Semen quality of 324 fertile Japanese men

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BACKGROUND: A number of studies have indicated regional differences in semen quality. To examine the current status in Japan, we undertook a cross-sectional study on the semen quality of fertile Japanese men for comparison with recent European results. METHODS: Semen parameters of 324 fertile men from the Kawasaki/Yokohama area were investigated. The semen parameters were compared with those published for fertile men from four European cities, Copenhagen, Paris, Edinburgh and Turku. RESULTS: When adjusting for confounders such as ejaculation abstinence period and age, the lowest sperm concentrations were detected in men from Kawasaki/Yokohama followed by men from Copenhagen, Paris, Edinburgh and Turku, but only the differences between men from Kawasaki/Yokohama and men from Edinburgh and Turku were significant (P = 0.0008 and P < 0.0001, respectively). Total sperm count, percentage of motile sperm and percentage of normal sperm observed in Kawasaki/Yokohama were significantly lower than those from all European centres except for motile sperm in men from Paris. CONCLUSIONS: Japanese fertile men had a semen quality at the level of Danish men, who have been reported to have the lowest among investigated men in Europe. The low level of semen quality of the fertile Japanese men may be due to lifestyle or other environmental factors; however, ethnic differences caused by different genetic variation or combinations cannot be ruled out by this study.

Key words: fertile men/reproductive function/semen quality

Introduction

In the past decade, stimulated by a meta-analysis showing a major decline in sperm counts over the last 50 years (Carlson et al., 1992), many retrospective studies have been conducted and some reported a significant reduction in semen quality over time, while others reported no change. This is still inconclusive as to the global temporal trends, but regional differences in semen quality have been reported for some areas in the USA and Europe (Fisch et al., 1996; Vierula et al., 1996; Jørgensen et al., 2001, 2002; Swan et al., 2003). For example, the European study of fertile men showed that sperm concentration of Danish men was only 74% of that of Finnish men (Jørgensen et al., 2001). Only a small number of such studies have been conducted in Asia or other non-Western countries. A study from Sapporo, Japan, did not find any change in semen volume and sperm concentration between 1975–1980 and 1998 when evaluating semen samples from healthy volunteers (Itoh et al., 2001). However, selection bias in the inclusion of study subjects and an uncontrolled effect of variable abstinence periods might have affected the results, and thus the results cannot be compared reliably with recent results from Europe. Therefore, we undertook a study of fertile Japanese men in order both to describe the current status of semen parameters of Japanese men from the Kawasaki/Yokohama area and to compare the semen parameters with the results from Europe. In a preliminary report of the ongoing study, presented at the 3rd Asian and Oceanic Congress of Andrology, we found that the first 255 included men from the Kawasaki/Yokohama area had a high sperm concentration when based on raw data that were unadjusted for confounding factors (Baba et al., 2000). Here, we report the results based on the final number of men included (n = 324) and take confounders into consideration, allowing for comparison with the previous European study.

Materials and methods

The investigation procedure described below was harmonized—regarding most aspects—with those of the previously published European study (Jørgensen et al., 2001). The method of assessment of semen volume was different from that in the European study, and therefore a data correction was made before statistical analyses (see ‘Data correction’).
Subjects
Male partners of pregnant women who attended the antenatal clinic at five hospitals in the Kawasaki/Yokohama area were invited to participate in our study. A total of 1284 fertile men were invited to participate and 324 (25.2%) agreed. The number of couples from each hospital was 141 from St Marianna University Hospital in Kawasaki, 50 from Seibu Hospital in Yokohama (a branch of St Marianna University Hospital), 26 from Toyoko Hospital in Kawasaki (another branch of St Marianna University), 25 from Asakawa Maternity Hospital in Yokohama, and 82 from Hori Maternity Hospitals in Yokohama. All hospitals were located in urban areas. Three study nurses approached pregnant women during their routine visits to the hospitals at gestational weeks 8–12 and invited their husbands to participate in this study.

