Screening of donor semen samples before acceptance in a donor sperm bank

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Disclosure

I have no commercial or other activities that may reflect on the contents of this lecture

Lecture objectives

• Give an short overview of different society guidelines for selection criteria for sperm bank donors
• Discuss the different pre-freezing and post-thaw semen parameter criteria for sperm bank donors
• Discuss factors that may influence donor semen quality
Selection criteria for semen donors

• General criteria
  – Society guidelines
• Semen quality criteria

International societies guidelines

American Society of Reproductive Medicine
– 2006 Guidelines for Sperm Donations (1)

Selection criteria of Donor
  – Good health status
  – Absence of genetic abnormalities
  – Legal age (<40 years)
    • Risk for increased rate of sperm aneuploidy
  – Selection of donors with established fertility is desirable - but not required
  – Psychological evaluation

ASRM, 2006
American Society of Reproductive Medicine – 2006 Guidelines for Sperm Donations (2)

Semen testing
• Several samples to be examined
• After a 2- to 5-day abstinence
• Examined within 1 to 2 hours after ejaculation
• No uniformly accepted minimum semen standards
• In general, the minimum WHO criteria for normal semen quality can be applied (WHO, 1999)

British Fertility Society (BFS) Guidelines – 2008 (1)
• Age
  – Upper limit = 40 years
  – If >40 increased infertility, miscarriages and congenital malformations
• Screening for fertility
  – Semen to be assessed according to WHO (1999) criteria
  – In general well described relationship between semen parameters and conception

British Fertility Society (BFS) Guidelines – 2008 (2)
• Screening for fertility (continued)
  – This relationship does not seem to exist for use of thawed-cryopreserved sperm in IUI
  – BAS (1999) no recommended minimum acceptance criteria for donors based on semen quality or post-thaw survival rates
British Fertility Society (BAS) Guidelines – 2008 (3)

• Screening for fertility (continued)
  – Growing awareness of relationship between semen quality and sperm DNA integrity
  – Growing awareness of relationship between sperm DNA integrity and embryo quality

• Recommendation (2008)
  – Only those men with (pre-freeze) semen quality values above WHO (1999) normal values should be accepted as donors

ABA, ACE, BAS, BFS, RCOG, 2008

ESHRE (predictive) criteria for Donor semen (1998)

• Most predictive factor
  – Number of motile spermatozoa per straw
  – Number of motile spermatozoa inseminated
  – No absolute standards from semen examinations

Barratt et al., 1998

ESHRE (predictive) criteria for Donor semen (1998)

• Sperm functional tests
  – Hamster penetration test
  – CASA motility analysis

• Limitations
  – Number of pregnancies per donor

Barratt et al., 1998
Selection criteria for semen donors

• Semen parameters
  – Minimum requirements for donor semen
  – Pre-freeze and post-thaw results
  – Variability in donor semen quality

WHO 1999 and 2010 criteria for normal semen quality (ASRM, 2006; BAS, 2008)

<table>
<thead>
<tr>
<th></th>
<th>WHO 1999 (1)</th>
<th>WHO 2010 (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>≥2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Sperm concentration (10^6/ml)</td>
<td>≥30</td>
<td>15</td>
</tr>
<tr>
<td>Total number (10^9/ ejaculate)</td>
<td>≥40</td>
<td>39</td>
</tr>
<tr>
<td>Total motility (% a+b+c)</td>
<td>≥40</td>
<td>40</td>
</tr>
<tr>
<td>Progressive motility (% a + b)</td>
<td>≥50</td>
<td>32</td>
</tr>
<tr>
<td>Morphology (% normal)</td>
<td>≥44</td>
<td>4</td>
</tr>
<tr>
<td>Vitality (% live)</td>
<td>75</td>
<td>58</td>
</tr>
<tr>
<td>White blood cells (10^6/ml)</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

(1) WHO, 1999; Menkveld, 2010 (2) Cooper et al., 2009; WGO, 2010

Minimal semen quality required for semen donors

• Castilla et al., 2007
  – Sperm concentration - ≥ 21.3 (58.0) \(10^6/ml\)
  – Progressive motility - ≥ 50 (60) %
  – Progressive motile count - ≥ 42.6 (96.6) \(10^6/ml\)
    * (For a <10% probability that the actual value will be less than the minimum required value)
Minimum semen quality required for semen donors

