A decade of sperm washing: clinical correlates of successful insemination outcome

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BACKGROUND: Since 1999, we have treated HIV-positive men with sperm washing as part of a risk-reduction programme.

METHODS: Retrospective analysis of the sperm-washing database from the treatment of 245 couples with 439 cycles of intrauterine insemination assessed the effects of patient factors (age, maternal FSH, rank of attempt), markers of HIV-disease [time since diagnosis, CD4 count, viral load (VL), use of highly active antiretroviral therapy (HAART)], cycle factors (natural versus stimulated, number of follicles, fresh versus frozen sperm) and sperm parameters on clinical (CPR) and ongoing pregnancy rate (OPR).

RESULTS: Overall 111–245 (45.4%) couples achieved a clinical pregnancy (CPR: 13.5% and OPR: 9.6% per insemination) with no sero-conversions. The mean duration since HIV diagnosis was 5.8 years, 73% of men were on antiretroviral therapy, there was an undetectable VL in 64% and the median CD4 was 409 cells/mm3. A significantly decreased OPR and a non-significantly increased miscarriage rate (MR) was observed after the female age of 40. Similarly, there was a significant increased OPR and decreased MR for women with a mean cycle maternal FSH of <6.4 IU/l. There was no effect of VL, CD4 count, use of HAART or time since diagnosis on the outcome. Nor was there a difference in the OPR according to paternal age, rank of attempt, cycle regime or number of follicles. Semen volume, sperm concentration, total count and progressive motility and post-wash concentration, progressive motility and total motile count inseminated were significantly higher in successful cycles. The use of frozen sperm had a significant negative impact on outcome.

CONCLUSIONS: This study of the potential safe and successful reproductive options available to HIV-positive men demonstrates that maternal age and semen quality, rather than HIV factors, remain the most important determinants of cycle success.

Key words: assisted reproduction / HIV / IUI / sperm washing

Introduction

Many similarities exist between HIV and other once fatal diseases that afflict adults of reproductive age, for whom fertility treatment is rarely refused. In the developed world, the introduction and development of highly active antiretroviral therapy (HAART) during the past decade has transformed the lives of those infected with HIV and led to its redefinition as a chronic disease (Scandlyn, 2000) and with the continued improvements in HAART, projected life expectancy should approach that of negative controls (The Antiretroviral Therapy Cohort Collaboration, 2008). These changes mean that it is no longer justifiable to deny fertility treatment for HIV-positive adults, the majority of whom are of reproductive age (Gilling-Smith et al., 2001). In addition to improvements in life quality and expectancy, the use of selected antiretrovirals during pregnancy and at the time of delivery or during elective Caesarean section and the avoidance of breastfeeding are measures that have collectively led to a fall in vertical transmission risk from >30 to <2% (de Ruiter et al., 2008). As a result, increasing numbers of HIV-positive men and women are seeking advice on how to conceive with minimum risk of infecting their HIV-negative partner and prospective child or, in the case of concordant couples, of transmitting variant (drug resistant) viral strains to their partner or offspring.

Reproductive assistance to HIV-discordant couples can make a significant impact in prevention of viral transmission. Sperm washing, pioneered in Milan (Semprini et al., 1992) and involving sperm being washed free of seminal plasma and non-sperm cells (NSC) before insemination, rests on the observation that HIV is present as a free virus in the seminal plasma and as a cell-associated virus in the leukocytes or NSC but does not appear to be able to attach to, or infect, the spermozoa (Bagasra et al., 1994; Baccetti et al., 2001; Quayle et al., 1997, 1998). From 1999, as our unit became established as
the UK referral centre for sperm washing as part of a risk-reduction programme, there has been a year on year increase in total infectious cycles performed and we have performed the largest series of such inseminations in the UK (Gilling-Smith et al., 2006).

