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■ Original Research Article

Impact of Age on Semen Quality in Male Partners of Infertile Couple: A retrospective study in a tertiary Hospital in North-West Nigeria.

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ABSTRACT

Background: Spermatogenesis is known to persist well into old age. Nevertheless, advanced paternal age has been associated with significant reductions in pregnancy rates, and age-related decline in androgen secretion levels suggestive of impairment in sperm parameters. **Aim:** To determine association between age and semen quality in male partners of infertile couple attending fertility clinic in a tertiary Hospital in North-Western Nigeria. **Method:** This was a retrospective study. The laboratory semen analysis (SA) records of 452 male partners of infertile couples seen at the fertility clinic for a 3-year period (January 2019 to December 2021) retrieved were analyzed using SPSS version 23. All results with incomplete records of parameters of interest were excluded, and the semen parameters classified into normal and abnormal based on WHO 2010 standard criteria. **Result:** Data retrieval rate was 75.3%. The mean age of male Partners with normal & abnormal SA were 33.79±7.21 and 37.57±8.63 respectively. There were 261 (57.7%) abnormal SA. We found a negative correlation between age and semen volume, non-progressive motility (NPM), progressive motility (PM), vitality, morphology and sperm concentration ($p<0.05$), but no correlation between age, and sperm pH & immotile sperm for the normal & abnormal SA parameters combined. While with the normal & abnormal SA groups separated; only morphology had a significant negative correlation for the normal SA group. There was still significant negative correlation in NPM, PM, vitality, morphology & sperm concentration for the abnormal SA group. **Conclusion:** This study found that non-progressive motility, progressive motility, vitality, morphology and sperm concentration decreases significantly as age increases in men with abnormal SA, and only morphology decreased significantly with age in men with normal SA.

Keywords: Aging; Fertility; Infertility; Paternal age; Semen parameters

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INTRODUCTION

Infertility is failure to achieve conception after 12 months or more of regular unprotected sexual intercourse.[1] Various factors are associated with infertility among which male factor alone contributes 35- 40% of infertile cases.[2,3] The pathogenesis of male factor infertility is multifactorial, and can be as a result of any alteration to the normal physiology of reproductive organs that may affect sperm functions resulting in oligozoospermia (low sperm count), asthenozoospermia (loss of motility), teratozoospermia

(abnormal morphology), and azoospermia (sperms absence in ejaculation), which interferes with successful fertilization.[2,4]

It is well known that maternal age contributes significantly to human infertility following decline in functional oocytes in women by mid to late thirties, leading to a higher risk of infertility, pregnancy complications, miscarriages, congenital anomalies, and perinatal complications. While in men, it has been reported that spermatogenesis usually persists well into old age,[5] although an age-related decline in androgen secretion levels may suggest impairment in sperm parameters. Also, advanced male age has been associated with significant reductions in pregnancy rates.[6] A study demonstrated that men older than 35 years have half the chance of fathering a child within 12 months compared with men younger than 25 years of age,[7] but not withstanding children have been fathered by men over 90 years old.[8]

The effect of paternal age on semen quality and reproductive function is controversial for several reasons. First, there is no consensus on the definition for advanced paternal ageing. Secondly, the reports from various studies have conflicting results, especially for the most common semen parameters tested. Studies in recent times have demonstrated a direct correlation between aging and structural and functional changes of sexual organs and the endocrine system, therefore, suggesting an effect on sperm parameters and fertility.[9,10] It is important to understand that while semen analysis result may correlate with "fertility," the assay is not a direct measure of fertility.

The volume of the testes starts to decrease after 60 years of age. Gonadotropin levels increase and testosterone levels decrease with aging. The number of Leydig, Sertoli, and germ cells decreases with aging. Aging introduces vascular changes that lead to testicular fibrosis. Male germ cells undergo continuous DNA replication and division throughout an individual's life. Therefore, older men are at greater risk of having germ cells carrying mutations.[9] Increasing male age has also been shown to be associated with disorders like achondroplasia,[11] and autism[12] among many others.

