

Is The Quality of Donated Semen Deteriorating? Findings from a 15 Year Longitudinal Analysis of Weekly Sperm Samples

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ABSTRACT: **Background:** Studies suggest that global semen quality is declining, but the debate remains open owing to geographic variation.

Objectives: To evaluate temporal trends of sperm parameters – namely concentration, motility and total motile sperm count – in sperm donated during the period 1995–2009.

Methods: In a retrospective longitudinal cohort study we analyzed the sperm count and motility of 2182 semen samples provided on a weekly basis by 58 young, healthy, fertile, university-educated, paid donors.

Results: Despite the lowering of criteria for sperm parameters satisfactory for donation that were implemented in 2004, 38% of applicants for sperm donation are now rejected based on semen quality as compared to a third of applicants 10–15 years ago ($P < 0.001$). If the old strict criteria were in place 88% of candidates would be rejected today ($P < 0.0001$). Over the study period, the average sperm parameters dropped from a concentration of 106 ± 25 million spermatozoa/ml with $79\% \pm 4.3\%$ motility to 68 ± 14 million/ml with $66\% \pm 4.5\%$ motile sperm ($P < 0.0001$, $P < 0.0001$, respectively). The total motile sperm count per ejaculate also decreased, from 66.4 ± 18.2 million to 48.7 ± 12 million ($P < 0.005$). When the previous criteria were implemented for the analysis of the latest group of sperm donors, only 18% of donors had an acceptable sperm quality, with an average concentration of 87 ± 12 million spermatozoa/ml, $73\% \pm 2.6\%$ motile sperm and total motile sperm count of 53.1 ± 3.8 million per ejaculate – still significantly lower than 15 years ago ($P = 0.01$, $P = 0.003$, $P = 0.058$ respectively).

Conclusions: The rapid deterioration of sperm quality among fertile semen donors is alarming and may lead to cessation of sperm donation programs.

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ing sperm count over the past 50 years. Between 1938 and 1991, a significant decline in the average sperm concentration from 113 to 66 million/ml was recorded among men with no history of infertility [7]. A reanalysis of the studies included by Carlsen et al. has confirmed the observed trends [8].

During the last two decades two studies were published in Israel, but they reached opposite conclusions on the trend of sperm counts. A retrospective analysis of a single ejaculate of 188 sperm donors between 1980 and 1995 showed an increase in total motile sperm count along with a decline in the percentage of normal sperm morphology [9]. Later, a second retrospective study of 2638 semen samples provided by patients for intrauterine insemination during 1990–1999 showed a significant annual drop in sperm count and motility by 5.2 ± 0.9 million/ml and $0.5\% \pm 0.14\%$, respectively [10]. Since several well-controlled studies showed marked geographic differences in sperm counts among fertile European men, we wished to evaluate sperm quality in Israeli fertile men over time [11,12].

SUBJECTS AND METHODS

This was a retrospective longitudinal cohort study analyzing sperm quality of all semen donors for the years 1995–2009. The mode of recruitment of men and compensation for semen donation, and the method of semen analysis have remained the same during the past 15 years.

STUDY POPULATION

The study population comprised 58 semen donors. All the men were young (mean age \pm SD 25.2 ± 3.2 , range 20–37 years), healthy, unmarried, highly educated (college and university students), paid volunteers, living in the vicinity of Jerusalem at the time of sperm donation; 98% were Caucasian. The donors were recruited by posting ads on the university campuses and by word of mouth. The compensation for semen donors was relative to the cost of living in Israel during the study period.

Following interviews to rule out health problems and hereditary diseases, the donors underwent a complete physical examination. Blood tests for transmissible diseases (human immunodeficiency virus, syphilis, hepatitis B and C) and hereditary

During the past three decades several reports have suggested that the quality of semen in healthy men is globally declining [1-6]. A meta-analysis of 61 studies worldwide by Carlsen and colleagues [7] found a trend towards decreas-

disorders (Tay-Sachs, cystic fibrosis) were performed, as were a blood count and determination of blood group and Rh factor.

For the purpose of analysis the study population was divided into three groups, based on the time of enrollment: past (1995–1999) (n=20), interim (2000–2003) (n=16), and recent (2004–2009) (n=22).

DONATION OF SEMEN SAMPLES

Each candidate for donation provided, by masturbation, two ejaculate samples 2 weeks apart after 3–4 days of sexual abstinence. After meeting certain criteria of high sperm quality, the potential donor was considered qualified for donation. These criteria were based on a certain attrition rate of the sperm in the process of freezing, thawing and preparation for intrauterine insemination.

