

# Semen quality and age-specific changes: A study between two decades on 3729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city

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**Objective:** To compare the semen quality and age-specific changes in men between the 1980s and 2000s.

**Design:** Prospective study.

**Setting:** Andrology laboratory, University of Calcutta, India.

**Patient(s):** A semen sample was obtained from 3729 men presenting for infertility problems in two distinct decades, that is, between 1981–85 and 2000–2006.

**Intervention(s):** Subjects with sperm count  $>20 \times 10^6/\text{mL}$  without any extreme pathological disorders were selected. Samples having a major liquefaction problem were excluded.

**Main Outcome Measure(s):** A standard World Health Organization procedure for semen analysis was performed that included assessment of volume, sperm concentration, and percentage motility. The motility parameters were further classified into forward progressive motility and nonprogressive motility.

**Result(s):** The present large-scale study confirms a significant decline in the sperm motility parameters and seminal volume in the present decade. However, no change in overall sperm concentration was noted. A decline was seen in sperm motility with increasing age in both decades.

**Conclusion(s):** There are significant changes in sperm motility and volume between the two decades, and the age-related changes in semen parameters are also different in the two decades. (Fertil Steril® 2009; ■: ■–■. ©2009 by American Society for Reproductive Medicine.)

**Key Words:** Sperm count, motility, age, pollution

During the last few decades there has been an increasing amount of debate regarding the issue of declining semen parameters. Declining sperm count was first reported in 1974 by Nelson and Bunge (1), and this was thought to be due to changes in the environment and lifestyle. Since then, numerous works have been carried out that try to establish a connection between sperm function and environmental and occupational exposures (2, 3). In an urban population, the study has attained importance after the observations that human semen showed significant concentrations of environmental toxins such as heavy metals and chlorinated pesticides

(4). In 1992, Carlsen et al. reported a global decline in sperm density between 1938 and 1990 (5). Swan et al. (6) published a reanalysis of the studies included by Carlsen et al. In that analysis, they found significant declines in sperm density in the United States, Europe, and Australia. However, they found no decline in sperm density in non-Western countries, for which data were very limited.

Numerous studies have reported a similar decline (7–9). However, there are also reports denying this decline in some major studies conducted throughout the world (10–13), and some others that even showed an increase in total motile sperm count (14). Becker and Berhane (15) conducted a meta-analysis of 61 sperm count studies. A significant decline was found only in U.S. studies. Studies from specific sites have found declines in sperm counts, but a worldwide decline has not been demonstrated.

To carry out a systematic, scientific study to determine the decline in human semen quality is a difficult task (16). The majority of the studies conducted so far suffer from either one or many of the following defects: [1] selection bias of

Received October 29, 2008; revised January 13, 2009; accepted January 23, 2009.

D.M. has nothing to disclose. A.C.V. has nothing to disclose. M.P. has nothing to disclose. S.K.B. has nothing to disclose. A.K.B. has nothing to disclose. R.K.S. has nothing to disclose. A.A. has nothing to disclose.

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subjects, [2] inclusion of pathological disorders, [3] low number of study population, [4] improper statistical modeling, [5] regional and temporal differences, and [6] intertechnician variability in scoring.

Very few studies have been conducted in the Asian countries, especially on the Indian subcontinent. Hence, the global statistics are bound to be biased and inaccurate. Recently, Marimuthu et al. (17) conducted a study in India for the evaluation of trend in semen analysis for 11 years in 1176 subjects attending a fertility clinic. However, they did not find any overall decline in sperm count. Another important study was done in Mumbai, India, that indicated a decline in semen quality in a selected Indian population over a decade (18).

The present study is the first of its kind on the Indian subcontinent in which we compared two distant decades, the 1980s and the 2000s, on a large study population consisting of 3729 men attending an andrology lab with normal sperm count in the city of Kolkata (formerly Calcutta) in India. Emphasis was given not only to sperm count but also to the percentage motility, percentage forward progressive motility (FPM), seminal volume, and age-specific changes.

## MATERIALS AND METHODS

The study was carried out according to the guidelines and approval of the ethics committee of the University of Calcutta, India.

