Part I: The practice of sperm donation today

Semen characteristics and fertility tests required for storage of spermatozoa

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This review addresses critical issues in the selection of semen donors who are very fertile. Traditional semen parameters have been employed and are still used to assess pre- and post-freeze samples in order to discriminate between donors of high and low fecundity. The most predictive factor is the number of motile spermatozoa per straw and the number of motile spermatozoa inseminated. Nevertheless, no absolute standards for fertile samples can be derived from the basic semen examination. The employment of sperm function testing such as the hamster penetration test or computerized motility analysis has been shown to enhance moderately the prediction of fertility of semen samples however, further studies are necessary to determine if these improvements are clinically useful. The need to determine with a high degree of confidence the fecundity of donor semen is enhanced by limitations in the number of pregnancies allowed per donor. The recent publication of league tables in the UK has put extra pressure in clinics to use highly fertile donors. Spermatozoa are also cryostored for patients prior to cancer treatment. With the development of intracytoplasmic sperm injection every sample produced by cancer patients can be stored irrespective of the quality. However, several factors need to be elucidated to maximize the fertility of those patients. The establishment of regional centres in Europe will be a good starting point to deal with many of the issues raised in this review.

Key words: cancer patients/donor insemination/sperm cryostorage/sperm function testing

Introduction

The recruitment and selection of semen donors is a problematic procedure (Barratt and Cooke, 1993; Barratt, 1995; Craig et al., 1997). Only relatively few donors are recruited compared with those screened (Barratt, 1996). It is therefore critical that each procedure in the recruitment process is optimized. The aim of this manuscript is to determine if we can predict which semen donors are likely to be fertile compared with those who are likely to achieve poor success rates. Unfortunately, we have only limited data on several issues. For example, we still do not know what is the optimum number of motile spermatozoa to inseminate to achieve good pregnancy rates (10% live birth rate per cycle). In addition, despite the widespread use of putative sperm function tests, including computer-assisted semen analysis (CASA), there is only a limited amount of data to support their use in a donor insemination (DI) programme (European Society for Human Reproduction and Embryology, 1996). Semen banks often use different techniques to screen semen samples and different freezing protocols, making comparisons between studies difficult. In addition, we will discuss the semen characteristics of men who are referred for semen storage prior to treatment for cancer.
Relationship between traditional semen parameters and conception in intracervical and intrauterine insemination

The predictive value of the traditional semen analysis parameters, these being either pre or post thaw, in the context of a donor insemination programme has been addressed in a significant number of studies. A study conducted by the French CECOS Federation examined a large number of DI cycles ($n = 15364$; 1438 ejaculates) using intracervical insemination (ICI) (Mayaux et al., 1985). Using multivariate analysis they showed that the most predictive variable for conception was the post-thaw motility of the sample ($P < 10^{-7}$). Once post-thaw motility was considered, neither pre-thaw motility nor sperm concentration or morphology had any influence on the success rate.

Numerous studies indicate that the number of motile spermatozoa inseminated (NMSI) is one of the most important factor for DI success using either ICI or intrauterine insemination (IUI). However, NMSI was not described in the analysis in the above-mentioned study (Mayaux et al., 1985).

Byrd et al. (1990) found that optimal pregnancy rates are achieved by IUI if the NMSI is $6-15 \times 10^6/ml$ while a higher NMSI is needed for optimal pregnancy rates with ICI ($50-100 \times 10^6/ml$). The only other important factor that influenced the outcome of IUI was the post-thaw survival. If $<30\%$ of the spermatozoa retained their motility post-thaw, the pregnancy rate with IUI was 5.5\%, in contrast to post-thaw motilities of 30–50\% for which the pregnancy rate was 15.4\%. If the post-thaw motility of a sample was $>50\%$, the pregnancy rate almost double (27.2\%).

In a recent prospective study conducted by the CECOS Federation, the fecundity of cryopreserved semen was found to be closely related to the number of motile spermatozoa per straw (NMSS) when ICI was used and to the NMSI when either ICI, IUI or in-vitro fertilization (IVF) was used (Le Lannou et al., 1995). In the case of ICI higher pregnancy rates were observed when two inseminations were performed per cycle either on the same day or at 24 h interval (12.3 versus 7\% for one insemination). In these cycles two straws were inseminated and the conception rate increased according to the NMSS.

The conception rate for ICI rose from 9.1\% when the NMSS was $<4 \times 10^6$ to 13.8\% for NMSS of $4-8 \times 10^6$ and 17.2\% with NMSS $>8 \times 10^6$ ($P < 0.001$ between the higher and the lower NMSS). There was no relationship between NMSS and IUI or IVF. Pregnancies were achieved with IUI even when the NMSI dropped to $<0.5 \times 10^6$ spermatozoa. However, the optimum NMSI was set at $1.5–25 \times 10^6$ spermatozoa as both a higher and a lower number of spermatozoa decreased the conception rate ($P < 0.02$). It should be noted that overall, significantly higher pregnancy rates were achieved when IUI was performed (15\%) than when ICI was the treatment of choice (9.5\%; $P < 0.01$). Both of these relatively non-invasive techniques yielded lower pregnancy rates than IVF (18.3\%).

