Advanced Paternal Age and Semen Parameters of Sub-fertile Men Seeking Assisted Reproduction Technology in Nigeria

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Abstract

Background: The main aim of the present study was to investigate the influence of advanced paternal age on semen volume, liquefaction, sperm concentration, motility and sperm morphology among an indigenous urban black African population.

Materials and Methods: This was a retrospective and cross-sectional study carried out between 2010 and 2015 on a sample of 505 apparently healthy adult males who presented for assisted conception at Nordica Fertility Center in Nigeria for secondary infertility. Paternal age (years) was categorized into < 40, 40–44, 45–49, and > 49. Sperm was collected by masturbation and examined within 60 minutes of collection. Analysis was done using STATA 13 and the level of significance was set at p < 0.05.

Results: Overall means (± SD) of age (years) and BMI were 42.5 (7.2) and 27.4 (4.5) respectively. Means of seminal fluid volume (ml), liquefaction time (mins) and progressive motility (%) at age < 40 (2.3 ± 1.4 ml; 28.9 ± 7.5 and 39.9 ± 22.4 respectively) were significantly higher (t = 2.50, p - value = 0.007; t = -1.87, p - value = 0.03 and t = 1.76, p -value = 0.04 respectively) than that at age > 49 years. There was no significant difference in sperm count (> 10/ml in all age groups. There was no important statistical alteration in sperm cell morphologies regardless of age difference. Multivariate regression analysis indicated that paternal age significantly correlated with seminal fluid volume (r = -0.02, t = -2.65, p - value = 0.008) but not with motility or sperm count.

Conclusion: The study showed that paternal age may influence some semen parameters such as volume and liquefaction time. These may serve as useful points of consideration in assisted conception when dealing with male patients older than 49 years.

Keywords: Advanced paternal age; Semen volume; Sperm concentration; Motility; Morphology

Introduction

In recent times, focus has shifted from female-directed researches on infertility to studies on male factor infertility or the contribution of the male to infertility. Numerous studies have been carried out to establish the relationship between the age of the woman and her fertility potential; on the other hand, the effect of advancing age on male fertility is still unclear. Hence one of the most important topics in andrology is advanced paternal age and its influence on infertility, chromosomal abnormalities and miscarriages. Socioeconomic pressures, high rate of divorce and subsequent remarriage, adoption of women in managerial or executive positions and the new role of females in the society have led to deferred fathering of a child in many parts of the world [1–5]. Moreover, male remarriage, for any reason, as well as prevalence of polygamy, have contributed to interests generated in advanced paternal age. Late child wish, in some cases, are due to the desire for a male child, especially where all the children are females. The traditional African societal view regards male child as the breadwinner; guardian of culture and custom and the one to cater for his parents when they are elderly. Moreover, after the demise of a male household head, the son is expected to carry on the father’s name and lineage while the girls marry and change their family name. Late child wish is also seen, especially among clergies who could not have a child. In most African settings, especially Nigeria, having a male child to inherit properties is a common wish of parents. There are opinions that advanced age of the human male implicitly impacts on overall semen parameters including the morphology of the sperm cells leading to alterations in its fertility potential and eventually impacting the health of the off-spring [4]. It is a common knowledge that females, from the time of birth till the age of about 45 years, possess a predetermined quantity of oocytes which, when depleted, is characterized by menopause, the cessation of monthly menstrual bleeding. However, males' reproductive system continues to produce sperm cells and seminal fluid throughout their lives [4]. It is interesting to note that in Britain, the percentage of those who first attain fatherhood in the age range of 35–54 rose from 25% in 1993 to 40% in 2003 of overall births while, at the same time, the proportion of “first time fathers”, aged below 35 years dropped from 74% in 1993 to 60% in 2003 [5]. Similarly, in other parts of the developed world, comparable changes in increasing age of “fresh fatherhood” have been reported. For instance, a study noted from 1998 through 2008, the average age of those who became fathers for the first time rose by three years in Australia [6] and by two years in Germany [2]. Another study reported a 24% rise in birth rate for fathers aged 35–54 years since 1980 in the United States [7]. Effect of male age on fertilization of a viable egg is an important public health concern since an increase in the male population choosing to father a child in older ages is being observed [8]. A detailed literature review of paternal age and seminal fluid quality revealed decline in quantity of the semen, motility of sperm cells and proportion of morphologically normal sperm cells [9]. Studies have also shown that advanced paternal age has many implications on various aspects of the male reproductive system such as semen parameters, testicular functions, reproductive hormones, sperm DNA integrity, telomere length, de novo mutation rate, chromosomal structure, and epigenetic factors. Others, such as Alshahrani, et al. [18] however expressed divergent views that there are no significant age-related differences in these parameters, but that advanced paternal age is associated with sperm DNA damage. Ho, et al. [20] found no significant effect of paternal age and semen parameters on fertilization rate and pregnancy outcomes. Furthermore, studies have reported confounding factors such as duration of abstinence, average ejaculation frequency, time from sample collection to sample processing and seasonal weather changes in relation to semen parameters. In Africa, there is a paucity of information on paternal age and semen parameters, and few scientists study the male reproductive capacity and advancing
Materials and Methods