Several maternal, cycle and seminal determinants of success have been identified for intrauterine insemination (IUI) cycles. IUI outcome has been demonstrated to be improved with lower maternal age (Badawy et al., 2009; Duran et al., 2002), the number of pre-ovulatory follicles (Duran et al., 2002; van Rumst et al., 2008) and the use of ovarian stimulation for unexplained subfertility (Verhulst et al., 2006) and endometriosis (NICE, 2004). Although sperm motility (Montanaro Gauci et al., 2001), morphology (Badawy et al., 2009; Van Waart et al., 2001) and post-preparation motility (Hendin et al., 2000; Stone et al., 1999) have also been shown to affect IUI outcome, total motile count (TMCI) inseminated is the marker most consistently shown to be a determinant of success (Badawy et al., 2009; Campana et al., 1996; Khalil et al., 2001; Ombelet et al., 1997; Stone et al., 1999; Toner et al., 1995; van der Westerlaken et al., 1998). Early data from the sperm-washing programme also suggested a possible effect of HIV parameters (viral load (VL) and CD4 count) on cycle success (Nicopoulous et al., 2004).

In order to better advise patients of factors that may improve their outcome, we have carried out a retrospective analysis of our database to define whether there are clinical predictors of success in IUI/spERM washing in HIV-positive men.

Materials and Methods

Sperm-washing work-up

Pretreatment work-up is discussed fully elsewhere (Gilling-Smith et al., 2006). In brief, a full fertility and sexual health screen is performed on both partners to define the optimum treatment modality, exclude HIV co-infection and treat any genital lesions or infections that may increase the risk of viral transmission (Fleming and Wasserheit, 1999).

Our recommendations are that all patients should receive careful pre-conceptual counselling, both together and individually before embarking on treatment (Gilling-Smith and Almeida, 2003), where the nature and risks of sperm washing, the impact of possible treatment failure, the practicalities of coping with a child when one parent is HIV positive and the possibility of having to cope as a single parent are all discussed. In particular, it is mandatory that both partners understand sperm washing to be a risk-reduction method and not a risk-free method as technically, the virus could still be present in the washed sample at a titre below the detection limit of the HIV assay. Although there have been no reports of seroconversion in the female partner when semen is correctly processed in the 3315 cycles published thus far by the CREATHe network (Centre for Reproductive Assisted Techniques for HIV in Europe) (Bujan et al., 2007), the possibility of viral infection of the woman and subsequent child still exists and the alternative risk-free option of donor insemination should be discussed and appropriate consent should be taken from both partners that includes confirmation of this information.

Treatment cycle

All couples offered IUI require evidence of at least one patent tube and semen parameters suitable for IUI according to our lab criteria. If there is evidence of spontaneous ovulation (mid-luteal progesterone >30 ng/ml), the couple are initially offered the natural cycle IUI. They are scanned from Days 8–9 of their cycle to ensure correct timing of insemination, and scans are performed every 2–3 days thereafter to track the developing follicle. Once the lead follicle is 17 mm in diameter, either spontaneous ovulation occurs (detected by ovulation predictor kits using early morning urine samples; Assure, Conception Technologies, CA, USA) or human chorionic gonadotrophin is given by subcutaneous injection if necessary. IUI is subsequently performed 24 h after a positive Luteinising Hormone surge on an ovulation predictor kit, or 40 h post-hCG injection.

If the female partner is anovulatory, or following 3–6 failed natural cycles, insemination is performed in conjunction with ovulation induction using either clomiphene (first line) or gonadotrophins (second line).

Our analysis included all cycles performed in the 10-year period and updated the cycles originally included in our preliminary analysis in 2004 (Nicopoulous et al., 2004).

Semen collection and preparation

The process of sperm washing involves centrifuging the ejaculated semen in a 45–90% colloidal silica density gradient to separate progressively motile, HIV-free sperm from the infected NSC and seminal plasma that remain in the supernatant.

Semen samples for insemination are obtained by masturbation into sterile plastic containers after a period of abstinence of at least 3 days and specific precautions as described elsewhere performed to minimise risk to staff and minimal cross-contamination risk to uninfected gametes and embryos (such as handling of samples within a separate high security laboratory) (Gilling-Smith et al., 2005). Samples are allowed to liquefy at room temperature for 30 min prior to analyses. Semen parameters are assessed as outlined by World Health Organisation criteria (WHO, 1992). Most recent serum VL, CD4 count and medication history are confirmed at the time of production of the sample for insemination.

