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

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Summary

Two different protocols for ovulation induction used in phase I of the *in vitro* fertilization (IVF) programme at Tygerberg Hospital are presented. Previous experience with gonadotrophins and clomiphene citrate was applied in the development of the protocols. By means of experience gained during ovulation induction it was possible to establish critical values for the parameters of follicle maturity, which are used to determine the optimal time for follicle aspiration. Ultrasonically measured follicle size, critical serum oestradiol levels for each mature follicle and cervical mucus scoring were the parameters used. Fifty-one ova were obtained during 29 of the 34 attempts at follicle aspiration. Only 5 of the ova were immature. At least 1 mature ovum was obtained at 80% of all laparoscopies. Twenty-three embryos were transferred to 15 patients, and 3 pregnancies occurred. As a result of this programme 2 babies were born — the first in South Africa by IVF and embryo transfer.

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Successful *in vitro* fertilization (IVF) depends on the collection of mature ova from ovarian follicles.[1] Therefore accurate timing regarding oocyte development, visible ovaries at laparoscopy and a satisfactory technique for the collection of oocytes are mandatory.

Spontaneous cycles

The first successful pregnancy following IVF and embryo transfer (ET) occurred after the aspiration of an ovum during a spontaneous, unstimulated cycle.[2] A possible advantage of using spontaneous cycles is that the hormone-balanced luteal phase enhances the chances of implantation of the embryo in the endometrium.[3] There are several disadvantages of follicle aspiration in spontaneous cycles:

1. The exact moment of the mid-cycle luteinizing hormone (LH) surge has to be known; this requires LH assays, which are usuallly performed on urine specimens,[4,5] although a rapid

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immunoassay has been developed for use on blood samples.[1] Urine specimens are collected at 2 - 4-hourly intervals and the hormone assay takes 2 hours, so that a heavy burden is placed on the laboratory staff. Laparoscopic aspiration of follicles takes place 26 - 28 hours after the beginning of the LH surge.[4]

2. Since the exact moment for follicle aspiration cannot be planned, this procedure may have to be performed at an inconvenient time.

3. The LH surge may be missed in approximately 14% of cases.[3]

4. Only 1 embryo can be transferred during a cycle. The pregnancy rate is considerably increased by the transfer of 2 -3 embryos.[6]

Stimulated cycles

Although the first attempts at using stimulated ovarian cycles were unsuccessful,[7] ovulation induction is practically routinely performed in all present IVF and ET programmes.[8] Two different regimens were in use at the time that the Tygerberg programme was planned; these formed the basis of the protocols used in phase I of the Tygerberg programme and will be discussed later.

Patients and methods

Thirty-four patients were selected for IVF during the period 3 June - 31 October 1983. All the patients had irreversible tubal damage. Some patients had additional causes of the infertility, including immunological factors and male partners in whom the results of semen analysis did not stringently comply with the prerequisites listed in the accompanying article on p. 751 of this issue.

Two different protocols for ovulation induction were evaluated simultaneously. In protocol I human menopausal gonadotrophin (HMG) was used, and in protocol II clomiphene citrate (CC) was used.

Protocol I

In the early stages of the IVF and ET programme at Tygerberg Hospital it was impossible to make use of serial LH assays as an aid to determining the optimal time for follicle aspiration because of technical problems. It was therefore decided to evaluate a protocol based on that of Jones *et al.*[6]

HMG administration and monitoring of response. Serum oestradiol (E_2) assay, cervical mucus evaluation, and ultrasound examination of the ovaries were performed according to methods described previously.[9] Treatment and monitoring commenced on the 4th day after a menstrual period had started (day 4). Pelvic ultrasound examination was performed before the first dosage of HMG was administered to identify any cystic pelvic structures which might cause confusion in the interpretation of follicle follow-up data. Blood for E_2 assay was collected at 08h00. Two ampoules of HMG were given

daily at 16h00 when the E_2 value for that morning was available. If the E_2 value more than doubled over a period of 24 hours the dosage of HMG was reduced.

Human chorionic gonadotrophin (HCG) administration. A dose of 10 000 U HCG was administered at 20h00 on the day that the E_2 value for each follicle > 16 mm reached the level of at least 1 200 pmol/l and the cervical mucus score was at least 15 out of 18.

Follicle aspiration. Laparoscopy for follicle aspiration was planned for 08h00, 36 hours after HCG was administered. This time was convenient for the whole team and ensured that a theatre and an anaesthetist were always available.

