

Is Male Infertility a Concern of Time? Comparison of Semen Parameters over a Five Year Period among Sub-fertile Men Visiting a Fertility Centre in Chennai, Tamil Nadu, India

S PRAMODITA¹, VM ANANTHA EASHWAR², GB PAVITHRA³, TIMSI JAIN⁴, YOGESH MOHAN⁵

ABSTRACT

Introduction: Semen parameters, especially sperm count, concentration, progressive motility and volume have been said to be diminishing over time among men in developing countries like India.

Aim: To compare the semen parameters (semen concentration, normal forms, progressive motility and defects) of sub-fertile males visiting Advanced Reproductive Care (ARC) fertility Centre in Chennai.

Materials and Methods: This cross-sectional study was conducted in Advanced Reproductive Care, Inc. (ARC) International Fertility and Research Centre, Chennai, Tamil Nadu, India, from the period between January 2015 to December 2019. Total 50 semen samples were selected randomly each year bringing the total to 250 samples which were included in the study. Masturbation was the major method of semen collection. The semen was analysed for the various parameters like semen concentration, normal forms,

progressive motility and defects in the laboratory under microscope on wet preparation of slides as per the standard guidelines. The various semen parameters along with the age of the participants were entered in Microsoft Excel and analysed by Statistical Package for the Social Sciences (SPSS) version 22.0.

Results: Total 250 semen reports were included in the study. Subjects belong to age group between 20 to 60 years. It was found that there was a statistically significant mean difference among progressive motility (25.14 ± 16.37 in 2015 to 17.30 ± 11.41 in 2019; p -value=0.009), semen concentration (78.1 ± 61.15 in 2015 to 65.96 ± 57.85 in 2019; p -value=0.001) among the semen samples over the years. There was a decreasing trend with the semen parameters, being lower than optimal over the years.

Conclusion: The study findings suggested that there was a decrease in semen parameters over the years and further studies have to be carried out to find the various environmental and lifestyle factors which could be responsible for the same.

Keywords: Azoospermia, Semen analysis, Sperm motility, Subfertility

INTRODUCTION

Infertility is one of the upcoming worldwide problems as it affects 15% of married couples worldwide [1]. It is a stressful state for the couple. There is a common misconception, where women are blamed, but male fertility issues have been alarming over a period of years. There is a prevalence of 15-20% in the general population, and of which the male factor contributes to 20-40% [2,3].

According to World Health Organisation (WHO) the overall prevalence of primary infertility in India ranges between 3.9% to 16.5% [4]. There is also discrepancy among the infertility rates among different states within India [5]. WHO conducted preliminary study which was multicenter study which showed that 45% of sub-fertile men were found to be affected by oligozoospermia or azoospermia [6]. Sperm count and motility have been diminishing over time. A decrease of 2% per year in sperm count over past 23 years has been reported in a study of 1350 passion sperm donors. The fact is half of the cases of infertility may be related to male factor. Worldwide sperm count has estimated to have dropped by 50% since 1930. A meta-analysis of studies published in human reproduction update reveals a 54.2% drop in sperm count in the period of 1973 to 2011 in western countries. In 1940, young men produced an average of 100 million sperm cells/ml of semen. Current men have been observed to produce much less [7].

There are various factors which may be responsible for rising cases of male infertility in the country. These may be attributed to exposure to insecticides in the agricultural workplaces, exposure to X-rays and exposure to toxic chemicals which could likely cause

disruption in the endocrine factors such as xenoestrogens. Due to modernisation, most of them practice sedentary lifestyle in the workplace. Due to the frequent use of laptops, men tend to place them on their thighs which can cause overheating of the testis, rise in psychosocial problems like depression, anxiety, substance abuse like tobacco smoking, early onset diabetes due to sedentary lifestyle factors are some of major factors which contribute to rising cases of infertility in India [8].

Despite the consistency across multiple studies, two major gaps remain in this literature [9,10]. First, there is little data regarding long-term trends in sperm motility, morphology and important markers of sperm function especially in South India. With the above background, the study was conducted with the aim to investigate whether there is a decline in semen parameters like concentration, progressive motility, morphology and those were analysed and compared between 2015 to 2019 among men seeking treatment for infertility in a tertiary care center.

