Male subfertility and assisted reproduction: the quest for the ultimate treatment strategy
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IUI in male subfertility: are we able to select the proper patients?

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Abstract

There is at this time no indication as to which semen parameters from the fertility workup discriminate between couples with male subfertility who will and will not benefit from intrauterine insemination (IUI). This study evaluated the predictive capacity of semen parameters (both pre- and post-wash) and antisperm antibodies (ASA) obtained during the fertility workup on IUI outcome in couples with male subfertility in a retrospective cohort study. It included 290 couples, who underwent 722 IUI cycles. The overall ongoing pregnancy rate was 9% per cycle. Model I, with female age, duration of subfertility, secondary subfertility, the presence of anovulation, cervical hostility and cycle number had an area under the curve (AUC) of 0.59. Adding the presence of ASA to this model improved the AUC to 0.65 (model II). Further addition of the post-wash total motile count (TMC) to the model with ASA (model III) improved the AUC to 0.67. Using the models to exclude couples from IUI due to low expected pregnancy rates would increase the pregnancy rate to 11% per cycle with model I and to 14% per cycle for model II and for model III. In conclusion, in the selection of patients with male subfertility for IUI, the use of prediction models including ASA can increase the efficiency of IUI.

**Key-words:** intrauterine insemination, male subfertility, ongoing pregnancy, semen parameters, prognostic factors.
Introduction

Decision making and patient counseling in reproductive medicine for either intrauterine insemination (IUI), in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in male subfertility is often difficult. The various semen parameters that are combined in the standard semen analysis (i.e. sperm count, motility, morphology, total motile count) and the semen parameters after processing have been extensively investigated as to their relationship with IUI outcome

Most studies however, report on semen parameters at the time of the actual insemination, which obviously eliminates these semen parameters as a tool for counselling before the start of treatment. The studies that report on the semen parameters during fertility work-up lack a multivariable approach which takes into account all variables known to influence IUI outcome. This limits their use in daily clinical practice.

At present, there are no studies on IUI in male subfertility that provide data to aid the clinician in identifying those couples that will benefit from IUI and couples that will not. We therefore initiated the present study to evaluate the prognostic and clinical value of all parameters obtained during the fertility work-up in a cohort study among consecutive couples undergoing IUI for male factor subfertility. We specifically investigated the additional value of presence of ASA and the post-wash total motile count (post-wash TMC) on baseline characteristics of the couple.

Material and methods

Patients

The study included data from all couples diagnosed with male subfertility that underwent IUI between January 1997 and December 2004 in the Academic Medical Centre, Amsterdam, The Netherlands. Male subfertility was defined as more than one semen analysis that did not meet the WHO criteria for concentration, motility and/or morphology (i.e. concentration of \( \leq 20 \times 10^6/\text{ml} \), progressive motility of \( \leq 50\% \), and \( \leq 30\% \) spermatozoa with normal morphology) \(^{13}\). Couples undergoing IUI with heterologous semen were excluded from the study. All couples had been trying to conceive for at least 12 months. Ovulation was assessed using a basal body temperature curve, mid-luteal progesterone concentration and/or transvaginal sonography. Tubal patency was assessed by hysterosalpingography.
and/or laparoscopy. Couples were considered to be candidates for treatment with IUI if the woman was ovulatory, with or without ovulation induction, and if she had at least one patent tube, either at hysterosalpingography or at laparoscopy. All male patients had at least two semen analyses during the fertility work-up. Similar to other authors, the mean for sperm concentration, motility and morphology of all the semen analyses per patient was used for statistical evaluation $^{10,14,15}$. In all patients, a post-wash TMC was performed during the fertility work-up. Until 2001 it was standard procedure not to perform IUI when there were less than $1 \times 10^6$ motile spermatozoa in the ejaculate after preparation. In 2001 the cut-off value was changed to more than $3 \times 10^6$ motile spermatozoa for eligibility for IUI $^{16}$. General patient information collected included patient's age, subfertility being either primary or secondary, subfertility diagnosis, duration of subfertility, ovulatory status and semen parameters. Cycle-specific information that could be obtained before the start of an IUI cycle included the use of ovarian stimulation, the type of stimulation and cycle number. An ongoing pregnancy, defined as positive fetal cardiac activity of at least one fetus at 12 weeks gestation, was considered as the primary outcome of this study.

