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Original Article

Biochemistry Section

Effects of Paternal Age on Semen Quality and Status of Fertility at a Tertiary Care Hospital, West Bengal, India: A Cross-sectional Study

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ABSTRACT

Introduction: Modern couples are inclined to postpone conception as a result of societal pressures and expectations during the last several decades. The impact of age on spermatogenesis in men is not much studied. While there are no known limits to the age at which men can father the children, the implications of Advanced Paternal Age (APA) are poorly understood.

Aim: To assess the relationship between advancing age and aberrant sperm parameters in the male partners of sub fertile couples in West Bengal, India.

Materials and Methods: This cross-sectional hospital-based study was carried out in the Department of Physiology, R.G. Kar Medical College and Hospital, West Bengal, India, from February 2020 to January 2021. A total of 56 male subjects of couples, who have been screened as subfertile, in the age group of 21-40 years were enrolled in the study. Semen analysis was done by light microscopy using a Makler counting chamber after three days of abstinence according to the World Health Organisation (WHO) 5th edition 2010 guidelines. The data was evaluated with the use of an Microsoft Excel sheet and Statistical Package for the Social Sciences (SPSS) software version 20.0 by employing a Chi-square test.

Results: In the present study, the study population consisted of 56 male partners of subfertile couples with a mean age of 29.68±5.87 years. Thirty one subjects (55.4%) out of 56 subjects had abnormal semen parameters. Most common abnormality detected was asthenozoospermia (n=10, 17.9%) followed by oligoasthenozoospermia (n=9, 16.1%) and oligozoospermia (n=6, 10.7%). There was significant association of APA with aberrant semen parameters.

Conclusion: While asthenozoospermia was the most common semen abnormality, oligoasthenozoospermia was mostly found among the males of higher age groups. Clinicians could advise genetic counselling and DNA fragmentation assay to potentially prevent mishaps of delayed paternity.

Keywords: Asthenozoospermia, Male infertility, Oligoasthenozoospermia, Semen analysis

INTRODUCTION

Clinically, infertility is defined as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. In India, the prevalence of primary infertility ranges from 3.9% to 16.82% [2]. The male component represents roughly 40-50% of the overall infertility [3]. Male infertility is indicated by decreased sperm concentration, motility, or aberrant sperm morphology [4]. Semen analysis is the simplest and most cost-effective test for detecting and providing information on male factor infertility.

Infertility is a severe worldwide issue that affects not only the health of couples but also has economic, demographic, and socio-cultural ramifications. Infertility approximately affects 8-12% of couples worldwide [5,6]. The incidence of infertility is highest in nations with high fertility rates, a concept known as barrenness amid plenty [7].

The availability of oocytes and the coming onset of menopause simultaneously constrain the window of fertility in women. Men, on the other hand, tend to be relatively untouched by the concept of this reproductive cascade. In fact, males have long been considered impervious to the implications of ageing on fertility in both a physiological and social sense. Official accounts of elderly men fathering children have boosted awareness and acceptance of Advanced Paternal Age (APA) in modern times, as advances in assisted reproductive technologies have changed the course of reproductive medicine [8-10].

Rates of unexpected births have declined and parental age at first birth has increased due to higher career and educational goals, as well as better life expectancy and near universal availability of contraceptives [11]. Additionally, the success of Invitro Fertilisation (IVF) has provided many couples with a reproductive security blanket, ensuring that if traditional conception techniques fail, the technology will save them [11].

Statistics on maternal age are readily available particularly from birth certificates, but paternal information is frequently lacking, and the age of the father is not reported in 14% of all births [12,13]. As there is no uniform definition, it is difficult to present statistics on APA. The current mean paternal age is 27 years, and the accepted APA threshold at conception is >40 years of age [14]. Thus, there is indeed a need for comprehensive data collection, guidelines, and outcome research on APA.

Hence, the present study was conducted to assess the relationship between APA and aberrant sperm parameters in the male partners of subfertile couples in the Indian population.

MATERIALS AND METHODS

A cross-sectional hospital-based study was carried in the Department of Physiology, R.G. Kar Medical College and Hospital (tertiary care institution), Kolkata, West Bengal, India, from February 2020 to January 2021. Study was commenced after taking Institutional Ethical Committee approval (IEC no is RKC/122). Prior to inclusion in the study, informed consent of the subjects was obtained and confidentiality was adequately maintained.

