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The relative chance of pregnancy following tubal or uterine transfer procedures

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In a 7-month study period, a total of 113 pregnancies were generated in 380 women (30%) undergoing transfers in one of four assisted conception procedures: gamete intrafallopian transfer (GIFT), pronuclear stage tubal transfer (PROST), tubal embryo stage transfer (TEST), and in vitro fertilization and embryo transfer (IVF-ET). It was shown that both the pregnancy rate per transfer procedure and the number of pregnancy sacs arising per embryo transferred were significantly higher among the groups having tubal transfer ($P < 0.001$). There were no significant differences in the pregnancy or implantation rates among the three groups having tubal transfer procedures when the GIFT results were adjusted for a 72% fertilization rate noted in the combined IVF-ET and PROST groups. Early pregnancy wastage showed a similar pattern among the four groups and, overall, 67% of pregnancies advanced beyond 20 weeks. Fertil Steril 49:858, 1988

The PIVET Medical Centre in vitro fertilization and embryo transfer (IVF-ET) program was started in 1980 and began generating pregnancies through 1981.[1] However, despite a large experience and increasing expertise, the clinical pregnancy rate per year has ranged from only 12.1% to 19.1% of cases transferred.[2] During sessional observations, pregnancies may cluster to generate transient high rates, but even the longest established IVF units still generally report pregnancy rates of around 20% or less on large series,[3–5] although one group consistently reports higher rates.[6] Following the initial report on gamete intrafallopian transfer (GIFT),[7] the method was introduced at the PIVET Medical Centre in late 1985 for nontubal causes of infertility.[8] An initial comparative trial between IVF-ET and GIFT indicated improved pregnancy rates for the latter, which was subsequently adopted as the preferred method for all nontubal causes of infertility. In oligospermic cases, a successful modification of the original protocol was developed.[9] In four subcategories of infertility, a further technique was developed known as pronuclear stage tubal transfer (PROST), and this also has generated higher pregnancy rates than comparable cases managed by IVF-ET.[10] Furthermore, pregnancies arising from the two techniques involving tubal transfer had an improved outcome over that of IVF-ET.[11]

In the light of these findings, a synchronous study was undertaken to determine the relative chance of pregnancy per oocyte or embryo transferred in the GIFT, PROST, and IVF-ET procedures, as well as a small number of observations following the transfer of cleaving embryos to the fallopian tubes (TEST; tubal embryo stage transfer).[10]

MATERIALS AND METHODS

The series documents all cases managed within the IVF-related assisted conception program at the

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PIVET Medical Centre in the first 7 months of 1987. This study period was chosen because it represented a time of maximum stability with respect to personnel and methodology. All procedures were performed on a rostered basis by one of the four experienced gynecologists, and each managed all oocyte recoveries and transfer procedures for that particular day. Cases were treated as they arose and were unselected during this series. The individual gynecologist managed a similar number of each procedure. Similarly, five embryologists were rostered on a daily basis through the course of this study, and there were no significant changes within the work patterns or techniques applied in the dedicated IVF laboratory during the study period. All culture media were made up on a weekly basis by one laboratory member and passed strict quality control tests before being used for any assisted conception procedure. This included the successful culture (> 90%) of mouse embryos from the fertilized 1-cell stage through to expanded blastocysts. The stimulation protocol, timing of human chorionic gonadotrophin (hCG) trigger, luteal support treatments, and all monitoring was conducted similarly for the three groups, and no bias was noted in these areas. The protocols for each procedure are outlined as follows:

Gamete Intrafallopian Transfer

One hundred eighty-six women (age range, 22 to 46 years; mean, 32.62 ± 4.86) underwent GIFT. The indication was all nontubal infertility, apart from the four categories managed by PROST or TEST. A proportion of oligospermic patients were included for management with the modified technique if more than 300,000 motile spermatozoa could be recovered from preliminary test semen samples by the overlay technique. Certain cases of tubal infertility (i.e., previous reconstructive tubal microsurgery) were included for management by GIFT if tubal patency had recently been demonstrated and at least one fallopian tube was shown to be mobile and freely accessible at preliminary laparoscopy. Women were admitted to the hospital on an outpatient basis and the husbands produced their semen specimen 2 hours prior to the procedure, which was undertaken under general anesthesia with endotracheal intubation. Oocytes were normally recovered at laparoscopy, although occasionally the transvaginal technique was applied for recovery from an inaccessible ovary, usually on the left side where adherent sigmoid colon occasionally precluded laparoscopic access. The gametes were prepared as previously described,[8,9] selecting oocytes with the highest grading,[12] and transferred exactly 4 cm into the fallopian tube via a Teflon catheter (William Cook, Melbourne, Victoria, Australia) inserted through the fimbrial end at laparoscopy. Patients were discharged home 4 hours after recovery from anesthesia and no restrictions were placed on their subsequent activities.

