Characteristics of semen parameters in a selected population of Indian men over a period of 10 years

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Several reports have appeared in literature on the reported decline in semen quality globally. The Institute for Research in Reproduction, Mumbai, has been analysing semen from fertile as well as infertile men for over two decades. In view of the concerns expressed in the literature, we carried out a retrospective study on the data available with us to find out whether or not similar fall in semen quality can be observed in our studies as well. The preliminary results of the analysis of the data obtained from 1986–1995 is presented. The data from a total of 1505 semen analyses from men enrolled for different programmes was analysed. For comparison, data from proven fertile men were grouped in two groups, one carried out before 1990 ($n = 106$) and the other after 1990 ($n = 60$). The data based on four seminal parameters: volume, sperm count, motility and morphology of the sperms is reported in this communication. The results are reported as mean for each year as well as mean for two year groupings. The data shows a significant decrease ($P < 0.05$) in sperm count (43%) and a departure from normal sperm morphology (30%) over a period of time. No significant changes were seen in the volume and motility. In the fertile group as well, there is a similar reduction in sperm count and sperm morphology. The percent of all men in the category of > 100 million/ml sperm count decreased with a concomitant increase in the < 20 million/ml group. The preliminary analysis indicates a decline in semen quality in our group of men over a decade.

PUBLICATIONS in a variety of journals including medical, environmental and lay press that human semen quality, the world over, is decreasing, has evoked a lot of concern. A number of studies on this suggest that sperm counts in human are declining[1–13] coupled with increasing incidence of testicular cancers and congenital abnormalities of male reproductive function[12,14]. The issue however is controversial[15–18] and debatable due to poor availability of records, quality control, quality of semen analysis, and method of statistical analysis[19,20]. This Institute has been analysing semen from fertile and infertile men since the last two decades. The present study reports the data of semen analysis over a period of ten years, 1986–1995. This paper presents preliminary analysis of the data based on retrospective study conducted by our laboratory.

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This study used the semen samples (IRR Data) of 1505 males enrolled at the infertility clinic of the Institute over a 10 year period for *in vitro* fertilization and embryo transfer programme. In addition, data obtained from men of proven fertility (from 1986–1990, $n = 106$), (1990–1995, $n = 60$) was also analysed.

Uniform methodology according to the WHO manual (1992)[21] was adopted. Sperm morphology was studied following stringent criteria standardized at the Institute[22]. The age, occupation, and place of stay of the mean were recorded. The four semen attributes: volume, sperm count motility, and morphology were determined. The samples were collected in a sterile beaker with 3–5 days of sexual abstinence by masturbation. The volume of the sample was measured in a graduated centrifuge tube. The liquefaction time was noted and viscosity was measured. Motility was graded into: rapid linear, slow linear, nonprogressive and immotile. Sperm count was estimated using a haemocytometer according to the WHO manual[21]. Sperm morphology was studied by using Papanicolaou stained smears for atleast 200 spermatozoa, and percentage of normal and abnormal forms was evaluated using the criteria standardized at the Institute. The data available for the year 1996 was also analysed for understanding further continuing trend of semen quality.

Mean with standard deviation for each parameter and also for a 2 year group (1986–87, 1988–89, 1990–91, 1992–93, 1994–95) was calculated. The data is expressed as percent of men for each parameter for every 2 year. Statistical analysis was performed using Students's *t*-test.

No significant changes were noticed in the volume and percent motility. However as shown in Figure 1 the mean sperm count over the years fell by 43%—a statistically significant ($P < 0.05$) decrease from 65.71 million/ml to 28.78 million/ml. Sperms with normal morphology decreased by nearly 30% which was significant ($P < 0.05$). The mean values were 33.5% and 9.03% normal forms in 1986 and 1995 respectively. Percent of men in different categories of volume, motility, morphology and sperm count is given in Figure 2. Here too, volume and motility did not show much of a change. But, the morphology and sperm count showed a definite decrease in the percent of normal from (> 30%) with a corresponding increase in < 10% normal forms over a period of time. The percentage of men showing sperm count < 20 million/ml increased and > 100 million/ml decreased over a period of time.

