Longitudinal changes in semen parameters in young Danish men from the Copenhagen area

Elisabeth Carlsen1,4, Shanna H.Swan2, Jørgen Holm Petersen1,3 and Niels E.Skakkebæk1

1University Department of Growth and Reproduction, GR 5064, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen, 2Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark and 3Department of Family and Community Medicine, University of Missouri, Columbia, MO, USA

To whom correspondence should be addresseed. E-mail: ecarlsen@rh.dk

BACKGROUND: Several recent studies have reported low sperm concentration in young men recruited from the general population, but it is unknown whether the semen quality of these young men reflects that of more mature men or is reduced due to relative immaturity. We conducted a longitudinal follow-up study to address this question. METHODS: We followed 158 young men (median age = 19.1 years at entry) for up to 4 years and requested quarterly semen samples (total 1838 semen samples) and yearly genital examinations. We examined longitudinal changes in sperm concentration, semen volume, percentage of immotile sperm and percentage of morphologically normal sperm. We used general linear models in which each man served as his own control which also controlled for age, smoking, urogenital infections or disorders, fever and abstinence time. RESULTS: We found no evidence that sperm concentration, total sperm count or percentage of morphologically normal sperm changed appreciably during the 4 years of follow-up. Semen volume appeared to increase slightly with age, perhaps due to greater acceptance of the study protocol by participants. Sperm motility also improved somewhat, although this may, at least in part, reflect a trend in motility measurement. CONCLUSIONS: In this analysis of 1838 semen samples from 158 young men from the Copenhagen area, sperm concentration, total sperm count and sperm morphology did not change significantly during 4 years of follow-up, suggesting that previously reported low sperm concentration and poor sperm morphology among young Danish men are unlikely to be the result of immaturity.

Key words: abstinence/age/fever/sperm

Introduction

Recent studies have reported surprisingly low sperm concentration and a reduced number of morphologically normal sperm among young Danish men recruited from the general population (Andersen et al., 2000) compared with previous studies of fertile Danish men (Jørgensen et al., 2001a) and men from couples attempting pregnancy (Bonde et al., 1998a). Similarly, reduced sperm count and reduced numbers of morphologically normal sperm in young men have also been reported recently in other countries (Selevan et al., 2000; Jørgensen et al., 2002). Studies have shown a cohort effect for sperm concentration, with men born more recently having lower concentration than men of the same age in earlier years (Irvine et al., 1996; Bonde et al., 1998b). However, whether the low counts and the lower number of morphologically normal sperm seen in these men is a reflection of a cohort effect, due to relative immaturity, or both, could not be determined from previous cross-sectional studies. It would be expected that men over 18 years of age would be sexually mature, since spermarche has been shown to occur in early adolescence (13–14 years of age) (Nielsen et al., 1986; Schaefer et al., 1990). Furthermore, one report from Poland found that semen quality appears normal ~21 months after the first ejaculation (Janczewski and Bablok, 1985).

To determine whether immaturity could account, in whole or in part, for the low sperm concentration and poor morphology seen in young Danish men, we followed a subpopulation of the men included in a previous study (Andersen et al., 2000), using a protocol that requested a semen sample every 3 months for up to 4.5 years. Here we report on changes in semen parameters and testicular volume during the follow-up period, after controlling for age at study entry and other covariates. To our knowledge, this is the first study to obtain repeated measurements of semen parameters over an extended period in a sample of young men recruited from the general population.

Materials and methods

Study design

The present study follows a subpopulation from our previous study of young men recruited from the general population (Andersen et al., 2000). In that study, men were recruited when they appeared for the compulsory medical examination for the military draft that is...
required of young Danish men who have no known severe chronic diseases (85% of young Danish men). The initial study included a physical examination, blood and semen samples, and completion of a brief questionnaire including demographics, life style factors and medical history (Andersen et al., 2000). In that study, men were recruited from two cities in Denmark (Copenhagen and Alborg) in the first phase of the study (September 1996–April 1997) and only from Copenhagen in the second phase (September 1997–April 1998). During the first phase, 292 men were recruited from Copenhagen, and most of these men (84%) had asked to receive the results of their semen analysis. To maximize the participation rate, this group of 246 men was selected as the target population for the current study. Participants were asked to provide quarterly blood and semen samples and to undergo a yearly physical examination. Follow-up data were collected between June 1997 and May 2001. Subjects were paid 400 DKr (~US$60 per sample) as compensation. The local ethics committee approved the study and all men participated after informed consent.

