Changes of Sperm Parameters Along Time Among Groups of Different Qualities

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ABSTRACT: **Background:** Male infertility is solely responsible for approximately 20% of all infertility in couples. Various factors have been proposed as having a negative effect on sperm quality; however, the reasons for the global decline in sperm parameters during the last few decades are still controversial.

Objectives: To investigate the fluctuations of semen parameters (sperm concentration, motility, and morphology) in three sperm quality groups and to examine the trends of those parameters in the same men over time.

Results: Our data showed deterioration in all semen parameters assessed in the group of men originally considered as having normal semen values according to the 2010 criteria of the World Health Organization. In contrast, we found significant improvement over time in all semen parameters in the group of men with severe oligo-terato-asthenozoospermia.

Conclusions: Our results suggest that, although there were changes in sperm quality over time in the groups assessed, the clinical significance is negligible and does not necessarily justify a change in the therapeutic approach to infertility or sperm cryopreservation.

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KEY WORDS: male infertility, oligo-terato-asthenozoospermia (OTA), semen analysis, semen fluctuations, sperm parameters

Male infertility is solely responsible for about 20% of all infertility in infertile couples [1]. The male infertility factor is usually defined by abnormal values of semen parameters. However, even if results of semen analysis are normal, other male factors may be present [2]. Conventional semen analysis involves the measurements of several semen parameters, including volume and pH, as well as sperm parameters. The analysis is used to determine whether these parameters fall within the normal range for fertile men [3]. Examination of several samples is essential due to day-to-day variations in semen quality [4]. Sperm concentration, motility, and morphology are the most important parameters assessed by conventional semen analyses. However, these variables describe only visible features of sperm cells and do not allow any prediction of their genetic constitution [5].

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Various factors have been proposed as having a negative effect on sperm quality, including aging [6,7], cigarette smoking and alcohol consumption [8,9], cell phone usage [10,11], working conditions, diet, and environmental exposures to endocrine disruptors or to toxic factors; the latter mainly in industrialized countries [12,13]. However, a global decline in sperm parameters during the last decades is still a matter of controversy [13-15].

This retrospective cohort study was conducted to investigate the fluctuations in semen parameters in three sperm quality groups and to examine changes in those parameters over time.

METHODS

STUDY GROUP

The study included 1833 male subjects who underwent semen analysis at the Andrology Laboratory at the Institute for the Study of Fertility, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center between 1991 and 2010. Records of semen analysis were collected in a computerized database, which stored the data of male individuals who had undergone several semen analysis tests during follow-up visits ranging from 1 to 10 years. The study group represented a heterogeneous population in terms of age, place of residence, reasons for referral, health status, and etiology of fertility impairment. The institutional review board at the Sourasky Medical Center approved the study in accordance with the Helsinki Declaration of 1975.

SEMEN ANALYSIS

Semen samples were collected by masturbation into a sterile plastic container after 2–5 days of abstinence. The men were instructed to deliver the sample within 1 hour after ejaculation. The analyzed semen parameters included volume, liquefaction, pH, and viscosity as well as sperm concentration, motility, viability, and morphology. To determine the concentration and motility of the sperm cells, 10 µl of semen was loaded into the Makler Counting Chamber (Sefi Medical Instruments, Haifa, Israel) and examined at a magnification of ×200 under phase contrast illumination. Percent motile sperm was graded A (rapidly progressive), B (slow or sluggish progressive), or C (non-progressive) and calculated after counting at least 100 sperm cells in 4 to 6 randomly chosen fields. Sperm morphology was

evaluated following Papanicolaou staining and scored as normal or abnormal using the strict criteria of Kruger et al. [16]. All semen samples were processed and analyzed by experienced laboratory technicians at the same laboratory according to a standardized protocol. The laboratory successfully participates in various quality control exercises (UK NEQAS, External Quality Assessment Schemes).

STATISTICAL ANALYSIS

Statistical analyses, processing, and calculations were performed by the Statistical Laboratory at Tel Aviv University using IBM Statistical Package for the Social Sciences statistics software, version 22 for Windows (SPSS, IBM Corp, Armonk, NY, USA). The square root values of sperm concentrations were used to obtain normal distribution of the data. Associations between sperm concentration, overall motility, and normal morphologic characteristics were evaluated by Pearson's correlation coefficient. To describe changes in each parameter as a function of follow-up time, we used mixed models analyses, which took into account the repeated measures of the data. Finally, we used the McNemar test to assess the shifts of subjects between cutoff values across time for each parameter.

RESULTS

The study group included a total of 1833 men, of whom 1408 had two repeated semen analyses and 425 men had three to seven semen analyses, over a period of 10 years. The minimum time interval between two subsequent analyses was 1 year with a mean of 3.47 ± 1.94 years and median of 3 years. The men who comprised the study cohort were divided into three groups according to sperm concentration, overall sperm motility, and percentage of sperm cells with normal morphology [Table 1]. Group assignment was made separately for each parameter according to the results of the first documented semen analysis. The distribution of the subjects among the three sperm quality groups is shown in Figure 1. Pearson's correlation coefficients between sperm concentration, overall motility, and normal morphology varied between 0.38 and 0.55. There was a statistically significant correlation among all three sperm parameters ($P < 0.001$).

