Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi: 10.1016/S2305-0500(14)60017-8

Effect of age and abstinence on semen quality: A retrospective study in a teaching hospital

Priyadarsini Sunanda[1,2], Babita Panda[1*], Chidananda Dash[1], Rabindra N. Padhy[2], Padmanav Routray[3]

[1]*Centre for Human Reproduction, IMS & SUM Hospital, Siksha 'O' Anusandhan University, K8-Kalinga Nagar, Bhubaneswar 751003, Odisha, India*

[2]*Centre Research Laboratory, IMS & SUM Hospital, Siksha 'O' Anusandhan University, K8-Kalinga Nagar, Bhubaneswar 751003, Odisha, India*

[3]*Aquaculture Production and Environment Division, Central Institute of Freshwater Aquaculture, Bhubaneswar-751002, Odisha, India*

ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form 24 February 2014
Accepted 26 February 2014
Available online 20 June 2014

Keywords:
Age
Abstinence
Semen quality

ABSTRACT

Objective: To elucidate the effect of age and sexual abstinence on semen quality (semen volume, total count, progressive motility, vitality and morphology). **Methods:** A total of 730 semen samples were analyzed. Subjects were grouped according to the age (20-29, 30-34, 35-39 and 40-50) and abstinence (2-3, 4-5 and 6-7). Semen parameters were evaluated following WHO standard criteria. **Results:** Analysis of 730 semen samples showed negative correlation of progressive motility (r=-0.131, P< 0.01), vitality (r=-0.173, P< 0.01), morphology (r=-0.324, P< 0.01) with age. With increase in age percentage of progressive motility, vitality and normal morphology in mean values declined after the age group of 35-39 to 40-50 years, but no change in volume and count were observed. Increase in abstinence with individual days significantly affected semen volume (H= 20.65, P< 0.001), count (H= 36.67, P<0.01), progressive motility (H= 13.53, P<0.05) and vitality (H= 15.33, P< 0.01). But, no effect was found on sperm morphology. Mann Whitney U test confirmed the changes in semen volume, total count and vitality in paired grouping from 2-7 days (P<0.05), but changes in sperm motility were observed after 5 days of abstinence in each paired group upto 7 days (P<0.05). Mean values of semen parameters among three abstinence groups (2-3, 4-5 and 6-7 days) also showed similar result. **Conclusions:** In the present study, age negatively affected progressive motility, vitality and morphology of human sperm. Semen samples showed intra varied results within WHO amended abstinence period.

1. Introduction

Infertility is the inability to contribute conception after 12 months of unprotected intercourse; this may be due to male, female or some unexplained factors. Males alone contribute 35% to 40 % of infertile cases[1, 2]. Pathogenesis of male infertility is multi-factorial[3] and any alteration to normal physiology of reproductive organs may affect sperm functions resulting in oligozoospermia (low sperm count), asthenozoospermia (loss of motility), teratozoospermia (abnormal morphology), azoospermia (absence of sperms in the ejaculate) and in severe form, oligoasthenoteratozoospermia (OAT) that causes problem for a successful/fruitful fertilization[2]. Apart from the most conventional causes, age and abstinence were found to have significant role in sperm physiology.

Males have the advantage over females that they can contribute to conception even after the age of 40 and upto an age beyond 40 years of sexual maturity[4]. However, in advanced ages, degenerative changes in germinal epithelium[5], decreased number of Leydig cells[6], and their functions affect spermatogenesis through decrease in testosterone level[7], starting at the age of 30 years[8]. In animal studies, it was found that aging of males affect the sperm quality and spermatogenesis[9]. An age related decrease in nitric oxide level has shown to be associated with erectile dysfunction in older age people. In addition,

*Corresponding author: Prof (Dr.) Babita Panda (Obst & Gyn), Head of the Unit, Centre for Human Reproduction, Institute of Medical Sciences & SUM Hospital, K8- Kalinga Nagar, Bhubaneswar-751003, Odisha, India.
E-mail: chrimsivf@gmail.com

semen quality also affects the implantation loss to the fertilized embryo[10] and possible autosomal dominant disorders by wrongly selecting a sperm among the semen pool of aged males[11].

Testicular volume and histopathological changes were strongly associated with the age of males that result in alteration of sex hormone production affecting the sperm physiology. However, the threshold age of sperm production in males is yet to be defined[12, 13]. Further, in the changing life style scenario, delayed parenthood is an upcoming problem and targeted couples need an assisted reproduction procedure to have a child[14]. Some retrospective studies in USA, China and India showed age related effects in semen parameters following the WHO guideline[11, 15, 16]. Here, the varied representations are ambiguous to find the optimal age/s for the treatment of infertility.

Apart from age, abstinence plays an important role in the maintenance of sperm quality, recommended by World Health Organization (WHO) for assisted reproduction. In other words, evaluation of sperm count depends on both interval between the ejaculations and their frequency in hours[17]. There are reports of positive correlation of volume and total count with abstinence period[18-20]. Further, some studies also showed the inverse correlation of motility with abstinence period[21]. In between WHO specified period of sperm collection (2-7 days) there is a variation of sperm quality[22, 23], whereas, other studies showed no significant effect of abstinence[24]. Hence, there is an ambiguity of semen collection, quality determination and the rate of success in fertilization within the assigned period. As sperm gains motility following the addition of secretions from accessory glands (seminal fluid), the variations of semen parameters depend on the quantitative and qualitative function of respective reproductive organs. So, more studies can suggest the particular abstinence period to obtain better semen quality in different geographical regions.