Comparable data were presented by Hurd et al. (1993) who collated data from a prospective trial for DI using either ICI, IUI or a combination of intratubal and intrauterine insemination (ITI/IUI). They demonstrated that although similar numbers of motile spermatozoa were inseminated in all three methods (ICI: $26 \pm 13 \times 10^6$; IUI: $27 \pm 14 \times 10^6$; ITI/IUI: $25 \pm 15 \times 10^6$) the pregnancy rate per cycle was significantly higher for IUI (18.6\%; $P = 0.02$) than ICI (3.8\%) or ITI/IUI (7.3\%).

Notably, higher numbers of motile spermatozoa are used for insemination either with IUI or ICI in the American studies in comparison with the French. This was attributed to differences in the method used for sperm cryopreservation (specifically 0.25 ml straws for the former and vials of $\geq 1$ ml for the latter).

However, it is obvious that although data regarding the basic semen parameters, those being pre- or post-freeze, can predict to an extent the success of donor insemination, it is a relatively insensitive method as a basis for donor selection. It would be sensible however, to only use donors with a reasonable number of motile spermatozoa post-freeze. Using the CECOS data, this figure would be $>4 \times 10^6$ motile spermatozoa per 0.25 ml straw. One important question to address is ‘will the use of sperm function testing in the selection of donors improve pregnancy rates?’

What is the role of sperm function tests?

There are some data to support the use of sperm function tests (hamster oocyte fusion, acrosome...
reaction, quantitative motility) in the selection of donors for a donor insemination programme (Richardson and Aitken, 1993). For example, Irvine and Aitken (1986) demonstrated that a combination of data describing movement characteristics of human spermatozoa and their capacity for sperm-oocyte fusion (using the hamster test) was shown to predict the fertility of cryostored samples with >80% accuracy. However, there is a paucity of data on sperm function testing related to success of donor ejaculates using in-vivo conception as the outcome. The vast majority of data is related to in-vitro fertilizing capacity and as such may not be as relevant to the in-vivo situation. An overall examination of the limited data would support the use of selected sperm function tests (e.g. acrosome reaction ability and sperm-oocyte/zona binding) to eliminate some donors who may have good characteristics but may be defective in one or more aspects of sperm function. Bearing in mind the time required and expense to perform sperm function testing along with the paucity of data on their clinical benefit it is inappropriate to perform this on every semen sample. It would be prudent however to examine one or two samples of each donor if they had above the minimum number of motile spermatozoa acceptable. Richardson and Aitken (1993) have, on several occasions, stated the case for regional sperm banks in the UK where advanced testing of semen would be performed. Whilst this approach is sensible and practical, market forces preclude the concept as a reality at least in the near future.

**Computer-assisted semen analysis**

Based only on scientific evidence i.e. a positive effect on pregnancy rates, it would be difficult to justify the routine use of CASA in a DI programme. However, there is considerable proof to support the predictive value of CASA motion measurements for in-vivo fertility. One of the most important features is the concentration of motile cells. Many studies, using CASA, repeatedly find this to be the most important parameter for predicting success (Barratt et al., 1993; Tomlinson et al., 1993; Krause, 1995). Therefore it would be acceptable to use CASA for the recruitment and selection of donors. The implementation of CASA is a difficult and traumatic procedure in the andrology laboratory (Clements et al., 1995). In our laboratory we have been using the Hamilton Thorn (HTM) for 3 years to screen semen samples for DI. We believe we have addressed many of the issues of machine performance (Clements et al., 1995). Critical use of the HTM has allowed us to improve our quality control of donor samples and provided us with quantitative data to manage our programme, i.e. we have adopted a screen of a minimum post-thaw motile concentration per straw (0.25 ml) of $2.5 \times 10^6$. Under this value we do not accept samples. Using this regime we have a consistent pregnancy rate in our clinic of >14% per cycle. However, despite these criteria we still observe a highly noticeable difference in donor fecundity (Figure 1). In our own clinic, data obtained from CASA of either fresh or post-thaw samples is of limited value in predicting the potential fecundity of a donor, or even of selected samples used for insemination. Above the threshold of semen characteristics prescribed in our donor selection programme there is little to relate increasing concentration, motility or cryopreservation success to increasing fecundity (Table I). Although, for example, the mean percentage of motility (whether fresh or post-thaw, progressive or total) is significantly related to fecundity, such parameters are of little value in prediction for an individual donor.
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Table I. Characteristics of 33 donors which were used in donor insemination treatment. Each donor provided at least 15 semen samples and donors were used in at least 15 treatment cycles.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration F (x 10^6/ml)</td>
<td>144</td>
<td>73–241</td>
</tr>
<tr>
<td>Motile concentration F (x 10^6/ml)</td>
<td>120</td>
<td>49–211</td>
</tr>
<tr>
<td>Motile concentration PT (x 10^6/ml)</td>
<td>27</td>
<td>12–59</td>
</tr>
<tr>
<td>Motility F (%)</td>
<td>81</td>
<td>65–95</td>
</tr>
<tr>
<td>Motility PT (%)</td>
<td>35</td>
<td>21–69</td>
</tr>
<tr>
<td>Progressive motility F (%)</td>
<td>74</td>
<td>54–92</td>
</tr>
<tr>
<td>Progressive motility PT (%)</td>
<td>28</td>
<td>16–56</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>41</td>
<td>29–63</td>
</tr>
<tr>
<td>Fecundity (%)</td>
<td>12</td>
<td>3–38</td>
</tr>
</tbody>
</table>