Pronuclear Stage Tubal Transfer

PROST was applied to 123 women (age range, 25 to 46 years; mean, 31.83 ± 4.36) for infertility due to oligospermia or asthenospermia, and in those situations where either partner carried significant antispermatozoal antibodies, particularly combined IgA and IgG in semen, or significant levels of any circulating antibody subtype in the female.[13] PROST also was applied for cases that had two or more previous failed GIFT treatments and an additional group that was having ovum donation. In all cases in this series, oocytes were recovered by the transvaginal ultrasound-directed technique under light-mask and airway anesthesia. Husbands produced their semen specimens approximately 4 hours after oocyte recovery. Thereafter, the insemination and subsequent culture was undertaken as for IVF-ET with dissection of the coronal coat from all oocytes at 12 to 18 hours after insemination. Selected pronuclear stage oocytes (those derived from higher grade oocytes preferred) were transferred at laparoscopy into the fallopian tubes via a Teflon catheter in a similar manner to the GIFT technique, employing general anesthesia and endotracheal intubation. Patients were discharged home 4 hours after recovery from anesthesia, with no restrictions placed on their subsequent activity.

In Vitro Fertilization-Embryo Transfer

IVF-ET was applied only in those cases where there was tubal disease causing bilateral tubal obstructions, inability to gain access to the fallopian tubes for cannulation, or absent fallopian tubes (n = 131; age range, 27 to 44 years; mean, 34.62 ± 4.26). All oocyte recoveries were undertaken using the transvaginal ultrasound-guided technique, which was performed under light-mask and airway anesthesia. Similarly to the PROST procedure, husbands produced their semen samples approximately 4 hours after oocyte recovery and inseminations were performed after overlay separation of the motile spermatozoa. The coronal coat

was removed by fine-needle dissection 12 to 18 hours after insemination to confirm the pronuclear stage, and all pronuclear oocytes were transferred to fresh culture medium for an additional 24 to 30 hours. Selected embryos were transferred to the uterus using a double catheter technique at 40 to 48 hours after insemination, when the majority of embryos were at the 4-cell stage.[14] The ET procedure was performed in the combined lithotomy/10 degrees Trendelenburg position without anesthesia. Ten milligrams of oral diazepam was given 2 hours prior to the procedure. Patients rested for 4 hours and then proceeded home, with the instruction to avoid intercourse and to minimize physical activities over the ensuing 5 days.

Tubal Embryo Stage Transfer

TEST is regarded as an extension of the PROST procedure. It applies particularly to the ovum donation program and involves the transfer of early cleavage stage embryos into the fallopian tubes at laparoscopy under general anesthesia with endotracheal intubation. The 3 cases reported (age range, 28 to 36 years; mean, 32.67 ± 4.16) had received donated excess oocytes from other women undergoing IVF-ET or GIFT, which had been fertilized and cryopreserved at the 4- and 8-cell stages between 8 and 20 months previously. On subsequent thawings, such embryos are considered suitable for transfer if ≥50% of blastomeres appear morphologically normal. The culture techniques are the same as for IVF-ET, and the cryopreservation methodology has been reported elsewhere.[15] Thereafter, the transfer procedure is the same as PROST.

Pregnancy Diagnosis

Pregnancy was diagnosed by an elevated serum β-hCG level on or after day 16 of the luteal phase, and the demonstration of a significant rise at least 3 days thereafter. All cases had an ultrasound assessment (Diasonics DRF400, Milpitas, CA) performed between the seventh and eighth weeks. Pregnancies with falling hormone levels and no detectable gestational sac on ultrasound were considered biochemical pregnancies and were not curettaged. Pregnancies that reached the stage of a defined intrauterine gestational sac at 7 weeks without a viable uterine fetus were regarded as blighted ovum pregnancies. They were differentiated from pseudosacs by the finding of a double hyperechoic rim completely encircling the transonic sac.[16] Pregnancies that miscarried after ultrasound detection of fetal heart movement were diagnosed as miscarriages. Ectopic gestations were diagnosed very early by elevated β-hCG levels without a definite intrauterine gestational sac detectable on ultrasound at 7 weeks. All pregnancies failing to reach 20 weeks' gestation are included in the early pregnancy wastage data.