### Subject Enrollment

To avoid selection bias and error, we selected only those men having count  $>20 \times 10^6/\text{mL}$ . Since the morphology assessment procedure was not homogeneous, this parameter was omitted from the study. Samples having a major liquefaction problem were also omitted. The semen analysis was carried out according to World Health Organization (WHO) guidelines (19), which were first published as a manual in 1980 (there were four editions of the manual altogether, in 1980, 1987, 1992, and 1999) in the andrology laboratory, University of Calcutta. Since it is a decade-based study, there is no possibility of seasonal variations. To avoid regional variation, the study population belonged to the city of Kolkata. A total of 1752 men constituted the study population in the 1980s, while data on 1977 men were collected in 2000s. However, it should be borne in mind that the results of the study only reflect the semen quality and age-related changes of men attending an andrology laboratory with infertility-related problems and thus should not be extrapolated to the condition of the general population. Nonetheless, since the choice of subjects was constant throughout and extreme pathological disorders were eliminated by selecting only  $>20 \times 10^6/\text{mL}$  sperm count, the study definitely gives an idea of the changing semen quality between the decades.

In the last few years, there has been an ever-increasing amount of discussion about the influence of the environment on man. The long-term side effects of environmental risk fac-

tors on human reproduction have been a central issue in these discussions. Kolkata has been designated as one of the most polluted cities in the world (20). Hence, the present study is also of high relevance for finding out whether there is any overall correlation between semen parameters and environmental risk factors. Thus we have also given a survey account of the pollution status of Kolkata during these decades showing the amount of risk factors present as well as an account of the increase in vehicular pollution.

**Subjects** Semen samples were obtained from the male partner of the couple attending the andrology laboratory for infertility-related problems. A total of 1752 men constitute the study population in the 1980s (from 1981 to 1985), while data on 1977 men were collected in the 2000s (from 2001 to 2006). The mean age ( $\pm$ SD) in the study populations are 33.24 ( $\pm$ 6.126) and 35.17 ( $\pm$ 5.043) in decades 1 and 2, respectively.

**Semen analysis** Each subject produced a semen sample by masturbation into a sterile wide-mouthed plastic specimen container. The men were instructed to abstain from ejaculation for 2–5 days before producing the semen. The sample was allowed to liquefy at 37 °C for 20 minutes before analysis. Measurement of both sperm concentration and motility was done in a prewarmed (37 °C) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel).

A standard WHO procedure (19) for semen analysis was performed that included assessment of volume, sperm concentration, and percentage motility. The motility parameters were further classified into FPM and nonprogressive motility.

### Statistical Analysis

Statistical analysis of the data was performed using S-Plus 2000, MINITAB Statistical Software (Version 13.31; Minitab Inc., State College, PA) and Microsoft Excel 2003 (Microsoft, Redmond, WA). The distributions of the variables (i.e., semen parameters) were examined separately for both decades, the 1980s and 2000s, and were found to be approximately normal for some parameters but not for all. Usual transformations, like log transformation and square root transformation, were considered, but they did not show much improvement. Hence, differences between semen parameters in the two decades were examined both by means of paired *t*-test (parametric test) and Mann-Whitney *U*-test (nonparametric test).  $P < .05$  was considered statistically significant. The Pearson product moment correlation coefficient was used to measure the degree of linear relationship between age and the semen parameters (or some transformation, where necessary) for the two decades.

## RESULTS

The results clearly suggest that there has been a significant decrease in semen volume (in milliliters), overall sperm motility (percent), and FPM (percent) in the 2000s when compared with the 1980s.

The overall sperm concentration ( $\times 10^6/\text{mL}$ ) did not show any significant change between the two decades. Apart from semen volume, all the other semen parameters showed a significant correlation with increasing age.

In both the decades, overall motility (percent) as well as FPM (percent) decreased with the increase of age, but the concentration in both decades increased with the increase of age. However, the rate of decrease of motility (percent) as well as FPM (percent) with increasing age is greater in the 2000s. Again, the rate of increase of concentration ( $\times 10^6/\text{mL}$ ) with increasing age was higher in the 2000s.

The semen characteristics of the study populations, that is, concentration, volume, motility, and FPM for the two decades are presented in Table 1 and in Figure 1A–1D. A significant decline in the semen volume, sperm motility, and percent FPM was seen in the 2000s as compared with the 1980s. Distributions of age in the samples from the two decades are shown in Figure 2. The range of age was 22–62 years. The mean age ( $\pm\text{SD}$ ) in the study population was  $33.24 \pm 6.13$  and  $35.17 \pm 5.043$  years in the 1980s and 2000s, respectively.