F = fresh; PT = post-thaw; motility defined as >0 μm/s; progressive motility defined as ≥10 μm/s.

success. Perhaps the wide inter-sample variation shown by donors helps to explain why this is so.

In a very recent study the use of CASA (HTM) was compared with manual semen parameters for predicting successful and unsuccessful ejaculates in a DI programme (Macleod and Irvine, 1995). There was a significant increase in predictive value when using a HTM compared with manual analysis (overall correct classification, 85 versus 78%). Motility parameters [amplitude of lateral head displacement (ALH) and average path velocity (VAP)] and morphometry parameters were significantly related to conception. However, it was not possible to derive clinically useful threshold values for prediction of success using CASA. It is likely that CASA will be shown to be useful in the management of donors but further data is required if clinical decisions based on CASA parameters are to be used.

How many samples should we examine before we accept/reject a donor?

The large inter-sample variation shown by donors suggests that it might be reasonable to analyse several samples before acceptance on the basis of semen characteristics. However, in our own experience recalling selected potential donors (i.e. those whose semen analysis placed them above World Health Organization (WHO) normal limits but below the threshold of acceptance for donors, or those who were acceptable on fresh but not post-thaw criteria) was of dubious value, providing a gain of 14% (6/44) in donors at the expense of re-screening 26% (87/332) of potential donors. Selection on the basis of one semen analysis and test-freeze was therefore 86% efficient in selecting donors compared to using two. As a consequence, in our clinic if a potential donor produces a sample below the acceptance criteria then no repeat sample is required.

Number of samples stored

In the UK this is a critical issue. Under the Human Fertilisation and Embryology Authority regulations (HFEA, 1990), only 10 live births are allowed per semen donor. This figure may only be exceeded under exceptional circumstances. Therefore, with a constraint such as this, it is essential that only a limited number of semen samples are stored. In our clinic we store a maximum of 300 0.25 ml straws which represents on average 20 ejaculates. As our donors donate between one and three times per week (Table II) a donor only donates on average for 10 weeks. Obviously the number of ejaculates being stored for 10 live births depends on the live birth rate in the clinics where the ejaculates are used. Low live birth rates mean that more ejaculates are needed and consequently the turnover of donors (i.e. the rate of recruitment) is a lot slower. In contrast, high success rates mean that donor recruitment needs to be very efficient (a Red Queen situation). A similar if not stricter guideline regarding the number of live births resulting from the gametes of a single donor has

Table II. Calculation of the approximate number of donor ejaculates required for 10 live births using various live birth rates.

<table>
<thead>
<tr>
<th>Live birth rate</th>
<th>Straws per live birth</th>
<th>No. ejaculates</th>
<th>No. weekly donations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>80</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

*Two ejaculates per week.

Assumption: four straws (0.25 ml) per cycle and 16 straws per ejaculate.
been established in France (Lansac, 1996) where the number has been limited to five. Therefore, if the issue of restriction of the number of live births per donor in Europe is to be considered then each sperm bank needs to be aware that this will limit the number of ejaculates stored and therefore have major implications for donor recruitment.

