The relationship between fertility potential measurements on cryobanked semen and fecundity of sperm donors

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Sperm penetration assay (SPA) scores obtained from cryobanked semen were correlated with therapeutic insemination (TI) fecundity in a group of established sperm donors, thereby evaluating the efficacy of the SPA in screening donors for sperm banking. While the SPA has been used to separate fertile from infertile males, we altered assay conditions to use frozen semen and to distinguish performance among fertile donors. Three frozen ejaculates from TI pregnancy-proven donors were analysed. Of 905 TI cycles, 275 recipients achieved 95 pregnancies. There were no significant relationships between fecundity and donor semen, washed sperm parameters, sperm recoveries or recipient age. A significant relationship was revealed between mean SPA scores (range 8.7–66.6 penetrations/ovum) and donor fecundity (range 0.04–0.16, P < 0.03). Sperm concentration was varied in an effort to establish the most sensitive test condition. Using 0.25 × 10⁶ motile spermatozoa/ml, a highly significant relationship was observed (P < 0.002). The four donors with the lowest SPA scores achieved the four lowest fecundities. It is concluded that a modified SPA can be used on frozen donor semen to estimate donor fertility potential. If applied routinely in donor semen banking, poor quality applicants could be excluded, thereby increasing pregnancy rates while decreasing donor screening costs.

Key words: fecundity/frozen donor semen/sperm banking/sperm penetration assay/therapeutic insemination

Introduction

Therapeutic insemination (TI) by donor is established as a highly effective and well-accepted means to achieve conception. With the advent of the AIDS pandemic, programmes offering TI by donor have abandoned the use of fresh semen because of the potential risk for transmitting infectious diseases (Sidhu et al., 1997; Barratt et al., 1998). Unfortunately, reliance on quarantined, cryopreserved semen has resulted in decreased cycle fecundity compared with fresh semen (Barratt et al., 1998).

The recruitment of donors for TI is an increasingly difficult process; at Oregon Health Sciences University (OHSU) only one of eight men initially evaluated is accepted as a donor (Mixon et al., 1989) similar to the 70–87% drop-out or failed screening rates reported by others (Sidhu et al., 1997). The three principal reasons for this low acceptance rate are; lack of interest after the initial phone interview or after filling out the intake questionnaire; secondary to serology or family medical history; and on the basis of semen quality or post-thaw survival. Once admitted to a TI by donor programme, donors are often selected by recipients on the basis of physical characteristics, educational levels, hobbies and general interests as well as a history of reported pregnancy within a programme. Ideally, sperm banks should initially select donors on the basis of fertility potential rather than establishing fertility or non-fertility on the basis of reported pregnancies once in a programme (Thyer et al., 1999). For this reason and because of the expense and effort involved in recruiting donors and maintaining a sperm bank, it is important to look for a reliable, prognostic test for selecting those donors that will give the best performance.

The sperm penetration assay (SPA) has been used for fertility potential measurement and male factor infertility evaluation for well over a decade (Overstreet et al., 1980; Binor et al., 1981; Blazac and Overstreet, 1981; Wolf and Sokoloski, 1982; Martin and Taylor, 1983; Wolf et al., 1983; Irvine and Aitken, 1986; Crister et al., 1987; Kuzan et al., 1987; Smith et al., 1987; Corson, 1990). Irvine and Aitken (Irvine and Aitken, 1986) reported a significant difference in the SPA scores of individual frozen donor ejaculates that produced a cycle pregnancy compared to those that did not. Their fecundity correlation improved when sperm movement criteria were included. However, the accuracy and utility of the SPA as a prognostic indicator of donor fecundity in the context of TI by donor with frozen semen has not been established (Barratt et al., 1998).

The biological basis for the SPA is dependent on two factors, the ability of the hamster egg to fuse with and subsequently decondense the head of human spermatozoa and the requirement that spermatozoa must undergo capacitation before fusing with the egg plasma membrane. Thus, the SPA provides a measure of the ability of the spermatozoon to complete physiological events that are required for normal fertilization (Yanagimachi et al., 1976; Aitken, 1994).

