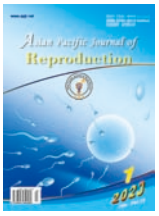

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Impact of chronological ageing on semen parameters in southern Indian men visiting infertility centre: A retrospective study

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ABSTRACT

Objective: To investigate the association between age and semen parameters among male partners of subfertile couples.

Methods: This retrospective study analyzed the semen of 1 523 infertile men aged 26 to 50 years. Data were extracted from GarbhaGudi IVF Centre database from January 2019 to September 2020. The basic semen parameters were interpreted according to the WHO manual 2021, 6th edition. Semen parameters in different age groups were compared.

Results: Total and progressive motile sperms were significantly higher in the age group of 26-30 years compared to other age groups (P<0.05). Normal sperm count was significantly higher in the age group of 26-30 years compared to the age groups of 41-45 years and >46 years (P=0.001). However, sperm head defects, neck and midpiece defects, tail defects, and cytoplasmic droplets showed statistically insignificant difference in all the age groups (P>0.05). Semen viscosity showed no statistical difference in all the age groups compared to the reference age group of 26 to 30 years.

Conclusions: Higher age can lead to a significant decrease in normal sperms and motility in subfertile men. Hence, male partner age should be considered as one of the major determining factors for reproductive outcomes.

KEYWORDS: Age; Semen analysis; Sperm count; Sperm morphology; Motility; Spermatozoa; Infertility; Male

1. Introduction

Infertility has emerged as a significant public health issue in recent years. A systematic review of 277 health surveys worldwide found that about 48.5 million partners have infertility[1]. In India, the reported prevalence of primary infertility is between 3.9%-16.8%[2]. Studies documented that male factors contribute to 40% of infertility cases, female factors contribute to 40%, and male and female factors contribute to 20% of infertility cases[3]. Male infertility has been caused by disease conditions affecting multiple systems[4]. Genitourinary diseases such as testicular failure, cryptorchidism, varicocele, *etc*., are responsible for male infertility[5,6]. All these factors may cause infertility by affecting semen quality and quantity. At the micro-level, they may manifest as oligozoospermia, asthenozoospermia, necrozoospermia, teratozoospermia, or a combination[5].

Various lifestyle factors such as obesity, alcohol, smoking, recreational drugs, and radiation exposure affect sperm quantity and quality[5]. There has been a considerable increase in the average age at marriage and the average age at childbirth of both male and female partners. Age is one factor that needs to be understood more deeply because many men prefer to become parents later[7]. Few studies have reported increasing age in men associated with poor semen quality and adverse fertility outcomes[8,9].

Semen analysis has been performed as a routine laboratory test in couples dealing with infertility. Usual sperm parameters include semen volume, viscosity, pH, motility, vitality, concentration, total

Significance

Age-related negative effects on sperm parameters have been demonstrated in many studies. The present study investigated the effect of chronological ageing on semen parameters and found sperm morphology is a better predictor for male fertility, followed by sperm motility. This study suggests that men who choose to delay fatherhood may reduce their chance of a successful pregnancy outcome.

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sperm count, and stained morphology[10]. A retrospective semen analysis of 1 219 male partners of subfertile couples treated at a rural tertiary hospital in India had reported a significant negative impact of higher age on volume, count, motility, and morphology[11]. Mishra and co-workers assessed temporal trends of male infertility in 6 466 fertile men and 7 020 infertile men between 1979 and 2016[12]. Seminal quality showed a decreasing secular trend with increasing age in infertile men compared to fertile men. Only a handful of published articles have examined the relationship between decreasing semen parameters and aging, especially in India[1]. Also, there is a possibility of heterogeneity based on socio-demographic parameters of the local population and the geographical location. Hence, we hypothesize that, although age negatively impacts on sperm parameters, not all such parameters are negatively affected. Therefore, the current study was designed to evaluate and compare the relationship between age and semen quality characteristics, including sperm concentration, morphology, and motility.

2. Subjects and methods

2.1. Subjects

The present study was a non-interventional retrospective cross-sectional study involving analysis of 1523 semen analysis reports performed from January 2017 to September 2019 at the Reproductive Health Center. The sample size was calculated based on the formula for a cross-sectional study, 85% power, and 0.4 ratios. Subfertile men with >26 and <50 years (according to the Indian Council of Medical Research, 2017 guidelines) of age were included, and if there was an incomplete semen report, missing data, or ejaculatory dysfunction, were excluded from the present study.