Semen quality has been commonly regarded as a measure of male fecundity, and changes in semen quality can occur after exposure to toxic agents or from host factor such as age. It is important to provide more evidence and clarity on the association between advanced paternal age and reduced semen quality. Hence, this retrospective study was conducted to determine the impact of age on semen quality in male partners of infertile couple attending fertility clinic in our centre. This information will be useful in the determination of couple fertility prospects, and in educating the general public on aging and fertility. The specific objectives were to; assess for the mean or median values of semen parameters (pH, volume, non-progressive motility, progressive motility and immotile, morphology, vitality, and sperm concentration) for men with normal and abnormal values, determine the correlation between age and semen parameters for men with normal and abnormal values

MATERIALS AND METHODS

This was a retrospective, analytical cross-sectional study. Semen analysis records from the study centre microbiology laboratory, of male partners of infertile couples seen at the fertility clinic for a 3-year period (January 2019 to December 2021) were retrieved. Samples collected by masturbation or coitus interruptus for those who could not masturbate, following 3-4 days of abstinence were transferred to the laboratory and examined within 1 h of collection. The volume of the seminal fluid was measured. The pH was determined by dropping sample on pH paper and viscosity determined using Pasteur pipette. The counting chamber was used for the count and sperm motility was determined by applying a drop of the sample onto a slide, then examined under the microscope with appropriate lens, & motility graded as; progressive motility, non-progressive motility and immotile. Sperm morphology and vitality were determined by using pap and Haematoxylin and Eosin (H&E), staining technique.

A single seminal fluid analysis results of all male partners of infertile couples (infertility due to female factor/male factor/combination of the two or unknown reasons) were considered. All results with incomplete records of the semen parameters of interest were excluded. Also, results that the age of the patient could not be retrieved were also excluded. Records of semen analysis done over the 3-year period of study were retrieved using a proforma. The classification of all semen parameters into normal and abnormal was done using the WHO 2010 standard criteria.[2]

Statistical analysis of retrieved data was done using Statistical Package for the Social Sciences, version 23.0, SPSS Inc, Chicago, Illinois, USA (SPSS). Quantitative variables were compared using unpaired t-test and all the data expressed as means ± standard deviation (SD), median and interquartile range, or percentage. Spearman's correlation (non-parametric) or Pearson correlation (parametric) was used to find out significant correlation between age and various semen parameters. Statistical significance defined as a p value ≤ 0.05, and confidence intervals set at 95%. The findings are presented in tables below. The results were categorised into normal and abnormal SA report based on the WHO 5th edition[2] standard references for sperm concentration, morphology, progressive motility and total motility. All three falling within the normal limit was categorised in this study as normal semen analysis (SA) and any falling below is abnormal SA.

This study was conducted following proper ethical clearance from the Research Ethics Committee.

RESULTS

About 2000 semen analysis requests were made during the period of study, but only 30% (600) results were available out of which four hundred and fifty-two with complete required data were retrieved and analyzed, giving us a retrieval rate of 75.3%. The mean age for all study participants was 35.98±8.27 years with a range of 19-70 years, while for those with normal and abnormal semen analysis (SA) results it was 33.79±7.21 years and 37.57±8.63 years respectively (p<0.001). There were 191 (42.3%) normal SA and 261 (57.7%) abnormal SA. The most dominant age group was 31-40 years among those with normal SA (48.2%) and abnormal SA (52.2%). Masturbation was the most frequent method of sperm collection recorded among both normal SA 118 (61.8%) and abnormal SA 141 (54.0%). Majority of the semen analyzed were hypo-viscous in both groups. This baseline information on semen collection & age of participants are summarised as shown in table-1.

