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Pregnancies following pronuclear stage tubal transfer

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Pronuclear stage tubal transfer (PROST) is a technique that involves in vitro fertilization (IVF) of oocytes, followed by the transfer of pronuclear oocytes into the fallopian tubes. It has been developed for its prognostic value of confirming fertilization in couples with oligospermia or asthenospermia and enabling fertilization in cases with antispermatozoal antibodies (ASAB). PROST has provided useful diagnostic information in the management of couples who have failed to conceive in other treatment programs and has particular advantages over IVF for those receiving fresh donated oocytes for ovarian failure. Fourteen pregnancies resulted from 52 transfers, providing a pregnancy rate of 27% per transfer. The pregnancy rates were higher than a matched IVF series in the male factor and female ASAB groups and reached statistical significance for the ovum donation group. It is anticipated that both pregnancy rates and fetal wastage will be improved over conventional IVF and embryo transfer for the described infertility groups. Fertil Steril 48:851, 1987

The gamete intrafallopian transfer (GIFT) technique was introduced at the PIVET Medical Centre in December 1985. The technique was run in conjunction with the existing in vitro fertilization (IVF) program and, at the end of the first 6-month period, a significantly higher proportion of GIFT patients conceived (overall 27% compared with 15%, $P < 0.001$).[1] Furthermore, those pregnancies achieved from GIFT were significantly more likely to proceed beyond 20 weeks.[2] Because of these findings, the current aim at PIVET is to transfer IVF cases to the GIFT program whenever the female partner has at least one accessible and patent fallopian tube.

It is recognized, however, that the GIFT technique is unsuitable for cases of severe oligospermia/asthenospermia where the necessary higher numbers of motile sperm cannot be obtained from the ejaculate[3] and also in those cases where the female has circulating antispermatozoal antibodies (ASAB). In the latter group, a recently published case report indicated that fertilized oocytes transferred to the fallopian tubes could achieve an ongoing pregnancy.[4] It was therefore considered that IVF may have certain advantages, at least in achieving oocyte fertilization for those categories of infertility. In addition, it was thought that IVF had certain diagnostic advantages in patients who had repeated failures in the GIFT program or unexplained failure to conceive in other programs, such as the donor insemination program. A fourth category also provided concern in the GIFT program: those patients having ovum donation. Where this was performed as a direct donation of fresh oocytes, it meant that both donor and recipient were together in the hospital ward on the same day, making it difficult to ensure confidentiality.

It was therefore decided to explore the concept of pronuclear stage tubal transfer (PROST) whereby oocytes are fertilized in vitro and subsequently transferred into the fallopian tubes at the pronu-

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clear stage (18 hours after insemination) for the four categories.

MATERIALS AND METHODS

All infertile couples attending the PIVET Medical Centre were fully evaluated and managed according to a documented protocol.[5] The PIVET IVF unit is accredited by the Fertility Society of Australia[6] and fulfills all requirements, including ethical approval for IVF and related procedures by the Committee for Human Rights, University of Western Australia. Couples were included in the PROST program, according to the groups detailed below, when it was known that the wife had at least one laparoscopically accessible and patent fallopian tube. Semen samples were classified as being normospermic ($\geq 12 \times 10^6$ motile spermatozoa/ml), moderately oligospermic ($5.1–11.9 \times 10^6$ motile spermatozoa/ml), or severely oligospermic ($\leq 5 \times 10^6$ motile spermatozoa/ml) according to the criteria of the World Health Organization.[7] Antispermatozoal antibodies (IgA, IgG, IgM) were identified using the indirect immunobead test[8] in serum of the female partner and semen of the male partner. The details of the four groups of patients studied were as follows.

Group 1: Poor Sperm Quality (Male Factor)

PROST was carried out on 55 couples where the male partner was demonstrated to have a significant identifiable problem. In all cases, the total progressively motile sperm density was $<5 \times 10^6$/ml. All couples previously had IVF treatments with known difficulty in obtaining satisfactory washed sperm preparations and had reduced oocyte fertilization rates. Ten of these cases had antispermatozoal antibodies in the semen (3 with IgA and 7 with IgA and IgG in combination). Altogether, 8 couples chose to have the oocytes collected divided into two lots: for insemination with the husband's or donor spermatozoa (split fertilization).