Puresperm (Nicadon, Sweden) solution is diluted to 45–90% using a sperm buffer medium (Cook®; Queensland, Australia) and the solutions are warmed to room temperature in centrifuge tubes before use. The ejaculate is layered over the prepared density gradients and centrifuged at 1200 rpm for 20 min. Following centrifugation, the supernatant is aspirated, and the sperm pellet at the bottom is re-suspended in a fresh medium and centrifuged (‘washed’). This series of ‘washes’ was classically performed three times to maximise the clearance of NSC, before preparation of a final swim-up. From September 2008, a modified protocol of two washes in all, with a swim-up only performed if the patient is not on HAART or the sample is prepared with significant debris (white blood cells etc.), was adopted. This was introduced to maximise the post-wash sperm yield with no increase in the residual virus demonstrated (Vourliotis et al., 2009).

Following the approximate 45 min wash procedure, as a quality control for the procedure, an aliquot of washed sperm (~100 ml) is subsequently tested for detectable HIV RNA prior to the sample being used for treatment. A nucleic acid sequence-based amplification (NASBA; Biomerieux, Basingstoke, Hampshire, UK) was initially used and from June 2007, a ROCHE PCR assay has been performed in view of the improved time-efficiency (45–60 min preparation for PCR compared with 3.5 h on PCR machine). This assay has a detection limit of >25 HIV-1 RNA copies per 10⁶ sperm. From 2004, it became mandatory for couples to freeze a washed, negative sample as a back-up in case residual HIV is found in a post-wash sample that would otherwise necessitate cycle cancellation.

In the lithotomy position, the washed tested sperm is injected using an insemination catheter (Cook, Australia) into the uterine cavity and a pregnancy test is performed 2 weeks later. HIV-testing of the female partner and children born is recommended at 3–6 months after the last treatment and delivery, respectively.
Predictors of success were divided into patient/cycle related, HIV-related and semen-related factors. For comparisons between groups, continuous variables were assessed using the Shapiro–Wilk test of normality to assess distribution. As none of the factors assessed were normally distributed, the non-parametric Mann–Whitney U-test was used to compare groups.

For further analysis of effect on IUI outcome, seminal parameters, CD4 count, VL, duration of infection, use of duration of use of antiretroviral therapy, maternal age, stimulation regime and the number of follicles were also assessed as categorical variables and their effects were assessed using Fisher’s exact test and χ² tests.

The primary outcome was the rate of ongoing pregnancy (OPR) defined as the viable pregnancy beyond 24 weeks gestation (OPR). Secondary outcomes were the rates of hCG-positive pregnancy (PR), defined as a serum or urinary PR test and clinical pregnancy (CPR), defined as viable intrauterine pregnancy confirmed on first-trimester ultrasound scan, and the miscarriage rate (MR), defined as the proportion of positive pregnancies failing to reach 24 weeks gestation.

The predictive value of continuous variables on the pregnancy outcome were further assessed using the receiver operator curve (ROC) analysis, with sensitivity plotted against 1-specificity. The curve shows each possible decision threshold, the percentage of abnormals (cycle failure) correctly diagnosed (i.e. true positives) against the percentage of normals (cycle success) incorrectly diagnosed as abnormal (i.e. false positives) and areas under the curve (AUC) were calculated.

All statistical analysis was performed using analyse it statistical software for Microsoft Excel.

Table I outlines the characteristics of the 245 couples evaluated for treatment over the decade 1999–2008, of whom 151 proceeded to IUI. The majority of referrals came from genitourinary medicine physicians in charge of overall HIV care (63.1%). Of the remainder, 5.0% were self-referrals and 8.6, 8.1, 7.7, 5.9, 5.0 and 1.4% came from tertiary fertility units, general practitioners, haematologists (all for haemophiliacs patients), general gynaecologists and chest physicians, respectively. Of note, 13 of the 19 that came from other fertility centres were diagnosed during the routine work-up for subfertility (5.0% of total cohort).

Only 38.4% of couples referred were London-based, a further 52.7% were from the rest of the UK and 8.9% were from overseas (eight were European, six were African, four were Asian and three were from North America).