Table 1: Baseline information on semen collection and age of participants

Variables	Normal SA	Abnormal SA	
Age (Years) distribution	2 (1.0%)	3 (1.1%)	
<20	67 (35.1%)	45 (17.2%)	
21-30	92 (48.2%)	136 (52.2%)	
31-40	23 (12.0%)	51 (19.5%).	[t=-4.93,
41-50	7 (3.7%)	25 (9.6%).	p < 0.001,
51-60	0 (0.0%)	1 (0.4%).	95% CI (-5.2 to
>60	33.79±7.21 years	37.57±8.63 years	-2.275)]
Method of sperm collection	118 (61.8%)	141 (54.0%)	
Masturbation	73 (38.2%)	120 (46.0%)	
Coitus interruptus			
Viscosity			
Hypo-viscous	91 (47.6%)	130 (49.8%)	
Normo-viscous	55 (28.8%)	56 (21.5%)	
Hyperviscous	45 (23.6%)	75 (28.7%)	

Table 2: Semen Parameters

Semen parameter	Normal	Abnormal
Morphology	336 (74.3%)	116(25.7%)
Motility	195 (43.1%)	257(56.9%)
Concentration	252(55.8%)	200(44.2%)

Overall, about a quarter 116 (25.7%) of the SA recorded abnormal morphology (teratospermia), while 336 (74.3%) had normal morphology. Slightly above half 257 (56.9%) had abnormal motility (asthenospermia), while 195 (43.1%) had normal motility. Majority of the SA had normal sperm concentration 252 (55.8%), while 200 (44.2%) had low sperm concentration (oligospermia), as shown in table 2. The mean/median values of the semen parameters recorded for men with normal and abnormal SA have been summarized in table-3 above.

Table 3: Mean ± SD or median & IQR values, of semen parameters for men with normal & abnormal SA

Variables	Normal SA	Abnormal SA
pH	7.8 ± (SD 0.41)	7.9 ± (SD 0.41)
Volume(mls)	2.79 ± (SD 2.16)	2.74 ± (SD 1.59)
Nonprogressive motility (%)	10.00 (IQR 7)	2.00 (IQR 10)
Progressive motility (%)	55.00 (IQR 25)	2.00 (IQR 15)
Immotile (%)	30.00 (IQR 25)	55.00 (IQR 80)
Vitality (%)	65.00 (IQR 20)	5.00 (IQR 25)
Morphology (%)	87.00 (IQR 10)	53.00 (IQR 82)
Sperm concentration (sperm/ml x10^6)	62.60(IQR 49.80)	2.00 (IQR 14.6)

Table 4: Correlation Between Age and Semen Parameters for Both Groups Combined

Variable	Spearman (rho) Or Pearson (r) Correlation	p-value
pH	r = -0.44	p = 0.347
Volume (mls)	r = -0.11*	p = 0.021
Non-progressive motility (%)	rho = -0.16**	p = 0.001
Progressive motility (%)	rho = -0.26**	p<0.001
Immotile (%)	rho = -0.80	p= 0.91
Vitality (%)	rho= -0.26**	p<0.001
Morphology (%)	rho = -0.16**	p= 0.001
Sperm concentration (sperm/ml x10^6)	rho = -0.26**	p<0.001

There is a statistically significant negative correlation (using the Pearson or Spearman's correlation) between age and semen volume, non-progressive motility, progressive motility, vitality, morphology and sperm concentration. This means that there is a decrease in these parameters with increase in age. While there is no significant correlation found between age and semen pH and non-motile semen as shown in table-4 above.

Also, there was statistically significant negative correlation found between age of men and SA quality using independent t-test [t=-4.93, p<0.001, 95% CI (-5.2 to -2.275)], with the mean age of those with normal SA (33.79±7.21 years) being significantly lower than that of those with abnormal SA (37.57 ±8.63 years).

Table 5 above, shows the correlation between age and SA parameters for those with normal and abnormal results separately. There was no significant correlation in pH for both groups. Unlike in table 4, semen volume had no significant correlation with age (p=0.754) between normal SA (2.79 ± 2.16) and abnormal SA (2.74 ± 1.59). The normal SA group only had a significant negative correlation between age & morphology (rho= -0.15*, p=0.03), while the abnormal SA group had significant negative correlation between age and non-progressive motility, progressive motility, vitality, morphology & sperm concentration. Both groups had no significant negative correlation between age & immotile sperm.