Inclusion criteria: Male subjects aged between 21-40 years, of the couples who have been primarily screened as subfertile at the Out-Patient Department of Gynaecology and Obstetrics, R.G. Kar Medical College and Hospital, Kolkata, India were included in the study. Subfertility is described as any form of reduced fertility with prolonged time of unwanted non conception [15]. Infertility has two types: primary infertility refers to couples who have not become pregnant after at least one year having sex without using birth control methods. Secondary infertility refers to couples who have been able to get pregnant at least once, but now are unable [16].

Exclusion criteria: Subjects having urological/ postsurgical complications, history of testicular injury, congenital defects (e.g., cryptorchidism, testicular atrophy), venereal diseases or any form of active orchitis, and those who were unwilling to undergo the tests, were excluded from the study.

Sample size calculation: As per Zargar AH et al., prevalence of infertility due to male factor exclusively, i.e. male infertility in India is 23% [17].

$$\text{Sample size (n)} = \frac{(Z_{1-\alpha/2})^2 \times p \times (100-p)}{L^2},$$

where, $(Z1-\alpha/2) = 1.96$

L = precision in absolute terms=9

p=23

$$= \frac{(1.96)2 \times 23 \times 77}{81} = 83.99 \sim 84 \text{ subjects (approx.)}$$

Calculated sample size was 84 subjects; however, authors were able to include 56 subjects for the study due to the Coronavirus Disease 2019 (COVID-19) pandemic situation. Hence, total of 56 study subjects were incorporated in the study by non random purposive sampling.

Study Procedure

A detailed history of the couple's infertility (primary or secondary), age, occupation, medical history, and any surgical intervention was recorded on a predesigned proforma. Each of the male partners of the subfertile couples was carefully clinically examined with proper medical history as per case record proforma. The clinical examination included genital examination, anthropometric measurements and blood pressure measurement to confirm the absence of any of the conditions mentioned in the exclusion criteria. Body Mass Index (BMI) was calculated using height and weight measurements to be categorised according to the BMI categories in Asian populations [18]. Finally, the subjects were asked to provide semen samples for semen analysis.

After three days of abstinence, semen was collected in a sterile container at the hospital. According to World Health Organisation (WHO) 5th edition 2010 standards [4], the sample was examined for volume and pH before being further evaluated by light microscopy using a Makler counting chamber for sperm concentration, motility and morphology.

a. **Volume:** The volume of the ejaculate is measured by a graduated centrifuge tube, which serves both as the measuring cylinder and the storage vessel of the sample.

b. **pH:** The pH of the sample is recorded with the help of litmus paper, by evenly spreading one drop of semen onto the pH paper and after 30 seconds, the colour change is compared with the calibration strip.

c. **Microscopic examination:** Fixed volume of semen e.g., 10 µL, was taken on to a clean glass slide with micropipette and covered with a 22×22 mm coverslip to provide a chamber which allowed the spermatozoa to swim freely. The weight of the cover slip spreads the sample evenly. After stabilising for one minute, wet preparation was examined under a binocular compound light microscope. It was ensured that no air bubbles were trapped or formed between the slide and the coverslip.

d. **Motility:** Viewing fields were selected at least 5 mm away from the edge of the coverslip, to avoid observation of effects of drying on motility. The slide was examined under 400X magnification with phase-contrast optics after the sample had stopped drifting. The slide was systematically scanned to view a minimum of 5 High Power Fields (HPF) and at least 200 spermatozoa were evaluated. Initially the Rapidly Progressive (RP) cells were scored, then the Non Progressive (NP) cells and finally the Immotile (IM) spermatozoa were counted. Percentages of each motility category of cells were calculated and recorded.

e. **Assessment of sperm concentration:** The sperm concentration of the semen sample was evaluated by the means of Makler counting chamber. It is a simple-to-use device for rapid and accurate sperm concentration evaluation from undiluted specimens.