The majority of the female partners were nulliparous (53.1%), although 37% had conceived with their current partner, seven of whom had done so following the diagnosis of HIV. Two had undergone donor insemination cycles ending in first-trimester miscarriage, two had conceived following the use of unprotected intercourse (ending in term deliveries, one in a serodiscordant couple and one in a seroconcordant couple), one had undergone sperm washing and IUI in Milan, one had had in vitro fertilization with sperm washing in France (both term deliveries) and one had undergone experimental serum VL monitoring and timed intercourse during periods of apparent undetectable VL (term delivery).

In total, 60 couples had other co-infectious morbidity, most commonly coexisting Hepatitis C in the male partner in 26 couples (16 of whom derived both infections through treatment of Haemophilia) and HIV seroconcordance in 23 couples. The majority of men were unable or unwilling to pinpoint timing/mode of transmission but where they could, a sexual cause predominated in 37.3%. There were 26 men who had been infected haematologically, 22 of whom were haemophiliacs and the remainder had received transfusions for other reasons. Eight men were infected via intravenous drug abuse and infected needle use, three via needle stick injuries, and one suggested possible trauma and exposure at the time of an assault to have caused transmission.

Table I also demonstrates the HIV profiles of the treated male cohort. The mean duration between HIV diagnosis and referral was almost 5.8 years and 66.9% of men were on HAART at referral with a further 6.1% starting at some point during either investigation or treatment within our unit. The majority was well and stable at referral but in 13 couples (5.0% of total cohort).

Table II outlines the aetiological factors, cycle characteristics and outcome of the 439 IUI cycles commenced in 151 couples between 1999–2008 (median rank of attempt—2; range 1–11). Of these cycles, 429 proceeded to insemination. Of the 10 cancelled, 9 were as a consequence of positive post-wash virus prior to the adoption of a mandatory frozen back-up and one as a consequence of a NASBA kit failure over the same time period.
were 34.2 years and 6.7 IU/l, respectively. The majority of treatments distorting the cavity in 7 cycles (1.6%).

17 cycles (3.9%), endometriosis in 16 cycles (3.6%) and fibroids not allowed by anovulation in 47 cycles (10.7%), unilateral tubal factor in the remainder, where a factor was identified, mild male factor subfertility of frozen sperm.

For residual HIV post-wash tests necessitating cancellation or the use of sperm, (and of the remaining 437 cycles, there were 16 (3.7%) positive NASBA* indicates presence of virus particles after washing.

Overall, there were two kit failures (one proceeded to use frozen sperm), and of the remaining 437 cycles, there were 16 (3.7%) positive for residual HIV post-wash tests necessitating cancellation or the use of frozen sperm.

Of the 439 cycles, 326 were performed on couples where no abnormality was found during the fertility work-up (74.3%). Of the remainder, where a factor was identified, mild male factor subfertility (suitable for IUI) occurred most commonly (54 cycles; 12.3%) followed by anovulation in 47 cycles (10.7%), unilateral tubal factor in 17 cycles (3.9%), endometriosis in 16 cycles (3.6%) and fibroids not distorting the cavity in 7 cycles (1.6%).

The mean maternal age and FSH level at the time of insemination were 34.2 years and 6.7 IU/l, respectively. The majority of treatments (56.2%) used a natural cycle with 78.7% of cycles having only one follicle at either natural ovulation or hCG trigger.

Our IUI treatment cycles demonstrated a PR, CPR and OPR per insemination of 14.2, 13.5 and 9.6%, respectively (3.4% multiple pregnancy rate) with no seroconversions demonstrated.

Predictors of cycle success

Patient/cycle related

PRs of 18.1, 13.2, 16.1 and 8.8% and OPRs per insemination of 9.7, 9.2, 13.3 and 1.8% were demonstrated for maternal ages of <30, 30–34, 35–39 and ≥40, respectively (Table III). Although the PR and CPR was not significantly different across the groups, there was a statistically significant decrease in the OPR (10.8 versus 1.8%; P = 0.03) and borderline significant increase in the MR (28.6 versus 80%; P = 0.07) in cycles in women ≥40. However, the low number in the miscarriage groups makes statistical analysis difficult and may explain the relatively high MR in the low age group (≤30) and the relatively low MR in the 35–40 age group.