Physical examination
Genital examinations were performed yearly during the study period (median four times) by one of three study physicians. Testicular size was estimated using Prader’s orchidometer with graded sizes (1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20 and 25 ml) and by ultrasound using a sector scanner (Aloka SSD-1100) with a 7.5 MHz transducer. Scans were performed in longitudinal and transverse planes and testicular volume was calculated after measuring the longest axis (A) and the two perpendicular dimensions (B and C) using the formula for an ellipsoid: volume = 4/3\(\pi \times A \times B \times C \times 1/8\) (Lenz et al., 1993). For larger testes, when it may not be possible to measure testicular length directly by ultrasound and physical examination, the measurements were estimated by extrapolation.

Semen analysis
All semen samples were obtained by masturbation into a wide-mouthed plastic container in a separate room close to the semen analysis laboratory. Sexual abstinence of 48 h was requested, but samples were accepted regardless of abstinence period, and the reported abstinence time was recorded. Semen samples with abstinence times >1 month (720 h) or <4 h were excluded (n = 14). We analysed semen samples according to the World Health Organisation’s (WHO) 1992 guidelines (World Health Organization, 1992). At the first visit, semen volume was estimated by subtracting a single estimate of the weight of an empty container from the weight of the sample plus container. For all later visits, containers were pre-weighed. Due to this change in methodology, we excluded semen volume and total sperm count (derived from semen volume) from the first sample, while sperm concentration, morphology and motility were included for all samples. Furthermore, if sample volume was ≤0.1 ml, it was assumed that part of the sample had been lost and the sample was excluded (n = 5).

Sperm concentration was determined using the Bürker-Türk counting chamber and calculated as the mean of counts of four predetermined squares in the counting chamber, using a single dilution of the semen sample. If <10 sperm were counted in any square, the number of sperm in the whole chamber was counted twice and averaged. Only one sample was aozoospermic and this was excluded. Total sperm count was calculated as the product of semen volume and sperm concentration.

Morphology was scored on Papanicolaou-stained slides following WHO methods (World Health Organization, 1992) and 200 sperm were scored on each slide. Here we report the percentage of sperm scored as morphologically normal (% normal). For men who provided at least four semen samples, the first two, the middle and the last samples were scored, otherwise, all samples were scored. In addition, morphology was evaluated in all semen samples from 20 randomly chosen participants. The first semen sample from each man was scored morphologically when all men had completed their first visit, whereas all remaining samples were scored at the conclusion of the study.

Time to semen analysis (in hours) was calculated as the difference between the time at which the evaluation of sperm motility was initiated and the time of ejaculation. Sperm motility was graded according to the WHO classification scheme with the following modifications: rapid (rapid forward progressive motility), slow (forward motility but not rapid progressive), non-progressive motility (not forward motility) and immotility. The motility assessment was performed in triplicate and the average calculated for 100 sperm in each count. Only the percentages of sperm graded as rapid progressive, motile, non-progressive motile and immotile are reported here.

Our laboratory monitors an external quality control programme for sperm concentration assessment, in which our laboratory blindly sends five semen samples to other laboratories 10 times a year (Jørgensen et al., 2002) and the same quality control for sperm concentration was performed in our laboratory. Sperm concentration, motility and morphology were evaluated by, respectively, four, five and three technicians. Not all technicians participated throughout the study period. Covariates indicating which technician performed the analyses were included in all multivariable models.