The objective of the present work was to correlate SPA
scores obtained from cryobanked semen with corresponding fecundities in a group of established sperm donors, thereby evaluating the efficacy of the SPA in screening potential donors for sperm banking. It should be emphasized that the SPA was performed after the initial donor screening on the frozen aged semen and not on the fresh specimen. Hopefully, this approach would account for any hidden cryo damage that might have occurred during the freeze–thaw process. This investigation was made possible by the optimized SPA quality control technology that routinely measures sperm penetrating ability from frozen–thawed semen. Since the processing conditions of the optimized SPA were originally developed to distinguish the infertile from fertile population rather than the relative performance among just the fertile population, conditions were also altered here in an effort to provide enhanced assay sensitivity.

**Materials and methods**

**Design**

Frozen semen (three different ejaculates) from 11 pregnancy-proven donors whose samples were used in at least 35 cycles of intrauterine insemination (IUI) were analysed for relationships between donor fecundity and SPA scores. The sperm concentration employed in the SPA was also varied in an effort to establish the most sensitive test condition. While all recipients were carefully screened to identify pathological complications, the potential confounding influence associated with undiagnosed female pathology cannot be ignored. However, in the present study the effect of variations in female reproductive pathology on donor fecundity was minimized, since semen from each donor was routinely used to inseminate many different recipients (Irvine and Aitken, 1986). We obtained legal documentation from all donors, permitting us to use their frozen semen for IUI or experimental purposes, therefore IRB approval was not specifically required.

**Screening protocol**

Eleven donors underwent a standardized screening protocol, including physical examination, and review of medical, family and infectious disease history. All donors met minimum semen criteria defined as >70% normal morphology (as defined by the World Health Organization, 1992), sperm count >60×10⁶/ml, post-thaw motility (demonstrating some forward progression) >25% and at least 50% cryosurvival. The storage age for donor samples tested ranged from 1.1 to 5.1 years (mean 3.4 years). The majority of IUI patients underwent initial evaluations at OHSU. They were carefully screened to eliminate any detectable pathology. Patients with risk factors for tubal disease were screened with hysterosalpingography and were referred for treatment or to in-vitro fertilization (IVF) if there was evidence of tubal occlusion. Ovulatory dysfunction was treated with either clomiphene citrate or gonadotrophin therapy. This study includes all IUIs performed from October 1990 to February 1998. All inseminations were performed at OHSU on the day after a luteinizing hormone (LH) surge was detected by a urine predictor kit. Pregnancy was defined as a positive pregnancy [urine or serum human chorionic gonadotrophin (HCG)] test or a verbal report from the patient if she was followed by an outside provider.

**Statistical analysis**

Data sets were analysed using statistical software from SAS Institute Inc. (Gary, NC, USA). A Pearson correlation coefficient or a linear regression probability <0.05 was considered to be significant. SPA run values were included in this study only if the two control values included in assay passed established Westgard rules of compliance (Westgard et al., 1981). Westgard defined seven rules for accepting or rejecting assay outcomes based on a comparison of control values with the standard deviation of the prior corresponding controls plotted on a Levey–Jennings chart. After every SPA test run all the previously obtained values for a control series are used to recalculate a new or ‘running’ standard deviation. By adding the previous run control value, the increased number of values improves the definition of acceptable limits.

**Sperm cryopreservation**

Semen samples, collected by masturbation, were liquefied for approximately 30 min at 37°C before a semen analysis was conducted. Samples were frozen by one of two methods.

Conventional method (n = 861)

One volume of semen was diluted with 3 volumes of cryoprotectant, 211 mmol/l TES (N-tris [hydroxymethyl] methyl-2-amino-ethane sulphonic acid), 96 mmol/l Tris (hydroxymethyl-aminomethane), 11 mmol/l dextrose, and 1% penicillin–streptomycin solution to which was added 20% fresh hen yolk, TEST Yolk Buffer (TYB) (Irvine Scientific, Santa Ana, CA, USA) modified by the addition of glycerol to a concentration of 29.6%. The final glycerol concentration in the semen/cryoprotectant mixture was 7.4%.