During the period 1995–2003 the following strict criteria were applied: semen volume > 2 ml, sperm concentration > 70 million/ml, and progressive sperm motility percentage > 70% (70/70). Beginning in early 2004 the criteria for an acceptable donor were changed to sperm concentration > 50 million/ml and progressive sperm motility percentage > 60% (50/60). This change was based on the clinical observation of a decline in the number of eligible donors.

After qualification as a semen donor, each man donated an average of 3.1 ejaculates per month for 12 consecutive months (mean range 29–55 semen samples per donor). Each

Table 1. Characteristics of the study population

	Past sperm donors 1995–1999 (n=20)	Interim sperm donors 2000–2003 (n=16)	Recent sperm donors 2004–2009 (n=4)	P value	Recent sperm donors 2004–2009 (n=22)	P value
	Strict criteria (70/70)				New criteria (50/60)	
Age (yrs) Mean ± SD	24.7 ± 3.4	25.6 ± 2.9	29.5 ± 5.2	NS	25.4 ± 3.4	NS
Range	20–33	21–31	26–37		20–37	
Place of birth						
Israeli born (%)	55	33	0	NS	38	NS
Non-Israeli born (%)	45	67	100		62	

ejaculate was provided after 3–4 days of sexual abstinence. Sperm volume, concentration and motility for each ejaculate were recorded. The weekly donated sperm samples (n=2182) served as the platform for this study. Monthly average semen parameters per person (n=696) were compared longitudinally.

ANALYSIS OF SEMEN SAMPLES

The specimen was incubated to liquefy at 37°C and analyzed within 1 hour. The volume of seminal fluid was determined by a graduated pipette. The concentration of sperm was determined with a Mackler counting chamber: 6 µl of gently

Table 2. Decline in sperm quality parameters in 15 years

	Past sperm donors (n=20)	Interim sperm donors (n=16)	Present sperm donors (n=4)	P value	Present sperm donors (n=22)	P value
	Strict criteria (70/70)				New criteria (50/60)	
Mean sperm conc. (million/ml)	106 ± 25	88 ± 9.5	87 ± 12	0.01	68 ± 14	< 0.0001
95% CI of the mean	69–156	74–103	74–102		50–90	
Median sperm conc. (million/ml)	102	87	87	< 0.0001	65	< 0.05
Q1-Q3	91–122	81–96	79–96		58–75	
Total sperm motility (%)	79% ± 4.3%	75% ± 4.3%	73% ± 2.6%	0.003	66% ± 4.5%	< 0.0001
95% CI of the mean	73–88	66–81	70–76		60–74	
Median sperm motility (million/ml)	79	77	74	< 0.0001	66	< 0.02
Q1-Q3	76–82	72–79	72–75		63–69	
Mean TMSC (millions)	66.4 ± 18.2	54.3 ± 16.7	53.1 ± 3.8	NS	48.7 ± 12	< 0.005
95% CI of the mean	46.9–110	32.1–87.1	48.6–57.5		33.4–71.5	
Median TMSC (millions)	63.3	49.8	53.1	NS	47	< 0.01
Q1-Q3	52.2–71.4	42.8–66.4	50.1–56.1		39.7–54.6	
Rejection rate	10/30	8/24	32/36	< 0.0001	14/36	< 0.001
Pregnancy rate*	100%	100%	100%	NS	82%	< 0.05

* Pregnancy rate is defined as achievement of a pregnancy by each sperm donor
TMSC = total motile sperm count, CI = confidence interval

Figure 1. Distribution of the monthly mean sperm count [A] and mean total motile sperm count [B] of 696 specimens donated during the years 1995–2009.



mixed semen were placed in the chamber and spermatozoa were counted with a Zeiss microscope with phase optics at a magnification of x100 and x200. The percentage of progressive motile spermatozoa was calculated from the ratio of the number of rapidly moving sperm to the total number of sperm counted, according to the 1993 classification system of the World Health Organization [13]. Morphology score was not used as a parameter due to high inter-observer variation [14]. During the study period, all sperm analyses were performed in the same laboratory with the same equipment and by the same laboratory personnel. Tests of laboratory quality assurance were done periodically to ensure the validity of sperm analyses over time.

The pregnancy rate was defined as at least one live birth outcome from each donor when sperm was used for intra-uterine insemination.