- Björndahl et al., 2010
  - Sperm concentration \(>50 \times 10^6/\text{ml}\)
  - Progressive motility (a+b) \(\geq 40\%\)
  - Morphology (% normal) \(\geq 14\%\)\(^{(1)}\)

\(^{(1)}\) WHO, 1999

Requirements for Post-Thaw semen quality

Number of motile spermatozoa per straw (NMSS)

- Barratt et al., 1998
  - No relationship between NMSS and IUI or IVF pregnancy rates
  - Minimal NMSS = \(2.5 \times 10^6\)
- Mortimer, 1994
  - \(5 \times 10^6\) progressive motile spermatozoa/straw

- Yogev et al., 2004
  - \(8 \times 10^6\) progressive motile spermatozoa/straw
    - (Will ensure minimum of \(4 \times 10^6\) progressive motile spermatozoa/ 0.5 ml straw)
- Castilla et al., 2007
  - To ensure \(8 \times 10^6\) progressive motile spermatozoa, actual numbers needed per straw must be:
    - Progressive motility of 25\% = 28\%
    - Sperm concentration of \(32 \times 10^6/\text{ml} = 37 \times 10^5/\text{ml}\)
      - (for a <10\% probability that the actual value will be less then the minimum required value)
Requirements for Post-Thaw semen quality

- **Post-thaw survival (PTS) and pregnancy rate**
  - PTS ≤ 30% = 5.5%
  - PTS of 30-50% = 15.4%
  - PTS >50% = 27.2%

- **Number of motile spermatozoa inseminated (NMSI)**
  - NMSI of <0.5x10^6 = Pregnancies possible
  - NMSI between 1.5-25x10^6 = Acceptable pregnancy rates
  - Optimal pregnancy rate with NMSI = 6-15x10^6/ml

Barratt et al., 1998

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Post-thaw semen quality variability between donor sperm banks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total concentration (10^6/ml)</td>
<td>164.8±3.6</td>
<td>97.3 – 264.0</td>
<td>86.7</td>
</tr>
<tr>
<td>Total motility (% a+b+c)</td>
<td>65.5±6.0</td>
<td>46.0 – 78.0</td>
<td></td>
</tr>
<tr>
<td>Motile count (10^9/ml)</td>
<td>125.6±31.7</td>
<td>65.6 – 207.0</td>
<td></td>
</tr>
<tr>
<td>Progressive motility (% a+b)</td>
<td>38.9±4.4</td>
<td>29.0 – 48.0</td>
<td>44.1</td>
</tr>
<tr>
<td>Progressive motile count (10^9/ml)</td>
<td>74.1±19.1</td>
<td>41.5 – 122.7</td>
<td>114.3</td>
</tr>
<tr>
<td>Morphology (% normal - SC)</td>
<td>20.3±2.1</td>
<td>16.5 – 25.0</td>
<td>39.7</td>
</tr>
</tbody>
</table>

Carrell et al., 2002

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Variation in donor fertility

McGowan et al., 1983 (1)
- 177 donors – good semen quality
- 4 year follow-up
- Variation in pregnancy results
  - <5 to >20 pregnancies per 100 inseminations
- Analyzed semen parameters of 25 most fertile and 25 least fertile
Variation in donor fertility

McGowan et al., 1983 (2)

<table>
<thead>
<tr>
<th></th>
<th>25 most fertile</th>
<th>25 least fertile</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration (10⁶/ml)</td>
<td>130 ± 67.8</td>
<td>104 ± 52</td>
<td>N/S</td>
</tr>
<tr>
<td>Motility - before freezing (%)</td>
<td>69 ± 4.3</td>
<td>68 ± 4.8</td>
<td>N/S</td>
</tr>
<tr>
<td>Motility – post thaw</td>
<td>52 ± 7.1</td>
<td>49 ± 6.2</td>
<td>N/S</td>
</tr>
<tr>
<td>Morphology (% normal)</td>
<td>76 ± 8.3</td>
<td>58 ± 9.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Recommendation: Discard donors with no pregnancies in 12 cycles

Source of variability between donors, specimens and measurements

Centola et al., 1992
- 12–47% due to specimen to specimen by donor
- 12–41% due to measurement variability
- Also variability within donor semen samples with regard to freezibility

There was a relationship between motile sperm count after semen dilution and post-thaw motile sperm count
Good motile sperm count after semen dilution was a good indicator of semen freezibility

Rate of donor acceptance

Only one out of ten men initially evaluated will qualify as a sperm bank donor
- Reasons
  - Lack of interest after interview
  - Issus for completing questionnaires, serology testing, medical history
  - Low semen quality
  - Poor post-thaw semen quality