IUI-Ready method (n = 44)

Semen was centrifuged at 350 g for 20 min through a 50–70–90% Percoll (Pharmacia, Uppsala, Sweden) gradient. The pellet was washed once and resuspended in IUI-Ready Cryoprotectant [Tyrode’s solution with albumin and pyruvate (TALP), HEPES–6% glycerol–2% sucrose] to approximate a motile sperm concentration of 40×10⁶/ml. In both methods, diluted samples were transferred to pre-labelled Nunc (Nalge Nunc International, Naperville, IL, USA) cryotubes (including a test vial to be thawed for cryosurvival), placed on wands submerged in a beaker of water at ambient temperature and held at 4°C for 1½ h. Samples were then exposed to liquid nitrogen vapours for 30 min and immersed in liquid nitrogen for 5 min prior to placement in permanent storage locations. A minimum of 24 h after freezing, a test vial was thawed, the sperm motility determined, and the number of motile spermatozoa per freeze vial calculated. This laboratory found in a recent study that fecundity rates using conventional compared to IUI ready methods were statistically identical (Mixon et al., 1998).

**Sperm thaw and preparation for IUI**

One hour before the scheduled insemination, vials were removed from liquid nitrogen and placed at room temperature for 5 min, then at 37°C for 10 min. Conventionally frozen samples were washed by one of two methods.

Wash/resuspension method

The thawed semen was mixed with two volumes of wash medium [human tubal fluid (HTF)-HEPES + 1% human serum albumin (HSA)] in a 15 ml conical centrifuge tube, centrifuged for 7 min at 350 g before the resulting pellet was resuspended in 2 volumes of wash medium and centrifuged in the same manner. The final pellet was resuspended in 0.4 ml of wash medium.

Gradient centrifugation method

The thawed semen was layered on top of a 50–70–95% Percoll (Pharmacia) gradient and centrifuged at 350 g for 25 min. The resulting pellet was resuspended in 3.0 ml of wash medium, centrifuged at 350 g for 7 min. The final pellet was resuspended in 0.4 ml of wash medium.

**Measurements on donor cryobanked semen and fecundity**
medium. For both methods, the total number of motile spermatozoa in the final preparation was calculated.

Sperm thaw and preparation for SPA
A single vial from three different ejaculates from each donor were thawed in a 4°C waterbath. All vials tested in the SPA were frozen by the conventional method (above). An aliquot was immediately analysed to determine post-thaw motility and the rest of the sample was held for an additional 18 h at 4°C in preparation for the SPA. The next morning the 4°C semen samples were thermal shocked by the rapid addition of 6 ml 37°C Biggers, Whitten, and Whittingham’s buffer containing 0.3% human serum albumin (BWW-0.3% HSA) (Biggers et al., 1971). Samples were centrifuged at 300 g for 10 min, aspirated and resuspended in 3.0 ml of BWW-0.3% HSA before distribution into six 12×75 mm culture tubes, and centrifuged at 300 g for 10 min. Swim-up spermatozoa were harvested after a 90 min incubation at 37°C, centrifuged again at 300 g for 10 min, and resuspended to normalize the final concentration to 5.0 (or 1.0, 0.5, or 0.25 when indicated)×10⁶ motile spermatozoa/ml.

