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A prospective randomized study of the optimum timing of human chorionic gonadotropin administration after pituitary desensitization in in vitro fertilization

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Objective: To determine if there is an optimum time for the administration of human chorionic gonadotropin (hCG) after pituitary desensitization with gonadotropin-releasing hormone agonists (GnRH-a) has been achieved before ovarian stimulation for in vitro fertilization (IVF).

Design: Prospective randomized study.

Patients: Two hundred forty-seven patients undergoing an IVF treatment cycle who were randomly divided into three groups.

Interventions: All patients were administered subcutaneously buserelin acetate 500 μg/d from day 1 of the menstrual cycle. After pituitary desensitization had been achieved at least 14 days later, ovarian stimulation with human menopausal gonadotropin was commenced. Ovarian stimulation, cycle monitoring, oocyte recovery, and IVF and embryo transfer (ET) techniques were identical in all three groups. Patients in group 1 (n = 79) had hCG administered when the mean diameter of the largest follicle had reached 18 mm, at least two other follicles were >14 mm, and serum estradiol (E_2) levels were consistent with the number of follicles observed on ultrasound. Patients in groups 2 (n = 84) and 3 (n = 84) had hCG administered 1 day and 2 days, respectively, after the above criteria had been reached.

Results: The mean day of hCG administration ($P < 0.01$), maximum serum E_2 concentration ($P = 0.06$), number of days of serum E_2 rise ($P = 0.03$), and mean diameter of the largest follicle ($P < 0.0001$) were significantly different. There were, however, no significant differences in the mean number of preovulatory and medium size follicles, number of oocytes recovered or embryos transferred. There were also no significant differences in the oocyte recovery, fertilization and cleavage rates, in the number of embryos frozen, or in the pregnancy rates per initiated cycle and per ET.

Conclusions: There is no significant advantage in the precise timing of hCG administration after pituitary desensitization with GnRH-a. Fertil Steril 1992;57:1259–64

Key words: Timing of human chorionic gonadrotropin, long-buserelin acetate, in vitro fertilization

All successful in vitro fertilization (IVF) programs currently use pharmacological agents to stimulate the ovaries to induce the development of multiple preovulatory follicles. During conventional ovarian stimulation with gonadotropins, either alone or in combination with clomiphene citrate (CC), accurate timing of human chorionic gonadotropin (hCG) administration is critical because of the fear of a premature spontaneous surge of luteinizing hormone (LH) and even of premature ovulation that may lead to cancellation of the treatment cycle. Despite close

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monitoring of follicular growth with repeated pelvic ultrasound examinations and hormonal assays, it has been reported that 15% to 30% of treatment cycles are canceled before the stage of oocyte recovery on this account (1).

Since the original description of the use of gonadotropin-releasing hormone agonists (GnRH-a) and gonadotropins for ovarian stimulation in IVF (2), the method has gained widespread popularity, and many IVF programs currently use the technique as the main method of ovarian stimulation. Because by using this combination spontaneous LH surges and spontaneous ovulation are prevented, there is, therefore, greater flexibility allowed for the timing of hCG administration. Many IVF centers have used this to extend the follicular phase of the treatment cycle to allow oocyte recoveries to be performed during weekdays only or even on certain days of the week. It remains to be established, however, whether there is an optimum time for hCG administration when GnRH-a are used to desensitize the pituitary before ovarian stimulation for IVF.

MATERIALS AND METHODS

Two hundred forty-seven patients who were referred to the Hallam Medical Centre for IVF were recruited for the study that had Institutional Review Board approval. They were prospectively randomized by drawing serially numbered sealed envelopes, which randomly allocated them to groups 1, 2, or 3. All patients were administered the GnRH-a, buserelin acetate (D-Ser(tBu)6,PRo9-NHEt) LH-releasing hormone (Suprefact; Hoechst, Hounslow, United Kingdom) starting on the 1st day of the menstrual period. The GnRH-a was administered by subcutaneous (SC) injection in a dose of 500 μg/d, and after 14 days of administration the serum estradiol (E_2) concentration was measured and a pelvic ultrasound (US) examination performed. If pituitary desensitization had been achieved, as shown by a serum E_2 concentration < 100 pmol/L and the absence of follicular activity on pelvic ultrasonography, the administration of human menopausal gonadotropin (hMG, Pergonal; Serono, Welwyn Garden City, United Kingdom) was commenced and SC buserelin acetate 200 μg/d continued until, and including, the day of hCG (Profasi; Serono) administration. If pituitary desensitization had not been achieved after 14 days of treatment, administration of buserelin acetate was continued until desensitization was achieved, and then treatment with hMG was commenced. The standard dose of hMG used was three ampules per day, but four and five ampules per day were given if the patient was over the age of 35 and 40 years, respectively. Monitoring of follicular growth was achieved with daily US scans and serum E_2 measurements. The dose of hMG was increased if there was poor follicular growth.