Protocol II

Disadvantages of using HMG for ovarian stimulation include the high cost of these injections and the long time that patients have to be available for active monitoring. A second protocol was therefore evaluated especially to reduce the time of stay near Tygerberg Hospital for those patients coming from far away.

Ovulation induction. A dose of 100 mg CC was administered daily from day 5 to day 9.

Monitoring. As from day 8 the same examinations as used in protocol I were performed.

HCG administration. A dose of 10 000 U of HCG was administered as soon as the E_2 value for each follicle > 16 mm reached 1 500 pmol/l *or* when the E_2 value increased above 3 500 pmol/l.

Follicle aspiration. Laparoscopy was scheduled to take place 36 hours after the HCG administration. Figs 1 and 2
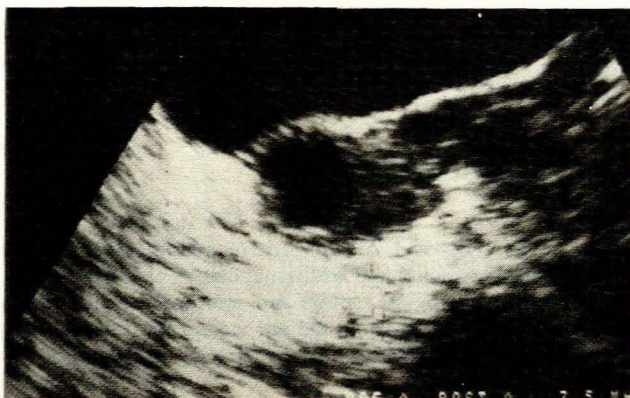

Fig. 1. The ultrasonic image of a mature ovarian follicle with a cumulus mass prior to follicle aspiration.
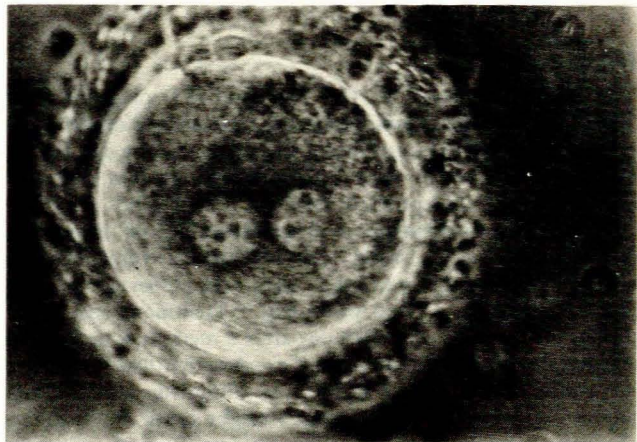

Fig. 2. A fertilized mature oocyte with two pronuclei.

show the ultrasonic image of a follicle before follicle aspiration and a fertilized ovum.

Results

Table I summarizes the results of protocol I and Table II the results of protocol II. Criteria for evaluation of maturity of ova are discussed in an accompanying article (p. 754 of this issue).

TABLE I. RESULTS OF PROTOCOL I	
No. of patients	18
No. of follicles > 16 mm	31
Unsuccessful attempts at aspiration	3
Ova obtained	
Mature	23
Immature	4
No. of embryos transferred	10
No. of patients who underwent ET	7
No. of pregnancies	2

TABLE II. RESULTS OF PROTOCOL II	
No. of patients	16
No. of follicles > 16 mm	42
Ovulation detected before planned laparoscopy	2
Unsuccessful attempts at aspiration	2
Ova obtained	
Mature	23
Immature	1
No. of embryos transferred	13
No. of patients who underwent ET	8
No. of pregnancies	1

Fifty-one ova were obtained by 29 of the 32 laparoscopic follicle aspirations performed in protocols I and II. From 80% of all patients at least 1 mature ovum was obtained. Although 25 ova were fertilized, only 23 embryos were transferred in 15 patients because 2 embryos appeared to be abnormal. Three pregnancies were confirmed by rising ß-HCG levels, but only 2 were confirmed ultrasonically. Both pregnancies went to term, and these were the first babies to be born after IVF and ET in South Africa.

Discussion

Motivation for protocol I

As previously mentioned, it was impossible to perform serial LH assays to help determine the optimal time for follicle aspiration. It was therefore decided to evaluate a protocol based on that of Jones et al.[6] The Tygerberg protocol differs from that of Jones et al. in the following ways:

1. The quantity of follicles is not taken into account in the interpretation of E_2 values in the Jones et al. protocol. A critical E_2 value for each follicle > 16 mm is the primary indicator for the timing of HCG administration in the Tygerberg protocol.

