Duration of sexual abstinence: epididymal and accessory sex gland secretions and their relationship to sperm motility

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BACKGROUND: The data on the association between the abstinence period and sperm motility are conflicting.

METHODS: Ejaculates from 422 men assessed for infertility were analysed according to the World Health Organization (WHO) guidelines. Seminal plasma neutral α-glucosidase (NAG), prostate-specific antigen (PSA), zinc and fructose were measured. Three groups were defined according to the length of sexual abstinence: G2–3 (2–3 days), G4–5 (4–5 days) and G6–7 (6–7 days).

RESULTS: The total percentage of progressively motile spermatozoa was significantly higher in G4–5 compared with G2–3 and G6–7 (medians 55 versus 47 and 42%; P < 0.039 and P < 0.001, respectively). The percentage of spermatozoa with tail defects was significantly higher in G6–7 compared with G2–3 and G4–5 (medians 14 versus 10 and 10%; P = 0.011 and P = 0.002, respectively). NAG was significantly lower in G2–3 compared with G4–5 and G6–7 (medians 23 versus 34 and 34 mU/ejaculate; P < 0.001 and P = 0.001, respectively). The same trend was found regarding zinc (medians 6 versus 8 and 8 μmol/ejaculate; P = 0.001 and P = 0.005, respectively).

CONCLUSIONS: Within the time interval recommended by the WHO (2–7 days), the length of the abstinence period is associated with sperm characteristics and should be taken into consideration when interpreting results of semen analysis.

Key words: fructose/NAG/PSA/sexual abstinence period/sperm motility

Introduction

Following spermatogenesis and maturation during epididymal transit, spermatozoa are stored in the cauda epididymis and remain immotile until the time of ejaculation (Eddy and O’Brien, 1994). Motility is induced at ejaculation when spermatozoa are mixed with secretions from various male accessory sex glands (Lindholmer, 1974).

Sperm motility is believed to be one of the most important parameters in evaluating the fertilizing ability of the ejaculated spermatozoa (Bongso et al., 1989; Eimers et al., 1994; Donnelly et al., 1998). Moreover, recent data have indicated that sperm motility characteristics obtained by computer-aided sperm analysing systems (CASAs) may also serve as predictive parameters of fertility (Larsen et al., 2000; Hirano et al., 2001).

Several factors have been shown to influence semen parameters, one of which is the length of sexual abstinence. Although there is general agreement that semen volume and sperm concentration increase with prolonged sexual abstinence (LeLannou et al., 1986; Blackwell and Zaneveld, 1992; Pellestor et al., 1994), the issue of sperm motility is still contradictory (Mortimer et al., 1982; Sauer et al., 1988; Check et al., 1991; Magnus et al., 1991; Blackwell and Zaneveld, 1992; Pellestor et al., 1994).

The results of seminal investigation play an important role for clinical decisions regarding the strategy for infertility treatment. Therefore, it is of a crucial importance to minimize the impact of variation in sample collection conditions on the final result of this test. According to the manual of the World Health Organization (WHO), which is the most accepted guideline for performing semen analysis, a 2–7 day abstinence period is recommended prior to semen analysis for infertility investigation (World Health Organization, 1999). However, in the recommendations by the European Society of Human Reproduction and Embryology (ESHRE) and the Nordic Association for Andrology (Kvist and Bjorndahl, 2002), standardization of abstinence time to 3–4 days is strongly advised.

Apart from the lack of proper information about the correlation between the length of the sexual abstinence period and sperm motility, the possible mechanism of such an association is also unresolved. Epididymal and accessory sex gland secretions play a crucial role for proper sperm function (Mann, 1964; Tremblay et al., 1979; Lilja, 1985; Lilja et al., 1989; Lee et al., 1989; Kret and Milad, 1995; Robert and Gagnon, 1996). However, only one study addressed the issue of the length of sexual abstinence seen in relation to the epididymal and accessory sex gland secretions (Cooper et al., 1993).
Therefore, the aim of this study was to determine the association between the length of sexual abstinence periods and the epididymal and accessory sex gland secretions and their relationship to sperm motility as assessed manually and by use of CASA.