In general, retrospective data implies the declined sperm counts through ages[25-27]. In India, diminished sperm quality were reported in Kolkata, Bombay, Bangalore, Jodhpur and some other places in South India[16, 28]. So, in the present study, semen parameters were analyzed in a clinical set up of Eastern India, from 2010-2013 with reference to paternal age and abstinence period.

2. Materials and methods

2.1. Subjects

Patients attending Infertility Unit of a tertiary healthcare centre and teaching hospital, for evaluation of infertile couples, were taken as subjects. Seminal fluid analysis was done for the male partners of 730 couples attending the Infertility unit from 2010 to 2013. Male partners having the complain of infertility between age 20-50 years and sexual abstinence 2-7 days were included in the study; patients (142/872) with diabetes, infectious diseases, hydrocele, hernia, varicocele, addiction to tobacco, alcohol and azoospermia cases were excluded.

2.2. Sample collection and semen analysis

Semen samples were collected by masturbation into wide-mouth plastic container, in a room close to the andrology laboratory. The patients were advised to keep the abstinence period around 2-7 days. Samples were analyzed within 30-60 min, after liquefaction at 37 ℃. Semen parameters like sperm count/mL, percentage of motile spermatozoa, percentage of normal spermatozoa were analyzed along with the presence of pus cells in semen according to the WHO standard criteria[2].

About 6-10 μL of semen was put on to a microscopic slide and sperm motility was analyzed under bright field microscope under 400× magnification. At least 200 sperms were counted, and the mean value from duplicate measurements was represented. Sperm counts were done by using Neubauer's haemocytometer with requisite dilutions (1:2, 1:5, 1:20), as per the WHO manual, 2010[2]. Diluted semen samples were mixed properly and 10 μL of the sample was transferred to Neubauer's chamber for counting of spermatoza. Replicate dilutions were prepared to get correct values. Sperm morphology was assessed in Papanicolau-stained smears (Haematoxylene, Orange-G and EA-50 stain) using light microscopy (Olympus India Ltd) under oil immersion at 1 000× magnification.

Vitality of sperms was estimated by the hypo-osmotic-swelling (HOS) test that was performed by mixing equal volumes of semen and hypo-osmotic solution, prepared from 7.35 g sodium citrate and 13.5 g fructose in 1 000 mL distilled water. The mixture was incubated for 30 min at 37 ℃, from which an aliquot of 10 μL was immediately examined at the 40x magnification. The percentage of swollen (vital) sperm was assessed by counting a minimum of 200 spermatozoa.

2.3. Statistical analysis

Semen quality parameters were analyzed by the use of [a]*Chi*-square test, [b]Pearson's correlation and [c]Kruskal-Wallis *H* test were applied, whenever needed. For each parameter where a statistically significant variation was found with respect to abstinence, pair-wise comparison was performed by [d]Mann-Whitney *U*-test. For the same, Statistical programme for the Social Sciences (SPSS Inc., version 20.0) was used.

3. Results

Out of 730 subjects, 315 (43.2%) were found to be normozoospermic, 263 (36.02 %) oligoasthenozoospermic, 98 (13.4%), and 54 (7.4%) asthenozoospermic. Semen parameters showed different ranges like volume from 1.5-6.5 mL, total count 1-209 millions, Progressive motility 0-65 %, vitality 8%-88 % and percentage of normal sperms (morphology)

between 5% to 37 %. Further, means of the semen parameters were 2.5 mL, 48.8 millions, 35.2 %, 75.6 %, 31.3 % respectively (Table 1).

In comparison to WHO reference values, 12.05 % of samples showed below standard criteria of the semen volume. Similarly for total count, progressive motility, vitality and morphology were 50.8%, 43.4%, 12.32% and 1.90 % respectively showed below WHO reference values (Table 2). *Chi*- square test was applied to compare the normal reference values among different age groups of 20-29, 30-34, 35-39, and 40-50 years except normal morphology, as subjects below <4 % reference values were very low. The difference in the subjects below standard criteria (WHO) among the age groups were found to be significant for progressive motility (χ^2 = 9.6, ^aP< 0.05) and vitality (χ^2 = 14.28, ^aP< 0.01) only.

Further, the mean values of semen parameters in different age groups were represented in Figure 1. Mean values of semen volume were almost equal in all age groups and for total sperm count it declined only after 40 years of age. With increase in age progressive motility, vitality and normal morphology found to decline slowly, that begin at the age group 35-39 years, but the best quality of semen was observed at the age group 30-34 years. Pearson's correlation was applied to find out significant changes between age and semen parameters. Progressive motility (r=-0.131, ^bP< 0.01), vitality (r=-0.173, ^bP< 0.01) and normal morphology (r=-0.324, ^bP< 0.01) were found to be negatively correlated with increasing age (Figure 2). But significant changes were found for semen volume and total count.