Group 2: Female Antispermatozoal Antibodies

Eight women had PROST because of the presence of antispermatozoal antibodies in their serum. Four had IgA, two had IgA and IgG in combination, and two had IgA, IgG, and IgM together. All had demonstrated fertilization, but failed to conceive in previous IVF-ET (embryo transfer) treatment cycles. Antispermatozoal antibody-free donor serum was used as a replacement for the patient's own serum in the culture medium used for fertilization.

Group 3: Poor Response in Previous Treatment Cycles

Fifteen couples were brought into the PROST program because of poor results in previous treatment cycles. The problems were as follows: (1) seven had not conceived following three attempts at GIFT treatment; (2) four had not conceived following two attempts at GIFT treatment and the failure to fertilize supernumerary oocytes; (3) four had not conceived on the donor insemination program, despite insemination of spermatozoa from donors of proven fertility during a minimum of seven treatment cycles.

Group 4: Women Receiving Donated Oocytes Because of Ovarian Failure

Four women have so far been included in this group. The four had premature ovarian failure as diagnosed by markedly elevated serum FSH concentrations (>50 IU/l) and no response to attempted ovarian stimulation using hMG. Each had transcervical embryo transfers on three to five previous occasions following the fertilization of donated oocytes. Exogenous steroids were administered to the women using the replacement therapy schedule of sequential oral estrogen (Progynova, Schering, Berlin, Federal Republic of Germany) and intravaginal suppositories of progesterone described by Lutjen et al.[9] Oocytes were obtained from women on the GIFT program following the signed consent of the donor couple. Confidentiality was a condition of consent for both donor and recipient. Oocytes were fertilized with spermatozoa from the recipient's husband and transferred into the fallopian tubes of the recipient at the pronuclear stage.

All women in this study had follicle growth stimulated by the administration of clomiphene citrate (CC; Clomid, Merrell Dow Pharmaceuticals, Inc., Cincinnati, OH), human menopausal gonadotrophin (hMG; Pergonal, Serono, Rome, Italy), or a combination of CC/hMG. The response to treatment was monitored daily from day 2 of the menstrual cycle by the measurement of serum estradiol (E_2), progesterone (P), and luteinizing hormone (LH) by radioimmunoassay. From day 8, daily transabdominal ultrasound follicle tracking was performed and oocytes were collected approximately 34 to 36 hours after the administration of

10,000 IU of human chorionic gonadotrophin (hCG; Primogonyl, Schering, Berlin, Federal Republic of Germany) or the onset of a spontaneous LH surge. In the majority of cases, oocytes were collected by transvaginal ultrasound-directed techniques performed under light mask and airway general anaesthesia.[10]

Culture conditions and the preparation of spermatozoa were carried out according to the methods described by Yovich and Stanger.[11] Oocytes were inseminated with 100,000 motile washed spermatozoa (increased for oligospermics, where possible) 4 to 6 hours after collection. If fertilization was shown by the presence of two pronuclei within oocytes 18 hours after insemination, then a maximum of three pronuclear oocytes were transferred into the fallopian tubes according to the GIFT procedure. The transfer was achieved by means of a 16-gauge Teflon catheter (Cook, Melbourne, Australia). Those cases of conventional IVF-ET used for the comparisons with PROST treatment had procedures performed according to the fully described techniques at PIVET.[12] Pregnancy was diagnosed 16 to 19 days after oocyte collection by rising levels of β-hCG in the serum and confirmed approximately 5 weeks later by ultrasound.

RESULTS

Eighty-two treatment cycles were managed and 52 cases reached pronuclear stage transfer. Fourteen pregnancies were achieved, giving an overall pregnancy rate of 26.9% per transfer. No significant differences were noted between the groups with regard to the ages of the males or females or the duration of infertility of the couples (Table 1). Table 2 shows that an average of 4.2 oocytes were collected per cycle, with no significant difference between the groups of patients. The fertilization rate in the male factor group was significantly lower with husband's spermatozoa (46/222, 20.7%) than with donor spermatozoa (21/31, 67.7%) ($P < 0.005$).

Table 3 shows the PROST treatment outcome with respect to fertilization failure (total oocytes) and pregnancy. Total fertilization failure was seen in the male factor group and in those with a poor history. Total fertilization failure with husband's spermatozoa occurred in 31 of 55 cases in the male factor group and 4 of 15 cases in the group with a poor previous history. In the former, 28 pronuclear stage transfers were carried out, although fertilization with husband's spermatozoa was successful in only 24 couples. The additional 4 transfers followed fertilization using donor spermatozoa. This was similar to the poor history group, where 4 transfers followed fertilization using donor spermatozoa as planned (husbands azoospermic, failed donor insemination) but 4 had failed fertilization from normospermic husbands, with one achieving a fertilized oocyte from donor spermatozoa in a split fertilization attempt. However, no pregnancies occurred.