Collection, processing and storage of semen for quality control
Semen samples that contained over 200×10⁶ motile spermatozoa were collected from individuals with established SPA levels and diluted with an equal volume of TYB containing 8% glycerol (Johnson et al., 1995). The sample was aliquoted into 0.5 ml French straws and sealed with putty. Straws were placed horizontally in a Petri dish, cycles (3.3 mean inseminations per recipient, range 1–7) were thawed and placed in medium with 1 mg/ml hyaluronidase. The motile spermatozoa employed by others to achieve fertilization were also aspirated and resuspended in 3.0 ml of BWW-0.3% HSA before distribution into six 12×75 mm culture tubes, and centrifuged at 300 g for 10 min. Swim-up spermatozoa were harvested after a 90 min incubation at 37°C, centrifuged again at 300 g for 10 min, and resuspended to normalize the final concentration to 5.0 (or 1.0, 0.5, or 0.25 when indicated)×10⁶ motile spermatozoa/ml.

Hamster ova preparation
Golden Syrian hamsters received ovarian stimulation after which the oviducts were excised and transferred to the laboratory in 0–4°C BWW-0.3% HSA (Johnson et al., 1990). Cumulus masses were retrieved and placed in medium with 1 mg/ml hyaluronidase. The zonae pellucidae were then enzymatically digested in medium with 1 mg/ml trypsin. Following multiple washes, 20 zona-free ova were added to each 50 µl drops containing motile spermatozoa, covered with silicone oil and incubated at 37°C for 3.5 h in 95% humidified air. For incubation, they were washed to remove loosely bound spermatozoa, carefully placed on a glass slide, and topped with a coverslip. Phase-contrast microscopy at ×400 was used to confirm the presence of decondensed sperm heads associated with tails, interpreted as a positive penetration. Most patient specimens tested by the optimized SPA will have had all ova penetrated by multiple spermatozoa. Donor scores were expected to average 35 penetrated spermatozoa in each ovum incubated (Johnson et al., 1990). Consequently, SPA scores were expressed as the mean number of penetrations/ovum (p/o, total penetrations/total number ova incubated, previously described as sperm capacitation index or SCI).

Results
Characteristics of frozen donor spermatozoa
Semen samples from 11 sperm donors with ages ranging from 23 to 44 years collected and analyzed here were frozen between 1989 and 1996. Initially, relationships between semen or washed sperm characteristics and donor fecundity were evaluated. The following parameters were analysed: concentration; percent motility; total motile/ejaculate (sperm concentration×volume×percent motility); post-thaw recovery of motile spermatozoa; and post-thaw swim up recovery of motile spermatozoa. The mean total motile spermatozoa per ejaculate was 267×10⁶ (±82 SD, range 152–368×10⁶); the post-thaw motility averaged 37.1% (range 24.7–40%); and the mean cryosurvival (Table I, frozen vial motile/original vial motile) was 57.0% (±6 SD, range 42–63%). The yield of motile spermatozoa from each of the three donor samples was also determined after SPA processing: thaw, overnight incubation at 4°C, a thermal shock wash step, and a 90-min swim-up. There were no statistically significant relationships between the original semen parameters or any of the above monitored motility parameters or sperm recoveries and fecundity (Pearson correlations, P > 0.1).

Donor and recipient characteristics
Each donor participated in at least 35 TI by donor cycles (mean = 82, range 35–134) (Table II). Donor fecundity (number of pregnancies divided by the number of cycles) varied from a low of 0.04 to a high of 0.16. A total of 275 individual recipients experienced 905 donor insemination cycles (3.3 mean inseminations per recipient, range 1–19). Of the 905 inseminations, six were followed by a second insemination in the same cycle from the same donor. Only data from the first IUI have been included. Most recipients (85%) used one donor. The other 15% ‘crossed-over’ using a total of two or more of the donors included in the study. The mean donor recipient age at the time of each insemination averaged 35.1 years and ranged, for each donor, from 33.3 to 36.6 years (Table II). The mean donor motile spermatozoa per insemination averaged 2.82×10⁷ (range 2.12–3.57) and 95 pregnancies resulted with an overall fecundity of 0.105. All inseminations delivered well over a minimum threshold of motile spermatozoa employed by others to achieve fertilization (Byrd et al., 1990; Campana et al., 1996; Berg et al., 1997). While not significant (P = 0.08), a trend was apparent when a linear regression of the means of inseminated motile spermatozoa was plotted versus fecundity. The only statistically significant relationship between any of the monitored parameters and fecundity was the mean number of IUI cycles (linear regression, P = 0.03).