Covariates
We included a term for age at study entry, as well as the age of the participant at each visit to separate a possible cohort effect from an effect of maturation during the study period. All participants had completed a questionnaire during their first visit that included: date of birth, past history of proven fertility and smoking status at entry. Since only five men had a history of proven fertility, this variable could not be examined further. The initial examination provided data on body mass index (BMI), which was updated at each physical examination. A history of fever in the preceding 3 months was noted at each study visit. In addition, the season of sample collection was examined and categorized as: spring (March–May), summer (June–August), autumn (September–November) and winter (December–February).

A ‘genital disease’ group was defined comprised of men with a history of presence of urogenital infections or disorders at entry (n = 37). This group was subdivided further into: men with a history of cryptorchidism (whether a late testicular descent was spontaneous or a result of hormonal treatment or surgical replacement, n = 16); men with a history of urogenital infection (epididymitis, chlamydia, gonorrhoea and cystitis, n = 7); men with a history of varicocele or clinical varicocele at examination (greater than grade 1, n = 12); and men with a history of testicular torsion (n = 3). One man had a history of both urogenital infection and a clinical varicocele. The genital disease group used in the final analysis was based on data at entry, which do not include the few additional abnormalities detected during follow-up examinations (n = 5). The genital disease group was examined separately but, since age effects for this group were similar to those for men without genital abnormalities, results are presented for both groups combined.

Statistical analyses
All semen parameters except the percentage of morphologically normal sperm were log-transformed to stabilize variances and to normalize the data. Changes in semen parameters in relation to age were described in a general linear model, which included...
subject-specific terms to account for between-subject variability of semen parameters. The effect of age was analysed as a continuous function using piece-wise linear function (age less than or greater than 23 years, but other cut-off levels were also tested) as well as a linear function over the whole age span. Year of birth (1971–1979) was included initially in the model to examine a possible birth cohort effect but, since none was found, this term was not retained. Similarly, season was examined in preliminary models but, since no consistent seasonal effects were observed in these data, the term was not included in final statistical models. Because testicular volume may be an intermediate variable between age and semen parameters, it was not included in final statistical models. Duration of abstinence, smoking, fever and laboratory technician were included in all parameters, it was not included in these models. Duration of abstinence was included in motility calculations only. The effect of abstinence time was modelled as a continuous function using piece-wise linear function, one piece for the first 4 days of abstinence, one for abstinence time of 4–6 days and another for abstinence time of >6 days. All analyses were carried out using the Statistical Package for Social Sciences (SPSS) for Windows release 10.0 and SAS (SAS Institute Inc., 1996).

Results

Of the 246 young men who were asked to participate in this follow-up study, 158 (64%) agreed and provided at least one sample during follow-up. These men delivered a total of 1838 semen samples (mean 11.6 per man) between September 1996 and May 2001. While most men gave quarterly samples in the follow-up period, some men participated less frequently. Most men (n = 87) gave at least 13 samples, while 33 gave 10–12, 18 gave 7–9 and 20 men gave <7 samples.

We compared our subpopulation with all young men recruited from the Copenhagen area (n = 592) in Andersen et al. (2000) and with the group of young men who were asked but declined to participate (non-participants, n = 88) with respect to the following variables as reported at first visit: age, proven fertility, genital disorders, smoking status, individual semen parameters and abstinence time (Table I). The three groups were comparable with respect to age and BMI. However, there were fewer smokers, somewhat more men with genital disorders and fewer with proven fertility among participants. Sperm concentration was also somewhat higher among participants. Table II shows sperm concentration, percentage of immotile sperm and percentage of morphologically normal sperm in the first semen sample by number of years of follow-up. There was a tendency towards higher sperm concentration among men with longer follow-up, although it was not statistically significant. However, we used a model in which each man serves as his own control. Therefore, this trend did not bias our results because the age effect was estimated for each man, regardless of length of follow-up, and then in effect averaged.

Age and year of birth

The median age of participants at first visit was 19.1 years (range 18.2–26.1 years; 25 and 75% quartiles, 18.8 years and 20.0 years, respectively). At first visit, 42.4% were younger than 19 years and only 8.9% were older than 20 years. The majority of men (72.8%) were born in 1977 and 1978.

