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The role of gamete intrafallopian transfer (GIFT) in the treatment of oligospermic infertility

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Gamete intrafallopian transfer (GIFT) was used to treat 32 couples where the male partner was oligospermic ($<12 \times 10^6$ motile spermatozoa per milliliter of semen). Initially, 100,000 motile spermatozoa were transferred per fallopian tube and no pregnancies were achieved in 11 cases. The technique was then modified so that a maximal number of motile spermatozoa were transferred (range, 0.11 to 0.90×10^6 spermatozoa) and 6 of 21 (29%) pregnancies resulted, with 325,000 spermatozoa being the lowest number associated with pregnancy. It appears that the modified GIFT technique, whereby an increased number of motile spermatozoa are replaced with the oocytes, is an effective therapy in the treatment of oligospermic infertility. Fertil Steril 48:608, 1987

Since the first report by Asch et al.,[1] gamete intrafallopian transfer (GIFT) has been demonstrated to be a successful form of treatment for normospermic couples in which the female partner has patent and accessible tubes.[2-4] Furthermore, in a preliminary report on the use of GIFT in the treatment of oligospermic infertility,[5] one of two women became pregnant following the transfer of four oocytes and 100,000 motile spermatozoa; however, the pregnancy miscarried within the first 2 months of gestation. Unfortunately, our early experience in treating oligospermic infertility with the conventional GIFT technique was not so encouraging.[2] The present report further examines the usefulness of GIFT in treating oligospermic couples, with the principal aims being to: (1) describe the poor success rate achieved when only 100,000 motile spermatozoa were replaced into each fallopian tube; and (2) determine the value of a modified GIFT technique in which an increased number of spermatozoa are replaced.

MATERIALS AND METHODS

Infertile couples attending PIVET Medical Centre were evaluated according to a systematic protocol.[6] The male partners were classified according to the criteria of the World Health Organization[7] as being moderately oligospermic (5.1 to 11.9×10^6 motile spermatozoa per milliliter of semen) or severely oligospermic ($\leq 5 \times 10^6$ motile spermatozoa per milliliter of semen). Antispermatozoal antibodies are measured in the serum of both partners: cervical mucus and seminal plasma. None of the patients included in this report had antispermatozoal antibodies.

All women in this study had follicular growth stimulated by the administration of clomiphene citrate (CC; Clomid, Merrell-Dow Pharmaceuticals, Inc., Sydney, Australia), human menopausal gonadotropin (hMG; Pergonal, Serono Pharmaceuti-

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cals, Inc., Rome, Italy), or a combination of CC and hMG. The response to treatment was monitored daily from day 2 of the menstrual cycle by the measurement of 17β-estradiol (E_2), progesterone (P), and luteinizing hormone (LH) using radioimmunoassays, and from day 8 of the cycle using ultrasound. This monitoring technique detects the earliest E_2 rise to apply the 6-day rule.[8] Ovulation was triggered by the occurrence of an endogenous LH surge or the administration of 10,000 IU human chorionic gonadotropin (hCG; Primogonyl, Schering AG, Berlin, West Germany) at an appropriate time[9] and oocytes collected approximately 35 hours later via laparoscopy or an ultrasonically guided needle when ovarian access was restricted.

Husbands produced a semen sample by masturbation 2 hours before the oocyte collection. Motile spermatozoa were then isolated using the procedure of the in vitro fertilization (IVF) program, being either an overlay technique[10] or a sedimentation method.[11] The latter was particularly useful for oligospermic samples and involved placing the washed pellet of cellular matter and debris from the semen, suspended in 1 ml human tubal fluid (HTF) medium[12] supplemented with 20% serum, into a culture well (four-well, multidish, Nunc, Inter Med, Denmark). This mixture was left for 1 hour to allow the debris to settle to the bottom of the well. The supernatant was then taken off; this contained the motile spermatozoa. However, care was taken to recover the maximum possible number of spermatozoa without removing any of the sedimented debris and compromising the quality of the preparation. The final preparation of spermatozoa was kept in a humidified atmosphere of 90% N_2, 5% O_2, and 5% CO_2 at 37°C until required. In the initial series of patients, the final suspension of motile spermatozoa was adjusted to 100,000/50 μl, whereas, in the later series, the spermatozoa recovered were concentrated into a volume of 0.3 ml to give a final concentration of >100,000/50 μl.

