Constant decline in sperm concentration in infertile males in an urban population: experience over 18 years

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Objective: To investigate semen quality in infertile men from an urban population over an 18-year period.

Design: Retrospective study.

Setting: Andrology clinic at a city university hospital.

Patients: A total of 9,327 men were referred to the clinic for infertility investigation. After excluding those with azoospermia, 7,780 samples were evaluated.

Intervention(s): Semen samples were analyzed within half an hour of production using computer-automated semen analysis and phase microscopy.

Main Outcome Measure(s): Sperm concentration, morphology, and motility; semen pH and leukocyte concentration; and patient age.

Result(s): The median patient age over the study period was 31.6 years, with an increase from 30.8 years in 1986 to 34.4 years in 2003. The median sperm concentration was 10.25 million/mL for the entire period, with a decline from 27.75 million/mL in 1986 to 4.60 million/mL in 2003. The median proportion of normally shaped spermatozoa was 15%, and the proportion of motile spermatozoa was 21%. The pH increased from 7.4 in 1986 to 7.9 in 2003, and the median leukocyte concentration was 1.50 million/mL.

Conclusion(s): A constant decline in median sperm concentration was found in the infertile men. The reason for this remains unclear, although the high pH and leukocyte concentration indicates the involvement of infection.

The need for artificial insemination has increased steadily over the past years, while the reasons for infertility are unchanged. In 20% of cases, infertility has a male cause alone, in 39% a female cause alone, and in 26% both partners are symptomatic; in 15% of cases no cause can be found (1–4).

The first report of decreasing sperm concentration in the human male was published by Carlsen et al. in 1992 (5). Many subsequent studies showed contradictory results. Some found no change in sperm concentration (6–8). Others found regional differences (9–11). The overall trend in the literature is a slight and constant decline in sperm concentration (10, 12–15). The reason for this remains unclear.

Different populations were investigated in these papers, including an unselected population (8, 13, 14), infertility patients (6, 16, 17), and semen donors (12, 15, 17). In addition, some of the studies involved only small numbers of patients, and in most cases 1,000 or fewer semen samples were analyzed. There are few reports of larger numbers, one example being the study of Andolz et al. (16), which had 22,759 participants.

The aim of this study was to investigate semen quality in patients who attended an andrology clinic evaluation over a period of 18 years.

MATERIALS AND METHODS

In this retrospective study, all semen samples collected at the andrology clinic in the Department of Urology, Medical University of Vienna, between 1986 and 2003 were investigated. Vienna is the capital of Austria, with about 1.7 million inhabitants, and should therefore represent an urban area. Patients were instructed not to ejaculate for at least 3–5 days before bringing in the semen sample. They were provided with a plastic cup with a wide opening and asked to bring the sample to the department within half an hour of producing it. If this was not possible they ejaculated in a special room in the hospital. All samples were produced by masturbation.

Analysis was performed by only two specially trained laboratory workers a maximum of 1 hour after ejaculation. All specimens were investigated according to World Health Organization (WHO) laboratory manuals (1–3). Data were gathered for sperm concentration, morphology, and motility, semen pH and leukocyte concentration, and patient age.
Microscopic analysis for sperm concentration and sperm motility was carried out using a computer-automated semen analysis system (Hamilton Thorne motility analyzer 05028 74; Hamilton Thorne Research, Danvers, MA) with ×400 magnification. To study sperm morphology, object slides (Testsimplets; Waldeck GmbH, Münster, Germany) precolored with methylene blue and cresyl violet acetate were used. One drop of the semen sample was placed on the object slide and was incubated at room temperature for 2 hours and then examined under ×600 magnification by the laboratory technician. The pH was measured using Merck (Merck KGaA, Darmstadt, Germany) indicator paper strips (working range pH, 6.5–10.0). To measure sperm vitality, one drop of semen was mixed with one drop of 0.5% eosin and incubated for 2 minutes at room temperature. It was then examined under ×600 magnification again by the laboratory technician. Leukocytes were analyzed as round cells by phase microscopy without using a peroxidase reaction according to the WHO guidelines. To differentiate leukocytes from immature germ cells, precolored object slides (with methylene blue and cresyl violet acetate), as described above, were used.