2. Cervical mucus scoring and karyopyknotic indexing do not play a major role in the evaluation of follicle maturity in the Tygerberg protocol, but a mucus score of at least 16 out of 18 is a prerequisite for HCG administration.

3. HCG is administered 18 hours after the last HMG injection and not after 50 hours, as in the Jones et al. protocol.

The changes in the Jones protocol were due to the highly successful regimen of ovulation induction with gonadotrophins practised by J.A.M.H.v.S. at Tygerberg Hospital.[11] The average E_2 value for each follicle > 16 mm and the mucus score at the time of HCG administration were determined in 15 cycles in which pregnancy occurred. The following values were considered ideal to copy in planning the timing of HCG administration: (i) average E_2 value for each follicle > 16 mm on the day of HCG administration — 1 307 pmol/l (standard deviation 434 pmol/l); and (ii) cervical mucus scores in all cases > 16 out of 18.

Motivation for protocol II

The motivation for the critical level of 1 500 pmol/l for each follicle > 16 mm is found in Fig. 3.[10] These values were obtained from anovulatory patients who received CC for ovulation induction. The average E_2 level for each follicle > 16 mm was found to be 1 550 pmol/l 2 days before ovulation. Trounson et al.[12] use approximately the same critical value. A previous study at Tygerberg Hospital,[10] as well as other studies,[13,14] have pointed out that CC adversely affects cervical mucus. This parameter was therefore not taken into account in scheduling HCG administration.
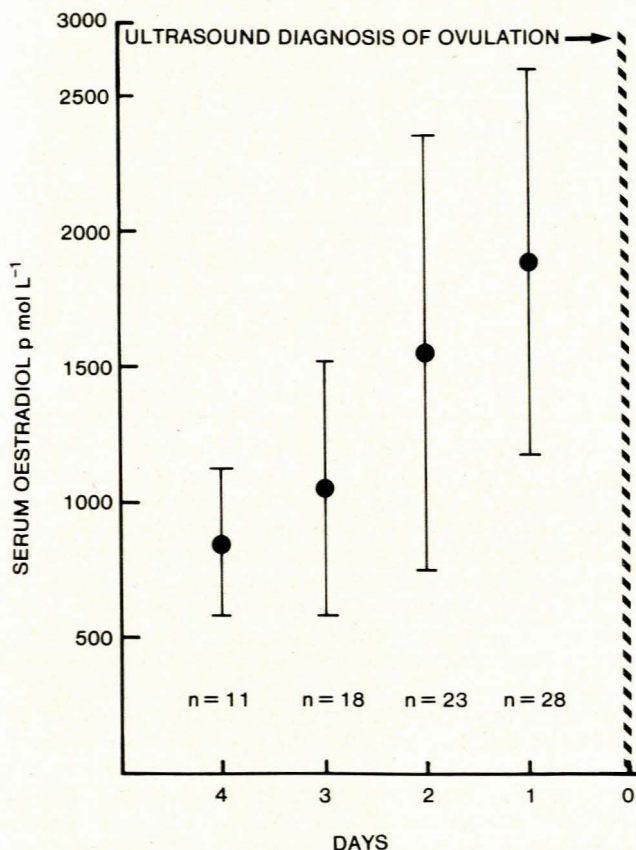

Fig. 3. Serum E_2 levels per follicle > 16 mm in clomiphene-stimulated cycles on the 4 days before the diagnosis of ovulation by ultrasound.[10]

Forty per cent of patients in protocol I underwent ET in comparison with 50% of those in protocol II. This difference may be coincidental since the number of patients is not adequate for statistical analyses.

The concept of a 'critical E_2 level per mature follicle' as the most important parameter of follicle maturity is probably the key to success in this study. The 'critical E_2 levels' were obtained in patients from the infertility unit at Tygerberg Hospital who underwent ovulation induction for anovulation. The same technique for monitoring follicles with ultrasound was used as well as the same cervical scoring system and E_2 radio-immunoassay. Therefore these values could be applied directly in ovulation induction for IVF.

A more aggressive approach to ovarian stimulation will undoubtedly produce more ova. This may increase the chances of a successful pregnancy. However, as the number of follicles produced increases it will become increasingly difficult to judge follicle maturity and the optimal time for ovum aspiration.

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