Diurnal variation of semen quality in human males

A.Cagnacci¹, N.Maxia and A.Volpe

Department of Obstetrics and Gynaecology, University of Modena, via del Pozzo 71, 41100 Modena, Italy

¹To whom correspondence should be addressed at: Istituto di Clinica Ginecologica e Ostetrica, via del Pozzo 71, 41100 Modena, Italy. E-mail: cagnacci@unimo.it

The possibility of a diurnal variation in semen quality was tested in 54 human males attending our infertility clinic. Of the enrolled subjects, 24 were normozoospermic and 30 were suffering from oligo- and/or asthenozoospermia. Seminal fluid was collected by masturbation twice by each subject, once in the morning (7:00–7:30 a.m.) and once in the afternoon (5:00–5:30 p.m.). Abstinence from sexual intercourse for 3–4 days before each of the two collections was requested. Semen parameters were evaluated independently by two biologists before and after pellet swim-up. Beside similar macroscopic parameters, specimens collected in the afternoon showed a higher number (P < 0.01) and concentration (P < 0.01) of spermatozoa. Also, immediately (P < 0.05), and at 1 h (P < 0.02) and 2 h (P < 0.01) after pellet swim-up, the number of spermatozoa with progressive linear motility was higher in the afternoon than in the morning. These data are the first documenting a diurnal rhythm in sperm quality which may contribute to the reported variability in semen parameters, and may prove useful for spontaneous and assisted conceptions. Key words: circadian rhythms/fertility/humans/spermatozoa

Materials and methods

The study, approved by the Institutional Review Board, was carried out in 54 males recruited among those attending our infertility clinic. Of these subjects, 24 were normozoospermic and 30 had oligo- and/or asthenozoospermia. After providing their informed consent, all subjects were requested to maintain a constant daily lifestyle for 20 days. All experiments were carried out between October and January. For 20 days, each subject recorded in a diary their sleeping and eating patterns, and their daily consumption of coffee, alcoholic beverages and cigarettes. During the last 10 days (after 3 days of sexual abstinence), subjects were randomized to collect spermatozoa in the morning (7:00–7:30 a.m.) or afternoon (5:00–5:30 p.m.). After a further 3 days of sexual abstinence, the morning and afternoon spermatozoa collection regimens were reversed. The interval between the last ejaculation and time of morning or afternoon sperm collection was maintained as constant as possible by each subject. Differences in sexual abstinence between the morning and afternoon collections never exceeded 4 h, as documented by subjects’ diaries. World Health Organization (WHO, 1992) procedures for sperm collection, analysis and definitions were followed. Sperm morphology was evaluated by Shorr staining (WHO, 1992). Semen samples were obtained at the laboratory. Spermatozoa were collected by masturbation in a sterile, wide-mouthed plastic container and kept at a temperature of about 25°C; analyses were performed within 60 min of collection. In order to obtain a maximally objective evaluation of semen parameters, each specimen was evaluated independently by two biologists. As, in our department, semen analysis is performed both in the morning and in the afternoon, it was possible to blind the two biologists with regard to the provenance of the semen, and the design of the study. After conjunct macroscopic evaluation of the semen parameters (volume, colour, viscosity and pH), the microscopic characteristics of each specimen were evaluated. Sperm concentration was measured in a specimen fraction using a Neubauer chamber, while sperm motility was evaluated using a Makler chamber. Sperm motility was defined as rapid progressive linear motility, sluggish linear progressive motility, non-progressive motility or immobile, in accordance to the respective categories a, b, c and d, as defined by WHO (1992).

