



## 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.

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In many countries the increase in the number of infertility units and the number of couples seeking treatment indicates widespread acceptance of assisted reproduction as a strategy for treatment of involuntary childlessness. Because the treatment itself is costly, it is imperative that prospective participants in assisted reproduction programmes be given accurate indications of their probability of having a healthy live baby following treatment.

In 1984 the first pregnancy using gamete intrafallopian transfer (GIFT) was achieved in a woman with unexplained infertility.<sup>1</sup> The development of GIFT was based on the assumption that transfer of oocytes and spermatozoa to the Fallopian tubes would bring about optimal conditions for fertilisation and early embryo growth, resulting in higher quality embryos with a higher implantation rate.

The GIFT procedure has substantially enhanced the prospects of many couples entering assisted reproductive technique (ART) programmes, and numerous publications have confirmed its usefulness.<sup>2</sup> The pregnancy rate (PR) with

this technique has been reported to range between 27% and 48%,<sup>2</sup> and in 1996, 29% of deliveries per retrieval were reported by the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry.<sup>3</sup>

As several factors may influence the likelihood of pregnancy from GIFT, it is difficult to assess the contribution of a single factor. Some of the factors that have been investigated include oocyte maturity,<sup>4</sup> number of oocytes transferred,<sup>5</sup> age,<sup>6</sup> sperm characteristics,<sup>4,7</sup> fertilisation of excess oocytes<sup>8</sup> and aetiology.<sup>9,10</sup>

These considerations prompted us to conduct a multivariate analysis of factors affecting the probability of pregnancy in the GIFT programme at the Tygerberg Hospital fertility centre.

### Materials and methods

#### Patients

Using results from our computerised databank we retrospectively analysed a total of 863 GIFT cases seen 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

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laboratory after 3 - 4 days of abstinence. Semen analyses were performed according to the World Health Organisation (WHO) criteria<sup>11</sup> and sperm morphological features were evaluated according to the WHO 1999 criteria (Tygerberg strict criteria).<sup>11</sup> Teratozoospermia was diagnosed if the semen analyses showed < 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).<sup>7</sup>

### Sperm preparation

Semen obtained 2 hours before the GIFT procedure was allowed to liquefy, 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 Veeck<sup>12</sup> 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  $\beta$ -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  $\chi^2$  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<sup>13</sup> (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 21.43%



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 (< 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

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

Variable	Pregnant		<i>p</i> -value
	Number	Percentage	
Oocyte maturity			0.001
Metaphase I	95/853	11.1	
Metaphase II	300/853	35.2	
Number of oocytes transferred			0.001
0	2/19	10.5	
1	1/25	4.0	
2	27/86	31.4	
3	240/603	39.8	
4	27/109	24.8	
5	2/5	40.0	
6	1/5	20.0	
7	0/1	0	
Number of stimulated cycles			0.017
1	188/491	38.2	
2	64/193	33.7	
3	28/76	36.8	
4	8/42	19.1	
5	6/25	24.0	
> 6	6/26	23.1	

used to assess the goodness of fit of the model.

With regard to age, patients < 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 × 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).

## Discussion

Although a variety of factors may influence pregnancy outcome in a GIFT programme, the results of this study suggest that the most important factors are female age and

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

Sperm morphology (%)	Overall pregnancy rate		<i>p</i> -value
	Women < 38 years	Women > 38 years	
0 - 4	27.1% (35/129)	15.0% (3/20)	0.017
5 - 14	40.0% (181/452)	24.4% (19/78)	
> 14	38.6% (51/132)	29.0% (9/31)	



Table III. Analysis of maximum likelihood estimates: overall pregnancy rates

Variable	p-value	Odds ratio	95% CI
Age $\geq 38$ yrs	0.0039	1.87	1.22 - 2.85
TSC (after swim-up)	0.0488	1.01	1.00 - 1.03
Morphology $< 5\%$	0.0327	1.58	1.04 - 2.42
GIFT MI	0.0640	0.54	0.28 - 1.04
GIFT MII			
1 x MII	0.3562	2.32	0.39 - 13.88
2 x MII	0.0309	5.20	1.16 - 23.25
3 x MII	0.0069	7.51	1.74 - 32.42
4 x MII	0.0242	5.78	1.26 - 26.59

TSC = total swim-up count; MI = metaphase I; MII = metaphase II.