Table I. Characteristics at first visit of three study populations

<table>
<thead>
<tr>
<th></th>
<th>Men from the Copenhagen area included in Andersen et al. (2000)</th>
<th>Participants in the follow-up study</th>
<th>Non-participants in the follow-up study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>592</td>
<td>158</td>
<td>88</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.0 (18.8; 19.9)</td>
<td>19.1 (18.8; 20.0)</td>
<td>18.9 (18.7; 19.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3 (20.8; 24.1)</td>
<td>22.8 (21.4; 24.2)</td>
<td>22.3 (20.7; 24.6)</td>
</tr>
<tr>
<td>Smoker</td>
<td>36.5%</td>
<td>28.0%</td>
<td>35.2%</td>
</tr>
<tr>
<td>Genital disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>81.2%</td>
<td>76.6%</td>
<td>84.1%</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>9.8%</td>
<td>10.1%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Varicocele</td>
<td>5.3%</td>
<td>7.6%</td>
<td>5.7%</td>
</tr>
<tr>
<td>Urogenital infection</td>
<td>4.0%</td>
<td>4.4%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Testicular torsion</td>
<td>0.7%</td>
<td>1.9%</td>
<td>0</td>
</tr>
<tr>
<td>Proven fertility</td>
<td>7.4%</td>
<td>3.2%</td>
<td>10.2%</td>
</tr>
<tr>
<td>Sperm concentration (10⁶/ml)</td>
<td>41.0 (19.0; 77.2)</td>
<td>45.0 (18.9; 80.8)</td>
<td>34.0 (19.0; 59.8)</td>
</tr>
<tr>
<td>Immotile sperm (%)</td>
<td>37.0 (29.7; 45.0)</td>
<td>37.7 (29.7; 44.3)</td>
<td>36.7 (31.3; 47.0)</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>40.0 (33.5; 45.5)</td>
<td>41.0 (34.5; 46.5)</td>
<td>41.0 (34.5; 45.5)</td>
</tr>
<tr>
<td>Abstinence time (h)</td>
<td>61.0 (51.5; 85.0)</td>
<td>60.0 (58.0; 84.0)</td>
<td>61.5 (46.3; 85.8)</td>
</tr>
</tbody>
</table>

*Median (25%; 75% quartiles) for continuous variables or percentage of subjects with characteristic.

Table II. Median semen parameters at study entry in relation to duration of follow-up

<table>
<thead>
<tr>
<th>Years of follow-up</th>
<th>n</th>
<th>Sperm concentration (10⁶/ml)</th>
<th>% immotile sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2.0</td>
<td>20</td>
<td>26.0</td>
<td>39.0</td>
</tr>
<tr>
<td>2.0–2.5</td>
<td>7</td>
<td>39.0</td>
<td>38.0</td>
</tr>
<tr>
<td>2.5–3.0</td>
<td>8</td>
<td>83.0</td>
<td>37.0</td>
</tr>
<tr>
<td>3.0–3.5</td>
<td>11</td>
<td>55.0</td>
<td>35.0</td>
</tr>
<tr>
<td>3.5–4.0</td>
<td>55</td>
<td>46.0</td>
<td>36.0</td>
</tr>
<tr>
<td>&gt; 4.0</td>
<td>7</td>
<td>48.0</td>
<td>38.5</td>
</tr>
<tr>
<td>All men</td>
<td>158</td>
<td>45.0</td>
<td>37.7</td>
</tr>
</tbody>
</table>

*Number of men in the study followed for the given number of years.
Table III. Semen parameters in the first semen sample by genital disease (median and quartiles)