Table III also shows the pregnancy outcome when cycles are divided into quartiles by FSH levels, demonstrating a significant decrease in the OPR and an increase in the MR across the quartiles with increased FSH (P = 0.02 and P = 0.03, respectively; χ²-test). Similarly, there was a significant increase in the OPR (12.7 versus 6.2%; P = 0.03) and a decrease in the MR (21.2 versus 50.1%; P = 0.04) using the cut-off for the mean cycle maternal FSH of <6.4 IU/l.

There was no significant difference in any outcome according to the cycle regime (OPR of 11.2 and 8.5% for natural versus stimulated cycles, respectively) or number of follicles (10.5 versus 8.1% for 1 versus >1, respectively). Although the OPR was lower in those cycles where an additional fertility factor was identified (6.8 versus 10.9%), this difference failed to reach statistical difference. Dividing by aetiology, there was a 7.4% (4/54), 6.4% (3/47), 6.3% (1/16) and 0% (0/17) OPR for mild male factor, anovulatory, endometriosis and tubal factor. Similarly, there was no impact of paternal age or rank of attempt on the outcome (OPR of 12.8, 6.9, 11.4 and 7.7% for the first, second, third or fourth or more attempts).

HIV related

Table IV demonstrates the pregnancy outcome according to HIV parameters. Cycles are divided into quartiles by the CD4 count with no significant difference across the four groups (χ²-test P > 0.05) or comparing the individual groups (Fisher’s exact test; all P > 0.05) for the primary outcome of OPR or any secondary pregnancy outcomes. Similarly, using the median CD4 as a cut-off (450 cells/mm³), there was no difference in the OPR (11.2 versus 9.2%).

PR (13.7 versus 15.5%), CPR (12.1 versus 15.1%), OPR (9.7 versus 10.1%) and MR (29.4 versus 34.9%) were similar in cycles where the HIV-positive partner had detectable or undetectable VL, respectively.

Similarly, there was no significant difference in PR (12.5 versus 14.9%), CPR (11.5 versus 14.2%) and the OPR (10.6 versus 9.2%) between those off and on HAART, respectively. Although not reaching statistical significance, the MR was higher in those using HAART (15.4 versus 38.3%).

Semen related

Pre-preparation semen volume (3.2 versus 2.6 ml), total sperm count (183.5 versus 149.8 million), progressive motility (49.7 versus 44.8%),
post-wash concentration (15.7 versus 12.3 million/ml), progressive motility (89.1 versus 77.8%) and TMCI (7.2 versus 5.4 million) were all significantly higher in successful cycles compared with unsuccessful cycles (Mann–Whitney test, all \( P < 0.05 \)).

Using WHO reference values as cut-offs, the primary outcome of OPR was higher with concentrations \( > 20 \) million/ml (10.5 versus 7.3%), total counts \( > 40 \) million (10.7 versus 5.7%), progressive motility \( > 50\% \) (15.2 versus 7.7%) or with abnormal morphology \( > 85\% \) (9.8 versus 8.3%), but these increases did not reach clinical significance. However, both PR and CPR were significantly higher using the concentration (CPR 15.6 versus 8.3%), count (15.3 versus 7.6%) and motility cut-offs (23.2 versus 9.4%).

Using median values across the cycle cohort, there was a significant increase in the OPR with concentrations \( > 55 \) million/ml (14.3 versus 8.1%) and total counts \( > 130 \) million (15.7 versus 5.7%).

OPRs of 12.1, 12.3, 9.9 and 4.3% were demonstrated for TMCI values (post-preparation volume \( \times \) concentration \( \times \) percentage motile/100) of \( > 10, 5–10, 2–5 \) and \( < 2 \) million, respectively, with a significant reduction in the OPR with TMCI \( < 2 \) million (4.3 versus 11.2%, \( P = 0.04 \)).

Frozen sperm was used in 77 of 429 inseminations performed (17.9%), as a consequence of either a positive post-wash PCR test or couple preference (as a consequence of either geographical difficulties, social commitments or difficulties in producing a sample on the day of insemination). The PR (16.7 versus 3.9%; \( P = 0.003 \)), CPR (15.8 versus 3.9%; \( P = 0.005 \)) and OPR per insemination (11.2 versus 2.6%; \( P = 0.02 \)) were all significantly higher in cycles performed using fresh sperm. TMCI was also significantly higher in the fresh cycles (mean/median of 6.3/4.5 and 2.9/1.6, respectively).