Once oocytes had been collected and graded,[13] the gametes were transferred to the fallopian tube at laparoscopy. The GIFT catheter was a 50-cm (length), 16-gauge, end-hole Teflon catheter (William Cook, Melbourne, Australia). The catheter was then loaded with: (1) HTF media with 20% pooled serum to fill the dead space; (2) 5 μl air; (3) 25 μl of medium with oocytes; (4) 5 μl air; (5) 50 μl of spermatozoal suspension; and (6) 5 μl air.

The fallopian tube was then catheterized as described by Molloy et al[4] and the gametes transferred. Pregnancy was diagnosed 16 to 19 days after transfer by a rising concentration of β-hCG in serum and confirmed about 5 weeks later by ultrasound.

RESULTS

Table 1 provides the raw data for the semen profiles of the moderate and severely oligospermic groups, using the standard or modified techniques of insemination. The semen profiles included spermatozoal density, motility count, and morphology assessment on both initial and washed preparations. The data means of the original samples were compared by Student's *t*-test. In addition, the oocyte numbers and quality grading were noted and the results ranked for comparison by the Wilcoxon-Mann-Whitney nonparametric method. In all respects of semen and oocyte factors, the groups proved to be well matched because no significant differences were revealed between them when analyzed with respect to those having the standard or modified insemination technique.

The pregnancy rates in the oligospermic couples are shown in Table 2 and categorized according to use of the standard or modified GIFT technique. Applying the standard method of transferring 100,000 motile spermatozoa per tube, no pregnancies were obtained from 11 cases. When an increased number of motile spermatozoa were transferred, six pregnancies were achieved from 21 attempts (29%). Four pregnancies were ongoing singleton pregnancies now delivered healthy, one was an ectopic pregnancy, and one was a blighted ovum.

The number of motile spermatozoa transferred per tube using the modified technique is shown in Figure 1, with pregnancies being achieved when a range of 0.36 to 0.69 $\times 10^6$ motile spermatozoa were transferred. There is no difference in the number (geometric mean ± limits of 1 standard error of the mean [SEM]) of motile spermatozoa transferred per tube for pregnant (0.46 [0.43 to 0.51]) and nonpregnant (0.42 [0.37 to 0.49]) patients.

Therefore, spermatozoal preparations from oligospermic patients should contain > 100,000 spermatozoa to achieve pregnancies. In this study, the lowest number associated with pregnancy was 325,000 motile spermatozoa per milliliter.

DISCUSSION

Extensive experience has been gained in the treatment of oligospermic infertility by IVF-em-

Table 1 Semen Characterization of Individual Patients Participating in This Study