Pearson correlation coefficients were calculated to study the nature of the dependence of the sperm parameters on age for both decades. The correlation coefficients and the associated *P*-values are shown in Table 2. Significant correlations were observed between age and all the semen parameters. However, the linear relationship was not significant in both decades for volume. Linear regressions for concentration, motility, and FPM on age are shown in Figure 3A–3F. We found that in both decades, overall motility (percent) as well as FPM (percent) decreased with the increase of age. However, in both decades we see an increase in sperm concentration with advancement of age.

The difference in the rate of change of semen parameters with increase in age between the two decades was calculated. An annual decrease of 0.415% in motility with age was observed in the study population in the 2000s versus an annual decrease of 0.314 percent (error,  $\pm 0.108\%$ ) in 1980s. Simi-

larly, the FPM had a lower decrease of 0.319% (error,  $\pm 0.107\%$ ) per year with age in 1980s as compared with a decline of 0.376% (error,  $\pm 0.179\%$ ) per year in 2000s.

Interestingly, the rate of increase in sperm concentration with increase in age was less in the 1980s (0.735; error,  $\pm 0.216\%$ ) compared with the 2000s, during which it was 0.921 (error,  $\pm 0.356\%$ ).

## DISCUSSION

The outcomes of the present study should be analyzed both from the global perspective as well as in the context of the changing milieu of the city of Kolkata between these two decades. However, as mentioned before, the result of the study reflects the changing semen quality of men attending an andrology laboratory with infertility-related troubles and thus should not be equated with the condition of the general population. Even so, as the choice of subjects was constant throughout and extreme pathological conditions were eliminated by selecting only those patients with a sperm count  $> 20 \times 10^6/\text{mL}$ , the study definitely gives an idea of the changing semen quality between the decades. Before analyzing the outcome of the results, a brief account of the survey conducted on the pollution status of the city of Kolkata should be taken into consideration.

The data published by the World Bank show that as of 2002, Kolkata was the third most polluted city in the world based on the content of particulate matter (20). Although proper statistics of the environmental conditions of the 1980s in Kolkata are difficult to obtain, according to a survey conducted by National Environmental Engineering Research Institute, Nagpur (21), the oxides of nitrogen ( $\text{NO}_x$ ) and carbon monoxide (CO) concentrations in the ambient air have steadily increased in Kolkata from the 1970s to 2000.

Transport is now the dominant source of CO,  $\text{NO}_x$ , and lead (Pb, and other heavy metals) in Kolkata through the growth in motor vehicle traffic in recent years. Transport emissions have risen from an estimated 1825 tons per annum in 1970 to 25,550 tons per annum in 1990 in Kolkata (22).

**TABLE 1**

**Semen characteristics in the two decades.**

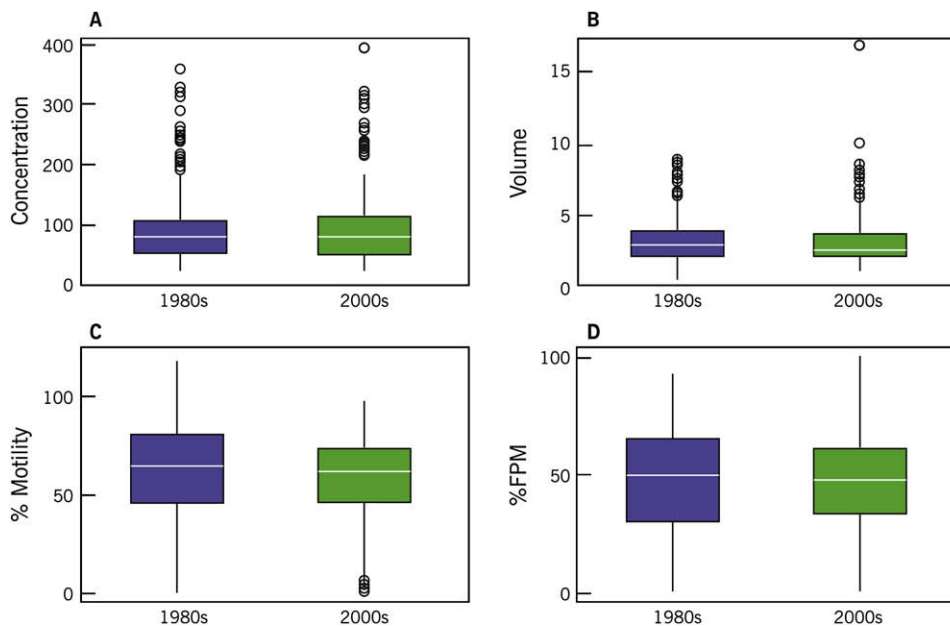
Semen parameters	1980s			2000s			Nonparametric (Mann-Whitney) test <i>P</i>
	Mean (SD)	Median	IQR	Mean (SD)	Median	IQR	
Concentration, $\times 10^6/\text{mL}$	83.91 (46.55)	80.00	50–105	87.01 (53.50)	77.00	47–111	.403
Volume, mL	2.9715 (1.395)	2.700	2.0–3.7	2.705 (1.192)	2.500	2.0–3.5	.000
Motility, %	60.682 (23.40)	65.00	45–80	57.893 (19.84)	60.00	46–73	.000
FPM, %	47.539 (23.16)	50.00	30–65	46.732 (20.65)	47.00	33–60	.012