The eligibility criteria for the male participants were as follows: age 20–45 years at the time of invitation, residence in the local area surrounding the hospital at which he was recruited, and both the man and his mother had to be born in Japan. In addition, the woman’s current pregnancy had to be achieved by normal sexual relations and not as a result of fertility treatment. Cryptorchidism, orchitis, epididymitis, genital tract surgery (including varicocelectomy), chemotherapy, radiotherapy or chronic illness, previous treatment for infertility or reduced fertility, unwanted pregnancy or prolonged time to pregnancy were not criteria for exclusion. To allow for the possible influence of seasonal changes on the semen characteristics, the study period covered one full calendar year including January to December 1998.

Questionnaires
Both the men and their pregnant partners completed a questionnaire that was also used in the European study (Jørgensen et al., 2001). As the original questionnaire was in English, it was translated into Japanese in advance. The translated questionnaire was back-translated to English to control for translation errors. The questionnaire included information on age and previous or current diseases including known history of fertility.

Physical examination
Physical examination of the male participants was performed by one of four urologists on the day the man delivered the questionnaire and his semen sample. Evaluations of testes disposition, varicocele and Tanner stages of pubic hair were performed with the men in the standing position. For assessment of testis size, all examiners used the same type of wooden orchidometer (Pharmacia & Upjohn, Denmark). Standardization and quality control of the physical examination among four urologists was achieved during daily examinations of infertility patients.

Semen samples
Semen samples were obtained by masturbation and ejaculated into a clean collection container. All men were asked to abstain from ejaculation for a period of ≥48 h. The period of ejaculation abstinence was calculated as the time between the current and previous ejaculations as reported by the men. Semen samples were collected at the laboratory except for ~22% of samples that were collected at homes due to the convenience of the participants. In these cases, participants were asked to keep the samples at ≥20°C and <37°C during transport to the laboratory. In the laboratories, the semen samples were kept at 37°C until analysis. Samples were liquefied at 37°C and volume measured by aspirating the entire sample into a graduated syringe. We made plans for this study based on the European protocol, but during the study, we realized that the method of semen volume assessment which we adopted (volume measurement by aspiration) had been different from that recommended in the European protocol (weighing). On noting this problem, we decided to make corrections at the end of the study without changing the method in the middle of the study in order to avoid inconsistency of the data (see ‘Data correction’).

For the assessment of sperm motility, 10 μl of well-mixed semen was placed on a clean glass slide (which had been kept at 37°C), and covered with a 22x22 mm coverslip. The preparation was placed on the heating stage of a microscope (37°C), and immediately examined at a total magnification of ×400. The microscope field was scanned systematically, and the sperm were classified as either motile (WHO motility classes A, B or C) or immotile (WHO motility class D), in order to report the proportion of motile sperm (World Health Organization, 1992). The motility assessment was repeated on a second 10 μl aliquot of semen, and the average value was calculated for both samples.

For the assessment of sperm concentration, each semen sample was thoroughly mixed for ≥10 min in a rotation device. An aliquot of the sample was put into the diluent using a positive displacement pipette and mixed for a further 10 min. The diluent consisted of 50 g NaHCO3, 10 ml 40% formaldehyde and distilled water up to 1 litre. The sperm concentration was assessed using a Bürker–Türk haemocytometer as a counting chamber. One drop of the diluted specimen was transferred to each chamber of the haemocytometer, which was allowed to stand for 5 min in a humid chamber before the cells were counted at a total microscope magnification of ×400. Only sperm with tails were counted.

Smoke was prepared for morphological evaluation, fixed with a mixture of absolute alcohol (2/3) and acetone (1/3), and then sent to Paris for a modified Shorr stain (WHO, 1992), and assessment of sperm morphology according to criteria described previously (David et al., 1975).