Four women with primary ovarian failure received donated oocytes at the appropriate stage of the induced cycle. Transfer of the pronuclear oocytes occurred on day 14 of the cycle in the two nonconception cycles and days 15 and 17 in the two conception cycles. Women undergoing transfer of oocytes in the IVF program had 4-cell and 8-cell embryos transferred between days 16 and 18 of the cycle (day 14 = LH surge + 1).

During the course of the PROST study, the outcome for male factor and female ASAB cases was compared with cases of IVF handled simultaneously. Table 4 shows a matched series from the male factor group, selecting those with sperm density < 5 million progressively motile spermatozoa per milliliter. There was a similar rate of failed fertilization, but the pregnancy rate per case and

Table 1 Patient Profile and Subfertility Category for the First 82 Cases of PROST Treated at PIVET Medical Centre

Group[a]	No. couples	Age		Duration of infertility
		Male	Female	
		yr	*yr*	*yr*
Male factor	55	34.3 ± 1.2	30.2 ± 0.8	4.7 ± 0.7
Female ASAB	8	31.2 ± 1.0	30.7 ± 0.7	4.8 ± 1.6
Poor history	15	33.8 ± 1.3	32.5 ± 2.1	6.1 ± 0.5
Ovarian failure	4	29.5 ± 4.6	28.5 ± 3.9	6.0 ± 0.4
Total	82	32.2 ± 0.9	30.6 ± 0.7	5.4 ± 0.5

[a] Groups compared by Student's *t*-test, no significant differences.

Table 2 Summarizes Oocyte Recovery and Fertilization Profile for Four Subfertility Categories Managed by PROST

Group	Treatment cycles	Oocytes collected	AV/cycle	Fertilization	
				Husband	Donor
				(%)	(%)
Male factor	55	253	4.6	46/222 (20.7)	21/31 (67.7)[b]
Female ASABs	8	32	4.0	16/32 (50)	—
Poor history	15	53	4.2	40/48 (83.3)	3/5 (60)
Ovarian failure	4	15[a]	3.8	10/15 (66.6)	—
Total	82	353	4.2	112/317 (35.3)	24/36 (66.7)[c]

[a] From donor.
[b] $P < 0.005$, $\chi^2 = 17.88$.
[c] $P < 0.005$, $\chi^2 = 10.06$.

per embryo transfer was apparently better in the PROST series, just falling short of statistical significance. The pregnancy outcome for the female ASAB group is shown in Table 5. The PROST method was shown to be equally effective as IVF. It was not possible to obtain a suitable matched series for the poor history series. Table 6 compares the total experience of ovum donation patients (for ovarian failure) in IVF (PIVET, 1984 to 1986) compared with the PROST study. There is a significant improvement in the pregnancy rate by PROST and the finding was similar when embryos (4-cell or 8-cell) were transferred to the fallopian tubes in the procedure designated as TEST (tubal embryo stage transfer). The TEST data is of particular interest because embryos were transferred to the woman at the same stage and time frame as for the IVF group, with the only differences relating to the technique and site of transfer.

DISCUSSION

At the PIVET Medical Centre, a significant difference in pregnancy rates has been shown between the IVF and GIFT programs.[1,13] This may be due to some benefits in the intratubal environment or simply because of minimization of the extra corporeal exposure of gametes and embryos. In the GIFT program, gametes are transferred after a short period on the same day as oocyte recovery, whereas in the IVF program, embryos are transferred 2 days later. However, it was recognized that certain cases of nontubal infertility may not be managed best by the GIFT procedure and that there were certain benefits in the IVF technique that should be applied to those cases in order to diagnose fertilization, perhaps to allow the application of in vitro techniques to enhance fertilization, to be able to select fertilized oocytes for transfer, to add donor-fertilized oocytes where low numbers of patient-fertilized oocytes were obtained and, specifically in the case of ovum donation for ovarian failure, to avoid donor/recipient contact. This led to the development of the PROST technique at PIVET.[14]