Note: IQR = Interquartile range.

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## FIGURE 1

A graphical comparison of the semen characteristics (A–D) in the two decades using box plots.



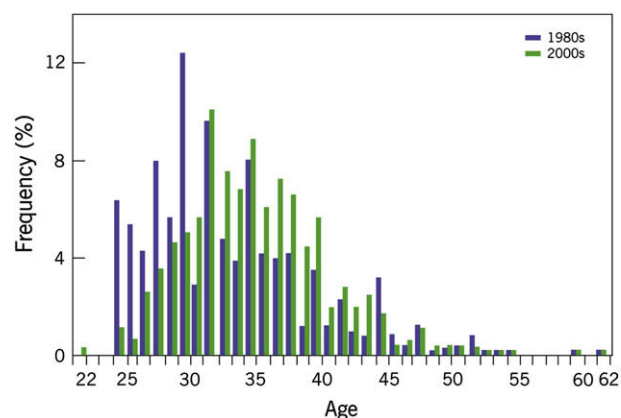
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The 2000s also showed an incredible rise in the number of auto-rickshaws (motor-driven three wheelers) in Kolkata, which increased the vehicular pollution in the city manifold because they use a highly polluting mixture of solvent and kerosene, which is a much cheaper alternative to standard fuels or liquid propane gas. An investigation carried out on behalf of a leading daily (the *Telegraph*, India) confirms the use of highly toxic fuel mixtures in auto-rickshaws (23).

According to a recent report by Centre for Science and Environment, the concentration of respirable particulate matter (RPM) in the city of Kolkata is 1.5 times the national standard (24). The level of hazardous  $\text{NO}_x$  in the ambient air is  $65 \mu\text{g}$ , which is 5 points above the national standard. Regarding the ambient air quality status of the 2000s, we have data from the Central Pollution Control Board, India, as well as from the West Bengal Pollution Control Board, India. According to the data of the Central Pollution Control Board, India, in the 2000s,  $\text{NO}_x$ , RPM, and suspended particulate matter (SPM) are all in high or critical states in Kolkata (25).

## FIGURE 2

Distributions of age in the samples from the two decades: 1980s and 2000s.



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The West Bengal Pollution Control Board also substantiates the fact that in the 2000s, SPM, RPM, and  $\text{NO}_x$  levels were above the normal range in the city (26). Another major concern is the excessive contamination of toxic chemicals through food intake. Since associations among pesticide exposure, semen parameters, and reproductive outcomes have been widely studied (27, 28), this factor needs to be looked into based on this city's background. A pilot study conducted by the pollution board in Kolkata revealed that vegetables grown on land irrigated with water that carries untreated sewage in Kolkata were contaminated with lead, cadmium, and nickel (29).

There have been several publications in recent times on the issue of the effect of environmental pollution, especially air pollution, on human sperm function. Some of these works mention a direct effect of pollution on seminal parameters, while others showed insult at the genomic level with no change of seminal parameters (30, 31). Earlier reports

**TABLE 2****Correlation between semen parameters and age in the two decades.**

Parameters	1980s		2000s	
	Correlation with age	P	Correlation with age	P
Concentration, $\times 10^6/\text{mL}$	0.097	.001	0.111	.010
Volume, $\text{mL}^{\text{a}}$	-0.049	.085	-0.076	.085
Motility, %	-0.080	.005	-0.114	.008
FPM, %	-0.084	.003	-0.090	.036

<sup>a</sup> Values were log transformed.*Mukhopadhyay. Change in semen quality in two decades. Fertil Steril 2009.*

showed that a negative effect of the concentration of heavy metals on both sperm concentration and motility can affect the fertility status of the male (32, 33). Another study highlighted the damage at the genomic level due to air pollution but without any change in seminal parameters (34).