Statistics

The pregnancy rates were compared using chi-square analysis in contingency tables, applying Yates correction where required. The age distribution of patients in the various groups is documented with the ranges and means (± standard deviation [SD]) and were compared by Student's *t*-test.

RESULTS

A total of 113 pregnancies arose by one of the assisted conception methods following oocyte recovery in 443 women (25.5%) and 380 women undergoing a transfer procedure (29.7%). There was no significant difference in the age distribution of the patients in the various groups. The pregnancy rates for the four treatment modes are documented in Table 1. The pregnancies are reported with respect to the number of women becoming pregnant following a transfer procedure, and also the number of gestational sacs arising per embryo transferred. The pregnancy rate per transfer for IVF-ET is significantly lower than the other treatments ($\chi^2 = 28.39$, 3 df; $P < 0.001$). No significant differences were shown among the three groups having tubal transfer procedures.

There were also significantly fewer pregnancy sacs arising per embryo transferred in IVF-ET compared with all other treatments ($\chi^2 = 31.87$, 3 df; $P < 0.001$). In comparing the three tubal transfer techniques, it was shown that the number of gestational sacs arising per oocyte transferred in GIFT was significantly less than the number of pregnancy sacs arising from PROST. However, if this was adjusted to take into account the projected number of embryos arising from GIFT, there were no significant differences in sacs per embryo among the GIFT, PROST, and TEST procedures. The fertilization rate of oocytes in the combined IVF-ET and PROST groups was 588 of 812 (72.4%). If this figure is applied to the GIFT series, the 76 pregnancy sacs are then assumed to arise

Table 1 Results of All Patients Treated by Four Assisted Conception Techniques at PIVET Medical Centre, January to July 1987 Inclusive

IVF-ET							
Total collections	131						
Pregnancy rate/transfer	14/112	(12.5%)					
Sacs/embryo transferred	15/377	(4.0%)					
			Embryos transferred				
		1	2	3	4	5	
Pregnancy rate[a]		0/15	1/14 (7.1%)	3/25 (12%)	6/31 (19.4%)	4/27 (14.8%)	
Pregnancy sacs arising[a]			1 (3.6%)	3 (4.0%)	6 (4.8%)	5 (3.7%)	
GIFT							
Total collections	186						
Pregnancy rate/transfer	66/184	(35.9%)					
Sacs/oocyte transferred	76/707	(10.8%)					
Sacs/embryo[b] transferred	76/512	(14.8%)					
			Oocytes transferred				
		1	2	3	4	5	
Pregnancy rate		0/1	2/12 (16.6%)	6/22 (27.3%)	50/129 (38.9%)	8/20 (40%)	
Pregnancy sacs arising		0	2 (8.3%)	7 (10.6%)	56 (10.9%)	9 (11.0%)	
PROST							
Total collections	123						
Pregnancy rate/transfer	30/81	(37.0%)					
Sacs/embryo transferred	36/211	(17.1%)					
			Embryos transferred				
		1	2	3	4	5	
Pregnancy rate		3/18 (16.7%)	10/23 (43.5%)	7/17 (41.2%)	9/19 (47.4%)	1/4 (25%)	
Pregnancy sacs arising		3 (16.7%)	11 (23.9%)	10 (19.6%)	9 (11.8%)	3 (15.0%)	
TEST							
Total collections	3						
Pregnancy rate/transfer	3/3	(100%)					
Sacs/embryo	3/10	(30%)					
			Embryos transferred				
		1	2	3	4	5	
Pregnancy rate			1/1 (100%)		2/2 (100%)		
Pregnancy sacs arising			1 (50%)		2 (25%)		

[a] IVF-ET < all others; $P < 0.001$.
[b] Adjusted values, assuming fertilization rate = 72.4% (588 oocytes fertilized from total of 812 oocytes recovered for IVF and PROST).

from 512 embryos, providing an adjusted sac rate of 14.8% of embryos.

There were no significant differences in the proportion of pregnancy sacs arising per oocyte or embryo transferred when the rates in each group were compared with respect to the number transferred. The rates proved remarkably constant throughout for the IVF-ET, GIFT, and PROST groups.

