In-vitro fertilization in Western Australia

A viable service programme

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ABSTRACT: A service programme of in-vitro fertilization (IVF) and embryo transfer (ET) has been established in a small unit in Western Australia after a successful pilot study which was undertaken in 1981. The key features of the programme include simplified monitoring of the follicular phase of stimulated cycles; oocyte retrieval and ET are both undertaken during routine daytime work schedules. The results of the first eight months of the programme are presented, during which 13 further pregnancies were generated and 10 healthy infants were delivered (seven boys and three girls). In the last session in which 49 patients took part a mean of 2.3 mature preovulatory oocytes were collected by means of a double-lumen aspiration/flushing needle from 98% of 48 patients who reached the laparoscopy stage. Ninety-four per cent of 48 patients proceeded to embryo transfer by means of a double-catheter technique; the pregnancy rate was 20.8% per laparoscopy or 22.7% per embryo transfer.

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During 1981, a pilot study to establish the feasibility of an in-vitro fertilization (IVF) programme in Perth, Western Australia, was undertaken in the King Edward Memorial Hospital. Using a simplified technique of patient monitoring (ultrasound tracking of follicle development only), and having at its disposal a highly restricted budget and access to an operating theatre on only one day per week, the study produced one successful pregnancy from 42 attempts (in five of which raised levels of human beta-chorionic gonadotrophin (HCG) were demonstrated) and culminated in the delivery of Western Australia's first IVF baby in July 1982.[1]

The pilot study confirmed the need for improved monitoring of the follicular phase, and for daily access to an operating theatre. Subsequently, the programme had continued in association with the Programmed In-Vitro Fertilization and Embryo Transfer (PIVET) laboratory in the Cambridge Private Hospital, Perth. The operating theatre is accessible each morning seven days a week, and the IVF laboratory is accommodated within the theatre complex. The programme was directed towards the use of HCG to control the initiation of luteinization and the final stages of oocyte maturation. This paper reports the results of the first eight months of the programme (from June, 1982, until February, 1983), which was designed to fit in with the routine running of the hospital.

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Patients and methods

The majority of patients selected for IVF and embryo transfer (ET) had either obstructive fallopian tube disease or absent tubes. In the latter months of the study, patients from other infertility therapy programmes (such as patients with endometriosis, who had a partner with oligospermia, who had heterologous sperm antibodies, or in an occasional case, had unexplained infertility), in whom conventional treatments had proved unsuccessful, were also included. Preliminary investigations in all patients included at least three months of assessment of menstrual charts to determine the mean cycle length; mid-luteal estimations of progesterone and prolactin levels to confirm the potential for spontaneous ovulation; preliminary laparoscopy to ensure that at least 25% of one ovary was accessible; normal results of a Papanicolau smear and endocervical swab; negative results of hepatitis B screening; and a preliminary semen appraisal which included density, morphological assessment, and the preparation of a motile fraction suitable for in-vitro insemination.

In all patients, ovarian function was stimulated with clomiphene citrate (150 mg/day) on days 2 to 6 of the menstrual cycle. In addition, human menopausal gonadotrophin (HMG, Pergonal, Serono, Italy) was administered if the serum oestradiol-17β (E2) level on day 8 was lower than 135 pg/mL, or if a poor response to clomiphene had been observed (less than two mature follicles detected by ultrasound, or a low E2 level) in a previous cycle. Generally, HMG was administered as a single intramuscular injection of three ampoules/day (225 u of follicle-stimulating hormone (FSH); occasionally this dose was increased to a maximum of seven ampoules per single injection/day (525 u FSH) to obtain a satisfactory response indicated by daily monitoring. From day 8, all patients attended for a blood test before 9 a.m. each day. Subsequently, the patients' cervical scores[2] were recorded and ultrasound scanning for the assessment of ovarian follicles was performed. The levels of progesterone (P4), luteinizing hormone (LH), and E2 were measured each day (the levels of E2, by a non-extraction double-antibody radioimmunoassay [Mallingkrodt, Melbourne, Australia]; those of P4, by Coat-a-Count solid-phase