Medical records of men aged 27–70, who presented on account of infertility at three Nigerian cities - Lagos, Asaba and Abuja - were examined retrospectively. These men were being evaluated towards providing assisted reproduction technology (ART) necessitating the need for them to provide semen for analysis. The patients for this study consulted between 2010 and 2015 at the Andrology Unit of Nordica Fertility Center. Of the initial 588 patients, 35 (6.0%) declined to provide their semen for analysis, the main reason being that they were in a religious fasting period; 16 (2.7%) absconded and 32 (5.4%) sperm samples were discarded because 14 had inadequate data, time of collection was not specified in eight and days of abstinence were not provided by 10 men. In general, most of the patients consulted because their wives were not getting pregnant, after marriage or again after her first pregnancy despite regular and constant sexual intercourse for at least 24 months, while few just came for male child preference.

Inclusion criteria

All consenting adult males with primary and secondary infertility who presented for fertility assessment; those who were currently or within two weeks prior to analysis, not on any medication, particularly anabolic steroids, antibiotics and antimalarials, or any medication that would have interfered with spermatogenesis were included into the study.

Exclusion criteria

Those with history of sexually transmitted diseases were excluded from the study. Men with past or current history of undescended testis, surgical operations on the testis, urethral disease, chronic liver disease, HIV infection, Renal failure, TB of the Genitourinary system and Diabetes Mellitus.

Semen sample collection

A convenient private room, adjacent to the laboratory was provided for patients to produce semen samples by masturbation after a minimum/maximum of 2-7 days sexual abstinence period. Patients were initially counseled on the need for accuracy in the collection of semen, on the kits (wide-mouth measuring cylinder) to be used for the collection and on the need to report any loss of semen during collection. Each patient has a medical record file with his data such as name, age (or date of birth), days of abstinence, date and time of collection, if there was any loss in semen volume to be used for the collection and on the need to report any loss of semen. Semen samples were evaluated for sperm volume, on the kits (wide-mouth measuring cylinder) and the commencement of seminal fluid were assessed but not used in further analysis. Abnormal semen parameters were defined as earlier detailed [23] and paternal age was stratified (< 40, 40–44, 45–49, 50–54, 55–59 and ≥ 60 years old). Statistics were analyzed using STATA 13, and logistic regression or ANOVA was used to determine significance. The assumed cut-off age in this study was 40 years.

Results

Semen Analysis

All semen samples were received in the laboratory within 30–60 minutes of production. After liquefaction for 30 minutes or maximum of 60 minutes, semen samples were evaluated for sperm count, motility and morphology.

Sperm volume: The volume of the ejaculate was determined by directly reading it on a wide-mouth graduated cylinder provided for each patient [23].

Sperm concentration: Sperm concentration analysis was performed as a function of sperm numbers against volume in 10 squares of the chamber [23] counting at least 200 spermatozoa and expressed as 10^6 spermatozoa/ml.

Sperm motility: Replicate wet samples were assessed under 200X magnification to classify approximately 200 spermatozoa as (i) rapidly forward, fast progressive motility, (ii) moderately forward, slow progressive motility, (iii) jerky non-progressive motility and (iv) immotile/no movement and the motility was expressed as percentage at room temperature [23].

Sperm morphology: Smears were made according to the sperm concentration and the papanicolaou staining method was used to determine the morphology of the sperm cells which were characterized as morphologically normal or abnormal and the final morphology was expressed as percentage. At least 200 spermatozoa were counted fewer than 100X oil immersion objective [23].