Table 5: Correlation between Age and Semen parameters for men with normal & abnormal SA separated

	Normal SA		Abnormal SA	
Variables	Spearman (rho) or Pearson (r) correlation	p-value	Spearman (rho) or Pearson (r) correlation	p- value
pH	r = -0.06	p=0.35	r = -0.06	p=0.29
Volume (mls)	r = -0.10	p=0.16	r = - 0.12	p=0.55
Non progressive motility (%)	rho = 0.04	p=0.55	rho = -0.15**	p = 0.02
Progressive motility (%)	rho = -0.01	p=0.94	rho = -0.20**	p=0.001
Immotile (%)	rho = -0.03	p=0.67	rho = -0.09	p=0.11
Vitality (%)	rho = 0.03	p=0.63	rho = -0.21**	p=0.001
Morphology (%)	rho = -0.15*	p=0.03	rho = -0.16**	p=0.01
Sperm concentration (sperm/ml x10^6)	rho = 0.08	p=0.27	rho = -0.18**	p=0.004

*Statistically significant values

DISCUSSION

This study found 261 (57.7%) abnormal SA, which is higher than 31.8% from south-west Nigeria [13], lower than 64% from south-east Nigeria [14], but similar to 58% from Sokoto [15] in the same North-west region as this study. The differences may be due to negative effect of sociocultural practices & climatic differences on male fertility, such as less health seeking behaviour/health access, and hot weather in the north as compared to southern Nigeria. The temperature of the scrotum is 2–4 °C lower than the core body temperature [16], and rise in environmental temperature is one of the factors that causes a rise in scrotal temperature, which affects process of spermatogenesis resulting in male infertility.

While the higher record from south-east, may be due to the high level of large- and small-scale industrial activities in the region which expose men to negative impacts of environmental chemicals from industrial waste, pesticides, insecticides, herbicides, food additives and pollutants such as, polychlorinated biphenyls, which leads to decline in spermatogenesis and semen quality. [17] Also, social factors like alcohol consumption may have contributed. In the above discussion we hypothesised that the factors highlighted could be responsible for the differences observed. This information could be used in the future to design prospective studies that aim to discover factors that could affect semen quality.

The mean age of men with abnormal semen parameters in this study was approximately 38 years and was comparable with the findings in a study done in Italy. [18] The men with normal SA were significantly younger than those with abnormal SA parameters (33.79±7.27 and 37.57±8.63 respectively, $p < 0.001$). This was in contrast with a cross-sectional study in Iran that shows that, though the infertile men with abnormal SA were older than those with normal SA, but there was no statistically significant difference (p=0.73) between their mean age.[19] This may probably be because other factors apart from age such as infection, co-morbidities, drugs, cigarette smoking were responsible for the abnormal SA. Seminal fluid pH contributes to sperm quality and function, because acidic pH has been demonstrated to have adverse effects on the sperm cell activity. [20,21] A pH <7.2 suggests blockage of seminal vesicles and pH >8.0 suggests an infection[2].

In this study, the mean seminal fluid pH of 7.8 in men with normal parameter and pH 7.9 in those with abnormal parameter showed no significant difference, and agrees with findings in other studies in Africa.[20,22,23] The mean semen volume in this study was also within WHO acceptable normal range and comparable to findings in Birnin Kudu within same region of Nigeria.[24] A semen volume of less than 1.5mls is abnormal, and may warrant the need for further investigations such as urine microscopy to rule out retrograde ejaculation and other pathologies. The sperm vitality is an important determinant of male fertility as this gives more information about the live sperm cells. The median vitality of 65% obtained among those with normal values in this study is comparable to the value obtained among fertile couples in Egypt [25], while the median vitality of 5% recorded among those with abnormal result is not surprising, as it is contributory to their abnormal result.

There was no significant correlation found between the age of the men in this study and the seminal fluid pH value, which is similar with the findings in Italy and Turkey.[16,26] The volume of seminal fluid usually reduces with age due to decline in the accessory gland function. This study demonstrated a negative correlation and significant association between age and mean volume of seminal fluid for normal and abnormal SA combined and this agrees with the findings in other studies within and outside the country.[10,27,28,29].

