 ±2.46 vs. 20.13 ±2.64 respectively, P<0.05). Immotile of sperm (Grade D) were increases in all groups of ages. High percentage of Teratozoospermia (disrupted morphology more than 70%) was showed in all groups but without significant. Although there is no statically significant in sperm concentration between four groups of ages but we noted that sperm concentration was positively correlated with PH, sperm motility grade A, grade B and grade C, (It was negatively associated with sperm motility grade D) and sperm abnormality.

**Conclusion:** Our observations emphasize the men with these finding on semen analysis alone will predict to be infertile. Such data will be useful in helping physicians counsel patients with adequate percentage motility (>40%) and normal morphology, to proceed with ICSI as the initial assisted reproductive procedure to enhance the likelihood of good outcomes.

**Keywords:** Infertility, Male factor, Semen analysis, Paternal age.

## INTRODUCTION

Lack of conception following one year of continuous unprotected sexual contact, is the clinical definition of "infertility". Worldwide, the prevalence of infertility is between 10 and 15 percent, and it has increased in recent years. (1). Male factors are involved in about 50% of infertility cases, with 20% of those being pure "male factor" infertility cases, and around 30% of cases of infertility caused by both the female and male factors. (2). Nowadays, "traditional semen analysis" is the principal method used to evaluate and diagnose male infertility, which looks at the volume, concentration, vitality, and morphology of the sperm. (3). When sperm parameters fell below the World Health Organization's (WHO) recommended level, males were considered infertile. (4). The most prominent of these are impaired sperm motility (astheno-zoospermia), low sperm concentration (oligo-zoospermia), and poor sperm morphology (teratozoospermia). Disturbance in control mechanisms involving "Pre-testicular, testicular, and posttesticular causes" which affects sperm count, motility, and morphology (5). As it's known, compared to most other bodily fluids, semen has a much higher buffering capacity. As a result, the pH of the seminal fluid may be crucial in maintaining the viability and quality of the sperm as well as for achieving fertilization (6). According to the WHO, a healthy semen must have a pH between 7.2 and 8.0. (4). In patients with oligospermia or asthenospermia, the pH of the semen has been found to be lower than 7.2. (7). Semen volume and other seminal markers of epididymal, prostatic, and seminal vesicle activity are some additional parameters that are less clearly linked to infertility.

(8). To be infertile, however, is a diagnosis given to about 15% of males with a normal semen analysis. (9). According to several studies, semen analysis parameters decreased globally as paternal age increased. (10) Over the past several decades (11). As said by certain research, paternal ages greater than (40, 50 and 60 years) (12, 13, 14) are associated with decreased pregnancy rates and a higher likelihood of miscarriages (15). Begueria et al., (16) were been revealed that sperm volume decline, concentration rises and motility reduces with every five years of male age. So, based on the available literature, “the semen analysis is the gold standard for assessing male factor infertility” and based on the results semen analysis, infertile, indeterminate, or fertile patients can be classified (17). We identified the remaining gap in our knowledge of a potential association between the basic semen characteristics altered with paternal age.

## Materials and methods Patient Evaluation

Eighty-four men enrolled in this study; they were presented at the Kamal Al- Samarra hospital in Baghdad / Reproduction and Infertility Unit, from September 2020 through April 2021; an informed consent form was signed by each patient. All data were assured for privacy and confidentiality. All 84 people who met the inclusion requirements were divided into 4 groups based on their ages for analysis: 1st group are presented within age (n=19, 25-31) years, whereas the 2nd group were with age (n=40, 32-40 years) and 3rd, 4th groups are presented (n =18, 41-50; n=7, 51-60 years respectively). Full history was taking: primary or secondary infertility with their present partner, duration of marriage, drugs, surgical history, and smoking. As part of the protocol before the ICSI procedure, these males were denoted for semen analysis in the lab.

### Semen collection and evaluation

Semen analysis was performed using standard laboratory manual procedures according to WHO criteria (Fifth Edition) (4). Specimens were collected into a sterile container by masturbation after 3 to 4 days of sexual abstinence, allowed to liquefy at 37 C° and evaluated within 1 hour of collection for detection the following parameter : using a graduated disposable pipette, appearance volume was determined , A pH paper was used to estimate pH, by utilizing a microscope, a cover slip was put to distribute a tiny drop on a glass slide to assessed motility , morphology and concentration .Parameters were considered normal when semen volume > 1.5 mL, PH 7.2-7.8, sperm concentration > 15 million/mL, progressive motility > 40% and normal morphology > 4%.