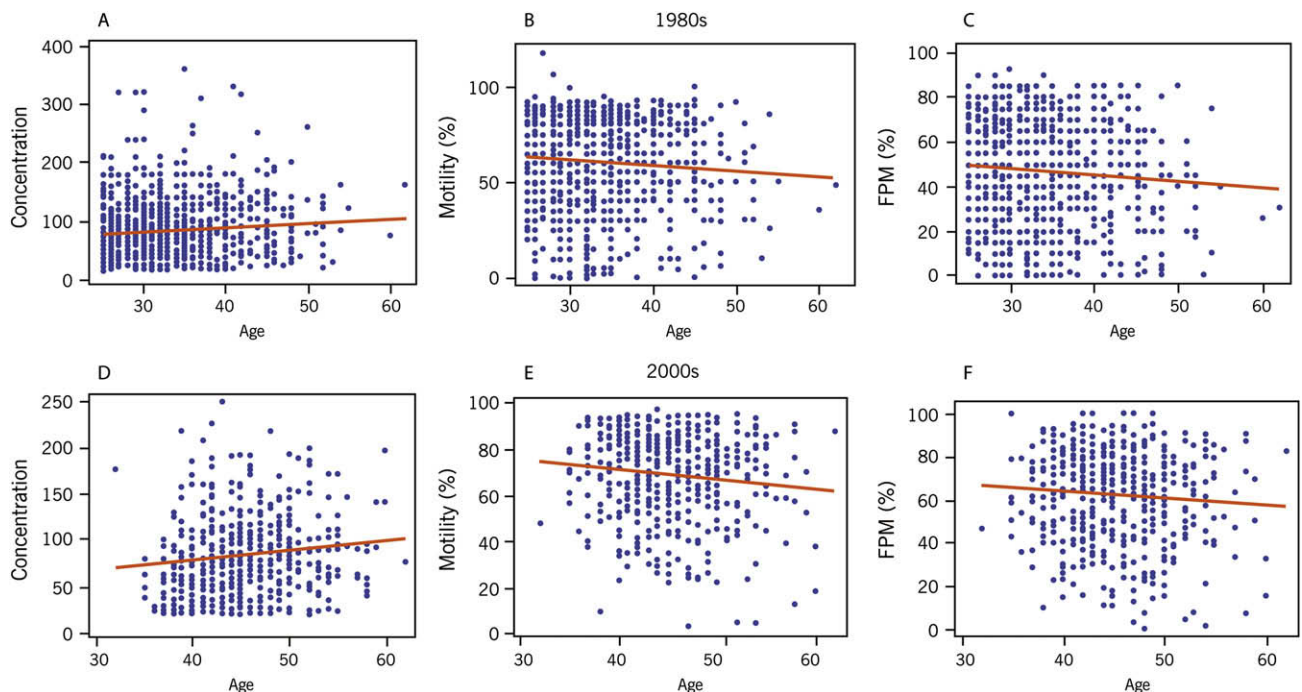
In the present study, the overall sperm concentration did not show any significant change between the two decades. This result is in agreement with some of the previous studies mentioned before (30, 31) and also with major large-scale studies conducted throughout the world regarding the decline of semen quality (10–13).

The results of the present study clearly suggest that there has been a significant decrease in semen volume, overall sperm motility, and FPM in the 2000s when compared with the 1980s.

The decline in seminal volume is in accordance with several previous studies (10, 35, 36). The anti-androgenic effect of the environmental toxicants and xenoestrogens may have a detrimental effect on the functioning of accessory sex glands (seminal vesicle, prostate, and bulbourethral and urethral glands), which through their secretions control the seminal volume. The decline in the motility parameters due to pollution effect is also highlighted in some previous studies

**FIGURE 3**

Scatter diagrams and linear regression lines of concentration, motility (%), and FPM (%) on age in the two decades 1980s (A–C) and 2000s (D–F).

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**TABLE 3****Slopes of the fitted regression lines.**

Semen Parameters	1980s		2000s	
	Slope	SE	Slope	SE
Concentration, ×10 <sup>6</sup> /mL	0.735	0.216	0.921	0.356
Motility, %	-0.304	0.108	-0.415	0.156
FPM, %	-0.319	0.107	-0.376	0.179

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(31). The alarming concentrations of NO<sub>x</sub> in the ambient air may play a principal role in the pathophysiology of sperm motility. Normozoospermic fertile men exhibited NO concentrations that were significantly lower than those of asthenozoospermic infertile men (37). Although endogenous NO can be beneficial to sperm motility (38), when generated in excessive amounts, it can lead to sperm toxicity and a decrease in motility (39). This pathogenic effect on sperm motility may be a result of free radical-generated oxidative stress (40) and a disturbance of the redox equilibrium.