The pregnancy outcome from the four groups is shown in Table 2, which includes the age distribution of the conceiving women. There were no significant differences among the mean ages within the various groups. Early pregnancy wastage ranged from 19.4% in the PROST series to 33.3% for IVF-ET. However, these differences were not statistically significant.

DISCUSSION

A relevant comparison among different techniques of assisted conception should take into account three main variables: the age of women being treated, the underlying infertility history, and the

Table 2 Outcome of All Pregnancy Sacs Generated by Four Assisted Conception Techniques at PIVET Medical Centre, January to July 1987 Inclusive

Treatment mode	No.; age range	Outcome		
		Early pregnancy wastage		Ongoing[a]
	(mean ± SD)			
IVF-ET	15; 29–42 (36.2 ± 4.02)	1 Biochemical 1 Blighted ovum 2 Ectopics	(33.3%)	(66.7%)
GIFT	76; 22–42 (32.7 ± 4.67)	4 Biochemical 13 Blighted ova 3 Miscarriages 5 Ectopics	(32.9%)	(67.1%)
PROST	36; 22–38 (29.7 ± 3.36)	2 Biochemical 2 Blighted ova 2 Miscarriages 1 Ectopic	(19.4%)	(80.6%)
TEST	3; 28–36 (32.7 ± 4.16)	1 Ectopic	(33.3%)	(67.7%)

[a] >20 weeks gestation.

number of embryos transferred. In this study, the age range and average age of patients was similar in the four groups. The underlying infertility history differed among them, but the treatment indications for each individual group are quite different and preclude a controlled study at this stage of development for assisted conception procedures at the PIVET Medical Centre. However, from other reports, the tubal group appears to be relevant for comparative studies,[4,17] and the data presented here indicate a significant benefit of tubal transfers over uterine transfers for cases of nontubal infertility. We have therefore adopted this principle, as well as clarifying the indications for PROST and TEST, having previously demonstrated benefits for certain subcategories of nontubal infertility over the GIFT method.[10] Given that these techniques represent state-of-the-art developments in assisted reproduction, it is relevant to compare the chance of pregnancy and the chance of each individual embryo implanting. This provides information that may point to important technical and physiologic differences that have an implication for future developments in human reproductive research and clinical management.

The most striking result is the significantly higher pregnancy rate arising from tubal transfer procedures when compared with the transfer of embryos into the early postovulatory uterine cavity. The data becomes more striking when the comparison is made for pregnancy sacs arising per embryo transferred. In this study, both the pregnancy rate per transfer and the proportion of embryos successfully implanting in IVF-ET are lower than we have reported previously,[18] and which have been reported from other centers.[19] A clear explanation of this is not readily available, but the three factors that bear on it—embryo quality, uterine receptivity, and ET techniques—have been considered carefully. The only identifiable changes in the past 3 years have related to ovarian stimulation methodology and the ET procedure. Currently, human menopausal gonadotrophin (hMG) injections are continued up to the final morning prior to hCG trigger in the evening. This has led to increased oocyte numbers recovered, whereas fertilization rates and embryo quality grading have remained the same. However, it is possible that the lack of a coasting phase prior to hCG trigger may create a high circulating estradiol/progesterone ratio, which might retard appropriate decidual development within the uterine cavity. This phenomenon has been reported,[20] but has not yet been formally analyzed within this study. However, such factors are unlikely to be relevant here because the ovarian stimulation protocol was exactly the same for the four groups studied. With respect to ET technique, the only difference over the past 3 years has been the transfer of embryos in culture medium rather than 50% patient's serum. Our initial comparative studies showed no significant differences, but this may need re-evaluating. Over a 7-year period, the implantation rate of all embryos transferred at PIVET has averaged 8.0%, and it should be noted that, even if the IVF-ET pregnancy rates and embryo implantation rates were double that observed

in this study, the rates would still be significantly different from the tubal transfer procedures.

