Original Article

Effect of age on semen parameters

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Abstract

Aim: This study was designed to evaluate the effect of age on various semen parameters. **Settings:** Womens Center and Hospitals Pvt. Limited, Coimbatore. **Design:** Observational cross-sectional study was conducted from October 2020 to March 2021. **Study subjects:** Male partners of 366 infertile couples attending the infertility clinic at Womens Center, Coimbatore. **Materials and methods:** Semen samples collected by masturbation were evaluated for parameters such as volume, sperm concentration, motility, and morphology as per the standard World Health Organization 2010 (5th edition) guidelines. **Outcome measures:** Comparison of volume, concentration, normal morphology, and motility in different age groups: <30, 30–35, 36–40, and >40 years. **Results:** No significant association of volume, concentration, and normal morphology with age was observed. However, there was a significant decline in motility with age. **Conclusion:** In the present study, motility was the only semen parameter that had a significant negative association, whereas all the other parameters did not exhibit any change with advancing age.

Keywords: motility, semen analysis, and semen parameters

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INTRODUCTION

Male factors alone or in combination with female factors contribute to 50% of the total infertility cases. The female biological clock has been well established as having a negative effect on fertility. However, the risks of abnormal pregnancies and heritable effects associated with advancing paternal age are poorly understood.

It has been observed in recent years that there has been a shift in the average paternal age of the first child for a variety of reasons. As couples delay childbearing, it is becoming increasingly important to determine whether the advanced paternal age of infertile couples is associated with diminished semen quality.

Semen analysis is a primary step in the laboratory evaluation of the infertile male. It includes the assessment of the semen volume, sperm concentration, motility, and morphology using World Health Organization (WHO) criteria.

Some studies have shown that with increasing paternal age, semen volume, sperm motility, and the percentage of normal morphology tend to decrease.[1,2] However, other studies had conflicting conclusions and there is no consensus among various studies.

Thus, this study is planned to evaluate various semen parameters in different age groups of men attending an infertility clinic.

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MATERIALS AND METHODS

This observational cross-sectional study was conducted from October 2020 to March 2021.

Sample size

Sample size of 468 was calculated based on the study of Kumar *et al.*[3] In their study carried out in central India, they evaluated the semen analysis of 1219 participants categorizing them into five subgroups (group 1: 21–28 years; group 2: 29–35 years; group 3: 36–42 years; group 4: 43–49 year, and group 5: 50–60 years) . Pearson's correlation was applied to find out significant changes between age and semen parameters.

Even though the calculated sample size was 468, due to the coronavirus disease 2019 pandemic situation, the patient load was not up to the expected level. Hence, only 366 male partners of infertile couples were recruited into the study.

Study subjects

Male partners of infertile couples attending the infertility clinic at womens center, Coimbatore. Written informed consent was taken from the participants before recruiting them in the study and collecting their clinical specimens. A detailed history including information on lifestyle habits and occupation besides medical and surgical history was documented as per the proforma. Results of female partner evaluation were also noted from the couple's file.

All participants were assessed for their serology status for viral/bacterial infections including human immunodeficiency virus (HIV)-1 and HIV-2, hepatitis B surface antigen, hepatitis C virus, and venereal disease research laboratory test before semen analysis.

Inclusion criteria

All male partners of couples who presented for infertility evaluation were included in the study.

Exclusion criteria

- Azoospermia
- Men with ejaculatory and erectile dysfunction
- Recent febrile illness (in the preceding 3 months)
- Chemotherapy, radiation therapy, any reproductive surgery in the last 6 months
- History of hormonal therapy and antioxidant therapy in the last 6 months

Semen collection

Subjects were instructed to report to the center for semen analysis after 2 to 5 days of abstinence. Semen was collected by them through masturbation into a clean sterile, wide-mouth sample collection container. In case of loss of part or complete sample because of spillage or other reasons, patients were instructed to report and come for a repeat sample. If any participant failed to provide a sample in the clinic, he was offered to bring the sample from home provided the sample could reach the laboratory within half an hour of collection time.

Semen analysis

The samples were kept at room temperature for about 20 to 30 minutes for liquefaction, after which semen analysis was performed according to the standard WHO 2010 (5th edition) guidelines.[4] After liquefaction, the viscosity of the sample was estimated by gently aspirating it into a Falcon pipette, allowing the semen to drop by gravity and observing the length of thread.

The general appearance of the sample was also noted. Semen volume was estimated by Falcon graduated pipette. The pH value was measured using pH paper and compared with a calibration strip.