MATERIALS AND METHODS

This cross-sectional study was conducted in Advanced Reproductive Care, Inc. (ARC) International Fertility and Research Centre, Chennai, Tamil Nadu, India, from the period between January 2015 to December 2019. Informed consent was obtained from each study participant before including them for the study. Institutional Ethical Committee (IEC) approval was obtained from Private Medical College in Kancheepuram District (IEC No: SMC/IEC/2020/03/392).

Inclusion criteria: Sub-fertile men who sought treatment for infertility were included in the study.

Exclusion criteria: Men suffering from infertility due to congenital diseases were excluded from the study.

Universal sampling technique was followed, in which 250 sub-fertile men who sought fertility treatment in year 2015 to 2019 were included based on inclusion, exclusion criteria and available logistics.

Procedure

Semen parameters of 250 sub-fertile men who sought fertility treatment in the year 2015 to 2019 at the same center on account of infertility were analysed for sperm count, motility, morphology, semen volume and the results were compared. Semen reports from January 2015 to December 2019 with 50 sample chosen randomly each year were compared. Approximately, 50% of those contacted by the research nurses were enrolled. The final study sample included 250 semen reports, after excluding reports of men who were having azoospermia. Only semen reports of morphologically normal sperm were assessed.

Semen collection: Masturbation was the main method of semen collection. The samples were analysed by senior Embryologist, techniques for analysis and the equipment used remained the same throughout semen collection.

Semen Analysis

The semen samples were collected from the infertile subjects after a prerequisite of 48 hours of sexual abstinence. Sperm analysis was performed according to the WHO guidelines by preparing slides in wet preparation to assess semen parameters like progressive sperm motility and semen concentration. Sperm morphology was assessed with the help of morphology kit. It was divided into five parts such as normal forms, head defects, neck and mid piece defects, tail defects and cytoplasmic droplets which were assessed by traditional fixation and sequential staining procedure of semen slides [11]. Based on the sperm concentration, the infertile subjects were classified into normozoospermia (>15 million sperm/mL), oligoasthenozoospermia (≥ 32 million sperm/mL), and azoospermia (no spermatozoa) [11]. Authors analysed data from the years 2015 to 2019 to investigate the secular trends in semen parameters. Parameters like concentration, progressive motility, morphology was analysed and compared between years 2015 to 2019 to show if there is any decline in semen quality of individuals seeking infertility treatment over a period of 5 years. The normal values of the various semen parameters are given in [Table/Fig-1].

Semen parameter	Normal values
Volume	1.5 mL
Concentration	>15 M/mL
Progressive motility	>32%
Normal forms	>4%

[Table/Fig-1]: Normal semen parameters [11].

STATISTICAL ANALYSIS

The data was entered in Microsoft Excel and analysed by using Statistical Package for the Social Sciences (SPSS) version 22.0. Analytical tests used was t-test to compare the mean difference and Analysis of Variance (ANOVA) test to compare multiple groups.

RESULTS

A total of 250 semen reports were included in the study. They belong to age group between 20 to 60 years. Among 50 semen samples in each group, most of the subjects belonged to 25-35 years. In the year 2015, among 50 samples, 70% subjects had tail defects more than 20%. [Table/Fig-2].

On analysis of semen parameters from the year 2015 to 2019, it was found that there is a significant difference in mean of progressive motility and concentration of sperm which was found to