The lower reference limit according to WHO laboratory manual is as follows [4]:

- Volume- 1.5 mL (95% CI: 1.4-1.7)
- Sperm concentration- 15 million spermatozoa/ml (95% CI: 12-16)
- Total motility (progressive +non progressive motility)- 40% (95% CI: 38-42)
- Progressive motility- 32% (95% CI: 31-34)
- Sperm morphology- 4% normal forms (95% CI: 3-4)
- Presence of RC- <10/HPF.
- Normozoospermia (Nzs): Total number (or concentration) of spermatozoa, and percentages of progressively motile and morphologically normal spermatozoa, equal to or above the lower reference limit.
- Oligozoospermia (Ozs): Total number (or concentration) of spermatozoa below the lower reference limit.
  - 5-15 million sperms/mL of seminal fluid → mild-moderate oligozoospermia
  - <5 million sperms/mL of seminal fluid → severe oligozoospermia.
- Asthenozoospermia (Ass): Percentage of progressively motile spermatozoa below the lower reference limit.
- Oligoasthenoteratozoospermia (OATS): Total number or concentration of spermatozoa, and percentages of progressively motile and morphologically normal spermatozoa, below the lower reference limit.
- Azoospermia (Azs): No spermatozoa in the ejaculate.
- Leucocytospermia (Lcs): Presence of leukocytes in the ejaculate above the threshold value.

STATISTICAL ANALYSIS

The data was evaluated with the use of an Microsoft Excel sheet and Statistical Package for the Social Sciences (SPSS) software version 20.0. Data was presented in percentage. The Chi-square test was used for univariate analysis with a 95% degree of confidence limit. A p-value <0.05 was taken to indicate a significant difference.

RESULTS

The mean age of the subjects in this study was 29.68±5.87 years, with an age range of 21-39 years. Average duration of marriage was

5.8 years with majority 32 (57.1%) married for 0-5 years. Majority of the participants in the study 38 (67.9%), sought consultation due to primary infertility. Nineteen (33.9%) of the study subjects had normal BMI [Table/Fig-1].

Characteristics		n (%)
Age group (years)	21-25	15 (26.8)
	26-30	18 (32.1)
	31-35	12 (21.4)
	36-40	11 (19.6)
Body mass index	Normal	19 (33.9)
	Overweight	12 (21.4)
	Preobese	17 (30.4)
	Obese type 1	8 (14.3)
Duration of marriage (years)	0-5	32 (57.1)
	6-10	16 (28.6)
	11-15	6 (10.7)
	16-20	2 (3.6)
Infertility	Primary	38 (67.9)
	Secondary	18 (32.1)

[Table/Fig-1]: Baseline characteristics of the study subjects.

In the present study, the mean ejaculate volume of the subjects was 3±1.2 mL, mean pH was 7.4±0.32, mean total motility was 48.9±20.2% and the mean sperm concentration was 49.7±42.6 million/mL. Out of the total (56) study population, 25 (44.6%) were normozoospermic, 10 (17.9%) had asthenozoospermia and 6 (10.7%) had oligozoospermia. Leucocytospermia was seen in 3 (5.4%) subjects and azoospermia was seen in only 1 (1.8%) subject. Multiple semen parameter derangements were detected in 11 (19.6%) study subjects. Out of these 11 cases, 9 (16%) subjects had oligoasthenozoospermia, and 2 (3.6%) subjects had OATS [Table/Fig-2].

Semen parameters	n	Percentage
Normozoospermia	25	44.6%
Asthenozoospermia	10	17.9%
Oligozoospermia	6	10.7%
Leucocytospermia	3	5.4%
Azoospermia	1	1.8%
Oligoasthenozoospermia	9	16.1%
Oligoasthenoteratozoospermia	2	3.6%

[Table/Fig-2]: Frequency distribution of abnormal semen characteristics.

On stratifying the study subjects according to their age to the quality of semen, authors observed that 13 (52%) of all normozoospermics were found in the 26-30 years of age; 4 (40%) of all asthenozoospermics were found in the 21-25 years of age; 3 (50%) of all oligozoospermics were found in the 31-35 years of age; 5 (55.6%) of all oligoasthenozoospermics were found in the 36-40 years of age; the sole case of azoospermia was found in the 26-30 years of age; and the oligoasthenoteratozoospermic subjects were found equally both in the 21-25 years and 31-35 years of age [Table/Fig-3].

The present study showed the age wise distribution of semen abnormalities, it was observed that advancing age of the subjects showed statistically significantly higher rates of semen abnormalities (p-value=0.013) [Table/Fig-4].