**IUI protocol**

Intrauterine insemination was performed in spontaneous cycles as well as in hyperstimulated cycles. From January 1997 to December 1998 patients underwent ovarian stimulation using clomiphene citrate as the standard protocol. From January 1999 ovarian stimulation was performed with recombinant-FSH, follitropine (Puregon®, Organon, Oss, The Netherlands or Gonal-F®, Serono Benelux BV, the Hague, The Netherlands). Follicle growth was monitored by transvaginal sonography in all patients during the IUI cycle. In case of IUI in a spontaneous cycle, the endogenous luteinizing-hormone surge was detected by a urinary semi quantitative monoclonal antibody kit (OvuQuick, Quid San Diego, CA, USA). Semen was inseminated 20-30 hours after this detection. Human chorionic gonadotropin (Pregnyl, Organon, Oss, The Netherlands) was administered in a single dose of 10,000 IU when one follicle had at least a diameter of 18mm and no LH surge was detected in the urine. Semen was inseminated 40 hours later. A total of 0.3 ml suspension of processed spermatozoa was introduced into the uterine cavity with a catheter (International Medical, Zutphen, The Netherlands). The cycle was canceled if more than 3 follicles of $\geq 16mm$ were present.
Semen analysis and processing

Semen analysis was performed according to WHO guidelines. Anti-sperm antibodies on the surface of the spermatozoa in the ejaculate were detected by the mixed agglutination reaction (MAR), with a cut-off for a positive test result, i.e. positive ASA, of 10% of spermatozoa with anti-sperm antibodies on the surface.

Patients had a minimal sexual abstinence of two days and analysis of the semen was performed within 1 hour of ejaculation. Data on the maximum sexual abstinence period were not obtained, since that was not a routine question in our semen analysis protocol. After liquefaction, volume, concentration and motility were determined. Semen was diluted 1:1 with culture medium (Ham’s-F10 (Invitrogen, Breda, The Netherlands) or Human Tubal Fluid (Cambrex, Verviers, Belgium) supplemented with pasteurized plasma protein solution (Sanquin, Amsterdam, The Netherlands) and subjected to density gradient centrifugation using 70% Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) or 70% PureSperm (Nidacon, Gothenburg, Sweden) (650x g for 10 min.). Then the pellet was washed once with culture medium (180x g for 10 min.) and, depending on the sperm concentration, resuspended in 1-2 ml of culture medium. The sample was then incubated for 1h at 37 °C / 5 % CO₂ during which the motile spermatozoa were allowed to swim to the bottom of the tube (“swim-down”). Finally, the pellet was washed again (180x g for 10 min.) and the volume, concentration and motility were assessed and the post-wash TMC was calculated. All measurements were performed with the Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel).

Data analysis

All analyses were performed at cycle level, i.e. each cycle was considered as a separate unit of analysis. All cycles that resulted in an insemination were included in the analysis. For each of the continuous variables, female age, male age, duration of subfertility and the semen parameters, we assessed whether the association with the occurrence of pregnancy was linear or not. In each of these continuous variables we constructed a spline function to assess the association between the variables and the occurrence of ongoing pregnancy. A spline function expresses the probability of pregnancy as a function of the continuous variable and is constructed using logistic regression analysis. Based on these spline functions, linearity was assessed and in absence of linearity the continuous variables were redefined where necessary, taking into account the
association between these variables and the occurrence of pregnancy.

Subsequently, we described the potential prognosticators for IUI outcome, and assessed the association between these characteristics and the ongoing pregnancy rate in a univariable analysis by calculating odds ratios and 95% confidence intervals (CI). We used logistic regression analysis to evaluate whether the presence of ASA or the post wash TMC during fertility work-up could improve the prognostic capacity of the couples’ baseline characteristics to predict a pregnancy occurring after IUI. To do so, we first developed a logistic regression model incorporating the relevant clinical characteristics of the couple, i.e. female age, male age, duration of subfertility, primary or secondary subfertility, presence of tubal pathology or anovulation, and semen parameters and the cycle-specific characteristics that were available before the actual start of the IUI cycle, i.e. cycle number and the method of ovarian hyperstimulation.

