FACTORS ASSOCIATED WITH DIZYGOTIC TWINNING AFTER IVF TREATMENT WITH DOUBLE EMBRYO TRANSFER

HUM REPROD. 2012 OCT;27(10):2966-70.

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ABSTRACT

Background
Dizygotic twin pregnancies after IVF treatment are the result of multiple embryos transferred into the uterine cavity, followed by successful double implantation. Factors that increase the chance of multiple implantation after IVF are relatively unknown. The present study aimed to investigate whether features of body composition, such as maternal height, weight and body mass index (BMI) are associated with an increased chance of dizygotic twinning after IVF with double embryo transfer (DET).

Methods
This study was conducted using data from a large Dutch nationwide cohort that comprised 19,861 women who had IVF or ICSI treatment between 1983 and 1995 (OMEGA study). First ‘fresh’ IVF and ICSI cycles with DET resulting in a delivery of a singleton or twin (living as well as stillborn) were selected. A multivariable logistic regression analysis was performed, with the delivery of a singleton or twin as the dependent variable and height, weight, BMI, maternal age, number of retrieved oocytes, use of alcohol, smoking, highest level of education and parity as independent variables.

Results
Of the 6598 women who completed their first IVF or ICSI cycle, 2375 had DET, resulting in 496 deliveries of 371 singletons and 125 twins. Multivariable regression analysis revealed that tall women (>1.74 cm) and women with a high number of retrieved oocytes (>8) had an increased chance of dizygotic twinning [OR: 1.8 (95% CI: 1.0–3.4) and OR: 2.2 (95% CI: 1.3–3.8), respectively].

Conclusions
Our data demonstrate that tall stature and increased number of retrieved oocytes independently increase the chance of dizygotic twinning after IVF with DET.
INTRODUCTION

Dizygotic twinning is the result of multiple ovulation and fertilization, followed by double implantation. It has been demonstrated that spontaneous dizygotic twinning is often associated with increased levels of basal FSH. Major factors increasing dizygotic twinning rates are a higher maternal age, increased parity and genetic inheritance. Associations with socio-economic status, seasonal and geographical variation and ethnic origin have also been reported. It has been shown that features of body composition (in particular maternal height but also body mass index (BMI)) associate with natural dizygotic twinning. This is supported by the fact that countries with a taller female population have higher twinning rates.

With IVF treatment, multiple ovulation and fertilization are artificially induced. Dizygotic twin pregnancies after IVF treatment are the result of multiple embryos transferred into the uterine cavity followed by successful double implantation. Patient and treatment characteristics associated with dizygotic twinning after IVF with double embryo transfer (DET) are factors involved in the process of multiple implantation and still remain relatively unknown.

In IVF treatment one of the main challenges lies in finding a balance between high pregnancy rates and low multiple pregnancy rates. Aside from helping a couple to establish a pregnancy, another important goal of IVF physicians is to help couples achieve a carefree pregnancy and deliver a healthy baby at full term. As a consequence, avoiding multiple pregnancies is more and more emphasized, since these pregnancies have higher risks of pre-eclampsia and preterm or immature delivery. Ideally, to make IVF treatment more tailor-made for the individual IVF patient, a prediction model based on specific patient and treatment characteristics should be developed as a support in the choice for single embryo transfer (SET) or DET.

Therefore, it is important to investigate which patient and treatment characteristics are associated with an increased chance of dizygotic twinning after DET, which depends on a combination of maternal and embryonic-related factors. A previous study indicated that both maternal vascular endothelial growth factor-A (VEGF-A) levels and BMI are positively associated with the chance of multiple implantation after IVF treatment with DET.

The aim of the present study was to further investigate whether features of body composition, such as maternal height, weight and BMI are associated with an increased chance of dizygotic twinning after IVF with DET.
MATERIAL AND METHODS

Study population
For this study we used data from a large Dutch nationwide retrospective cohort of women who received IVF or ICSI treatment: the OMEGA study. The main purpose of the OMEGA study was to examine the late effects of hormone stimulation in IVF-treated women, with an emphasis on the development of gynaecological tumours. A more detailed description of the study population, study procedures and data collection is given by Klip et al. (2001), de Boer et al. (2003) and van Leeuwen et al. (2011). In short, the OMEGA project was a nationwide cohort study. It was started in 1995 and comprised 19,861 women who had suffered with subfertility for more than 1 year when first attending the IVF clinic received at least 1 IVF or ICSI treatment cycle in one of the 12 IVF hospitals in the Netherlands between 1 January 1983 and 1 January 1995.