The means (± sd) of their age and body mass index (BMI) were 42.5 (7.2), [23] were included in the study. The means (± sd) of duration of marriage and years trying to conceive were 7.2 (4.1) years and 5.7 (3.5) years respectively (Table 1). Majority (340, 67.3%) had fathered at least a child and the main reasons for visiting infertility clinic were (i) their wives were not getting pregnant again (318, 63.0%) and (ii) low sperm count (172, 34.0%) as examined in other laboratories. The Table also shows that male factor, as the cause of infertility at three Nigerian cities - Lagos, Asaba and Abuja - were examined retrospectively. These men were being evaluated towards providing assisted reproduction technology (ART) necessitating the need for them to provide semen for analysis. The patients for this study consulted between 2010 and 2015 at the Andrology Unit of Nordica Fertility Center. Of the initial 588 patients, 35 (6.0%) declined to provide their semen for analysis, the main reason being that they were in a religious fasting period; 16 (2.7%) absconded and 32 (5.4%) sperm samples were discarded because 14 had inadequate data, time of collection was not specified in eight and days of abstinence were not provided by 10 men. In general, most of the patients consulted because their wives were not getting pregnant, after marriage or again after her first pregnancy despite regular and constant sexual intercourse for at least 24 months, while few just came for male child preference.

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Other semen parameters that were recorded included days of abstinence, time of semen production, time of examination, liquefaction time (minutes), color, viscosity, sperm concentration (×10^6/ml) and motility. Sperm morphology indices that were examined included small tapering head (STHD), double amorphous head defect (DAHD), mid-piece defect (Mpd), tail defect (TD) and immature forms (IFs). Fus cells and epithelial cells in the seminal fluid were assessed but not used in further analysis. Abnormal semen parameters were defined as earlier detailed [23] and paternal age was stratified (< 40, 40–44, 45–49, 50–54, 55–59 and ≥ 60 years old). Statistics were analyzed using STATA 13, and logistic regression or ANOVA was used to determine significance. The assumed cut-off age in this study was 40 years.

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Results

In all, 505 apparently healthy adult consecutive males, urban residents, who reported at Nordica Fertility Clinic for Assisted Reproduction Technology (ART) and whose mean (± sd) age was 42.5 (7.2), were included in the study. The means (± sd) of duration of marriage and years trying to conceive were 7.2 (4.1) years and 5.7 (3.5) years respectively (Table 1). Majority (340, 67.3%) had fathered at least a child and the main reasons for visiting infertility clinic were (i) their wives were not getting pregnant again (318, 63.0%) and (ii) low sperm count (172, 34.0%) as examined in other laboratories. The Table also shows that male factor, as the cause of infertility was observed in only 101 (20.0%) of the study subjects. Those who ever smoked cigarettes (372, 73.7%) and those who ever drank alcohol (392, 77.6%) far outnumber those who never did.

The means (± sd) of their age and body mass index (BMI) were 42.5 (7.2) years and 27.4 (4.5) respectively (Table 2). The overall mean days of sexual abstinence before producing semen by ejaculation was 3.9 (4.0) while the mean duration of time between semen production and analysis was 36.1 (17.9) minutes. The
mean duration of days of abstinence by normal weight men was significantly longer than that by obese men (t = 1.68, df = 217.6, p-value = 0.05). All seminal fluids examined were normal whitish-gray colored. There was no significant difference in the proportion of those ≥ 60 years and not extend to 60+. All seminal fluids examined were normal whitish-gray colored. There was no significant difference in the proportion of those ≥ 60 years.

