Factors affecting pregnancy outcome in a gamete intrafallopian transfer (GIFT) programme

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Objective. To identify the factors that most significantly affected pregnancy rates in a gamete intrafallopian transfer (GIFT) programme.

Methods. A total of 863 GIFT cycles were analysed retrospectively. The variables found to be associated significantly with pregnancy were then used to obtain multivariate analysis using logistical regression.

Results. Overall and ongoing pregnancy rates were significantly better in patients ≤ 38 years than in patients > 38 years (37.3% and 28.4% v. 23.7% and 11.0% respectively), and age was positively associated with success after GIFT (odds ratio [OR] 1.87, 95% confidence interval [CI]: 1.22 - 2.85).

Metaphase I (MI) oocytes were negatively associated with pregnancy (OR 1.54, 95% CI: 0.28 - 1.04). The highest pregnancy rates occurred when 3 metaphase II (MII) oocytes were transferred (39.8%, OR 7.51, 95% CI: 1.74 - 32.42). With regard to sperm morphology, overall pregnancy rates of 25.5% (≤ 4% normal forms) and 37.2% (> 4% normal forms) were obtained. Morphology of > 4% normal forms was positively associated with pregnancy (OR 1.58, 95% CI: 1.04 - 2.42).

Conclusion. The results of this study suggest that the most important factors influencing pregnancy rates in a GIFT programme are the woman’s age and those factors pertaining to the characteristics of the gametes. Considering the emotional and financial costs it is important to relate this information to all prospective participants in a GIFT programme.

Materials and methods

Patients

Using results from our computerised databank we retrospectively analysed a total of 863 GIFT cases from January 1995 to December 1999. The women were aged 22 - 47 years. Couples seeking enrolment for GIFT were comprehensively evaluated. The main indications for GIFT (female patients) included unexplained infertility, ovulatory dysfunction and endometriosis.

Semen analysis

Semen samples were obtained by masturbation at the...
During the GIFT procedure, laparoscopy and follicle aspiration < 14% normal spermatozoa. According to the spermatozoon morphology three categories were identified: 0 -4% normal forms (p-pattern or poor prognosis pattern), 5 - 14% normal forms (g-pattern or good prognosis pattern) and > 14% normal forms (normal pattern).7

Sperm preparation

Semen obtained 2 hours before the GIFT procedure was allowed to liquify, diluted 1:2 with Ham’s F10 (supplemented with 10% maternal serum) medium (GIBCO, Grand Island, NY) and washed twice using centrifugation at 300 g for 10 minutes before routine swim-up.

Ovulation induction

A combination of clomiphene citrate and human menopausal gonadotropin (HMG) was used to achieve ovarian hyperstimulation. Follicular growth was monitored by serial ultrasound measurements and luteinising hormone (LH) determinations. Human chorionic gonadotropin (HCG) was given when the dominant follicle was 18 mm or more in diameter and at least two other follicles of 16 mm or more were present. Aspiration of the follicles was performed according to a standard procedure 36 hours after HCG administration.

GIFT procedure

During the GIFT procedure, laparoscopy and follicle aspiration were done under general anaesthesia. The maturity of the oocytes retrieved was determined according to the criteria of Veeck2 as being either metaphase I (MI) or metaphase II (MII). Usually 3 - 4, preferably MII oocytes, were transferred with spermatozoa by means of a catheter into the Fallopian tubes (2 cm from the fimbrial end). Five hundred thousand to 750 000 sperm per oocyte were transferred in each patient.

Pregnancy

A biochemical pregnancy was diagnosed by the presence of β-HCG in the woman’s serum on day 12, with a significant rise (doubling every 48 hours) over the 4 days following the GIFT procedure (overall pregnancy rate). In this study an ongoing pregnancy was defined as the number of babies born per treatment cycle.

Statistical analysis

Variables were screened for their association with pregnancy using χ² tests or Student’s t-tests as appropriate. Those found to be significant (p < 0.05) were then used to obtain multivariate analysis using logistical regression. Logistical analysis is particularly useful for two reasons. First, it provides a means for estimating the odds ratio (OR) of each predictor variable. The OR of a given variable with regard to pregnancy is a measure of the likelihood of pregnancy when the variable is present compared with the likelihood of pregnancy when the variable is absent. Second, the estimated probability of pregnancy for particular combinations of predictor variables can be calculated. Using the variables female age, number of ova transferred, total sperm count before and after swim-up, forward progression, sperm morphology, quality of oocytes (MI or II) and number of cycles performed, stepwise logistical regression was used to select the most appropriate model for a more detailed investigation. Multiple logistical regression, using maximum likelihood estimates, was used in the detailed investigation to model pregnancy rates on the following risk factors: female age, total sperm count after swim-up, sperm morphology, and quality of oocytes used in GIFT. The CATMOD (categorical modelling) procedure from SAS® (SAS Institute, Cary, NC) was used for calculations. The likelihood ratio test was used to assess the goodness of fit of the model. OR and 95% confidence intervals (CIs) for model parameters are reported. This model was used for overall pregnancy rates.

