Unexplained subfertility
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Citation for published version (APA):
Tjon-Kon-Fat, R. I. (2017). Unexplained subfertility: Illuminating the path to treatment
Can we identify subfertile couples that benefit from immediate in vitro fertilisation over intrauterine insemination?

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European Journal of Obstetrics & Gynecology and Reproductive Biology 2016; 202:36-40
ABSTRACT

Objective: Available treatment options in couples with unexplained or mild male subfertility are intrauterine insemination with controlled ovarian hyperstimulation (IUI-COH) and in vitro fertilisation (IVF). IUI-COH is a less invasive treatment that is often used before proceeding with IVF. Yet as the IVF success rates might be higher and time to pregnancy shorter, expedited access to IVF might be the preferred option. To identify couples that could benefit from immediate IVF over IUI-COH, we assessed whether female age, duration of subfertility or prewash total motile count (TMC) can help to identify couples that would benefit from IVF over IUI-COH.

Study design: We performed a secondary data-analysis of a multicentre open-label randomised controlled trial in three university and six teaching hospitals in the Netherlands. 116 couples with unexplained or mild male subfertility were randomised to one cycle of IVF with elective single embryo transfer with subsequent frozen-thawed embryo transfers or 3 cycles of IUI-COH. The primary outcome was an ongoing pregnancy within 4 months after randomisation. Our aim was to explore a possible differential effect of specific markers on the effectiveness of treatment. We chose to therefore assess female age, duration of subfertility and TMC as these have previously been identified as predictors. For each prognostic factor we developed a logistic regression model to predict ongoing pregnancy with that prognostic factor, treatment and a factor-by-treatment interaction term.

Results: Female age and duration of subfertility were not associated with better ongoing pregnancy chances after IVF compared to IUI-COH (p-value for interaction = 0.65 and 0.26, respectively). Only when TMC was lower than 110 (x10^6 spermatozoa/mL), the probability of ongoing pregnancy was higher in women allocated to IVF (p-value for interaction = 0.06).

Conclusion: In couples with unexplained or mild male subfertility, a low TMC might lead to higher pregnancy rates after IVF than after IUI-COH. This finding needs to be validated in a larger trial before it can be applied in clinical practice.
INTRODUCTION

Unexplained or mild male subfertility affects up to half of all couples who have not achieved a pregnancy after 12 months of unprotected intercourse (Brandes et al., 2010). The majority of these couples will ultimately conceive naturally (Brandes et al., 2011). Therefore, treatment should only be given to couples with low chances on natural conception (Steures et al., 2006; van der Steeg et al., 2007).

Available treatment options are intrauterine insemination (IUI) with controlled ovarian hyperstimulation (COH) and in vitro fertilisation (IVF). IUI-COH is a less invasive treatment option that is often used before proceeding with IVF. Yet as the IVF success rates might be higher than that with IUI-COH and time to pregnancy shorter, expedited access to IVF might be the preferred option.

The Fast Track and Standard Treatment (FASST) trial compared standard treatment of three cycles of IUI with clomiphene citrate, three cycles of IUI with gonadotrophins and up to six cycles of IVF to an accelerated treatment skipping the three cycles of IUI with gonadotrophins (Reindollar et al., 2010). They found that an accelerated approach to IVF resulted in a shorter time to pregnancy with fewer treatment cycles. This raises the question whether there are couples that would have benefited from receiving IVF immediately instead of IUI-COH.

Existing prediction models have evaluated factors that predict the probability of pregnancy after IVF and IUI-COH (Leushuis et al., 2009). As these models estimate pregnancy chances for each treatment per se, they do not take into account any added benefit of one treatment over the other.

To identify couples that would likely benefit from immediate IVF instead of IUI-COH, we need to take into account factors that predict pregnancy chances after IVF and IUI-COH. Important predictors for pregnancy chances after IVF and IUI-COH are female age, duration of subfertility and total motile count (TMC) (Leushuis et al., 2009; van Loendersloot et al., 2010; Custers et al., 2007). Advanced female age and duration of subfertility are important negative prognostic factors for pregnancy chances (Leushuis et al., 2009). The absolute risk of not conceiving increases with female age because of the decreasing ovarian reserve. Couples with a longer duration of subfertility could have subtle functional abnormalities in oocyte and/or sperm function, which we could bypass by using IVF over
IUI-COH. Couples with a lower TMC might benefit more from IVF, as concentrated motile spermatozoa are brought into contact with the oocyte in vitro.