With increasing sexual abstinence, volume of semen

Table 1
Description of indices of semen quality.

Semen quality	Volume (mL)	Total count (×10^6)	Progressive motility (%)	Vitality (%)	Normal Morphology (%)
Range	1-6.5	1-209	0-65	8-88	3-37
(Mean±SE)	2.50 ±0.05	48.80±1.40	35.20±0.68	75.60 ±0.57	26.31±0.28
WHO Reference values	≥1.5	≥39	≥32	≥58	≥4

Table 2
Percentage of subjects below WHO reference values of semen parameters among different age groups.

Age group	N	Volume (mL)	Total count (×10^6)	Progressive motility (%)	Vitality (%)	Normal morphology(%)
20-29	102	12.70	55.80	40.20	9.80	2.94
30-34	284	9.50	46.50	38.70	8.09	1.76
35-39	236	13.60	50.40	45.00	16.80	1.30
40-50	108	14.80	58.30	55.60	21.30	2.80
Total	730	12.05	50.80	43.40	12.32	1.90

N: No. of samples

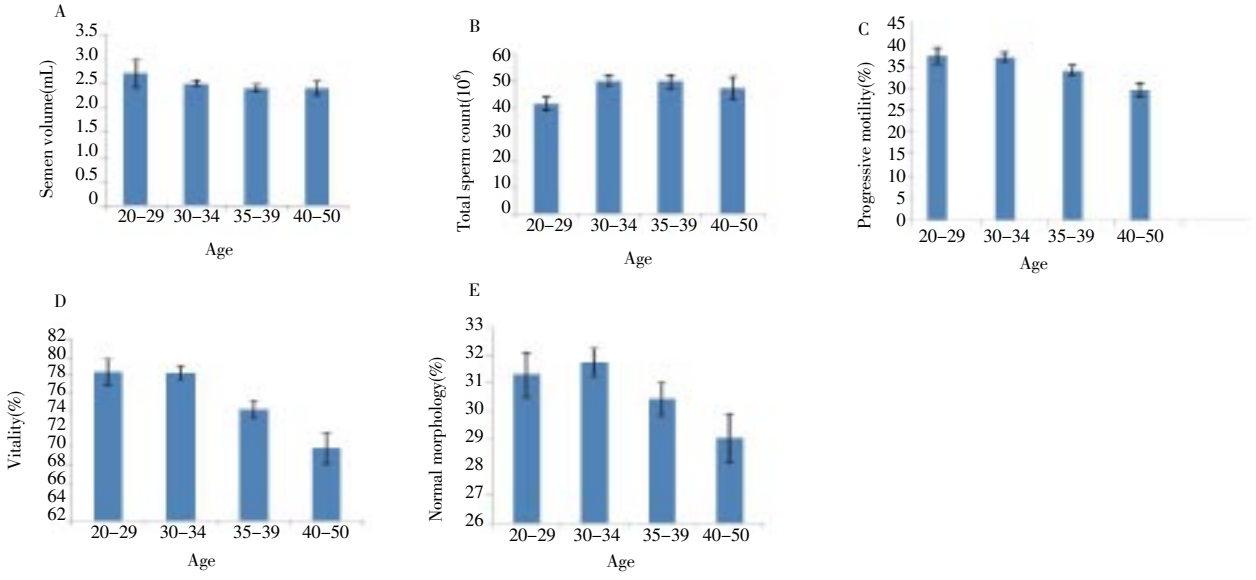

Figure 1. Comparison of semen parameters in different age groups(Mean ± SE).