2.2. Semen analysis

After 20 min of liquefaction, the sperm count, volume, viscosity, pH, total motility, progressive motility, non-progressive motility, immotile, abnormal, and normal sperm morphology were evaluated, according to World Health Organization (WHO) guidelines (2010[13]. The concentration was determined using a Makler chamber (Sefi-Medical Instruments, Haifa, Israel). The motile sperm with path velocities greater than 25 mm/s and greater than 80% linearity were identified as progressively motile sperm using a light microscope (Labomed Opti CX compound microscope with halogen 6v-20w illumination, USA). Sperm morphology was determined by standard protocol as per WHO after staining of fixed semen smears (Diff quick method, Repro labs).

2.3. Statistical analysis

Data analysis was performed using SPSS Software version 22 (v. 17.0; SPSS Inc, Chicago, IL, USA). Data were expressed as mean and interquartile range. The data were subjected to Kolmogorov–Smirnov test. The non-parametric test, Kruskal Wallis test and *post hoc* test were used to compare different age groups. A *Chi*-square test was used to compare the categorical parameters. *P* value less than 0.05 was considered statistically significant.

2.4. Ethics statement

The present study protocol was reviewed and approved by the Institutional Ethics Committee (GEC/GGIRH19_2/ 26052020). The data were collected from the existing medical records. All the data were blinded for personal information during the analysis of the data to maintain confidentiality.

3. Results

3.1. Baseline characteristic of the infertile men

The present study included a total of 1523 men aged between 26 and 50 years. The study was carried out after obtaining approval from the institutional ethics committee. Subfertile men with >26 and <50 years (according to the Indian Council of Medical Research, 2017 guidelines) of age were included in the study. The body mass index of the males was (27.4±6.5) and they had (5.7±1.5) years of married life. The number of patients aged between 31 and 35 years had an increased proportion of diabetes, hyperthyroidism, and hypothyroidism as compared to other age groups. Furthermore, a higher proportion of smokers, alcohol-intake, and tobacco chewers were observed in the age group of 31 to 35 years (Table 1).

3.2. Impact of age on percentage of normal sperm, live sperm (vitality) sperm concentration, semen volume and pH

The normal sperms were found be significantly higher in the age group 26 to 30 years as compared to age group 41 to 45 years (P=0.001), and >46 years (P=0.001). There was no statistically significant difference of live sperms among different age groups. The effects of chronological age on sperm concentration, semen volume and pH were insignificant among the different age groups (P>0.05). The sperm concentration among all groups of the infertile men was ≥40 million/mL of semen and was statistically insignificant (P>0.05). The volume of semen sample was ≥2.5 mL in the infertile men and pH was pH≥7.4. However, there was no statistically significant difference in volume and pH of semen sample among different age groups (P>0.05) (Figure 1).

3.3. Impact of age on sperm motility

The effects of chronological age on total sperm motility and various sperm motility defects are shown in Table 2. The total motile sperms were significantly higher in the age group 26 to 30 years as compared to age group 36 to 40 years (P=0.007), 41 to 45 years (P=0.001) and age group of >46 years (P=0.001) (Figure 2A). The progressive and non-progressive motile sperms were found to be statistically significant between the age group of 26 to 30 and 36 to 40 years, 26 to 30 and 41 to 45 years, 26 to 30 years and >46 years (P<0.05) (Table 2; Figure 2B, 2C). However, there were no

statistically significant differences in non-motile sperms among the different groups (P>0.05) except between the age group between 41 to 45 and >46 years (P<0.05) (Figure 2D).

3.4. Impact of age on sperm morphology

The effects of chronological age on sperm morphology are shown in Table 2 and Figure 3. The distribution head defects in sperms were similar in all age groups (P>0.05), the interquartile range in >46 years of age was less than other age groups (Figure 3A) and left skewness was observed in the data distribution. On contrary, right skewness was observed in neck and midpiece defected sperms and found to be statistically insignificant among all the age groups (P>0.05) (Figure 3B). The tail defects and cytoplasmic defects were not found to be significant across the different age groups (P>0.05) (Figure 3C, 3D).

3.5. Impact of age on semen viscosity

The semen viscosity showed no difference in the statistical outcome in all the age groups compared to the reference age group 26 to 30 years (Table 3).

The interquartile range of sperm concentration was 37–52, total motility was 38–79, progressive motility was 19–30, non-motile sperms were 20–35, normal sperms were 1–2, head defects were 10–36, neck and midpiece defects were 3–21, tail defects were 2–14, cytoplasmic droplets were 2–5, vitality was 0.9–1.2, and volume was 1.2–1.5.