Sperm function evaluation (SPA)
Quality control
It is important to account for the degree of interassay variation inherent in the SPA in comparing values from multiple tests, as scores within these limits are equivalent. As a routine part of SPA validation and ongoing quality control, thawed aliquots from cryostored single ejaculates were employed in each assay. High and low scoring controls were run in parallel with each patient test. A running standard deviation about a running mean was used to define acceptable testing limits. A single ejaculate may be aliquoted and used in 15–28 assays allowing a determination of interassay variability (Johnson et al., 1990, 1995). For the assays conducted during this study, 13 individual frozen control ejaculates (with a range of 8–27 trials each)
produced mean scores covering the full diagnostic range from 4.5 to 56.1 p/o (Figure 1). All runs included in the present study were considered ‘in control’, since they satisfied established Westgard rules of compliance. The coefficients of variation (CV) for these controls revealed a familiar inverse relationship with mean penetration values, similar to earlier studies (Johnson et al., 1990, 1995): three low means (<12 p/o) had an average CV of 28%, eight medium means (>12 and <40 p/o) had an average CV of 16%; and two high means >40 p/o had an average CV of 9%.

**Donor evaluation**

Three different ejaculates from each donor were tested in the SPA at a conventional sperm concentration of $5 \times 10^6$/ml (Table I). Inter- and intra-donor variations were similar to those reported for fresh donor semen (Johnson et al., 1990, 1995). Despite intra- and inter-assay variation, a wide but consistent range in mean SPA scores was found with a low of 8.7 and a high of 66.6 p/o. Further, a linear regression of the mean penetration rates against fecundity revealed a significant relationship ($n = 11$, $P < 0.03$; Figure 2).

### Table I. Sperm cryosurvival and sperm penetration assay (SPA) scores on three frozen ejaculates from 11 donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>% Sperm cryosurvival</th>
<th>SPA score $b$ (Mean ± SD)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Ejaculate 1</td>
<td>Ejaculate 2</td>
</tr>
<tr>
<td>1</td>
<td>42 ± 7.8</td>
<td>14.3</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>61 ± 7.2</td>
<td>43.1</td>
<td>52.6</td>
</tr>
<tr>
<td>3</td>
<td>47 ± 10.0</td>
<td>9.4</td>
<td>13.1</td>
</tr>
<tr>
<td>4</td>
<td>57 ± 7.6</td>
<td>9.9</td>
<td>38.2</td>
</tr>
<tr>
<td>5</td>
<td>57 ± 15.5</td>
<td>34.4</td>
<td>28.0</td>
</tr>
<tr>
<td>6</td>
<td>59 ± 13.4</td>
<td>70.3</td>
<td>68.6</td>
</tr>
<tr>
<td>7</td>
<td>60 ± 3.5</td>
<td>32.5</td>
<td>40.5</td>
</tr>
<tr>
<td>8</td>
<td>63 ± 6.6</td>
<td>49.7</td>
<td>36.8</td>
</tr>
<tr>
<td>9</td>
<td>59 ± 9.3</td>
<td>36.8</td>
<td>40.1</td>
</tr>
<tr>
<td>10</td>
<td>61 ± 4.6</td>
<td>74.4</td>
<td>60.4</td>
</tr>
<tr>
<td>11</td>
<td>61 ± 4.6</td>
<td>50.8</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*aAverage of three ejaculates ± 1 SD (thawed % motility/initial % motility) $\times$ 100.

*bAverage penetrations/ovum.