GENERAL LINEAR MIXED MODEL ANALYSIS

The findings from the samples collected during a total follow-up of ≤ 10 years were analyzed and the effect of time on semen parameter measurements was examined for all participants.

Table 1. Cutoff points in sperm quality defining the three study groups

	Group A	Group B	Group C
Concentration (10^6/ml)	› 15	› 5 and ≤ 15	≤ 5
Overall motility (%)	› 40	› 20 and ≤ 40	≤ 20
Normal morphology (%)	› 10	› 4 and ≤ 10	≤ 4

There were significantly lower concentrations and overall lower motility values over time in group A, while there was a significant increase in those parameters over time in groups B and C [Table 2] [Figure 2A, 2B] ($P < 0.001$). Sperm morphology was significantly reduced over time in groups A and B, while it was significantly increased over time in group C [Table 2] [Figure 2C] ($P < 0.001$).

Finally, we examined whether there was any change in group assignment over time among the three sperm quality groups. The results showed that most of the subjects remained in their original group according to their concentration, overall motility, and normal morphology (76%, 70%, and 61%, respectively). No difference was found between the deteriorating and improving groups in sperm concentration over time (11% and 13%, respectively). However, there was a significant difference between deteriorating and improving groups in declined motility (16% and 14%, respectively) and declined morphology(24% and 15%, respectively, McNemar test, $P < 0.001$).

Figure 1. Distribution of subjects among the sperm quality groups. Assignment of participants to quality groups was according to their first semen analysis

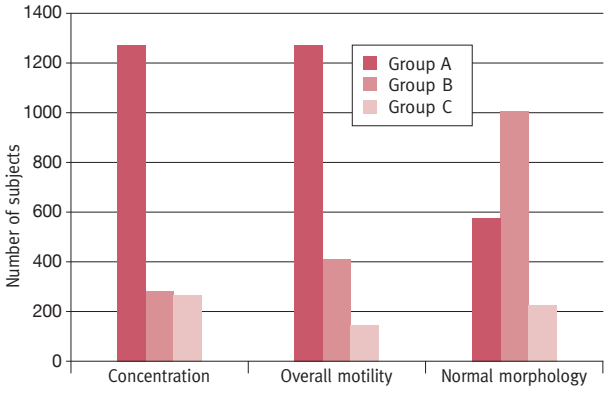
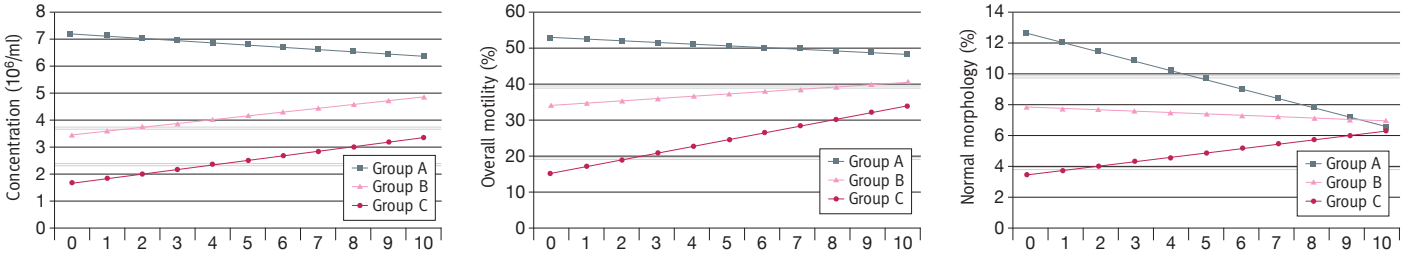


Table 2. Estimates of fixed effects. Grouping was made according to sperm quality. The square root values of sperm concentrations were used to obtain normal distribution

	Concentration		Overall motility		Normal morphology	
	Estimate	Time	Estimate	Time	Estimate	Time
Group A	7.18	-0.08	52.88	-0.44	15.97	-1.02
95%CI	(7.07, 7.30)	(-0.11, -0.05)	(52.33, 53.44)	(-0.62, -0.26)	(15.52, 16.41)	(-1.15, -0.90)
Group B	3.44	0.14	34.22	0.66	9.07	-0.21
95%CI	(3.07, 3.82)	(0.03, 0.24)	(32.54, 35.91)	(0.11, 1.21)	(8.15, 9.97)	(-0.47, 0.046)
Group C	1.66	0.16	15.11	1.56	3.52	0.29
95%CI	(1.29, 2.04)	(0.06, 0.26)	(12.87, 17.36)	(0.92, 2.20)	(2.51, 4.52)	(0.00, 0.59)