Patient	Vol	Initial count TC[a]	MC[b]	PMC[c]	% Motile	Washed count TC	MC	PMC	% Motile	Normal morphology (%)	No. of oocytes	Quality of oocytes 1	2	3	Sperm inseminated
										%					
Patients with severe oligospermia using standard technique															
1	2.1	1.3	1.05	0.9	80.8 (++)	1.5	1.05	0.8	70	60	2		1	1	100,000
2	1.4	4.3	1.5	0.6	35 (++)	3.4	1.0	0.4	29	52	5		2	3	100,000
3	3.8	9.5	4.3	2.6	45 (++)	0.9	0.4	0.3	44	64	4		2	2	100,000
4	4.0	16.3	3.3	1.6	20 (++)	0.9	0.3	0.15	33	80	6		4	2	100,000
Mean	2.83	7.85	2.54	1.43	45.2	1.68	0.69	0.41	44.0	64	4.25				
± SEM	0.64	3.29	0.76	0.45	12.93	0.59	0.20	0.14	9.22	5.89	2.12				
Patients with severe oligospermia using modified technique															
5	4.0	9.0	2.0	0.3	22 (++)	1.6	0.5	0.1	31	26	4	1	3		250,000
6P[d]	4.0	4.6	1.7	0.8	42 (++)	1.4	1.4	0.9	100	60	2		1	1	500,000
7P	3.0	8.0	5.0	4.0	62 (++)	5.6	3.8	2.9	68	70	4		1	3	325,000
8	8.5	0.5	0.2	0.1	40 (++)	3.8	1.7	1.2	45	39	5	2	2	1	150,000
9P	9.0	7.5	1.5	1.05	20 (++)	5.0	1.0	0.5	20	60	4		1	3	380,000
10	3.0	1.7	5.0	3.0	29 (+)	13.2	4.9	2.0	37	40	2			2	500,000
11	4.8	7.0	4.0	3.0	57 (++)	0.8	0.3	0.11	38	60	4		2	2	110,000
12	4.7	0.15	0.15	0.15	100 (++)	0.05	0.05	0.025	50	50	9	1	3	5	900,000
13	3.0	1.0	3.0	1.8	30 (++)	1.4	0.75	0.66	54	40	3			3	400,000
Mean	4.89	5.38	2.50	1.57	44.67	3.65	1.6	0.93	49.22	49.44	4.11				
± SEM	0.76	1.26	0.63	0.48	8.44	1.36	0.53	0.31	7.84	4.70	1.37				
Patients with moderate oligospermia using standard technique															
14	3.9	21	7	5	33 (++)	1.3	0.9	0.7	69	70	5		1	4	100,000
15	3.5	18	10	7	55 (++)	1.2	0.95	0.85	89	50	2		2		100,000
16	4.0	30	9	1.8	30 (++)	3.0	1.4	1.0	47	66	6	1	5		100,000
17	3.8	17	9	5	55 (++)	9.6	6.0	4.5	62	80	2		2		100,000
18	3.5	21	7	5	33 (++)	1.3	0.9	0.7	69	70	4		1	3	100,000
19	1.3	15	7.5	0.75	50 (+)	1.45	0.4	0.04	28	70	3	1	2		100,000
20	2.3	13	7	5	54 (++)	6.8	4.1	3.6	60	50	4		2	2	100,000
Mean	3.19	19.29	8.07	4.22	44.29	3.52	2.09	1.60	60.57	65.14	3.71				
± SEM	0.38	2.10	3.05	0.82	4.40	1.26	0.80	0.64	7.22	4.22	0.56				
Patients with moderate oligospermia using modified technique															
21	2.5	10	5.8	2.3	58 (++)	2.6	1.7	0.7	65	45	4		1	3	750,000
22	3.4	24	9	1.0	37 (++)	2.1	1.2	0.96	57	70	4			4	490,000
23	3.0	16	11	7.0	69 (++)	9.7	5.2	4.6	54	40	3			3	450,000
24	3.5	60	7	3.8	12 (+)	8.0	4.3	2.1	54	11	5			5	800,000
25	4.4	15	11	9.0	73 (++)	3.1	2.9	2.7	93	57	7		7		250,000
26P	5.0	21	10	5.5	48 (++)	16.0	6.9	4.5	43	60	1			1	500,000
27	3.5	22	8	3.0	36 (++)	8.3	5.1	4.7	61	59	9			9	415,000
28	2.2	22	7	4.2	32 (++)	7.0	2.6	1.9	37	59	6		2	4	500,000
29P	2.3	13	9	8.0	69 (++)	9.6	8.6	4.7	88	38	5		3	2	450,000
30	1.0	14	10	7.0	71 (++)	3.1	2.3	2.0	74	75	5		2	3	500,000
31P	4.0	20	6	4.8	30 (++)	6.1	3.0	2.2	50	70	4		3	1	690,000
32	4.2	12	7	3.5	58 (++)	4.6	3.1	2.5	67	70	8		5	3	500,000
Mean	3.25	20.75	8.4	4.93	49.42	6.68	3.9	2.80	61.92	54.50	5.08				
± SEM	0.32	3.81	0.53	0.70	5.75	1.16	0.63	0.42	4.85	5.28	0.63				

[a] TC, total count.
[b] MC, motile count.
[c] PMC, progressively motile count; graded motility (+ to +++).
[d] P, pregnancy achieved.