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Age (years)</th>
<th>Abstinence time (h)</th>
<th>Sperm concentration (10^6/ml)</th>
<th>% immotile sperm</th>
<th>% normal morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>No genital disease</td>
<td>121</td>
<td>19.2 (18.8–20.1)</td>
<td>60.0 (58.0–84.0)</td>
<td>52.0 (21.0–85.8)</td>
<td>37.0 (29.2–43.8)</td>
<td>41.0 (35.1–46.3)</td>
</tr>
<tr>
<td>Any genital disease</td>
<td>37</td>
<td>19.0 (18.7–19.7)</td>
<td>61.0 (54.5–95.0)</td>
<td>22.0 (10.7–61.0)</td>
<td>38.3 (32.0–46.3)</td>
<td>39.0 (34.0–46.5)</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>16</td>
<td>18.8 (18.7–19.2)</td>
<td>60.0 (38.3–107.5)</td>
<td>20.0 (9.6–59.5)</td>
<td>37.0 (27.0–49.0)</td>
<td>39.0 (27.0–50.0)</td>
</tr>
<tr>
<td>Varicocele</td>
<td>12</td>
<td>19.2 (18.9–20.2)</td>
<td>60.0 (59.0–78.8)</td>
<td>41.0 (10.0–96.3)</td>
<td>40.7 (34.7–46.0)</td>
<td>41.0 (36.0–50.0)</td>
</tr>
<tr>
<td>Urogenital infection*</td>
<td>7</td>
<td>19.6 (18.7–22.7)</td>
<td>61.0 (51.0–73.0)</td>
<td>20.0 (15.0–95.0)</td>
<td>37.7 (27.0–46.7)</td>
<td>42.0 (34.0–46.0)</td>
</tr>
<tr>
<td>Testicular torsion</td>
<td>3</td>
<td>18.9 (18.6–20.2)</td>
<td>83.0 (78.0–180.0)</td>
<td>18.0 (0.6–39.0)</td>
<td>38.3 (35.0–71.0)</td>
<td>34.0 (34.0–37.0)</td>
</tr>
</tbody>
</table>

*One man with both varicocele and a history of urogenital infection was included in both groups.

Genital diseases

Semen parameters at study entry are shown in Table III by category of genital disorders. Comparing unadjusted median sperm concentrations in men with and without genital diseases, sperm concentration was considerably lower for the genital disease group (median 22 × 10^6/ml versus 52 × 10^6/ml; P = 0.007).

Abstinence time

Although we requested that duration of abstinence be at least 48 h, 30% of the semen samples were delivered after <48 h of abstinence. The median duration of abstinence was 59 h (range 4–720). The average duration of abstinence was found to decrease by 2.3 h per year of age [95% confidence interval (CI) −4.4 to −0.3 h]. We analysed the effect of abstinence time on sperm concentration using a continuous piece-wise linear function and found a steep linear increase for 0–4 days, a less marked increase between days 4 and 6 and no significant change after 6 days, although this estimate is uncertain since only 4.6% of semen samples were delivered after an abstinence time >6 days. Sample volume, total sperm count and percentage of rapid progressive motile sperm increased significantly, and percentage of non-progressive motile and immotile sperm decreased significantly with increasing abstinence time for 0–4 days. The percentage of morphologically normal sperm was not affected by abstinence time (Table IV).

Febrile illness

We compared sperm concentration in samples that were delivered within 3 months of a febrile episode with other samples from the same man using the general linear model and controlling for confounders as described above. Of the 1838 semen samples, 87 (4.7%) were preceded by a febrile episode. Sperm concentration was significantly lower following a febrile episode (−29.4%; 95% CI −51.92 to −10.2), Fever also caused a significant decrease in the percentage of morphologically normal sperm (−3.4%; 95% CI −5.4 to −1.3) and total sperm count (−28.1%; 95% CI −39.7 to −14.2), whereas the percentage of non-progressive motile and immotile sperm increased following an episode of fever (15.4%; 95% CI 2.0–30.6, and 6.1%; 95% CI 0.2–12.3, respectively). However, there was little change in the percentage of rapid progressive motile sperm following fever (−3.9%; 95% CI −23.6 to 20.9). A variable denoting febrile illness in the 3 months prior to sample collection was included in all multivariate models.