immunoassay [Diagnostic Products, Los Angeles, California]; and those of LH, by double-antibody radioimmunoassay [Diagnostic Products, Los Angeles, California]). The ultrasound (Diasonics DRF1, Nuclear Enterprises, Milpitas, California) measurement of the total uterine length was recorded together with follicular size calculated as the mean of three transonic diameters in different planes. When the E2 level reached approximately 400 ng/mL per large (1.8 cm or greater) leading follicle (Tables 1 and 2), ovulation was triggered with 5000 units of HCG (Schering, Berlin, West Germany). The cervical score and day of expected ovulation, determined by applying the formula of McIntosh et al. (mean length of the follicular phase = 0.770 [mean length of cycle] − 7.685)[3] were used as modulating guides. Data in Tables 1, 2 and 3 are reported as mean values and standard error limits.

Patients admitted to hospital for the HCG trigger injection had a blood sample taken during the evening, just before the injection; oocyte recovery was scheduled for 36 hours after the HCG injection in those patients whose follicular phase LH levels remained within two standard deviations of the mean. If an LH surge was detected in the day −2 morning blood sample, oocyte recovery was scheduled for the following morning if the surge was considered to be in an early stage (indicated by the only marginally elevated P4 level [between 0.5 and 2.0 ng/mL]), estimating the collection time to be within 30 hours of the onset of the LH surge. It can be seen from Table 3 that P4 levels greater than 2 ng/mL on day −1 indicate impending ovulation; hence, if the P4 value is above this level, the follicle is well luteinized, the LH surge is in a late stage, and ovulation is impending. The oocyte recovery was cancelled in such patients in our programme. If an LH surge started later on day −2 (as demonstrated by an LH level rise in the blood sample taken before the HCG injection), oocyte recovery was rescheduled to between 26 and 32 hours after the HCG injection. Such patients were known to be early in the surge, as the P4 levels in the day −2 morning blood samples were not elevated. In our series, laparoscopy was performed between 8 a.m. and 1 p.m. in most patients; in those who were in the early stage of an LH surge (12%), laparoscopy was rescheduled to times ranging from 8 a.m. to 5 p.m.

Patients who were undergoing ovum aspiration and then proceeding to embryo transfer were admitted to hospital for a total stay of five days. The embryo-culture laboratory is adjacent to the operating theatre and adjoins a semen collection room which is entered from outside the hospital. Semen collections are placed immediately in a hatchway and a buzzer/light system alerts staff members; the sample is received and placed on a reciprocating rocker in the incubator at 37°C without delay.

Aspiration technique

We developed a double-lumen needle for the combined aspiration and flushing of follicles. The final model is a 30-cm stainless steel needle of 16 wire-gauge diameter (outer diameter, 1.63 mm) for aspiration with a 23-gauge (outer diameter, 0.61 mm) stainless steel needle for flushing attached by silver solder along its full length. The two needle tips are honed to a single bevel and Teflon tubing is attached to the proximal ends — one leading to a flushing syringe and another leading to the collection chamber (16-mL Falcon tube No. 2001). A vacuum pressure (40–80 mmHg), produced by a suction bottle, is used to aspirate the follicles, and is controlled by a foot pedal. After the initial aspiration, the follicle is flushed up to three times with modified Tyrode's solution,[4] containing 5% of maternal serum and 40 units/mL of heparin (Commonwealth Serum Laboratories, Melbourne, Australia). Once the oocyte has been detected with the stereomicroscope, the aspirating needle is withdrawn from the follicle and is washed through with approximately 5 mL of heparinized medium before proceeding to the next follicle. Upon completion of the procedure, the needle is immediately cleaned by multiple distilled water flushings; contact with any detergent is avoided.