To prevent erroneous exclusion of potential prognosticators, all factors with a p-value of <0.30 after stepwise logistic regression were included in the final model. The model performance was assessed by construction of a Receiver Operating Characteristic (ROC)-curve and calculation of the accessory area under the curve (AUC). We then added the presence of ASA (model II) and subsequently the post-wash TMC (model III) to this model and repeated the ROC-analysis. The three ROC-curves were compared for statistically significant differences using a non-parametric approach. The reliability of the models was estimated with the Hosmer-Lemeshow test for goodness-of-fit.

Eventually, we performed internal validation with bootstrapping to reduce the overfit of the created models. Bootstrapping is a technique to create equal virtual populations. We bootstrapped 200 times and in each of these new datasets the same multivariable logistic regression was assessed. A shrinkage factor was calculated by analyzing the differences of the prognostic models.

To assess the clinical value of the presence of ASA and the post-wash TMC during the fertility work-up we calculated the predicted pregnancy rates of the prediction model with ASA and the predicted pregnancy rates of the prediction model with ASA and the post-wash TMC and compared them to the observed pregnancy rates. We plotted these predicted pregnancy rates and assessed whether the addition of the post-wash TMC influenced the predictive capacity of model II.

Finally, we assessed the efficiency of these models, i.e. which prediction model combined the optimal pregnancy rate with the lowest exclusion of couples
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For IUI at the lowest cycles needed to perform for an ongoing pregnancy. The efficiency of the three models was tested on the same patient data set.

Results

We included 290 couples who underwent 722 consecutive cycles: 66 couples underwent only one cycle of IUI, 60 underwent two cycles, 54 three cycles, 43 four cycles, 32 five cycles, 23 six cycles, five seven cycles, five eight cycles and two nine cycles. Among these 722 cycles a total of 81 clinical pregnancies occurred, resulting in 67 ongoing pregnancies. The other 14 clinical pregnancies resulted in 12 miscarriages (15%) and two ectopic pregnancies. This represented an ongoing pregnancy rate per inseminated cycle of 9% and an ongoing pregnancy rate per couple of 23%. Among the ongoing pregnancies there were seven twin pregnancies and two triplet pregnancies, i.e. 13% of all ongoing pregnancies were multiple pregnancies.

All continuous variables, except the post-wash TMC during the fertility work-up, showed a linear relationship with the ongoing pregnancy rate occurring after IUI. The post-wash TMC showed a positive linear association with the ongoing pregnancy rate up to a cut-off value of 6 million. Above this cut-off value there was no association between the post-wash TMC and ongoing pregnancy (Figure 1). As a result of this spline analysis, the post-wash TMC was redefined as a continuous variable up to 6 million, whereas all values > 6 million were defined as 6 million.

Figure 1 Relationship between post-wash TMC and ongoing pregnancy rate in couples undergoing IUI for treatment of male subfertility.

The baseline characteristics of the patients are listed in Table 1.
<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics and uni- and multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline patient characteristics</strong></td>
</tr>
<tr>
<td><strong>Univariable analysis</strong></td>
</tr>
<tr>
<td>OR</td>
</tr>
<tr>
<td>Female age</td>
</tr>
<tr>
<td>Male age</td>
</tr>
<tr>
<td>Secondary subfertility</td>
</tr>
<tr>
<td>Duration of subfertility</td>
</tr>
<tr>
<td><strong>Semen characteristics before preparation</strong></td>
</tr>
<tr>
<td>Volume (ml)</td>
</tr>
<tr>
<td>Total sperm count (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
</tr>
<tr>
<td>Normospermia (%)</td>
</tr>
<tr>
<td>Total Motile Count (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Anti-sperm antibodies (ASA)</td>
</tr>
<tr>
<td><strong>Semen characteristics after preparation</strong></td>
</tr>
<tr>
<td>Total sperm count (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
</tr>
<tr>
<td>Total Motile Count 6x10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Female subfertility diagnosis</strong></td>
</tr>
<tr>
<td>Tubal subfertility</td>
</tr>
<tr>
<td>Anovulation</td>
</tr>
<tr>
<td>Cervical hostility</td>
</tr>
<tr>
<td><strong>IUI cycle information</strong></td>
</tr>
<tr>
<td>Cycle number</td>
</tr>
<tr>
<td>Recombinant FSH</td>
</tr>
<tr>
<td>Clomiphene citrate</td>
</tr>
<tr>
<td>No stimulation</td>
</tr>
</tbody>
</table>