Table 1. Characteristics of infertile men in the different age groups.

Characteristics	Age group, years				
	26-30 (n=251)	31-35 (n=715)	36-40 (n=407)	41-45 (n=116)	>46 (n=34)
Smoking, n(%)					
Ever	17 (6.8)	52 (7.3)	40 (9.8)	9 (7.8)	4 (11.8)
Current	22 (8.8)	71 (9.9)	38 (9.3)	10 (8.6)	3 (8.8)
Alcohol intake, n(%)					
Ever	64 (25.5)	170 (23.8)	121 (29.7)	21(18.1)	8 (23.5)
Current	19 (7.6)	47 (6.6)	20 (4.9)	10 (8.6)	2 (5.9)
Tobacco chewing, n(%)					
Ever	1 (0.4)	4 (0.6)	2 (0.5)	0 (0.0)	0 (0.0)
Current	4 (1.6)	14 (2.0)	4 (1.0)	3 (2.6)	1 (2.9)
Diabetes, n(%)	36 (14.3)	128 (17.9)	68 (16.7)	18 (15.5)	4 (11.8)
Hypertension, n(%)	105 (41.8)	255 (35.7)	160 (39.3)	49 (42.2)	12 (35.3)
Hypothyroid, n(%)	21 (8.4)	61 (8.5)	41 (10.1)	12 (10.3)	3 (8.8)
Hyperthyroid, n(%)	3 (1.2)	6 (0.8)	5 (1.2)	1 (0.9)	0 (0.0)
Asthma, n(%)	1 (0.4)	6 (0.8)	11 (2.7)	2 (1.7)	0 (0.0)
Epilepsy, n(%)	1 (0.4)	5 (0.7)	3 (0.7)	2 (1.7)	0 (0.0)

Ever smokers, defined as abstinence from smoking for at least 15 years on the day before the start of treatment; current smokers, defined as smoking >100 cigarettes/lifetime, or smoking >100 cigarettes/lifetime but abstinence from smoking for less than one year on the day before the start of treatment. Ever alcohol intake defines no alcohol consumption in their life time, and current alcohol intake defines occasional consumers of alcohol. Ever tobacco chewing defines no tobacco use in their lifetime, and current tobacco chewing defines tobacco chewing twice a day.

Table 2. Comparison the semen parameters of infertile men between the reference age group of 26-30 years and other different age groups.

Semen parameters	Age groups, years	Mean rank	P value
Total motility	26-30 (Reference)	834.7	
	31-35	800.5	>0.999
	36-40	715.4	0.007
	41-45	631.8	0.001
	>46	416.6	0.001
Progressive motility	26-30 (Reference)	826.5	
	31-35	799.5	>0.999
	36-40	714.7	0.020
	41-45	654.4	0.005
	>46	438.4	0.001
Non-progressive motility	26-30 (Reference)	767.8	
	31-35	781.5	>0.999
	36-40	755.6	0.010
	41-45	689.7	0.001
	>46	635.8	0.001
Normal sperms	26-30 (Reference)	785.7	
	31-35	798.9	>0.999
	36-40	742.2	>0.999
	41-45	630.9	0.013
	>46	493.8	0.002

Table 3. Comparison of semen viscosity among different age groups.

Viscosity	Age group, years					*Chi* square	*P* value
	26 to 30 (*n*=251)	31 to 35 (*n*=715)	36 to 40 (*n*=407)	41 to 45 (*n*=116)	≥46 (*n*=34)		
Normal, *n*(%)	230 (91.7)	664 (92.9)	358 (88.0)	104 (90.0)	32 (94.1)	8.51	0.07
High viscous, *n*(%)	21 (8.3)	51 (7.1)	49 (12.0)	12 (10.0)	2 (5.9)		