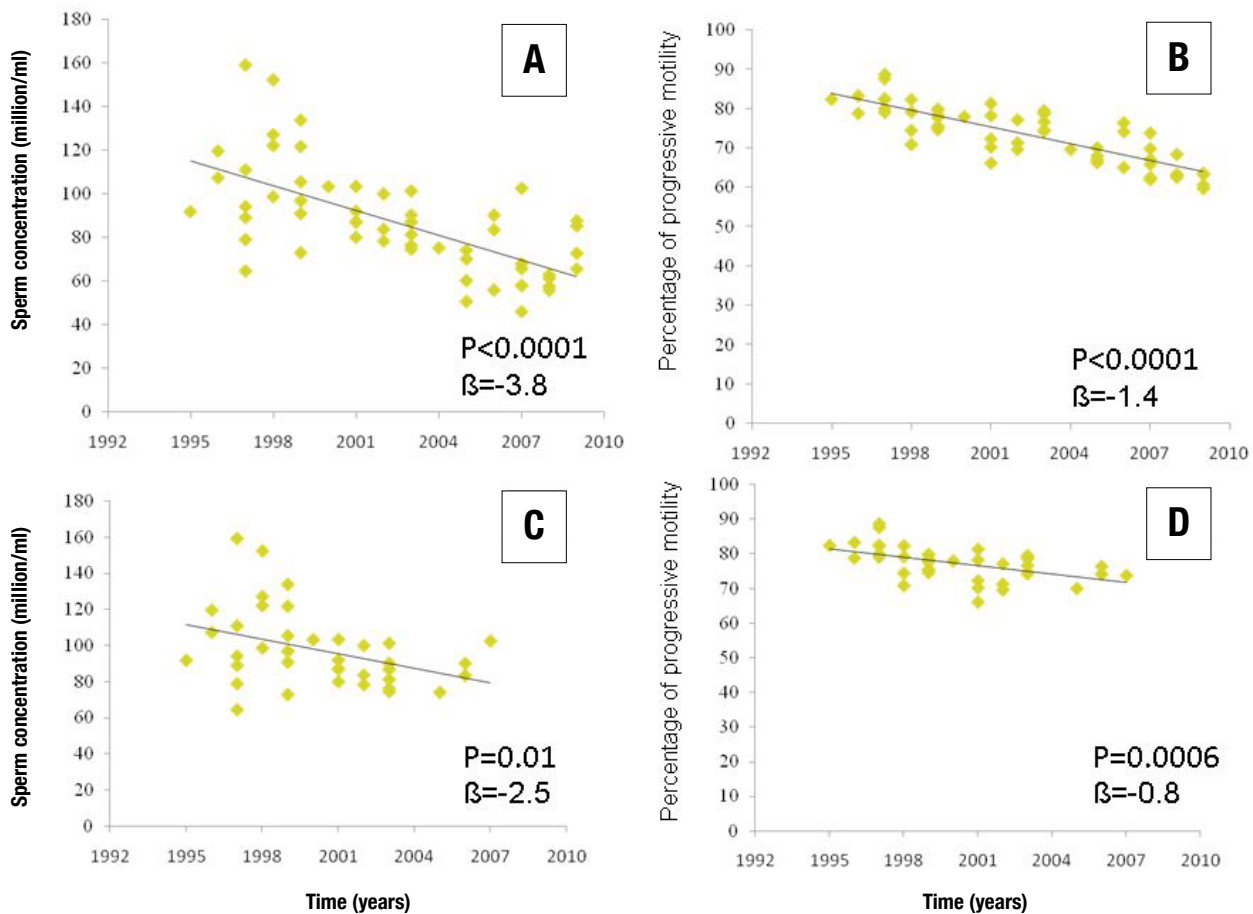
STATISTICAL ANALYSIS

Means and standard deviation were calculated for sperm concentration, motility and TMSC. Kruskal-Wallis test was used to compare medians among the three donation periods (past, interim and recent) [Tables 1 and 2]. Median scores test was used to compare the median values among the groups [Table 2]. Linear regression analysis was used to assess the changes in semen characteristics over the 15 years of the study. Dependent variables for each donor were the annual mean of sperm concentration and annual mean of percentage motility (total of 58 observations).

For the final analysis we used a general linear model for repeated measurements of the 12 monthly average samples

TMSC = total motile sperm count

Figure 2. Linear regressions of mean sperm concentrations [A & C] and motility [B & D] plotted over time. Both when using the new criteria (50/60) [A & B] and the old criteria (70/70) [C & D] for sperm count and motility, sperm parameters deteriorated significantly



of 58 donors including time of donation and donor's age and birth place as covariates.