Our study group performed a randomised pilot trial comparing one cycle of IVF with elective single embryo transfer (eSET) to 3 cycles of IUI-COH in treatment-naive women (Custers et al., 2011). To assess whether female age, duration of subfertility and TMC can indeed be used to identify subgroups of couples that would benefit from immediate IVF over IUI-COH, we undertook a post hoc analysis of this trial data.
MATERIALS AND METHODS

Study design, patients and interventions
The Single Embryo Transfer or IUI (SETI) trial (ISRCTN86744378) was a multicentre open-label randomised controlled trial, performed between November 2006 and February 2009. The SETI protocol was approved by the institutional review board of the University of Amsterdam. The background, methods, baseline characteristics of the randomised patients, and results have been reported elsewhere (Custers et al., 2011). In brief, the SETI trial included 116 couples with unexplained or mild male subfertility. Eligible were couples with a female age up to 38 years, a duration of subfertility of at least 12 months and a TMC of at least 3x10⁶. Unexplained subfertility was defined as a TMC of more than 10x10⁶ spermatozoa/mL and exclusion of a cervical factor (with the use of the post-coital test). Mild male subfertility was defined as a TMC of 3-10x10⁶ spermatozoa. All couples had an unfavourable prognosis for natural conception, this was defined as a probability of natural conception within the next twelve months of less than 30%, as calculated with the validated synthesis model of Hunault (van der Steeg et al., 2007).

All couples had undergone a basic fertility workup including assessment of ovulation, a tubal patency test and semen analysis. We did not have information on body mass index from the participants of the SETI trial. Female age, duration of subfertility, and TMC were documented. Duration of subfertility was defined as the time from when the couple started actively trying to conceive to inclusion in the study. TMC was calculated for unprocessed semen samples by multiplying the concentration of the semen (10⁶mL⁻¹), progressive motility (%) and volume (mL). Whenever TMC was measured twice for a couple, we used the average of TMC of the two semen samples for our analysis.

Couples were randomly allocated to either one cycle of IVF with elective single embryo transfer (IVF-eSET) with subsequent frozen-thawed embryo transfers, or 3 cycles of IUI-COH within 4 months after randomisation. In the IVF-eSET group, couples underwent controlled ovarian hyperstimulation with recombinant-FSH after down-regulation in a long agonist protocol. Treatment was continued until at least three follicles >18 mm had developed. After ovulation induction cumulus–oocyte complexes were recovered by ultrasound-guided retrieval 36 h thereafter. Embryos were scored daily with the use of validated morphologic scoring criteria. According to the eSET policy, one good-quality embryo was selected for transfer on day 3; if no good-quality embryo was present, double-embryo transfer was performed.
In the IUI-COH group, couples underwent ovarian stimulation with 50–75 IU recombinant-FSH. The goal of the ovarian stimulation was to achieve the growth of one to (maximally) three dominant follicles. Women were inseminated 36–40 h after ovulation induction with hCG. If more than three dominant follicles (≥15 mm) were present the cycle was cancelled.

The primary outcome of the trial was an ongoing pregnancy conceived within 4 months after randomisation, defined as a viable intrauterine pregnancy at ultrasound at 12 weeks’ gestation. This trial also looked at the number of multiple pregnancies, clinical pregnancies, live births and financial costs. Follow-up was continued until live birth.

**Statistical analysis**

Our aim was to explore a possible differential effect of specific markers on the effectiveness of treatment. We chose to therefore assess female age, duration of subfertility and TMC as these have previously been identified as predictors.

There were no missing data for ongoing pregnancy or female age, but there were some missing data for TMC (2.6%) and for duration of subfertility (1.7%). To increase the statistical power of the modelling and to control the possibility of bias of complete case analysis, we imputed missing values using single imputation.

For each prognostic factor we developed a logistic regression model to predict ongoing pregnancy with that prognostic factor, treatment and a factor-by-treatment interaction term. The latter term expresses to what extent the treatment effect varies with the factor. In this context, we defined that prognostic factors can be useful for treatment selection if they show a biologically plausible qualitative interaction with treatment. Given the exploratory nature of our analysis, we used a more liberal p-value of 0.1 (Selvin, 1996). Any marker showing an interaction with treatment at a p-value lower than 0.1 was further investigated for its potential as a treatment selection factor, by calculating the population benefit of using that marker to guide the choice of treatment, in terms of the increase in the pregnancy rate (Janes et al., 2011). All analyses were performed based on the intention-to-treat principle. We used R for Windows (Version 3.0.1; R Foundation for Statistical Computing, Vienna, Austria) for the analysis and TreatmentSelection package (version 1.0.2) to evaluate individual treatment selection factors (Janes et al., 2014).
RESULTS

The SETI trial included 116 couples, of which 58 were allocated to IVF-eSET and 58 to IUI-COH. Baseline characteristics of the couples were comparable (Table I). The ongoing pregnancy rate at 4 months after randomisation was 14 (24%) in the IVF-eSET group and 12 (21%) in the IUI-COH group (RR 1.17, 95% CI 0.60–2.3).