Results

A total of 863 GIFT procedures were performed during the study period. The mean age of the female patient population was 32.4 years (range 22 - 47 years). The overall pregnancy rate was 34.8% (300/863), with 25.4% (219/863) ongoing pregnancies. Some of the demographic categories do not show a total of 863 cases due to missing data.

The main indications for GIFT were unexplained infertility (62%), endometriosis (16%), ovulatory dysfunction (10%) and the remaining 12% (immunological causes, adhesions, minimal tubal damage, uterine and cervical causes) were grouped together because of smaller numbers. The overall pregnancy rates in the various groups were 35.6% (179/504, unexplained), 41.6% (55/132, endometriosis), 39.5% (34/186, anovulation) and 28% (28/100, all other cases) respectively. There were no significant differences in pregnancy rates within the different groups (p = 0.088).

In this series there was a significant difference in pregnancy rates according to female age. The overall pregnancy rate for women aged 38 years and younger was 37.3% (269/721) compared with 23.7% (31/131) in the group older than 38 years (p = 0.0028). This trend was also noted in the ongoing pregnancy rates for women aged 38 years and younger and those above 38 years (28.4% versus 11% respectively, p = 0.0001). When female age was further categorised into 20 - 24, 25 - 29, 30 - 34, 35 - 39 and > 40 years a significant negative linear trend toward lower pregnancy rates with advancing age was observed (29.4%, 45.3%, 39.3%, 27.9% and 21.4%).
respectively, \( p = 0.001 \).

The maturity (MI versus MII) of the oocytes transferred showed a directly significant association with pregnancy rates (\( p = 0.001 \)) (Table I). MI oocytes were selected for transfer only when standard stimulation protocols resulted in limited numbers of higher quality oocytes. The mean number of eggs transferred was 3, with a range of 0 - 7. Nineteen pregnancies occurred after spontaneous ovulation and subsequent transfer of spermatozoa only to the Fallopian tubes (0 transferred) (Table I). The number of oocytes transferred affected the pregnancy rates (\( p = 0.001 \)) as shown in Table I. The highest rate of success per cycle (39.8%) was achieved when 3 MII oocytes were transferred. The twin pregnancy rate in the group receiving 2 oocytes was 10%. The multiple pregnancy rate in the 3 oocyte group was 15% for twins and 1.6% for triplets. In the group receiving 4 oocytes, 23% were twin pregnancies with 4% triplet pregnancies. No quadruplets occurred in this study and there were no multiple pregnancies in the 5, 6 and 7 oocyte groups (small numbers). The ectopic pregnancy rate in this study was 1.66%. The number of transfer cycles affected the pregnancy rates, with a significant trend towards higher rates of success within the first three transfer cycles (\( p = 0.017 \)) (Table I).

Sperm morphology was a significant factor contributing to the probability of success after GIFT. According to sperm morphology three categories were identified: 0 - 4% normal forms (p-pattern), 5 - 14% normal forms (g-pattern) and > 14% normal forms (n-pattern). Significant differences occurred in the overall pregnancy rates in the p-pattern group (PR = 25.2%) when compared with the g-pattern (PR = 37.7%; \( p = 0.0064 \)) and n-pattern (PR = 36.8%; \( p = 0.0379 \)) groups. Within the two female age groups (\( \leq 38 \) years, > 38 years) the ongoing pregnancy rates were also significantly influenced by sperm morphology (\( p = 0.017 \)) (Table II).

Using the variables found to be significantly associated with pregnancy, stepwise logistical regression was used to select the most appropriate model for a more detailed investigation. Multiple logistical regression, using maximum likelihood estimates, was used to model pregnancy rates on the following risk factors: female age, total sperm concentration after swim-up, sperm morphology, maturity of oocytes and number of oocytes transferred (Table III). The likelihood ratio test was used to assess the goodness of fit of the model.

With regard to age, patients \( \leq 38 \) years of age had a significantly better chance of success than those > 38 (OR 1.87, 95% CI: 1.22 - 2.85). The main egg and seminal variables significantly associated with success after GIFT were sperm morphology > 4% normal forms (OR 1.58, 95% CI: 1.04 - 2.42) and the number of mature oocytes transferred (3 x MII oocytes, OR 7.51, 95% CI: 1.74 - 32.42). The transfer of MI oocytes was negatively associated with pregnancy (OR 0.54, 95% CI: 0.28 - 1.04). Total sperm concentration after swim-up also significantly influenced success with GIFT (OR 1.01, 95% CI: 1.00 - 1.03).