Materials and methods

Subjects

The study was based on semen samples obtained from 422 consecutive non-azoospermic men (median 34 years, range 21–64 years) undergoing infertility assessment at the Fertility Centre, Malmö University Hospital between May 2002 and November 2003. Serum levels of FSH, LH, testosterone, inhibin B and sex hormone-binding globulin (SHBG) were available for 107 of these 422 subjects. Sperm concentration and percentage motile sperm were significantly lower in this subgroup compared with the 315 men from whom the hormone data were not available. However, in these 107 men, there was no difference in the levels of any of the reproductive hormones or age between the groups defined according to the length of their abstinence period (see below).

Semen samples

The ejaculates were obtained by masturbation after 2–7 days of sexual abstinence (median 4 days). Only completely collected semen samples were included. For men delivering more than one sample during the study period, only the first ejaculate was included in the analysis.

Semen analysis

Thirty minutes after ejaculation, 450 μl of the ejaculate were taken off using a common air displacement pipette and mixed with 50 μl of benzamidine (0.1 mol/l) in order to stop the biochemical processes involved in liquefaction. The mixture was centrifuged for 20 min at 4500 g, and the seminal plasma was decanted and stored at −20°C until analysed for the activity of neutral α-glucosidase (NAG), and the concentrations of prostate-specific antigen (PSA), zinc and fructose (see below). After liquefaction, within 1 h after ejaculation, the ejaculates were analysed for the following characteristics: semen volume, sperm concentration, sperm motility (the motility grade; a, b, c and d) and sperm morphology. All semen tests were performed according to the WHO semen manual (1999). Thereafter, CASA motility using the CRISMAS (Image House, Copenhagen, Denmark) system was performed as described before (Elzanaty et al., 2002) with the following modification. Motility analysis was performed in 20 μl Microcell chambers (Leja, Oslo, Norway) instead of a 10 μl Makler chamber. The CASA motility was classified as follows: motile spermatozoa [curvilinear velocity (VCL) ≥ 25 μm/s], locally motile spermatozoa (VCL 5–25 μm/s) and immotile spermatozoa (VCL < 5 μm/s). CASA was performed in 375 of the 422 semen samples. The 47 samples with no CASA done did not differ from the remaining 375 regarding the length of the abstinence period.

Biochemical analysis

Biochemical markers of epididymal function (NAG), prostatic function (PSA and zinc) and seminal vesicles function (fructose) were assessed as described before (Elzanaty et al., 2002). Total α-glucosidase activity was first measured using a commercially available kit (Episcreen™; Fertipro, Gent, Belgium) according to the instructions given by the manufacturer; thereafter, the NAG activity was estimated by the use of the corresponding table provided by the manufacturer. The concentration of PSA in seminal plasma was determined with the PROSTATUS™ kit from (Wallac Oy, Turku, Finland). The concentration of zinc in seminal plasma was determined by a colorimetric method (Makino et al., 1982). The concentration of fructose in seminal plasma was determined with a spectrophotographic method, essentially as described in Wetterauer and Heite (1976). Because of low semen volume, the biochemical markers were only measured in 401 of the 422 semen samples. The 21 samples with no biochemical analysis done did not differ from the remaining 401 regarding the length of the abstinence period.

Statistical methods

Statistical analysis was performed using the SPSS 11.0 software (SPSS Inc., Chicago, IL). The period of sexual abstinence was calculated (expressed as whole days) from the date and time of previous ejaculation, which was asked for in the questionnaire completed by the patients. The subjects were divided according to the length of sexual abstinence into three groups: G2–3 (2–3 days), G4–5 (4–5 days) and G6–7 (6–7 days). For each parameter, the variation between the three abstinence period groups was analysed primarily by means of Kruskal–Wallis test. For each parameter where a statistically significant variation was found, pair-wise comparison was performed by Mann–Whitney U-test. No adjustment for seasonal variation in semen parameters was done. However, the sample deliveries and the length of the sexual abstinence period were evenly distributed throughout the study period. Finally, the proportion of men who fall below the WHO standards regarding semen volume, sperm concentration or percentage motile spermatozoa were compared between the different abstinence groups using Fisher’s test. Morphology data were not included in this analysis since the WHO manual does not define any reference range for this parameter. A P-value < 0.05 was considered as statistically significant.

