The hormonal and morphological events of stimulated cycles in an in vitro fertilisation programme

J. V. VAN DER MERWE, A. GRACE, E. SNYMAN

Summary

A better understanding of the physiological processes involved in the stimulated cycle for successful harvesting of mature eggs in an in vitro fertilisation programme is a prerequisite for obtaining good pregnancy rates. The hormonal levels of oestradiol, luteinising hormone (LH) and follicle-stimulating hormone correlated with ultrasonographic studies of follicular growth, were followed during an in vitro fertilisation cycle giving an overall pregnancy rate of 32% per transfer procedure.

The combined use of clomiphene citrate (Clomid; Mer-National) and human post-menopausal gonadotrophin (Pergonal; Script Intal) produces an average of 5.96 follicles per patient. Human chorionic gonadotrophin (HCG) was given prior to the oestrogen peak, and no spontaneous LH peak occurred before the day of the HCG injection. Timing of this injection needs to be synchronised with definite hormonal and ultrasonography values.

After the Bourne Hall meeting (1981)1 scientists active in the field of in vitro fertilisation (IVF) and embryo transfer (ET) came to the conclusion that laboratory techniques and other technical aspects of this new procedure had been standardised, and further improvement in the success rate of this new modality would come from a better understanding of the physiological processes involved in the menstrual cycle, and manipulation thereof, and study of the biology of ET and implantation as well as successful early embryonic development.

It was the experience of Steptoe and Edwards2 and of the group in Melbourne, Australia3 that only spontaneous cycles were successful. Ovulation induction regimens, however, had been shown to be just as successful in achieving pregnancies.3 One definite advantage of the stimulated cycle is that the number of oocytes and, therefore, embryos for transfer is increased. Recent reports from successful IVF programmes have shown a progressive increase in the chances of establishing a pregnancy with increasing numbers of embryos transferred to the patients.3 However, the stimulated cycle creates an abnormal milieu and might not be the best cycle in which to establish an ongoing pregnancy. This is further suggested by the relatively high pregnancy rate on transfer of single embryos after freezing.6 Whether this abnormal hormonal environment affects the processes involved in oocyte maturation, or influences the luteal phase and therefore, irrespective of the quality of the embryo transferred, results in a suboptimal environment leading to a poor outcome, is unknown. Luteal phase insufficiency has been shown to occur in stimulated cycles for IVF.7 Because both natural and stimulated cycles have been monitored, knowledge of the hormonal and morphological events involved in follicular growth and specific time of ovulation has increased.8 11 This article deals with the hormonal changes and follicular growth as measured by ultrasonographic tests during a cycle with a high pregnancy rate, making use of a combined clomiphene citrate (CC) (Clomid; Mer-National) and human postmenopausal gonadotrophin (HMG) (Pergonal; Script Intal) ovulation induction regimen. These data could serve a useful purpose in evaluating the response of patients during IVF attempts.

Patients and methods

During the period 5 May - 10 July 1984, 44 patients were accepted into the IVF programme of the department of Obstetrics and Gynaecology, University of Pretoria and H. F. Verwoerd Hospital. The treatment of 13 patients was cancelled, due to either poor response to the ovulation regimen (12 patients) or premature ovulation as seen on ultrasonography (1 patient). The average age of the remaining 31 patients was 32 years. By far the majority of patients (24) had a tubal factor to account for infertility. In the remaining patients infertility was due to endometriosis (1), a male factor (2), or an unknown factor (4). All patients were thoroughly screened and underwent a complete fertility work-up and psychological evaluation by a social worker before being accepted into the programme.11 A qualified nurse went through the technical aspects of the procedure before informed consent was obtained from the couple.

Ovulation was induced by 50 mg CC and 1 ampoule HMG per day from day 5 through to day 9. Depending on individual responses a further 1 - 2 ampoules of HMG were given from day 10 onwards. This varied between 1 and 2 ampoules per day for a maximum of 3 days. The average number of ampoules per patient for the cycle was 6.26 (range 5 - 9).

