Monthly variation in human semen quality in male partners of infertile women in the tropics

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The aim of the study was to determine whether there were significant ‘seasonal’ variations in the rhythm of sperm parameters (i.e. semen volume and sperm density) of men who reside in the tropics. A total of 7656 semen analysis results from the department of obstetrics and gynaecology of a tertiary general hospital was analysed. These samples were obtained as part of an initial screening for male partners of couples with problems trying to conceive who were attending a fertility clinic from 1991 to 1995. The subjects’ mean semen volume and sperm density of 2.9 ml and 26.9 $10^6$/ml respectively were within the World Health Organization reference values. There were no significant month-to-month variations in the adjusted (for age of subject, year of test and technologist who performed the analysis by analysis of co-variance, ANCOVA) mean semen volume and sperm density over the 5 year period. Variations in semen volume and density observed in the temperate climates are not seen in this study, which was conducted in subjects staying in the tropics. This observation may be related to relative constant temperature and hours of light exposure among men who reside in the tropics. As this study is, so far, published data from only one country, Singapore, it may not truly reflect the actual situation for individuals who are residing in the tropics.

Key words: daylight/semen volume/sperm density/temperature

Introduction

There is overwhelming evidence that human semen quality varies with season in the northern hemisphere. Levine (1999) reviewed 11 studies on seasonal variation of sperm parameters and concluded that sperm concentration was lowest during the summer season (July, August, September). The extent of the summer reduction in sperm concentration was similar for studies conducted above 45° latitude (Edinburgh, Lille, Basel, Calgary) and those conducted at lower latitudes (New York, Houston, New Orleans) (Levine, 1999). More recently, Centola and Eberly (1999), using only computer-assisted semen analyses of 2065 samples conducted by one person, reported that percentage of rapid progressive spermatozoa and straight-line velocity were significantly lower in the spring.

What causes these seasonal changes in semen quality? Climatic heat was proposed to be a possible causative factor in earlier studies (Levine et al., 1988; Politoff et al., 1989). However, later reports suggested that heat may not be as important a factor as photoperiod (Synder, 1990; Levine et al., 1992). So far, it appears that all the reports on seasonal variation of human semen quality were conducted in the temperate countries. There are no reports of large semen quality data of men from the tropics. Men residing in the tropics are exposed to almost constant hours of daylight throughout the year. Data on variation in semen quality through the year for these men would be useful for testing the importance of photoperiod on spermatogenesis. The objective of this study, therefore, was to determine if there is a circannual rhythm of sperm parameters of men in the tropics.

Materials and methods

This study is a case series. All semen analyses were evaluated between 1991 and 1995 at the fertility clinic in an obstetrics and gynaecology department of a general hospital in Singapore. The first semen specimens from male partners of couples who were undergoing initial screening for infertility were used in the analysis. No information was available on the clinical status of the men who were attending the fertility clinic as some of the men only contributed their semen for analysis during the initial screening and were not seen at the clinic. A total of 7628 semen analyses were obtained from the database records. The age range was between 20 and 50 years. There were 75 men whose samples were azoospermic and thus were removed from the study. Therefore, 7553 semen analysis results were used in all the subsequent analyses.

Semen collection and analysis

The men were asked to collect their semen at home in the morning by masturbation into a sterile wide-mouth plastic container, after
3 days of abstinence. The samples were brought to the laboratory within 1 h of collection. Time of ejaculation, abstinence period, spillage (if any), and fever during the last 3 months were recorded by the subject. All semen samples were processed and analysed by experienced laboratory technologists at the Fertility Clinic of the Singapore General Hospital immediately upon receiving the samples. Seven laboratory technologists were involved in the semen analysis from 1991 to 1995. Volume, total sperm density, sperm viability, proportion of progressively motile spermatozoa and proportion of normal and abnormal sperm forms were examined according to the World Health Organization’s guidelines for the examination of human semen (WHO, 1992). For the objective of this study, only the data for sperm density and semen volume were used for the analysis.