OR= odds ratio CI=confidence interval; <sup>a</sup> continuous from 1-6 million; <sup>b</sup> P<0.30
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In the univariable analysis none of the baseline characteristics was statistically significant associated with an ongoing pregnancy. From the pre-wash semen parameters only the presence of ASA was significantly associated with the occurrence of pregnancy (OR 2.8 95% CI 1.5-4.9). The MAR test detected ASA in 33 couples who underwent 102 cycles of IUI. The average percentage of bound spermatozoa was 54% (20%-100%). In the group of patients with ASA, there was no association between increasing percentages of bound sperm and IUI outcome (data not shown).

The post-wash TMC during the fertility work-up also showed a positive relationship with an ongoing pregnancy, although not statistically significant (OR 1.1 95% CI 0.96-1.4).

In the multivariable analysis none of the baseline characteristics reached statistical significance. Again, presence of ASA was the only variable that showed a significant association with an ongoing pregnancy (OR 3.0 95% CI 1.6-5.5). The fertility work-up model that was constructed (model I) with stepwise multivariable logistic regression contained female age, secondary subfertility, duration of subfertility, the presence of anovulation and/or cervical hostility, cycle number and the absence of ovarian hyperstimulation and had an area under the ROC-curve of 0.59 (95% CI 0.52-0.66) (Table 2).

<table>
<thead>
<tr>
<th>Model</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.59</td>
<td>0.52-0.66</td>
</tr>
<tr>
<td>II</td>
<td>0.65</td>
<td>0.58-0.72</td>
</tr>
<tr>
<td>III</td>
<td>0.67</td>
<td>0.60-0.74</td>
</tr>
</tbody>
</table>

* a difference between the AUC’s model I and model II: 0.06 95% CI -0.01 to 0.12
* b difference between the AUC’s model II and III: 0.02 95% CI -0.01-0.05
* c difference between the AUC’s model I and III: 0.08 95% CI 0.02 to 0.15

When we added ASA to the model (model II), the AUC improved to 0.65 (95% CI 0.58-0.72), a difference that was not statistically significant (0.06 95% CI -0.01 to 0.12). Further addition of the post-wash TMC during the fertility work-up (model III) improved the AUC (0.67 95% CI 0.60-0.74). This improvement was statistically significant when compared to the AUC of model I (0.08 95% CI 0.02 to 0.15), but not when compared to model II (0.02 95% CI
In order to check for possible bias with the analysis of multiple cycles, we also limited our analysis to first cycles only. In that analysis we found slightly better AUC’s: Model I 0.63 (95% CI 0.52-0.74), model II 0.66 (95% CI 0.55-0.78), model III 0.70 (95% CI 0.59-0.80). These AUC’s were not statistically significantly different from the AUC’s from the multiple cycles analysis. The goodness-of-fit tests (Hosmer-Lemeshow) were 0.29, 0.63 and 0.43 for the three models, respectively, indicating a good overall goodness of fit for all models. Internal validation by bootstrapping showed 18% overfitting of model I, 20% overfitting of model II and 16% overfitting of model III, indicating that shrinkage factors of 18%, 20% and of 16%, respectively, should be applied. Further calculations were done after correcting for the overfitting of the models.

**Additional value ASA**

We investigated the additional value of ASA using a different approach. We plotted the predicted probabilities for an ongoing pregnancy based on model I, versus the predicted probabilities for an ongoing pregnancy based on model II. From this plot, it is possible to calculate a change in clinical management that is caused by the addition of ASA to the diagnostic work-up if one assumes that the probability of an ongoing pregnancy in an IUI program has to be higher than 10% per cycle.

From Figure 2, we can derive that model I predicted a probability of pregnancy above 10% in 273 cycles (38% of all cycles).

The observed pregnancy rate in these 273 cycles was 11%. Addition of ASA did not alter the predicted rate in 178 (25% of all cycles) of the 273 cycles, and the observed pregnancy rate in these 178 cycles was 13%. In the remaining 95 cycles (13% of all cycles) that had a predicted probability above 10% with model I, addition of ASA caused a decrease in the predicted probability towards < 10%. The observed pregnancy rate in this group was 8% per cycle. The addition of ASA identified a subgroup of patients that would not benefit from IUI if model I would have predicted an ongoing pregnancy rate >10% per cycle.