Monitoring of the response as described previously11 was done from day 10 onwards. Human chorionic gonadotrophin (HCG) (Profasi; Script Intal) was given at 20h00 on the day that the average diameter of the dominant follicle was at least 20 mm in the presence of a good serum oestradiol value (preferably above 3000 pmol/l and in the vicinity of 1000 pmol per large follicle, i.e. average diameter > 15 mm). Laparoscopic oocyte aspiration was performed under general anaesthesia 36 hours after the HCG injection. Transfer of embryos was done 52 - 56 hours after aspiration. This procedure as well as management of the oocytes and embryos has previously been described.11

Blood for hormone determinations (oestradiol, luteinising hormone (LH) and follicle-stimulating hormone (FSH)) was taken daily. Serum oestradiol determinations were performed daily using the 125I-oestradiol direct radio-immunoas say kit (Radioisotope Service, Switzerland); LH and FSH determinations were done in

Department of Obstetrics and Gynaecology and Reproductive Biology Research Unit, University of Pretoria and H. F. Verwoerd Hospital, Pretoria

J. V. VAN DER MERWE, M.MED. (O. & G.), F.C.O.G. (S.A.), M.D.
A. GRACE, M.B. CH.B.
E. SNYMAN, B.Sc. HONS
batches using the Amerlax LH RIA kit (Amersham UK) and the double-antibody FSH method (Diagnostic Products Corporation, USA). The average number of follicles as well as the average diameter of each follicle (calculated by obtaining three diameters in two planes) were determined daily with a sectorscan (ATL 850 A Squibb Medical Systems).

Results

Results for all 31 patients involved in the programme are presented in Table I. The average number of follicles, the average diameter of all follicles as well as the dominant follicle are illustrated in Figs 1, 2 and 3. Results are given from day -3 to day +2 with day 0 arbitrarily defined as the day of the HCG injection. The average diameter of all follicles showed a progressive increase from day -3 to day +2, with an average growth tempo of 1.3 mm per day for all follicles (Fig. 1).

<table>
<thead>
<tr>
<th>TABLE I. RESULTS FOR 31 PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Oocytes harvested</td>
</tr>
<tr>
<td>Fertilisable oocytes</td>
</tr>
<tr>
<td>Oocytes divided</td>
</tr>
<tr>
<td>Embryos transferred</td>
</tr>
<tr>
<td>Pregnancies</td>
</tr>
<tr>
<td>Average oocytes/patient</td>
</tr>
<tr>
<td>Average embryos transferred/patient</td>
</tr>
</tbody>
</table>

As illustrated in Fig. 2, the average diameter of all follicles on the day of HCG injection was 15.5 (± 1) and on the day of aspiration 17.6 (± 0.4). However, the dominant follicle had an average growth tempo of 2.3 mm per day up to the day of HCG injection, and 1.9 mm per day thereafter (Fig. 3). Thus from Figs 2 and 3 it can be seen that the average growth tempo of dominant follicles was nearly double that of the average growth of all follicles over the same period.

Serum oestradiol levels are represented graphically in Fig. 4. The oestradiol surge was seen on day +1 with a peak of 4892 ± 314 pmol/l and declined to 2367 ± 164,5 pmol/l on the day of aspiration. The average increase in blood hormone levels per day from day -3 to day +1 was 611 pmol/l. If the serum oestradiol per patient on day 0 was divided by the number of large follicles (≥ 15 mm in diameter) for each patient on that day, the average serum oestradiol per large follicle on the day of the HCG injection was 1806 ± 150 pmol/l.

The serum gonadotrophin levels are illustrated in Figs 5 and 6. No patient on the combined LH/HCG regimen had an endogenous LH surge before the HCG injection. FSH levels declined from day -3 to day +2 from an average of 18.2 ± 2.9 IU/l on day -3 to 14.3 ± 1.3 IU/l on day +2.

Laparoscopy for aspiration of oocytes was performed on average on day 14.2 ± 1.2. Of the 104 oocytes harvested only one was dismamre and therefore not inseminated. Although an average of 5.9 follicles per patient were present on ultrasonography, on the day of aspiration an average of 3.4 oocytes was found per patient (57.6%). Of the 103 oocytes that were inseminated, 62 (60%) divided and were transferred in 25 patients (an average of 2.5 embryos per patient) (Table I).

An elevated β-HCG level was found in 8 patients (biochemical pregnancy rate per transfer of 32%). Two of these pregnancies were only biochemical pregnancies, 1 patient had triplets which aborted at 8 weeks and 2 other patients aborted single pregnancies in the 10th week of pregnancy. The remaining 3 pregnancies were uneventful and normal babies were delivered at term. Most of the embryos in these 8 patients were at the four-cell stage at the time of transfer.
Fig. 3. Average diameter of the dominant follicle from day -3 to day +2 (mean ± SD).