### Table II. Donor intrauterine insemination (IUI) characteristics: number of recipients and their mean age, number of IUI cycles, spermatozoa inseminated, total pregnancies with resulting fecundity

<table>
<thead>
<tr>
<th>Donor no.</th>
<th>No. of recipients $a$</th>
<th>Mean recipient age (years) $b$</th>
<th>IUI cycles</th>
<th>Total motile sperm/IUI $c$</th>
<th>No. of pregnancies</th>
<th>Fecundity $d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>35.3 ± 4.5</td>
<td>46</td>
<td>22.8 ± 5.0</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>36.6 ± 5.9</td>
<td>54</td>
<td>27.4 ± 5.9</td>
<td>3</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>34.2 ± 5.0</td>
<td>35</td>
<td>21.2 ± 7.4</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>33.3 ± 4.9</td>
<td>69</td>
<td>27.7 ± 8.3</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>34.5 ± 5.2</td>
<td>79</td>
<td>25.7 ± 6.9</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>34.3 ± 5.1</td>
<td>79</td>
<td>32.3 ± 8.5</td>
<td>7</td>
<td>0.09</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>35.5 ± 5.1</td>
<td>118</td>
<td>33.1 ± 9.1</td>
<td>12</td>
<td>0.10</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>35.0 ± 4.7</td>
<td>134</td>
<td>26.9 ± 6.2</td>
<td>17</td>
<td>0.13</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>35.3 ± 3.9</td>
<td>88</td>
<td>25.2 ± 7.0</td>
<td>12</td>
<td>0.14</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>35.6 ± 5.5</td>
<td>108</td>
<td>35.7 ± 9.1</td>
<td>15</td>
<td>0.14</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>36.0 ± 3.9</td>
<td>95</td>
<td>31.7 ± 7.5</td>
<td>15</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*a275 different recipients, some of whom used more than one donor, account for the total of 324.

*bMean ± 1 SD recipient age at the time of each IUI.

*cMean ± 1 SD total motile spermatozoa inseminated/IUI.

*dFecundity = no. of cycles/no. of pregnancies.

**Figure 1.** Sperm penetration assay (SPA) scores on multiple frozen aliquots from single ejaculates to monitor quality control (QC). Despite intra- and inter-assay variation, a wide but consistent range in mean SPA scores was found with a low of 8.7 and a high of 66.6 p/o. Further, a linear regression of the mean penetration rates against fecundity revealed a significant relationship ($n = 11$, $P < 0.03$; Figure 2).
Figure 2. Mean donor sperm penetration assay (SPA) scores on frozen semen plotted against IUI fecundity. Each mean (not shown) was derived from an SPA performed on three different frozen ejaculates from 11 donors at $5 \times 10^6$ motile spermatozoa/ml, the conventional sperm concentration. Each set of vertically aligned symbols shows triplicate results from each donor. The line was generated by linear regression analysis of the means ($P < 0.03$).

Figure 3. Frozen sperm penetration assay (SPA) scores dependency on tested sperm concentration. Each concentration includes SPA scores from frozen ejaculates on each of 11 donors. Lines connect each donor series (different symbols from top to bottom correspond to table donor nos 10, 6, 2, 11 9, 8, 7, 5, 4, 3, 1) tested at 5.0 (conventional), 1.0, 0.5 and $0.25 \times 10^6$ motile spermatozoa/ml. A single datum for one donor was not completed.

Altered sperm concentrations

Because of the possibility that sperm–egg interactions follow zero-order kinetics at conventional sperm concentrations, minimizing differences between high scoring donors, a sperm concentration dependency experiment was conducted (Figure 3). Penetrations may become independent of increasing spermatozoa concentrations because binding sites become saturated or a limitation exists in the number of spermatozoa that the ovum can fuse with or decondense. Therefore, ejaculates from each donor were tested at 1.00, 0.50 and $0.25 \times 10^6$ motile spermatozoa/ml as well as at the conventional concentration. Two donor groups were apparent in a visual assessment of the results. One, comprising two doners, showed a marked tendency to plateau with high penetration scores at all but the lowest sperm concentration. Two these donors (nos 6 and 10) also had the highest SPA scores and as a group differed statistically from the second group, comprised of nine donors ($P < 0.02$, one-way ANOVA).

