Thai Journal of Obstetrics and Gynaecology
March 1999, Vol. 11, pp. 29-33

GYNAECOLOGY

The first series of the simplified IVF-ET Programme in Songklanagarind Hospital

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ABSTRACT

Objective To study the outcomes of the in vitro fertilisation-embryo transfer programme in Songklanagarind Hospital.
Design Descriptive study.
Setting Government infertility clinic.
Patients Twenty-eight infertile couples indicated for assisted reproduction.
Interventions Twenty-eight female partners were stimulated using a long protocol and ultrasonographic monitoring. The retrieved oocytes were transported from the operative room to the laboratory by a mobile Earle-HEPES culture system. They were then inseminated in vitro and the embryos were cultured under a mineral oil layer. The three best embryos were transferred to the uterine cavity 48 hours after retrieval. The serum hCG was assayed 2 weeks later.
Main outcome measures Fertilisation rate, implantation rate, and clinical pregnancy rate.
Results Twenty-six oocyte retrieval cycles in this series resulted in 64.1% fertilisation rate, 16% implantation rate, and 34.6% clinical pregnancy rate (per retrieval cycle). No complication or abortion was reported.
Conclusion The outcomes of the first series of the IVF-ET programme in Songklanagarind Hospital were satisfactory.

Key words: In vitro fertilisation - embryo transfer, Assisted reproduction, Infertility, Songklanagarind Hospital

The first series of the simplified IVF-ET Programme in Songklanagarind Hospital

The in vitro fertilisation - embryo transfer (IVF-ET) has become the mainstay of modern infertility management for more than two decades. The IVF-ET procedure has been performed routinely in many infertility units worldwide, including many centers in Thailand. Because of limitation in resources, the IVF-ET Programme in Songklanagarind Hospital has developed slowly and is probably the latest medical

school that established this kind of service. The standard IVF-ET programme requires a high quality laboratory such as a cell or tissue culture laboratory, high grade materials and instruments, and experienced scientists in order to achieve and maintain good results. All of these items seem to be limited in the Songklanagarind IVF Programme. However, we have tried to apply our instruments and simplified the procedure with little disturbance to quality. Following is the first, small series report of our IVF-ET Programme which resulted in a satisfactory clinical pregnancy rate.

Materials and methods

From July 1997 to December 1998, 28 infertile couples were enrolled for IVF-ET treatment. The infertile couples were evaluated completely with basic infertility investigations and were all candidates for the IVF-ET cycle. A counseling programme was provided for the couples regarding the method, risks and success rate of the procedure.

The female patients were stimulated with a long protocol, starting with nasal gonadotrophin releasing hormone agonist (GnRHa; Suprefact, Hoechst) on day 23-25 of the preceding cycle. When pelvic ultrasonography revealed adequate ovarian suppression, human menopausal gonadotrophin (hMG; Humegon, Organon) was administered intramuscularly along with GnRHa starting at 225 IU per day. The growth of the follicles was monitored with pelvic ultrasonography starting five days later and then every other day. Dosage of hMG was adjusted according to the growth of the follicles. On day 5 of hMG, the dose was continued if the follicles reached 8-10 mm in diameter. If not, the additional dose of 75-150 IU of hMG was given. When at least two leading follicles had reached 18 mm in diameter or more, 10,000 IU of human chorionic gonadotrophin (hCG; Pregnyl, Organon) was injected intramuscularly. The estradiol level was not measured before the hCG injection. HMG and GnRHa was discontinued after the hCG injection. The oocyte retrieval was performed 34-35 hours later transvaginally using a 16 G, double lumen, aspiration needle (Cook, Australia) with ultrasound guidance.

The oocyte retrieval was performed in the operation theatre in a building next to the laboratory room with balanced general anesthesia. We used a common regulated suction system (110-120 mmHg) in the operative room for aspiration and a hand-manipulated syringe for flushing. The flushing medium was the heparinized, phosphate buffer saline. The follicular aspirates were examined quickly in the normal environment of the operative room. The oocyte-cumulus complexes were selected and transferred to the laboratory in the Earle-HEPES culture medium (made in house). The culture medium was in the conical tubes (Falcon) and placed in a warm waterbath (37°C). In the laboratory room, the oocyte-cumulus complexes were washed and transferred to the Earle culture media drops supplemented with 10% maternal serum, covered with mineral oil (embryo tested, Sigma), in the CO_2 incubator (5% CO_2 in air).

