Development of an In Vitro Fertilization and Embryo Transfer Programme : Preliminary Experience

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Abstract : *From October 1986 to August 1988, a total of 256 cases of infertile women with ages ranging from 26 to 42 years were admitted for the IVF & ET programme. Bilateral tubal occlusion and pelvic endometriosis were the most common indications. A combination of clomiphene citrate and hMG (211 cases) or hMG alone (45 cases) were used for superovulation. The average estradiol levels on the day of hCG administration was 1738 ± 761 pg/ml. Oocytes were retrieved by either laparoscopy (78 cases) or vaginal ultrasound guidance (178 cases). One hundred and five cases were cancelled (41%) due to poor response and premature LH surge. A total of 542 oocytes were collected and 312 oocytes were fertilized giving a fertilized rate of 57.5%. Embryos at 2 or 4 cells stage were transfered in 151 cases chemical and clinical pregnancies were diagnosed in 30 cases. (Thai J Obstet Gynaecol 1991;2:1-6.)*

Key words : in vitro fertilization, embryo transfer programme

The birth of the first test-tube baby on July 25, 1978 was considered a medical breakthrough of reproductive technology that involved superovulation, oocyte recovery, extracorporeal fertilization and replacement of fertilized egg[1]. This milestone was the culmination of many years of studies in animals that involved various rodents and the mammalian system[2]. The techniques of human in vitro fertilization embryo replacement have generated tremendous interest in both biologists and clinicians. The techniques are constantly changing and improving but important clinical information is now being obtained that offers many new approaches to the study and treatment of various aspects of tubal infertility that were not possible in the past[3-6]. In this report, we describe the development and experience of the in vitro fertilization and embryo transfer programme at Human

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Development

An extensive experiment of culturing 2-cell stage of mouse embryo (Balb-C/CD-1) began in mid 1985. Two-cell stage of mouse embryos which were fertilized in vivo, were further cultured in vitro and allowed to grow up to blastocyst. After achieving development to blastocyst in more than 70%, it was then decided to start on human oocytes fertilize in vitro[7]. The successful in vitro fertilization of a human oocyte in our laboratory was done on March 26, 1986. The first attempt of transferring 4-cell stage human embryo was done on June 12, 1986 but failed to concieve. The first birth from our IVF & ET programme in Thailand was a healthy male infant on August 15, 1987[7].

Patients and treatment protocol

From October 1986 to August 1989 a total of 256 infertile women with ages ranging from 26 to 42 years were admitted to the IVF & ET programme. The couples had a duration of infertility of 2 years or more (mean 8.4 ± 3.2 SD: range 2-17 years). All patients had previously undergone investigation for their infertility problems including semen analysis, laparoscopy, hysterosalpingography and hormonal assessment. Infertile couples attending our clinic can be classified into four categories 1) pure tubal factors, 2) pelvic endometriosis with previous surgery, 3) male factors, and 4) mixed infertility.

The majority of cases (211 patients) were treated with clomiphene citrate (CC) (Clomid, Merrel Dow, USA) 100 mg in divided doses from days 3 to 7 of the menstrual cycle and 150-225 IU of human menopausal gonadotropin (hMG) (Pergonal, Serono, Switzerland or Humegon, Organon, Holland) given intramuscularly, started on day 7 until follicular maturation was reached. Forty-five patients were superovulated with 2-3 ampules (150-225 IU) of hMG per day starting on days 3 of the menstrual cycle onwards until hCG was administered. With both treatments serum estradiol (E2) and follicular development were being monitored daily from day 6 onwards. A constant rise of E2 over 5-6 days coupled with at least 2 leading follicular diameter of 17-18 mm or more, 10000 units of human chorionic gonadotropin (hCG, Profasi, Serono, Switzerland or Pregnyl, Organon, Holland) were administered intramuscularly at 08:30 pm. 34-36 hours after hCG was given, oocytes were retrieved by either laparoscopy (78 cases) or ultrasonically guided transvaginal puncture (178 cases). The average estradiol levels on the day of hCG administration of CC/hCG treatment were 1738 ± 761 pg/ml (range 316 - 3691 pg/ml). Each mature follicle was aspirated with single lumen needle (Swemed, Sweden) with constant pressure (100-120 mmHg). Aspirating fol-

licles were flushed with Ham's F-10 medium. The oocytes were immediately washed and classified as previously described[8] and then transferred into a Falcon tube containing 1 ml of Ham's F-10 supplemented with 10% heat inactivated preovulatory patient's serum. The tubes containing oocytes were placed in an incubator under an atmosphere of 5% CO_2 in air for 4-6 hours prior to insemination.

