Time to pregnancy in relation to semen quality assessed by CASA before and after sperm separation

A.G. Andersen1,2,4, S. Ziebe1, N. Jørgensen2, J. H. Petersen2,3, N. E. Skakkebæk2 and A. Nyboe Andersen1

1 The Fertility Clinic, 2 Department of Growth and Reproduction, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen Ø and 3 Department of Biostatistics, University of Copenhagen, Blegdamsvej 3, DK-2100 Copenhagen N, Denmark

4 To whom correspondence should be addressed at: Department of Growth and Reproduction, Copenhagen University Hospital, Section GR-5064, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. E-mail: agma@get2net.dk

BACKGROUND: In order to provide a reference for infertile men, we defined normal values of semen quality in a population of fertile men, using computer-assisted semen analysis (CASA) before and after sperm separation. Additionally, we investigated the relationship between semen quality and time to pregnancy (TTP).

METHODS AND RESULTS: Semen samples were obtained from 315 proven fertile men. The median sperm concentration in fresh samples was $10^7 / c9061 \times 10^6 / ml$ (5–95 percentiles: $16–322 / c9061 \times 10^6 / ml$), the median percentage of motile sperm cells was 65% (14–87%) and the median percentage of progressively motile cells was 37% (5–64%). After density gradient sperm separation, the median total sperm count was $46 / c9061 \times 10^6$ (4–350 / c9061 \times 10^6), the median percentage of motile sperm cells was 77% (16–95%) and the median percentage of progressively motile cells was 63% (11–84%). No significant associations were found between TTP and sperm counts or sperm motility, either before or after sperm separation. This may be due in part to the fact that the study comprised couples with proven fertility.

CONCLUSION: We have defined semen parameters, including the results of density gradient separation, in a population of normal fertile men which may be of interest in the evaluation of semen samples from infertile men.

Key words: computer-assisted semen analysis/density gradients/fertile men/time to pregnancy

Introduction

The predictive value of semen analysis after density gradient separation in relation to fertilization rates has been assessed in several studies using IVF procedures (Yue et al., 1995; Parinaud et al., 1996; Sukcharoen et al., 1996; Hammadeh et al., 1997; Prakash et al., 1998). Assessment of sperm quality after density gradient separation has also been shown to predict the pregnancy rates after intrauterine insemination (IUI) (Swanson et al., 1995; Kelly et al., 1997). So far, studies describing semen quality after density gradient separation have all been conducted in subfertile or infertile populations undergoing treatment, while normal fertile males have never been the targets of such studies. The World Health Organization (WHO) recommends that in order to determine normal ranges for semen variables ‘...specimens should be evaluated from men who have recently achieved a pregnancy, preferably within 12 months of the couple ceasing contraception...' (World Health Organization, 1992). To our knowledge, no such normal values for sperm counts after density gradient separation exist, although many fertility clinics, at least in Scandinavia, assess sperm quality primarily by the results of sperm preparation techniques.

In many studies it has been shown that in an assisted reproduction programme, the information obtained from computer-assisted semen analysis (CASA) has a predictive value in relation to oocyte fertilization (Aumann et al., 1989; Liu et al., 1991; Sukcharoen et al., 1996). Moreover, a predictive value of CASA has been found in relation to pregnancy rates after IVF (Paston et al., 1994; Donnelly et al., 1998), and IUI (Irvine et al., 1994; Macleod & Irvine, 1995). However, as is the case for density gradient separation, most previous studies have been based on couples attending fertility clinics, while the literature gives very little information on CASA of semen samples from known fertile men.

The primary objective of the present study was to define normal values of semen quality in fertile men, as assessed by the Hamilton-Thorn Motility Analyzer before and after density gradient separation, in order to provide a reference for studies of infertile men. Time to pregnancy (TTP) is believed to be a sensitive measure of fecundity (Joffe, 1996). Therefore we also investigated the association between TTP and the outcome of CASA, and tested whether density gradient separation affected the predictive value.