Variable	2015	2016	2017	2018	2019
Age (years)					
<25	1 (2%)	1 (2%)	0	1 (2%)	1 (1%)
25-35	25 (50%)	26 (52%)	25 (50%)	26 (52%)	21 (42%)
>35-45	19 (38%)	20 (40%)	19 (38%)	16 (32%)	25 (50%)
>45	5 (10%)	3 (6%)	6 (12%)	7 (14%)	3 (6%)
Total	50	50	50	50	50
Concentration (M/mL)					
<50	20 (40%)	27 (54%)	27 (54%)	18 (36%)	23 (46%)
50-100	14 (28%)	16 (32%)	22 (44%)	19 (38%)	15 (30%)
>100-150	13 (26%)	4 (8%)	1 (2%)	12 (34%)	7 (14%)
>150	3 (6%)	3 (6%)	0	1 (2%)	5 (10%)
Total	50	50	50	50	50
Volume (mL)					
<2	21 (42%)	20 (40%)	24 (48%)	22 (44%)	20 (40%)
2-4	28 (56%)	18 (36%)	25 (50%)	22 (44%)	26 (52%)
>4	1 (2%)	12 (24%)	1 (2%)	6 (12%)	4 (8%)
Total	50	50	50	50	50
Progressive motility (%)					
<15	15 (30%)	6 (12%)	23 (46%)	11 (22%)	27 (54%)
15-30	21 (42%)	20 (40%)	23 (46%)	16 (32%)	16 (32%)
>30-45	7 (14%)	15 (30%)	4 (8%)	16 (32%)	6 (12%)
>45	7 (14%)	9 (18%)	0	7 (14%)	1 (2%)
Total	50	50	50	50	50
Normal forms (%)					
<3	32 (64%)	45 (90%)	47 (94%)	46 (92%)	50 (50%)
3-6	16 (32%)	2 (4%)	3 (6%)	2 (4%)	0
>6	2 (4%)	3 (6%)	0	2 (4%)	0
Total	50	50	50	50	50
Head defects (%)					
<50	46 (92%)	45 (90%)	50 (50%)	30 (60%)	48 (96%)
≥ 50	4 (8%)	5 (10%)	0	20 (40%)	2 (4%)
Total	50	50	50	50	50
Neck and mid piece defects (%)					
<20	4 (8%)	7 (14%)	8 (16%)	14 (28%)	8 (16%)
≥ 20	46 (92%)	43 (86%)	42 (84%)	36 (72%)	42 (84%)
Total	50	50	50	50	50
Tail defects (%)					
<10	2 (4%)	16 (32%)	7 (14%)	19 (38%)	17 (34%)
10-20	13 (26%)	20 (40%)	28 (56%)	29 (58%)	20 (40%)
>20	35 (70%)	14 (28%)	15 (30%)	2 (4%)	13 (26%)
Total	50	50	50	50	50
Cytoplasmic droplets (%)					
<2	43 (86%)	50 (50%)	35 (70%)	50 (50%)	30 (62%)
2-4	2 (4%)	0	14 (28%)	0	16 (32%)
>4	5 (10%)	0	1 (2%)	0	4 (8%)
Total	50	50	50	50	50

[Table/Fig-2]: Semen parameters and related variables of the study samples from 2015 to 2019.

be statistically significant at 95% confidence interval (p -value <0.05) [Table/Fig-3].

In order to find out between which years the mean difference was found to be more significant; comparison was done between the years 2015 to 2019 on different semen parameters. When compared with the semen concentration in the year 2015, statistically significant mean difference was found with semen concentration in the years 2016 and 2017. The semen concentration in 2017 had statistically significant mean difference when compared with values in 2018 and

Semen parameters	Year of study					F-value	p-value
	2015	2016	2017	2018	2019		
Semen volume (mL)	2.20±0.87	2.97±2.16	2.29±1.02	2.68±1.3	2.50±1.28	2.344	0.055
Progressive motility (%)	25.14±16.37	30.90±13.545	18.26±9.45	28.14±15.05	17.30±11.41	3.443	0.009
Concentration of sperm (M/mL)	78.1±61.15	57.72±45.70	44.44±29.564	69.84±43.03	65.96±57.85	10.01	0.001
Normal forms (%)	2.90±1.67	2.92±7.42	2.18±0.896	1.80±2.080	1.28±0.640	1.985	0.097

[Table/Fig-3]: One-way ANOVA analysis of semen parameters of men from the year 2015-2019. p-value <0.05 was considered as statistically significant

2019 [Table/Fig-4]. Regarding progressive motility, when the values in 2015 were compared, it was found that statistically significant mean difference found with the values obtained in the years 2016, 2017 and 2019 [Table/Fig-5].

Semen concentration	Mean difference	p-value
2015		
2016	20.380	0.038
2017	33.660	0.001
2018	8.260	0.398
2019	12.140	0.215
2016		
2015	-20.380	0.038
2017	13.280	0.175
2018	-12.120	0.215
2019	-8.240	0.399
2017		
2015	-33.660	0.001
2016	-13.280	0.175
2018	-25.400	0.010
2019	-21.520	0.028
2018		
2015	-8.260	0.398
2016	12.120	0.215
2017	25.400	0.010
2019	3.880	0.691
2019		
2015	-12.140	0.215
2016	8.240	0.399
2017	21.520	0.028
2018	-3.880	0.691

[Table/Fig-4]: Multiple post-hoc comparisons (LSD) of Semen concentration of men from the year 2015-2019. p-value <0.05 was considered as statistically significant