Semen quality		Age category (years) 21-25	26-30	31-35	36-40	Total (n)
Semen quality	Nzs	7 (28%)	13 (52%)	2 (8%)	3 (12%)	25
	Ass	4 (40%)	1 (10%)	3 (30%)	2 (20%)	10
	Ozs	1 (16.7%)	1 (16.7%)	3 (50%)	1 (16.7%)	6
	Lcs	1 (33.3%)	1 (33.3%)	1 (33.3%)	0	3
	Oas	1 (11.1%)	1 (11.1%)	2 (22.2%)	5 (55.6%)	9
	Azs	0	1 (100%)	0	0	1
	OATS	1 (50%)	0	1 (50%)	0	2
Total (n)		15	18	12	11	56

[Table/Fig-3]: Frequency distribution of study subjects according to their semen quality into different age groups.
Values are presented as n (%); Nzs: Normozoospermics; Ass: Asthenozoospermics; Ozs: Oligozoospermics; Oas: Oligoasthenozoospermics; Azs: Azoospermic; OATS: Oligoasthenoteratozoospermic

Age category		Semen parameters Normal n (%)	Abnormal n (%)	Total (n)	p-value
	21-25	7 (46.7%)	8 (53.3%)	15	**0.013**
	26-30	5 (27.8%)	13 (72.2%)	18	
	31-35	2 (16.7%)	10 (83.3%)	12	
	36-40	3 (27.3%)	8 (72.7%)	11	
Total		25	31	56	

[Table/Fig-4]: Association of age with the presence of semen abnormalities.
p-value in bold font indicates statistically significant value (Chi-square test)

DISCUSSION

Present hospital-based study was conducted to investigate semen parameters in order to determine male factor infertility and its relationship with advancing age of males. The most prevalent anomaly found in the study population (17.9%) was asthenozoospermia. Similar to this result of present study, Bodal VK et al., and Garg J et al., observed asthenozoospermia as the most prevalent semen abnormality in their investigations, with a prevalence of 17% and 14.3%, respectively [19,20]. However, Bhaduri N et al., and Kulkarni SN and Kulkarni NV found the prevalence of asthenozoospermia in their study population to be 4.4% and 3.6%, respectively [21,22].

Oligoasthenozoospermia was seen in 16.1% subjects, which is similar to that reported by Bodal VK et al., [19]. However, studies by Garg J et al., and Kulkarni SN and Kulkarni NV reported the prevalence of the same as 6.8% and 4.1% [20,22]. Oligozoospermia was seen in 10.7% cases which is similar to Bodal VK et al., and Garg J et al., [19,20]. Azoospermia and OATS were detected in 1.8% and 3.6%, respectively. Studies by Garg J et al., and Kulkarni SN and Kulkarni NV found the prevalence of these semen abnormalities to be 10.9% and 7.3%, and 34.4% and 22.1%, respectively [20,22]. In the present study, overall prevalence of aberrant sperm parameters was 55.4%, which was similar to studies conducted by Bodal VK et al., and Kulkarni SN and Kulkarni NV [19,22]. However, this higher percentage of male infertility with respect to accepted national prevalence is possibly due to small sample size and variation in geographical factors [19,22].

In this study, mean age of the study population was 29.68±5.87 years. Subjects in the age group of 26-30 years had higher frequency of normal semen quality. Oligozoospermics were seen mostly in the 31-35 years age group; while asthenozoospermia was seen majorly in the age group of 21-25 years. Oligoasthenozoospermia was seen in the 36-40 years of age group. Eventually, subjects in the age groups 31-35 years and 36-40 years had higher incidences of semen abnormalities. Therefore, it can be concluded that advancing age of the subjects showed statistically significant (p-value=0.013) higher rates of semen abnormalities (especially oligoasthenozoospermia). The findings of this present study were supported by a study conducted by Kidd SA et al., who found that increase in male age

is associated with a decline in semen volume, sperm motility and sperm morphology [9]. In another study conducted by Asha A, it was found that age is intimately linked to the decrease in the motility of sperm and vitality, while the least effect is observed on the number of spermatozoa [23].