Statistical analysis was carried out using SAS software (SAS/STAT User’s Guide, Version 8 1999; SAS Institute, Cary, NC). The median and 5% and 95% percentiles were used for the description of time trends because of the apparent skewness of the underlying data. Associations of parameters with time were tested using the nonparametric Spearman correlation coefficient.

RESULTS
A total of 9,327 semen samples were analyzed from 1986 to 2003. Of these, 1,547 showed azoospermia and were not investigated. Consequently, 7,780 semen specimens were included in this retrospective study. The annual number of samples varied between 216 and 731 (mean, 432).

The results of the analysis are shown in Table 1. The median age of the men over the entire 18-year period was 31.6 years (range, 21.9–45.2 years). For the last 4 years (2000–2003) the median age was 33.9 years, slightly higher than in previous years. The youngest median age (29.9 years) was seen in 1988 (range, 22.2–44.3 years).

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of patients</th>
<th>Age (y)</th>
<th>Sperm concentration (million/mL)</th>
<th>No. of sperm with normal morphology (%)</th>
<th>Sperm motility (WHO a+b%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>519</td>
<td>30.8 (21.8–44.8)</td>
<td>27.75 (0.25–99.00)</td>
<td>10 (0–35)</td>
<td>14 (0–48)</td>
</tr>
<tr>
<td>1987</td>
<td>488</td>
<td>31.3 (22.6–45.8)</td>
<td>25.50 (1.75–72.50)</td>
<td>13 (0–43)</td>
<td>30 (0–55)</td>
</tr>
<tr>
<td>1988</td>
<td>413</td>
<td>29.9 (22.2–44.3)</td>
<td>20.00 (1.50–63.00)</td>
<td>13 (0–40)</td>
<td>28 (0–54)</td>
</tr>
<tr>
<td>1989</td>
<td>346</td>
<td>31.2 (23.4–42.8)</td>
<td>18.13 (1.25–65.75)</td>
<td>17 (0–44)</td>
<td>29 (0–50)</td>
</tr>
<tr>
<td>1990</td>
<td>357</td>
<td>30.7 (22.4–43.5)</td>
<td>16.75 (1.00–62.25)</td>
<td>19.5 (0–45)</td>
<td>26 (0–48)</td>
</tr>
<tr>
<td>1991</td>
<td>396</td>
<td>30.6 (21.3–43.7)</td>
<td>15.25 (0.75–63.00)</td>
<td>19 (0–44)</td>
<td>29 (0–51)</td>
</tr>
<tr>
<td>1992</td>
<td>453</td>
<td>31.5 (22.0–44.9)</td>
<td>18.00 (0.75–63.25)</td>
<td>23 (0–48)</td>
<td>29 (0–54)</td>
</tr>
<tr>
<td>1993</td>
<td>731</td>
<td>30.2 (22.9–43.9)</td>
<td>10.50 (0.50–69.5)</td>
<td>19 (0–48)</td>
<td>21 (0–48)</td>
</tr>
<tr>
<td>1994</td>
<td>615</td>
<td>31.0 (23.1–44.4)</td>
<td>10.50 (0.50–74.10)</td>
<td>18 (0–47)</td>
<td>21 (0–51)</td>
</tr>
<tr>
<td>1995</td>
<td>531</td>
<td>31.6 (20.7–45.8)</td>
<td>10.60 (0.50–101.50)</td>
<td>14 (0–39)</td>
<td>18 (0–48)</td>
</tr>
<tr>
<td>1996</td>
<td>562</td>
<td>31.9 (20.8–43.7)</td>
<td>14.60 (1.50–131.80)</td>
<td>9 (0–39)</td>
<td>20 (0–51)</td>
</tr>
<tr>
<td>1997</td>
<td>529</td>
<td>32.3 (22.0–46.7)</td>
<td>12.30 (1.70–132.50)</td>
<td>7 (0–40)</td>
<td>18 (0–50)</td>
</tr>
<tr>
<td>1998</td>
<td>489</td>
<td>32.9 (22.4–44.6)</td>
<td>2.50 (0.20–59.90)</td>
<td>16 (0–42)</td>
<td>18 (0–58)</td>
</tr>
<tr>
<td>1999</td>
<td>382</td>
<td>32.8 (20.4–47.0)</td>
<td>2.20 (0.30–18.70)</td>
<td>16 (5–41)</td>
<td>15 (0–48)</td>
</tr>
<tr>
<td>2000</td>
<td>290</td>
<td>33.9 (22.3–46.5)</td>
<td>3.15 (0.50–17.70)</td>
<td>16 (5–45)</td>
<td>15 (0–58)</td>
</tr>
<tr>
<td>2001</td>
<td>242</td>
<td>33.8 (21.8–48.2)</td>
<td>3.50 (0.70–14.90)</td>
<td>64 (0–83)</td>
<td>17 (0–74)</td>
</tr>
<tr>
<td>2002</td>
<td>216</td>
<td>33.5 (20.1–46.8)</td>
<td>3.15 (0.50–16.20)</td>
<td>54 (0–74)</td>
<td>18 (2–67)</td>
</tr>
<tr>
<td>2003</td>
<td>221</td>
<td>34.4 (22.7–46.8)</td>
<td>4.60 (0.30–21.10)</td>
<td>63 (0–78)</td>
<td>26 (4–75)</td>
</tr>
<tr>
<td>Overall</td>
<td>7,780</td>
<td>31.6 (21.9–45.2)</td>
<td>10.25 (0.50–78.45)</td>
<td>15 (0–59)</td>
<td>21 (0–54)</td>
</tr>
</tbody>
</table>