Season

Samples were distributed fairly uniformly by season: 388 semen samples in spring (21.1%), 433 in summer (23.6%), 552 in autumn (30.0%) and 465 in winter (25.3%). There was no significant association between season and any semen parameter; however, there was a suggestion of a higher percentage of morphologically normal sperm and lower sperm concentration during the summer months, whereas semen volume was somewhat lower during the winter months.

Longitudinal changes in semen parameters with age

The relationship between age and each semen parameter was examined as a linear function over the entire age span, as well as by including two piece-wise linear functions for age less than (1503 samples) and above 23 years of age (335 samples).

There was no significant change in sperm concentration, total sperm count or percentage of morphologically normal sperm with age (Table IV). Overall, we found a significant increase in semen volume and in the percentage of rapid progressive motile sperm as well as a significant decrease in the percentage of non-progressive and immotile sperm with age (Table IV).

However, when analysed as two continuous piece-wise linear functions, the increase in semen volume was significant only for men <23 years of age (mean change per year 2.7%; 95% CI 1.0–4.4). After 23 years of age, there was little change (0.7%; 95% CI −2.8 to 4.3). Similarly, the decrease in percentage of non-progressive and immotile sperm was only significant for men ≥23 years of age (mean change per year −10.6%; 95% CI −13.0 to −8.1, and −1.7%; 95% CI −3.0 to −0.4, respectively), and the increase in rapid progressive motile sperm was only significant for men up to 23 years of age (mean change per year 8.3%; 95% CI 3.0 −13.8). For men older than 23 years of age, changes were of similar magnitude for each of the three motility classes but were no longer significant (−4.1%; 95% CI −8.9 to 1.0, −2.2%; 95% CI −4.7 to 0.4, and 2.5%; 95% CI −6.3 to 12.2) perhaps because of small numbers.

We also examined semen parameters in relation to age separately in men with and without genital disorders but saw no significant differences between these groups with respect to the effect of age.
There was a strong correlation between testis size measured by orchidometer and ultrasound ($R = 0.82$; $P < 0.001$). The mean orchidometer volume of both testes increased significantly with age (0.39 ml/year; 95% CI 0.18–0.60; $P < 0.001$) after controlling for subject, current BMI and physician. By ultrasound, however, the mean volume of both testes decreased significantly with age ($-0.66$ ml/year; 95% CI $-0.83$ to $-0.49$; $P < 0.001$).

**Discussion**

In our 4 year follow-up study of longitudinal changes in semen quality, which included 1838 semen samples from 158 young men (median age of 19.1 years at entry), we found no significant changes in sperm concentration, total sperm count or percentage of morphologically normal sperm. There was a small increase in semen volume and in the percentage of rapid progressive motile sperm and a decrease in the percentage of non-progressive motile and immotile sperm with age, significant only for men aged ≤ 23 years. The changes in testicular size with age as measured by orchidometer and ultrasound were inconsistent.

Our study was designed to examine the effect of ageing on semen quality in young men of ages comparable with those included in studies of military recruits (Andersen et al., 2000; Selevan et al., 2000; Jørgensen et al., 2002). We believe that the 4 years included in this analysis are the critical ones to examine a maturation effect in such men. Extending the study further would have done little to increase statistical power, since it is unlikely that, had an increase been seen, it would have continued at the same rate as men continued to age further. This is supported by the fact that the point estimate for an age effect was reduced for all semen parameters in men older than 23 years compared with younger men (data not shown). We chose, therefore, to study a large number of samples collected during the 4 year period we consider most relevant to address the study question.