The increasing concentrations of heavy metals (chiefly Pb) due to rise of vehicular pollution and food sources (29) can also hamper overall semen parameters. Hernández-Ochoa et al. (41) studied environmental exposure of lead on sperm quality and found that sperm concentration and motility associated negatively with the concentration of Pb in spermatozoa and that semen volume associated negatively with the concentration of Pb in seminal fluid. Eibensteiner et al. (42) found that sperm motility decreased with increasing Pb concentrations in blood in a simple linear regression in a study conducted among traffic police in Arequipa, Peru. Our recent research findings indicate that DNA damage due to oxidative stress can be one of the major causes of the decrease in sperm motility (43). Recently, Izawa et al. conducted a study on the toxic effect of diesel exhaust particles (DEPs) on spermatogenesis in the mouse. Their results clearly demonstrated that DEPs suppress testicular function, especially spermatogenesis and sperm motility, and showed that these effects may be aryl hydrocarbon receptor dependent (44).

Another important aspect of the present study is the analysis of the age-specific changes of the observed semen parameters and a comparative study of these changes in the two decades. Apart from semen volume, all the other semen parameters showed a significant correlation with increasing age. In both the decades studied, overall motility (percent) as well as FPM decreased with the increase of age. These results are supported by earlier reports (45–47). A recent large-scale study was conducted by Levitas et al. (47) to examine the relationship between age and semen parameters in 6022 men with normal sperm concentration. According to their findings, sperm motility was found to be inversely related to age, with a peak motility at age <25 years and lowest motility at age ≥55 years.

Declined sperm motility might be due to age-dependent changes in epididymal and accessory sex gland function (48, 49). However, in the present study, an interesting finding is that sperm concentrations in both decades increased with increasing age. This result is unique in the sense that it is contrary to other research findings that showed either no change of sperm count with age (50) or a decrease in sperm count with age (51, 52). Kidd et al. (53) reviewed the literature on the association between male age and semen quality and concluded that increased male age is associated with a decline in semen volume, sperm motility, and sperm morphology but not with sperm concentration. However, a study done by Nieschlag et al. (54) to evaluate reproductive functions in young fathers and grandfathers found that the sperm density was higher in the older group.

It is evident that the result of the present study, which highlights an increase in sperm concentration with age in both the decades, requires a thorough investigation since the abstinence period was comparable for all age groups (2–5 days). It is to be noted that the increasing number of spermatozoa found with increasing age corresponds with decreasing motility parameters (as found in the present data) and with increasing DNA damage (55) and abnormal morphology (unpublished data). This may result in the increase in the spontaneous abortions with the increase in the age of the male partner that has been highlighted in some studies (56). Although further studies are needed, one of the possible reasons behind the increase in concentration along with the production of defective sperm with increasing age may be due to the weakening of the apoptotic machinery with advancing age (55). This may result in a failure to eliminate defective spermatozoa, and thus there may be an accumulation of abnormal and healthy germ cells in the final ejaculate.

The present study also shows that the rate of increase of sperm concentration with increasing age was higher in the 2000s. The possible reason for this may be the fact that in the aging males in the 2000 series (2001–2006), in addition to the advancing age, the increased environmental pollutants, which cause oxidative stress, may have further weakened the apoptotic machinery. A study conducted on mice of different age groups revealed that oxidative stress significantly increased apoptotic metaphase spermatocytes in young mice, whereas it severely reduced testicular apoptosis in older mice (57).

In conclusion, our study confirms the preceding studies that there is a significant decline in the sperm motility parameters as well as in the seminal volume in the present decade when compared with earlier decades. However, no change in overall sperm concentration is noted, which also substantiates a section of previous findings. There has also been a decline in motility with increasing age in both decades, which confirms the results of prior surveys. The increase in sperm count with age in both the decades is an interesting finding of the present study, which may be explained by the apoptotic theory but needs further investigation at the molecular level.

*Acknowledgement:* The authors thank Ms. Madhumita Bhattacharjee, B.Sc., Vidyasagar University, India, for her assistance.

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