Results of phase I of the *in vitro* fertilization and embryo transfer programme at Tygerberg Hospital

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Summary

Phase I (3 June - 31 October 1983) of the *in vitro* fertilization (IVF) programme at Tygerberg Hospital is outlined in this article. This programme led to the birth of the first IVF baby in South Africa after fertilization and embryo transfer (ET) took place at this institution. The baby was born on 29 April 1984; a second baby was born on 20 June 1984.

During phase I 24 laparoscopies were performed; 32 oocytes were obtained from 56 follicles, of which 62,5% were fertilized. Eleven ETs were performed, which led to 3 pregnancies. An ongoing pregnancy rate of 18% per ET and a pregnancy rate of 12,5% per laparoscopy were achieved.

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The technique of *in vitro* fertilization (IVF) and embryo transfer (ET) has not only captured the imagination of many, but has also brought new hope to thousands of childless couples in our country and all over the world. Although IVF is applicable to different problems in infertility,[1] in our clinic it was decided to concentrate on patients with tubal problems and whose male partner was fertile.

The preliminary work on laboratory methods and mice studies was started in September 1982. The ovulation-induction programme was launched simultaneously. The aim was to be able to grow 2-cell mice embryos to the blastocyst stage at a constant tempo, and to use the mouse-oocyte system as a method of quality control.[2] Two-cell mouse embryos were used to test tubes, Teflon tubing, needles, culture dishes and other equipment to be used in the human IVF programme. Daily pH, carbon dioxide and oxygen pressure and temperature measurements were performed on growth medium in culture dishes, simulating the conditions in which human IVF was to be carried out. The same dishes were also used to grow 2-cell mouse embryos. Osmolarity was also checked daily in culture dishes and tubes after 24 and 48 hours. Changes in osmolarity were also studied in the laminar air-flow cabinet over a period of time, and the changes documented. This preliminary work, although time-consuming, provided insight into laboratory factors that could influence fertilization of the human oocyte.

The first follicle aspiration was performed in April 1983 so that we could master the technique and visualize oocytes in the laboratory. Our first patient for IVF was scheduled for 3 June 1983. At that stage we were convinced that follicle aspiration and detection of the oocyte was not a problem. Quality control in the laboratory was excellent and the principles for the ovulation-induction programme were laid down as accurately as possible.

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Patients and methods

Screening of patients

Women under 40 years of age were accepted for the programme. Screening laparoscopies were performed to evaluate the accessibility of the ovaries in all cases. In all cases a tubal factor was involved in the couple's infertility; male partners were all fertile according to our laboratory's criteria: sperm count/ml $\geqslant$ 10 million, motility $\geqslant$ 30%, forward progression $\geqslant$ 2,0, and normal cell morphology $\geqslant$ 20%[3] (patients with a normal morphology rate of 15-19% were occasionally accepted).

Ovulation induction

Either clomiphene citrate 100 mg from day 5 to day 9 was given or low-dose human menopausal gonadotrophin (HMG) was started on day 3 of the cycle. All patients underwent a preliminary ultrasound examination to exclude ovarian cysts that could hamper follicular follow-up. Oestradiol (E_2) estimations were started on day 3 in patients receiving HMG and on day 5 in those on the clomiphene regimen. Daily ultrasound examinations were commenced when the E_2 value was $>$ 500 pmol/l (a Philips SDU 7 000 was used for follicular measurement). When the leading follicle reached a diameter of 18 mm measured in two planes with an E_2 level of 1 000 pmol/l per follicle $>$ 16 mm in diameter, human chorionic gonadotrophin (HCG) was given intramuscularly at 20h00 or 21h00 and a laparoscopy was performed 36 hours later. On the day of laparoscopy an ultrasound examination was performed to make sure that ovulation had not taken place, because luteinizing hormone (LH) estimations were not performed in phase I of our programme (for more details see the accompanying article by Van Schouwenburg and Kruger on page 759 of this issue).

Laparoscopy

Laparoscopy was performed 36 hours after HCG injection. No instruments or antiseptic were allowed to enter the vagina or uterus. One hundred per cent CO_2 was used to induce pneumoperitoneum[4] with the standard three-puncture technique,[5] and a Bourn-Hall aspiration needle was used. The collection device was a tissue-culture tube (Falcon 3033). Aspiration was controlled by a wall-suction apparatus adjusted to 100 mmHg (controlled by an assistant).