Results

Statistically significant variation between the three abstinence period groups was found for the following parameters: sperm concentration, total sperm counts, rapidly progressive spermatozoa, total fraction of progressively motile spermatozoa, total number of motile spermatozoa, CASA motile, CASA immotile, straight line velocity (VSL), amplitude of lateral head displacement (ALH), linearity (LIN), percentage spermatozoa with tail defects, semen volume, total NAG activity and total amount of zinc. For the remaining parameters, the inter-group variation was not statistically significant. Pair-wise comparisons between G2–3, G4–5 and G6–7 gave the following results.

The time of abstinence and sperm parameters

Sperm concentration and total sperm count were significantly higher in G4–5 compared with G2–3 (P = 0.010 and P < 0.001, respectively) but were not different from G6–7. The percentage spermatozoa with tail defects was significantly higher in G6–7 when compared with G2–3 (P = 0.011) and G4–5 (P = 0.002) (Table I).

The percentage of rapidly progressive motile spermatozoa (a) was significantly lower in G6–7 compared with G2–3 (P = 0.006) and G4–5 (P = 0.001). The total percentage of progressively motile spermatozoa (a+b) was significantly higher in G4–5 compared with G2–3 (P = 0.010 and P < 0.001, respectively).
higher in G₄–₅ compared with G₂–₃ (P = 0.039) and G₆–₇ (P < 0.001), and lower in G₆–₇ compared with G₂–₃ (P = 0.038). Furthermore, the total number of motile sperm was significantly higher in G₄–₅ compared with G₂–₃ (P = 0.001). The CASA percentage motile spermatozoa was significantly higher in G₄–₅ compared with G₂–₃ (P = 0.008) and G₆–₇ (P = 0.011); the opposite trend was found regarding CASA percentage immotile spermatozoa (P = 0.008 and P = 0.026, respectively). Furthermore, the CASA motility characteristic VSL was significantly higher in G₂–₃ compared with G₆–₇ (P = 0.012). ALH was significantly higher in G₄–₅ compared with G₂–₃ (P = 0.009), whereas LIN was higher in G₂–₃ compared with G₄–₅ (P = 0.012) (results are summarized in Tables I and II).

The time of abstinence and seminal parameters

Seminal volume was significantly lower in G₂–₃ compared with G₄–₅ (P = 0.010) and G₆–₇ (P = 0.023), G₄–₅ and G₆–₇ not differing from each other (Table I). The total activity of NAG was significantly lower in G₂–₃ when compared with G₄–₅ (P < 0.001) and G₆–₇ (P = 0.001), the two latter groups not differing from each other. The same trend was found regarding amounts of zinc (P = 0.001 and P = 0.005, respectively) (Table III).

Finally, a significantly higher fraction of men with seminal volume, sperm concentration and percentage motile spermatozoa below the WHO reference range was found in G₂–₃ (84 out of 124; 68%) (P = 0.001) as compared with G₄–₅ (54 out of 75; 72%) (P = 0.001) as compared with G₄–₅ (103 out of 223; 46%). On the other hand, G₂–₃ and G₆–₇ did not differ from each other (P = 0.63).

Discussion

The main conclusion of our study is the finding that even within the abstinence period time interval recommended by the WHO semen manual (1999), subgroups defined by a more narrow time window differ significantly when regarding the percentage of motile sperm and sperm motility characteristics. Our data also indicate that the abstinence time covaries with epididymal and accessory sex gland secretions as well as the percentage of spermatozoa with tail defects.

Although in G₄–₅, as compared with the two other groups, a significantly higher proportion of men fulfilled the WHO criteria considering seminal volume, sperm concentration and percentage motile spermatozoa, the results of the current study do not allow us to pinpoint any subinterval within the range of 2–7 days as ideal from the sperm motility point of view, but rather to conclude that significant differences exist within this time frame.