Quality control of sperm concentration assessment
One technician from the laboratory at the Department of Urology, St Marianna University School of Medicine participated in an external quality control programme for sperm concentration assessment coordinated by the Department of Growth and Reproduction Copenhagen, Denmark (Jørgensen et al., 2001, 2002) during the study period. Each month 5 blinded samples were sent from the Danish laboratory. Fresh samples from normal semen donors had been preserved by addition of 10 μl of a 3 mol/l sodium azide solution per 1 ml of the ejaculate after liquefaction and finally 600 μl of semen were sent by mail in 1 ml cryotubes. Thus, assessment of sperm concentration was performed 4–8 days after the semen preparation, including in the Copenhagen centre. The results were reported to the Copenhagen centre for statistical analysis. This external quality control study was planned for international comparative studies of semen quality (Jørgensen et al., 2001, 2002), where it was necessary to monitor the inter-laboratory variation in assessment of sperm concentration. The quality control data from the Kawasaki centre were used to correct the sperm concentration data of the fertile Japanese men to allow comparison with the results from the European study (Jørgensen et al., 2001). Internal quality control was simultaneously carried out using a part of the same quality control samples (n = 15) to detect inter-technician variation among the four technicians at the Kawasaki centre who undertook the semen analysis in this study. The coefficient of variation between the four technicians was 7.83%.

Data correction
Before using multivariate models to adjust for covariates of semen quality, we made two corrections of the semen data from the fertile Japanese men in order to make them comparable with the European data.

The raw data of semen volumes from the fertile Japanese men were corrected applying the formula:

\[
\text{corrected volume} = \text{observed volume} + 0.49
\]
with which differences in the method of semen volume assessment (aspirating semen into a graduated syringe for the present study versus volume measurement by weighing for the European study) was conciliated. The value 0.49 is the mean value of differences between the volumes measured by weighing and those measured by aspirating, which were obtained from another set of semen samples (n = 102).

The quality control study of assessment of sperm concentration showed a significant difference between the Kawasaki centre and the Copenhagen centre (reference laboratory) \( P < 0.001 \), and a linear increasing trend in sperm concentration estimate over the study period, which was revealed as an increase up to a maximum of 42% relative to the reference laboratory. To describe this time trend, several statistical models were investigated; simple linear correction was found to be the best to describe the changes. Thus, the raw data were then corrected with a formula:

\[
\text{log(corrected sperm concentration)} = \text{log(observed sperm concentration)} - 0.42(\text{days/365}),
\]

in which ‘days’ signifies a date of semen analysis represented as the number of past days after the first day in the study period of 1 year. Corrected total sperm count was obtained from multiplying the corrected sperm concentrations by the corrected semen volume.

**Statistical analysis**

Between-hospital differences in self-reported previous diseases and diseases detected during clinical examination, men’s year of birth, year of investigation and season of investigation were tested with a Fisher’s exact test. A Kruskal–Wallis test was used to test between-hospital differences in men’s age, duration of abstinence and duration from ejaculation to assessment of motility.

Semen volume, sperm concentration and total sperm counts were normalized by natural logarithmic transformation before analysis to correct for the markedly skewed distribution. Multivariate regression analyses were carried out to compare the Japanese men with men from the four European centres. In these analyses the general level of each centre was estimated while adjusting for known confounders, including age and abstinence time. Age and abstinence time entered the model as piecewise linear functions (linear splines); for example, one straight line for abstinence <48 h, another straight line for abstinence periods 48–96 h, etc. Season did not enter the model because it was not related to any semen parameters investigated. The final models were subjected to standard checks of the residuals.

The statistical analyses were performed using the statistical packages SAS version 8.1 and SPSS version 9.0.