bryo transfer (ET) at PIVET Medical Centre.[14] The present study evaluates the use of GIFT in treating oligospermic couples. Despite the achievement of a pregnancy by Asch et al.[5] using the conventional GIFT technique, we were unable to obtain a pregnancy in the first 11 oligospermic patients treated, at a time when 4 of 9 pregnancies were achieved in normospermic couples under similar conditions.[2] Data from our IVF program[10,14] and from studies using zona-free hamster ova[15]

Table 2 Incidence of Pregnancy for Oligospermic Couples in a GIFT Program, When Either the Standard (100,000) or Modified (>100,000) Number of Motile Spermatozoa Are Transferred

Technique	Semen quality	Oocytes transferred				Total
		1	2	3	≥4	
Standard	Moderate oligospermia	0/1	0/2	0/1	0/3	0/7
	Severe oligospermia	—	0/1	—	0/3	0/4
Modified	Moderate oligospermia	1/1	—	0/1	2/10	3/12
	Severe oligospermia	—	1/2	0/1	2/6	3/9

suggest that the proportions of spermatozoa that are capable of fertilizing oocytes are reduced in oligospermic samples. It was therefore decided that a simple and effective modification to GIFT might be the transfer of an increased total number of motile spermatozoa. Interestingly, pregnancies were quickly achieved once this modified procedure was used[16] and the success has been sustained in the present report. A similar effect has been described for IVF, where an increased fertilization rate is achieved for oligospermic couples when oocytes are inseminated with 500,000 motile spermatozoa,[17] however, this is not always observed, and Mahadevan and Trounson[18] found that fertilization of oocytes was not improved when an increased number of spermatozoa were used for the insemination of oocytes.

It has been shown that the chance of achieving pregnancies by the GIFT technique is related to the number of oocytes transferred to the fallopian tubes.[19] In particular, the transfer of three or four oocytes is significantly better than the transfer of only one or two oocytes. The data in Table 2 is presented with respect to oocyte numbers transferred and indicates that the likelihood of pregnancy was more closely related to sperm preparation modification because there was no significant disparity between the two groups with respect to oocyte numbers transferred. Furthermore, there was no obvious disparity between the two groups with respect to the quality of oocytes transferred.

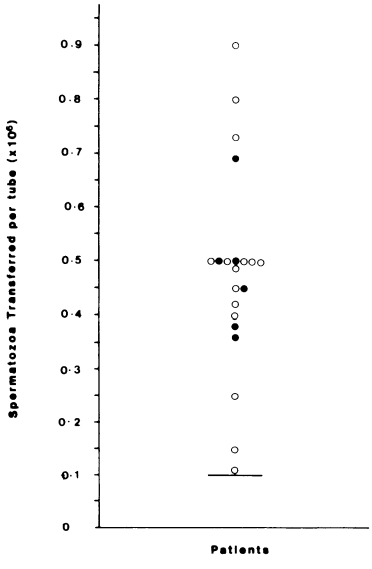

Figure 1 The number of motile spermatozoa transferred per fallopian tube in oligospermic couples treated using the modified GIFT technique. ○, not pregnant; ●, pregnant.

In our IVF-ET program, fertilization failure is seen in approximately 30% of oligospermic patients.[14] The uncertainty of whether fertilization occurs in vivo in women who do not become pregnant following a GIFT treatment cycle has led to the development of PROST (Pronuclear Stage Tubal Transfer),[16,20] whereby the fertilization of oocytes is confirmed in vitro and pronuclear stage oocytes are transferred into the fallopian tubes.

The progress of the pregnancies is encouraging, with 27 of 33 being ongoing pregnancies. Although it is a small series, the rate of fetal wastage appears similar to the overall outcome of GIFT pregnancies in the PIVET Medical Centre program.[21] More importantly, however, this rate seems lower than for artificial insemination by husband (AIH; 45.6% pregnancy loss) or IVF (58.0% pregnancy loss), which are the two techniques providing the main therapeutic options for oligospermic couples.

In summary, the present study has shown that: (1) no pregnancies were achieved when oligospermic couples were treated with the conventional GIFT technique in which 100,000 motile spermatozoa are transferred into each fallopian tube; and (2) a modified GIFT technique, where an increased number of motile spermatozoa (minimum 325,000 in this series) are replaced with the oocytes, is an effective therapy in the treatment of oligospermic infertility.

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