The husband's semen was collected $1\frac{1}{2}$ hours after oocytes recovery. Liquified semen was washed twice in culture medium and the final sperm pellet was over-layered with 2-3 ml of fresh medium. After incubation for 15-30 min, the top 1 ml of the over lay was collected which contained the motile good quality sperm for insemination. Two incubated oocytes in the tube were inseminated with 0.1 x 10^6 ml spermatozoa and fertilization was confirmed at pronuclear check after 18-20 hours of insemination. The fertilized egg was then transferred into a 4-well dish containing fresh medium. Eggs that were not fertilized were re-inseminated. Follow up was done 24 hours after culture, the embryos at 2 to 4-cell stage (maximum of 4 embryos/patient) were transferred into the uterus using a Cook's catheter.

The patients were placed in dorsal lithotomy position with head tilted down. The external genitalia was cleaned with normal saline and prepped. The cervix was exposed with a bivalve speculum and the ectocervix was gently cleaned of cervical mucus with small swabs soaked with normal saline then swabbed with culture medium. The outer sleeve of the Cook's catheter was passed through the cervical canal and held at mark. The embryos were then loaded into the internal catheter with 30 µl of culture medium supplemented with 50% serum. The catheter was then passed through the outer catheter into the uterine cavity and fixed. The outer catheter was gently withdrawn for about 5 cm and both catheter were fixed at this position for one minute. The embryos were expelled by depressing the one ml syringe by 0.02-0.03 ml. The catheters were gently withdrawn and promptly checked microscopically to ensure the embryo had not been retained. Half an hour later the patient was transferred maintaining the position at the time of transfer and allowed home 4-6 hours later. The luteal phase was supported by administration of 1500 IU of hCG on day of transferring embryos and on day 4,7,10 and 13 after the embryo was transferred. Progesterone 50 mg (Schering AG, Germany) was injected intramusculary for 5 consecutive days starting on the day of oocyte recovery. Pregnancy was confirmed by assaying serum β-hCG 14 days after embryo transfer. At 7-8 weeks pregnancy was further confirmed by ultrasound. Amniocentesis in most of the cases was performed at 16-20 weeks of gestation.

Results

The age distribution of the IVF

patients (n=256) is given in Table 1. Of the 256 patients, 62% were more than 35 years. Only 9% of the patients who were in their twenties went through the IVF treatment programme. The analysis of the four main categories of referal were tubal occlusion/ adhesion (54.3%), pelvic endometriosis (31.3%), oligospermia (10.5%), and idiopathic (3.9%). Details of the patients treated for 256 cycles are given in Table 2. In all, 105 treatment cycles were cancelled at different stages due to poor response 38 cases (14.8%), failure to retrieve oocytes 10 cases (3.9%), failure to fertilize oocytes 21 cases (8.2%), or premature LH surge 36 cases (14%). It was possible to recover oocytes obtained for fertilization and trasfer of embryos for 151 treatment cycles. A total of 542 oocytes were collected and 312 fertilized giving a fertilization rate of 57.5%. An average of 2.0 embryos were transfered per patient. The pregnancy outcome and the type of pregnancy are given in Table 3. A total of 18 patients had β-hCG>20 mIU/ml 14 days after the embryo transfer. However, 6 pregnancies were lost due to abortion during the first trimester and ectopic pregnancy. Two healthy infants were born and 4 are on going pregnancies (>16 weeks).

Table 1 Age distribution of the IVF & ET patients (N=256)

Age (years)	No. of patients (%)
< 29	22 (9.0)
30-34	73 (28.9)
35-39	123 (46.9)
> 40	38 (15.1)

(Mean 35 ± 3.5, min = 26, max = 42)

Table 2 Details of treatment cycles (n=256)

Categories	No. of patients	(%)
Cancellation	105	(41)
Poor response	38	(14.8)
Failed to retrieve	10	(3.9)
Failed to fertilize	21	(8.2)
Premature LH surge	36	(14)
E2 peak (pg/ml)	1738 ± 761	
No. of oocytes collected	542	
No. of oocytes/puncture	2.8	
No. of oocytes fertilized	312	
Fertilization rate	57.5	
No. of embryo transfer/case	2.0	

Table 3 Success of 151 embryo transfer

Outcome	No. of cases
Chemical pregnancies (B-hCG>20 mIU/ml)	18
Clinical pregnancies	12
Abortion (2-3 m)	4
Ectopic	2
Term pregnancies	2
On going (>16 weeks)	4
Total	12

Discussion

A large proportion of the patients (62%) who went through our IVF & ET programme were older than 35 years and it is well documented that the IVF & ET prognosis for such patients is poor. A recent study demonstrated that the age of the patient was significantly affected by

their response to stimulation, and also the outcome of treatment[9]. Comparing women >35 years, 27% failed to reach oocyte recovery compared with 15% <35 years. Women over the age of 35 years had a reduced chance of implantation which supports previous findings[10].

As shown in the results, the stimulation protocol used for most of the patients was clomiphene citrate and hMG. Furthermore, the occurrence of premature LH surges in some patients making it difficult to time the oocyte collection which can result in the recovery of poor quality oocytes and failed fertilization. Premature luteinization and the spontaneous LH surge have been shown to reduce the successful outcome of IVF cycle[11,12]. In the past five years GnRH has been introduced to inhibit endogenous gonadotropin output and subsequently augment follicular growth with hMG[13].