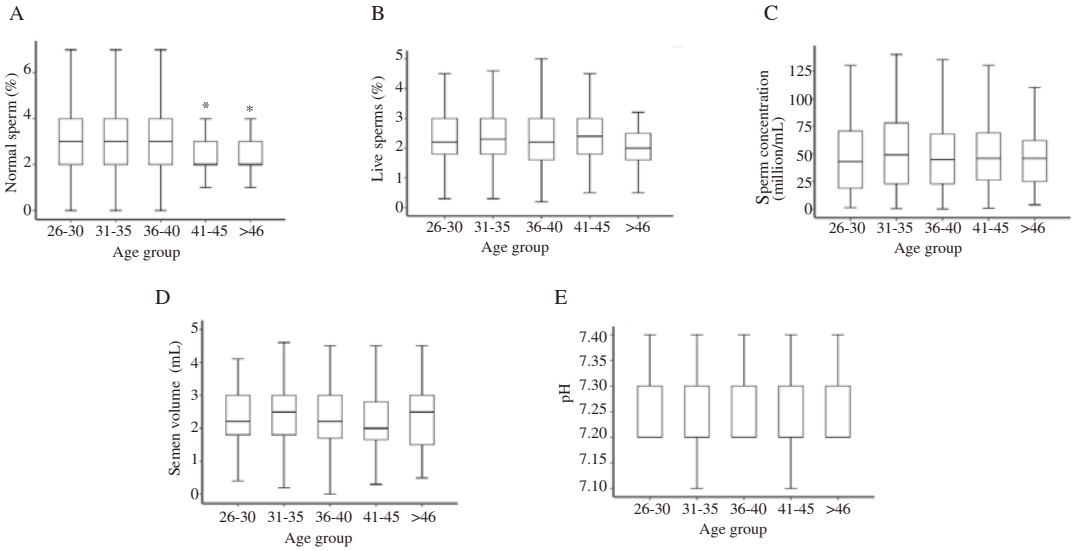

Figure 1. Effect of age on normal sperm (A), live sperm (B), sperm concentration (C), semen volume (D), and semen pH (E) in different age groups among infertile men. *P=0.001: compared to the reference age group of 26-30 years.

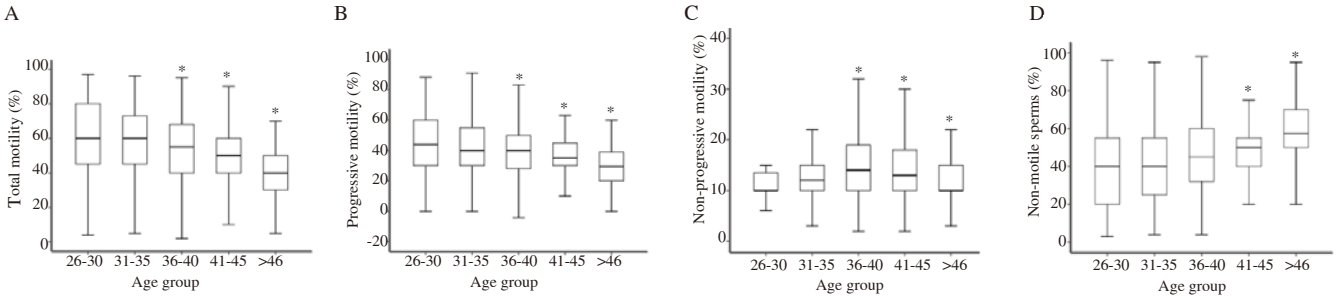

Figure 2. Effect of age on percentages of total motility (A), progressive motile sperms (B), non-progressive motile sperms (C), and non-motile sperms (D) in different age groups among infertile men. *P=0.001: compared to the reference age group of 26-30 years.

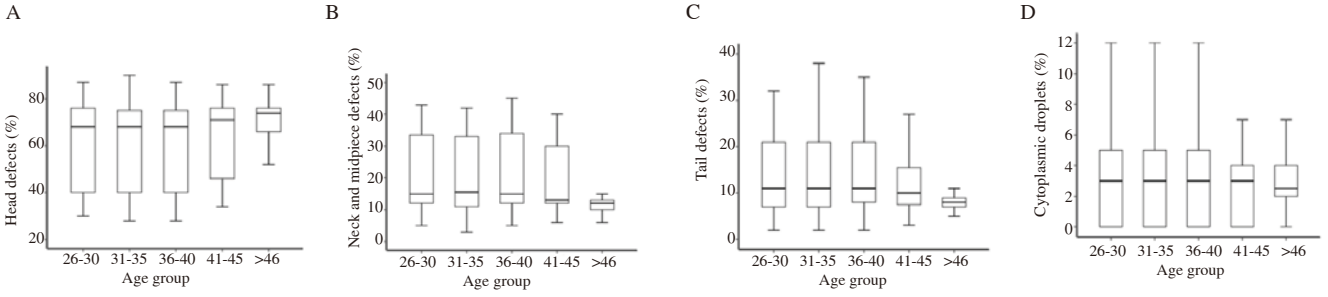

Figure 3. Effect of age on sperm morphology such as percentages of sperm head defects (A), neck and mid piece defects (B), tail defects (C), and cytoplasmic droplets (D) in different age groups among infertile men.