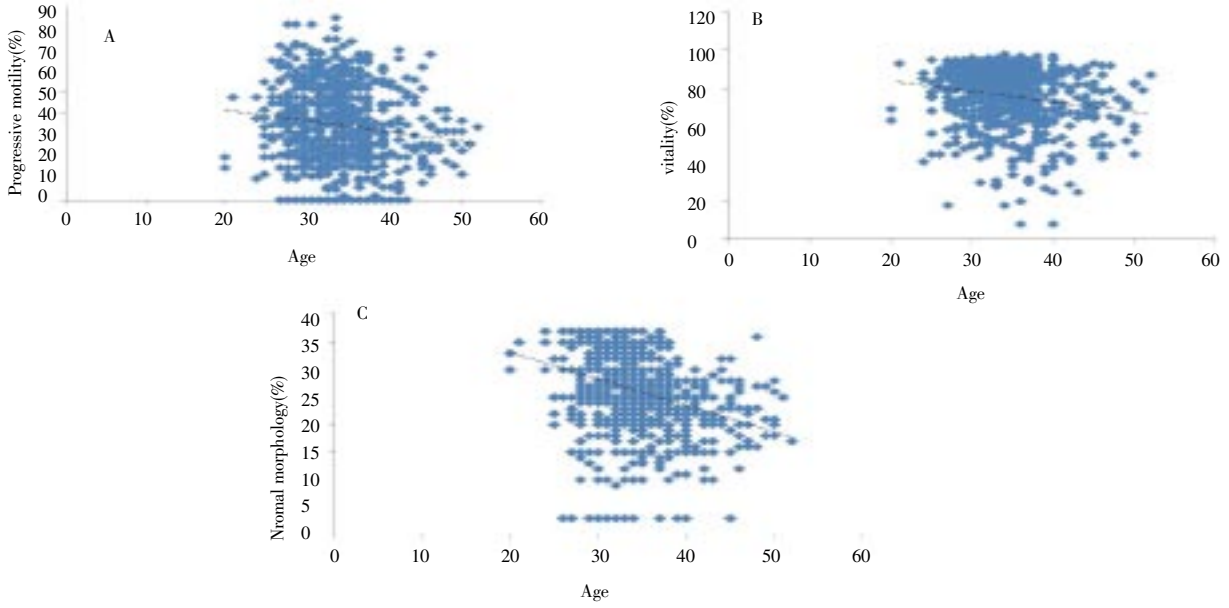

Figure 2. Scatter diagrams and linear regression lines of progressive motility (%), vitality and normal morphology (%) of sperm on age.

and total count showed increasing trend in mean values. Progressive motility was found to be same between 2–3 and 4–5 days, but a sudden decrease in motility was found after 5 days of abstinence (Figure 3). Kruskal–Wallis *H* test was applied to the semen parameters according to the individual days of sexual abstinence, where volume (H=20.65, $^{c}P<$ 0.001), total count (H= 36.67, $^{c}P<$ 0.001), progressive motility (H=13.53, $^{c}P<$ 0.05) and vitality (H=15.33, $^{c}P<$ 0.01) were found to be highly significant for abstinence of 2–7 days. No change was found for normal morphology of sperm with increasing abstinence period. Mann Whitney *U* test confirmed the changes in semen volume significantly in each paired grouping from 2 days upto 7 days ($^{d}P<$ 0.05) and total sperm count changed from 3 days of abstinence to 7 days ($^{d}P<$0.05). However, vitality was observed to be different between 2 and 4 days, 3 and 5 days, 4 and 5 days, 4 and 6 days in paired groups of sexual abstinence period ($^{d}P<$0.05). Significant changes in sperm motility were observed after 5 days of abstinence in each paired group upto 7 days ($^{d}P<$ 0.05).

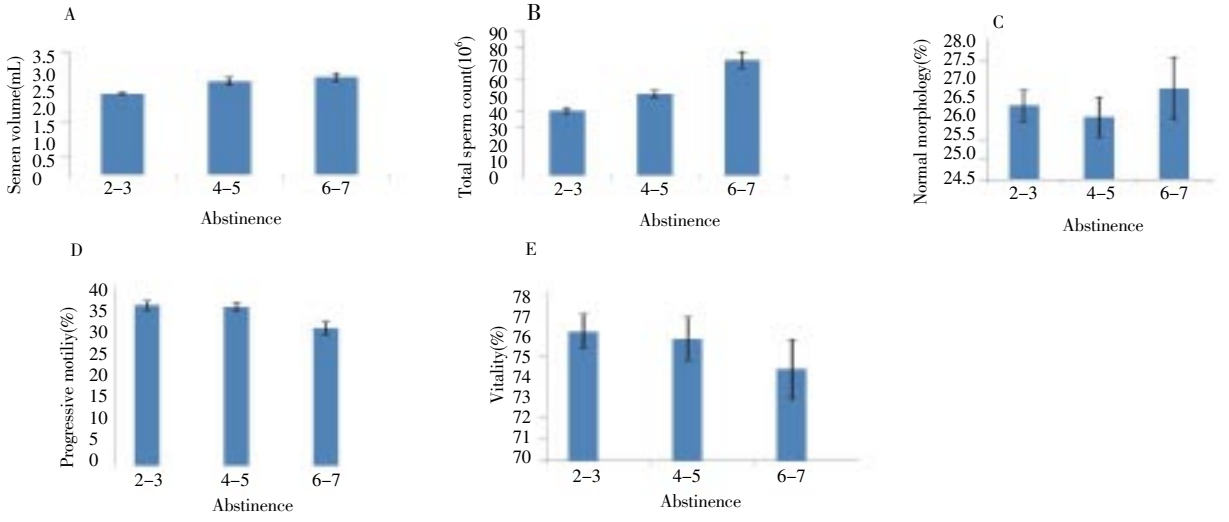

Figure 3. Comparison of semen parameters in different abstinence period (Mean ± SE).

4. Discussion

Semen quality analysis is the preliminary step to know the fertility status of males for assisted reproduction (WHO, 2010). Researchers have differently analyzed age and abstinence to find their effect on fertility potential[16, 21]. Here, age significantly affected the sperm quality, viz; progressive motility, vitality and morphology. Similar results were

obtained in Chinese populations[15]. It was partly supported by another retrospective study, [29] where the decreasing trend was observed with viability, morphology and volume. Whereas, contradictory findings on Cordoba (Argentina) population and sub-fertile patients of Arabian Gulf University reported the age related decrease in all the semen parameters [30,31].