To determine the concentration of sperm, 10 µl of thoroughly mixed semen was loaded on a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) under a light microscope at a magnification of 10×. The number of sperm cells in 100 squares was counted which gives the concentration of sperm in millions/ml. The motility parameter was graded according to the WHO classification (5th edition) into forward progressive motility, nonprogressive motility, and immotility. If round cells >1 million/ml noted, LeucoScreen test was performed using the LeucoScreen test kit.

Sperm morphology

Smears were prepared for sperm morphology and vitality assessment. Following fixation (air-dried) using an Eosin & Nigrosin staining kit (Krishco Medical Products, India), the slide was examined with bright field optics at 100× magnification with oil immersion, sperm morphology was assessed according to Kruger strict criteria.[5] At least 100 spermatozoa were counted from each sample. The results were expressed as the percentage of live sperms, dead sperms, normal spermatozoa, head defects, midpiece defects, and tail defects. The patients were divided into four groups based on their ages: <30, 30 to 35, 36 to 40, and >40 years.

Statistical analysis

The data were entered in Microsoft excel and were analyzed using SPSS software version 23. Categorical variables were presented as frequency and percentages, and continuous variables were presented as mean ± standard deviation. Independent sample *T* test was used to measure the association between the semen analysis parameters and different age groups. Bivariate analysis using Chi-squared/Fischer exact test was applied between categorical variables and outcome. $P < 0.05$ was considered as statistically significant.

RESULTS

Our study subjects were between the age of 26 and 48 years with a mean age of 32.69 ± 4.29. Majority (77%) were below the age of 35 years and only 6% were above 40 years of age [Table 1].

Distribution of study population according to semen parameters is represented in Figure 1. The semen volume in the samples ranged from 0.2 to 6 ml, and in nearly 30% samples, the volume was <1.5 ml. On the other hand, the sperm concentration in the samples ranged from 0.5 to 145 million/ml and 18% of subjects showed concentration of less than 15 million/ml. The percentage of samples showing normal morphology (>4%) was only18%. Sperm motility in the samples ranged from 0% to 95% with 7.7% of samples showing motility less than normal value of 40%.

Table 1: Distribution of study population according to age

Age group (years)	Number	Percentage (%)
<30	130	35.5
31–35	152	41.5
36–40	62	16.9
>40	22	6.1

As shown in Table 2, when comparing different age groups, the semen volume, concentration, and morphology were comparable and there was no statistically significant difference of these parameters with age. In contrast to the other parameters measured, sperm motility showed significant decline with age. The mean sperm motility in the study population was 60.99% with <30 years age group, showing the highest mean value of 62.8% and the 31 to 35 years age group not far behind with a mean value of 62.32%. The age group 36 to 40 years exhibited the lowest mean value of 54.49%.

As shown in Figure 2, semen volume, sperm concentration, and normal morphology have no significant association with age. However, as age increases, sperm motility decreases and the difference is statistically significant.

DISCUSSION

The outcome of our study reveals that age does not have a significant effect on semen volume, concentration, and normal morphology. However, age has a significant negative effect on motility.

Aging is a natural and irreversible process affecting all body parts including the reproductive system. Although the effect of maternal ageing on a couple's fertility and reproduction is well documented, the role of paternal age on the same is not studied enough. There are many studies suggesting that women over the age of 35 years have a higher risk of infertility, pregnancy complications, spontaneous abortion, congenital anomalies, and