### Statistical analysis

To determine how age groups affected study parameters, the Statistical Analysis System SAS tool was applied. (18). Significant comparisons between means were made using the Least Significant Difference-LSD test (Analysis of Variation - ANOVA).The correlations between parameters were evaluated by calculating Pearson’s correlation coefficient (r). Significance of differences estimated at two-tail P level <0.05, <0.01.

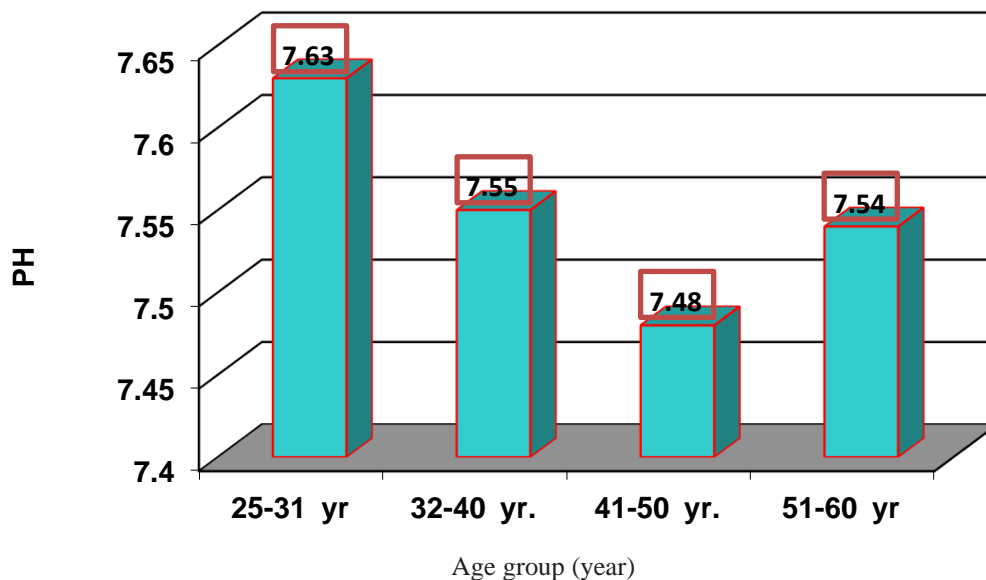
## Results Semen analysis parameters outcomes

Eighty-four males were willing to give semen sample to analysis. Although the distribution of seminal volume (ml) values decrease with advanced ages as shown in Table -1 but the results show non -significant differences (P>0.05) among different age groups (3.16 ±0.26; 3.00 ±0.23; 2.58 ±0.33 and 2.40 ±0.36 ml) respectively. The PH in 1st group (25-31) years were (7.63 ±0.05) shows significant increase differences (P<0.05) in comparison with 3rd group (7.48 ±0.03) at age 41-50 years. While group 2 (32-40 yr) shows non- significant differences (7.55 ±0.02) when compared with fourth group (51-60 yr) (7.54 ±0.02) .The second group (7.55 ±0.02) also shows non -significant variation with 1st group. The seminal volume and PH between different male ages are delineated in (Table 1) and (Fig 1).

**Table 1:** Comparison between difference Age groups in Seminal Volume and PH

Age group (year)	Mean ± SE	
	Seminal Volume(ml)	PH
25-31 (n=19)	3.16 ±0.26	7.63 ±0.05 a
32-40 (n=40)	3.00 ±0.23	7.55 ±0.02 ab
41-50 (n=18)	2.58 ±0.33	7.48 ±0.03 b
51-60 (n=7)	2.40 ±0.36	7.54 ±0.02 ab
LSD value	1.052 NS	0.119 *
P-value	0.461	0.050

Means sharing a common letter don not differ significantly. Others differ significantly \* (P≤0.05).