All assays were performed after the semen had liquefied and within 1 h of collection by masturbation. Samples that remained viscous were liquefied by mechanical pipetting with a large-bore disposable pipette. The volume was assessed by aspirating the semen into a 5 ml graduated micropipette with disposable tips (Oxford–Macrose, Swedesboro, NJ, USA). Sperm concentration was determined with a Neubauer haemocytometer. A 1:20 dilution was made using 50 µl semen and 950 µl sperm diluent solution. A 10 µl droplet was removed from the well-mixed sample and applied to the slide chamber, which was mounted with a glass coverslip. A second sample was applied to the other chamber. After the spermatozoa had settled, they were counted under phase-contrast microscopy. Only mature spermatozoa (with tails) were counted. Pinhead spermatozoa were excluded. If the difference between the two counts exceeded 10%, another haemocytometer was set up and the counts repeated.

Intra-specimen assays for all the above parameters consistently gave values within a 10% variance.

Statistical analysis
The sperm density distribution was skewed. Log transformation was first used to normalize the data but the transformed data was still slightly skewed to the left. However, with cubic root transformation of sperm density, normality of the distribution was achieved. The cubic root of sperm density data was used in all subsequent analysis involving sperm density. We have also calculated the geometric mean of sperm density as the geometric mean was used for comparison with other studies.

The monthly variations in the semen parameters (i.e. sperm density and semen volume) from 1991 to 1995 were studied. The year-to-year variations in the semen parameters could confound the results since there were significant differences in the mean sperm density for the various years (see Table II). Similarly, the inter-technologists variations and age of the subjects would also need to be considered. Analysis of co-variance (ANCOVA) using the general linear model procedure was used to determine mean sperm density and semen volumes adjusting for age of subject, the technologist performing the semen analysis and year of examination. The data were then plotted, with the adjusted mean value and the 95% confidence limits, to show the distribution of the various semen parameters over the 12 calendar months. The Bonferroni Post Hoc test was used to compare the semen parameters between the calendar months. Statistical analysis was carried out using the Window version of SPSS 9.1 on a personal computer (SPSS, 1999).

Discussion
To our knowledge, this is the first study that analysed the monthly fluctuation of semen quality in men from the tropics. Although the data were obtained from men who were attending the infertility clinic it does not imply that all the men were infertile. The present findings (Table I) were compared with our two other published studies (Chia et al., 1998, 2000). The infertile men in the 2000 study, among other inclusion criteria mentioned in the study, had more than 1 year of failed attempts then mentioned in the study, had more than 1 year of failed attempts plotted, with the adjusted mean value and the 95% confidence interval by calendar months. Although there were month-to-month fluctuations in the mean semen volume these differences were not significant. Similarly, there were no significant differences in the month-to-month adjusted (for age of subject, year of test and technologist who performed the analysis) mean sperm density (Figure 3).

Table 1 shows the distribution of the semen volume and sperm density of the studied population in comparison with two other studies of Singapore men, an infertile and a fertile group. The data for the semen volume for this study were comparable to the other two studies. These two studies were conducted in the same clinic and under identical analysis procedure. As for sperm density, the present study data were much better than the infertile group although they were slightly poorer than the fertile group. This fertile group had their semen collected when their wives were pregnant at the time of the study. The subjects’ mean sperm density of 26.9×10^6/ml is within the normal sperm density of 20×10^6/ml as recommended by the WHO’s criteria (WHO, 1999). Figure 1 shows the cumulative percentages of the sperm density for the three groups of men. The present study data fall within the two groups and are better than the infertile men’s sperm density.

There were no significant differences in the mean semen volumes over the 5 year period. The year-to-year differences for the mean semen volume were only about 0.1 ml. However, there were differences in the mean sperm density over the 5 year period. It was constant for 1991 and 1992, going up in 1993 and coming down in 1994 and 1995. No significant trend was noted in the mean sperm density over the 5 years. The mean sperm density was highest in 1993. There were significant differences when 1993 sperm density was compared to other years, except for 1994 (1993 versus 1991, P = 0.009; versus 1992, P = 0.001 and versus 1995, P = 0.017)(Table II).