**Receiver operator curve analysis**

ROC analysis demonstrated AUC of 0.55 (95% confidence interval (CI) 0.46–0.63), 0.56 (95% CI 0.47–0.65) and 0.58 (95% CI 0.50–0.66) for

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**Table III** Patient/cycle factors as predictors of insemination outcome.

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>72</td>
<td>18.1% (13)</td>
<td>15.3% (11)</td>
<td>9.7% (7)</td>
<td>46.2% (6/13)</td>
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<tr>
<td>30–34</td>
<td>152</td>
<td>13.2% (20)</td>
<td>13.2% (20)</td>
<td>9.2% (14)</td>
<td>30.0% (6/20)</td>
</tr>
<tr>
<td>35–39</td>
<td>143</td>
<td>16.1% (23)</td>
<td>15.4% (22)</td>
<td>13.3% (19)</td>
<td>17.4% (4/23)</td>
</tr>
<tr>
<td>≥40</td>
<td>57</td>
<td>8.8% (5)</td>
<td>8.8% (5)</td>
<td>1.8% (1)</td>
<td>80.0% (4/5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum FSH (IU/l)</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5.1</td>
<td>102</td>
<td>13.7% (14)</td>
<td>12.7% (13)</td>
<td>8.8% (9)</td>
<td>35.7% (5/14)</td>
</tr>
<tr>
<td>5.2–6.4</td>
<td>102</td>
<td>18.6% (19)</td>
<td>18.6% (19)</td>
<td>16.7% (17)</td>
<td>10.5% (2/19)</td>
</tr>
<tr>
<td>6.5–7.6</td>
<td>105</td>
<td>12.4% (13)</td>
<td>12.4% (13)</td>
<td>7.6% (8)</td>
<td>38.5% (5/13)</td>
</tr>
<tr>
<td>≥7.7</td>
<td>106</td>
<td>12.3% (13)</td>
<td>10.4% (11)</td>
<td>4.7% (5)</td>
<td>61.5% (8/13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated</td>
<td>177</td>
<td>15.3% (27)</td>
<td>14.1% (25)</td>
<td>8.5% (15)</td>
<td>44.4% (12/27)</td>
</tr>
<tr>
<td>Natural cycle</td>
<td>224</td>
<td>14.7% (33)</td>
<td>14.3% (32)</td>
<td>11.2% (25)</td>
<td>24.2% (8/33)</td>
</tr>
<tr>
<td>1 Follicle</td>
<td>315</td>
<td>14.6% (46)</td>
<td>14.3% (45)</td>
<td>10.5% (33)</td>
<td>28.3% (13/46)</td>
</tr>
<tr>
<td>&gt;1 Follicle</td>
<td>86</td>
<td>16.2% (14)</td>
<td>14.0% (12)</td>
<td>8.1% (7)</td>
<td>50.0% (7/14)</td>
</tr>
</tbody>
</table>

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**Table IV** HIV factors as predictors of insemination outcome.

<table>
<thead>
<tr>
<th>CD4 count (cells/mm³)</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;320</td>
<td>91</td>
<td>16.5% (15)</td>
<td>15.4% (14)</td>
<td>11.0% (10)</td>
<td>33.3% (5/15)</td>
</tr>
<tr>
<td>320–450</td>
<td>96</td>
<td>12.5% (12)</td>
<td>12.5% (12)</td>
<td>11.5% (11)</td>
<td>8.5% (1/12)</td>
</tr>
<tr>
<td>450–620</td>
<td>102</td>
<td>15.7% (16)</td>
<td>15.7% (16)</td>
<td>8.8% (9)</td>
<td>43.8% (7/16)</td>
</tr>
<tr>
<td>≥620</td>
<td>97</td>
<td>16.5% (16)</td>
<td>14.4% (14)</td>
<td>9.3% (9)</td>
<td>43.8% (7/16)</td>
</tr>
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<table>
<thead>
<tr>
<th>Viral load (copies/ml)</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
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<tbody>
<tr>
<td>Undetectable</td>
<td>278</td>
<td>15.5% (43)</td>
<td>15.1% (42)</td>
<td>10.1% (28)</td>
<td>34.9% (15/43)</td>
</tr>
<tr>
<td>Detectable</td>
<td>124</td>
<td>13.7% (17)</td>
<td>12.1% (15)</td>
<td>9.7% (12)</td>
<td>29.4% (5/17)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HAART treatment</th>
<th>No. of cycles</th>
<th>PR/insemination (%)</th>
<th>CPR/insemination (%)</th>
<th>OPR/insemination (%)</th>
<th>Miscarriage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>316</td>
<td>14.9% (47)</td>
<td>14.2% (45)</td>
<td>9.2% (29)</td>
<td>38.3% (18/47)</td>
</tr>
<tr>
<td>No</td>
<td>104</td>
<td>12.5% (13)</td>
<td>11.5% (12)</td>
<td>10.6% (11)</td>
<td>15.4% (2/13)</td>
</tr>
</tbody>
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maternal/cycle factors of female age, rank of attempt and female serum FSH. AUC of 0.52 (95% CI 0.42–0.62), 0.53 (95% CI 0.44–0.62) and 0.59 (95% CI 0.49–0.7063) were demonstrated for HIV factors of the CD4 count, years since diagnosis and years on HAART. The CIs of all of these factors crossed 0.50, the point at which the predictive ability of a parameter is no better than chance alone.