Table 1: Socio-demographic Characteristics of study subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Item</th>
<th>Freq. (%)</th>
<th>Mean (± sd)</th>
<th>Mean (± sd)</th>
<th>Mean (± sd)</th>
<th>Mean (± sd)</th>
<th>Color</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>All</td>
<td>505</td>
<td>42.5 (7.2)</td>
<td>3.9 (4.0)</td>
<td>36.1 (17.9)</td>
<td>505 (100.0)</td>
<td>0 (0.0)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>&lt; 40</td>
<td>178</td>
<td>35.3 (3.1)</td>
<td>3.9 (5.7)</td>
<td>35.2 (18.6)</td>
<td>178 (100.0)</td>
<td>0 (0.0)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td></td>
<td>40–44.9</td>
<td>148</td>
<td>41.7 (1.4)</td>
<td>3.7 (3.1)</td>
<td>35.5 (16.9)</td>
<td>148 (100.0)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td></td>
<td>45–49.9</td>
<td>101</td>
<td>47.0 (1.4)</td>
<td>3.6 (1.5)</td>
<td>37.9 (17.3)</td>
<td>101 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>≥ 50</td>
<td>78</td>
<td>54.5 (4.5)</td>
<td>4.3 (2.8)</td>
<td>36.8 (18.9)</td>
<td>78 (100.0)</td>
<td>0 (0.0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td></td>
<td>BMI Kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.5–24.9 (Normal)</td>
<td>147</td>
<td>22.9 (1.4)</td>
<td>4.0 (3.2)</td>
<td>36.2 (17.4)</td>
<td>147 (100.0)</td>
<td>0 (0.0)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td></td>
<td>25.0–29.9 (Overweight)</td>
<td>240</td>
<td>27.3 (1.3)</td>
<td>4.0 (5.1)</td>
<td>36.9 (18.5)</td>
<td>240 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>≥ 30 (Obese)</td>
<td>118</td>
<td>33.4 (4.5)</td>
<td>3.5 (1.5)</td>
<td>34.1 (17.2)</td>
<td>118 (100.0)</td>
<td>0 (0.0)</td>
<td>1 (0.9)</td>
</tr>
</tbody>
</table>

Table 2: Seminal fluid characteristics and means of age, duration of abstinence and time interval between ejaculate production and seminal fluid analysis relative to paternal age differentiation and BMI groupings. Data should stop at > 49 years and not extend to 60+.

Semen volume (ml), liquefaction time (mins), sperm motility and sperm concentration (x10⁶/ml) was analyzed as illustrated in Table 2. Figure 1 specifically illustrates the differences in means of semen volume in different age groups. In the overall study sample, an insignificant marginal increase was detected in the mean semen volume of men aged 40–44 years (100.0) 0 (0.0) 100 (99.0) 1 (0.1) 0 (0.0) -

volume of men aged 55–59 years (1.5 ± 1.2) and of those aged ≥ 60 years (1.4 ± 0.8) were significantly lower (t = 2.7, df = 27.2, p-value = 0.006 and t = 2.7, df = 9.3, p-value = 0.01 respectively) than those of men aged < 40 years (2.3 ± 1.4). Mean sperm motility among men aged < 40 years (39.9 ± 22.4) was significantly higher when compared only to men aged 55–59 years (t = 2.1, df = 25.2, p-value = 0.02). Probably due to a small sample size, there was no noteworthy difference in sperm motility at age < 40 (n = 178) compared to age ≥ 60 years (n = 8), though the mean of sperm motility was the least (28.3 ± 22.1) in this oldest group.

Figure 2 shows frequency distribution of paternal age and abnormal semen parameters as defined by WHO [23]. The proportion of men who produced < 2 ml of sperm volume was highest in age groups 55–59 and ≥ 60 years and least among those aged 40–44 years; production of sperm concentration of < 20 x 10⁶/ml was most prominent among those aged 55–59 years and motility < 50% was observed more in the last age groups.

Further analysis was conducted on sperm morphology in six


Table 3: Mean semen volume, liquefaction time, sperm motility and sperm counts relative of males in all patients including those with azoospermia and those without azoospermia relative to age.

Discussion

Where the topic of semen and semen parameters or quality is deliberated upon, it is usually in connection with the degree of fecundity and procreation. The present study examined the association of advanced paternal age and some selected semen parameters among normal and apparently healthy black indigenous African men from Nigeria. In all, five hundred and five seminal fluid samples were collected and examined strictly by WHO criteria and further analyzed relative to advanced paternal age. This study noted indications of substantial decrease in some seminal fluid parameter which were supposed to be age-dependent. There was apparently no specific age effect on the observed mean concentration of morphologically defective sperm cells in older age groups compared to those younger than forty years. It is noteworthy to observe that some seminal fluid parameters such as volume, motility and sperm concentration were in fact elevated at age 40–44 years before declining at later ages. Liquefaction time rose steadily, though a transient stabilization was observed between the ages of 40–49 years. The observed increase in liquefaction time may be related to elevated semen viscosity noted in older men. Sperm cells may not be able to swim freely in semen with elevated viscosity. The findings in this study support those for semen parameters in prior clinical studies of infertile patients and sperm donors [9]. This study also shows that racial ethnicity may not have much influence on semen volume as earlier studies were conducted among Caucasians while this investigation was among indigenous blacks in Africa, though this claims still needs further clarification. As with a prior study,
atrophy of the gonads and other accessory organs such as the prostate gland [9]. Furthermore, conditions such as undescended testis, orchitis, mumps and sexually transmitted diseases (STDs) affecting the genitourinary tract (GUT), metabolic and cardiovascular diseases or environmental toxins are possible causative agents which may influence reduced seminal fluid volume as age advances [2].