Our finding of decreased sperm motility with a short abstinence period was in agreement with previous reports regarding men assessed for infertility (Mortimer et al., 1982) as well as normal men (Cooper et al., 1993). In the study of Cooper et al., where the subjects were asked for multiple ejaculations for depletion of extra-gonadal reserves prior to the actual period of abstinence, the short length of the abstinence period was associated with a lower level of NAG activity and amount of zinc, which seems in accordance with our finding (Cooper et al., 1993). Thus, it has been found that the final sperm function is dependent on epididymal transit where spermatozoa maturation occurs (Bedford, 1990; Amann et al., 1993). The transit time through the epididymis

### Table I. Semen volume, sperm concentration, total sperm count, manually assessed sperm motility and sperm morphology according to different sexual abstinence periods from 422 men assessed for infertility

<table>
<thead>
<tr>
<th>Variables</th>
<th>G₂–₃ (n = 124)</th>
<th>G₄–₅ (n = 223)</th>
<th>G₆–₇ (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>3.6 (1–13)</td>
<td>4 (1–13)</td>
<td>4 (1–10)</td>
</tr>
<tr>
<td>Sperm concentration (10⁶/ml)</td>
<td>28 (0.1–345)</td>
<td>47 (0.1–245)</td>
<td>38 (0.1–314)</td>
</tr>
<tr>
<td>Total sperm count (10⁹/jaculate)</td>
<td>110 (1–922)</td>
<td>188 (0.4–1051)</td>
<td>162 (0.4–1258)</td>
</tr>
<tr>
<td>a (%)</td>
<td>16 (0–70)</td>
<td>16 (0–79)</td>
<td>8 (0–69)</td>
</tr>
<tr>
<td>a + b (%)</td>
<td>47 (2–88)</td>
<td>55 (0–93)</td>
<td>42 (0–85)</td>
</tr>
<tr>
<td>b (%)</td>
<td>27 (2–61)</td>
<td>29 (0–66)</td>
<td>24 (0–77)</td>
</tr>
<tr>
<td>c (%)</td>
<td>14 (0–36)</td>
<td>14 (0–44)</td>
<td>13 (0–46)</td>
</tr>
<tr>
<td>d (%)</td>
<td>37 (0–85)</td>
<td>30 (1–100)</td>
<td>40 (0–100)</td>
</tr>
<tr>
<td>Total motile sperm (10⁶/jaculate)</td>
<td>53 (0.04–10)</td>
<td>118 (0–746)</td>
<td>74 (0–943)</td>
</tr>
<tr>
<td>Normal form (%)</td>
<td>6 (0–15)</td>
<td>5 (0–19)</td>
<td>4 (0–16)</td>
</tr>
<tr>
<td>Tail defect (%)</td>
<td>10 (2–47)</td>
<td>10 (2–51)</td>
<td>14 (6–44)</td>
</tr>
</tbody>
</table>

Values are given as median (range). Statistical analysis was done using the Mann–Whitney test. Values with the same sign in the superscripts are statistically different (*, †, ‡ P < 0.05).

### Table II. Computer-aided sperm motility (CASA) according to different sexual abstinence periods from 375 men assessed for infertility