However, no significant association between age and volume in the separate groups. There was a statistically significant negative correlation between the age of the patients and sperm non-progressive and progressive motility in those with abnormal SA, which means that as age increases, the sperm cell motility decreases. This agrees with the findings from other studies within and outside Nigeria.[27,30,31] Morphology refer to the size and shape of sperm cell and it is an important determinant of sperm fertilisation properties during natural and assisted reproductive technology (ART). The findings in this study showed a negative correlation between age and sperm morphology and this was comparable to the findings in the studies done within Nigeria and in Europe. [24,32,33]

Sperm vitality has little effect on semen quality especially when the sperm motility is greater than 39%, however when the total motility is less than 40%, there is need to check vitality which becomes important to discriminate between immotile dead sperm and immotile live sperm. We found a negative association between age and sperm vitality of men with abnormal SA and this is in keeping with findings from other studies.[28,29,32]

This study also shows significant negative association between aging and sperm concentration and this is similar to the findings of other studies.[28,29,34,35,36] which is thought to be due to decline in testosterone level with age. While the systematic review by Johnson et al., reported a statistically significant age-associated declines in semen volume, percentage motility, progressive motility, and normal morphology, except sperm concentration[10], but did recognise a gradual decrease in sperm concentration over time. The decline in SA quality with age was linked to oxidative stress causing inflammation and endocrine dysfunction that affects spermatogenesis.

In addition, the findings in this study agreed with another study in India which showed that total sperm count, morphology and motility decrease with age, from 35 years old,[37] but differs from the findings in Ebonyi south-east Nigeria that reported no significant relationship between age and abnormal semen parameter.[23] They found that abnormal SA was more in urban men who consume alcohol and those with primary infertility. These confounders were not explored in this study and this may be the reason for the difference in the results.

This study looked for impact of age on semen quality for those with normal and abnormal SA separately, and found statistically significant negative correlation between age and; non-progressive motility, progressive motility, morphology, vitality and counts among men with abnormal SA, which was not so in their counterpart with normal SA. This finding supports two possible impressions; firstly, that not just age contributes to the abnormal SA, which is why there is no significant correlation with age among those with normal SA. Secondly, that there is a decrease in semen quality in both groups with increase in age, as depicted by the negative correlation, but more significant in those with abnormal SA even though structural and functional changes of sexual organs and the endocrine system with age occurred in both groups [9,10]. This is supported by findings in other studies that show association between age and abnormal seminal fluid parameter. [19,28,38]

Our findings indicate that male age needs more recognition as a potential contributor to poor semen quality. The findings from this study could be used in counselling couples with infertility about their fertility potential. Understanding the effects of age on specific parameters can also be used in selecting the appropriate ART techniques for infertile couples. Early evaluation for male partners for infertility, and to create awareness of the impact of age on male fertility in the society

Limitations

1. This study included withdrawal method of semen sample collection for those unable to masturbate, which may result in incomplete semen samples, leading to inaccurate measurement of volume and sperm count.
2. This study used a single semen analysis result which may not be the true picture of the semen quality if a repeat sample was done after 3 months, bearing in mind that an abnormal finding in the first SA could be due to error during sample collection or during analysis in the laboratory.
3. Being a retrospective study, the effect of confounders was not explored.

CONCLUSION

This study found that non-progressive motility, progressive motility, vitality, morphology and sperm concentration decreases significantly as age increases in men with abnormal SA, and only morphology decreased significantly with age in men with normal SA.

Recommendations

a. A future prospective multicentre longitudinal study can be conducted on infertile men and fertile men to determine the association between age and semen quality in both groups. The study should determine the effects of confounders such as lifestyle factors (smoking, alcohol, etc), environmental factors (occupational exposures to radiation, toxins, heat, etc), hormonal factors (following a full hormonal assessment of reproductive hormones) and genetic factors.
b. Future studies can also be done to determine the underlying basis or mechanisms involved in the observed changes in semen parameters with age. This understanding could identify potential

therapeutic targets that can be used to develop therapeutic agents (such as antioxidant therapy, nutrient supplementation, etc) based on the findings

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