**Figure 1:** Comparison between difference Age groups in PH.

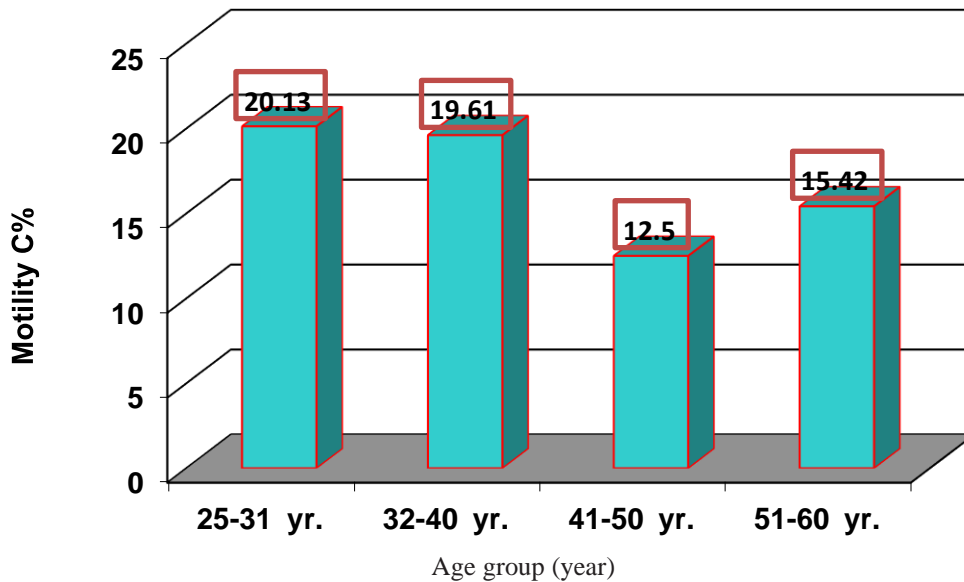
Interesting, all men in this study regardless of age were asthenozoospermia (having overall reduced sperm motility below the 40%). In the same regard the rustles demonstrated there was no significant differences between four different groups of age in Rapid linear progressive (Grad A) ( $8.00 \pm 2.61$ ;  $8.41 \pm 1.44$ ;  $6.38 \pm 2.90$  and  $6.42 \pm 4.46$ ), progressive movement (Grade B) ( $17.46 \pm 3.29$ ;  $20.81 \pm 1.83$ ;  $14.55 \pm 3.27$  and  $14.55 \pm 3.27$ ). Furthermore, the non-progressive motility of sperm (Grade C) in group of age

41-50 years were significantly lower differences versus the group of age 25-31 years ( $12.50 \pm 2.46$  vs.  $20.13 \pm 2.64$  respectively,  $P < 0.05$ ). While between 2nd and 4th groups were also affected but the differences were not statistically significant ( $19.61 \pm 1.45$  vs.  $15.42 \pm 3.83$  respectively). In contrast, we noticed an increase about immotile of sperm

(Grade D) in group of (41-40 and 51-60 years,  $66.56 \pm 7.01$ ;  $66.56 \pm 7.01$ ) than at age (25-31 and 32-40 years,  $54.73 \pm 6.43$ ;  $50.02 \pm 3.65$ ) but without significant differences, data are depicted in (Table 2) and (Fig. 2).

**Table 2:** Comparison between difference Age groups in Motility of sperm

Age group (year)	Mean $\pm$ SE			
	Grad A%	Grad B%	Grad C%	Grad D%
25-31 yr.	$8.00 \pm 2.61$	$17.46 \pm 3.29$	$20.13 \pm 2.64$ a	$54.73 \pm 6.43$
32-40 yr.	$8.41 \pm 1.44$	$20.81 \pm 1.83$	$19.61 \pm 1.45$ ab	$50.02 \pm 3.65$
41-50 yr.	$6.38 \pm 2.90$	$14.55 \pm 3.27$	$12.50 \pm 2.46$ b	$66.56 \pm 7.01$
51-60 yr	$6.42 \pm 4.46$	$14.57 \pm 5.27$	$15.42 \pm 3.83$ ab	$63.57 \pm 10.84$
LSD value	7.919 NS	9.670 NS	7.506 *	19.60 NS
P-value	0.897	0.281	0.0498	0.125
Means sharing a common letter don not differ significantly. Others differ significantly * ( $P \leq 0.05$ ).				



**Figure 2:** Comparison between difference Age groups in Motility C.

Additionally, the results of the study concerning sperm concentration reveal non – significant differences between four groups and were within normal range as showed in Table3(32.60 ±7.62; 39.75 ±3.69; 37.11 ±7.39 and 27.42 ±11.61 million /ml respectively, P>0.05). Abnormal morphology of sperm (Teratozoospermia) were recorded high percentage in all groups, but show non- significant differences (83.80 ±3.02; 78.43 ±2.44; 83.44 ±4.23 and 83.85 ±5.98 % respectively, P>0.05).