**Issues of league tables**

In the UK individual centre success rates (pregnancy and live birth rates) are published. A league table has been developed by the accumulation of success rate data from individual clinics. Despite the existing argument regarding the correctness and value of the published document (Deech, 1996; Jacobs and Abdalla, 1996) it will in theory enable the patient to choose the centre which they perceive to have the best success rates. It is therefore critical to each centre to maximize the pregnancy rates in order to remain competitive (in the UK, IVF and DI are mainly in the private sector). It is likely that this will place greater pressure on the selection of donors, i.e. the most fertile ones will be the most frequently used (until they have had 10 live births). In such an atmosphere it is tempting to select donors who have the greatest number of motile cells and inseminate patients several times with excessive numbers of spermatozoa in the mistaken belief that more must be better. Several studies have proven that this is not the case. In a donor insemination programme no differences in pregnancy rate per cycle was observed when women were inseminated twice around the time of ovulation with either one or two 0.25 ml straws; each straw containing a minimum of $2.7 \times 10^6$ progressively motile spermatozoa (Corrigan et al., 1994). Interestingly, the number of motile spermatozoa post-thaw needed to achieve a pregnancy in the aforementioned study is comparable to the optimal number quoted for successful artificial insemination by husband (AIH). Campana et al. (1996) looked at factors affecting the outcome of IUI using washed husband’s semen and showed that there is no difference in the pregnancy rate per cycle if $1-5 \times 10^6$ or $>1 \times 10^6$ motile spermatozoa are inseminated. The pregnancy rate however was significantly reduced if $<0.5 \times 10^6$ spermatozoa (1.8% per cycle) were inseminated in comparison with $>1-5 \times 10^6$ (7.1%). The lower cut-off point for NMSI in order to achieve a pregnancy with IUI using fresh prepared partners sample was set to $1 \times 10^6$ by a study of similar nature (Horvarth et al., 1989). This may signify that once spermatozoa survive the freezing/thawing process they are equally capable of fertilization with their fresh counterparts.

However, the truth is we do not have, and never will have, absolute thresholds for motile sperm concentrations and subsequent pregnancy rates. Nevertheless, the above studies re-enforce our belief that a reasonable number of motile spermatozoa ($5 \times 10^6$) should be inseminated per treatment with two treatments per cycle carried out. No doubt other clinics achieve comparable success rates with a lower threshold. We do not know if using sperm function testing (e.g. acrosome reaction test) would significantly improve our success rates. The problem is that to test these ideas often requires using hundreds of cycles and at present many clinics are not able to maintain a consistent treatment regime over the number of years required to perform these trials. In any such trial the end point must be fecundity.

**Storage of patient samples**

There is a paucity of data on this subject. One problem we encounter in the storage of samples for patients with cancer is the time constraints. We often have only 1 or 2 days to organize everything. Education of cancer specialists is therefore an important objective.

It is encouraging that following treatment of such patients, pregnancies have been reported after IVF or zygote intra-Fallopian transfer (ZIFT), with significantly low numbers ($<1 \times 10^6$) of frozen/thawed motile spermatozoa (Tournaye et al., 1991; Sanger et al., 1992). The use of intracytoplasmic sperm injection (ICSI) will improve their management as even non-motile spermatozoa can be injected to the oocyte. We hence store almost every semen sample we are presented with (Table III). Many issues over the storage of these samples must be addressed, e.g. nature, amount and timing of counselling; effect of cancer and/or drugs on sperm function and DNA. Much more research is required on these issues. For instance, Auger et al.
(1996) have recently demonstrated that patients with testicular cancer have low semen quality and they appear to have reduced spermatogenesis in the contralateral testis. However, as long as their semen sample is cryopreserved prior to orchidectomy then the post freeze semen parameters are comparable to non-cancer controls. A European network would be a good starting point to act as a focus of expertise to which centres can turn.

In addition, we have had several requests to store samples from patients who are almost dead, i.e. on a life support machine, or have died very recently. This is again a complex issue. For example, in the UK the patient needs to give written consent before producing a sample, without which we cannot use the sample to inseminate the partner (HFEA, 1990). Centres receiving such requests must comply with the national guidelines if they are present.

Conclusions

In conclusion there are no clinical thresholds for the recruitment of donors which are absolute. In the near future when more data is available it is likely that CASA will add to the selection of donors as will the selected use of sperm function tests. At the end of the day each centre should monitor their own pregnancy rates and if they are low then an examination of semen characteristics should be an appropriate place to investigate in order to improve success. We believe the most sensible option is to set up regional sperm banks throughout Europe.

References


Table III. Semen characteristics for 29 patients attending for sperm storage prior to chemotherapy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration F (×10⁶/ml)</td>
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</tr>
<tr>
<td>Motile concentration (×10⁶/ml)</td>
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</tr>
<tr>
<td>Progressive motility F (%)</td>
<td>41</td>
<td>0–71</td>
</tr>
<tr>
<td>Motility F (%)</td>
<td>49</td>
<td>0–80</td>
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<td>Motile concentration F (×10⁶/ml)</td>
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<td>0–26</td>
</tr>
<tr>
<td>Progressive motility PT (%)</td>
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<tr>
<td>Motility PT (%)</td>
<td>15</td>
<td>0–46</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>36</td>
<td>0–146</td>
</tr>
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</table>

F = fresh; PT = post-thaw; motility defined as >0 μm/s; progressive motility defined as ≥10 μm/s.