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  $\leq 38$  and  $> 38$  years respectively,  $p = 0.0028$ ) are consistent with the results of previous reports of lower pregnancy rates in older women.<sup>14-16</sup> This trend was also noted in the ongoing pregnancy rates for women aged  $\leq 38$  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  $\leq 38$  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.*<sup>17</sup> applied GIFT to a population of women  $\leq 40$  years of age, they achieved a clinical pregnancy rate of only 9.6% per transfer as opposed to 27.3% for those  $< 40$  years of age. The reported ongoing pregnancy rates in this study of 28.4% and 10.7% in patients  $\leq 38$  and  $> 38$  years respectively, are consistent with the data reported by Penzias *et al.*<sup>17</sup> suggesting that GIFT does not overcome the effects of ageing.

Despite the ability to achieve pregnancy in women  $\geq 38$  years of age and older, these women are at higher risk of adverse maternal and fetal events, e.g. higher ectopic pregnancy rate<sup>3</sup> and a spontaneous miscarriage rate as high as 50%.<sup>18</sup> Considering the total pregnancy rate of 23.7% achieved in our study in women older than 38, the low ongoing pregnancy rate (10.7%) in this group might be related to the higher incidence of these adverse events. Therefore, older women enrolling for GIFT must be informed about the significantly increased risk of failure compared with younger patients.

Sperm count, motility, and the percentage of normal morphological features have been the traditional criteria for semen quality. The results of our study confirm significantly lower pregnancy rates when  $\leq 4\%$  normal forms were present ( $p = 0.0064$ ). Sperm morphology of  $> 4\%$  was also significantly associated with success (OR 1.6, 95% CI: 1.04 - 2.42). It was shown that if strict criteria are used to evaluate sperm morphology, this parameter could be used to predict the

chances of *in vitro* fertilisation (IVF) and subsequent pregnancy outcome. Appraisal of GIFT for male factor infertility must include semen properties in order to evaluate the effectiveness of this method. In a recent structured review of published literature on the predictive value of normal sperm morphology as an indicator of male fertility potential in the IVF situation, Coetzee *et al.*<sup>19</sup> reported normal sperm morphology as an integral part of the standard semen analyses and the most cost-effective means of evaluating the male factor. In the prediction of pregnancy, 82% and 75% of the studies produced positive predictive values when using the 5% and 14% thresholds respectively. Our study confirms the importance of sperm morphological characteristics as a predictor of fertilisation success in a GIFT programme. The fact that sperm morphology plays a significant role in pregnancy outcome in a GIFT programme must be explained to the patients during counselling before the GIFT procedure.

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.

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 transferred when standard stimulation protocols yielded limited numbers of mature oocytes, the pregnancy rates with 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 MII oocytes (PR = 39.8%). The transfer of 3 mature oocytes was associated with a better chance of success (OR 7.51) than when 2 (OR 5.20) or 4 (OR 5.78) mature (MII) oocytes were transferred. The transfer of only 1 mature oocyte was not associated with success and cannot be justified ( $p = 0.3562$ , OR 2.32, 95% CI: 0.39 - 13.88). In a study previously performed at our unit, Matshe *et al.*<sup>20</sup> also reported higher ongoing pregnancy rates in patients younger than 38 years when transferring 3 oocytes than when transferring 4 (28.8% versus 20.6%,  $p < 0.005$ ). However, the multiple pregnancy rates were shown to be higher in the group receiving 4 oocytes (33% versus 14%,  $p < 0.005$ ). For patients older than 38 the ongoing and multiple



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.<sup>20</sup> 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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