In the 437 cycles (61% of all cycles), in which model I predicted a probability of an ongoing pregnancy < 10% per cycle, the observed pregnancy rate was 8%. Addition of ASA did not change this probability in 369 cycles to >10%, and the observed pregnancy rate in these cycles was 6% per cycle. In the remaining 68 cycles (9% of cycles) which had a low predicted probability with model I, model II predicted a pregnancy rate >10%. The observed pregnancy rate in this group was 18% per cycle, indicating that the addition of ASA was
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helpful in identifying a subgroup that could still benefit from IUI if model I predicted <10% per cycle.

Figure 2 Predicted probabilities

Correlation between predicted probability of an ongoing pregnancy between the model without ASA (Model I) and the model with ASA (Model II), after correcting with the shrinkage factor. Cycles with an ongoing pregnancy (solid circles); cycles without an ongoing pregnancy (open circles). Numbers indicate the number of cycles in each quadrant and the number of pregnancies in each quadrant, while percentages indicate the observed rate of an ongoing pregnancy in each quadrant.

Additional value of the post-wash TMC

Although the post-wash TMC did not increase the AUC of model II, it did increase the AUC of model I. So we investigated the additional value of the post-wash TMC using a different approach. We plotted the predicted probabilities for an ongoing pregnancy based on model II, versus the predicted probabilities for an ongoing pregnancy based on model III. From this plot, it is possible to calculate a change in clinical management that is caused by the addition of the post-wash TMC to the diagnostic work-up if one assumes that the probability of an ongoing pregnancy in an IUI program has to be higher than 10% per cycle.

From Figure 3, we can derive that model II predicted a probability of pregnancy above 10% in 246 cycles (34% of all cycles).

The observed pregnancy rate in these 246 cycles was 14%. Addition of the post-wash TMC did not alter the predicted rate in 219 (90%) of the 246 cycles, and the observed pregnancy rate in these 219 cycles was 16%. In the remaining 27 cycles (4% of all cycles) that had a predicted probability above
10% with model II, addition of the post-wash TMC caused a decrease in the predicted probability towards < 10%. The observed pregnancy rate in this group was 3.7% per cycle. Although this would cause a more accurate change in allocation to a group with a poor prognosis, only 4% of cycles, i.e. 13 couples, would benefit from the addition of the post-wash TMC.

Figure 3 Predicted probabilities
Correlation between predicted probability of an ongoing pregnancy between the model with ASA (Model II) and the model with ASA and the post-wash TMC (Model III), after correcting with the shrinkage factor. Cycles with an ongoing pregnancy (solid circles); cycles without an ongoing pregnancy (open circles). Numbers indicate the number of cycles in each quadrant and the number of pregnancies in each quadrant, while percentages indicate the observed rate of an ongoing pregnancy in each quadrant.

In the 464 cycles (66% of all cycles), in which model II predicted a probability of an ongoing pregnancy < 10% per cycle, the observed pregnancy rate was 6%. Addition of the post-wash TMC did not change this probability in 441 cycles to >10%, and the observed pregnancy rate in these cycles was 6% per cycle. In the remaining 23 cycles (5% of cycles) which had a low predicted probability with model II, model III predicted a pregnancy rate >10%. The observed pregnancy rate in this group was 4% per cycle, indicating that the addition of the post-wash TMC in this subgroup was not helpful.

Efficiency of the models
The data derived from figure 2 and 3 were used to construct Table 3 where all three models and the selective use of model III (in case model II
predicted a chance of an ongoing pregnancy >10% and model III predicted a chance of an ongoing pregnancy <10%) were incorporated.