Spearman correlation coefficient

| P   | 0.001 | 0.001 | 0.001 | 0.001 |

Note: Results are given as median and (range).

The median sperm concentration during the study period was 10.25 million/mL (range, 0.50–78.45 million/mL). The highest median sperm concentration was found in 1986 (27.75 million/mL; range, 0.25–99.00 million/mL), and the lowest in 1999 (2.20 million/mL; range, 0.30–18.70 million/mL). The sperm concentration fell to <5.00 million/mL after 1997. The sperm concentration range was 0.20–59.90 million/mL from 1998 to 2003 and 0.25–132.5 from 1986 to 1997 (see Fig. 1). As the decline varies during the 18 years, the decline from 1986 to 1997 was statistically significant, with a Spearman correlation of \( r = -0.099, P = .0001 \), and after the year 1998 the correlation was \( r = 0.0826, P = .0004 \). The overall decline was statistically significant with a Spearman correlation of \( r = 0.345, P = .0001 \).

The median percentage of motile (WHO a+b) sperm was 21% (range, 0–54%). The median percentage of normally shaped sperm cells was 15% (range, 0–59%; \( r = 0.38, P = .0001 \)). The percentage for the years 1986–2000 was similar, after which time it increased to a maximum of 64% in 2001.

The median concentration of leukocytes was 1.50 million/mL (range, 0.25–8.75 million/mL). Over the study period there was a trend toward lower numbers (\( r = -0.385, P = .0001 \)).

The median pH was 7.9 (range, 7.3–8.5), with a steady increase (\( r = 0.499, P = .0001 \)) during the 18-year period.

**DISCUSSION**

This study found a decreasing sperm concentration in men seeking evaluation for infertility in Vienna over the 18-year period 1986–2003 (Fig. 1). The decrease was particularly evident in the years before 1999, after which time the sperm concentration remained relatively constant, although low. From 1999 onward the number of samples investigated per year (\( n = 216–382 \)) was lower than that examined in previous years (\( n = 346–731 \)). However, the smaller number of samples is unlikely to have influenced sperm concentration. A change in the patient population might be a cause, but only men referred to the clinic for infertility were investigated. The median sperm concentration was >20 million/mL in only 2 years (1986 and 1987), so the population with oligospermia was relatively homogeneous. Indeed, it is remarkable that the sperm concentration remained stable within the range 2.5–4.6 million/mL for 6 years.

A change of laboratory worker may have introduced bias. However, the sperm concentration was low before the new technician started work in 2000 and no marked difference was found in subsequent years. The fact that only two individuals carried out the analysis over such a long period may be considered an advantage. Both workers were equally trained.

Few data are available concerning sperm concentration in infertile men (6, 16). These papers report contradictory results concerning a change over time in the semen parameters measured, although the results of the studies are suitable for comparison with our data. In both cases, the study design was the same, that is, a retrospective study of semen samples collected as part of infertility investigations. In contrast with our results, however, Berling and Wölner-Hanssen (6) found an increase in sperm concentration (from a mean of 45.95
Decline in sperm concentration

There may be bias in this study, as only 718 specimens were analyzed. Andolz et al. (16) investigated 20,411 men producing sperm and found a mean sperm concentration of 44 million/mL with a statistically significant decline (0.2%) in semen volume and a nonsignificant increase (0.04%) in sperm concentration.