In group 1, hCG 10,000 IU was administered when the mean diameter of the largest follicle had reached 18 mm, there were at least two other follicles > 14 mm, and serum E_2 concentrations were consistent with the number of follicles observed on US. In groups 2 and 3, the same dose of hCG was administered 1 day and 2 days, respectively, after the above criteria had been reached.

Transvaginal US-directed oocyte recovery (3) was performed 35 hours after hCG administration. One to three pre-embryos were transferred approximately 48 hours after oocyte recovery.

The age of the patient, number of previous IVF attempts, number of years of infertility, day of hCG administration, maximum serum E_2 concentration, diameter of the largest follicle on the day of hCG administration, number of preovulatory and medium size follicles, number of oocytes collected, number of cases of ovarian hyperstimulation syndrome, number of embryos frozen and the oocyte recovery, fertilization, cleavage, implantation and pregnancy rates (PRs) per initiated cycle and per embryo transfer (ET) were recorded. The results of the three groups were compared using ANOVA and χ^2 tests.

RESULTS

There were 79 patients in group 1 and 84 each in groups 2 and 3. There were no significant differences in the indications for IVF in the three groups (Table 1). The mean age of the patients, number of previous IVF attempts and years of infertility were comparable in the three groups (Table 2). The mean day of hCG administration ($P < 0.01$), maximum serum E_2 concentration ($P = 0.06$), number of days of serum E_2 rise ($P = 0.03$), and the mean diameter of the largest follicle ($P < 0.0001$) were significantly different in the three groups (Table 2). The mean

Table 1 Indications for IVF in the Three Groups of Patients*

	Group 1	Group 2	Group 3
Tubal damage	32	39	34
Unexplained infertility	17	23	20
Male factor infertility	20	15	26
Endometriosis	5	4	3
Failed donor insemination	5	3	1

* No significant differences in all categories.

Table 2 Ovarian Response to Standard and Prolonged Stimulation by 1 or 2 Days

	Group 1* (n = 79)	Group 2† (n = 84)	Group 3‡ (n = 84)	Probability
Age (y)	32.44 ± 4.26 (23 to 44)‖	33.61 ± 3.96 (21 to 43)	33.07 ± 3.97 (23 to 41)	NS§
No. of previous IVF attempts	0.92 ± 1.30	0.80 ± 1.03	0.68 ± 1.08	NS
Length of infertility (y)	4.76 ± 3.11	5.81 ± 3.08	4.80 ± 2.91	NS
Abandoned for poor response	8	3	6	NS
Day of hCG administration	10.45 ± 1.27	10.86 ± 1.23	11.12 ± 1.45	$P < 0.01$
Maximum E_2 concentration (pmol/L)	6,943 ± 2,871	6,895 ± 3,159	8,001 ± 3,790	$P = 0.06$
No. of days of E_2 rise	6.26 ± 1.13	6.87 ± 1.05	7.30 ± 1.29	$P = 0.03$
Diameter of largest follicle (mm)	19.47 ± 1.51	20.47 ± 1.13	22.47 ± 1.42	$P < 0.0001$
No. follicles > 14 mm	9.33 ± 4.69	8.72 ± 3.51	10.21 ± 5.05	NS
No. follicles 12 to 13 mm	5.48 ± 3.38	5.82 ± 5.73	5.32 ± 4.34	NS
No. follicles aspirated	15.34 ± 6.75	14.38 ± 7.75	15.49 ± 8.0	NS
No. of oocytes collected	12.45 ± 6.17	11.38 ± 7.04	12.01 ± 6.88	NS

* Standard stimulation.
† Prolonged stimulation by 1 day.
‡ Prolonged stimulation by 2 days.
§ NS, not significant.
‖ Values are means ± SD with ranges in parentheses.

number of follicles aspirated and oocytes collected in the three groups were 15.34 ± 6.75, 14.38 ± 7.75, and 15.49 ± 8.0 and 12.45 ± 6.17, 11.38 ± 7.04, and 12.01 ± 6.88, respectively. These differences were not statistically significant. The mean oocyte recovery (79.79% ± 16.5%, 77.41% ± 20.1%, and 77.55% ± 19.1%, respectively), fertilization (69.07% ± 28.7%, 78.90% ± 28.4%, and 72.14% ± 27.1%, respectively), and cleavage (76.77% ± 25.3%, 83.14% ± 23.7%, and 80.29% ± 26.9%, respectively) rates were comparable in the three groups (Table 3). There were three, five, and three cases of failed fertilization in the three groups, respectively. The mean number of embryos transferred in the three groups were virtually identical (2.58 ± 0.99, 2.56 ± 1.05, and 2.55 ± 1.02 in groups 1, 2, and 3, respectively). The PRs per initiated cycle and per ET were 26.82% and 32.35% in group 1, 25.31% and 28.98% in group 2, and 33.76% and 38.80% in group 3, respectively. These differences were not statistically significant. There was also no significant difference in the first trimester abortion rates, which were 19.05%, 23.8%, and 14.81% in groups 1, 2, and 3, respectively.

