Evolution of semen quality in North-eastern Spain: a study in 22 759 infertile men over a 36 year period

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Introduction

There is continuing controversy over whether sperm quality has changed over time. Some studies have reported a decline in sperm quality (Nelson and Bunge, 1974; Leto and Frensilli, 1981; Bostoff et al., 1983; Bendvold, 1989; Carlsen et al., 1992; Auger et al., 1995; Irvine et al., 1996; Adamopoulos et al., 1996; Van Waeneghem et al., 1996) while others could not detect any changes (MacLeod and Wang, 1979; Tummon and Mortimer, 1992; Suominen and Vierula, 1993; Fisch et al., 1996; Paulsen et al., 1996; Berling and Wöllner-Hanssen, 1997).

The controversy over sperm counts began with a meta-analysis by Carlsen et al. (1992) which showed an important decline in sperm concentration between 1940 and 1990. Some studies have criticized both the retrospective design and the mathematical analysis used in this study (Brake and Krause, 1992; Bronwich et al., 1994; Olsen et al., 1995; Fisch and Goluboff, 1996).

Some authors (Fisch et al., 1996; Paulsen et al., 1996; CECOS et al., 1997) suggest that for an accurate assessment of changes in sperm quality, the geographic location must be taken into account. Such geographical differences might be related to ethnic, genetic or environmental factors (Danish Environmental Protection Agency, 1995; Adamopoulos et al., 1996; Bujan et al., 1996).

However, these apparent variations could also be affected by the methods of semen analysis, data processing or population selection (Lipshultz, 1996). An alternative way to investigate possible changes in semen parameters would be to assess by retrospective analysis data from a single laboratory, using the same technique and based on a large population over an extended period of time as the one presented here.

We started this study to investigate whether sperm quality has changed among men in infertile couples referred to our laboratory during the last 36 years (from 1960 to 1996). The purpose was to retrospectively investigate changes in semen parameters (volume, concentration, motility, morphology) of 22 759 men with fertility problems, from North-eastern Spain, living in the greater Barcelona area (rural and urban), and evaluated in a single laboratory by the authors, since 1960.

The possibility of a selection bias due to geographical differences or related to the fact that men in infertile couples do not represent a random sample of the general population, especially because many couples were infertile due to a female factor are recognized. Nevertheless, this population includes the largest number of individuals analysed so far, and the observations over 36 years represent an extended period of time.

Materials and methods

Subjects

From a total population of 22 759 men studied because of infertility in our laboratory, 762 men (3.3%) were excluded because of age and/or because the period of sexual abstinence was unknown. Of the total population 1364 men had azoospermia (6.0%). The changes observed in the semen parameters analysed in this large population showed no evidence of a deteriorating sperm quality, although a statistically significant decline was observed in the percentage of normal spermatozoa.

Key words: Semen analysis/semen quality/time-related change
area from North-eastern Spain who worked in technical or business positions, manufacturing jobs, agriculture and students.

Sample collection
Seminal samples were collected by masturbation into wide-mouth glass or plastic containers, supplied by the laboratory, after 3–7 days of sexual abstinence.

Semen analysis
The following semen parameters were analysed: volume, sperm count, total sperm count, percentage of motile spermatozoa, motile sperm count, percentage of normal spermatozoa and normal sperm count. All analyses were carried out by the authors and the same laboratory technician. Samples were analysed within 30–60 min, after liquefaction. The volume was determined by drawing up the entire sample into a 10 ml graduated and contrasted glass tube.

For sperm counts, a Bürker or Bürker–Türk haemocytometer (Andolz and Bielsa, 1995) was used with appropriate dilution, depending on the initial impression of sperm concentration (1:10; 1:20; 1:50). Diluting fluid was 10 ml of sodium saline (9 g/l NaCl) or phosphate buffer (0.15 M NaCl) with 100 µl of formaldehyde (35%). Diluted seminal samples were mixed before transferring a drop to the two chambers of the haemocytometer. After about 2–3 min in a moist chamber to allow for the sedimentation of the cells, the spermatozoa were counted under a light microscope at ×250 magnification. The number of sperm cells in three of the nine squares on the diagonal of the reticle in each chamber were counted, and the mean value was calculated (World Health Organization, 1992).

To determine the percentage of motile spermatozoa, a 20 µl drop of gently mixed semen (37°C) was placed on a glass slide (37°C) under a coverslip (20×20 mm). The slide was placed on a heating stage (37°C) and observed at ×400 magnification under phase contrast. At least 100 spermatozoa were counted, and the mean value from duplicate measurements was calculated. If the coefficient of variation between the two aliquots was >5%, the determination was repeated in two different aliquots (Andolz and Bielsa, 1995). The percentage of motile spermatozoa was calculated from the ratio of the number of rapidly and slowly moving spermatozoa (grades a and b, according to the WHO classification, 1992) to the total number of spermatozoa counted.