The male partners were asked to collect their semen by masturbation on the day of oocyte retrieval. The semen was analyzed to assess the concentration, motility, and morphology. A sperm preparation was performed with discontinuous Percoll gradient (40% / 90%) centrifugation method.[1]

After 3-4 hours of incubation, the oocyte-cumulus complexes were inseminated at the concentration of 100,000 spermatozoa/ml in the Earle media drop under mineral oil layer. The oocytes were then incubated further in the incubator.

Sixteen to seventeen hours later, the oocyte-cumulus complexes were partially denuded with a hand-pulled pipette in order to be examined under microscope. The two-pronuclei zygotes with second polar body were selected and transferred to the Earle culture medium with 15% maternal serum for further incubation under the mineral oil layer. Forty-eight hours after oocyte retrieval, the three best embryos were selected and transferred back into the uterine cavity transcervically using the Earle culture medium supplemented with 50% maternal serum. The rest of the embryos were cryopreserved for later use.

The patients were administered 50 mg of progesterone (Proluton, Schering) intramuscularly daily starting from the day of oocyte retrieval. The serum beta hCG was assayed in each patient twelve days after embryo transfer. The patients with a positive result (serum beta hCG > 10 mIU/ml) would received pelvic ultrasonography 4 weeks after the procedure to define clinical pregnancy. The luteal support was continued until 10 weeks of pregnancy for the positive cases.

Results

The clinical characteristics of patients were described in table I. From 28 stimulation cycles, 2 cycles were canceled due to poor response and hyperstimulation. Twenty-six oocyte retrieval were performed and resulted in 256 oocytes collected (table II). Sixty-four percent of these oocytes could be fertilised normally and 92% of the zygotes resulted in cleaving embryos. The mean number of the transferred embryos was 2.58. There were embryos for transfer for every treatment cycle.

Table 1. Clinical characteristics of female patients

Patients (N)	28
Age (year)	34.3±3.4
Duration of infertility (year)	5.7±3.0
Causes of infertility	
Tubal occlusion	15
Cornual part	*6*
Distal part	*7*
Post tubal sterilisation	*2*
Pelvic adhesion	*5*
Endometriosis	*5*
Oligozoospermia	1
Unexplained infertility	2
Total	**28**

Table 2. Results of treatment cycles

Stimulation cycle (N)	28
Oocyte retrieval cycle (N)	26
Collected oocytes per retrieval (N)	3-22
mean ± SD	9.8±5.0
Total collected oocytes (N)	256
Fertilisation rate (%)	64.1
Cleavage rate (%)	92.1
Transferred embryos (N)	2.58±0.7
Clinical pregnancy rate (%) per retrieval	34.6
Implantation rate (%)per embryo	16.4

Majority of the cleaving embryos were classified as grade II embryos (grade I: symmetrical blastomeres and no anucleate fragment, grade II: <10% fragment and/or asymmetrical blastomeres, grade III: 10-25% fragment, grade IV: >25% fragment). Nine pregnancies (7 singletons and 2 twins) were diagnosed. This resulted in a 34.6% clinical pregnancy rate (per retreival) and a 16% implantation rate (per embryo). No complications or miscarriages were reported.

Discussion

The demand from infertile couples for the assisted reproduction technology and the academic progression of this science forces every tertiary medical center to develop its own programme. With the advance of technology, the knowledge has been established with many of successful methods being reported elsewhere. However, the ART programme needs intensive investment and expertise. Therefore, application and modification is neccessary to make the programme feasible in a limited place.