**Results**

**Population characteristics and semen parameters of the fertile Japanese men**

The self-reported information regarding age, previous fertility and diseases of the 324 participating men is shown in Table I. None of the men reported having or having had cryptorchidism, hypospadias or testicular cancer. Although the ages of the men and the frequencies of first pregnancies differed significantly between the five hospitals, the frequencies of previous subfertility (waiting time to pregnancy of >1 year), treatment for infertility and other diseases did not differ between the groups of men from five hospitals. Therefore, the men included from the five different clinics were analysed as one single population. Table II summarizes the findings of the physical examinations of the men. None of the men suffered from severe genital abnormality, although one man previously had had one testis removed due to testicular torsion. A left varicocele was observed in 21.0% of the men (14.2% grade 1, 4.3% grade 2, and 2.5% grade 3). No right varicocele was observed.

Standardized semen parameters of the fertile Japanese men are shown in Table III as expected calculated semen parameters of a recently proven fertile, 30 year old man, having ejaculation abstinence periods of both 96 and 216 h. These values are based on the results from the regression analyses after taking the confounders into account.

The ejaculation abstinence period was 208/134 (mean/median) h with the 5th–95th percentile 64.5–708.0 h. Increasing abstinence period had an increasing effect on semen volume (\( P = 0.006 \)), sperm concentration (\( P < 0.0001 \)) and total sperm count (\( P < 0.0001 \)) up to 9 days (216 h), after which no further effect was observed for these parameters. The effects of abstinence period represented as increasing rate per hour below 216 h on sperm concentration were 1.00534/h for 0–96 h and 1.00508/h for 96–216 h, and those on semen volume were 1.00253/h for 0–96 h and 1.00130/h for 96–216 h. Age >30 years had a decreasing effect on percentage of motile sperm (\( P = 0.004 \)) but no effects on any other semen parameters. The mean/median duration from semen ejaculation to assessment of motility was 43/40 min, 5th–95th percentile 20–80 min. The frequency of motile sperm was higher in the samples assessed before 40 min after ejaculation than after, but the difference was not significant (\( P = 0.13 \)). Semen parameters could not be shown to differ between the four seasons of the investigation period.

**Association between sperm concentration and other parameters in the fertile Japanese men**

Regression analyses showed that increasing sperm concentration had an increasing effect on sperm motility up to \( 40 \times 10^6/\text{ml} \), no further effect up to \( 200 \times 10^6/\text{ml} \) and a decreasing effect thereafter. The men with oligozoospermia (unadjusted sperm concentration <20×10^6/ml) had lower number of motile sperm (mean/median: 46.7/49%) than the men with higher sperm concentrations (mean/median: 56.5/57%, \( P = 0.0006 \)). Oligozoospermic men also had lower testicular volumes (mean/median: 20.0/20 ml versus 21.4/22 ml for the left, \( P = 0.03 \); and 20.3/20 ml versus 21.7/22 ml for the right, \( P = 0.02 \)). Sperm concentration was lower in men with varicocele than those without varicocele (mean/median: 71.8/61.0×10^6/ml versus 102.8/78.8×10^6/ml, \( P = 0.004 \)) regardless of the grade of the varicocele, whereas sperm motility was not affected (56.1 and 53.7%, men with varicocele and no varicocele respectively, \( P = 0.22 \)). The frequencies of Chlamydia infection and thyroid disease were 1.2 and 1.9% respectively. Comparisons of semen parameters in men with and without previous Chlamydia infection and men with and without previous thyroid disease did not differ significantly (semen volume: \( P = 0.48 \), sperm concentration: \( P = 0.93 \),...
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Comparison of semen parameters between the Japanese and the European fertile men

To compare the semen quality of the fertile Japanese men with that of European men, we had access to the database of the European study (Jørgensen et al., 2001). After data corrections and adjustments for covariates, the comparisons were performed between the relative value of semen parameters of men from the Kawasaki/Yokohama area in Japan and those from Copenhagen (Denmark), Paris (France), Edinburgh (Scotland) and Turku (Finland) using multivariate models. Table IV shows that the sperm concentration was higher in men from Copenhagen, Paris, Edinburgh and Turku centres than in men from the Kawasaki/Yokohama area by 4, 10, 28 and 47% respectively. These differences between men from the Kawasaki centre were not significant when compared with men from Copenhagen (P = 0.55) and Paris (P = 0.23), but significant for men from Edinburgh (P = 0.0008) and Turku (P < 0.0001). For the total sperm count, percentage of motile sperm and percentage of normal sperm, men from the Kawasaki centre had significantly lower levels than those from all European centres except for motile sperm of men from Paris.