Sperm morphology was assessed in Papanicolaou-stained smears in the first decade (1960–1969), but in the last period (1970–1996) all samples were examined using the staining technique, described by us (Bielsa et al., 1994). There was a good correlation between these two methods and ours was easier and provided much more information about the cells. For morphology assessment we used the criteria described by Jöel (1959) and MacLeod (1964) and the classification proposed by David et al. (1975).

Statistical analysis
The data were analysed using SPSS statistical computer software, version 7.5 (SPSS Inc, Chicago, IL, USA). Categorical variables are presented as percentages. All continuous variables are presented as mean ± SD. When their distribution departed from abnormal distribution, median and first and third quartiles are presented. To aid comparison with other authors, e.g. Berman et al. (1996), they are also presented the geometric mean and the 95% confidence interval.

After testing the assumptions on residuals: linearity, equality of variance, independence of error, normality, as well as the examination of the plot of residuals that permitted detection and investigation of outliers, it was concluded that multiple linear regression models were appropriate to assess the effect of age, days of abstinence and calendar year (independent variables) on semen characteristics. Log-transformation (base 10) was required for semen volume, sperm count, total sperm count, percentage of sperm motility, motile sperm count, percentage of normal spermatozoa and normal sperm count. The multiple coefficient of determination ($r^2$) is presented for each model along with $P$ value and the slope of the independent variable of interest which represented the yearly change in percentage. The $\chi^2$ for trends was used to test whether the percentage of azoospermic men decreased from the 1960s through the 1990s.

Results
Semen characteristics
Table I shows the semen characteristics of the 20 411 men included in the study. The seminal volume was 4.1 ± 1.8 ml (mean ± SD); percentage motile spermatozoa was 32.4 ± 16.9% and percentage normal spermatozoa was 49.1 ± 18.0%. For sperm count, total sperm count, motile sperm count and normal sperm count, the geometric mean (95% CI) was calculated and the values were: 27.8 (27.1–28.4); 103.5 (101.0–106.0); 8.8 (8.5–9.0) and 12.9 (12.5–13.2) respectively (Table II).

| Table I. Semen characteristics of 20 411 men studied because of infertility |
|-----------------------------|-----------------------------|
| Characteristic               | Mean (SD)                   |
| Semen volume (ml)            | 4.1 (1.8)                   |
| Sperm count ($\times 10^6$/ml$^3$) | 44.0 (74.0)                |
| Total sperm count ($\times 10^6$)$^4$ | 166.6 (286.4)             |
| Motile spermatozoa (%)       | 32.4 (16.9)                 |
| Motile sperm count ($\times 10^6$/ml$^4$) | 14.3 (31.1)               |
| Normal spermatozoa (%)       | 49.1 (18.0)                 |
| Normal sperm count ($\times 10^6$/ml$^4$) | 21.2 (42.5)               |
| Age (years)                  | 31.9 (5.4)                  |
| Abstinence period (days)      | 4.7 (1.0)                   |

Table II. Log-transformed and geometric mean of abnormal distributed semen data in 20 411 fertile men

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Semen volume
Multiple linear regression analyses after adjustment for age and abstinence period revealed a 0.2% ($P < 0.001$) yearly decline in seminal volume of the 20 411 men included in the study (Figure 1A). Although statistically significant, the weak decline in seminal volume is considered clinically irrelevant ($r^2 = 0.006$) (Table III).

Age and period of abstinence influenced seminal volume.
Table III. Adjusted percentage of changes per year in semen characteristics of 20,411 men studied because of infertility

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<th>Percentage change</th>
<th>95% CI</th>
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<tr>
<td>Semen volume (ml)</td>
<td>0.006</td>
<td>$-0.2^a$</td>
<td>$-0.3$ to $-0.09$</td>
</tr>
<tr>
<td>Sperm count ($10^6$/ml)</td>
<td>0.004</td>
<td>$+0.04^b$</td>
<td>$-0.3$ to $+0.4$</td>
</tr>
<tr>
<td>Motile spermatozoa (%)</td>
<td>0.003</td>
<td>$+0.4^a$</td>
<td>$+0.2$ to $+0.5$</td>
</tr>
<tr>
<td>Normal spermatozoa (%)</td>
<td>0.248</td>
<td>$-3.6^a$</td>
<td>$-3.7$ to $-3.6$</td>
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As converted from the antilog of the regression coefficients. CI = confidence interval.
$P$ values adjusted for age and abstinence period: $^aP < 0.001$; $^b$not significant.

The volume decreased significantly with age (0.5%; $P < 0.001$), but increased with the period of abstinence (2.9%; $P < 0.001$).

**Sperm count**

There was a non-significant increase (0.04%) over the 36 year period in the spermatozoa group (Figure 1B) (Table III). The sperm count increased significantly both with increasing age (0.7%; $P < 0.004$), and increasing period of abstinence (10.0%; $P < 0.001$). There was a non-significant yearly decline (0.1%) in the total sperm count.