Table 3 Cycles needed to perform

<table>
<thead>
<tr>
<th>Model</th>
<th>Couples treated</th>
<th>Couples not treated (%)</th>
<th>Number of Pregnancies</th>
<th>IUI Cycles performed</th>
<th>Pregnancy rate per cycle</th>
<th>Pregnancy rate per couple</th>
<th>CNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No model</td>
<td>290</td>
<td>0</td>
<td>67</td>
<td>722</td>
<td>9%</td>
<td>22%</td>
<td>11</td>
</tr>
<tr>
<td>Model I</td>
<td>118</td>
<td>172 (60)</td>
<td>32</td>
<td>279</td>
<td>11%</td>
<td>27%</td>
<td>8.7</td>
</tr>
<tr>
<td>Model II</td>
<td>99</td>
<td>191 (66)</td>
<td>35</td>
<td>246</td>
<td>14%</td>
<td>35%</td>
<td>7</td>
</tr>
<tr>
<td>Model III</td>
<td>92</td>
<td>198 (68)</td>
<td>35</td>
<td>242</td>
<td>14%</td>
<td>38%</td>
<td>6.9</td>
</tr>
<tr>
<td>Selective</td>
<td>86</td>
<td>204 (70)</td>
<td>34</td>
<td>219</td>
<td>16%</td>
<td>40%</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Couples treated= couples counselled for IUI
Couples not treated=couples not counselled for IUI
CNP=cycles needed to perform

The efficiency of all four models was analyzed. If no prediction model was applied, i.e. selection of couples was based on our inclusion criteria for this study, a total of 290 couples underwent 722 cycles of IUI with 67 ongoing pregnancies resulting in an ongoing pregnancy rate of 9% per cycle and 22% per couple. From these data we derived the number of cycles needed to perform of 11 cycles for one ongoing pregnancy.

If model I would have been applied, a total of 118 couples would have undergone 279 cycles of IUI with 32 ongoing pregnancies resulting in an ongoing pregnancy rate of 11% per cycle and 27% per couple. The number of cycles needed to perform to achieve one ongoing pregnancy based on model I would be 8.7 cycles. A total of 172 couples (60%) would have been advised to undergo IVF based on model I at a loss of 35 pregnancies with IUI.

If model II would have been applied, a total of 99 couples would have undergone 246 cycles of IUI with 35 ongoing pregnancies resulting in an ongoing pregnancy rate of 14% per cycle and 35% per couple. The number of cycles needed to perform to achieve one ongoing pregnancy based on model II would be 7 cycles. A total of 191 couples (66%) would have been excluded from IUI at a loss of 32 pregnancies. Model III was comparable in its efficiency to model II. With the selective use of model III in case model II predicted an ongoing pregnancy rate >10% per cycle, and model III <10%, a total of 86 couples
would have undergone 219 cycles of IUI with an ongoing pregnancy rate of 16% per cycle and 40% per couple. The number of cycles needed to perform for one ongoing pregnancy was 6.4. A total of 204 couples (70%) would be excluded from IUI with this strategy at a loss of 33 pregnancies.

**Discussion**

This study is the first to assess all baseline and semen characteristics during the fertility work-up, i.e. before the start of treatment, in order to distinguish between couples with male subfertility that will benefit from IUI and those who will not. We demonstrated that female age, duration of subfertility, secondary subfertility, the presence of anovulation and cervical hostility, cycle number and the absence of ovarian hyperstimulation are independent factors associated with IUI outcome in couples with male subfertility. Addition of the presence of anti sperm antibodies (ASA) to a baseline model including these factors generated a significant increase in discriminative capacity (as determined by the AUC). Addition of the post-wash TMC during the fertility work-up did not generate a significant increase in discriminative capacity. When determining the prognostic value of a prediction model in subfertility, however, comparing the AUC's is not the most accurate method of assessment. It is not possible to achieve anywhere near 100% sensitivity, because the prediction of an ongoing pregnancy will never be 100% for any subgroup of patients.