Table I Baseline characteristics of couples allocated to IVF-eSET or IUI-COH

<table>
<thead>
<tr>
<th>Factor</th>
<th>IVF-eSET (n=58)</th>
<th>IUI-COH (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (years), median (IQR)</td>
<td>34.1 (31.4-35.9)</td>
<td>34.4 (32.2-36.4)</td>
</tr>
<tr>
<td>Duration of subfertility (years), median (IQR)</td>
<td>2.3 (1.9-2.8)</td>
<td>2.2 (1.8-3.0)</td>
</tr>
<tr>
<td>TMC (×10^6), median (IQR)</td>
<td>79.4 (27.4-154.6)</td>
<td>47.0 (27.6-118.3)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; SD, standard deviation; TMC, total motile count

Female age was not associated with benefit from IVF-eSET compared to IUI-COH in terms of ongoing pregnancy (p-value for interaction = 0.65) (Table II; Figure 1). Duration of subfertility had a weak association with benefit from IVF-eSET compared to IUI-COH (Figure 1). Couples who had a duration of subfertility shorter than 2.3 years benefited more from IUI-COH while those with a longer duration of subfertility benefited more from IVF-eSET, but this difference was not statistically significant (p for interaction = 0.26; Table II).

A lower TMC was associated with a higher benefit from IVF-eSET (p-value for interaction = 0.06; Table II). The probability of an ongoing pregnancy after IVF-eSET was 24% and independent of TMC value. In contrast, the probability of an ongoing pregnancy after IUI-COH was associated with the TMC, starting with 10% for low TMC values, increasing to 80% for higher TMC values (Figure 1). Couples with a TMC lower than 110 (x10^6) were more likely to benefit from IVF-eSET; couples with TMC over 110 (x10^6) were more likely to benefit from IUI-COH.

Table II The association between potential factors and ongoing pregnancy

<table>
<thead>
<tr>
<th>Factors</th>
<th>IUI-COH (OR 95% CI)</th>
<th>IVF-eSET (OR 95% CI)</th>
<th>P for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (years)</td>
<td>1.07 (0.86-1.37)</td>
<td>0.99 (0.81-1.24)</td>
<td>0.65</td>
</tr>
<tr>
<td>Duration of subfertility (years)</td>
<td>0.84 (0.43-1.29)</td>
<td>1.40 (0.66-2.91)</td>
<td>0.26</td>
</tr>
<tr>
<td>TMC (×10^6)</td>
<td>1.01 (1.00-1.02)</td>
<td>1.00 (0.99-1.01)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

TMC, total motile count

Which couples benefit from immediate IVF? I 89
Based on this preliminary finding one could suggest a TMC-based treatment selection strategy for these couples. In such a strategy, all subfertile couples with TMC less than 110 (x10^6) will undergo IVF-eSET and the others will be managed by IUI-COH. We estimate that in a population of subfertile couples similar to the SETI trial participants about 63% will have a TMC lower than 110 (x10^6). By applying the TMC-based treatment strategy, the ongoing pregnancy rate would be 28.6%, which is 5.9% higher than a strategy of treating all with IUI-COH (95% CI: 0–18.3%) and 6.1% higher than a strategy of treating all with IVF-eSET (95% CI: 0–16.2%).

\[ \text{Probability of ongoing pregnancy} \]

\[ \text{Female age (years)} \]

\[ \text{Duration of subfertility (years)} \]

\[ \text{Total Motile Count (TMC)} \]

Figure 1 Female age, duration of subfertility and TMC and the probability of ongoing pregnancy after IVF-eSET or IUI-COH
COMMENT

We assessed whether female age, duration of subfertility and TMC could be used in the decision to offer IVF or IUI-COH in couples with unexplained or mild male subfertility, based on data collected in a randomised trial. Our results indicate that TMC is a potential treatment selection factor. Couples with a TMC lower than 110 \((x10^6)\) could benefit from IVF, while those with a TMC over 110 could benefit from IUI-COH. There was no significant association between female age or duration of subfertility and chances on ongoing pregnancy after IVF or IUI-COH.