The study of analysis done on two groups of proven fertile men, one before the year 1990 and the other after 1990 is depicted in Figure 3. The distribution of volume in the normal range (i.e. 1.5–4.5 ml) did not change significantly. While significant decrease in percent of men having a sperm count of > 100 million/ml was observed, an increase was observed in percent of men

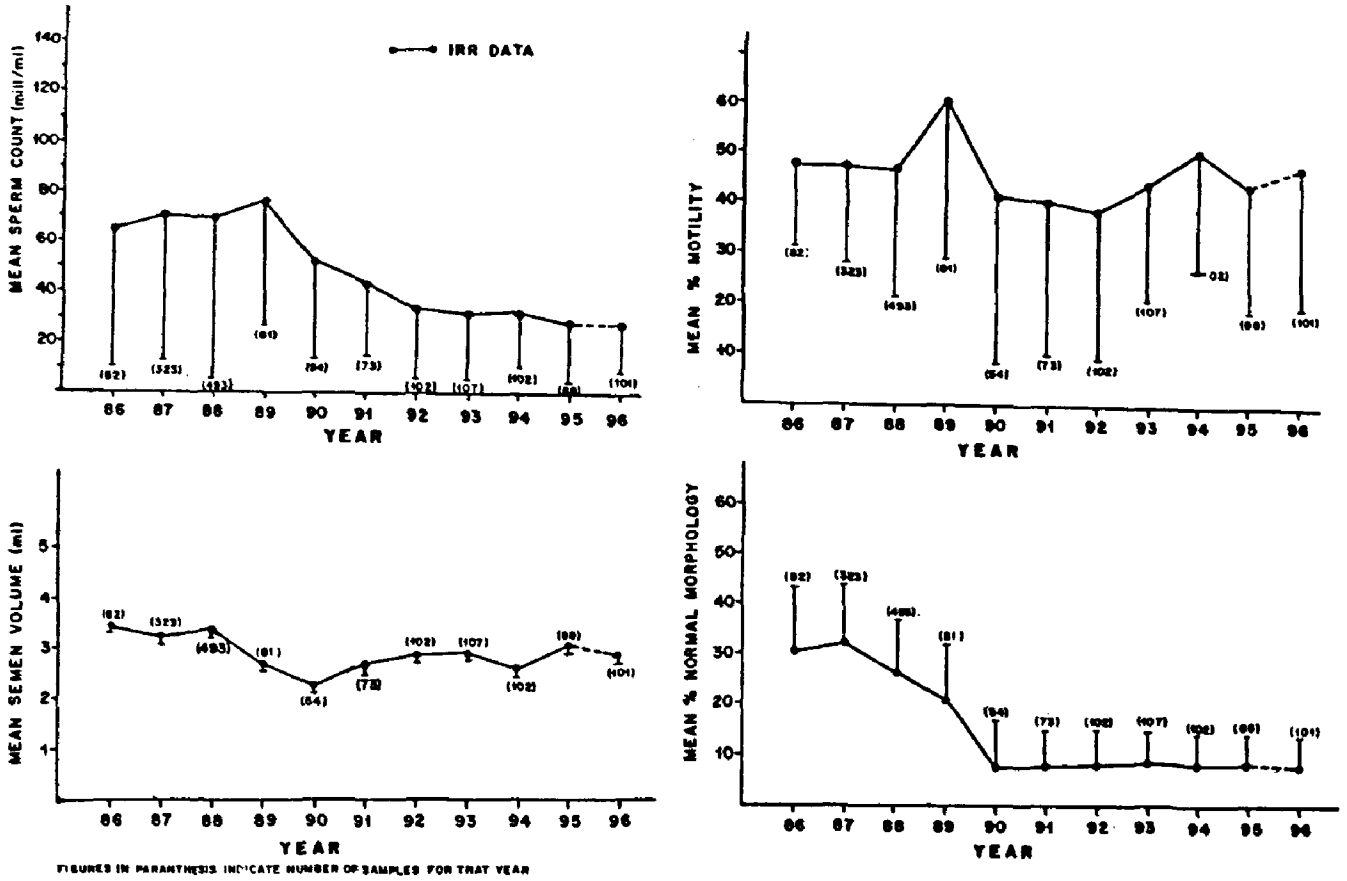

Figure 1. Semen parameters: semen volume, sperm motility, morphology and count (mean) over the years (1986–1995 and 1996).