Data storage and analysis was performed using SAS 9.1e package (SAS Institute Inc, Cary, NC, USA). All P values were two-tailed and $P < 0.05$ was considered statistically significant.

RESULTS

During the 15 year study period 90 men applied to be semen donors. A third (35.5%) of the applicants were rejected based on predetermined sperm quality criteria. The profile of the sperm donors has not changed over the years regarding mean age and place of birth, as seen in both the recent group that met the 50/60 criterion ($n=22$) and the smaller subgroup of recent donors who met the 70/70 criterion ($n=4$) [Table 1]. Of interest is the finding that all the recent candidates

who met the previous, stricter criteria for semen donation were not born in Israel. Figure 1A shows the distribution of the monthly mean sperm concentration of 696 specimens donated over the years. In the past (1995–1999) 91.9% of the samples (194/211) exceeded the strict criterion of > 70 million/ml sperm concentration, whereas in the present study only 48.7% of donated samples (128/263) were of that quality. The mean sperm concentration decreased from 106 ± 25 million/ml in the past group of donors to 68 ± 14 million/ml in the recent group. During the same period the percentage of motile spermatozoa declined from $79\% \pm 4.3\%$ to $66\% \pm 4.5\%$ ($P < 0.0001$ for both) [Table 2].

Figure 1B shows the distribution of the mean TMSC per ejaculate of all specimens donated over the years. In the past (1995–1999) 83% of the samples (160/193) were above the arbitrary 40 million TMSC as compared to only 55% (145/264) in

the present study. The mean TMSC per ejaculate decreased from 66.4 ± 18.2 million in the past group of donors to 48.7 ± 12 million in the recent group ($P < 0.005$) [Table 2].

Despite the 2004 lowering of criteria for an acceptable sperm donation, 38% of the recent candidates for sperm donation (14/36) were rejected on the basis of sperm quality as compared to only one-third of applicants (18/54) before 2004 ($P < 0.001$). Had the strict criteria been left in place, 88% of potential sperm donors (32/36) would have been rejected [Table 2]. Interestingly, while sperm quality remained high, beyond the 50/60 criterion, the total pregnancy rate has dropped significantly ($P < 0.05$).

When the previous criterion (70/70) was implemented for the analysis of the recent group of sperm donors (in 2004), only 18% of donors (4/22) had an acceptable sperm quality with an average concentration of 87 million spermatozoa/ml and 73% motile sperm, still significantly lower than the semen quality observed 10–15 years ago ($P = 0.01$, $P = 0.003$, respectively). The mean TMSC in this group decreased to 53.1 ± 3.8 million per ejaculate, yet this drop did not reach statistical significance ($P = 0.058$). In this analysis the pregnancy rate remained high and was comparable among the three groups [Table 2].

Linear regression analysis revealed that when using the new criterion (50/60) the mean sperm concentration decreased by 3.8×10^6 and sperm motility by 1.4% annually ($P < 0.0001$ for both) [Figure 2 A & B]. When using the old criterion (70/70), the annual decrease was 2.5 million and 0.8% for sperm concentration and motility ($P = 0.01$, $P = 0.0006$; respectively) [Figure 2 C & D]. Similarly, the TMSC decreased by 2 million spermatozoa per year when using the 50/60 criterion ($P < 0.0001$) and 2.8 million annually when using the previous (70/70) criterion ($P < 0.005$).

During a single year of semen donation the monthly average sperm concentration, motility and TMSC per person remained fairly constant with a small variability rate of -0.4% to +2.5%, -0.28% to +0.14%, and 1.2% to 2.4%, respectively.

In our sperm bank a TMSC of 20 million is the minimal sperm dose for intrauterine insemination. Analyzing the mean number of sperm doses provided monthly by past (11 ± 3.9 doses), interim (9 ± 3.3 doses) and recent donors (7.3 ± 2.8 doses) reveals a significant drop ($P = 0.0014$).

ANOVA analysis of the 696 mean semen samples disclosed that the time of donation (past, interim, recent) was the major factor in determining sperm quality. We can conclude that a statistically significant difference exists between the time groups for both sperm concentration and motility ($P < 0.0001$) as well as TMSC ($P < 0.01$).

Sperm parameters were not found to correlate with the groups' mean age ($P = 0.2$, $P = 0.7$ for sperm concentration and motility respectively) or birth place ($P = 0.7$, P

$= 0.15$ for sperm concentration and motility, respectively). Controlling for age and birth place to exclude these factors as potential confounders did not alter our findings.