Fertilization and culture

After detection and classification into mature or atretric,[5] the oocyte in its cumulus mass is washed twice in modified Tyrode's solution before being transferred to 1 mL of fertilization medium (modified Tyrode's solution containing 7.5% heat-inactivated maternal serum either in a 5-mL plastic culture tube [Falcon No. 2003], or in a Nunc four-well multichamber culture dish [No. 64673]). Spermatozoa are added two to six hours later — initially at concentrations of 0.5×10^6/mL to 1×10^6/mL, but later at reduced concentrations of between 2×10^4/mL to 20×10^4/mL. Semen is prepared by means of an overlay technique,[6] which is modified for oligospermic samples (those containing fewer than 20×10^6 sperms/mL).[7] In such cases, the entire semen specimen is used in the initial dilution and centrifugation, and the sample is overlaid in similar fashion, but incubated for a longer period (up to 60 minutes). The entire overlay is then removed, counted and, if necessary, centrifuged lightly before resuspension to give a concentration of 1×10^6/mL of motile sperms. Oocytes are removed from the fertilizing tube 14 to 18 hours after insemination, and are dissected from their cumulus and coronal coats by means of a combination of a finely drawn micropipette and sharp needle dissection. The appearance of the oocyte, and the number of pronuclei and polar bodies are documented before transfer into growth medium (modified Tyrode's solution with 15% heat-inactivated maternal serum).

Embryo transfer

Embryo transfer is usually scheduled 44 to 48 hours after insemination, when embryos are usually at the four-cell stage. Occasionally, transfer was undertaken on the third day, when embryos were noted to consist of between eight and 16 cells. All transfers were undertaken in the laboratory during the day. After oral premedication with diazepam (20 mg), patients were placed in the lithotomy position with 20° head-down tilt, the cervix was exposed with a bivalve speculum, cervical mucus was gently wiped away with a dry swab, and the external os was bathed with modified Tyrode's solution. A double-catheter technique is employed for the embryo transfer. The outer catheter is of pliable 5-French size polyurethane tubing and traverses the cervical canal for a distance of 4 cm; the inner catheter conveys the embryo in a 50-μL segment of growth medium. The latter is an open-ended 3-French medical grade Teflon tube with a bevelled end (W. Cook, Melbourne, Australia). It is inserted to within 1 cm of the uterine fundus, which is determined by appropriate markers on the inner and outer catheters, and compared to the previous uterine sounding and ultrasound estimation of uterine length. After transfer, patients remain in the lithotomy position for 10 minutes, then return to their beds where they remain in the head-down tilt position for a further eight hours. After a total period of 24 hours' bed rest, patients are discharged from hospital and advised to minimize physical activities for a further five days, and to avoid sexual intercourse until the outcome of the cycle is known.

Results

Within our series, E2 estimations (Table 1) and ultrasound (Table 2) were used as the primary indices of follicle maturation and growth, while P4 levels (Table 3) were used as an index of luteinization, signifying the stage of LH surge and the likelihood of ovulation before laparoscopy.

Assuming the optimum time for the HCG triggering of ovulation to be at an E2 value of about 400 ng/mL per large follicle, the actual day of laparoscopy for most patients proved to be similar to that predicted by the menstrual cycle formula of McIntosh et al.[3] The mean day of laparoscopy for most patients was 14.03 ± 0.23 of the cycle, while the expected day of ovulation, according to McIntosh et al. was

TABLE 1: Serum oestradiol-17β concentration in clomiphene-stimulated cycles and in clomiphene-HMG-stimulated cycles as an index of follicle maturation

Stimulation	Number of follicles	Oestradiol -17β (ng/mL)* Days before laparoscopy −6	−5	−4	−3	−2	−1
Clomiphene (n = 10)	1	243 ± 30	241 ± 25	265 ± 22	337 ± 33	426 ± 40	528 ± 22
Clomiphene (n = 10)	2	245 ± 20 (122)	280 ± 32 (140)	426 ± 211 (213)	661 ± 86 (330)	762 ± 99 (381)	847 ± 125 (423)
Clomiphene (n = 10)	3	502 ± 32 (167)	588 ± 40 (196)	853 ± 41 (287)	1004 ± 52 (334)	1308 ± 86 (436)	1513 ± 130 (504)
Clomiphene + HMG (n = 7)	Variable ($\bar{x}$ = 4.8)	187 ± 18 (39)	247 ± 21 (51)	395 ± 15 (82)	525 ± 21 (109)	959 ± 92 (199)	972 ± 120 (202)