Plots of penetration rates at the 1.00 and $0.50 \times 10^6$ motile sperm concentrations versus fecundity were characterized by a tendency for penetration rates to increase with increasing concentration.

Discussion

Traditionally, routine semen analysis has been employed and still is used to assess pre- and post-freeze samples in order to discriminate between donors of high and low fecundity. However, the most predictive factor may be limited to the number of motile spermatozoa per straw and the number of spermatozoa inseminated (Barratt et al., 1998) as much doubt has been cast on the use of semen parameters alone for male fertility prognosis or fecundity assessment since the functional competence of spermatozoa is not measured. In the above study, Barratt used minimum thaw motile sperm threshold criteria to achieve an overall clinical fecundity rate >0.14 but reported a wide variation in individual donor fecundities. Indeed, in the present study, we failed to detect correlations between semen or washed sperm parameters and either donor fecundity or sperm functional competence. Our focus here was to apply a functional test of sperm fertility in an attempt to correlate SPA scores on cryobanked semen and fecundity in a group of established sperm donors and to evaluate the efficacy of the SPA in screening potential donors for sperm banking. Variation in fecundity due to female factors is thought to be equalized by the relatively large number of recipients per donor (mean 29, range 15–44). Therefore, fecundity is most probably regulated by the functional competence of post-thaw spermatozoa as measured by the SPA.
The testing of fertility potential of frozen semen has been reported previously (Irvine and Aitken, 1986; Crister et al., 1987) as has the use of frozen semen for quality control purposes (Smith et al., 1987; Johnson et al., 1995) where samples are run multiple times, thereby defining both inter-assay and intra-ejaculate variability. In the present study, these coefficients of variation for QC samples ranged from 9 to 31% and were consistent with prior reports (Johnson et al., 1990, 1995). Here we also established that SPA scores on three ejaculates from each of 11 donors showed a remarkable degree of consistency. The inter-ejaculate variability ranged from 11 to 67%, similar to the variability previously reported for fresh semen from four donors over time (21–42%) (Johnson et al., 1990).

The prognostic significance of the SPA in the evaluation of male fertility potential has been challenged. A number of investigators have reported on the positive correlation with male infertility (in vitro and in vivo) (Wolf et al., 1983; Smith et al., 1987; Johnson et al., 1990, 1991); however, others have been critical, indicating that the SPA is a poor indicator of sperm function (Corson, 1990) or has an unacceptably high percentage of false negative results (Kuzan et al., 1987; Corson, 1990). SPA studies performed on small numbers of patients may contribute to the conflicting results. Also, since laboratories do not follow a standardized protocol, or employ standardized quality control procedures such as the use of internal controls as described herein, it is virtually impossible to compare results between groups. The present study supports the thesis that the SPA provides a reliable and reproducible approach to the analysis of sperm function in vitro (Overstreet et al., 1980; Wolf et al., 1983; Smith et al., 1987; Johnson et al., 1990; Aitken, 1994).

Penetration scores generated by the improved SPA, as employed here, have been assigned clinically significant predictive values based on the testing of pregnancy proven donors (Johnson et al., 1990). A threshold value for the SPA was established at >5.0 p/o (derived from a value 2 SD below the fertile population mean at 35.0 p/o ± 15 SD, n = 30). In the present group of 11 pregnancy proven donors only two showed mean SPA scores near the threshold values defined above. Donor 1 had the lowest fecundity with a mean SPA score of 8.7 p/o (range 2.7–14.3) while donor 3 with the second lowest fecundity had a mean SPA score of 11.0 p/o (range 9.4–13.1). The remaining nine donors showed mean SPA scores between 23 and 66.6 p/o. This finding is consistent with the expectations of proven donors as measured on non-frozen semen (Johnson et al., 1990, 1995), but contradictory results have been reported by others (Binor et al., 1981; Blazac and Overstreet, 1981) using frozen donor semen for predicting fertility status.