Despite the large number of samples included in this study, the CIs for the effect of age on sperm concentration and total sperm count were fairly wide; 6.7 and 8.2% per year (subtracting the lower from the upper confidence limit) for concentration and total count, respectively (Table IV). Over 4 years, men’s sperm concentration may, therefore, have increased as much as 19.6% or decreased by 7.2%. This implies that over the 4 years of follow-up, men’s median sperm concentration at entry (45.0 × 10^6/ml) could have decreased to 41.8 × 10^6/ml or increased to 53.8 × 10^6/ml without the change being statistically significant. However, even this upper limit is lower than sperm counts in Danish men reported previously (Andersen et al., 2000; Jørgensen et al., 2002). The CI for the median percentage of morphologically normal sperm is quite a bit narrower (0.5%). Therefore, the median for the percentage of morphologically normal sperm at entry (41%) could only have decreased to 40.3% or increased to 41.2% without our observing a statistically significant effect. The relative size of these CIs is consistent with the much larger intra-individual coefficient of
variation for sperm concentration (57.2%) than for the percentage of morphologically normal sperm (9.8%) that we recently reported based on another sample of young Danish men (Carlsen et al., 2004). Therefore, we are fairly confident that our ‘negative’ conclusions with respect to age and sperm concentration and morphology are not a consequence of incomplete follow-up or inadequate sample size.

Febrile disease within the previous 3 months significantly lowered sperm concentration, total sperm count and the percentage of morphologically normal sperm and increased the percent non-progressive motile and immotile sperm. We previously reported similar findings in a longitudinal study of semen quality in a small group (n = 27) of somewhat older men (median age of 24.4 years) (Carlsen et al., 2003).

With respect to season, we found a non-significant, slightly longer, abstinence time during the winter period compared with the other three seasons. However, the seasonal effect was estimated in the multivariate analysis also adjusting for abstinence time, so differences in abstinence time for the different seasons did not influence the estimates of the seasonal effects on semen parameters. In our study, we saw no significant relationship between season and semen quality. Several cross-sectional studies have described seasonal variation in semen quality, with a tendency towards lower sperm concentrations during the summer months (Tjoa et al., 1982; Reinberg et al., 1988; Politoff et al., 1989; Saint Pol et al., 1989; Levine et al., 1990; Gyllenborg et al., 1999; Jørgensen et al., 2002) or in the autumn (Chen et al., 2003), whereas other cross-sectional (Ombelet et al., 1996; Chia et al., 2001) studies and some longitudinal studies did not find any effect of season on semen quality (Abbatichio et al., 1987; Mallidis et al., 1991).

As has been reported previously (Swan et al., 2003), we found that semen quality was reduced in men with a history of present or previous genital disease but these conditions did not modify the effects of age on semen quality. Therefore, the final statistical models are fitted to all men and genital diseases are included as covariates (Table IV).

This group of 158 men represented 64% of a larger group of 18- to 20-year-old men who participated in a cross-sectional study of semen quality in 1996–1997 (Andersen et al., 2000). In that study, the participation rate was ~17–18%. While these men are characterized by their decision to participate, they are likely to be more representative of the general population than those included in semen studies based on highly selected subgroups such as infertility patients, volunteers or pre-vasectomy subjects. In this population-based sample, median sperm concentration was 41.0 × 10^6/ml, which was similar to that in men of the same age (mean 19.4 years) from Norway (41.0 × 10^6/ml) (Jørgensen et al., 2002) and the Czech Republic (44 × 10^6/ml) (Selevan et al., 2000), but somewhat lower than that in Finland and Estonia (54 × 10^6/ml and 57 × 10^6/ml, respectively) (Jørgensen et al., 2002). We considered the possibility that the low sperm counts among these young men reflected a birth cohort effect, a hypothesis supported by several other studies (Auger et al., 1995; Irvine et al., 1996). An analysis of 10 Danish occupational studies reported a median sperm concentration of 52.0 × 10^6/ml for men born between 1970 and 1976 (mean age 22.8 years at sampling) compared with a median sperm concentration of 63.0 × 10^6/ml for men born between 1935 and 1949 (mean age 44.0 years at sampling) (Bonde et al., 1998b). We found, however, in the current 4 year follow-up study that sperm concentration did not vary by year of birth, although the range of year of birth was very narrow, with most men (72%) born in 1977 and 1978.