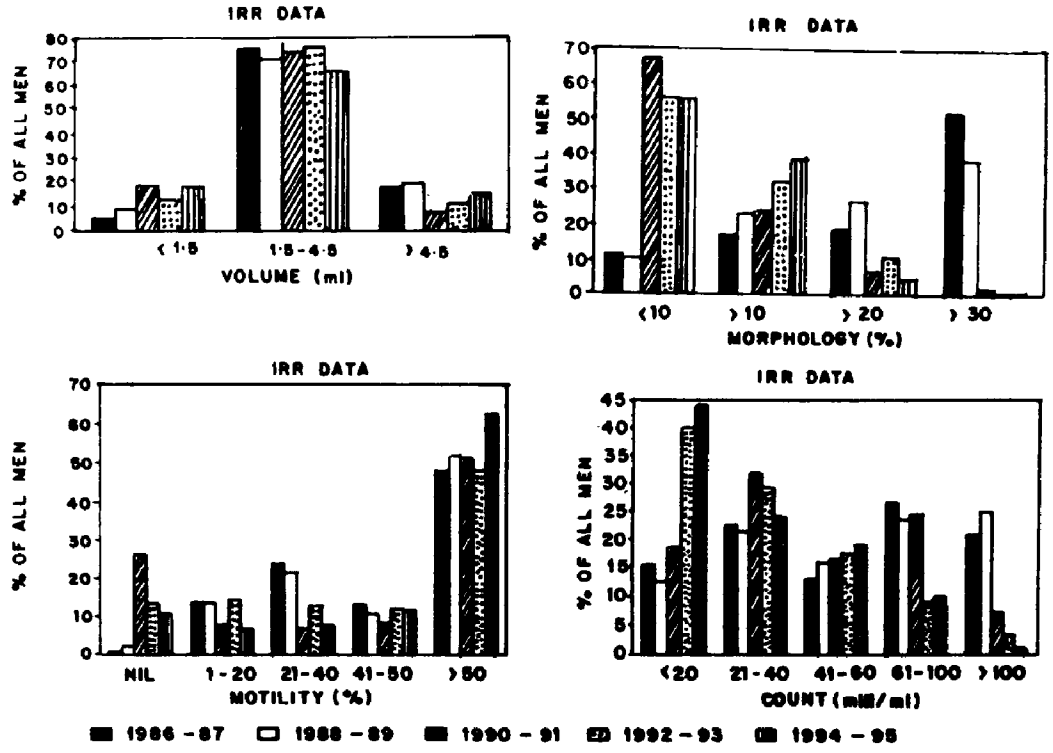

Figure 2. Per cent of men in different categories of volume, motility, morphology and sperm count from IRR.

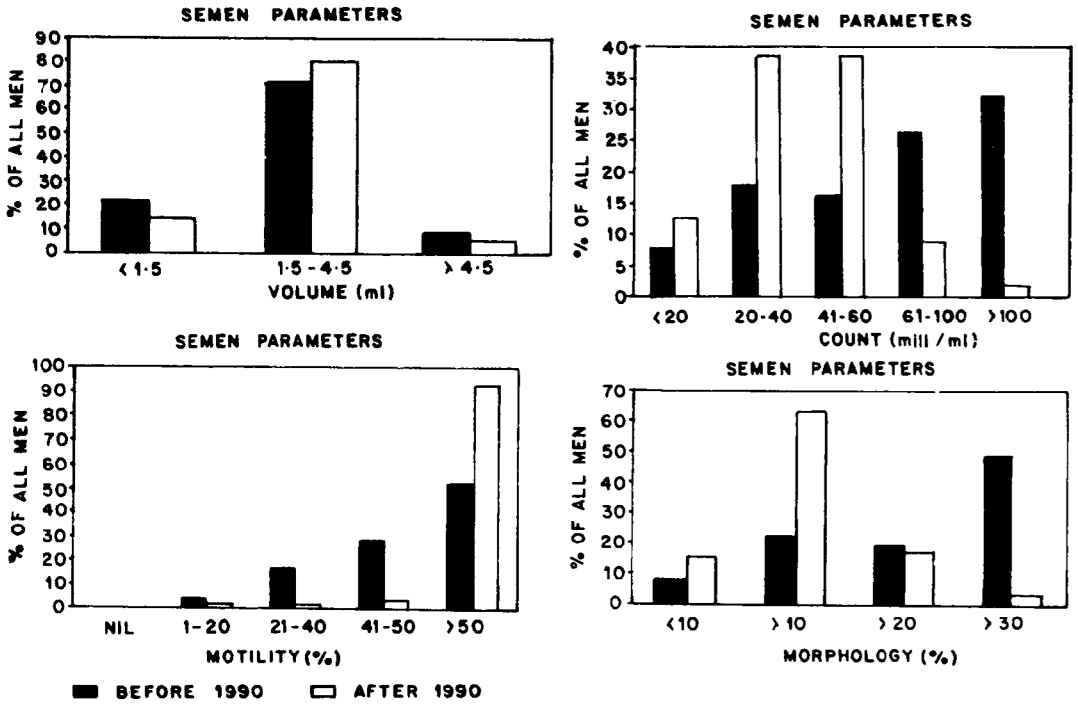

Figure 3. Semen parameters in proven fertile men, data of analysis done before 1990 and after 1990.

having a count of 20–60 million/ml. There was an increase in the number of oligozoospermic individuals (< 20 million/ml) in the later period. There was also an increase in percent of men with > 50% motility after 1990. A significant reduction in percent of men with > 30% normal sperms morphology was noticed. The mean count and morphology were significantly decreased ($P < 0.05$) after 1990 compared to before 1990. There was a striking drop in all the parameters after 1990 compared to the earlier period.