4. Discussion

Male infertility is caused by a variety of factors and conditions related to sperm function, morphology, and concentration, all of which make it difficult to fertilise an egg with sperm under normal circumstances. As a result, all factors that contribute to infertility may reflect sperm quality[14]. The present study did not show significant association between age and semen volume. A few population-based studies have found similar results in terms of sperm volume compared with age[11,15]. Nevertheless, evidence from past studies suggests that semen volume was significantly decreased with age[7,16]. Decreased seminal volume could be due to insufficient seminal vesicle fluid in the ejaculations[14]. The other factors affecting semen volume and sperm motility are prostatic changes, including smooth muscle atrophies[14].

Similarly, there was no significant association between sperm concentration and age. This outcome was comparable with previous studies which reported sperm concentration did not change with age[7,8]. On the contrary, research has shown that the sperm concentration can decline by 3.3% every year[17]. Kumar *et al* have found a negative association between age and sperm concentration and also reported that significant decrease in sperm concentration after the age of 35 years[18]. A study of 3 729 infertile men found a significant decline in other semen parameters whereas there was no change in the sperm concentration[19]. Further, A large study of 20 411 men reported a 0.7% statistically significant increase in sperm concentration per year of age. Over a 20-year period, this equates to a 14% rise in sperm concentration[20].

The present study showed that there was a statistically significant decrease in normal sperm morphology and motility as age advanced. A similar retrospective study was reported by Kumar *et al* showed a significant decrease in total sperm count, sperm motility, and morphology after 35 years[18]. Eskenazi and co-authors assessed the association between semen quality and age among healthy men. Their findings revealed a significant increase in volume, concentration, and motility over different decades[7]. Decreased sperm motility can be due to altered functions of the prostatic and seminal vesicle secretions. Epididymal transit also plays an important role in the maturation and progressive motility of spermatozoa[21,22]. Prostate-specific-antigen and α-glucosidase, markers secreted by the prostate and the epididymis, respectively, decrease with age and positively correlate with sperm motility[22,23].

Therefore, the present study found a significant relationship between increasing age and decreased sperm motility and morphology, whereas the semen volume, viscosity, and pH were not correlated with age. Evaluating the influence of age on semen parameters is complicated as it involves multiple confounding factors. The rate of biological aging may be quite different from individual to individual and also may be influenced by associated factors such as smoking, exercise, or the presence of comorbidities. Vascular diseases, accumulation of toxic substances, or infections of the male reproductive accessory glands may also affect reproduction.

The key limitation of the current study was its retrospective nature and purposive sampling with the possibility of selection and ascertainment bias, even though the semen analysis procedures were standardized. Additionally, our study population was single-cantered and only included those visiting the fertility clinic, limiting the generalizability of study findings. Moreover, in the present study, we were unable to determine the confounding factors and other lifestyle-related factors such as population's physical activity levels, dietary habits, nutritional intake, and general lifestyle habits. Despite these limitations, the study was conducted on a large cohort of subjects, hailing from large catchment areas, including rural and urban areas; hence, the findings can be generalized with reasonable caution. The current study did not include male patients with hydrocele, diabetes, infectious diseases, hernia, varicocele, tobacco addiction, alcohol addiction, and azoospermia.

In conclusion, this study reveals a significant relationship between chronological age and decreased sperm motility and morphology, whereas the semen volume, viscosity, vitality and pH did not show its impact on age. Future research investigating the male age and semen quality and fertility relationship must stress the methodological framework by designing population-based, large-scale prospective studies. Enrolling adequate samples all through the age spectrum, controlling the effects of potential confounding factors, and including appropriate comparison groups can aid in a more in-depth understanding of the influencing factors of semen quality.

Conflict of interest statement

The authors declare no conflicts of interest.

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Authors' contributions

Muhammed Asif was the principal investigator in this study, designed the study, collected the data, performed statistical analyses, analyzed the results, and drafted the manuscript. Asha S. Vijay supported the collection of data. Maheshwari, Syed Fyzullah, Usha Rani, and Swathi R helped in review of literature. Damodara Gowda KM designed the study, collected the data, analyzed the results, and drafted and revised the manuscript. All the authors approved the manuscript for publication.

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