Fig. 5. Serum FSH levels from day -3 to day +2 (mean ± SE).

Fig. 4. Serum oestradiol levels from day -3 to day +2 (mean ± SE).

Fig. 6. Serum LH levels from day -3 to day +2 (mean ± SE).
Discussion

It has been shown that more follicles develop with the combined use of CC and HMG than with CC or HMG separately. When the combination was used for ovulation induction purposes an average of 4.5 - 6.4 follicles per patient on day 0 were obtained compared with 2.3 - 3.7 follicles with CC alone and 4.3 - 4.7 follicles with HMG alone. In this study an average of 5.5 (± 0.3) follicles per patient were present on day 0 (Fig. 1).

One of the most critical factors determining the success rate, is the timely retrieval of an oocyte. For successful IVF a mature oocyte should be obtained minutes before spontaneous ovulation. Although immature oocytes can successfully be matured in vitro, fertilisation and cleavage rates seem to be better after in vitro maturation. The only parameters of possible oocyte maturation at this stage are ultrasonography of follicular size and serum oestradiol determinations as a function of follicular activity. These are used separately or in combination to determine the timing of HCG treatment so that oocyte maturation is obtained. Determining the onset of the LH surge gives additional information about follicle maturation and warns of ensuing ovulation, but it is seldom seen.

There is no unanimous policy as to the timing of the HCG injection. Some authors recommend that HCG should be given when the dominant follicle has an average diameter of 15 - 20 mm, while others recommend that the dominant follicle should be greater than 18 mm in diameter in the presence of a good oestradiol response. In a series of more pre-ovulatory oocytes were obtained and the fertilisation rates were higher (57% v. 28%) when HCG was given with a dominant follicle greater than 20 mm rather than 18 mm. Quigley et al. reported a cleavage rate of 72% v. 14% with the average diameter of the dominant follicle greater than 20 mm compared with smaller than 20 mm. In these authors' experience a cleavage rate of 46% was obtained when the average diameter of the dominant follicle on day 0 was 17 mm compared with a 62% cleavage rate in the present study. However, the induction regimen in the previous study was that of CC alone.

The size of the largest follicle can therefore be used to decide when HCG should be given, but the largest follicle is not necessarily the most oestrogenic. Furthermore, follicular size is not a clear indication that the oocyte can be fertilised, and only one of many oocytes may be destined to produce a fetus.

An alternative approach to the question of the timing of the HCG injection is to calculate the blood oestradiol levels as a function of all large follicles, which most authors define as having an average diameter of greater than 15 mm, although 16 mm is also accepted.

Lopata suggested that on the day of HCG injection a large follicle should correlate with a serum oestradiol production rate of 450 pg/ml. Thus it was suggested that when the largest follicle has reached a diameter of 18 mm or more, with an oestradiol increment in plasma of 1.5 nM for each large follicle, HCG should be given. Another suggestion was that the HCG should be administered if the plasma oestradiol level is over 500 pg/ml for each follicle greater than 14 mm in diameter. In the present study the serum oestradiol level was 1806 ± 150 pmol/1 for follicles with an average of 15 mm on day 0.

It should also be noted that a higher fertilisation rate is seen when the HCG is given prior to the plasma oestradiol peak rather than at or after the peak. In this study the oestradiol peak was seen on day +1 (Fig. 3).

Serum oestradiol, follicular growth (i.e. growth in diameter) and the number of follicles are correlated. This correlation seems to be important and deviations to either side influence the pregnancy rate negatively. Thus, if the serum oestradiol levels are too high, the luteal phase is shortened and a lower pregnancy rate results. Alternatively, a higher fertilisation rate (65.7%) was seen in a group with serum oestradiol levels greater than 500 pg/ml per follicle than in a group with levels below 500 pg/ml per follicle (59.8%). In the present study 2 of the pregnant patients with oestradiol levels of 6100 pmol/1 and 7400 pmol/1 on day 0 aborted after 6 weeks of pregnancy.