The correlation observed in this study between increasing fecundity and mean SPA scores was obtained at several sperm concentrations ranging from a low at 0.25 to a high at 5.0×10^6/ml, the standard concentration. The non-linear relationships between fecundity and mean SPA scores suggest that it may be efficacious to evaluate such a cohort of men with relatively high penetrating spermatozoa at lower inseminating sperm concentrations. At standard concentrations the number of penetrations per ovum may plateau secondary to the saturation of sperm binding sites on the oocyte plasma membrane or to saturation of the egg cytoplasm. Differences in the kinetics of capacitation may also be involved, which are known to vary among proven fertile donors (Wolf and Sokoloski, 1982; Martin and Taylor, 1983; Smith et al., 1987). By reducing the sperm concentration, penetration rates became more highly correlated to donor fecundity. Based on these findings, a reasonable SPA threshold value to distinguish potentially good from poor donors at a sperm concentration of 0.25×10^6 motile spermatozoa/ml can be proposed at 2 p/o. All four donors with a fecundity <0.06 had SPA scores <2 p/o and all seven donors with a fecundity >0.06 had SPA scores >2 p/o. It is interesting to note that the optimized SPA and the modified Micro-SPA (Johnson et al., 1991) as well as this ‘frozen donor’ SPA system have evolved to utilize different sperm concentrations. Thus, customization is required to position the assay discrimination value at the most sensitive portion of the penetration curve, to best separate men with different fertility potential.

The therapeutic donor insemination programme has provided a unique opportunity to investigate the predictive value of in-vitro tests of sperm function. We conclude that a standardized SPA protocol on cryopreserved spermatozoa can serve as a valuable supplement to the more traditional tests of semen quality in efforts designed to evaluate donor fecundity. The selection of high fecundity donors is a significant issue in maximizing TI by donor pregnancy rates. Currently there are no guidelines or minimum performance standards for donors. Some retrospective studies suggest dropping donors who had not produced a pregnancy by 12 cycles (McGowan et al., 1983). Thyer and co-workers (Thyer et al., 1999) analysed the first 40 cycles in 20 TI donors. The fecundity was constant with normal distribution in 20 cycles, but after only 15 cycles, 80% did not deviate by more than one quartile; donor performance at 15 cycles was predictive of performance at 40 cycles. They concluded that the individual analysis of donor fecundity every 15 cycles is important for sperm banks to consider and would justify dropping donors whose performances are poor.

A substantial cost benefit can be realized by both the cryobank and semen recipients if the SPA is used routinely to eliminate substandard donors. The cryobank would spend an additional fee for SPA testing; however, when performed as an initial donor screen such testing can preclude the costs and professional time invested in qualifying family medical history, physical examination and testing for infectious diseases. Moreover, as this technology evolves it may become possible to freeze fewer spermatozoa per vial from high SPA donor specimens. Diluting inseminations on the basis of sperm quality rather than quantity would mean fewer specimens would need to be collected and processed by the cryobank.

We can use the 11 donors in the present study and their 905 TI cycles as a model to predict the potential cost benefit for patients. The four poorest donors (fecundity 0.05 and SPA <2.0 p/o) collectively achieved 11 pregnancies in 204 cycles. If they were never used the remaining seven donors would achieve the same number of pregnancies in just 92 cycles (0.12 fecundity and SPA >2.0 p/o). Considering the demand
in the USA for donor insemination has been estimated to be >270 000 units per year (Crister, 1998), the overall cost benefit could be substantial.

In summary we have demonstrated a positive relationship between sperm function measurements on cryopreserved donor semen and corresponding donor fecundities. By modifying SPA conditions to use a lower concentration of motile sperma-
toza, the assay became more predictive of fertility potential. Since additional results are required before pre-screening donors becomes clinically relevant, we have initiated a prospec-
tive controlled study testing a broader spectrum of donors in our TI programme.

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