Until now, associations between prognostic factors and an outcome are used to calculate the probability of the outcome after treatment. If there are two treatment options available, like IUI and IVF, and we wish to decide which one of two treatment options is the best, we need to know the probability of that outcome after each treatment per se, and then compare the two probabilities (Janes et al., 2011; McLernon et al., 2014). The analysis presented here is one of the first to look at the potential effects of baseline prognostic factors in couples with unexplained or mild male subfertility on ongoing pregnancy rates after IVF compared to those after IUI-COH, thereby trying to identify any added benefit of immediate IVF over IUI-COH.

Our analysis was based on randomised trial data, hence baseline risk factors did not affect the choice of treatment. This gave us the opportunity to study the relationships between potential factors for treatment selection and ongoing pregnancy without the risk of selection bias. A potential weakness of the study is its limited sample size, making it difficult to detect smaller associations. We chose to therefore assess only three baseline risk factors for treatment selection.

We did not find an interaction between female age or duration of subfertility and treatment. One possible explanation is the inclusion criteria of this trial, as only women younger than 38 years and couples with a duration of subfertility of at least 12 months were eligible in accordance with the Dutch guidelines.

Our results suggest that the prewash TMC could be informative for the choice of treatment. A TMC below 110 million was associated with a lower chance of ongoing pregnancy after IUI-COH compared to IVF. TMC has long been used as a marker for success or failure after IUI-COH; yet other studies have shown lower cut-off values of <5 and <10 million to be associated with low pregnancy chances after IUI-COH but with no clear cut-off values.
for IVF (Ombelet et al., 2014; Merviel et al., 2010; van Voorhis et al., 2001). As these analyses were exploratory and hypothesis generating, a larger study is needed to confirm these results. The sample size necessary to detect significant results for TMC is 436 couples. We have included the formulae in a supplementary text that can be found at the end of the manuscript.

The original trial was not powered to detect effective stratification at the conventional 0.05 level for most plausible combinations of baseline variables and between subgroup differences. The main purpose of the analysis presented in this manuscript was to explore factors that could potentially be used for treatment selection, to generate hypotheses or stratification for further trials, rather than test hypotheses. Using a higher significance level than the conventional 0.05 is not unusual for identifying potentially useful markers. In the selection of potential predictors in multivariable prediction modelling a significance criterion of 0.20 is recommended, and even higher has been considered for prediction modelling (Steyerberg, 2009; Moons et al., 2012).

To explore factors that could potentially be used for treatment selection a national registry could provide more data than a randomised controlled trial. In daily practice physicians offer couples IUI or IVF based on the characteristics of the couple, such as age of the woman and duration of subfertility. This selection would be reflected in a national registry, making it difficult to explore potential factors that could affect pregnancy chances after treatment. In a clinical trial the random assignment of couples to either IUI or IVF, creates an ideal setting to look at baseline characteristics of the couple and their effect on pregnancy chances after treatment, independent of selection bias.

In the Forty and Over Treatment Trial (FORT-T) couples with the female partner aged 38–42 years old were randomised to receive either IUI with clomiphene citrate, IUI with gonadotrophins or immediate IVF (Goldman et al., 2014). Higher pregnancy rates were reported for the immediate IVF group. These data suggest that female age is able to select the best treatment in older couples, but we could not validate these findings in our study as we did not include women above 38 years of age.

In conclusion, we found that TMC could be a possible marker for the selection of treatment in couples with mild male or unexplained subfertility. Since this is based on an exploratory analysis of factors for treatment selection, validation of these findings in a larger trial is necessary before implementing these results in clinical practice.
SUPPLEMENTARY DATA

Sample size calculation for total motile count

The sample size estimation for a new dataset is done using the following formulae. We put in the formulae the observed 24% event rate of the in vitro fertilisation with elective single embryo transfer group ($p_1$) and 21% event rate of the intrauterine insemination with controlled ovarian hyperstimulation group ($p_2$). Marker variance ($\sigma^2$) is the variance of total motile count ($8168$). Power was set to 80% and type I error at 10% one-sided for detecting the interaction odds ratio (OR) of 0.99 or larger between the total motile count and treatment. In the formulae below $\delta$ is the logarithm of the interaction OR. This results in a sample of size 436 couples in each trial arm.

$$n = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2}{\sigma^2 \delta^2} \left( \frac{1}{p_1 (1 - p_1)} + \frac{1}{p_2 (1 - p_2)} \right)$$
REFERENCES