Study variables
Between 1997 and 1999 19,275 women received a study invitation letter, a health questionnaire and an additional form to ask each participant’s written informed consent for data abstraction from medical records. The health questionnaire inquired about women’s date of birth, weight, height, BMI, parity, reproductive history [number of pregnancies, pregnancy outcome (singleton or twin, living or stillborn and date of delivery), history of subfertility treatment, use of exogenous hormones, highest level of education and several other lifestyle factors (use of alcohol, smoking, etc.)]. Information from medical records was collected by specially trained research assistants. For each IVF treatment cycle, the following data were recorded: cause of subfertility [male factor, ovarian disorder, tubal factor, unexplained factor or ‘other’ (including for example: uterine abnormality and cervical factor)], date of start of first IVF cycle, type of IVF treatment, dosages and type of fertility drugs used in each phase of the menstrual cycle, number of retrieved oocytes, number of transferred embryos and whether the cycle resulted in a pregnancy. The response rate to the questionnaire was 71%. For 24% of the women, who returned the questionnaire and gave permission to obtain data from medical files, data from medical records could not be obtained due to limited project funding.
Outcome measure
In the current study, we made a selection of the participants in the OMEGA study; only the first completed IVF and ICSI cycles with DET that resulted in a delivery of a singleton or twin (both living and stillborn) were selected. In the event of miscarriage the number of lost fetuses was unknown; therefore, we could not include these pregnancies in our analysis. Instead the number of twin deliveries was included in the analysis as the number of ongoing implantations, since only a small number of twin pregnancies experience reduction after 12 weeks of gestational age.

Statistical analyses
Differences in patient and treatment characteristics between women conceiving singletons and twins were assessed using t-tests and χ² tests (TABLE I). The primary comparison was maternal height. For all other parameters we adjusted for multiple testing using the Bonferroni test. Factors associated with dizygotic twinning were investigated using multivariable binary logistic regression analyses using the likelihood ratio test (TABLE II). The other independent variables were BMI, weight, maternal age at the start of the first IVF cycle, number of oocytes retrieved at oocyte retrieval, use of alcohol, smoking, level of education and parity. To ensure a comparable number of women across categories, variables were divided into categories according to percentiles where applicable. Because BMI depends on weight and height, two separate regression models were run: the first only including BMI and the other instead of BMI including weight and height. For the statistical analyses, the statistical program Statistical Package for Social Sciences version 15.0 for Windows (SPSS, Chicago, IL, USA) was used. For the multivariable logistic analyses, a P<0.05 was used to detect the most important associated variables.
RESULTS

FIGURE 1 presents a flow diagram of the selection of patients. There were 8688 women with detailed data (questionnaires and medical records) on their first IVF or ICSI cycle. Totally, 6598 women completed their first IVF/ICSI cycle. Of these women, 2375 women had DET, resulting in 496 deliveries of 371 singletons and 125 twins. Of the included patients the majority (94.8%) started their IVF treatment between 1991 and 1996.

TABLE I demonstrates the patient and treatment characteristics of both women who delivered singletons and women who delivered twins. In 96% of the cycles human menopausal gonadotrophin was used to achieve controlled ovarian hyperstimulation. Maternal height (P = 0.043) and the number of retrieved oocytes (P = 0.004) were significantly different between the two groups.

Multivariable logistic regression analysis for multiple pregnancy (TABLE II) revealed that maternal height and number of retrieved oocytes at oocyte retrieval were independently significantly associated with dizygotic twinning. Women with a high number of retrieved oocytes (>8) were 2.2 (95% CI: 1.3–3.8) times more likely to have a twin pregnancy compared with women with a relatively low number of retrieved oocytes (<8). The women in the highest quartile for height (>174 cm) were 1.8 (95% CI: 1.0–3.4) times more likely to have a twin pregnancy compared with women in the lowest quartile for height (<164 cm). In the analysis there was no significant interaction between the number of retrieved oocytes and maternal height (P = 0.13). The other patient and treatment characteristics included in the analysis were not significantly associated with the chance of dizygotic twinning after IVF treatment with DET (see footnote TABLE II).
FIGURE 1 Selection of patients