**Table 3:** Comparison between difference Age groups in Sperm concentration and Abnormality

Age group (year)	Mean ± SE	
	Sperm con. x 10 <sup>6</sup> / ml	Abnormality%
25-31 yr.	32.60 ±7.62	83.80 ±3.02
32-40 yr.	39.75 ±3.69	78.43 ±2.44
41-50 yr.	37.11 ±7.39	83.44 ±4.23
51-60yr.	27.42 ±11.61	83.85 ±5.98
LSD value	20.762 NS	12.003 NS
P-value	0.645	0.523
NS: Non-Significant.		

Lastly, by using Pearson’s correlation coefficient of studied parameters with seminal volume and sperm concentration were investigated in these 84 semen samples. Actually, there was non- significant correlation between seminal volume and studied parameter including PH, the motility of sperm grad A , grad B, grad C, grad D, sperm abnormality and concentration. Indeed, it was noted a strong positive relationship between sperm concentration with PH, sperm motility grad A, grade B and grade C (correlation coefficients r were 0.24, 0.66, 0.61and 0.25 respectively). Interestingly, it was noted that elevated significantly sperm motility grad D and sperm abnormality when sperm concentration decreased (r values were - 0.67 and - 0.41 respectively all P<0.01) as shown in Table 4.

**Table 4:** Correlation coefficient between parameters study

Parameters	Correlation coefficient-r	
	Seminal Volume.	Sperm con.
PH	0.06 NS	0.24 *

Motility A%	-0.04 NS	0.66 **
Motility B%	-0.02 NS	0.61 **
Motility C%	0.15NS	0.25 *
Motility D%	-0.03 NS	-0.67 **
Abnormal	-0.02 NS	-0.41 **
Seminal Vol.	---	-0.07 NS
Sperm con.	-0.07 NS	---
* (P≤0.05), ** (P≤0.01).		

## Discussion

Infertility is a widespread health issue and (continues to be a growing problem as more men and women delay reproduction until later in life). According to Chandra et al., (19), up to 15% of couple's difficulty to maintaining or getting pregnant. Among those having reproductive problems, thirty to fifty percent of couples undergoing infertility have some male factor involved, and as many as ten to twenty percent have male factor as the only "detectable cause" (20). According to our opinion, semen analysis alone will constitute suitable documents for evaluation of "male fertility".

The possible impact of decreasing semen parameters at various age groups is being examined for the first time in this study among infertile Iraqi men donating ICSI procedure. Most studies on male aging suggest that semen parameters, such as seminal volume, sperm concentration, and motility, tend to decline with age (21). Therefore, patient in the current study according to their ages, divided into 4 groups; (19 patients with age 25-31years, 40 patients between the ages of (32-40) years, (18 patients at age 41-50) years and (7 patients were 51-60 years). Majority of our patient had seminal volume within normal rang but we observed a noticeable decrease with age but without statically significant, also we detected there was no correlation between overall seminal volume and studied parameter. Our results agreement with those of a study carried out in Punjab, where the majority of patients (74.24%), the semen volume were sufficient (22). According to our thinking, the length of sexual abstinence prior to the collection of seminal fluid samples is crucial, and seminal volume has minimal effect on the cause of male infertility.

All semen samples in the present study had a PH falling within the supposedly normal range but results revealed that the mean ( $\pm$ SD) in groups at age 25-31 years significantly increased in comparison in groups at age 41-50 years ( $7.63 \pm 0.05$  vs.  $7.48 \pm 0.03$ ). Our finding was disagree with the result of Haugen & Grotmol (23) was noted that the semen pH in their population was consistently higher than the WHO reference values (pH to be 8.2). Basic semen analysis includes determining the pH of the ejaculate. Much more so than the majority of other bodily fluids, semen pH have a very high buffering capacity and it's a matter of discuss. The pH of seminal plasma should be between (7.2 and 7.8). (4). Since seminal vesicles account for over 60% of the pH of the ejaculate, an acidic ejaculate with a pH below 7.2 may be a sign that the seminal vesicles are blocked, on the other hand, the prostate contributes with about 30%, that mean prostatic infections are typically linked to a pH that is alkaline, around 8 (4). Therefore, seminal plasma's numerous components, particularly ions, are required in order to maintain the buffering system with a normal pH range, which is necessary for other semen parameters like (motility and count). In light of our results, must be extending sample size especially older patient because with advancing age the fluid secretion from prostate and seminal vesicles may become more basic.