Before follicle aspiration the surgeon and assistant had to wash their surgical gloves in 4 x distilled de-ionized water to reduce the chances of contamination of the follicular fluid. Special care was taken not to touch the tip of the aspiration needle.[6] The ovary was stabilized by grasping the ovarian or round ligament. Suction was applied just before puncturing the follicle and the latter was then entered. After the follicle had collapsed and bloodstained fluid had entered the Teflon tubing, the suction apparatus was dislodged and the follicular fluid handed to the embryologist in the adjoining laboratory. The system was then rinsed with HEPES buffer and the rinse was also handed to the laboratory personnel.

The aspirate was transferred to a big Petri dish (Falcon 3003) and screened macroscopically. If the cumulus mass was detected, the presence of an oocyte was confirmed with the aid of a dissecting Zeiss microscope; if the cumulus mass could not be detected macroscopically, the entire Petri dish was thoroughly screened with the above microscope. If no ovum could be detected the follicle was rinsed by the surgeon with HEPES T6 (HT6) to which heparin 40 IU/ml had been added, and aspiration was repeated. Maturation of the oocytes recovered was classified according to criteria laid down by Testart *et al.*[7]

Laboratory methods

Glassware

The glassware was prepared according to a method described by Whittingham.[8] The Pasteur pipettes used routinely were always rinsed three times before use in the laboratory.

The medium

In phase I of our programme Whittingham's T6 growth medium was used. The medium was freshly prepared weekly, the osmolarity adjusted to 285 mmol/kg and the medium filtered by Millipore filtration (0,2 μm filter) with a pH of 7,9-8. After gassing the medium in a 5% CO_2-in-air incubator for 24 hours the pH is 7,4 on the Autocal PHM 83 pH meter. The medium is tested weekly with 2-cell mice embryos, 90% of which have to reach the blastocyst stage. If this standard is not reached, the medium is freshly prepared and retested.

For quality control the following were routinely measured daily: (*i*) percentage CO_2 in the incubator, tested with a Fyrite gas analyser; (*ii*) pH of the medium in a Falcon 3037 Petri dish, simulating the conditions in which the oocytes and spermatozoa are kept; (*iii*) osmolarity, recorded in a Falcon 3037 Petri dish after 24 hours (vapour pressure osmometer; Westcor Inc. 5100C); (*iv*) temperature in the incubator (it had to be 37°C); and (*v*) humidity, which should be 95-98%.

Insemination medium consisted of 90% T6 and 10% heat-inactivated maternal serum. The serum was prepared as described by Leung *et al.*[9] The oocyte was incubated for 5-6 hours before insemination in a culture dish.[10] The central well was filled with 1 ml insemination medium with 5 ml T6 in the surrounding well to keep osmolarity constant.[11]

Growth medium also consisted of 10% heat-inactivated maternal serum plus 90% T6. The oocyte was transferred 17-20 hours after insemination. The oocytes were dissected with a No. 30 needle and fertilization was documented. The next morning (± 40 hours after insemination) the embryos were inspected to observe cleavage.

Semen preparation

Semen samples were obtained 1-3 hours before insemination. The semen was allowed to liquefy at room temperature, and 1 ml semen was mixed with 2 ml insemination medium. Centrifugation was performed at 200 *g* for 10 minutes and the supernatant fluid discarded. The same procedure was repeated and the supernatant fluid again discarded. One millilitre of insemination medium was used for the final step and the semen was placed in the incubator (5% CO_2 in air) for 30 - 45 minutes.[10] A final count was performed and 100 000 motile spermatozoa per ml of insemination medium were added.

Embryo transfer

In phase I of our IVF programme the ET technique of the Norfolk group was followed closely.[12] The embryo or embryos were transferred 45-48 hours after insemination. The procedure took place in the theatre next to the laboratory. No premedication was given to the patients before transfer but all of them had been examined by T.F.K. during a previous cycle and they were well prepared and knew exactly what to expect.[13]

The patients were put into the knee-chest position if the uterus was anteverted and in the lithotomy position if the uterus was retroverted. The gynaecologist scrubbed as for a routine operation but did not wear gloves. The patient was positioned and draped with sterile towels, and the cervix was cleaned with T6. The embryo was then brought to the gynaecologist to be transferred. The embryologist closely followed the methods outlined by Jones *et al.*,[12] and 60-90 μl of fluid was injected into the uterus. The embryos were transferred in growth medium.

The patient rested in bed for 4 hours — on her stomach if the uterus was anteverted and on her back if the uterus was retroverted. She was discharged the next day. No antibiotics or progesterone at all were administered to the patients after transfer. No intercourse was allowed for 10 days and ß-HCG estimations were performed on the 10th day after transfer as well as on day 14.