*Mean values ± 1SE (E2 concentrations per follicle in parentheses).
Ovulation was induced in response to the combined day −2 parameters, one of which being E2 concentration; HCG was administered midway between day −2 and day −1.

14.75 ± 0.35. Overall, patients who were considered close to spontaneous ovulation were admitted to hospital for an HCG trigger injection when the diameters of the leading follicles were 1.8 cm or greater. This correlated with a mean cervical score of 7.4 for clomiphene and 7.1 for clomiphene/HMG cycles on day −2 (Table 2).

Progesterone levels of 0.5 ng/mL or lower were rarely associated with ovulation, but levels higher than 0.5 ng/mL on the day of HCG administration (day −2) were observed in six patients who had at least one ruptured follicle at the time of laparoscopy (Table 3). Approximately 12% of patients admitted to hospital had a spontaneous LH surge and in a third of these one or more follicles were dispersed at the time of laparoscopy, although oocytes were recovered in 60% of patients.[8]

A fertilization rate of more than 90% of mature oocytes was maintained throughout the programme and appeared independent of the concentration of spermatozoa within the range of 2×10^4 to 2×10^5/mL. In Table 4, the overall results of oocyte recovery, fertilization, and pregnancy rates are documented in relation to programme sessions which were of 10 to 13 weeks duration, interspersed with two to four weeks adjournment. Session 1 was regarded as the introductory period for laboratory staff members and the general development of experience with ultrasound and hormonal monitoring. During Session 2, patient

TABLE 2: Ultrasound estimation of follicle diameter and cervical mucus score as indicators of follicular maturation

Stimulation	Days before laparoscopy −6	−5	−4	−3	−2	−1	Number of patients
Clomiphene							
Follicle diameter (cm)							
1 follicle	1.5 ± 0.07	1.7 ± 0.09	1.8 ± 0.09	1.9 ± 0.15	2.0 ± 0.08	2.2 ± 0.32	10
2 follicles	1.2 ± 0.09	1.2 ± 0.20	1.5 ± 0.13	1.7 ± 0.20	1.9 ± 0.20	2.1 ± 0.30	10
3 follicles	–	–	1.3 ± 0.03	1.6 ± 0.15	1.9 ± 0.13	2.0 ± 0.35	10
Mean	1.2 ± 0.05	1.3 ± 0.03	1.5 ± 0.08	1.7 ± 0.17	1.9 ± 0.15	2.0 ± 0.35	30
Cervical mucus score*	3.7 ± 0.09	4.0 ± 0.90	4.2 ± 1.02	5.3 ± 1.20	7.4 ± 1.90	8.7 ± 2.10	30
Clomiphene + HMG							
Follicle diameter (cm)							
Variable number							
($\bar{x}$ = 4.8)	0.7 ± 0.09	1.2 ± 0.12	1.4 ± 0.25	1.5 ± 0.20	1.6 ± 0.35	1.7 ± 0.33	7
Cervical mucus score*	4.7 ± 0.90	5.1 ± 0.10	5.6 ± 0.75	6.9 ± 0.82	7.1 ± 1.20	10.7 ± 0.91	7

Mean values ± SE.
* Maximum score = 12.