Semen specimens were then processed by sperm washing and the swim-up technique (Agarwal et al., 1994). Specimens were washed twice with HTF medium (Irvine Scientific, Santa Ava, CA, USA) at a semen:HTF ratio of 1:2–1:4, and centrifuged at 600 g for 10 min. The sperm pellets were resuspended in 2.0 ml of HTF, transferred in equal parts to two clean round-bottomed test tubes, and centrifuged at 60 g for 5 min. Motile spermatozoa were allowed to swim up during incubation of test tubes (which were held at a 45° angle) in 5% CO₂ at 37°C for 1 h. Supernatants were aspirated into a clean
test tube and centrifuged at 600 g for 10 min. The final supernatants were aspirated and discarded. Sperm pellets were resuspended in 0.5 ml of HTF with 10% human albumin. Each of the two biologists re-evaluated semen parameters at the end of the procedure and after 1 and 2 h.

![Figure 1. Mean (± SE) concentration of spermatozoa with rapid progressive linear motility, observed in specimens of 54 individuals (24 normozoospermic, 30 oligo- and/or asthenozoospermic) collected in the morning (7:00–7:30 a.m.) and afternoon (5:00–5:30) (baseline). Concentrations of spermatozoa with rapid progressive linear motility observed immediately after, and at 1 and 2 h after pellet swim-up are also reported. *P < 0.05; †P < 0.025; ‡P < 0.01 compared with corresponding morning value.](image-url)

### Table I. Mean (± SE) sperm number per ejaculate (total), per ml (concentration) and with different motility (percentages calculated on corresponding total number and shown in parentheses) observed in 54 males, with specimens collected in the morning (7:00–7:30 a.m.) and afternoon (5:00–5:30 p.m.). Mean (± SE) number of recovered spermatozoa at pellet swim-up and number with different motility immediately after, and at 1 and 2 h after pellet swim-up, are also reported.

<table>
<thead>
<tr>
<th></th>
<th>Basal Total (×10⁶)</th>
<th>Basal Concentration (×10⁹/ml)</th>
<th>After swim-up Immediately</th>
<th>After swim-up 1 hour</th>
<th>After swim-up 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. (×10⁶)</td>
<td>127.8 ± 15.5</td>
<td>37.7 ± 4.1</td>
<td>14.9 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility (% of sperm)</td>
<td>Rapid progressive</td>
<td>28.3 ± 4.9 (–22)</td>
<td>11.6 ± 1.6 (–78)</td>
<td>9.2 ± 1.4 (–62)</td>
<td>9.6 ± 1.7 (–64)</td>
</tr>
<tr>
<td></td>
<td>Sluggish progressive</td>
<td>38.5 ± 4.9 (–30)</td>
<td>3.1 ± 0.4 (–21)</td>
<td>4.0 ± 0.5 (–27)</td>
<td>4.6 ± 0.6 (–31)</td>
</tr>
<tr>
<td></td>
<td>Non-progressive</td>
<td>13.4 ± 1.8 (–10)</td>
<td>0.0 ± 0.2 (–8)</td>
<td>1.2 ± 0.2 (–8)</td>
<td>2.3 ± 0.4 (–15)</td>
</tr>
<tr>
<td>Immotility</td>
<td>47.5 ± 5.4 (–37)</td>
<td>0.2 ± 0.08 (–1)</td>
<td>0.2 ± 0.08 (–1)</td>
<td>0.4 ± 0.1 (–3)</td>
<td>0.4 ± 0.1 (–3)</td>
</tr>
<tr>
<td>Rapid + sluggish</td>
<td>71.4 ± 10.2 (–56)</td>
<td>14.8 ± 1.9 (–100)</td>
<td>13.3 ± 1.8 (–89)</td>
<td>14.2 ± 2.1 (–95)</td>
<td></td>
</tr>
<tr>
<td><strong>Afternoon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. (×10⁶)</td>
<td>161.9 ± 22.9c</td>
<td>48.3 ± 5.6f</td>
<td>16.9 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (×10⁹/ml)</td>
<td>Rapid progressive</td>
<td>36.2 ± 7.7 (–22)</td>
<td>13.2 ± 1.8 (–78)</td>
<td>11.8 ± 1.7 (–70)b</td>
<td>10.2 ± 1.6 (–60)b</td>
</tr>
<tr>
<td></td>
<td>Sluggish progressive</td>
<td>45.4 ± 5.3 (–28)</td>
<td>3.2 ± 0.2 (–19)</td>
<td>4.2 ± 0.3 (–25)</td>
<td>5.0 ± 0.5 (–29)</td>
</tr>
<tr>
<td></td>
<td>Non-progressive</td>
<td>19.0 ± 2.8 (–12)c</td>
<td>0.0 ± 0.1 (–3)b</td>
<td>0.4 ± 0.1 (–3)b</td>
<td>1.2 ± 0.2 (–8)b</td>
</tr>
<tr>
<td>Immotility</td>
<td>61.2 ± 7.9 (–38)</td>
<td>0.0 ± 0.0 (–0)</td>
<td>0.0 ± 0.0 (–0)</td>
<td>0.0 ± 0.0 (–0)</td>
<td>0.0 ± 0.0 (–0)</td>
</tr>
<tr>
<td>Rapid + sluggish</td>
<td>88.2 ± 13.9 (–55)</td>
<td>16.4 ± 2.0 (100)</td>
<td>16.0 ± 1.9 (–96)</td>
<td>15.3 ± 1.9 (–91)</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.02; ‡P < 0.01 compared with the corresponding morning value (Wilcoxon signed-rank test).