In comparing the three tubal transfer methods, at first it appeared that the chance of pregnancy from TEST (cleaving embryos) and PROST (pronuclear embryos) was better than GIFT. However, the adjusted pregnancy rate for GIFT (converting oocytes transferred to the estimated number of fertilized oocytes using the known IVF/PROST fertilization rate of 72.4%) shows no differences in the chance that any one embryo may implant. This implies that the IVF procedure per se does not reduce the potential of an embryo to implant. Initially, when the GIFT results were known to be improved over those of IVF-ET, it was thought that there may be some benefit if gametes were able to be fertilized in vivo rather than in vitro. This possibility was suggested by animal studies[21] showing reduced size of implantation sites and reduced progeny after IVF, as opposed to in vivo fertilization, where all gametes had been previously handled in vitro. However, our data indicate that the techniques applied for human IVF are not in themselves a cause for the low implantation rates and higher pregnancy wastage seen after IVF-ET. When in vitro fertilized oocytes are transferred to the fallopian tubes, there is a 17% chance that any individual embryo will implant (it is too early at this stage to draw conclusions from the TEST data to know whether the implantation rate will remain higher for cleaving embryos). With respect to the question of a possible influence of accompanying oocytes or embryos, the data throughout this study indicate that the implantation rate is constant in the IVF-ET, GIFT, and PROST groups, regardless of numbers transferred, implying that embryos do not appear to be helping each other implant.

The data concerning pregnancy outcome do not show significant differences in this comparative study. We have previously reported on 1034 pregnancies arising in subfertile women and have shown that the mean early pregnancy wastage before 20 weeks is 27% (similar to this study), and certain differences could be found in the pattern of early pregnancy wastage among different treatment groups.[11] For example, there appeared to be a higher biochemical pregnancy rate following IVF-ET. Ectopic pregnancy rates were high (around 5%) among all groups where female subfertility factors were present, but the rate was higher in the GIFT series, and this related to underlying known or suspected tubal disease. However, the overall benefits of GIFT with respect to pregnancy rates and outcome have led us to counsel patients toward the consideration of GIFT, even though the ectopic rate may be twice that following IVF-ET.

In evaluating the optimum number of oocytes or embryos for transfer, the data indicate a difference for IVF-ET and the tubal transfer procedures. In the former, a maximal effect was seen when four embryos were transferred. It is apparent that the chance of multiple pregnancy is quite low even following the transfer of four or five embryos, but there is unlikely to be any benefit from transferring more than four, although the chance of a quadruplet pregnancy is only three per million, given a 4% implantation rate for embryos. As far as the tubal transfer procedures are concerned, the PROST data indicates that the pregnancy rate is plateaued following the transfer of two embryos. With GIFT, however, the plateau effect is not reached until four oocytes (equivalent to three embryos) are transferred. At this stage, we advise a maximum of three pronuclear oocytes to be transferred in PROST, but four unfertilized oocytes is the optimum choice for GIFT. This compromise should provide the optimum balance between a satisfactory pregnancy rate and excessive multiple pregnancies. In GIFT, the expected quadruplet risk is 15 per million, and the triplet risk for PROST is between 5 and 8 per thousand. It is not possible to draw meaningful conclusions at this stage from the data relating to the TEST procedure. However, the early results are encouraging and suggest that the method will prove at least as effective as PROST, hence we are currently advising patients that a maximum of three embryos may be the optimum number for transfer to minimize the risk of a high-multiple pregnancy.

In considering the reasons why tubal transfer techniques are associated with a higher pregnancy rate than uterine transfer, three possibilities emerge: (1) the tubal environment may provide the appropriate milieu for preimplantation embryo development in a fashion superior to that found in the uterine cavity or which can be achieved in the laboratory incubator; (2) there may be a discrete but unidentified tubal factor that improves embryo quality; and (3) the early postovulatory uterine environment may be hostile. This assumes that embryos transferred to the tubal environment remain there for several days until the uterine environment becomes favourable (e.g., by day 5).

At this stage we remain uncertain which factor is operating, but it appears relevant to return embryos to the in vivo environment at an early rather

than late stage because less than 30% of fertilized oocytes will develop to expanded blastocysts in vitro.[22] Where possible, the tubal environment should be used. It may not be necessary to cannulate the fallopian tube at laparoscopy, and the recently described technique of tubal cannulation via the cervix[23] should be considered as long as there is no underlying tubal disease. For those cases that can only be managed by IVF-ET, the findings of this study suggest that methods should be explored for improving the early postovulatory uterine environment. One possibility is the use of exogenous progesterone given immediately after oocyte recovery[24] and which might be contributing to the higher pregnancy rates reported from Norfolk[6] over that of other long-experienced IVF units. However, while the implantation rates following the uterine transfer procedure remain significantly lower than those following tubal transfer procedures, it is recommended that all nontubal infertility being considered for IVF should be offered the GIFT, PROST, or TEST programs rather than IVF-ET.

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