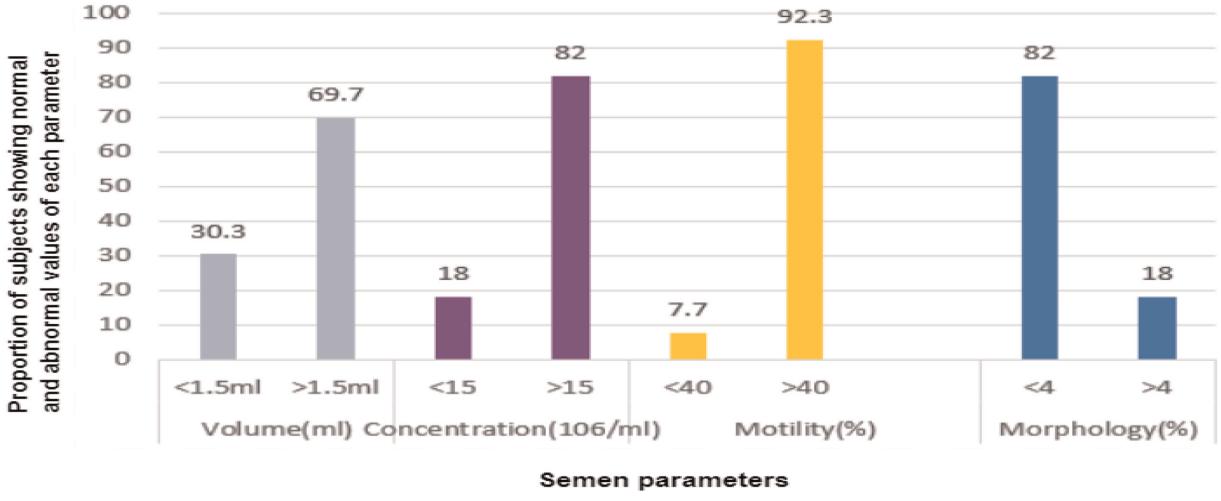

Figure 1: Distribution of normal and abnormal semen parameters in the study population.

Table 2: The mean ± standard deviation (SD) values of all semen parameters in different age groups

Parameters	*N*	<30 years	31–35 years	36–40 years	>40 years	Chi-squared value	*P*-value
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Volume	130	2.44 ± 1.33	2.291 ± 1.18	2.268 ± 1.14	2.31 ± 1.20	3.432	0.330
Sperm concentration	130	57.33 ± 37.00	61.32 ± 36.40	50.97 ± 36.29	52.70 ± 35.68	1.088	0.780
Motility	130	62.8 ± 13.01	62.32 ± 14.33	54.49 ± 16.96	58.03 ± 19.76	19.437	<0.001
Normal morphology	118	1.99 ± 1.66	2.04 ± 1.83	2.00 ± 1.56	1.92 ± 2.04	1.582	0.664

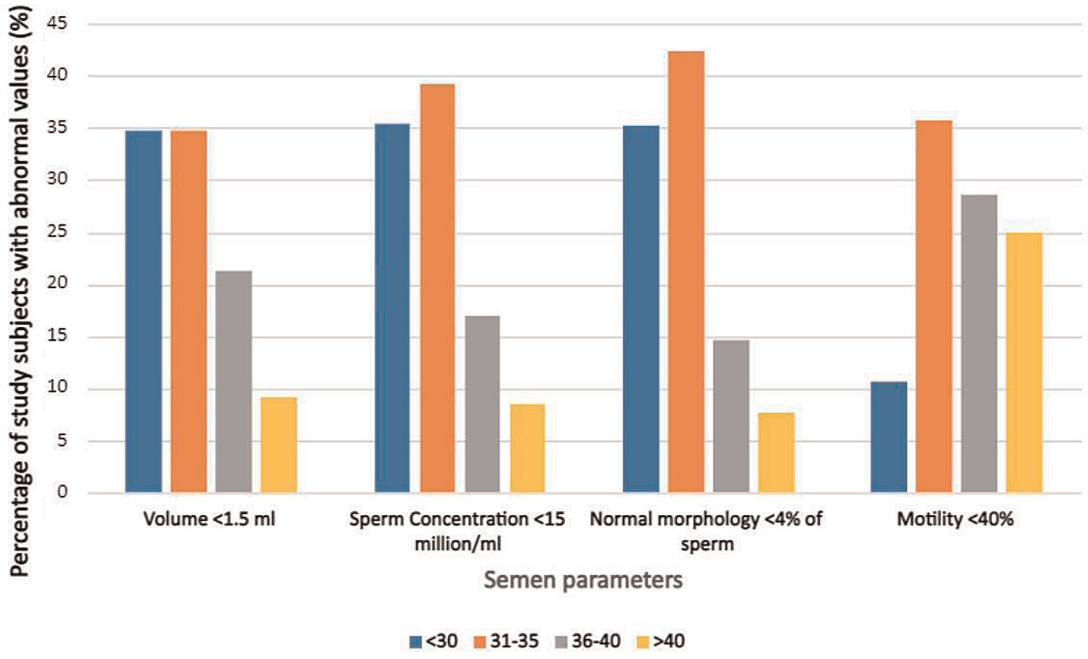

Figure 2: Percentage of study subjects with abnormal values of various semen parameters.

perinatal complications.[6] However, literature on the impact of paternal age on a couple's fertility remains unclear. Although spermatogenesis continues until late in life, and according to theory, it enables men to father a child even at a very advanced age, male fertility also seems to decline with age. Male reproductive organs undergo both minor and major changes as time passes, thereby leading to deterioration in semen parameters, hormone profile, and testicular cytological structure.[7]

Our results are in accordance with those of Winkle *et al.* who analyzed the semen parameters in 320 unselected patients and 84 normozoospermic controls, and their findings suggest that sperm motility decreased with increasing age, whereas concentration and normal morphology did not decline in aging men.[8]