Interestingly, all men in our study were asthenozoospermia (reduced sperm motility below the 40%), despite the results, appeared no significant differences regarding the (rapid linear progressive Grad A) and (progressive movement Grad B) of sperm, but significant decreased differences were observed in non-progressive motility of sperm (Grade C) in group at ages 41-50 years in comparison at age 25-31 years. In contrast, we observed an increase in immotile of sperm (Grade D) at age (41-40 and 51-60 years) than at age (25-31, 32-40 years) but without statically significant. Disagreement with current results, Walter et al (24) found association between decreasing semen parameters with advancing age. Sperm motility is important to travel a long distance to meet oocytes and begin fertilization. High sperm motility results from sperm maturation as they pass through the epididymis, which is influenced by epididymal proteins. Therefore, motility is a sign of post-testicular epididymal activity. (25). Consequently, Nadeem et al (26) established effect of cigarette with reduced testosterone levels on fertility, therefore decreased sperm count, motility, and semen quality. Our results established that smoking were 43 (51%) among all patients at different ages. However, coexistence of a smoking with another factor that adversely effects on semen and may be enough to effect of conception.

Concerning sperm concentration, our study demonstrated no- significant differences was observed at different male ages groups. Our result confirmed with studies carried out over an 11-year period at the National Institute of Health and Family Welfare in New Delhi to prove reports and speculating of a decline in male sperm counts. Throughout the whole study period, no year showed a noticeably decreased sperm count. (27). Our study reached a positive correlation between sperm concentration with “PH” ( $r = 0,024$ ;  $P = < 0.05$ ), “sperm motility” grade A, B, C ( $r = 0.66$   $P < 0.05$ ;  $r = 0.61$   $P < 0.05$  and  $r = 0.25$   $P < 0.01$  respectively), however it a strong negative correlation were found to be correlated with sperm motility grade D and sperm abnormality ( $r = - 0.67$  and  $r = - 0.41$  respectively all  $P < 0.01$ ).

At any time, there are two main aspects that determine a count of men sperm. His testicles have the specified number of Sertoli cells; and the period since the previous ejaculation (abstinence). Both obviously have significant effects on sperm count, but the main distinction is that abstinence is changeable whereas the number of Sertoli cells is established early in development (28). After adolescence, sperm production in the testes occurs continually; this is referred to as the "supply side" because it takes a sperm about 10 weeks to production. Rate in which these sperm are used up were determines through the frequency of ejaculation, and the amount of sperm in men's ejaculates at any given time is determined by the equilibrium between supply and demand. Studies have been suggested more than 40 million / ml a semen should ideally contain, in order to be considered within normal (29), this means if the concentration is less than 15 million / ml , the fertility may be reduced. In contrast, previous studies showed that pregnancy rates increase significantly as the total motile sperm count increases (TMSC) when greater than 5 million (30), whereas, other studies showed 8 million (31), Brash et al. also determined that the relationship became statistically significant only when the TMSC exceeded 20 million sperm (32). The variations between these studies may be explained to cause for infertility in the infertile couples analyzed, or the version of the WHO criteria that was utilized and prospective research designs.

Sperm morphology is another important, despite of we didn't identify the form of aberrant morphology such as two heads or two tails, which tests and the epididymis are responsible for. Along with our study, mean abnormal morphology (more than 70%) in all groups of age recorded high percentage but without significant differences (83.80, 78.43, 83.44 and 83.85 %) respectively. This contrasted with a study that found that aberrant morphology affected 53% of oligospermic males and abnormal motility affected 60% of them. Moreover , a study conducted between 1988 and 2007 in University Hospital of Marseille (France), which involved 10,932 semen specimen of male infertile couples established that the entire population showed declining in sperm concentration , total sperm count , rapid motility, and normal morphology (1.5 , 1.6, 0.4, 5.5 % per year , respectively) (33). Therefore, (sperm motility and morphology) have variable parameters throught time, and its relative levels rely on the person's current sperm count. Weaknesses and limitations in our investigation, however, were also present; from side we have been unable to an assessment of ICSI results that encompass (fertilization rate, blastocyst formation, implantation rate, and success of pregnancy) from these semen parameters analysis. Our study is also exclusive in being the first to study the effect of paternal age on seminal parameter characteristics in Iraqi men. An opportunity for future, more studies are needed to update the literature specifically expanding the sample size in older cohort of men to reflect the situation in Iraq.

## Conclusion

Our observations emphasize the semen analysis alone will predict male fertility .Such data will be useful in helping physicians counsel patients with adequate percentage motility (>40%) and normal morphology, to proceed with ICSI as the initial assisted reproductive procedure to increase the chance of good results. Prior to admission to ICSI, this requires adequate management of male partners and the eradication of any curable risk factors that could affect these parameters.

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