Results of the in vitro fertilization programme at Tygerberg Hospital, phases II and III


Summary

Phases II and III of the human in vitro fertilization programme at Tygerberg Hospital are presented. In phase II, 42 laparoscopies were performed and oocytes were obtained from 76% of the follicles aspirated, but with a fertilization rate of only 37%. The viable pregnancy rate per embryo transfer was 4%. Important changes took place in the programme, which led to a fertilization rate of 77% in phase III. Of the 78 patients subjected to laparoscopy, 65 (83%) reached the embryo transfer stage, resulting in a clinical pregnancy rate per embryo transfer of 23%, and with a 19% pregnancy rate per laparoscopy. The changes, methods and results of phases II and III are discussed.

Patients and methods

Patients accepted into the programme had tubal damage and their male partners had to be fertile, as outlined in our previous article. The ovulation induction programme in phase II was changed to a combination of clomiphene 100 mg and HMG 2 ampoules on alternate days, starting on the second day of clomiphene therapy. The HMG therapy was given 3 times, as discussed in a recent paper by Kerin et al. We used 50 mg less clomiphene per day than Kerin's group. Ultrasound examinations were performed on day 1 of the menstrual cycle and the cycle was cancelled if a cyst was detected. Thereafter ultrasound examinations were performed daily after the oestradiol (E2) values reached a level of 500 pmol/L. When a diameter of 18 mm was reached by one of the follicles with at least one other follicle more than 16 mm in diameter (measured in two planes with a Phillips ST 7000), the HCG 10 000 U was given intramuscularly. The laparoscopy was performed 36 hours later. This was a change from our previous method of giving low doses of HMG or clomiphene 100 mg as the only method of ovulation induction.

The laparoscopic technique also changed. The double-barrel Bourn Hall needle was replaced by the Monash needle, which is 14 cm in length, 17 mm in diameter and lined with Teflon. Flushing of the follicle took place only if the oocyte was not discovered in the follicular fluid or needle rinse. No heparin was used in phase III but the blood-stained fluid was transferred immediately to the laboratory. The needle was rinsed with 4-times distilled water after the procedure and every needle was relined after 4 aspirations. Dry heat was used to sterilize the needles.

A 3033 Falcon tissue culture tube was used to obtain the follicular fluid and these bottles were prewarmed in an incubator to 37°C in phase III of the programme. This did not take place in phase II. The follicular fluid was screened in the laboratory in 3033 Falcon Petri dishes. Care was taken to prewarm these dishes before use. The ovum was transported to a 3037 Falcon Petri dish as soon as possible after it was rinsed in T6 medium with no serum added. The rest of the methods followed were outlined in a previous article. Laboratory methods in phase II were as in phase I. Filtered T6 medium was used, made up once a week (filter 0.22 Gelman). The pH was 7.4 after 24 hours in a 5% carbon dioxide in air incubator (Forma Scientific 3157). The semen specimen was prepared by standard methods and described in a previous article. 100 000 motile spermatozoa were used per ml of insemination medium. Transfer to the growth medium took place after 20 hours. Oocytes were dissected and pronuclei documented (Table I).

In phase III the medium was changed to Ham F10 because the poor fertilization rate in phase II could possibly have been caused by deterioration of the purivate quality in the medium. Fresh purivate could not be obtained immediately. The osmolarity was 280 mOsm/kg and the pH after 24 hours 7.4. The serum in the growth medium was 20%. No filters were used because wetting-agent free filters could not be obtained immediately. Because very satisfactory results were achieved, with no infection to date, reversal to the use of filters did not occur.

All glassware was prepared as described by Whittingham. The Pasteur pipettes were rinsed 3 times before use and handling of oocytes.

In phase III, dissection of the oocyte was seldom performed after 20 hours but cleavage was observed 40 hours after insemination. In summary, the following changes took place: (i) T6 to Ham F10; (ii) osmolarity 285 mOsm/kg to 280 mOsm/kg; (iii) tubes and Petri

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In summary, the following points were regarded as important in phase III: (i) only the dorsal lithotomy position was used; (ii) Tomcat catheter used as transfer catheter; (iii) 30 - 60 ml fluid injected; (iv) 75% human serum used; and (v) change to the laboratory as a transfer area not only made it more convenient but reduced the time involved dramatically (Table II).

### Results

In phase II of the programme 42 laparoscopies were performed, 146 follicles aspirated, 112 oocytes obtained and 42 fertilized (37% fertilization rate per oocyte) (Tables III and IV). Embryos were transferred to 25 patients and 4 became pregnant. One had an ectopic pregnancy, two had biochemical pregnancies, and 1 patient became pregnant with twins after the transfer of 4 embryos. The babies were delivered on 17 September 1984 by caesarean section after 35 weeks' pregnancy. The first baby was a healthy female weighing 1 900 g with an Apgar score of 6 after 1 minute and 9 after 5 minutes. The second baby was a healthy male weighing 1 790 g with an Apgar score of 7 after 1 minute and 9 after 5 minutes. The postoperative course of both mother and babies was uncomplicated.

In phase III of the programme 78 laparoscopies were performed, 4 unsuccessfully; 341 follicles were aspirated and 288 oocytes obtained (84.5% oocytes per follicle) (Tables V and VI). The fertilization rate was 77.7% per oocyte and 83% of patients reached the embryo transfer stage. There were 17 pregnancies of which 15 were clinical. The clinical pregnancy rate per embryo transfer was 23% and per laparoscopy 19.2% (Tables V and VI).

### Discussion

The fertilization rate was 37% per oocyte in phase II of the programme. Because the fertilization rate per oocyte in phase I was 62%, we realized that something was radically wrong. Routine evaluation of the medium with two-cell mouse embryos did not detect any abnormality. The possibility existed that the quality of the purivate and its deterioration were the reason for the problem. Although T6 medium contains lactate this substance cannot sustain the development of the mouse oocyte and fertilized ovum in vitro. Fresh purivate was not...
Measurement of uterine cavity before ET

Preliminary examination early in the cycle; Tomcat catheter used to determine whether uterus can be entered easily; valsellum used if necessary

None

Always dorsal lithotomy

Dorsal lithotomy if uterus retroverted; knee-chest if in anteversion

Norfolk Teflon catheter

75% human serum + 10% T6

Up to 60-90 μl

6-6.5 cm in uterus; air-bubble technique

2-4 cell stage

42-46 hours

1-4 embryos

Dorsal lithotomy if uterus retroverted; knee-chest if in anteversion

Tomcat catheter

30-60 μl

6-6.5 cm in uterus; air-bubble technique

2-4 cell stage

42-46 hours

1-4 embryos; occasionally 5 embryos

As in phase II

None

In theatre ± 30 m from laboratory

In laboratory 2 m from incubator

None

None

Dorsal lithotomy if uterus retroverted; knee-chest if in anteversion

Norfolk Teflon catheter

75% human serum + 10% T6

Up to 60-90 μl

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Available immediately and a trial was run with Ham F10 medium, which resulted in an immediate improvement and excellent fertilization rates. The fertilization rate in phase III in which Ham F10 medium was used was 77.7% and compared well with rates quoted in the literature; these range from 60% to 90%.8-10 Although different media compare well in terms of fertilization and pregnancy rates,11, one point in favour of the Ham F10 medium is its simplicity of preparation. Trounson et al.11 have stated that a simple culture medium with relatively few chemical components has the advantage of ensuring quality control, since there is less chance of introducing unsuitable
We realize that there are numerous variables involved in the human IVF work, but with a dedicated team and precision as the keyword, success can be achieved.

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REFERENCES