The diagnostic value of PROST was highlighted by the confirmation of fertilization failure in 31 of 55 treatment cycles where a significant male factor was present. Such cases can then be counseled informatively as to the futility of further IVF, PROST, or GIFT attempts. Reduced fertilization rates were seen particularly in oligospermic men who had both IgA and IgG antibodies in their semen. This finding has been reported previously from PIVET.[1,3,15] In the eight couples who chose to have oocytes split for insemination with both husband and donor, four failed to fertilize by husband and therefore had donor-fertilized oocytes transferred, but the other four achieved fertilization by both donor and husband. Two of these elected to have all embryos transferred, while the other two elected husband-only fertilized oocytes. We have previously reported this in our IVF program,[16] in-

Table 3 Summarizes the Treatment Outcome with Respect to Fertilization Failure (Total Oocytes) and Pregnancy Rates/Transfer for Four Subfertility Categories Managed by PROST

Group	Treatment cycles	Fertilization failure with husband's sperm	Transfers	Pregnancies
				(%)
Male factors	55	31	28[a]	9 (32.1)
Female ASABs	8	0	8	3 (37.5)
Poor history	15	4	12[a]	0 (—)
Ovarian failure	4	0	4	2 (50.0)
Total	82	35	52	14 (26.9)

[a] Includes four transfers of oocytes fertilized with donor spermatozoa in each group.

Table 4 Comparative Treatment Outcomes with Respect to Fertilization Failure and Pregnancy Rates for Severe Male Factor Cases Managed by IVF and PROST During the Same Time-Frame

Case selection	Program	Cycles	Failed fertilization	Transfers	Pregnancies		
					No.	Case	Transfer
			(%)	(%)		%	%
Sperm density < 5 Million Progressively motile/ml	IV	29	12 (41)	17 (59)	2	7	12
	PROST	55	31 (56)	28 (51)	9	16	32

χ^2 (Yates correction) = 2.4, not significant.

dicating the need for careful counseling of patients prior to IVF or PROST programs so that the fate of embryos generated from the fertilization of oocytes with donor spermatozoa is considered fully. Couples may well choose that such embryos not be transferred if fertilization also occurs with husband's spermatozoa, and therefore the final disposal of all embryos must be decided within the ethical constraints governing the IVF unit and prior to embarking on the procedure.

The pregnancy results for PROST in the male factor group were apparently better than a similar series managed conventionally by IVF. It is likely that the pregnancy rate/transfer will prove significantly better as the number of cases managed by PROST increases. The data suggest that PROST is a better technique for managing cases of severe oligospermia and asthenospermia. These results may not be better than for oligospermia managed in the GIFT program, applying the modification described at PIVET, which requires increasing the motile sperm numbers transferred 4-fold.[17] If this cannot be obtained from the ejaculate, it appears that the GIFT pregnancy rate would be extremely low and, again, the PROST method should be considered. A number of techniques attempting to improve in vitro sperm motility or otherwise enhance fertilization by microinjection under the zona pellucida can only be considered in an in vitro environment. Where fertilization is achieved by such techniques, it is expected that PROST will provide the optimum pregnancy rates.

Table 5 Comparative Treatment Outcomes for Female Antisperm Antibody Subfertility Category Managed by IVF and PROST During the Same Time Frame

Program	Cycles	Transfers	Pregnancies
			(%)
IVF	20	20	4 (20.0)
PROST	8	8	3 (37.5)

χ^2 (Yates correction) = 0.9, not significant.

The therapeutic value of PROST was of particular interest in the group where the female partner had antispermatozoal antibodies. All eight couples had proven fertilization in previous IVF treatment cycles, applying the modification of supplanting ASAB-free donor serum in the culture medium.[18] The dual rationale for PROST was, first, to allow fertilization to occur in an environment free of antispermatozoal antibodies, since it has already been demonstrated that the fertilization rate in vitro is reduced if serum from women with antispermatozoal antibodies is included as a medium supplement.[18] Second, such patients usually have patent fallopian tubes and an improved chance of pregnancy was expected by minimizing the laboratory environment for gametes and embryos and, possibly, other benefits from early transfer to the intratubal environment. The pregnancy rate was higher (Table 5) than IVF, but not significantly so in this early series. It remains of interest that the female ASAB group does continue to have a higher rate of pregnancies in the IVF program than other categories.[1,16]

The use of PROST for patients with suspected fertilization problems, suggested by previous unsuccessful treatment cycles, was of particular interest from the diagnostic point of view. Fertilization was seen in the four patients with failed treatment by donor insemination, suggesting that the problem may lie with gamete transport in these cases. One case of failed fertilization occurred in the six