TABLE 3: Serum progesterone concentration as an indicator of impending ovulation

Stimulation	Progesterone (ng/mL) Days before laparoscopy −6	−5	−4	−3	−2	−1	Number of patients
No dispersed follicles at laparoscopy							
Clomiphene							
1 follicle	0.2	0.2	0.2	0.3	0.3	0.9	10
2 follicles	0.3	0.1	0.3	0.4	0.5	0.8	10
3 follicles	0.4	0.3	0.4	0.2	0.4	1.2	10
Clomiphene + HMG							
Variable	0.2	0.2	0.2	0.4	0.4	1.8	7
One or more dispersed follicles at ovulation							
Clomiphene	0.3	0.2	0.3	0.3	0.9	2.6	6

Oocyte recovery was scheduled 36 hours after the HCG trigger injection given in the evening of day −2.

TABLE 4: Summary of PIVET IVF programme, July, 1982, to February, 1983

	Session 1	Session 2	Session 3	Total
Patients accepted	40	54	49	143
Had undergone laparoscopy	39	52	48	139
Oocyte recovery performed	36 (92%)	47 (90%)	47 (98%)	130 (94%)
Embryo transfer performed	31 (80%)	40 (77%)	45 (94%)	116 (84%)
Number of pregnancies	2	1	10	13
Pregnancy rate per laparoscopy	5.1%	1.9%	20.8%*	9.3%
Pregnancy rate per embryo transfer	6.5%	2.5%	22.2%*	11.2%

*The pregnancy rates in Session 3 are significantly higher ($\chi^2 = 7.247$; $P < 0.01$) than those in Sessions 1 and 2 combined.

organization, the monitoring programme, the laparoscopic procedure and laboratory techniques were coordinated. However, we changed our approach to embryo transfer by utilizing the catheter system described by the Monash group (METS-1; W. Cook (Melbourne),[9] but failed to become proficient in its use, often creating bleeding. In Session 3, we returned to the familiar transfer technique, as described above.

Up to six embryos have been transferred together, and it can be seen from Table 5 that the pregnancy rate increased with multiple embryo transfers. Two twin gestations were confirmed in our series. Of the 13 pregnancies generated, five aborted spontaneously (38.5%) and the other eight patients have now been delivered; four by caesarian section for obstetric reasons and four per vaginum. All infants (seven boys and three girls) were healthy and had no apparent abnormalities at birth.

TABLE 5: Correlation of pregnancy rates with the number of embryos transferred

Number of embryos transferred	Number of patients	Number of pregnancies
1	13	1 (7.6%)
2	23	5 (21.7%)
3	4	2 (50.0%)
4	3	1 (33.3%)
5	–	–
6	1	1 (twins) (100.0%)

Discussion

While the service programme for IVF-ET at the PIVET Laboratory provided much more comprehensive facilities than those employed in the pilot study, it nevertheless was constrained within a rigid schedule in a busy surgical hospital. Oocyte collection based upon an endogenous LH surge requires relatively flexible access to an operating theatre. In general, our approach was to collect oocytes after an HCG trigger injection, but to be prepared to proceed to laparoscopy if an LH surge was detected in the pre-HCG serum sample and the plasma progesterone level was lower than 1.5 ng/mL, indicating an early stage of the surge. We found that oocyte recovery was cancelled, because of a premature LH surge, in fewer than 10% of patients; another 12% of patients were in an early stage of the surge and underwent oocyte collection the following day. In the latter group, mature oocytes were recovered from about 60% of patients, compared with more than 90% of those who had no evidence of an LH surge. We have considered it important to administer the HCG trigger injection as close as possible to the time of the spontaneous LH surge, in order to maintain a high fertilization rate and to generate morphologically normal embryos. The findings of Kerin et al., who applied a similar stimulation regimen,[10] support this concept. In fact, oocyte collection correlated well with the predicted times of ovulation calculated from previous menstrual histories and, to a lesser extent, with the cervical scores. Because of the desire to shorten the stay in hospital, to collect oocytes from HCG-controlled ovulation, and to reduce the complexity of preovulatory monitoring, the three-hour urinary LH-level estimations described by others[11,12] were not performed. Our current experience indicates that three-hour urinary LH assays confer no additional benefit within the constraints of a daytime schedule. The monitoring of follicle development by the daily measuring of serum E2 levels and by ultrasonic follicle measurements were undertaken during the morning, which minimized disruption of the patients' own schedules (allowing most of them to continue work if desired) and provided results which could be analysed in the early afternoon leaving ample time to reorganize the operating schedule for the following day if required.