Some earlier studies showed that sperm morphology may be regarded as sensitive gauge of the state of the germinal epithelium [9,19,30–32]. However, quite unexpectedly, sperm concentration was, in general, insignificantly higher among in older men in our study. This finding accords with what earlier studies reported [9,19,30–32]. However, Hossain, et al. [33] documented an inverse relationship between sperm motility observed in older men, a finding consistent with what Dondorn, et al. [29] Madenovic, et al. [29] and Eskenazi, et al. [7] reported. However, unlike the study of Nieschlag, et al. [24] which included a large sample size of men age over 60, the population of this age group in our study was quite small, which probably explains the insignificant difference when comparing the mean sperm motility in those aged ≥ 60 years and those aged < 40 years. Slow motility combined with increase liquefaction time and higher viscosity apparently spells doom for most viable sperm cells trying to reach the oocyte. Surprisingly, a subtle increase in sperm motility and in sperm concentration was observed at age 40–44 years compared to age < 40 years. This may be a pointer to the fact that the age bar may need to be raised by five years from < 40 to < 45 when deciding a possible cut-off point for advanced paternal age.

Quite unexpectedly, sperm concentration was in general, insignificantly higher among in older men in our study. This insignificant elevated sperm concentration seemed to be driven solely by the increase observed at age 40–54 years. This finding accords with what earlier studies reported [9,19,30–32]. However, Hossain, et al. [33] documented an inverse relationship between advanced paternal age and sperm concentration which is inconsistent with our findings.

The final major finding in our study was the increase noted in percentage of sperms with abnormal morphology. At age 40 years and above, an insignificant increase in the percentage of abnormal sperm cells with small tapering head defect, double amorphous head defect, tail defect and immature forms but not mid-piece defect or normal oval head, was observed in older men. This observation is similar to what other studies reported [25,28,34]. Some earlier studies showed that sperm morphology may be regarded as sensitive gauge of the state of the germinal epithelium of the testes [21,35]. Deterioration in sperm morphology may be related to defective function of the sperm cell [36].
Conclusion

Our findings suggest that, in general, advanced paternal age strongly influences quantity of seminal fluid volume, liquefaction time, and sperm motility time and this may impact on male fertility and management of male infertility considering rising paternal age.

Strengths and limitations

This study has some limitations that need to be addressed. Firstly, the sample was a convenient sample of men who consulted at Nordica Fertility Center for partial or complete infertility and who were involved in assisted reproduction technique (ART). Therefore, being a clinic-based assessment, the results may not be representative of all indigenous black men in Nigeria who are semi-or completely infertile. There was no information on men who did not participate in the study. Secondly, the sample size was skewed towards the younger age group. All in all - the number of men in age Groups above 50 was low compared to younger men and especially men aged ≥ 60 years had the least sample size. Therefore, this could have introduced a bias into the results of our study. Thirdly, being a retrospective study, raw data was extracted from the medical files of patients without any transformation, adjustment or weighting. This may have affected the methodological quality of data. Fourthly, the cut-off point of 40 years was arbitrarily designated and this may have reflected the elevated parameters observed in age 40–44 years. A probable cut-off point may have been either 45 or 50 years. Fifth, all study subjects were urban residents which excluded information on this topic from rural dwellers. Lastly, we did not categorize study subjects into smokers and non-smokers, drinkers and non-drinkers, though these variables were recorded for all. This would be addressed in another study. Perhaps, with careful adaptation, the significant intervention characteristics of this study can be made applicable to men in rural settings and to other subgroups of men such as heavy smokers, alcoholics and those with sexually transmitted diseases (STDs), who have similar level of risk. Further investigations on how extensive seminal fluid analysis can be adapted to diverse situations and sub-populations would be valuable.

Recommendations

We recommend a wider prospective study with bigger sample size of each age group and taking diverse lifestyles and other practices, such as use of alcohol and other medications, (including herbal teas, common in sub-Saharan Africa for sperm ‘boosting’ or improving male virility) into consideration. A multi-center African Study is also recommended.

Conflict of Interest

The authors declare no conflict of interest be it financial or in any other form.

References


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