FSH levels gradually dropped from day -3 to day 0, in accordance with the physiology of the normal menstrual cycle. The primary aim in the stimulated cycle is to increase both the level and the duration of FSH concentration in peripheral blood above threshold levels during the early follicular phase with the aim of facilitating further development of 3 or more antral follicles which have attained a stage of development for responding maximally to FSH. These selected follicles are not synchronous in their development even at this stage and eventually will be comparatively asynchronous both in terms of their ultimate size and their steroid synthetic capacity.

This may lead to disordered follicular and luteal endocrinology. Fertilisation, cleavage and implantation might therefore be jeopardised. Although it is difficult to evaluate the growth tempo and quality of developing early embryos it can be assumed that if embryos in the same patient are at different cleavage stages at 48 hours after insemination, this is probably due to asynchronisation. It is of interest to note in this regard that all pregnancies achieved in this series occurred after transfer of embryos in synchrony. Although there is no unanimous agreement as to the precise time between the temporal relationship of the oestradiol and LH peaks, the first-mentioned peak could occur 72 - 24 hours before the LH peak. In the present study it appears as if these two peaks occurred on the same day. The reason for this is that there was cross-reactivity between the HCG and LH in the radioimmunoassay. None of the patients in this series had an endogenous LH peak before the HCG injection.

In conclusion, it seems that the combination of CC and HMG as an ovulation induction regimen in IVF ensures multiple folliculogenesis with higher fertilisation and pregnancy rates. The HCG injection should be given when the average diameter of the dominant follicle is ≥ 20 mm with a serum oestradiol level of 1800 pmol/1 per large follicle. In this study, the HCG was given before the oestradiol peak and no endogenous LH surge was seen before day 0.

REFERENCES

Thiamine deficiency in black male hostel-dwellers
The need for thiamine supplementation of sorghum beer

J. VAN DER WESTHUYZEN, R. E. DAVIS, G. C. ICKE, J. METZ

Summary
Some indices of nutrition have been examined in hostel- and non-hostel-dwelling groups of industrially employed black males. Hostel-dwellers in the large metropolitan areas have to prepare their own food and many are accustomed to excessive alcohol intake, especially of sorghum beer. In the two groups studied, blood levels of vitamin B₁₂, folate, pyridoxal and albumin were similar, but erythrocyte thiamine levels were significantly lower in the hostel-dwellers. Although the proportion of subjects with elevated levels of γ-glutamyltransferase, an index of alcoholic liver disease, was similar in the two groups, thiamine-deficient hostel-dwellers had a greater proportion of elevated values suggesting that thiamine deficiency was related to both inadequate diet and excessive alcohol consumption. Fortification of sorghum beer with thiamine might prevent or reduce thiamine deficiency in this group. The cost would not materially affect the price of the beer.

In the RSA, a large number of black men have to live in hostels in urban areas away from their families for varying periods. When food is provided by a control facility in the hostel, nutritional intake may be satisfactory. However, when hostel-dwellers have to provide and prepare their own food, nutritional deficiencies are likely to occur, especially when nutritional needs are increased by regular physical labour. Among black migrant labourers living in hostels in the Pretoria-Witwatersrand-Vereeniging (PWV) area, adverse nutritional status is aggravated by habitual alcohol consumption, an increasing problem in the townships of the industrialised areas.¹ Much of this excessive alcohol intake is derived from the consumption of traditional (sorghum) beer. A pilot study was therefore undertaken of black labourers living under hostel conditions where they are required to provide their own food. In view of the possible combined adverse effect of poor diet and excessive alcohol consumption, the nutritional parameters examined included some which may

Department of Haematology, School of Pathology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg

J. VAN DER WESTHUYZEN, M.SC., PH.D.

Department of Haematology, Royal Perth Hospital, Perth, Western Australia

R. E. DAVIS, M.SC., PH.D., F.R.S.H.
G. C. ICKE, M.SC.

Reprint requests to: Dr J. van der Westhuizen, Dept of Haematology, South African Institute for Medical Research, PO Box 1036, Johannesburg, 2000 RSA.

¹ Among black migrant labourers living in hostels in the Pretoria-Witwatersrand-Vereeniging (PWV) area, adverse nutritional status is aggravated by habitual alcohol consumption, an increasing problem in the townships of the industrialised areas. Much of this excessive alcohol intake is derived from the consumption of traditional (sorghum) beer.