AUC of 0.58 (95% CI 0.49–0.67), 0.60 (95% CI 0.51–0.69), 0.61 (95% CI 0.52–0.69), 0.62 (95% CI 0.53–0.72), 0.65 (95% CI 0.56–0.74) and 0.66 (95% CI 0.58–0.75) were demonstrated for seminal factors of concentration, post-preparation concentration, TMCI, post-preparation motility, pre-preparation progressive motility and pre-preparation total count, respectively. Fig.1 demonstrates the ROC for the four parameters with best predictive value. There was no significant difference in AUC between these four parameters.

Discussion

We have demonstrated epidemiological and outcome data for a decade of provision of sperm-washing treatment and demonstrated, in contrast to early data, that in conjunction with female age, seminal parameters but not markers of HIV disease are important predictors of success for insemination cycles.

Despite the increased demand and continued cycle success, consistent with both (HFEA, 2007) and ESHRE data (Nyboe Andersen et al., 2009), some have questioned the role of sperm-washing in the management of HIV-positive men. The risk of HIV transmission has long been known to correlate with plasma VL and diminishes with HAART. Extrapolations from epidemiological and biological data led Swiss researchers to suggest that ‘HIV-positive individuals without additional sexually transmitted diseases and on effective antiretroviral therapy are sexually non-infectious’ (Vernazza et al., 2008).

Although biological data support the view that an undetectable VL can also be achieved in genital secretions when the serum VL is undetectable, it is clear that any correlation is weak (Kalichman et al., 2008) and compartmentalisation of virus as a possible consequence of a lack of penetrance of certain antiretrovirals into the genital compartment or the effect of co-existing infections is reported (de Ruiter et al., 2008).

A total of 293 of the 439 insemination cycles in our unit were performed on men with undetectable VL, of which 283 were confirmed to be on antiretroviral therapy. Of these, if we analyse the 186 seminal samples provided for fresh treatment cycles where complete data are available for both pre- and post-wash VL, 18 (9.7%) were found to have demonstrable virus (370–18 000 copies/ml) (Nicopoullos et al., 2009). Although reproductive counselling couples should include the option of natural conception, outlining the risks of timed unprotected intercourse where serum VL is detectable (Mandelbrot et al., 1997) and the lack of confirmation of safety with undetectable VL, our findings, that even in the healthiest cohort of HIV-positive men (undetectable VL on HAART) almost 10% have detectable seminal virus, do not support the statement that they are ‘sexually non-infectious’. We therefore continue to advocate sperm washing to maximise risk-reduction.

In addition to the continued minimisation of risk, continued efforts to maximise cycle outcome remain paramount. Despite our early findings of the role of HIV parameters such as VL, CD4 count and use of

Figure 1 ROC analysis for cycle outcome prediction. ROC analysis plotting sensitivity against 1-specificity for potential predictors of insemination outcome. Line of ‘no discrimination’ equates to area under curve (AUC) of 0.5 with improved predictive capability with increasing AUC. Predictors analysed as described in key.
HAART on the cycle outcome (Nicopoulos et al., 2004), re-analysis of a decade of treatment data now suggest that seminal parameters are the most important predictors of success.

