

## The in vitro fertilization of supernumerary oocytes in a gamete intrafallopian transfer program

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*Gamete intrafallopian transfer (GIFT) was performed on a series of 42 couples in whom four oocytes were replaced into the fallopian tubes and the remaining oocytes were inseminated with 100,000 motile spermatozoa. Fertilization in vitro was assessed 16 to 20 hours later. An overall pregnancy rate after GIFT of 40.0% (12/30) in normospermic couples and 16.7% (2/12) in oligospermic couples was seen. Supernumerary oocytes were submitted to in vitro fertilization (IVF) and the findings related to the likelihood of pregnancy. No correlation between IVF and the likelihood of GIFT pregnancies was found in either the oligospermic or normospermic couples. A similar proportion of pregnancies was seen in couples with or without evidence of fertilization of the supernumerary oocytes in both the oligospermic and normospermic couples. However, a high proportion of normospermic couples with no evidence of fertilization had only one oocyte remaining for insemination. These results suggest that the failure of low numbers of supernumerary oocytes to fertilize in vitro after GIFT relates to oocyte selection criteria and does not mean a reduced chance of conception in that treatment cycle. Fertil Steril 47:802, 1987*

Gamete intrafallopian transfer (GIFT) was originally described by Asch et al.<sup>1</sup> for the treatment of infertility in couples in whom the female partner has patent fallopian tubes. The potential usefulness of this technique is now being confirmed by other groups,<sup>2-4</sup> although conventional GIFT is inappropriate for various disorders and requires modification. Examples include the

transfer of an increased number of spermatozoa in cases of oligospermia<sup>5</sup> and the replacement of pronuclear oocytes to confirm fertilization in vitro when the husband is severely oligospermic<sup>5</sup> or the wife has antispermatozoal antibodies in serum.<sup>6</sup>

However, one of the main limitations of GIFT is the inability to determine whether fertilization occurs in vitro after the transfer of the oocytes and spermatozoa if pregnancy fails to ensue. The principal aims of the current study were (1) to correlate the in vitro fertilization (IVF) of supernumerary oocytes remaining after the GIFT attempt with the occurrence of pregnancy in that treatment cycle; (2) to determine whether the failure of any supernumerary oocytes to fertilize indicates a reduced chance of conception in the

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GIFT treatment cycle; and (3) to determine the value of using supernumerary oocytes in an IVF system to predict successful fertilization in vivo.

## MATERIALS AND METHODS

All patients included in the study had  $\geq 5$  oocytes collected, with any oocytes remaining after the GIFT attempt being inseminated with the husband's spermatozoa. The women in this study had follicular growth stimulated by the administration of human menopausal gonadotropin (hMG; Pergonal, Serono Laboratories, Inc., Rome, Italy) or a combination of hMG and clomiphene citrate (Clomid, Merrell-Dow Pharmaceuticals, Inc., Cincinnati, OH). The response to treatment was monitored daily from day 8 of the menstrual cycle by ultrasound and the measurement of serum  $17\beta$ -estradiol, progesterone, and luteinizing hormone by use of radioimmunoassay. Ovulation was triggered by the occurrence of an endogenous luteinizing hormone surge or the administration of 10,000 IU human chorionic gonadotropin (Primogonyl, Schering, Berlin, FRG) at an appropriate time,<sup>7</sup> and oocytes were collected by laparoscopy or an ultrasonically guided transvaginal route<sup>8</sup> as for the IVF program.

Semen samples were produced by masturbation 2 hours before the oocyte collection and motile spermatozoa prepared by an overlay technique to a final concentration of 2 to  $4 \times 10^6$ /ml. Semen samples were classified as normospermic ( $\geq 12 \times 10^6$  motile spermatozoa/ml) or oligospermic ( $< 12 \times 10^6$  motile spermatozoa/ml)<sup>9</sup> and had  $\geq 60\%$  normal forms.

Oocytes were examined with the dissecting microscope and graded according to the appearances of the oocyte, the coronal coat, and the cumulus cells.<sup>10</sup> Four mature oocytes were replaced per patient into the fallopian tubes at laparoscopy, together with approximately 100,000 motile spermatozoa, by use of a 50-cm, 16-gauge Teflon catheter (Cook, Melbourne, Australia) inserted 4 cm into each tube. In the case of oligospermic couples, the technique was modified so that the maximum number of motile spermatozoa recovered was transferred back into the fallopian tubes.<sup>5</sup>

Remaining oocytes, over and above the four transferred, were each inseminated with 100,000 motile spermatozoa 4 to 6 hours after the collection and pronuclei identified 16 to 20 hours later. Patients were counseled before the treatment cycle regarding the fate of supernumerary oocytes.

For those couples not choosing to dispose of the oocytes, resulting embryos were either cryopreserved, donated to an approved research program, or given to another infertile couple, according to the wishes of the patients and after the signing of appropriate consent forms. All of these options have been approved by the University of Western Australia's Committee for Human Rights.

The fertilization rates of supernumerary oocytes were compared with the rates obtained in the IVF program<sup>7</sup> when corresponding numbers of oocytes were collected. The IVF patients were all normospermic, and treatment cycles occurred over the same period as the GIFT cycles.