In the present study, means of the forward progressive motility decreased in older age groups. Similar result was obtained by Mukhopadhay *et al*. 2010[16] for motility only, but contradicted the trend of sperm concentration. As normal morphology of sperms is essential to gain motility during epididymal transit, it might be difficult to attain higher grade of motility, imperative for conception in older age males. There were reports of increased percentage of abnormal spermatozoa in older age groups than younger groups[32]. Further, detoriation of healthy germ cells in advanced age might be one of the reasons for loss of grade of motility[33]. Here, no significant change in semen volume was recorded, contradicting another study on older age patients undergoing IVF and ICSI, which showed no effect on semen parameters, rate of fertilization, miscarriages in

Table 3

A comparative study of age related changes in semen parameters.

No. of samples	Animal model/human	Significant findings	Reference
–	Rat	Decreased testosterone level, Leydig cell volume, Sertoli cell number per testes in older rats than younger ones.	[5]
–	Mice	Decreased seminal vesicle function, testosterone level, motility and Atrophied testes	[36]
–	Mouse	Decrease in germ cell number, loss of Sertoli cell′s polarity, degeneration of testes and increase in mononuclear phagocytes	[37]
–	Mice	Increased germline mutation with increased paternal age	[38]
–	Rat	Increase in paternal age was associated with pre-implantation loss, decrease in fetal weight, increase in neonatal death	[39]
–	Rat	Gene specific alteration of DNA methylation in germ cells	[40]
–	Brown –Norway rat	Decreased motility of sperm in cauda epididymis in older age Morphological anomalies such as mid-piece defects and cytoplasmic droplet in advanced age	[41]
833	Human	Reduced motility and higher abnormal morphology after 36 years of age	[42]
29	Human	Reduced motility and increased follicle stimulating hormone in older age men	[43]
97	Human	Decreased semen volume, progressive motility with increasing age from 22 to 80 years. Sperm concentration and total count Increased across the age decades	[33]
	Human	Decrease in semen volume and blastocyst embryo formation without any significant effect on other parameters	[10]
998	Human	Decrease in progressive motility, vitality and morphology	[15]
3698	Human	Decrease in vitality, volume and morphology Risk of disomies and chromosomal structural abnormalities in sperms of aged males	[29]
551	Human	Decrease in semen volume, sperm concentration, total count, total motile count and morphology Increased tail defects with increasing age	[32]
6022	Human	Top semen quality at the age of 30-35 years of age Sharp reduction in all parameters after 55 years	[44]
1364	Human	Reduction in semen volume , progressive motility, sperm concentration and total count with increasing age.	[45]
9168	Human	Age related decrease in volume, sperm count, motility, viability, morphology, fructose and alpha glucosidase activity	[30]
3729	Human	Decline in motility of sperm with increasing age Increasing trend of sperm concentration	[16]
Review	Human	Age related decrease in fertility rate and increase in DNA fragmentation in sperm, birth defects and chromosomal abnormalities in offspring.	[46]
975	Human	Age related changes in nuclear vacuoles and DNA damage	[47]
52	Human	Age related reduction in sperm density, motility, normal morphology, total antioxidant capacity and DNA integrity	[31]
4822	Human	Total sperm count and normal morphology declined after 40 years . Sperm motility and semen volume declines after 43 and 45 years respectively.	[48]
90	Human	Decline in enzymatic antioxidants and neutrophil with increase in age	[49]
730	Human	Decrease in progressive motility, vitality and morphology with increasing age. No significant changes were found in semen volume and total count	Present study

Table 4
A comparative study of abstinence related changes in semen parameters.

No. of samples	Significant findings	Reference
–	Increase in sperm number and Decrease in sperm motility and decrease in nuclear decondensation with increased abstinence period	[18]
195 (2–7 days)	Increase in semen volume from 2–7 days and no effect on sperm concentration	[50]
10 men (1, 2, 3, 4, 5 and 10 days)	Semen volume, sperm concentration and sperm total count increased with increasing abstinence Sperm acrosin remained same upto 5 days, but decreased after that upto 10 days Increase in morphologically normal sperms upto 5 days, but decreased after that.	[19]
6 men (2–18 days)	Increase in semen volume and sperm count with increasing abstinence period Decrease in motility and normal forms	[20]
422 (2–7days)	Semen volume and Sperm concentration increased after 4–5 days of abstinence than 2–3 days Highest progressive motility and total motile count were highest at 4–5 days N–alpha glucosidase and zinc were found to be higher at 4–5 days and 6–7 days than 2–3 days of abstinence.	[21]
6008 men, 9489 samples (1–14 days)	In oligozoospermic samples sperm count and motility increased upto 4 days, but declined in succeeding days. In normozoospermia count and motility showed an increasing trend upto 7 days. Normal forms were inversely related to abstinence period.	[22]
11 men with abstinence period 1, 3, 5 and 8 days (total 17 days)	Increase in semen volume and after sperm concentration 5–8 days in comparison to 1 day. No effect on motility and morphology of sperm.	[23]
419 semen samples (2–7 days)	Increase in semen volume and concentration after 4 days of abstinence and succeeding days. No effect on motility and total motile spermatozoa with increased duration of abstinence.	[24]
21 men , 210 samples (1–10 days)	Semen volume, sperm count and motility were negatively correlated with abstinence from2–10 days. Increase in progressive motility was from 2–4 days.	[52]
57 men, 1, 3–5 days and 18–30 hours of abstinence	No change in total motile sperm count between 3–5 days abstinence time and 18–30 hours abstinence time. DNA damage and the percentage of normal morphological sperm were significantly lower in 18–30 hours abstinence time	[54]
730 men, 2–7days	Semen volume and total count increased with increasing abstinence period. Sperm motility and vitality declined after 5 days of abstinence.	Present study