The IVF-ET programme is composed of two main parts, a clinical part and a laboratory part. The clinical part concentrates on the ovarian stimulation process. Gonadotrophins (Gn) and gonadotrophin releasing hormone agonists (GnRHa) are principle agents for stimulation. Many protocols have been reported with comparable results or slight superiority of the long protocol.[2,3] Although the long protocol utilizes a little more Gn and GnRHa than others, the long protocol has advantage of flexibility. Therefore, the clinician can schedule the patient programme easily.[4] This is very important for a regional center because patients have to travel a long distance to the clinic and the clinicians may be burdened with other jobs. We monitored the stimulated cycles with only transvaginal ultrasound. Our series resulted in 2 canceled cases due to poor response and hyperstimulation. Ultrasonographic monitoring without estradiol assay is a part of the simplified protocol, which has been reported with success,[5,6] because a rapid estradiol assay may not be available in every center. The clinician may need more experience to control the stimulated cycle with only ultrasound, but it is more convenient and costs less for the patients. Many clinics may have used this kind of monitoring with the clomiphene-stimulated cycle and it takes a little bit more skill to apply to GnRHa/Gn cycle. Fortunately, we have learned some from our established gamete intrafallopian transfer (GIFT) programme.

The laboratory part of IVF-ET is one of the most delicate procedures. Every step needs conditioned environment and meticulous technique. In addition to good instruments and laboratory, expertise is another crucial factor for the programme.[7] Every IVF lab has to experience a "learning phase" that may take either a short or long period depending on training and funding of the individual unit. The Songklanagarind IVF laboratory was stuck in the preparatory and learning phase for years. Our biggest problems were laboratory quality and the experience of personnel for gamete and embryo handling. Ideally, the IVF laboratory should be well-equipped like a cell/tissue culture laboratory.[7,8] Having such a laboratory requires a large budget possible only in larger hospitals. Some hospitals may have no choice and must do their best with limited available resources.

Our laboratory is a normal room with no conditioning facilities except an air conditioner. We could not control or provide an appropriate environment for a cell culture room. To compensate for this defect, we apply a close system for embryo culture with mineral oil layer to shield culture drops. A thin layer of oil is placed on top of the media drops to work as a barrier. It can slow down the CO_2 lose from the medium that affects the bicarbonate buffer system.[9] The oil barrier can stabilize the temperature of the media drops and prevent air-borne particles from contaminating the drops for a short while, enough time for oocyte or embryo handling outside the incubator. Furthermore, the mineral oil layer can act as a sink for the embryotoxic substance in the media drops.[10]

Another problem is the oocyte transfer from the operative room. Ideally, the laboratory and the operative room should be designed to work closely in order to facilitate the oocyte handling and transfer. Unfortunately,

we have to perform the oocyte retrieval under general anesthesia in an operative room located in another building. We apply a warm waterbath and the Earle-HEPES medium for the transfer system. This system works very well and should be applicable to any hospital of which the laboratory is separated from the oocyte retrieval room, or even satellite clinics. The bicarbonate buffer medium with transport incubator has been reported as well with satisfactory results.[11] However, the latter technique may increase cost. Some programme transports the oocytes in their own follicular fluid or flushing medium in an insulated box without examination.[5,12] With this technique, some oocytes may be missed.

This study shows the preliminary results of our simplified IVF-ET programme for an 18 month period. Most patients had either a tubal or a peritoneal factor as the main infertility problem. It was our policy to offer GIFT (gamete intrafallopian transfer) to infertile couples with patent uterine tubes as the first choice. This might be some advantage to our IVF programme because couples with tubal problems usually have a single problem. This series revealed a 64% fertilisation rate, a 16% implantation rate and a 34.6% clinical pregnancy rate which is satisfactory and comparable to other reports.[13-15] However, when more unexplained infertility cases are enrolled the programme should be reviewed again.

In summary, we report the first series of the Songklanagarind IVF programme with satisfactory results. We simplified the procedure and system to fit our condition successfully. However, our assisted reproduction programme still needs more effort for further development. More instruments and experience are essential to improve and maintain the results for more complicated cases. We are looking forward to extending our facilities to include a micromanipulation programme and standing as a reliable center in the South.

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