The preliminary analysis indicates a definitive trend in decline in semen quality. Further data obtained also indicates the continuation of this trend. Like previous studies[1–11,14] this too raises many questions and provides limited answers. Carlsen *et al.*[3] have reported a nearly 50% decrease in sperm count over a 50-year period. This study too shows a similar decrease (43%) in sperm count. Fishch and Goluboff[23] have reported that sperm counts vary dramatically among samples from different geographic locations. This point has to be kept in mind when analysing the data from different locations. The data presented here is not collected findings from different studies using different methodologies. The decrease in mean percentage of normal morphology over the later period suggests a deterioration not only in the quantity but also the quality of the sperms. We employed a stringent criteria for evaluation of sperm morphology which correlates with positive *in vitro* fertilization and as an indicator of fertilizing potential[22]. Though Carlsen *et al.*[3] reported a significant decrease in seminal volume from 3.40 ml to 2.75 ml, in our study we did not see a statistically significant change in the mean semen volume over a period of time. The parameters in fertile population, specially that of sperm count and morphology, did show a change with increase in < 20 million/ml group and a decrease in > 100 million/ml group. Sperm morphology also showed a downward trend, with a decrease in group with > 30% normal forms.

The semen samples were collected by masturbation and the interval of abstinence was 3–5 days. It is unlikely that these factors contributed to the observed decline in the sperm count. That the methodological error in dilution and volume of the sample loaded in the haemocytometer may cause apparent changes[24] is also unlikely, since most of the evaluations were carried out using the same dilutions and haemocytometer loading practice and observed by the same observer or under strict supervision. Therefore, any variation observed is due to genuine biological phenomenon and not due to errors related to measurement. The significant decrease in all the parameters after 1990 may be due to other factors like change in dietary habits, change in environment, etc. which need to be investigated further.

Various hypotheses have been put forward to explain the observed fall in the sperm counts. Exposure to estrogens[5] and estrogen-like substances[25–27] followed by increased exposure to a wide variety of dairy products and exposure to pollutants and fertilizers may be an important contributory factor. The type of population,

the methodology used, the quality control in the laboratory and the statistical method used also affect the findings. Detailed analysis using different statistical models will be reported later taking into consideration the methodology, quality controls, and population. The different statistical models like quadratic curve, spline fit, and stairstep model[18] will be used to reanalyse the mean sperm count and its temporal distribution. It is difficult to envisage how some artifact – rather than a true secular decline as seen in this preliminary analysis – might be responsible for the observed decline in the semen quality. Evidence[28,29] suggests that seasonality of birth rhythms has undergone significant changes in many countries, which is indicative of secular trends in human reproduction. The analysis of the data with respect to age, occupation and other parameters is underway.

The preliminary data analysis alerts us to conduct more studies both prospective and retrospective in the area of male reproductive function.

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The fate of *Mycobacterium tuberculosis* in activated human macrophages

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Human peripheral blood monocytes, that are unstimulated *in vitro*, permit free multiplication of intracellular *Mycobacterium tuberculosis* after 72 h in culture. There was no killing of bacilli in the intracellular environment even after *in vitro* activation of monocytes with a cocktail of lipopolysaccharide, phorbol myristate acetate, interferon gamma and tumour necrosis factor-alpha. We also tested the ability of adenosine triphosphate (ATP) in reducing the intracellular viability of mycobacteria. Infected monocytes upon ATP treatment underwent cell death, but no loss in the intracellular viability of *M. tuberculosis* or *M. smegmatis* could be observed.

TUBERCULOSIS remains an important global health problem with approximately one billion people presently infected with the disease[1]. During hundred years since the discovery of the tubercle bacillus, the increased understanding of bacteriological and pathological characters has resulted in important public health measures such as pasteurization of milk and BCG vaccination. However, it was chemotherapy that was introduced during the end of the last century which brought about tremendous decline in the mortality rates[2]. But the increasing number of multidrug resistant *M. tuberculosis* isolates from both AIDS and non-AIDS patients is an ominous trend that threatens the tuberculosis eradication programme[3]. Tuberculosis is actually on the rise again. The recrudescence of tuberculosis appears to have its roots in the AIDS epidemic, although declining control programmes, increased levels of homelessness and drug addiction are also contributing factors[2].

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