Pregnancies were diagnosed 16 to 19 days after oocyte collection by a rising concentration of  $\beta$ -human chorionic gonadotropin in the serum and confirmed about 5 weeks later by ultrasound.

Data were analyzed in  $2 \times 2$  contingency tables with Chi-square analysis or the Fischer's Exact probability test. The numbers of oocytes remaining were compared by Student's *t*-test. Differences were considered significant if  $P < 0.05$ .

## RESULTS

In the current series of patients, overall pregnancy rates of 40.0% (12/30) and 16.7% (2/12) were obtained in the normospermic and oligospermic couples, respectively, as shown in Table 1. Furthermore, there was no difference in the pregnancy rate when the couples were categorized according to whether one or more oocytes fertilized for both the normospermic and oligospermic groups.

However, for normospermic couples a significantly lower number of oocytes remained after the GIFT attempt and were inseminated in the group with no evidence of fertilization. This is shown in Table 2. This is explained by the high proportion (9/13, 69.2%) of couples with only one oocyte remaining after the GIFT attempt that did not fertilize (Fig. 1). The overall fertilization rate

**Table 1.** Pregnancy Rates in Couples With or Without One or More Oocytes Fertilized in Vitro

Semen quality	Pregnancies/treatment cycle		Significance
	No fertilization	Fertilization <sup>a</sup>	
Oligospermia	1/9 (11.1%)	1/3 (33.3%)	NS <sup>b</sup>
Normospermia	5/14 (35.7%)	7/16 (43.8%)	$\chi^2 = 0.20$

<sup>a</sup>One or more oocytes fertilized.

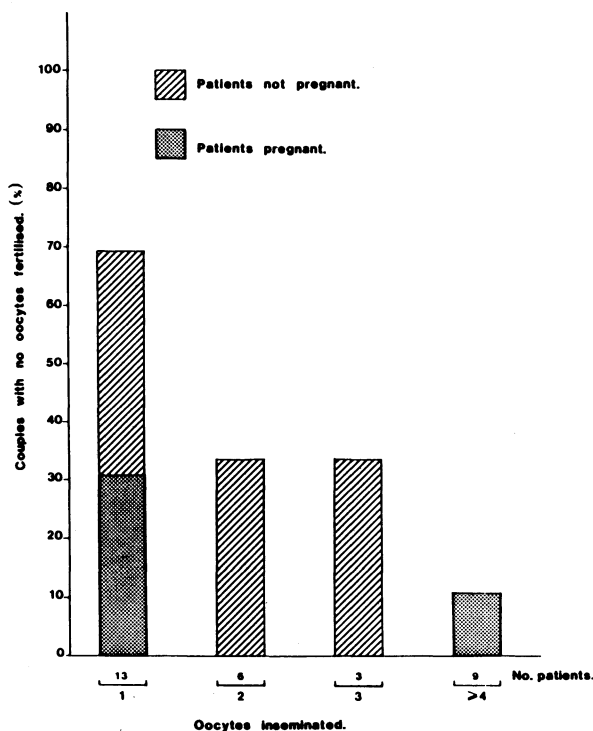
<sup>b</sup>Fischer's Exact probability test; not significant.

**Table 2.** Number of Oocytes (Mean  $\pm$  Standard Error of the Mean) that Were Remaining After the GIFT Attempt and Inseminated

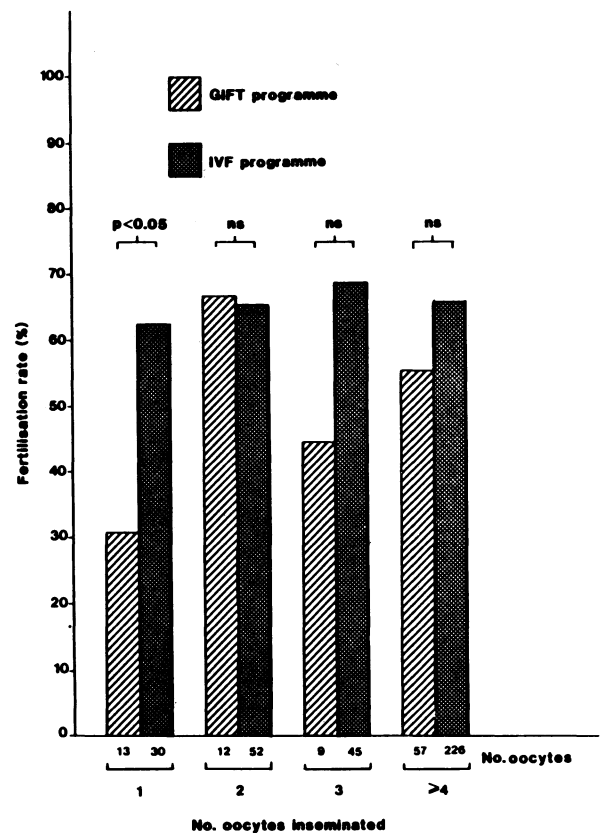
Semen quality	1 or more oocytes fertilized		Significance
	Yes	No	
Oligospermia	1.75 $\pm$ 0.41 (n = 3)	2.29 $\pm$ 0.52 (n = 9)	—
Normospermia	3.94 $\pm$ 0.65 (n = 16)	1.50 $\pm$ 0.28 (n = 14)	$P < 0.01$

of oocytes remaining and inseminated for normospermic couples has been compared with the fertilization of oocytes from women with normospermic husbands in the IVF program over the same period (Fig. 2). It was found that the fertilization rate in cases in which only one oocyte remained was significantly lower ( $P < 0.05$ ) than in the IVF cases in which only one oocyte was collected. However, no difference existed in the fertilization rates between the two programs when 2, 3, 4, or more oocytes were inseminated.