### Statistical analysis
All results were expressed as the mean of the values recorded by the two biologists. Statistical analysis of the data was performed by the Wilcoxon signed-rank test. The χ² test was used to exclude the null hypothesis that eventual variations in semen parameters were due to a random binomial distribution of the data.

### Results
The mean age of subjects was 31.5 ± 2.8 years, with a mean infertility duration of 3.8 ± 0.5 years. No significant difference was observed between the morning and afternoon collections in terms of sexual abstinence (45.6 ± 2.4 h for morning versus 43.2 ± 2.4 h for afternoon), time of going to bed (10:40 p.m. ± 0.24 h for morning versus 10:43 p.m. ± 0.30 h for afternoon), or time of awakening (6:34 a.m. ± 0.18 h for morning versus 6:37 a.m. ± 0.15 h for afternoon). The mean seminal fluid volume was 3.35 ± 0.1 ml in the morning and 3.43 ± 0.2 ml in the afternoon, with pH values of 7.82 ± 0.02 and 7.85 ± 0.03 respectively. Viscosity and agglutination were normal and similar at the two diurnal times, as well as sperm abnormalities (38.9 ± 1.4% in the morning versus 40.0 ± 1.2% in the afternoon). However, about 35×10⁶ fewer spermatozoa were observed in the morning specimens (Table I). Of the 54 subjects, 42 showed a higher sperm number and concentration in specimens collected in the afternoon, and this was significantly different from what was expected on the basis of a random binomial distribution of the data (P < 0.01). As a consequence of the higher sperm number, the concentration of rapid progressive plus sluggish progressive spermatozoa was greater in the afternoon than in the morning specimens (Table I).

After swim-up, the sperm number was slightly but not
significantly higher in the afternoon than in morning collections (Table I). However, the number of spermatozoa with rapid plus sluggish progressive linear motility was significantly higher in afternoon than in morning specimens, at both 1 h ($P < 0.02$) and 2 h ($P < 0.01$) after the pellet swim-up. Similarly, there were more rapidly progressive spermatozoa in the afternoon than in the morning, both in unprepared seminal fluid and at all the time points following pellet swim-up (Figure 1).

Afternoon versus morning differences were similarly evident in subjects with normozoospermia or oligo/asthenozoospermia.