FIRST IVF CYCLES OF WOMEN WITH DETAILED DATA FROM QUESTIONNAIRES AND MEDICAL RECORDS

N=8457 IVF / N=231 ICSI

EXCLUDED: UNCOMPLETED CYCLES:
- No oocyte retrieval
- No oocytes retrieved at oocyte retrieval
- Total fertilization failure

N=2040 IVF / N=50 ICSI

WOMEN WITH COMPLETED FIRST IVF OR ICSI CYCLE

N=6417 IVF / N=181 ICSI

EXCLUDED: NON DET

N=4127 IVF / N=96 ICSI

WOMEN WITH COMPLETED FIRST IVF OR ICSI CYCLE AND DET

N=2290 IVF / N=85 ICSI

EXCLUDED: NON PREGNANT WOMEN, ECTOPIC PREGNANCIES, MISCARRIAGES

N=1809 IVF / N=70 ICSI

WOMEN WHO DELIVERED SINGLETON OR TWIN

N=481 IVF / N=15 ICSI

Singletons n=371 (364 living, 7 stillborn)
Twins n=125 (122 living, 3 stillborn)
<table>
<thead>
<tr>
<th>Patient/treatment characteristic</th>
<th>Singleton (n=371)</th>
<th>Twins (n=125)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal height (cm)</td>
<td>169.3 (7.3)</td>
<td>170.7 (6.6)</td>
<td>0.043</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>63.9 (9.8)</td>
<td>64.8 (12.3)</td>
<td>0.424</td>
</tr>
<tr>
<td>BMI</td>
<td>22.3 (3.1)</td>
<td>22.2 (3.8)</td>
<td>0.884</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>31.5 (3.8)</td>
<td>30.7 (3.3)</td>
<td>0.035</td>
</tr>
<tr>
<td>Nulliparous/parous</td>
<td>278 (78.1)/78 (21.9)</td>
<td>100 (80.6)/24 (19.4)</td>
<td>0.549</td>
</tr>
<tr>
<td>IVF/ICSI</td>
<td>359 (96.8)/12 (3.2)</td>
<td>122 (97.6)/3 (2.4)</td>
<td>0.638</td>
</tr>
<tr>
<td>Cause of subfertility A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>137 (37.8)</td>
<td>47 (37.9)</td>
<td>0.991</td>
</tr>
<tr>
<td>Ovarian disorder</td>
<td>34 (9.4)</td>
<td>7 (5.7)</td>
<td>0.200</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>125 (34.2)</td>
<td>37 (29.8)</td>
<td>0.377</td>
</tr>
<tr>
<td>Unexplained</td>
<td>108 (29.1)</td>
<td>39 (31.2)</td>
<td>0.666</td>
</tr>
<tr>
<td>Other</td>
<td>153 (42.7)</td>
<td>49 (40.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>No of retrieved oocytes</td>
<td>10.4 (6.6)</td>
<td>12.5 (7.8)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>86 (24.4)</td>
<td>35 (28.9)</td>
<td>0.320</td>
</tr>
<tr>
<td>Middle</td>
<td>188 (53.3)</td>
<td>63 (52.1)</td>
<td>0.821</td>
</tr>
<tr>
<td>High</td>
<td>79 (22.4)</td>
<td>23 (19.0)</td>
<td>0.436</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>154 (41.5)</td>
<td>39 (31.2)</td>
<td>0.041</td>
</tr>
<tr>
<td>Use of alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>97 (26.9)</td>
<td>39 (32.0)</td>
<td>0.287</td>
</tr>
</tbody>
</table>

Values are mean ±SD or n (%). Chi-squared or independent t-tests were used as applicable.
A Patients could have more than one cause of subfertility.
B P<0.0045 (Bonferroni): there were no other statistically significant differences.
**TABLE II** Multivariable logistic regression analysis for singleton and twin pregnancies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of patients</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of oocytes retrieved</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>185</td>
<td>Reference</td>
<td>0.018 A</td>
</tr>
<tr>
<td>8-12</td>
<td>149</td>
<td>2.2 (1.3-3.8)</td>
<td>0.005</td>
</tr>
<tr>
<td>&gt;12</td>
<td>159</td>
<td>2.1 (1.2-3.6)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Maternal height (cm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;164</td>
<td>117</td>
<td>Reference</td>
<td>0.0032 A</td>
</tr>
<tr>
<td>164-169</td>
<td>121</td>
<td>1.0 (0.5-1.9)</td>
<td>0.43</td>
</tr>
<tr>
<td>170-174</td>
<td>127</td>
<td>0.8 (0.4-1.6)</td>
<td>0.52</td>
</tr>
<tr>
<td>&gt;174</td>
<td>128</td>
<td>1.8 (1.0-3.4)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