**Table I. Relation between selected variables and total pregnancy rate after GIFT**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Percentage</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte maturity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaphase I</td>
<td>95/853</td>
<td>11.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>300/853</td>
<td>35.2</td>
<td></td>
</tr>
<tr>
<td>Number of oocytes transferred</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>0</td>
<td>2/19</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1/25</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27/86</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>240/603</td>
<td>39.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>27/109</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2/5</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1/5</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0/1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Number of stimulated cycles</td>
<td></td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>1</td>
<td>188/491</td>
<td>38.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>4</td>
<td>8/42</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6/25</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>&gt; 6</td>
<td>6/26</td>
<td>23.1</td>
<td></td>
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</table>

**Table II. Influence of female age and sperm morphology on overall pregnancy rate**

<table>
<thead>
<tr>
<th>Sperm morphology (%)</th>
<th>Women ( \leq 38 ) years</th>
<th>Women &gt; 38 years</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 4</td>
<td>27.1% (35/129)</td>
<td>15.0% (3/20)</td>
<td>0.017</td>
</tr>
<tr>
<td>5 - 14</td>
<td>40.0% (181/452)</td>
<td>24.4% (19/78)</td>
<td></td>
</tr>
<tr>
<td>&gt; 14</td>
<td>38.6% (51/132)</td>
<td>29.0% (9/31)</td>
<td></td>
</tr>
</tbody>
</table>
those factors pertaining to the characteristics of the gametes. As expected, female age proved to be an important predictor of success. The data presented in this study (overall pregnancy rates of 37.3% and 23.7% in women ≤ 38 and > 38 years respectively, \( p = 0.0028 \)) are consistent with the results of previous reports of lower pregnancy rates in older women.\(^{15-17}\)

This trend was also noted in the ongoing pregnancy rates for women aged 38 years and younger and those above 38 years of age (28.4% versus 11% respectively, \( p = 0.0001 \)). Our study also shows that women of 38 years and younger have a significantly better chance (OR 1.87, 95% CI: 1.22 - 2.85) of success after GIFT than women older than 38. When Penzias et al.\(^{17}\) applied GIFT to a population of women ≥ 40 years of age, they achieved a clinical pregnancy rate of only 9.6% per cycle. However, this was higher in the group receiving 4 oocytes (33% versus 14%, \( p < 0.005 \)).

The recruitment and maturation of oocytes is one of the key steps in a GIFT programme and the expectation of pregnancy has been shown to be related positively to the number and quality of the oocytes transferred (Table I). With regard to maturity, MI oocytes were negatively associated with pregnancy (OR 0.54, 95% CI: 0.28 - 1.04). Although only 3 MI oocytes were significantly lower than with MII oocytes (11.1% versus 35.2%; \( p = 0.001 \)). Based on these findings and in the rare event of yielding only MI oocytes after stimulation it is important to consider diverting from GIFT and rather using IVF as a treatment option (after proper maturation of MI oocytes). Although the optimal number of oocytes to be transferred in GIFT is debatable and must be examined carefully in relation to the incidence of higher order multiple births, the optimal number according to the data presented is 3 MI oocytes (PR = 39.8%).

The introduction of intracytoplasmic sperm injection (ICSI) has meant that GIFT must be critically evaluated as treatment for severe male factor infertility. In our opinion patients with p-pattern morphology should be offered IVF/ICSI treatment due to its potential to achieve a higher pregnancy rate in this group.

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pregnancy rates did not differ significantly when 3 or 4 oocytes were transferred.

With the observed OR of 5.2 and 7.5 when 2 or 3 oocytes are transferred (Table III), the question arises whether in younger patients and patients undergoing GIFT for a second baby a policy of transferring only 2 oocytes (in cases with sperm morphology in the g- or n-pattern) is not a better option. This would most certainly affect the incidence of multiple pregnancies while still giving an excellent prognosis for pregnancy outcome.

The group of GIFT patients is heterogeneous and today the procedure offers a solution for several causes of infertility. From the results of this study it seems that female aetiology is not an important factor for predicting success in a GIFT programme. When comparing pregnancy rates according to aetiology no significant differences were found ($p = 0.088$). This implies that other variables are more important in predicting success after GIFT.

Worldwide, the GIFT procedure accounts for 13.5% of all assisted reproductive techniques. In South Africa during 1995, 71% of all ARTs were reported to be GIFT procedures. Although many factors may influence the likelihood of pregnancy from GIFT it appears from our study that egg and sperm characteristics together with female age are the most important predictors of success. This argument is further strengthened by the fact that no significant differences in pregnancy rates occurred among the different aetiological groups. In a clinic with such a high use of this technique it is important to consider the emotional and financial costs involved and to relate this information to couples entering ART programmes.

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References