Details of those women who then became pregnant even though the supernumerary oocytes did not fertilize are given in Table 3. Three of the five women had multiple pregnancies, and two women



**Figure 1**  
The incidence of normospermic couples with no evidence of fertilization when 1, 2, 3, 4, or more oocytes were inseminated.



**Figure 2**  
The overall fertilization rates for normospermic couples of oocytes remaining after GIFT when 1, 2, 3, 4, or more oocytes were inseminated, compared with fertilization of corresponding numbers of oocytes collected in the IVF program.

had all four oocytes replaced into one tube because of unilateral tubal occlusion.

## DISCUSSION

Pregnancy rates of around 30% to 40% may be expected after GIFT in normospermic couples, as described elsewhere<sup>1, 3</sup> and confirmed in the current study. However, if conception fails to occur in a given treatment cycle, then there is usually no indication as to the reason for the lack of success, and whether fertilization has occurred in vivo or not. The current study has shown that the insemination of supernumerary oocytes, in particular low numbers of oocytes, and their subsequent failure to fertilize in vitro does not mean that there is a reduced chance of conception in that treatment cycle. This is a particularly important finding, because patients with no evidence of fertilization can become very anxious. These patients can therefore be counseled to allay any fears.

**Table 3.** Details of Normospermic Couples with No Fertilization of Supernumerary Oocytes but in Whom Pregnancy Occurred from the GIFT Treatment Cycle

Patient	Female factor	Stimulation regimen	Oocytes			Pregnancies
			Collected	Transferred <sup>a</sup>	Remaining	
1	Negative PCT <sup>b</sup>	CC/hMG	8	2 + 2	4	Singleton
2	Negative PCT	CC/hMG	5	2 + 2	1	Singleton
3	Negative PCT	CC/hMG	5	2 + 2	1	Twins
4	Negative PCT	CC/hMG	5	0 + 4	1	Triplets
5	Failed AID <sup>d</sup>	CC/hMG	5	4 + 0	1	Twins

<sup>a</sup>Left tube and right tube.

<sup>b</sup>PCT, postcoital test.

<sup>c</sup>CC, clomiphene citrate.

<sup>d</sup>Artificial insemination by donor.

In the current study, it is accepted that the oocytes are subjectively assessed regarding maturity and often the better-looking oocytes transferred back into the fallopian tube. However, this is likely to be the case in a routine GIFT program when the chance of conception in that treatment cycle is to be maximized. For concrete diagnostic information, the value of using the heterologous ovum penetration test<sup>11</sup> to assess the likelihood of IVF requires further investigation. However, limitations are likely to exist with the use of zona-free hamster ova because a negative heterologous ovum penetration test does not mean that fertilization will not take place even though a positive test result is a good indication that fertilization will occur.<sup>11</sup> In the current study, the majority of discordant results in which fertilization did not occur in vitro but pregnancy ensued were seen when small numbers of supernumerary oocytes were used. Therefore, it would seem appropriate to evaluate further the use of larger numbers of supernumerary oocytes as a diagnostic tool. For this to be feasible, a more severe stimulation protocol would need to be employed than is routinely used in our GIFT program, or IVF-embryo transfer program in which an average of 5.0 and 5.3 oocytes are recovered when clomiphene citrate/hMG and hMG alone are used.<sup>12</sup> The stimulation used in the IVF program would thus still give only one or two supernumerary oocytes in a large proportion of cases.

The question of whether an IVF attempt should follow an unsuccessful GIFT attempt has been raised previously.<sup>3</sup> Nevertheless, one should perhaps resist abandoning GIFT too quickly because not all women could be expected to conceive at the first attempt, and there is bound to be a cumulative pregnancy rate with repeated attempts, as experienced in an IVF program.<sup>13</sup> Should an as-

essment of the ability of spermatozoa to fertilize oocytes need to be made, then it would seem more appropriate to use pronuclear stage transfer<sup>4</sup> as this is hoped to confirm fertilization and yet allow the very early embryo to develop within its natural tubal environment.

In conclusion, the current study has demonstrated that (1) there is no correlation between the fertilization of supernumerary oocytes and the incidence of pregnancy in the GIFT treatment cycle; (2) patients in whom no fertilization of supernumerary oocytes is seen should be counseled that there is not a reduced chance of conception; and (3) only the use of large numbers of supernumerary oocytes in an IVF system will prove useful in predicting successful fertilization in vitro.

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