Figure 2 shows the adjusted (for age of subject, year of test and technologist who performed the analysis) mean semen volume and the 95% confidence interval by calendar months. Although there were month-to-month fluctuations in the mean semen volume these differences were not significant. Similarly, there were no significant differences in the month-to-month adjusted (for age of subject, year of test and technologist who performed the analysis) mean sperm density (Figure 3).
**Table I.** Semen parameters in present study compared with two of our previous studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of samples</th>
<th>Means</th>
<th>SD</th>
<th>Range</th>
<th>Percentiles</th>
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<tr>
<td></td>
<td></td>
<td>25th</td>
<td>50th</td>
<td>75th</td>
<td></td>
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<td><strong>Present study</strong></td>
<td></td>
<td></td>
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<tr>
<td>Volume (ml)</td>
<td>7553</td>
<td>2.9</td>
<td>1.4</td>
<td>0.04–12.4</td>
<td>1.9 2.7 3.6</td>
</tr>
<tr>
<td>Density ((\times 10^6/ml))</td>
<td>7553</td>
<td>26.9</td>
<td>4.7</td>
<td>0.1–699</td>
<td>15.0 37.8 74.5</td>
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<td>Infertile men from Chia et al. (2000)</td>
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<tr>
<td>Volume (ml)</td>
<td>218</td>
<td>2.6</td>
<td>1.7</td>
<td>0.5–8.6</td>
<td>1.9 2.7 3.8</td>
</tr>
<tr>
<td>Density ((\times 10^6/ml))</td>
<td>218</td>
<td>14.8</td>
<td>5.1</td>
<td>0.1–227</td>
<td>6.7 19.1 41.9</td>
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<td>Fertile men from Chia et al. (1998)</td>
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<tr>
<td>Volume (ml)</td>
<td>243</td>
<td>2.4</td>
<td>1.3</td>
<td>0.2–8.7</td>
<td>1.3 2.2 3.0</td>
</tr>
<tr>
<td>Density ((\times 10^6/ml))</td>
<td>243</td>
<td>44.7</td>
<td>2.8</td>
<td>1.6–433</td>
<td>23.0 45.0 105</td>
</tr>
</tbody>
</table>

*a* Refers to geometric mean and SD.

**Figure 1.** Cumulative percentage curves of sperm density for the three groups of men, i.e. A = fertile (Chia et al., 1998); B = present study group; and C = infertile (Chia et al., 2000).

**Figure 2.** Adjusted mean semen volume for age of subject, year of test and technologist who performed the analysis by analysis of co-variance (ANCOVA). Note: *n* = number of samples in each month. CI = confidence interval.

**Figure 3.** Adjusted mean sperm density for age of subject, year of test and technologist who performed analysis by ANCOVA. Note: *n* = number of samples in each month. CI = confidence interval.

Our study group consisted of men from couples undergoing initial screening for inability to conceive. Generally, in approximately 30% of the cases, an important abnormality is identified in the man only (Howard, 1995). As such, the data, although taken from an infertility clinic, do not only reflect the sperm parameters of infertile men.

Another possible issue is that of selection bias, i.e. only those who could afford the services would come for the investigation. In some countries only a minor fraction of infertile couples seek medical assistance either because of cultural beliefs or financial reasons; this is not so in Singapore. Health care is affordable and readily accessible. Because of the norm of a two child family, most couples would seek medical assistance if they had a problem trying to conceive.

This hospital where the data were obtained is a general hospital which provides governmental financial subsidy for health services.

Effects of frequency of ejaculation on the results of semen...
analysis were eliminated in this study by the subjects having had a 3 day abstinence period, confirmed by the staff when the semen samples were collected.

There are other limitations inherent in this study. The clinical status of each of the subjects was not available. Different types of diseases, which this study was not able to look into, may influence the sperm parameters. As the samples were collected over a 5 year period, it was not feasible for only one technologist to perform all the seminal analysis. Inter-technologist biases could be introduced in the process. Similarly, there may be year-to-year fluctuations arising from inter-observer variation or laboratory measurement fluctuations. To minimize these possible confounders, data were adjusted for these factors when the month-to-month mean sperm parameters were studied.

No significant monthly differences were found in the mean semen volume and sperm density. These results were in contrast to those in a prospective study in San Antonio, Texas, USA, in which Levine et al. reported that men had significantly higher sperm density and percentage of normal sperm morphology in winter than in summer (Levine et al., 1990). Similar findings were reported in another prospective study of similar design but conducted in New Orleans, Louisiana, USA (Levine et al., 1992). Other retrospective studies, all in the temperate cities (Edinburgh, Lille, Bologna, Minnesota), have also reported reduction in sperm density during summer (Mortimer et al., 1983; Politoff et al., 1989; Campanello et al., 1990; Fisch et al., 1997).