OR: odds ratio, 95%CI: 95% confidence interval. Independent variables included in the analysis were: height and weight or BMI, maternal age, number of oocytes retrieved, use of alcohol, smoking, level of education and parity. Variables were divided into categories according to percentiles; body mass index (kg/m²): <20/20-25/25-27/>27, weight (kg): <60/60-67/>67, height (cm): <164/164-169/170-174/>174, number of oocytes:<8/8-12/>12, educational level: low (without completed vocational training)/middle (with vocational training)/high (with high vocational training, academic degree), parity: nulliparous/parous, use of alcohol: yes/no, smoking: yes/no A P <0.05.
In this study, we further explored the features of maternal body composition related to dizygotic twinning in a large nationwide cohort of patients who received IVF treatment with DET. We found that tall stature and increased number of retrieved oocytes both independently increase the chance of a dizygotic twin pregnancy. Although data were derived from the 1990s, our study is still able to indicate the fate of DET in relation to an important feature of maternal body composition: maternal height. Despite its limited size, to our knowledge this study is the most extensive one addressing this matter. As the emphasis of the current IVF practice is on the prevention of multiple pregnancies without compromising pregnancy rates, there is a need for identification of women with an increased chance of multiple implantation after DET. Multiple pregnancies are associated with an increased risk of adverse pregnancy outcome; therefore, the goal of IVF specialists should be to help a couple achieve a healthy, full-term, singleton pregnancy. This goal creates a need for further exploration of patient and treatment characteristics associated with dizygotic twinning, as was the aim of our present study, to be implemented in future predictive models to assist in the choice between SET and DET.

When evaluating our results, we have to bear in mind that for the current study we had to set the primary outcome as the delivery of a singleton or twin instead of the number of early implantations (6 and 12 weeks of gestational age), since data on early number of implantations (ultrasound investigations, miscarriages) was incomplete. We therefore could not analyse factors that increase the chance of successful first trimester multiple implantation as performed earlier by Lambers et al. [2007] and Tummers [2003]. Because only a small number of twin pregnancies experience reduction after 12 weeks and both live and stillbirths were included in our analyses, in our opinion our study is suitable to investigate the predictive factors in ongoing dizygotic twinning.

Our current findings confirm that body composition is associated with a higher chance of multiple implantation. Studies that investigated body composition and natural dizygotic twinning have also demonstrated a significant positive association with tall stature. In natural twin pregnancies, however, one cannot isolate the process of multiple implantation as is done in IVF treatment. Our results suggest that the general observation that twinning relates to tallness is the result of better implantation. The biologic association between maternal height and multiple implantation remains to be elucidated and seems to be independent of the response to the IVF treatment.
The number of retrieved oocytes significantly affects the chance of twinning after IVF with DET. It could be taken as a measure of ovarian sensitivity, which has been shown to be associated with increased implantation potential. Furthermore, a higher number of oocytes is likely to result in a higher number of embryos and, consequently, a higher chance of good quality embryos. In this way, the number of oocytes can be interpreted as a reflection of embryo quality. Unfortunately, information regarding embryo quality was not available in the OMEGA study. Therefore, we were not able to adjust for the effect of embryo quality on multiple implantation. However, even if it had been possible to recover data on embryo quality, it may not even be possible to join data from 12 different clinics, since most clinics use their own method of quality assessment.

In conclusion, our data demonstrate that tall stature and a high number of retrieved oocytes increase the chance of dizygotic twinning after IVF with DET. Future studies should develop predictive models to assist in the choice between SET and DET, optimizing pregnancy rates while minimizing multiple pregnancy rates, and making IVF treatment more tailor-made for the individual patient. Before applying maternal height into these prediction models, first external validation should be performed.

Acknowledgements
We are indebted to all women participating in the OMEGA project and owe special thanks to Dr H. Klip for her efforts in initiating this cohort. We are grateful to the research assistants who performed the initial extensive data abstraction from the medical files in all participating clinics. We thank the members of the OMEGA project, the medical registries and attending physicians from these clinics for providing the possibility for the initial data collection and the additional search that was performed for the present analysis.
REFERENCES