advanced age groups, except semen volume, and decreased rate of blastocyst embryo formation[10]. Further, animal studies demarcated histological changes in testes, anomaly in germ cells and seminiferous epithellium, preimplantation losses and increased frequency of spermatid mutations in the process of aging[34]. Damage of DNA was also found to be higher in aged males leading to spontaneous abortion[11].

Study of Zhu *et al*. 2011 showed the low reference value for motility (40.6%) and vitality (36.4%) in Chinese poulation. But in the present study, the percentage of subjects below standard reference values of WHO are very high in case of total count (50.8%) and progressive motility (43.4%). Good quality of semen was found in between the two initial age groups i.e., amid 20–34 years. Although age related changes in semen quality has been elucidated, in the present study semen parameters above the WHO certified criteria were very low, indicating declined sperm count and motility in the semen pool of 730 samples. As the whole process of spermatogenesis is regulated by hypothalamus–pituitary–Leydig cell endocrine axis and andogen dependent secretions, continuous exposure to environmental toxicants and today's lifestyle, might be one of the factors that alter the normal number and function of the germ cells, Sertoli cells and Leydig cells prior to the relative changes in aging [35]. A comparative study analyzing the age related changes in semen parameters has been given in Table 3. So is the possible positive predictive factor of age for the management of male infertility during the treatment correlating the standard physiological conditions.

Semen volume and total sperm counts were found to be significantly correlated with increase in sexual abstinence from 2–7 days. This is supported partly by other studies [23, 24, 50] for positive correlation of volume, sperm concentration and total count with abstinence. Here, progressive motility

and vitality of sperms also declined after 5 days of abstinence. Similar result was too reported from elsewhere for oligozoospermic samples, contradicting the result of normozoospermic that all semen parameters show increasing trend with abstinence period[22], corroborated by Pellester *et al*. [20]. As sperms are deposited daily into the Epididymis, increase in total count is obvious with increasing abstinence. So, oligozoospermic cases are suggested by clinicians to maintain the abstinence period for a couple of weeks to get an acceptable sperm count to achieve pregnancy[51]. On the other hand, daily ejaculation in oligozoospermics having<10 million sperm count achieved fatherhood with a good sperm quality[52]. Another study on daily ejaculation at a gap of 24 hour, reported the decrease in semen volume and count to an abstinence period of 10 days, where sperm progressive motility was of good quality after 2nd day, but declined after 5 days of abstinence[53]. Similar results on motility and vitality was also observed here also. A comparative study analyzing the abstinence related changes in semen parameters has been given in Table 4. It is known that sperms remains immotile during epididymal transit and get motility after in touch with the accessory gland fluids. Epididymis contains some inhibitory factors like lactate which diffuse into the sperm and lowers the pH inhibiting the motility of sperm. Outside the epididymis, it is reversed by the influx of Na^+ and HCO_3^- from the fluid of accessory glands[55]. Exposure to these inhibitory factors in epididymis for a prolonged period might be affecting either on metabolic activity of sperms or the opening of ion channels that is responsible to regain motility outside Epididymis.

As daily sperm production in human is 100-500 millions [17], increase in total count and high level of semen for a prolonged period in the sperm ducts that store it, may affect the motility and viability of sperms creating an oxidative stress condition through inflammation of the prostate gland[56]. Apart from these increased exposure to pollutants, endocrine disrupters (pthalates, polychlorinated biphenyles, xenoestrogens, pesticides, *etc*.), consumption of junk food, alcohol and smoking, badly affects the semen quality either by disrupting hormonal control or by DNA damage though huge production of reactive oxygen species. Disregulation of sex hormones and accessory sex glands (seminal vesicles, prostate and Cowper's glands) may affect the semen volume and potency of sperm for fertilization. These may be some of the possible reasons of the optimal activity of WHO amended semen parameters, in our 3 years retrospective data analysis.

In conclusion, present study predicted that aging negatively affect progressive motility, vitality and morphology of sperms and beyond 5 days of sexual abstinence sperm motility is reduced.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

SP is supported by a research fellowship from SOA University. Authors are thankful to Prof. DK Roy, Dean for consistent encouragement. Authors are also grateful to Er. Gopabandhu Kar, Managing Member, SOA University and Vice-Chancellor, SOA University for the financial support.

References

[1] Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. *Fertil Steril* 2006; **85**: 629-634.

[2] WHO. *WHO laboratory manual for the examination and processing of human semen.* 5th ed. Gevena: WHO; 2010.