At least two main modes of action may explain the age-dependent changes observed in semen quality [24]. First, there may be cellular or physiological changes in the genitourinary tract with aging. Autopsies of men revealed that there was age-related narrowing and sclerosis of the testicular tubular lumen, decreased spermatogenic activity, increased germ cell degeneration, and decreased number and function of Leydig cells. The decrease in sperm volume with age can be caused by insufficient seminal vesicles. Changes in the prostate that occur with aging, such as atrophy of smooth muscles, decreased protein and water content, can contribute to decreased semen volume and sperm motility. In addition, there may be age-related changes in the epididymis where the sperm acquires the capacity for rapid and progressive motility. Since the epididymis is a tissue sensitive to hormones, hormonal senescence can lead to decreased motility in older men. Additionally, older men may have a reduced ability to repair cell and tissue damage. Second, age offers a greater possibility of reproductive harm from exogenous exposure or disease.

Comparison of the present study with contrast studies are shown in [Table/Fig-5] [9,19-23].

Authors name and reference no.	Place and year of the study	Sample size	Prevalence
Bodal VK et al., [19]	Government medical college, Patiala Punjab, 2014	100	• Asthenozoospermia (17%) • Oligo-asthenozoospermia (13%) • Oligozoospermia (7%) • Azoospermia (6%)
Garg J et al., [20]	Lady Hardinge Medical College and Smt Sucheta Kripalini Hospital, Delhi 2020	400	• Asthenozoospermia (14.3%) • Oligozoospermia (13.8%) • Azoospermia (10.5%) • Teratozoospermia (10.5%)
Bhaduri (Bhattacharyya) N et al., [21]	Burdwan Medical college, West Bengal, 2015	161	• Oligozoospermia (19.9%) • Azoospermia (12.4%) • Asthenozoospermia (4.4%)
Kulkarni SN and Kulkarni NV [22]	MIMSR Medical College, Latur , Maharasshtra, 2017	220	• Asthenozoospermia (19.9%) • Oligozoospermia (18.6%) • Azoospermia (10.9%) • Oligoasthenoteratozoospermia (7.3%) • Oligoasthenozoospermia (6.8%) • Leucocytospermia (15.5%)
Kidd SA et al., [9]	Review of English language 2001	-	Decreases in semen volume of 3%-22%, decreases in sperm motility of 3%-37%, and decreases in percent normal sperm of 4%-18% were likely when comparing 30-year-old men to 50-year-old men. increased male age is associated with a decline in semen volume, sperm motility, and sperm morphology but not with sperm concentration.
Asha A [23]	SMS Hospital, Jaipur 2014	102	A significant decline was observed in sperm motility and vitality above the age of 30 years as compared to below than 30 years of age. A significant inverse correlation was found between sperm motility and vitality to age (31-45 years).
Present study	RG Kar Medical College , Kolkata, West Bengal, 2020-2021	56	• Asthenozoospermia (17.9%) • Oligozoospermia (10.7%) • Leucocytospermia (5.4%) • Azoospermia (1.8%) • Oligoasthenozoospermia (16.1%) • Oligoasthenoteratozoospermia (3.6%) • Subjects in the age groups 31-35 and 36-40 years had higher incidences of semen abnormalities. Hence, advancing age of the subjects showed significantly higher rates of semen abnormalities

[Table/Fig-5]: Comparison of the present study with other similar studies [9,19-23].

Over and above that, the children of fathers with APA also exhibit increased chances of genetic abnormalities (like Trisomy 21), cancers, autism and other psychiatric disorders [11]. However, no screening or diagnostic evaluation currently exists to specifically test for such disorders until after conception. Hence, it is recommended that older men desiring children of their own, should undergo genetic counselling to address particular concerns, and Deoxyribose Nucleic Acid (DNA) fragmentation assay should be included in their reproductive function analysis.

Limitation(s)

Certain limitations are to be mentioned with regard to this present study that the population selected to assess this link are only male partners of couples wishing to have children, and not the general male population. More importantly, the COVID-19 pandemic limited the scope of the study severely as there was a dearth of subjects for the study, leading to a small sample size.

CONCLUSION(S)

Male factor infertility is a common cause of infertility that has a substantial psychological and social impact on the couples who are affected. A substantial majority (55.4%) of the participants in the current research had aberrant sperm parameters. The most prevalent anomaly in the higher age groups was oligoasthenozoospermia. From this study, it can be affirmed that in the present environment, a man's reproductive age should be considered just as essential as a woman's age, but more research is needed to determine the strength of these correlations. This study will serve as guidance to clinicians when advising men on the decision to defer fatherhood and the dangers associated with late-life conception.

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