DISCUSSION

A longitudinal analysis demonstrated a significant drop in sperm concentration, percentage of motile sperm and TMSC recorded from donated samples collected over the past 15 years. In contrast to previous small cohort studies, in the present study the sperm parameters of each individual were fully analyzed on a weekly basis for a span of a year, minimizing the chance of drawing conclusions based on a few unrepresentative semen samples. The statistical superiority of evaluating multiple semen samples from each individual has been previously stressed by other researchers [15]. To the best of our knowledge, only the young Danish military conscripts study evaluated repeated measurements of semen parameters over an extended period [16].

In our study we observed a dramatic drop within only 15 years among fertile sperm donors in three parameters of sperm quality: mean TMSC per ejaculate, mean sperm concentration, and motility. Consequently, the number of sperm doses for intrauterine insemination per month dropped significantly. Moreover, had the criteria for sperm donor eligibility stayed unchanged the rejection rate would have risen to 88%, threatening the availability of sperm donation services for intrauterine insemination. The steep decrease in donated sperm quality within such a short time is alarming and as yet unexplained. Not age, birth place, duration of abstinence, or seasonality could serve as an explanation for the trend. The mode of recruitment of semen donors as well as their compensation did not change along the time period of this study. Unfortunately, lack of demographic data on the excluded men precludes a discussion of a potential association between their profile and sperm quality as well as profile differences between eligible and ineligible semen donors. Moreover, despite similar recorded demographic characteristics of eligible donors over the study period, the hypothesis that a different donor group has emerged of late is justifiable.

It is noteworthy that despite the deterioration in sperm quality over time, the individual's sperm parameters did not change significantly during the course of a year of semen donation. Stable sperm concentration was also reported by the Danish cohort during a 4 year follow-up period, suggesting a secular decrease in sperm production of the study population [16].

Despite the steep deterioration in sperm quality in our cohort the pregnancy rate of the study group remained high at $> 80\%$ but was significantly lower than previously recorded, suggesting that the subfertility limit may be imminent. Unfortunately, owing to a variable profile of semen recipients the useful parameter of time to pregnancy could not be investigated.

Sperm output, although a valuable tool for infertility inves-

tigation, is considered an unreliable surrogate for male fertility in population studies. In the absence of men worldwide willing to provide semen samples (if not concerned about their fertility), semen donors could be viewed as a unique group of young fertile men at the prime of sperm production available to study [17]. In 1951, a minimal sperm concentration of 20 million/ml was set as a statistically significant parameter for male fertility [18]. This limit was later endorsed by the World Health Organization as the lower limit of normal. In this study we estimated a declining rate of TMSc by 2.8 million per ejaculate per year and a sperm concentration drop of 2.5 million/ml per year, corresponding to an annual drop of 0.8% in sperm motility. If this downward trend persists we may predict that by 2030 this highly selective group of sperm donors will itself reach the subfertility sperm count of 20 million/ml. The prospect is that our program of semen donation for intrauterine insemination will soon be at risk of closure. Based on the estimations by Van Voorhis and team [19], this tipping point may be reached when the total motile sperm count is 10 million per sample.

Our study had some internal weaknesses that should not be overlooked: a) this was a retrospective analysis, subject to inborn biases, with all data being crude and none adjusted for potential confounders or factors known to impact semen quality; b) the number of subjects was relatively small; c) the large variation in sperm counts between individuals may bias the results; d) the group was highly selected and may therefore be unrepresentative of the normal population; and e) lack of information on lifestyle and habits of the study group precluded making assumptions of possible causes. Nonetheless, the strengths of the study should be mentioned: a) longitudinal cohort, b) long study duration, c) full and repeated sperm analyses, and d) the unique and high quality data collection.

The recorded rapid deterioration of sperm quality among semen donors in our unit may be alarming. This presumed trend can lead in the near future to closure of services of semen donation for intrauterine insemination based on low sperm quality and a switch to in vitro fertilization. An investigation of trends in quality of donated sperm in other Israeli units as well as plausible etiologies for the presumed drop in donated semen quality is needed.

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Cheerfulness and contentment are great beautifiers and are famous preservers of youthful looks

Charles Dickens (1812-1870), English writer and social critic regarded as the greatest novelist of the Victorian period and creator of some of the world's most memorable fictional characters. During his lifetime Dickens' works were popular, but it was in the twentieth century that his literary genius was fully recognized by critics and scholars. This year, 200 years since his birth, is witnessing a resurgence of interest in his works