Although ROC analysis did not suggest maternal age as a valuable overall predictor of the cycle outcome per se (AUC near 0.50), it is clear that IUI outcome in our cohort is significantly impaired at an age of over 40 (though it is stable below 40) as a consequence of both an impaired clinical pregnancy rate and increased MR. This likely effect of impaired ovarian reserve and oocyte quality is supported by similar impairment in pregnancy and increased miscarriage with increased FSH levels.

The lack of an increase in pregnancy outcome with ovarian stimulation or increased follicle number supports our current practice of initially offering couples the natural cycle IUI, unless they are anovulatory. Excluding cycles in anovulatory women, the outcome remains similar in cycles with 1 or >1 follicles and this also suggests that if no pregnancy is achieved after 3–6 cycles, it may be prudent to discuss the option of IVF with the couples rather than proceeding with ovulation induction. The exact number of cycles before considering further methods of assisted reproduction will depend on patient and clinician choice and availability of funding.

ROC analysis demonstrated the seminal parameters progressive motility, total count, post-preparation progressive motility and TMCI to have the highest predictive ability with no significant difference in the AUC between the four. This is supported by the significantly higher seminal parameters in cycles with an ongoing pregnancy. However, the AUCs of <0.8 demonstrated a limited clinical diagnostic value.

A lack of effect of HIV parameters on outcome may be explained by their effect on these semen parameters. Although, we have reported a positive and significant correlation between the CD4 count and raw sperm count, motility and morphology and post-wash concentration and total motile sperm count available for insemination (TMCI), no sperm parameters were significantly different between those with detectable or undetectable VL (despite significantly lower CD4 counts in the former) (Nicopoulos et al., 2009). This is a likely consequence of the detrimental effect of the use of HAART on sperm, supported by a negative correlation between duration of use and semen parameters. Therefore, any potential benefit of improved general health and CD4 count on seminal parameters and outcome is likely to be offset by the negative impact of HAART.

It would therefore be inappropriate to make recommendations with regard to the management of HIV disease (e.g. timing of antiretrovirals etc.) with a view to improving the outcome of fertility treatment. Disease control remains a paramount concern and appropriate management decisions should remain with the patient and gentitourinary medicine physicians. Future analysis that delineates the effects of different antiretrovirals on semen parameters and outcome may aid in decision-making.

In view of the effect of TMCI on ongoing pregnancy, mechanisms to improve the seminal parameters would be the more appropriate way forward and have already led to the adoption of the altered lab protocol to incorporate two rather than three wash cycles following density gradient centrifugation with a swim-up only required in insemination cycles when the man is not on HAART or has a detectable VL or when the sample prepares with significant debris. An analysis of cycles before and after the protocol change has demonstrated a significantly higher TMCI with the new protocol (8.6 versus 4 million sperm) and demonstrates that post-wash a significantly higher proportion of the raw total motile sperm remain available for transfer (21.6 versus 14.0%) (Vourliotis et al., 2009).

Similarly, the poor outcome in the 17.9% of cycles performed using frozen sperm, not previously demonstrated in insemination cycles, is a consequence of the effect of the wash/test process prior to freezing followed by the freeze/thaw on the TMCI. Therefore, mechanisms to encourage the use of fresh sperm wherever possible such as the use of GnRH antagonists to delay ovulation may further improve the cycle outcome.

In conclusion, we believe that sperm-washing remains the safest reproductive option for sero-discordant couples where the male is HIV-positive regardless of VL and HAART regime. Our analysis of clinical correlates of success demonstrates a detrimental effect on ongoing pregnancy of maternal age >40, suggests that IVF rather than IUI with ovarian stimulation may be more appropriate after multiple failed natural cycles (and in those over 40) and confirms that seminal parameters rather than markers of HIV disease best predict cycle outcome. When semen preparation leaves <2 million total motile sperm available for transfer, despite new laboratory protocols, the couple should be recommended to proceed to IVF/ICSI on future attempts and where possible the use of frozen samples should also be limited to IVF/ICSI where seminal parameters have less impact on outcome.

References


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