In this paper, sperm problems were not mentioned. Some patients were cancelled due to failed fertilization mainly associated with poor quality sperm (oligoasthenospermia). Fertilization at an acceptable rate was maintained for the normospermic group. Estimates of defective sperm function or sub-normal seminal characteristics in nearly 35% of infertile couples suggests that this is the defined cause of human infertility[14]. In addition, up to 20% of couples with unexplained infertility are unable to achieve fertilization due to defective interactions of gametes. This latter group may include those with defects in sperm/zona pellucida receptors; in some couples sperm binding occurs at the level of the zona pellucida but without penetration while with the others binding fails to occur.

One of the intrinsic problem of CC/hMG superovulation is prolonged hypersecretion of LH caused by CC which can lead to a degree of luteinization in the preovulatory cycle[11]. Furthermore, overexposure of the developing follicle to LH can lead to failure of implantation ascribed to decreased embryo fitness[15]. This may be due to premature initiation of terminal growth changes in follicle cells and oocytes, similar to those seen at midcycle following LH surge, but not sufficient to provoke follicular rupture [16,17].

The low pregnancy rate (take-home baby,7.4%) for IVF&ET reported in this paper may be due to our inadequate experience in the management of patients stimulation protocol, in vitro culture set up, media used and the postembryo transfer management of patients. However, we are hoping to improve our IVF&ET set up with the induction of GnRH agonist, better laboratory conditions and better management of patients following transfer.

References

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978;2:366-7.
2. Chang MC. Fertilization of rabbit ova in vitro. Nature 1959; 184:466-7.
3. Edwards RG, Steptoe PC, Purdy JM. Establishing full term human pregnancies

using cleaving embryos. Br J Obstet Gynaecol 1980; 87:737-56.

4. Lopata A, Johnston IWH, Hoult JJ, Spiers AI. Pregnancy following intrauterine implantation of an embryo obtained by in vitro fertilization of preovulatory egg. Fertil Steril 1980; 33:117-20.
5. Trounson AO, Leeton JF, Wood C, Webb J, Wood J. Pregnancies in human by fertilization in vitro and embryo transfer in the controlled ovulatory cycle. Science 1981; 212:681-2.
6. Edwards RG. Test-tube babies, 1981. Nature 1981; 293:253-6.
7. Virutamasen P, Witoonpanich P, Boonkasemsanti W, et al. Pregnancy following in vitro fertilization Chula Med J 1987; 31:911-7.
8. Marrs RP, Saito H, Yee B, Sato F, Brown J. Effect of variation of in vitro culture techniques upon oocyte fertilization and embryo. Development in human in vitro fertilization procedure. Fertil steril 1984; 41:519-23.
9. Fishel SB, Webster J, Faration B, Jackson P, Shelton K, Jonhson J. Establishing pregnancies after the follicular stimulation for IVF with clomiphene citrate and human menopausal gonadotropin only. Hum Reprod 1991; 6:106-12.
10. Fishel SB, Edwards RG, Purdy JM, Steptoe PC, Webster J, et al. Implantation abortion and birth after in vitro fertilization using the natural menstrual cycle or follicular stimulation with clomiphene citrate and human menopausal gonadotropin. J In Vitro Fertil Embryo Transfer 1985; 2:123-31.
11. Stanger JD, Yovich JL. Reduced in vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during follicular phase. Br J Obstet Gynaecol 1985; 92: 385-93.
12. Eibschitz I, Belaisch-Allart JC, Frydman R. In vitro fertilization management and results in stimulated cycles with spontaneous luteinizing hormone discharge. Fertil Steril 1986;45:231-6.
13. Rutherford AJ, Subak-Sharpe RJ, Dawson KJ, Margara RA, Franks S, Winston RML. Improvement of in virtro fertilization after treatment with buserelin an agonist of luteinizing hormone releasing hormone. Br Med J 1988;296:1765-70.
14. Hull MGR. Infertility : Nature and extent of the problem. In : Bock G, O'Connor MC.eds. Human Embryo Research : Yes or No? London : Tavitock Publications Ciba Foundation, 1986 : 24-38.
15. Howles CM, Macnamee MC, Edward RG, Goswamy R, Steptoe PC. Effect of high tonic levels of luteinizing hormone on outcome of in vitro fertilization. Lancet 1986; 2:521-5.
16. Winer-Sorgen S, Brown J, Tslutomer O, et al. Oocyte maturation inhibitor activity in human follicular fluid quantitative determintion in unstimulated and clomiphene citrate and human menopausal gonadotropin-stimulated ovarian cycles. J In vitro Fertil Embryo Transfer 1986; 3: 218-24.
17. Fowler RE, Fox NL, Edwards RG, Walters DE, Steptoe PC. Steroidogenesis by cultured granulosa cells aspirated from human follicle using pregnenolone and androgens as precursors. J Endocrinol 1975; 77:171-8.