Discussion

To our knowledge our results are the first to describe the current semen quality of Japanese fertile men, comparing these with results from a similar study in Europe. The Japanese fertile men had a sperm concentration almost at the same level as the Danish men, who had the lowest count among the

motility: P = 0.55 for Chlamydia infection; semen volume: P = 0.65, sperm concentration: P = 0.99, motility: P = 0.06 for thyroid disease).
European men studied while the Finnish men had the highest. The European study (Jørgensen et al., 2001) has pointed out that the detected regional differences in semen quality are associated with the regional incidences of testicular cancer (Skakkebæk et al., 1987, 1998; Møller and Skakkebæk, 1999). Denmark has been suggested to be the worst-case scenario regarding male reproductive health with Danish men having a low semen quality and a high risk of testicular cancer (Sharpe, 2001). The so-called testicular dysgenesis syndrome (TDS) hypothesis implies that exogenous, environmental factors may interfere with normal testicular development and lead not only to reduced sperm count and increased risk of testicular cancer in adult life but also at birth to increased risk of hypospadias and undescended testes at least when regarded at population-based levels (Skakkebæk et al., 2001). The Japanese men examined in this study showed low levels in semen quality like Danish men, but the incidence of testicular cancer is considerably lower among Japanese men (Adami et al., 1994; Toppari et al., 1996; Oshima et al., 2001), approximately at the same low level like Finnish men (Huyghe et al., 2003) who were found to have the best semen quality in the European study. The low levels of sperm counts of the Japanese men may thus reflect ethnic differences rather than a symptom of an impaired male reproductive health. While we have no available data from Japan to elucidate the possible influence of ethnicity on semen quality, some reports have shown lower testis parenchymal weight (Johnson et al., 1998), lower androsterone glucuronide levels despite similar serum testosterone levels (Lookingbill et al., 1991; Ross et al., 1992), lower testosterone production rate (Santner et al., 1998), and longer CAG repeats in the androgen receptor gene (Irvine et al., 1995) in Asian men compared with Hispanic or non-Hispanic white men. The finding by Chia et al. (1998) that Chinese, Malays and Indians have low sperm counts is in line with our findings, indicating low values in Asian men.

The abstinence period of the Japanese men (mean/median: 208/134 h) was longer than that of the fertile men from European cities (81/64 h for the Danish, 109/70 h for the Finish, 156/82 h for the Scottish and 157/96 h for the French; Jørgensen et al., 2001). Our finding of an increasing effect on semen volume, sperm concentration and total sperm count with increasing abstinence period is consistent with other investigations (Schwartz et al., 1979; Blackwell et al., 1992; Pell estor et al., 1994). However, increasing abstinence period had increasing effect on sperm concentration up to 96 h in men from four cities in Europe (Jørgensen et al., 2001), whereas the effect lasted up to 9 days in the Japanese men. We did not obtain any information on the ejaculation frequency up to the previous ejaculation to the delivered sample. However, this is also to be regarded as a minor factor, as shown by Carlsen et al. (2004) who found that most of the intra-individual variations in semen parameters could not be due to the ejaculatory frequency itself. In the previous European study (Jørgensen et al., 2001), a seasonal variation in sperm concentration (summer 70% of winter) and total sperm count (summer 72% of winter) were detected, but no significant differences between any of the four seasons were observed in this study of fertile Japanese men (data not shown). Some other previous studies from Europe and the USA have detected seasonal variations (Tjoa et al., 1982; Gyllenborg et al., 1999; Levine, 1999), however, studies from Australia, South Africa and Singapore did not find any seasonal variation (Mallidas et al., 1991; Omebe et al., 1996; Chia et al., 2001). There is a need for a specific, prospective study to confirm this situation in Japanese men.