It seems more in line with daily clinical practice to counsel patients with use of predicted probabilities of an ongoing pregnancy per cycle. Patients are not concerned about their chance relative to other couples (discrimination); instead, they are more interested in their predicted probability of pregnancy with IUI. Consequently, the clinical aim of the model is to differentiate between couples with a poor and couples with a good prognosis. The predicted probabilities based on the model including ASA were 100% accurate for those couples that had a predicted probability <10% per cycle. In those cases where this model would have advised IUI (predicted probability >10%) the addition of the post-wash TMC would have identified a very small subgroup of couples who would have a poor prognosis of pregnancy with IUI. This strategy would not lead to more efficient patient selection for IUI compared with the model with ASA alone.
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The finding that the parameters of the normal semen analysis had no impact on the prediction of IUI outcome can probably be attributed to the fact that the analysis was performed in a population of men already diagnosed as suffering from male subfertility. Thus, while the routine semen analysis is useful in identifying subfertile men, it has no capacity to predict IUI outcome in couples with male subfertility. The finding that the presence of anti-sperm antibodies was positively correlated with ongoing pregnancy after IUI might be explained by the fact that the semen preparation applied during intrauterine inseminations both enhances motility and elutes the antibodies from the acrosome region which in turn leads to better fertilization capacity of spermatozoa with ASA. ASA could also lead to impaired cervical mucus penetration, which is bypassed by intrauterine insemination.

The use of multiple cycles from a given patient in an analysis might lead to a lack of independence of data. A lower post-wash TMC could lead to earlier dropout and in later cycles higher values for the post-wash TMC would be relatively overrepresented. However, a reanalysis limited to first cycles did not change the direction and magnitude of the factors included in our model. The analysis on cycle level mimics daily practice more, as patients undergo cycles of IUI. Including cycle number in the prediction model will enable patient counselling per cycle and will provide the possibility of individual counselling as to how many cycles of IUI will be beneficial for that particular couple.

One could argue that the heterogeneity of our study population could lead to underestimation of the effect of sperm impairments on IUI outcome as we included couples with characteristics that could negatively influence IUI outcome, like one sided tubal pathology, female age > 35 years and longer duration of subfertility. Limitation of the analysis to couples with optimal baseline characteristics would not be in accordance with daily clinical practice and would therefore not only compromise statistical power, but in our opinion also weaken the external validity of the study.

Our study has also some limitations. Data collection was retrospective, and the post-wash TMC was used in the management of couples in the study. Couples for which the post-wash TMC during the fertility work-up showed less than 1 million progressively motile spermatozoa were excluded, since these couples were counseled to undergo IVF. This implies that the predictive capacity of the post-wash TMC as found in the present study might be underestimated.
due to selection bias. However, we feel that the cut-off value of 1 million is so low that it seems unlikely that the exclusion of these couples had a significant impact on the outcome of our study.

Another limitation of our retrospective analysis is the fact that the stimulation scheme was heterogeneous. Most women received ovarian hyperstimulation with gonadotropins, some with clomiphene citrate and some underwent IUI in a natural cycle. Hyperstimulation has been proposed as having additional value in IUI only in couples with a pre-wash total motile count $> 10 \times 10^6$\textsuperscript{24}.

Our results were comparable to a recent review that mentioned a not statistically significant odds ratio of 1.4 in favour of gonadotropins for IUI in male subfertility\textsuperscript{25}. In the proposed prediction model the absence of ovarian hyperstimulation has a negative association with the occurrence of an ongoing pregnancy. We found no positive association between the absence of hyperstimulation and a lower total motile count before and after processing (data not shown). In couples with cervical hostility IUI with ovarian hyperstimulation gives slightly, but not statistically significantly, better pregnancy rates than IUI in a natural cycle\textsuperscript{26}. In our population we found the absence of ovarian hyperstimulation to be an independent prognostic factor, as well as cervical hostility, thus establishing the fact that couples with semen impairments need a different approach than other couples with subfertility.

It was assumed that the alternative for couples with less favourable predicted probabilities on an ongoing pregnancy after IUI was IVF. For unexplained infertility there are arguments that it would be more efficient to combine IVF with ICSI on sibling oocytes in the first cycle after unsuccessful IUI to minimize the risk of total fertilization failure\textsuperscript{27}. For male subfertility it is not clear if such a strategy would be more efficient and therewith cost effective. External validation of our model is needed before it can be implemented in clinical practice\textsuperscript{28}. Despite the shrinkage that was performed, our model might still be overoptimistic, since its performance was only assessed in the population in which the model was developed.

In conclusion, this study shows that the combined use of baseline characteristics and ASA can prevent unnecessary IUI in 65% of the couples suffering from male subfertility, causing an increase in the pregnancy rate from 9% to 14% per cycle. A post-wash TMC during the fertility work-up will have very limited additional value.
IUI in male subfertility: are we able to select the proper patients?

References