According to the WHO laboratory manuals (1–3), the lower limit of a normal sperm concentration is 20 million/mL. It is therefore difficult to believe that in these two studies the male factor, or at least the mean sperm concentration, was the reason for infertility. It is more likely that the threshold values of a semen sample (sperm concentration, motility, and morphology) can be used to classify men as normo- or oligospermic rather than to predict fertility (17). It is also known that patients with values within the reference range may still be infertile, while those with oligospermia may have a chance of conception (18). Nevertheless, it has been published that a sperm concentration <3 million/mL has a contraceptive efficacy equivalent to a Pearl index of 1.4 (1).

Geographical differences in fertility are also described in the literature. One measure is the time to achieve pregnancy, which is prolonged in some regions. However, reports on the impact of semen parameters on the time to pregnancy are conflicting. Joffe (10) found that a decrease in sperm count was responsible for the results, while Jensen et al. (19) could not explain the geographical difference as being due to semen quality. Unfortunately, no mean semen concentration was published for either of these studies. Other investigators (9, 20) also found a geographical difference in semen quality, but the men investigated were fertile, with a mean sperm concentration of >20 million/mL.

In the present study no data are available for other factors influencing semen quality, such as profession, education, smoking, or level of stress. All patients were instructed to maintain sexual abstinence for at least 3–5 days, but it was impossible to check this retrospectively. Carlsen et al. (21) recently reported that ejaculatory frequency and the season affected sperm concentration, but such variations should not influence our results because of the long study period and large number of semen samples included.

Another parameter affecting semen quality is age. The greatest effect of age is on semen volume and sperm motility and morphology (22), with sperm concentration altering only marginally. Our data show on the one hand a slight increase in median age from 30.8 years (range, 21.8–44.8 years) in 1986 to 34.4 years (range, 22.7–46.8 years) in 2003 ($r = 0.107, P = .0001$) and on the other hand a constant decline in sperm concentration ($r = -0.345, P = .0001$) over the same period. Using the Spearman rho test, a negative correlation between age and sperm concentration could also be found ($r = -0.045, P = .0002$). Although this is statistically significant, the correlation with a coefficient of $r = -0.045$ is rather weak, so the influence of age on semen concentration must be considered as only marginal.

The pH and leukocyte concentration results are interesting. The median pH over the entire period was 7.9 (range, 7.3–8.5), which is at the upper limit of the normal range. The median number of leukocytes was 1.50 million/mL (range, 0.25–8.75 million/mL), also at the upper limit of the normal range. Leukocytes were identified without using the peroxidase reaction. Their concentration therefore varies significantly as it can be difficult to differentiate them from immature germ cells. A relationship between immature germ cells and leukocytes was reported by Tomlinson et al. (23, 25) and Kiessling et al. (24), who both suspected that leukocytes have a special role to play in the removal of abnormal germ cells from the ejaculate. Bearing in mind the technical difficulties in differentiating between the round cells, the number of leukocytes identified may have been falsely high. However, as the pH was also relatively high, an inflammatory or immunologic process in the genital organs might be suspected.

Reports of an association between leukocyte concentration and impaired semen quality are contradictory in the literature. The latest reports show that leukocytes may have a role in stimulating reactive oxygen species production, which may have a direct negative effect on sperm DNA integrity (26–28).

Semen culture has been performed routinely in our clinic, particularly since 2002, but pathogenic bacteria or a clinically apparent infection have seldom been found. This is in accordance with the observations of Rodin et al. (29), who point out that leukospermia is only a poor marker for the presence of bacteria. A bacterial infectious cause for the low semen leukocyte concentrations in our study is therefore questionable, although other pathogens, such as viruses or bacteria that are difficult to detect, were not investigated.

In conclusion, there has been a steady decline in sperm concentration in infertile men with oligospermia in Vienna over the last 18 years. Although a raised semen pH may indicate an infectious cause, no pathogen has been found in recent years when semen culture has been routinely performed. A change in study population can be excluded as the cause as only infertile men were studied.

REFERENCES