[3] Agarwal A, Sekhon LH. The role of antioxidant therapy in the treatment of male infertility. *Hum Fertil* 2010; **13**: 217-225.

[4] Amann RP. The cycle of the seminiferous epithelium in humans:a need to revisit? *J Androl* 2008; **29**: 469-487.

[5] Wang C, Leung A, Sinha-Hikim AP. Reproductive aging in the male brown-Norway rat: a model for the human. *Endocrinology* 1993; **133**: 2773-2781.

[6] Johnson L. Spermatogenesis and aging in the human. *J Androl* 1986; **7**: 331-354.

[7] Harman SM, Metter EJ, Tobin JD, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab* 2001; **86**: 724-731.

[8] Amaral S, Santos JR. Aging, mitochondria and male reproductive function. *Curr Aging Sci* 2009; **2**:165-73.

[9] Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, et al. The association of age and semen quality in healthy men. *Hum Reprod* 2003; **18**: 447-454.

[10]Dain L, Auslander R, Dirnfeld M. The effect of paternal age on assisted reproduction outcome. *Fertil Steril* 2011; **95**: 1-8.

[11]Kidd SA, Eskenazi B, Wyrobek AJ. Effect of male age on semen quality and fertility: a review of literature. *Fertil Steril* 2001; **75**: 237-248.

[12]Mahoud AM, Goemaere S, El-Gaerem Y, Van Pottelbergh I, Comhaire FH. Testicular volume in relation to hormonal indices of gonadal function in community dwelling elderly men. *J Clin Endocrinol Metab* 2003; **88**: 724-731.

[13]Sampson N, Untergrasser G, Berger P. The aging male reproductive tract. *J Pathol* 2007; **211**: 206-218.

[14]Zofnat WM, Auslandr R, Dirnfeld M. Advanced paternal age and reproductive outcome. *Asian J Androl* 2012; **14**: 69-76.

[15]Zhu QX, Meads C, Lu ML, Wu JQ, Zhou WJ, Gao ES. Turning point of age for semen quality: a population-based study in Chinese men. *Fertil Steril* 2011; **96**: 572-576.

[16]Mukhopadhyay D, Varghese AC, Pal M, Banerjee SK, Bhattacharyya AK, Sharma RK, et al. 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. *Fertil Steril* 2010; **93**: 2247-2254.

[17]Bjorndahl L, Mortimer D, Cristopher LBR, Castilla JA, Menkveld R. *Basic physiology: A practical guide to basic laboratory andrology*. Cambridge: Cambridge University Press; 2010, p. 5-31.

[18]LeLannou D, Colleu D, Boujard D, le Couteux A, Lescoat D, Segalen J. Effects of abstinence on maturity of human spermatozoa. *Arch Androl* 1986; **17**: 35-40.

[19]Blackwell JM, Zaneveld LJD. Effect of abstinence on sperm acrosin, hypoosmotic swelling, and other semen variables. *Fertil Steril* 1992; **58**:798-802.

[20]Pellestor F, Girardet A, Andreo B. Effect of long abstinence periods onhuman sperm quality. *Int J Fertil Menopausal Stud* 1994; **39**: 278-282.

[21]Elzanaty S, Malm J, Giwercman A. Duration of sexual abstinence: epididymal and accessory sex gland secretions and their relationship to sperm motility. *Hum Reprod* 2005; **20**: 221-225.

[22]Levitas E, Lunenfeld E, Weiss N, Friger M, Vardi IH, Koifman A, et al. Relationship between the duration of sexual abstinence and semen quality: analysis of 9 489 semen samples. *Fertil Steril* 2004; **83**: 1680-1686.

[23]Jonge CD, Fromboise ML, Bosmans E, Ombelet W, Cox A, Nijs M. Influence of the abstinence period on human sperm quality. *Fertil Steril* 2004; **82**: 57-65.

[24]Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. *Fertil Steril* 2004; **82**: 358-366.

[25]Andolz P, Bielsa MA, Vila J. Evolution of semen quality in North-eastern Spain: a study in 22,759 infertile men over a 36 year period. *Hum Reprod* 1999; **14**: 731-735.

[26]Itoh N, Kayama F, Tatsuki TJ, Tsukamoto T. Have sperm counts deteriorated over the past 20 years in healthy, young Japanese men? Results from the Sapporo area. *J Androl* 2001; **22**: 40-44.

[27]Seo JT, Rha KH, Park YS, Lee MS. Semen quality over a 10-year period in 22 249 men in Korea. *Int J Androl* 2000; **23**: 194-198.

[28]Gopalkrishnan K. Characteristics of semen parameters in a selected population of Indian men over a period of 10 years. *Curr Sci* 1998; **75**: 939-940.

[29]K€uhnert B, Nieschlag E. Reproductive functions of the ageing male. *Hum Reprod Update* 2004; **10**: 327-339.

[30]Molina RI, Martini AC, Tissera A, Olmedo J, Senestrari D, de Cuneo MF, et al. Semen quality and aging: analysis of 9.168 samples in Cordoba. Argentina. *Arch Esp Urol* 2010; **63**(3): 214-222.