A similar study which was a retrospective study by Gallo *et al.* found no correlation between male age and semen parameters such as semen volume, concentration, normal morphology, and motility in 439 couples.[9]

From our data, we did not find evidence that increasing age may affect concentration. This finding is supported by some other studies.[10-12] There are many studies that also reveal either a decrease or even an increase in sperm concentration with increasing age. For example, a study by Kumar *et al.* on 1219 male partners of infertile couples of rural tertiary care center of central India revealed a significant negative association of concentration with age.[3] Contrary to this, a study by Brahem *et al.* comprising semen samples of 140 infertile patients between 24 and 76 years of age and 50 fertile men between 25 and 65 years of age illustrated that with increased male age, sperm concentration increased.[13]

In addition to concentration, the present study reveals that the percentage of normal morphology is also to be unaffected with increasing age. Similar results were found by Winkle *et al.* in their study on 320 unselected patients and 84 normozoospermic controls in the year 2008.[8] This was further supported by studies conducted by Brahem *et al.*[13] There are some other studies that

Table 3: Various study results showing the effect of aging on semen parameters

Serial no.	Study	Year	No. of subjects	Significant findings
1.	Pino *et al.*[20]	2020	2681	Male age significantly declines semen volume, concentration, and motility
2	Shabani *et al.*[23]	2017	273	Age decreased sperm motility significantly
3.	Sunanda *et al.*[15]	2014	730	Age negatively affects progressive motility, vitality, and morphology of human sperm
4.	Oliveira *et al.*[12]	2014	1500	Significant reduction in the percentage of normal sperm, sperm progressive motility, and sperm vitality as age increased
5.	Stone *et al.*[19]	2013	5081	Sperm concentration and morphology declined after 40 years. Sperm motility fell after 43 years and volume after 45 years
6.	Mukhopadhyay *et al.*[16]	2009	3729	Volume and motility decreased with the increase of age, but the concentration increased with the increase of age
7	Plastira *et al.*[14]	2007	61 oligoasthenoteratozoospermia (OAT) patients and 49 men with proven fertility	Increased age in OAT patients is associated with an increase in sperm concentration, DNA fragmentation, as well as a decline in semen volume, sperm morphology, and motility
8.	Kidd *et al.*[11]	2001	Review literature	Evidence suggests that increased male age is associated with a decline in semen volume, sperm motility, and sperm morphology but not concentration

confirm that normal morphology declines with increasing age.[12,14,15]

Furthermore, our study showed no statistically significant change in semen volume with age which is supported by evidence provided by Sunanda *et al.* on 730 subjects in the year 2014.[15] This result contrasts with some data reported in other works of literature in which authors found that there is a pronounced decrease in semen volume with age.[16-20]

In contrast to other semen parameters, our study observed a statistically significant fall in sperm motility with aging. This is similar to many studies which indicate a fall in motility with advanced male age.[11,18,20] Moreover, a study by Sloter *et al.* revealed that sperm motility decreases by 0.8% per year and linear motion decreases by 0.2% per year.[21] On the other hand, there are many other studies that indicate no significant correlation between age and motility. For example, a study by Brahem *et al.*[13] and Li *et al.*[10] conclude that age does not have any impact on sperm motility.

Motility is a fundamental sperm property, it is necessary to enable the sperm cell to swim up the genital tract, penetrate the oocyte, and form the male pronucleus. Sperm motility appears to be very important not only for natural fertility but also in assisted reproduction, especially in the most advanced technique, Intracytoplasmic sperm injection, which allows fertilization with very few spermatozoa. In this case, it is of critical importance to have motile sperm cells, an unmistakable sign of their viability. Kasai *et al.*[22] demonstrated a higher fertilization and pregnancy rate in patients with higher sperm motility.

In recent years, there has been a shift in the average paternal age of the first child for a variety of reasons and therefore it is very important to understand the effect of age on sperm motility.

This study could serve to provide valuable knowledge not only in the determination of couple fertility prospects but also in the education of the general public about aging and fertility [Table 3].

Limitations

Study subjects in this study were male partners of infertile couples that might have caused some selection bias. So our study subjects were reproductively compromised individuals in comparison to other general population. In addition, our study took only age into consideration but there can be many other factors such as smoking, alcohol, occupation, pollution, obesity, drugs, other comorbid conditions (hypertension, diabetes), etc. that can have an influence on semen parameters and reproductive potential of a male. Hence, in the future, we can do a study taking these factors also into consideration.

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Conflicts of interest

There are no conflicts of interest.

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