Material and methods

Patients

From September 1996 to September 1997 male partners of pregnant women were recruited consecutively from the antenatal clinic at...
data analysis

For the fresh semen samples, the data on sperm concentration were logarithmically transformed, the data on total motility could not be transformed to fit the normal distribution, while the data on progressive motility were approximately normally distributed. After density gradient separation, data on concentration were logarithmically transformed, while data on total motility and progressive motility could not be fitted to the normal distribution.

The TTP data were analysed as a grouped version of a Cox-model (Farhein and Tutz, 1994). The model is estimated as a generalized linear model with a binomial distribution and a complementary log-log link function. Parameters are relative risk parameters with the usual interpretation known from the Cox-model. P-values and confidence intervals are based on the likelihood function. To avoid an effect of a change in behaviour after a long time of unsuccessful attempts, the TTP’s were censored at 13 cycles.

Differences in semen volume, duration of abstinence, and TTP between subgroups of men were tested by use of a non-parametric test (Mann–Whitney).

Statistical calculations were made using the statistical packages SPSS, release 8.0 and SAS, release 6.12 (SPSS A/S, Holte, Denmark).

Results

The male participants had a mean age in years of 31.6 ± 5.6 (SD), while the mean age of the pregnant women was 29.9 ± 3.6 years.

In Table I, sperm counts and motility parameters are given for the whole group of men (Table Ia), and for the two subgroups of men who had a duration of abstinence above and below 48 h, respectively (Table Ib,c). This distinction is made because men with a duration of abstinence >48 h had significantly better basal sperm concentration ($P < 0.025$) and better total sperm count after density gradient separation ($P = 0.032$) than those who had <48 h of abstinence. The duration of abstinence had no influence on the motility parameters.

After density gradient separation, the median total sperm count was $46 \times 10^6$ (5–95 percentiles: 4–350 $\times 10^6$), the median percentage of motile sperm cells was 77% (16–95%) and the median percentage of progressively motile cells was 63% (11–84%).

The distributions of sperm concentrations and total sperm counts in fresh samples (Figure 1a) and of total sperm counts after density gradient separation (Figure 1b) are shown for all men ($n = 315$). The participants were all fertile men, although some of them had very low sperm counts. To examine the lower normal values in more detail, the subgroup of men with total sperm counts $<40 \times 10^6$ after density gradient sperm separation was further subdivided. Altogether, 19 of the men (6%) had $<5 \times 10^6$ sperm after sperm separation, two men (0.6%) had $<2 \times 10^6$, and one man (0.3%) had $<1 \times 10^6$. The subgroup of men with very low sperm counts after density gradient separation ($<5 \times 10^6$) had significantly shorter durations of abstinence (58.9 versus 84.1 h; $P = 0.041$) and a significantly smaller semen volume (2.8 versus 4.0 ml; $P = 0.004$) than those with total sperm counts after sperm separation $>5 \times 10^6$.

Of the adjustment factors, the age of the woman was significantly associated with TTP. Women younger than 27 years had a significantly shorter TTP than women older than
Table I. Sperm counts and motility assessments among all men, and in the two subgroups of men with duration of abstinence above or below 48 h, respectively. For each parameter, the first line is the mean value with the standard deviation in parentheses, and the second line is the median value with the 5–95 percentiles in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fresh sample</th>
<th>After sperm separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) All men (n = 315)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (×10^6/ml)</td>
<td>139 (125)</td>
<td>107 (16–322)</td>
</tr>
<tr>
<td>Total sperm count (×10^8)</td>
<td>525 (483)</td>
<td>384 (56–1476)</td>
</tr>
<tr>
<td>Motile sperm cells (%)</td>
<td>59 (23)</td>
<td>65 (14–87)</td>
</tr>
<tr>
<td>Progressively motile sperm cells (%)</td>
<td>36 (18)</td>
<td>65 (14–87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 (5–64)</td>
</tr>
<tr>
<td>(b) Abstinence ≥48 h (n = 248)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (×10^6/ml)</td>
<td>144 (126)</td>
<td>113 (16–309)</td>
</tr>
<tr>
<td>Total sperm count (×10^8)</td>
<td>562 (503)</td>
<td>418 (57–1539)</td>
</tr>
<tr>
<td>Motile sperm cells (%)</td>
<td>60 (22)</td>
<td>66 (17–87)</td>
</tr>
<tr>
<td>Progressively motile sperm cells (%)</td>
<td>37 (18)</td>
<td>38 (7–66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 (5–64)</td>
</tr>
<tr>
<td>(c) Abstinence &lt;48 h (n = 67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (×10^6/ml)</td>
<td>110 (99)</td>
<td>94 (14–343)</td>
</tr>
<tr>
<td>Total sperm count (×10^8)</td>
<td>339 (274)</td>
<td>281 (34–910)</td>
</tr>
<tr>
<td>Motile sperm cells (%)</td>
<td>53 (27)</td>
<td>63 (5–88)</td>
</tr>
<tr>
<td>Progressively motile sperm cells (%)</td>
<td>32 (19)</td>
<td>37 (2–65)</td>
</tr>
</tbody>
</table>