**Motile spermatozoa**

The percentage sperm motility increased significantly by 0.4% ($P < 0.001$) in the spermatozoa group (Figure 1C) (Table III). The percentage of motile spermatozoa decreased significantly with age (0.3%; $P < 0.01$). In a similar fashion, motility decreased significantly (2.8%; $P < 0.001$) with increasing abstinence period. There was a non-significant yearly decline (0.2%) in motile sperm count.

**Normal spermatozoa**

Percentage of normal spermatozoa had a statistically significant decline in our population (3.6%; $P < 0.001$) (Table III) (Figure 1D). Abstinence period did not modify the percentage of normal spermatozoa, only age significantly decreased the percentage of normal spermatozoa (0.2%; $P < 0.001$). There was an adjusted 3.4% ($r^2 = 0.020$, $P < 0.001$) yearly decline in normal sperm count.

**Azoospermia**

Of the whole population ($n = 22,759$), 1364 men had azoospermia (6.0%) and 222 (1.0%) were not considered strictly azoospermic because spermatozoa were observed in the pellet after centrifugation. Of the 1364 men with azoospermia, five (0.4%) showed spermatogenic cells in their ejaculate. The percentage of azoospermic men over decades found in our laboratory was as follows: 1960–1969, 13.6%; 1970–1979,
6.5%; 1980–1989, 4.5% and 1990–1996, 2.5% (P < 0.001 χ² for trends). The mean (±SD) age of the azoospermic group was 32.3 ± 5.8 years (range, 17–71). The decrease in the percentages with time could be explained because, in more recent times, patients could have been examined elsewhere prior to study in our laboratory.

Discussion

The study of 20 411 men referred to our laboratory from 1960 to 1996 for fertility problems, showed no clinically important changes over time in semen parameters, except for morphology. Before the recent controversy, the hypothesis of declining sperm quality had attracted attention in the 1970s. However, MacLeod and Wang (1979) comparing two series of men evaluated because of ‘infertile marriage’ in 1951 (n = 1000) and in an 11 year study performed between 1966 and 1977 (n = 9000) found no significant differences in the sperm count. Ours findings are very similar to those obtained in this first large-scale study.

In contrast, our results do not agree with those of other authors who have reported a continuing decrease in sperm count over time (Nelson and Bunge, 1974; Leto and Frenssilli, 1981; Bostofte et al., 1983; Bendvold, 1989; Carlsen et al., 1992; Auger et al., 1995; Irvine et al., 1996; Adamopoulos et al., 1996; Van Waerelem et al., 1996).

In the same manner our results do not agree with those of Auger et al. (1995) in a study of 1351 fertile men in Paris, who reported a decrease in the percentage of motile spermatozoa by 0.6%, versus an increase in 0.4% (P < 0.001) in our group. On the other hand, the decrease in the percentage of normal spermatozoa (0.5%) observed by Auger et al. (1995) was lower than those observed in our population (3.6%). These authors showed a significant increase in the age of the sperm donors, from 32 years in 1973 to 36 years in 1992 (P < 0.001). This was not observed in our study (32.4 years in 1960 compared with 33 years in 1996).

Interestingly, the results from our group of 20 411 men are in agreement with those of Berling and Wölnér-Hanssen (1997) in fertile men from Southern Sweden, except for the increase in the percentage of normal spermatozoa. Similarly, the results obtained by Bendvold (1989) in infertile Norwegian men are comparable with those of our population (n = 20 411), except for the decrease in semen volume (r² = 0.006; statistically significant but not clinically important).

None of the changes in semen characteristics observed in this study of 20 411 men indicated deteriorating sperm quality. These changes were probably not clinically significant; the r² values generally being extremely small. The findings in our population of unselected infertile men agree with those of Fisch et al. (1996) who observed a slight increase in sperm count. Paulsen et al. (1996) also found a slight improvement of sperm concentration in sperm samples from healthy volunteers in Washington state, USA.

The only deteriorating characteristic was the percentage of normal spermatozoa which declined by 3.6% (r² = 0.248) per year from 1960 to 1996. This parameter varied over time depending on the classification criteria (Joël, 1959; MacLeod, 1964; David et al., 1975; World Health Organization, 1987, 1992) and evaluation experience. Nevertheless, this was not reflected in the normal sperm count because r² values were too small (r² = 0.020).

As it is very difficult to study a large sample of healthy men which represent the general male population, one has to rely on results from large published samples, among which this is the largest published so far. Furthermore, it provides data from a population living in different environmental conditions but in the same region of North-eastern Spain over an extended period of time.

In order to assess the selection bias, a recent report by Handelsman (1997) illustrates the magnitude of bias due to the use of self-selected volunteers for evaluating sperm output of healthy men in Australia.

Our results do not support the idea that sperm quality is decreasing. However, further prospective and multicentric studies, including representative samples of the general population will be needed to demonstrate whether sperm quality is really decreasing.

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