Monitoring of follicular development by daily E2 estimations (Table 1) shows a constant E2 level per follicle for clomiphene-stimulated cycles. On day −2, when it was decided to admit patients to hospital that evening for the HCG trigger injection, E2 levels were around 400 ng/mL per follicle. With clomiphene stimulation alone, the number of large follicles averaged 2.3, whereas the combination of clomiphene with HMG generated a mean of 4.8 large follicles. Seven women received additional HMG stimulation either because of a poor response to clomiphene during a previous cycle or because of an inadequate response during the treatment cycle determined by a low E2 level on day 8 of the menstrual cycles (lower than 135 ng/mL on day −6). Those who received an additional HMG injection also tended to have numerous small follicles (within the range of 1.2 to 1.6 cm) which were not commonly found in the group of patients who received clomiphene alone. The E2 levels per follicle were much lower in those in whom ovulation was stimulated with clomiphene/HMG. It was considered that, in these patients, the follicles may not have reached full maturation; however, the onset of spontaneous LH surge occurred on the day predicted from previous menstrual charts. In both groups, a rising cervical mucus score was noted to be a useful marker of an impending LH surge when it was greater than 7.0, but occasional patients failed to demonstrate a significant shift before the LH surge.

The oocyte recovery rate throughout the programme was consistently above 90% per laparoscopy, which was a considerable improvement on that achieved in the pilot study. This was attributed to the fact that the development of follicles was more likely to have been close to the stage of spontaneous dispersal, as well as to the advantages of the use of the combined aspiration/flushing needle.

While the overall pregnancy rate was around 10%, the Session 3 rate in excess of 20% appears to be a reproduceable achievement if two or more embryos are generated for transfer in each case (Table 5). In our series, 13 pregnancies have been achieved and 10 normal healthy infants have now been delivered with a predominance of boys — in contrast to the Monash report which documented seven girls among their first nine infants.[13] The spontaneous abortion rate of 38.5% is high, but is consistent with those reported by other groups.[14,15]

In the final group of 48 patients, 10 pregnancies gave a pregnancy rate of more than 20% per laparoscopy, and the chance of pregnancy was considered better if more than one embryo was transferred. While we would expect continuing improvement in conception rates associated with improved techniques of collection, culture, and embryo transfer, we are reluctant to implant more than three embryos at one time, as further experience has led to a triplet pregnancy (from a three-embryo transfer) and a quadruplet pregnancy has also occurred elsewhere (A. Speirs, personal communication). While it appears that the collection of more oocytes by using additional HMG stimulation would generate more embryos for transfer, our results suggest that the development of appropriate oocyte handling and embryo culture technology will generate a reasonable pregnancy rate from an average of 2.3 oocytes per case. It appears unreasonable to transfer more than three embryos and, therefore, the question of the dispersal of the excess embryos arises. This leads to the consideration of embryo cryopreservation technology, utilization of extra embryos for experimentation, and donation both of ova and of embryos.

The majority of patients who conceived by IVF-ET in our programme were those with tubal disease. However, four were women with patent fallopian tubes whose infertility was related to pelvic endometriosis, or to their partner's oligospermia,[7] or to the presence of heterologous sperm-immobilizing antibodies.[16] These findings are in accord with the expectations of those who feel that IVF may well apply in areas of infertility other than tubal disease.[17]

In conclusion, the results presented here indicate that an IVF-ET treatment programme can operate within the confines of a restricted oocyte collection schedule and within normal working hours. To achieve this, an HCG-controlled ovulation regimen was utilized in cycles stimulated with clomiphene or clomiphene/HMG and monitored by daily E2 and ultrasound estimations, and by cervical mucus changes. The day of oocyte recovery was found to correlate closely with the anticipated day of ovulation based on the previous menstrual history.

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