Results

Table I summarizes results in the first phase. Twenty-four patients had follicles aspirated, and in 18 of these oocytes were

TABLE I. RESULTS OF 24 LAPAROSCOPIES BETWEEN 1 JUNE AND 30 OCTOBER 1983

Total No. laparoscopies	24
Successful laparoscopies	18
No. of follicles	56
No. of oocytes	32 (57,14% of follicles)
No. of oocytes fertilized	20 (62,50%)
No. of oocytes/laparoscopy	1,3
No. of patients at transfer stage	11
Transfers/laparoscopy	11/24 (45,83%)
Pregnancies	3 (2 ongoing + 2 deliveries, 1 chemical pregnancy)
Pregnancies/laparoscopy (%)	12,5
Pregnancies/transfer (%)	27,27
Ongoing pregnancies/transfer (%)	18,18

recovered. A total of 32 oocytes were obtained, of which 20 cleaved (62,5% oocytes fertilized).

Eleven patients reached the transfer stage (46% of laparoscopies), and 3 patients became pregnant (Tables I and II). One patient had a rise in HCG on days 10 and 14 after transfer, with a value of 300 IU on day 18, but 1 day later she started menstruating. The other 2 patients went to term. The first patient had an uncomplicated antenatal period and the pregnancy reached 40 weeks. She went into labour spontaneously. At the end of the first stage two-fifths of the fetal head was still palpable above the pelvic brim, and a caesarean section was performed. A normal male infant (2850 g) was delivered, his Apgar scores being 10/10 at 1 and 5 minutes. Both mother and baby were discharged 8 days later in good health. The second baby was delivered at 41 weeks, also after an uncomplicated antenatal period. A vacuum extractor was used to deliver the baby because of a transverse position with a prolonged second stage. The baby's Apgar scores were 9/10 after 1 minute and 10/10 after 5 minutes. The baby was a normal male and the birth weight was 2830 g. No complications developed and both mother and baby were discharged after 1 week.

TABLE II. EMBRYO TRANSFER

No. of embryos at transfer	Patients	Pregnancies
1	4	1*
2	7	2
3	0	0

*Aborted.

Discussion

We believe that the use of the mouse oocyte system in our preliminary work as well as the use of this system as a routine method of quality control in the programme, the pH and osmolarity studies[14] and the removal of powdered surgical gloves from the programme, definitely played a role in our initial success.[6]

The ongoing pregnancy rate per ET is 18%. We realize that our figures are small, but this pregnancy rate is comparable with those of other groups.[15,16]

Our fertilization rate was 62% per oocyte. Trounson *et al*[16] quoted a fertilization rate of 40% in 1979 and of 65% in 1980. The Norfolk group achieved a 33% fertilization rate in 1981 in phase I of their programme, but this rate improved dramatically and they reported a fertilization rate of 90% for mature oocytes in 1981-1982.[17] Reasons mentioned for this improvement were a better-quality water as well as an alteration of the medium pH to 7,4.[18] Lately most leading groups are reporting a fertilization rate of 80% or more per oocyte.[16,19] This rate can be achieved with improvement of laboratory methods and the ovulation-induction programme.

At that time we could obtain only 1,3 oocytes per laparoscopy. This aspect of the work can be improved by using higher doses of HMG in the ovulation-induction programme and by improving the follicle-aspiration technique. Oocytes were obtained from 57% of follicles, and at 6 laparoscopies we were not able to retrieve oocytes. A good rate of retrieval is in the region of 80-90% per follicle.[4,19] In 1981 Wood *et al.*[4] already clearly pointed out that factors which may contribute to success in the collection of mature oocytes are: (*i*) exclusion of very difficult patients by preliminary laparoscopy; (*ii*) improved aspiration techniques; and (*iii*) improved surgical techniques. Their results improved from a 45% chance of obtaining an oocyte per follicle to above 80% within months.

Only 11 of the 24 patients (46%) subjected to laparoscopy reached the ET stage. Analysis of the literature makes it obvious that the level to be achieved must be at least 77%.[20]

Transfer of 2 or more embryos per patient at a constant rate can lead to a higher pregnancy rate. The Royal Women's Hospital group quote a pregnancy rate of 11% after transfer of 1 embryo, 18-22% if 2 or 3 embryos are transferred, and 38% if 4 are transferred.[21] The continuing pregnancy rate is higher if more than 1 embryo is transferred. Although the figures in our series are very small, it is interesting that our 2 full-term pregnancies resulted after transfer of 2 embryos per patient.

To conclude, the fact that we achieved a pregnancy rate of 18% per ET is a reflection of good quality control in the laboratory as well as of a sound ovulation-induction programme. The aim of this group is to increase the number of patients reaching the ET stage and to transfer more embryos per patient. In this way more childless couples could achieve pregnancy through IVF.

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