\(a,b P < 0.025.\)
\(c,d P = 0.032.\)

33 years [relative risk (RR) = 1.82; 95% confidence interval (CI) = 1.10–1.65; \(P = 0.017\)], and also women aged 27–30 years had shorter TTP than women older than 33 years (RR = 1.97; 95% CI = 1.27–1.56; \(P = 0.002\)). For the women aged 30–33 years, a non-significant tendency was seen towards a shorter TTP than for women older than 33 years (RR = 1.47; 95% CI = 0.95–1.56). Concerning cycle regularity, the presence of irregular menstrual cycles was significantly associated with increased TTP (RR = 0.50; 95% CI = 0.29–0.81; \(P < 0.01\)). A total of 30% of the women reported having suffered from a genital disease or some other pathology (pelvic infection, infection in the Fallopian tubes, cysts in the ovaries, chlamydia infection, gonorrhoea, vaginal discharge or fungal disease, endometriosis, thyroid disease, diabetes, fibroma in the uterus, appendicitis that ruptured) and this was tested in relation to TTP, but no significant effect was found (RR = 1.11; 95% CI = 0.82–1.49). Half of the men (n = 160) had previously been responsible for a pregnancy, with either the current or a previous partner, and 54% of the women had previously been pregnant. The effect of previous pregnancies on TTP was tested, but no significant effect was found for men (RR = 1.24; 95% CI = 0.93–1.66) or women (RR = 1.19; 95% CI = 0.89–1.59). Similarly, no significant impact on TTP was found from the women’s smoking habits (RR = 0.78; 95% CI = 0.58–1.04) or the frequency of sexual intercourse (RR = 1.09; 95% CI = 0.70–1.76).

No significant associations were found between TTP and any of the semen parameters. In Figure 2, scatter plots of TTP versus sperm concentration in fresh semen samples (Figure 2a), and TTP versus total sperm count after density gradient separation (Figure 2b) are given. Note that the Y-axes are cut off at a TTP of 50 months, while the X-axis in Figure 2a is cut off at a sperm concentration of 400×10^6/ml, and in Figure 2b the X-axis is cut off at a total sperm count of

---

Figure 1. Distribution of sperm counts in (a) fresh semen samples and (b) after density gradient sperm separation.
negative attitude from his wife/partner—due to uncertainty about the fatherhood—a given man would probably not wish to participate. Secondly, the participants had generally experienced a short TTP (median 2 months), and the suspicion of a different biological father is more obvious if a couple has been trying for a very long time and suddenly succeeds.

Some of the fertile couples rapidly achieved a pregnancy (0–3 months) in spite of very low sperm counts after density gradient sperm separation \(<2\times10^6\). This finding is in contrast with most studies of pregnancy rates after IUI, where conceptions rarely occurred when the women were inseminated with \(<2\times10^6\) motile sperm cells (Cruz et al., 1986; Byrd et al., 1987; Horvath et al., 1989; Stone et al., 1999). However, in accordance with the present study, a few other studies have reported the achievement of pregnancies in IUI-programmes after insemination of \(<1\times10^6\) sperm (Centola, 1997; Keck et al., 1998). Also, in studies of different populations of men, pregnancies have been reported with very low sperm counts (Horvath et al., 1989; Ombelet et al., 1997). Thus, underlining the importance of the female factor, it seems that as long as sperm are present there is no definitive threshold below which pregnancy is impossible.