We also considered the hypothesis that the low sperm concentrations and poor sperm morphology in these young men reflected sexual immaturity with respect to sperm production. In a longitudinal study of 134 teenage boys, Janczewski and Bablok (1985) found that normal mean values for semen volume, sperm concentration and sperm morphology were obtained within 12–14 months after first ejaculation, but sperm concentration increased until the twenty-third month after the first ejaculation. They also found that adult levels of the percentage of motile sperm were achieved 21–23 months after the first ejaculation. There was, however, no information regarding abstinence time, and the duration of follow-up was not given. In our study of young men aged 18–26 years at entry, we found no significant change in sperm concentration and total sperm count with age. To our knowledge, no study has found an increase in the number of sperm with age beyond the first few years of puberty. Most population-based estimates of sperm concentration are based on studies of men of reproductive age, and most of these do not include data on the age of the study population. For example, among the 56 studies of sperm concentration included in Swan et al. (1997), the mean or median age of the study population was given in only 22; the median of these estimates was 29 years. In a cross-sectional study of fertile men aged 21–50 years, Schwartz et al. (1983) found no significant difference in sperm concentration and total sperm count among men aged 21–25 years compared with the older age groups. They did, however, find that sperm morphology and sperm motility were significantly reduced in men aged 21–25 years compared with men aged 26–35 years. We saw no suggestion that the percentage of morphologically normal sperm changed with age, although only about one-third of semen samples in our study were analysed with respect to morphology. We found an increase in the percentage of rapid progressive motile sperm and a decrease in the percentage of non-progressive motile and immotile sperm, which was significant overall, and in men younger than 23 years.

The data on sperm motility, however, may be less robust than those on sperm concentration and sperm morphology due to a higher inter- and intra-observer variation (Jørgensen et al., 1997; Cooper et al., 2002; Brazil et al., 2004). We cannot be sure that our observed increase in sperm motility with age is not, at least in part, a result of a drift over time in the evaluation of motility. This hypothesis is supported by results from cross-sectional studies of semen quality in young men performed in our laboratory during the same time period in which an increase in the percentage of motile sperm comparable in magnitude with that we are reporting here was found, despite no increase in men’s ages (Jørgensen et al., 2001b)
Because of our quality control programme for sperm concentration, a trend in this variable due to laboratory variation is less likely. It is also less likely for morphology since one technician performed most of the analyses of sperm morphology at the close of the study (except for the first sample), although some bias could have been introduced due to the fact that the technician was not blinded to the men’s earlier morphology results. We found a small but significant increase in semen volume with age for men younger than 23 years of age, which has not been described before beyond the first few years of puberty (Janczewski and Bablok, 1985). We have accounted for abstinence time in these analyses, a factor that could otherwise have influenced semen volume. However, we have some evidence that the increase in semen volume over time may have been due to greater acceptance of the collection protocol by study participants rather than a result of increasing maturity. In a separate longitudinal study of young Danish men followed for a year, median semen volume in 10 samples (Carlsen et al., 2004), reflecting a greater increase in sample volume than the 2.3% we saw in the present study. In addition, of the five semen samples deemed to be incomplete due to small volume (<0.1 ml), three were obtained at the first visit. We found inconsistent changes in testis volume with age as measured by orchidometer and ultrasound. One explanation for the apparent increase by orchidometer measurement could be that the scrotal skin that is co-measured becomes thicker with age. Previous longitudinal measurements on adolescents have indicated that adult testis size was attained by the age of 17–18 years (Zachmann et al., 1974), but the study contained no measurements after the age of 19–20 years. The apparent decrease in ultrasound measurement of testes volume with age could be the result of increased experience of the physician performing the ultrasound measurement and, as a result, a decrease in the measurement of other structures. We have, however, no information regarding the inter- and intra-individual variation in the ultrasound measurements. In conclusion, this group of young men from the Copenhagen area, we found that sperm concentration, total sperm count and sperm morphology did not change significantly during 4 years of follow-up. We found small increases in semen volume and sperm motility with age for men aged ≤23 years. However, it is likely that these findings were the result, at least in part, of causes other than ageing. This study suggests that the previously reported low sperm concentrations and high proportion of morphologically abnormal sperm among young Danish men are unlikely to be the result of sexual immaturity.

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References


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