The causes of these seasonal changes in semen quality observed in the temperate countries but not in the tropics are unknown. Climatic heat and hours of daylight have been postulated as possible causes for seasonal fluctuation in the temperate countries—as temperatures are higher in summer and the daylight hours are much longer. If these hypotheses are true, there will not be any significant monthly difference in the semen quality in the tropics, given that temperature and daylight hour fluctuation are not large.

Normal spermatogenesis requires a temperature below that of the abdomen. The intrascrotal temperature is 2–3°C lower than the rectal temperature (Synder, 1990). High ambient air temperatures may inhibit thermal loss through the scrotum, leading to a rise in testicular temperature. Infertile men without varicocele have been noted to have significantly higher mean intrascrotal temperatures than men without fertility problems (Zorgniotti and Sealfon, 1988). Sperm production in humans is known to decrease when testicular temperature is raised by experimental techniques (Mieuss et al., 1987). In an epidemiological study by Chia et al. (1994), it was reported that ‘plant and machine operators (PMO) had an odds ratio (OR) of 1.93 (95% CI 1.12–3.30) for oligospermia compared with the non-PMO’. Workers exposed to excessive heat were found to be associated with a higher risk of oligospermia, OR = 2.72 (95% CI 1.12–7.41), in the PMO group. In a study to investigate the effects of climatic heat, Levine et al. (1992) collected semen from two groups of men, one engaged in predominantly indoor and the other in predominantly outdoor activity, both in summer and in winter. They reported there was no correlation between the number of hours worked outdoors during the summer and summer time measures of semen quality or any differences in these measures between summer and winter. They dismissed the hypothesis that the heat of the summer is detrimental to male reproductive capacity (Levine et al., 1992). There were no other reports to substantiate Levine’s observation.

The duration of daylight may affect semen quality. Under laboratory conditions, rhesus monkeys’ testis volume increases after exposure to shorter daylight (8 h of light, 16 h of dark) and decreases after exposure to longer daylight (16 h of light, 8 h of dark), with concomitant increases and decreases in plasma testosterone respectively (Chik et al., 1992). Levine has suggested that the summer suppression of spermatogenesis observed in the temperate countries may be due in part to the photoperiodic exposure affecting an endogenous circannual rhythm (Levine, 1994).

Singapore is located between latitudes 1° 09’N and 1° 29’N and longitudes 103° 36’E and 104° 25’E. The temperature is relatively stable throughout the year due to its close proximity to the equator (80 km north). The average maximum and minimum temperatures are around 32°C and 25°C respectively. The range for the daily mean bright sunshine hours for the last 10 years was from 5.1–5.9 h (Singapore, 1998). Whether the findings in the current study that there was no significant month-to-month variation in semen quality among Singapore men was related to lack of seasonal variation in climatic temperature and daylight duration is an intriguing question. If the conclusion that summer climatic temperature is not a determining factor of semen quality in man (Levine, 1994) is accepted, then the findings of this study lend support to the hypothesis that photo duration may be an important factor in human spermatogenesis.

In photoregulated, seasonally breeding mammals, the pineal gland and melatonin are key components in the neuro-endocrine pathway coupling day length to gonadotrophin release (Bronson, 1995). There is no doubt that melatonin secretion in humans is sensitive to light (Lewy et al., 1980). However, studies conducted on human melatonin secretion over the different seasons have been inconclusive (Lewy et al., 1980). Further studies on photoregulation of human spermatogenesis and the possible underlying neuro-endocrine pathways are needed.

As the current study is based on, so far, data obtained from only one country in the tropics, it may not truly reflect the actual situation for individuals who are residing in the tropics. There is a need for a multi-centre study involving other andrology centres to confirm these findings.

In summary, it has been shown that there were no significant month-to-month fluctuations in semen volume and sperm density among men who reside in the tropics. These results may be related to the minimal changes in temperature and daylight hours that the men were exposed to in the tropics.

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References