Progressive motility	Mean difference	p-value
2015		
2016	-5.760	0.033
2017	6.880	0.011
2018	-3.000	0.264
2019	7.840	0.004
2016		
2015	5.760	0.033
2017	12.640	<0.001
2018	2.760	0.304
2019	13.600	<0.001
2017		
2015	-6.880	0.011
2016	-12.640	<0.001
2018	-9.880	<0.001
2019	0.960	0.721

2018		
2015	3.000	0.264
2016	-2.760	0.304
2017	9.880*	<0.001
2019	10.840*	<0.001
2019		
2015	-7.840*	0.004
2016	-13.600*	<0.001
2017	-0.960	0.721
2018	-10.840*	<0.001

[Table/Fig-5]: Multiple post-hoc comparisons (LSD) of progressive motility of sperm of men from the year 2015-2019. p-value <0.05 was considered as statistically significant

The post-hoc comparison of other semen parameters like semen volume was not done as they did not have any statistically significant mean difference over the 5-year period.

DISCUSSION

Male infertility has been increasing alarmingly over the past few years due to causes unknown. Some may be attributed to changing lifestyle, workplace environment, exposure to toxic chemicals etc., [12]. It is necessary to identify and study the changing semen parameters over the years so that these findings can be better understood. The findings of the study done in an infertility center among 250 sperm samples collected and analysed over 5 years are discussed below.

The study findings suggested that semen parameters such as progressive motility and sperm concentration decreased over time when compared with 2015 and 2019. In a study done by Borges E et al., in Brazil, there was a time related decline of semen quality among patients suffering from infertility [13]. Auger J et al., observed that there was decline of sperm concentration and motility over the past 20 years [14]. Rolland M et al., demonstrated that there was significant reduction in sperm concentration over a period of 1989 to 2005 [15]. Similarly, Ajayi AB et al., found that there was a statistically significant 37% drop in sperm count and progressive motility over the past decade [8].

Contradictory findings were observed in studies conducted by Marimuthu P et al., in India and Vierula M et al., in Finland in which there was no significant difference in sperm parameters observed over the years [16,17]. In a study done by Marimuthu P et al., it was concluded that decline in sperm quality was not a global occurrence and it is found to be more prevalent in some parts of the world [12].

A study done by Merzenich H et al., when comparing meta-analyses of studies done on sperm quality over years found that there was a 50% fall in sperm quality observed over the years but found that the included studies were of heterogeneity as ethnical and geographical variations between different study areas could have played a role as they were not able to generalise the findings to the whole population as most were institution based studies [18]. There must be population based prospective studies to better understand the reality of sperm parameters over the years.

A study done by Adiga SK et al., in South India among 7770 subjects found that among those who visit for treatment of infertility, the quality of semen was found to be deteriorating over time and the causes of which were attributed were most probably due to nutritional, environmental lifestyle and economic factors [19].

Various causes have been attributed to decreasing sperm parameters over the years. Aging is one of the most important causes which have been attributed to infertility among men. Studies shows that sperm motility decreases with advancing age consistent with the study findings in which most of the study participants were aged above 35 years and progressive motility was found to be reduced when comparing 2015 with the year 2019 [20,21].

Limitation(s)

The study has limited external validity as the results of the study cannot be generalised to the population as it was a hospital-based study. Factors like lifestyle and social factors were not taken into account due to logistic limitations. If included they would have helped in finding the causal association of decreasing sperm trends if any, over the 5-year period.

CONCLUSION(S)

There was significant worsening of sperm counts and poorer mean progressive motility of sperms over the 5-years period from 2015 to 2019. Further research, mainly population-based studies are needed to understand the environmental, lifestyle and personal factors contributing to the decrease in semen parameters over time. Male infertility needs to be addressed with equal attention as given to female infertility, as both are equal contributors for infertility among married couples.

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PARTICULARS OF CONTRIBUTORS:

1. Undergraduate Student, Department of Community Medicine, Saveetha Medical College and Hospital, Thiruvallur, Tamil Nadu, India.
2. Assistant Professor, Department of Community Medicine, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
3. Postgraduate Student, Department of Community Medicine, Saveetha Medical College and Hospital, Thiruvallur, Tamil Nadu, India.
4. Professor and Head, Department of Community Medicine, Saveetha Medical College and Hospital, Thiruvallur, Tamil Nadu, India.
5. Professor, Department of Community Medicine, Saveetha Medical College and Hospital, Thiruvallur, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

VM Anantha Eashwar,
158, Washer Varadappa Street, Chennai, Tamil Nadu, India.
E-mail: eashwaranand@yahoo.in

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