Table 6 Pregnancy Outcome for Patients Having Ovum Donation for Ovarian Failure Treated by IVF-ET (PIVET, 1984–1986), PROST, and TEST (1986–1987)

Program	Transfers	Pregnancies
		(%)
IVF	41	2 (5)
PROST	4	2 (50)[a]
TEST	4	2 (50)[a]

[a] χ^2 (Yates correction) = 9.6; $P < 0.005$.

couples who had failed treatment by three cycles of GIFT. It is suggested that PROST is a useful therapeutic mode at this stage to select those patients who may have fertilization failure underlying the failure to conceive. For the one case that demonstrated fertilization failure in this group, we considered future treatments by PROST in an experimental protocol incorporating techniques to enhance IVF, such as spermatozoal microinjection and the use of 2-oxyadenosine or pentoxifylline. The remaining five patients who had failed to conceive on previous GIFT treatments and who had failed to fertilize supernumerary oocytes were selected for PROST rather than repeating GIFT treatment, primarily because they had been relatively poor responders to ovarian stimulation or had raised basal follicular phase LH levels. We have previously reported reduced fertilization of oocytes in such cases.[20] Of interest is that three of five such patients had failed fertilization. This was a surprisingly high proportion: we are no longer concerned with the failure of supernumerary oocytes to fertilize in GIFT treatments and the finding is not related to the chance of pregnancy in that treatment cycle.[21] However, the current data suggests that women who do not conceive from GIFT and repeatedly fail to fertilize supernumerary oocytes should be cycled through the PROST program for its diagnostic benefit. It is now a routine at PIVET Medical Centre that patients who fail to fertilize supernumerary oocytes on at least two occasions are cycled through the PROST program. Although no pregnancies were obtained in the series of 15 patients with a poor history, the observation is probably not significant and it would be expected that the failed donor insemination and failed GIFT treatment cases who demonstrated satisfactory oocyte fertilization rates would conceive by future GIFT treatments. However, one must consider that the demonstration of fertilization may not be sufficient in this group and they would be better served by TEST, which contributes the additional knowledge of cleavage and embryo development. One of the couples who had fertilization failure in a previous IVF treatment cycle with a suspected gamete disorder has had one of four oocytes fertilized in the PROST program. This demonstrates that there is some uncertainty in predicting the outcome of fertilization on the grounds of previous performance and confirms the experience of other workers.[22]

At PIVET, our previous experience with ovum donation for premature ovarian failure patients revealed rather poor results when treated by conventional IVF. The data was accumulated over an earlier time frame and includes embryos that had undergone variable periods of cryopreservation. However, the transfers were performed in accordance with the previously described schedule[9] to achieve synchrony and it was considered that the poor results were related to various embryo factors that might be improved by early tubal transfer. A significant improvement was demonstrated by PROST and the results were found to be equally effective for TEST where cleaving embryos are transferred into the fallopian tubes. The finding does imply that the tubal environment is significantly beneficial to pronuclear stage oocytes and embryos and is not simply a factor of laboratory exposure time. PROST was applied for the donation of fresh oocytes in order to maintain confidentiality between donors and recipients, as each would be hospitalized on different days. TEST was required for those recipients whose embryos were previously frozen, awaiting an appropriate stage of synchrony for transfer. This data indicates that the tubal environment is the preferred one for transfer of both pronuclear stage oocytes and early embryos.

The improved pregnancy rate of GIFT over IVF was consistent with animal studies,[23] indicating that the IVF and subsequent culture procedures are associated with implantation failure and significantly reduced numbers of progeny. The GIFT technique allows fertilization in vivo and it was not surprising to find an improved pregnancy rate over IVF. However, PROST entails fertilization in vitro with pregnancy results that are more in keeping with the GIFT results at PIVET and implies that the IVF process itself is not detrimental to the subsequent chances of implantation. The procedure of coronal dissection to identify pronuclear stage oocytes is also not detrimental. It may well be that prolonged culture in vitro may enhance the chance of successful implantation, but the tubal environment may also confer some benefit for gametes, pronuclear oocytes, and early embryos. With regard to diagnostic and therapeutic implications arising from the study, PROST has advantages over GIFT by allowing determination of fertilization or fertilization failure which is necessary for counseling of patients and consideration of future treatments. In addition, PROST allows the potential application of techniques to enhance fertilization. It also allows the selection of fertilized oocytes for transfer to improve the chance of preg-

nancy. Where split fertilization has been performed, the addition of donor-fertilized oocytes can be considered to improve the overall chance of pregnancy.

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