**Discussion**

To our knowledge, these are the first data documenting a diurnal variation in semen quality. Specimens collected in the afternoon showed a higher number and concentration of spermatozoa, particularly of spermatozoa with rapid plus sluggish progressive linear motility. These differences were evident both in unprepared seminal fluids and after pellet swim-up. Following the 74 days of spermatogenesis (Sharpe, 1994), mature spermatozoa accumulate within the vas deferens and cauda epididymidis, from which they are ejaculated. With the prolongation of sexual abstinence, although the concentration of spermatozoa increases, their quality deteriorates (Comhaire and Vermeulen, 1995). Sexual abstinence for 2–7 days is optimal to obtain a good quality and quantity of spermatozoa (Comhaire and Vermeulen, 1995). In the present study, sexual abstinence was within the optimum range and, more importantly, was similar between the morning and afternoon collections. Therefore, marked differences in sexual abstinence did not occur between the two collections, and any smaller difference was very likely inappropriate to induce a variation in sperm count of 35%, as was documented in our study. Indeed, a difference of 1 day in sexual abstinence is believed to induce a difference in sperm count of only 1–4%, associated with a reduction in sperm motility (Auger et al., 1995), which was not documented in our afternoon samples. Spontaneous variations in sperm concentrations (Comhaire and Vermeulen, 1995) may have undermined the validity of the present study, but the differences between the morning and afternoon specimens did not meet the criteria of a random binomial distribution of the data. The order in which the samples were collected also may have played a role in determining these results, but this risk was virtually eliminated by the study design, which was both randomized and crossover in nature. Finally, all evaluations were performed by two independent biologists, who were blinded with regard to the study design and specimen provenance.

As spermatogenesis is a long process (Sharpe, 1994), it is unlikely that diurnal rhythms in sperm quality may derive from diurnal variations in sperm production and maturation. Functional variations in the nerve–muscle mechanisms causing ejaculation (Setchell et al., 1994) are more likely to determine higher or lower concentrations of spermatozoa in seminal fluids. However, it was not only the number of spermatozoa that showed a diurnal rhythm, but also their motility. Immediately, but in particular at 1 and 2 h after pellet swim-up, the number and concentration of spermatozoa that had a rapid progressive linear motility was higher in afternoon than in morning specimens. This diurnal variation in sperm motility may derive from influences exerted on spermatozoa by seminal fluid components such as hormones, cytokines, nutrients, amino acids, electrolytes and as yet undefined substances derived either from blood or produced locally (Setchell et al., 1994).

No seminal fluid parameter is considered predictive for fertilization *in vitro*, except for the hamster oocyte zona pellucida penetration test (Liu and Gordon-Baker, 1992). However, sperm concentration, and the number of spermatozoa with rapid plus sluggish progressive motility, are important to define seminal fluid quality. A low concentration of motile spermatozoa is associated with a difficulty in achieving pregnancy both spontaneously and by intrauterine insemination (Steinberg and Rodriguez-Rigau 1983; Liu and Gordon-Baker, 1992; Dickey and Holtkamp, 1996), and often requires the use of costly assisted reproduction techniques (Liu and Gordon-Baker, 1992; Howards, 1995). The present data indicate that the number of spermatozoa with rapid plus sluggish progressive linear motility is higher by about $17 \times 10^6$ in specimens collected in the afternoon. Accordingly, spontaneous fertilization is more likely to occur with seminal fluids ejaculated at this time of day. Because the better quality of the afternoon specimens is maintained even when laboratory procedures for usual sperm preparation are employed, an important point in assisted reproduction procedures may also be highlighted. After sperm deposition into the vagina, there is an ~48 h umbrella of spermatozoa which is conserved in the cervix and tube, and which is available for oocyte fertilization upon ovulation (Harper, 1994). However, within only 5 min, the first spermatozoa arrive in the oviduct (Harper, 1994) and, following capacitation which may require <1 h (Yanagimachi, 1994), they may be ready for fertilization. Accordingly, the afternoon improvement in sperm quality, at a time of maximal ovulatory frequency, may represent an advantageous mechanism for spontaneous conception.

**References**


Received on December 19, 1997; accepted on September 29, 1998