<table>
<thead>
<tr>
<th>Variables</th>
<th>G₂–₃ (n = 111)</th>
<th>G₄–₅ (n = 201)</th>
<th>G₆–₇ (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motile sperm (%)</td>
<td>27 (0–71)</td>
<td>34 (0–86)</td>
<td>20 (0–84)</td>
</tr>
<tr>
<td>Locally motile sperm (%)</td>
<td>28 (7–88)</td>
<td>27 (4–53)</td>
<td>26 (0–53)</td>
</tr>
<tr>
<td>Immotile sperm (%)</td>
<td>45 (2–88)</td>
<td>33 (1–95)</td>
<td>51 (1–100)</td>
</tr>
<tr>
<td>VCL (µm/s)</td>
<td>59 (0–93)</td>
<td>63 (0–95)</td>
<td>61 (0–89)</td>
</tr>
<tr>
<td>VSL (µm/s)</td>
<td>25 (0–44)</td>
<td>23 (0–40)</td>
<td>22 (0–39)</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>38 (0–55)</td>
<td>39 (0–54)</td>
<td>37 (0–58)</td>
</tr>
<tr>
<td>ALH (µm)</td>
<td>1.7 (0–2)</td>
<td>1.8 (0–3)</td>
<td>1.8 (0–3)</td>
</tr>
<tr>
<td>LIN</td>
<td>42 (0–79)</td>
<td>38 (0–84)</td>
<td>39 (0–73)</td>
</tr>
</tbody>
</table>

Values are given as median (range). Statistical analysis was done using the Mann–Whitney test. Values with the same sign in the superscripts are statistically different (*, †, ‡ P < 0.05).

ALH = amplitude of lateral head displacement; LIN = linearity; VAP = average path velocity; VCL = curvilinear velocity; VSL = straight line velocity.
can be influenced by external factors such as sexual stimulus and ejaculatory frequency, inducing a sharp rise in intraluminal pressure, as a consequence of which the passage of spermatozoa is speeded up (Amir and Ortavant, 1968; Tischner, 1972) which might influence the maturity of spermatozoa. NAG is excreted mostly by the cauda epididymidis, and was found to correlate positively with the motility of spermatozoa (Viljoen et al., 1972) which might influence the maturity of spermatozoa. Its exact physiological role is not yet known; however, it might be important to provide the spermatozoa with optimal levels of energy (Tremblay et al., 2002). Recent studies have concluded that some of the CASA parameters, including VCL, VSL, (VAP), ALH and LIN provide a reliable estimation of the fertilizing ability of human spermatozoa both in vivo and in vitro (Donnelly et al., 1998; Larsen et al., 2000; Hirano et al., 2001). Our study demonstrated higher ALH values in semen samples obtained after 4–5 days of abstinence as well as higher VSL and LIN values in samples obtained after 2–3 days, pointing to a possible association between the length of the abstinence period and not only the percentage of motile spermatozoa but also the qualitative characteristics of their movements.

Our study has some limitations, which need to be pointed out. It was not based on the background male population but on patients referred due to infertility problems of the couple. Therefore, our results may not be representative for men in the general population but, on the other hand, the cohort selected by us is probably representative for the type of men delivering semen samples as a part of infertility work-up. This study has a cross-sectional design. Thus, a potential confounding factor which, therefore, cannot be excluded is the possibility that the subgroups with differing times of abstinence are not equal regarding the general status of their reproductive function. We did have access to hormone levels on patients referred due to infertility problems of the couple. Therefore, our results may not be representative for men in the general population but, on the other hand, the cohort selected by us is probably representative for the type of men delivering semen samples as a part of infertility work-up. This study has a cross-sectional design. Thus, a potential confounding factor which, therefore, cannot be excluded is the possibility that the subgroups with differing times of abstinence are not equal regarding the general status of their reproductive function. We did have access to hormone levels from ~25% of all subjects. Although this subgroup was not representative for the whole cohort, at least with regard to sperm concentration and motility, among those 107 men we could not find any correlation between their sexual hormone levels, the length of abstinence period or even age.

An additional factor which might affect ejaculate quality, i.e. the duration of pre-ejaculatory sexual arousal, was not estimated in our study. It has been found that the time taken to produce a specimen was positively correlated with sperm concentration but not with ejaculate volume (Pound et al., 2002).

In conclusion, our study demonstrated that among men delivering a semen sample as a part of an infertility investigation, significant differences in the number and percentage of motile sperm as well as in the motility characteristics exist, depending on whether the period of abstinence is 2–3,
4–5 or 6–7 days. This effect may be at least partly mediated through variation in secretions from the epididymis and prostatic gland as well as the morphology of the spermatozoa, particularly the percentage of tail defects.

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