[31]Omran HM, Bakhiet M, Dashti MG. Evaluation of age effects on semen parameters of infertile males. *Andrology* 2013; **2**: 106.

[32]Chen Z, Toth T, Bailey L, Mercedat N, Schiff I, Hauser R. Seasonal variation and age-related changes in human semen parameters. *J Androl* 2003; **24**: 226-231.

[33]Eskenazi B, Wyrobek A, Slotter E, Kidd S, Moore L, Young S, et al. The association of age and semen quality in healthy men. *Hum Reprod* 2003; **18**: 447-454.

[34]Gavrilov LA, Gavrilova NS. Human longevity and parental age at conception. In: Robine JM, Kirkwood TBL, Michel Allard. *Sex and longevity: Sexuality, gender, reproduction*. Parenthood: Springer-Verlag Berlin, Heidenberg; 2000, p. 7-31.

[35]Creasy DM. Pathogenesis of male reproductive toxicity. *Toxicol Pathol* 2001; **29**(1): 64-76.

[36]Parkening TA, Collins TJ, Au WW. Paternal age and its effects on reproduction in C57BL/6NNia mice. *J Gerontol* 1988; **43**: 79-84.

[37]Tanemura K, Kurohmaru M, Kuramoto K, Hayashi Y. Age-related morphological changes in the testis of the BDF1 mouse. *J Vet Med Sci* 1993; **55**: 703-710.

[38]Walter CA, Intano GW, McCarrey JR, McMahan CA, Walter RB. Mutation frequency declines during spermatogenesis in young mice but increases in old mice. *Proc Natl Acad Sci USA* 1998; **95**: 10015-10019.

[39]Serre V, Robaire B. Paternal age affects fertility and progeny outcome in the brown Norway rat. *Fertil Steril* 1998; **70**: 625-631.

[40]Christopher CO, Smiraglia DJ, Robaire Bernard. Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. *Proc Natl Acad Sci USA* 2003; **18**: 1775-1780.

[41]Syntin P, Robaire B. Sperm structural and motility changes during aging in the brown norway rat. *J Androl* 2001; **22** (2): 235-244.

[42]Schwartz D, Mayaux MJ, Spira A, Moscato ML, Jouannet P, Czyglik F, et al. Semen characteristics as a function of age in 833 fertile men. *Fertil Steril* 1983; **39**(4): 530-535.

[43]G.HaidI, A Jung, B^chill W. Ageing and sperm function. *Hum Reprod* 1996; **11**(3): 558-560.

[44]Levitas E, Lunenfeld E, Weisz N, Friger M, Potashnik G. Relationship between age and semen parameters in men with normal sperm concentration: analysis of 6022 semen samples. *Andrologia* 2007; **39**(2): 45-50.

[45] Maya WC, Berdugo J, Jaramillo ÁC. The effects of male age on semen parameters: analysis of 1364 men attending an andrology center. *The Aging Male* 2009; **12**(4): 100-103.

[46]Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. *Rev Urol* 2011; **13**(4): e184-e190.

[47]Silva LFI, Oliveira JB, Petersen CG, Mauri AL, Massaro FC, Cavagna M, et al. The effects of male age on sperm analysis by motile sperm organelle morphology examination (MSOME). *Reprod Biol Endocrinol* 2012; **10**:19.

[48]Stone BA, Alex A, Werlin LB, Marrs RP. Age thresholds for changes in semen parameters in men. *Fertil Steril* 2013; **100** (4): 952-58.

[49]Purandhar K, Seshadri S. Age associated variations in human neutrophil and sperm functioning. *Asian Pac J Reprod* 2013; **2**(3): 201-208.

[50]Padova G, Tita P, Briguglia G, Giuffrida D. Influence of abstinence length on ejaculate characteristics. *Acta Eur Fertil* 1988; **19**: 29-31.

[51]Gosálvez J, González-Martínez M, López-Fernández C, Fernández JL, Sanchez-Martin P. Shorter abstinence decreases sperm deoxyribonucleic acid fragmentation in ejaculate. *Fertil Steril* 2011; **96**: 1083-6.

[52]Van ZJA, Mankveld R, Relief AE, Van Niekerk MA. In: Hafez ESE. (ed.) *Oligozoospermia in human semen and fertility regulation in men*. Saint Louis: CV Mosby Company; 1976, p. 363.

[53]Valsa J, KP Skandhana, Gusania P, Khanc PS, Amithd S, Gondalia M. Effect of daily ejaculation on semen quality and calcium and magnesium in semen. *Rev Int Androl* 2013; **11**(3): 94-99.

[54]Sukprasert M, Wongkularb A, Rattanasiri S, Choktanasiri W, Satirapod C. the effects of short abstinence time on sperm motility, morphology and DNA mdamage. *Andrology* 2013: http://dx.doi.org/10.4172/2167-0250.1000107

[55]Sircar S. *Sperm transport and fertilization: Principles of medical physiology*. Germany: Thieme Medical Publishers;008; p. 562-569

[56]Whiting SK. Secrets to a healthy prostrate.....what you doctor does not tell you. The Institute of Nutritional Sciences. *Prostrate-Special Report 2004. www.HealthyInformation.com.*