The predictive value of the CASA results has been confirmed in relation to both oocyte fertilization (Liu et al., 1991; Macleod and Irvine, 1995) and pregnancy rates after IVF (Donnelly et al., 1998) and IUI (Irvine et al., 1994). Thus, it was of interest to test the association between the outcome of CASA and TTP. Surprisingly, no significant associations were found between TTP and sperm counts or motility parameters, neither in the fresh samples nor after density gradient separation. In contrast, in a recent study of couples of unknown fertility, the probability of conception increased with increasing sperm counts up to a sperm concentration of \(40\times10^6\) sperm (Bonde et al., 1998). However, the latter study was conducted in a population very different from the present, in that it comprised the full spectrum of fertility, including couples who did not conceive within the study period. Thus, our finding of lack of correlation between TTP and semen parameters among fertile men does not exclude the possibility that such correlations may exist in other populations of men, including men from the general population. Moreover, it is possible that the lack of association between TTP and semen parameters is to some extent due to the different technologies used in the present study. Slama et al. (2002) investigated a large population of proven fertile European men \((n = 942)\) using manual semen analysis only (Slama et al., 2002), and a significant association was found between the logarithm of the sperm concentration in the fresh semen samples and TTP \((RR = 1.09; 95\% CI = 1.00–1.19)\). When entering the logarithm of the sperm concentration before sperm separation in our study, we found \(RR = 0.98; 95\% CI = 0.84–1.15\). Slama’s results, being well within our confidence interval and thus consistent with our results, mean that a small effect cannot be ruled out.

Finally, it should be noted that all semen samples in the present study were also examined by conventional analysis in another laboratory, using a Bürkert-Türk counting chamber and otherwise evaluating the semen samples manually according to WHO (World Health Organization, 1992). The median

Discussion

We found a median sperm concentration in fresh semen samples of \(107\times10^6/ml\), and after density gradient separation the median total sperm count was \(46\times10^6\).

Surprisingly, 6% of the fertile men had a total sperm count after density gradient separation of \(<5\times10^6\). This subgroup of men had a short duration of abstinence and small semen volumes, and might not be representative of the general population of fertile men. The present population of fertile men might not be as motivated as other recommendations concerning a period of abstinence as infertile men, who deliver a semen sample as a part of infertility treatment. But also the well-known intra-individual variability in sperm counts possibly contributes to the finding of some men with low sperm counts in this fertile population.

The possibility that some of the men in the present study were not the biological fathers of their spouses’ foetuses cannot be excluded, although we do not find this explanation plausible. Firstly, participation required active signing in, and with a

---

**Figure 2.** Scatter plots and corresponding regression lines of (a) time to pregnancy versus sperm concentration in fresh semen samples and (b) time to pregnancy versus total sperm count after density gradient sperm separation

\(500\times10^6\). These cut-offs are made for illustrative reasons only, while all data were included in the analysis.
sperm concentration in the fresh semen samples was higher by use of CASA than by the conventional analysis (107 versus $61 \times 10^6$/ml), and this finding is in accordance with previous studies, which have also shown that CASA by use of the Hamilton-Thorn system provides higher sperm counts than manual analysis. Thus Macleod et al. found that among 1435 semen samples, the sperm concentration measured by the Hamilton-Thorn system was significantly higher than the concentration measured by manual analysis (Macleod et al., 1994). Additionally, Centola compared manual analysis with two different CASA systems and found that the Hamilton-Thorn system measured higher sperm counts than both manual analysis and the other CASA system (Centola, 1996). This would naturally also affect the results of density gradient separation, and the present results cannot be directly related to manual analysis.

In conclusion, we have described sperm counts and motility parameters, as measured by CASA, before and after density gradient sperm separation in a population of fertile men. In the present population large individual differences were found, but still these two methods may be of predictive value in subfertile populations. Our finding of no significant association between TTP and semen parameters in the present population does not exclude that such correlations may exist in other populations of men.

References


Received on July 6, 2001; accepted on September 18, 2001

Time to pregnancy and semen quality by CASA