95%CI = 95% confidence interval

Figure 2. Estimates of fixed effects. The graphs for each parameter were made according to the formulas obtained from the statistical model. The dotted lines represent the cutoff points of sperm quality that defines the three study groups. The square root values of sperm concentrations were used to obtain normal distribution



DISCUSSION

Many reports have claimed that the quality of human semen has declined over the past decades [17-19]. However, most studies were compromised by methodological difficulties. Samples tended to be small and subject to the selection biases inherent in fertility clinics. Moreover, most studies were based on data from diverse male subjects and without differential diagnosis within groups in terms of measures of semen quality. Although we encountered the same difficulties, the novelty of our current study lies in its examination of a variety of semen parameters with time in groups of men with different sperm quality. Our records contained data of semen analyses of men with follow-up ranging from 1 to 10 years. While our results showed that there was deterioration in all parameters assessed in males with normal semen values according to WHO classifications [3] (group A), they showed a significant improvement in all semen parameters in subjects with severe oligo-terato-asthenozoospermia (OTA) (group C). The consequences of variability in the results of those semen analyses can be explained by the phenomenon of regression toward the mean [20] (i.e., the process of spontaneous improvement in groups that initially presented measures below the normal threshold) as a reverse process of decline in subjects who had high values in their first semen analysis. However, the improvement in group C could also be explained by changes in lifestyle that the subjects may have made due to the results of their first semen analysis [21].

According to their first semen sample, most of our study population was assigned to group A, which consisted of subjects who had normal values for sperm concentration and motility. However, assessment of the morphology parameter showed that over half (56%) belonged to group B (normal morphology percentage of > 4 and ≤ 10). Since this study contains data collected over a period of 20 years, it was not in line with the current strict criteria for normal morphology [3], which defines normal morphology as being > 4%. Therefore, according to the new criteria, our group with values between 4 and 10 would also be considered as having normal morphology values. The usefulness of a sperm morphology index has often been challenged due to different classification systems, various slide preparation techniques, and inconsistency of analyses within and between laboratories [22]. The deterioration of normal sperm cell morphology in our groups A and B could also be explained on the basis of stricter requirements and different scoring systems.

Pearson's correlation was used to assess an association between all three sperm quality parameters. While a previous study found a correlation between sperm concentration and morphology [23], our data suggests a correlation exists between sperm concentration, motility, and morphology.

Intrauterine insemination is an effective treatment for male factor infertility when initial sperm motility is ≥ 30% and the total motile sperm count is $\geq 5 \times 10^6$. Lower initial values have a smaller chance of success and thus those cases should be treated directly with in vitro fertilization (IVF) [24]. To measure a clinical significance of the changes in semen quality over time, we examined whether there was any group reassignment over time. It emerged that the majority of the subjects remained in their original sperm quality group. Hence, a change in the proposed fertility treatment should not be considered. However, for those for whom there had been a change, it was toward reduction of the motility and morphology parameters ($P < 0.001$), which may have led those subjects to undergo IVF as the preferred fertility treatment.

Sperm cryopreservation is increasingly being used in patients with severe oligozoospermia under the assumption that there will be a progressive decline of testicular function over time. Cryopreservation is also appropriate to avoid a situation in which no sperm cells are found in the ejaculate at the time of oocyte retrieval [25]. Based on our current findings, we suggest that, due to the sperm quality improvement in group C over time, cryopreservation should be reconsidered in men with oligozoospermia. In addition, cryopreservation due to predicted sperm quality deterioration over time in groups A and B is not justified since the decrease in sperm quality over time is less deleterious than the reduction that is expected after freezing and thawing.

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Capsule

Curbing ILC2 enthusiasm

Atopic dermatitis is an allergic disease driven by type 2 immune responses in the skin. **Malhotra** and colleagues studied mouse models of dermatitis. They identified the tumor necrosis factor (TNF) family cytokine TNF ligand-related molecule 1 (TL1A) and its receptor death receptor 3 (DR3) as being critical in regulating cross-talk between skin-resident T regulatory cells (T_{regs}) and type 2 innate lymphoid cells (ILC2s) that drive skin inflammation. Retinoid-related orphan receptor α (RORα) drove expression of DR3 in T_{regs}. Upon deletion of RORα, skin-resident T_{regs} were unable to sequester TL1A, which drives effector functions of ILC2s. Thus, targeting the TL1A-DR3 axis may provide a route to treating dermatitis and other skin allergies.

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Eitan Israeli

"The ultimate measure of a man is not where he stands in moments of comfort and convenience, but where he stands in times of challenge and controversy. The true neighbor will risk his position, his prestige, and even his life for the welfare of others. In dangerous valleys and hazardous pathways, he will lift some bruised and beaten brother to a higher and more noble life"

Martin Luther King, Jr., (1929–1968), American Baptist minister and activist who became the most visible spokesperson and leader in the civil rights movement. He is best known for his role in the advancement of civil rights using the tactics of nonviolence and civil disobedience based on his Christian beliefs