The Japanese men showed the lowest total sperm counts when compared with the European men. The lowest total sperm counts in the Japanese men reflected their low semen volumes and low sperm concentrations. The lowest level of Japanese men was also observed for sperm motility and morphology. The interpretation of the motility finding is uncertain as the motility assessment is known to have a large inter-observer variation (Jørgensen et al., 1997) and quality control of motility assessment was not performed. The assessment of morphology was undertaken in the French laboratory, which also assessed the morphology for the European study. However, the influence of using different fixatives has not been ruled out as a possible confounder. The low levels in these parameters might, however, be one characteristic of Japanese males.

Comparative studies on semen quality have limitations in relation to many confounders such as population characteristics and methodology for semen analysis. In order to reduce such limitations, our study was designed in collaboration with the international coordinated studies of fertile men (Jørgensen et al., 2001), and protocols and quality control programmes were shared among the studies. In the international studies, male partners of pregnant women were chosen as study subjects because they appeared to constitute well-defined, demographically comparable groups in each of the participating countries. The participation rate in the present study was at an

### Table IV. Relative differences [% (95% confidence interval)] in semen parameters of fertile men

<table>
<thead>
<tr>
<th>Location</th>
<th>Sperm concentration</th>
<th>P</th>
<th>Total sperm count</th>
<th>P</th>
<th>Motile sperm</th>
<th>P</th>
<th>Normal morphology</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copenhagen/Kawasaki</td>
<td>104 (91–120)</td>
<td>0.5517</td>
<td>120 (105–139)</td>
<td>0.0171</td>
<td>108 (105–111)</td>
<td>&lt; 0.0001</td>
<td>117 (109–126)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Paris/Kawasaki</td>
<td>110 (94–128)</td>
<td>0.2299</td>
<td>122 (105–143)</td>
<td>0.0113</td>
<td>102 (99–105)</td>
<td>0.208</td>
<td>115 (107–124)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Edinburgh/Kawasaki</td>
<td>128 (111–148)</td>
<td>0.0008</td>
<td>142 (123–166)</td>
<td>&lt; 0.0001</td>
<td>113 (110–116)</td>
<td>&lt; 0.0001</td>
<td>117 (109–126)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Turku/Kawasaki</td>
<td>147 (127–170)</td>
<td>&lt; 0.0001</td>
<td>181 (155–211)</td>
<td>&lt; 0.0001</td>
<td>112 (109–115)</td>
<td>&lt; 0.0001</td>
<td>122 (114–132)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Difference = relative differences in semen qualities. For example, difference Copenhagen/Kawasaki of 104% for sperm concentrations shows that the fertile men in Copenhagen have a 4% higher sperm concentration than the fertile men from Kawasaki, and the difference Edinburgh/Kawasaki of 128% for sperm concentration shows that the fertile men in Edinburgh have a concentration of 128% compared with men from Kawasaki, i.e. 28% higher than the men from Edinburgh. For sperm motility, the ratio of motile to immotile sperm is 8% higher in Copenhagen than in Kawasaki. The percentage of morphologically normal sperm is 17% higher in Copenhagen than in Kawasaki.

Results are corrected for the confounders of age, ejaculation abstinence period, and for motility additionally for delay from time of ejaculation to assessment.
intermediate level compared to the European study and higher than in a recent US study (Swan et al., 2003).

In conclusion, our study showed that semen quality of fertile men from Kawasaki/Yokohama in Japan was at the same low level as Danish men from Copenhagen. However, in other respects Japanese men do not seem to have an impaired reproductive health like the Danes. Although a possible explanation for regional differences can be differences in lifestyle or other environmental factors, ethnic differences caused by different genetic variation or combinations is a likely contributing factor because Japan differs substantially from the Western countries in ethnicity, but also in lifestyle, even though there is the resemblance in environmental status as an industrialized and affluent society.

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References


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