Björnsdóttir et al., 2010
Possible negative influences on donor semen quality

• Environmental factors
  – Declining semen parameters
  – Lead
• Seasonal variations
• Effect of stress on semen quality of donors
• Effect of sperm functional tests

Declining semen parameters and donors semen quality

Conflicting reports in literature
• No declining effect of semen parameters over years
• Decline of semen parameters over years

No declining semen parameters

Costello et al., 2002
• Study period 1983 to 2001
• Linear regression results
  – Total sperm count ($r = 0.065; P = 0.17$)
  – Volume ($r = 0.002; P = 0.97$)
  – Sperm motility (found increase) (Spearman R = 0.194; $P = 0.001$)
• Conclusion
  – Semen quality of donors showed no decline
Decline in semen parameters

Horák et al., 2008

- Study period 1982 to 2004
- Upper Silesia (Poland – high polluted area)

<table>
<thead>
<tr>
<th>Year</th>
<th>Donors (n)</th>
<th>Cycles</th>
<th>Couples (n)</th>
<th>Pregancies (n)</th>
<th>Pregancies per cycle (%)</th>
<th>Pregancies per couple (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 - 1995</td>
<td>44 (Fresh samples)</td>
<td>1617</td>
<td>290</td>
<td>125</td>
<td>7.7</td>
<td>43.1</td>
</tr>
<tr>
<td>1996 - 2004</td>
<td>20 (Frozen samples)</td>
<td>1994</td>
<td>414</td>
<td>85</td>
<td>4.3</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Results – Needed more insemination and cycles per pregnancy (See Figure)

Effect of donor semen seminal plasma lead concentrations and IUI outcome

[Graph showing the relationship between mean seminal plasma lead concentration (μg/dL) and artificial insemination rate (%)]

Horák et al., 2008
Effect of seasons on donor semen quality
Yoge et al., 2004

- Fixed frozen semen aliquots (8-12x10^6/ml TPMSC) after thaw

<table>
<thead>
<tr>
<th>Number/season</th>
<th>Semen parameter</th>
<th>Highest</th>
<th>Lowest</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring (n=92)</td>
<td>Sperm concentration</td>
<td>March/Dec</td>
<td>September</td>
<td>0.030</td>
</tr>
<tr>
<td>Summer (n=97)</td>
<td>Morphology</td>
<td>March/Dec</td>
<td>September</td>
<td>0.038</td>
</tr>
<tr>
<td>Autumn (n=81)</td>
<td>Volume</td>
<td>No effect</td>
<td>No effect</td>
<td>N/A</td>
</tr>
<tr>
<td>Winter (n=97)</td>
<td>Motility</td>
<td>No effect</td>
<td>No effect</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Freezibility drops significantly between March to December
- Consequence – Need more straws (P=0.017) due to less TPMSC per straw available (P=0.002)

Effect of freezing and thawing on sperm functional abilities
Yoge et al., 2010

- Study design
  - 113 candidates tested to become semen donors
  - 16 active semen donors
  - Basic semen analysis, Hyaluronan-binding assay (HBA), sperm freezing and thawing

- Results
  - HBA was significant predictive for freeze-thaw outcome of ≥ 40%
  - 1 and 4 hour original motility >HBA for good freezability
  - Freeze-thaw had no influence on HBA

Conclusions
- Recruitment of semen donors for donor sperm banks is tedious due high out fall rate
- There are no universal semen parameter guidelines for the selection of donors for sperm banks around the world
- Selection of the best donor is complicated by variation of semen parameters from donors due to biological (environmental influences and donor semen variabiility) and analytical variability
- Will appear that ideal number = 5-10 X10^6 motile sperm per straw (0.5 ml)
Thank you for your attention

References (1)

Björndahl et al. Practical guide to Laboratory Andrology. Cambridge University Press. 2010
Carrell et al., Fertil Steril 78(1):16-21,2002
Castilla et al., Cell Tissue Bank 8:257-54,2007

References (2)

McGowan et al., Clin Reprod Fertil 2:269-274,1983
Menkeweld R. Asian J Androl 12:47-58,2010
Mortimer D. Practical Laboratory Andrology. Oxford University Press, 1994
Poland et al., Int J Fertil 31(1):229-31,1986
Yogev et al., Hum Reprod 19(4):880-5,2004
Yogev et al. Fertil Steril 93(1),2010