DISCUSSION

Maturation of oocytes begins during the latter part of fetal life, but the process is arrested at the diplotene stage of the first meiotic division, and it remains so into adult life until the preovulatory surge of LH occurs at midcycle of each menstrual cycle. The rise in LH levels leads to a reduction in the

Table 3 Outcome of Cycles After Standard and Prolonged Ovarian Stimulation by 1 or 2 Days

	Group 1*	Group 2†	Group 3‡
No. of viable oocytes	10.95 ± 5.56§	10.00 ± 6.14	10.63 ± 6.17
Oocyte recovery rate (%)	79.79 ± 16.5	77.41 ± 20.1	77.55 ± 19.1
Fertilized oocytes	7.62 ± 5.15	7.93 ± 5.61	7.63 ± 5.14
Fertilization rate (%)	69.07 ± 28.7	78.90 ± 28.4	72.14 ± 27.1
No. of cases failed fertilization	3	5	3
Cleaved embryos	5.86 ± 4.51	6.72 ± 5.19	6.28 ± 4.64
Cleavage rate (%)	76.77 ± 25.3	83.14 ± 23.7	80.29 ± 26.9
No. of cases of ovarian hyperstimulation syndrome	2 mild	3 mild 1 severe	6 mild
No. of ET	2.58 ± 0.99	2.56 ± 1.05	2.55 ± 1.02
Total embryos frozen	84 (15)‖	119 (20)	84 (19)
PR/initiated cycle (%)	26.82	25.31	33.76
PR/ET (%)	32.35	28.98	38.80

* Standard stimulation.
† Prolonged stimulation by 1 day.
‡ Prolonged stimuation by 2 days.
§ Values are means ± SD. Results are not significant in all parameters.
‖ Number of patients are in parentheses.

cyclic adenosine monophosphate content of the oocyte through disruption of its microanatomical links with cumulus granulosa cells, which, in turn, leads to resumption and completion of the first meiotic division of the oocyte (4). Approximately 36 hours later, ovulation occurs with release of an oocyte of appropriate maturity for fertilization. Timing of hCG administration, which is designed to mimic the LH surge, is therefore critical when the ovaries are stimulated in the presence of an intact hypothalamic-pituitary ovarian axis. Evidence obtained in the Rhesus monkey has shown that premature administration of hCG during follicular maturation leads to atresia of the dominant follicle and arrest of cyclic ovarian function (5). Similarly, when hCG is administered to women before maturation of the preovulatory follicle, ovulation is suppressed, and resumption of ovulatory menstrual cycles is delayed (6). On the other hand, if the administration of hCG is delayed, there may have been a preceding spontaneous LH surge, and if the interval between these two events is prolonged beyond 12 hours, subsequent embryo cleavage is impaired (7). Because of the necessity for this critical timing of hCG administration, close monitoring of follicular growth is, therefore, mandatory during conventional ovarian stimulation with CC and/or hMG. Unfortunately, despite this close monitoring, approximately 15% to 30% of treatment cycles have to be canceled because of an unscheduled premature discharge of LH (1).

Since the original description of the use of GnRH-a to block endogenous gonadotropin secretion followed by gonadotropic stimulation of the ovaries (2), numerous studies of the role of GnRH-a in assisted reproduction have been published. In general, it is agreed that the major advantage of this technique is that endogenous LH surges are almost completely eliminated (8). As a result of the greater flexibility afforded for the timing of hCG administration, a number of assisted conception programs have used this approach to simplify cycle programming so as to avoid weekend oocyte retrievals (9, 10) or even to schedule oocyte retrievals on as few as 3 working days per week (11). The safety of this approach has been reported by various groups of workers (12, 13). Nevertheless, it remains to be established whether there is an ideal time for hCG administration when the combination of GnRH-a and gonadotropins are used for ovarian stimulation for assisted conception.

In the present study, all patients underwent the traditional long protocol of GnRH-a administration in which ovarian stimulation with hMG was commenced only after pituitary desensitization had been achieved. The patients in the three groups were comparable in terms of age, number of previous IVF attempts, length, and etiology of infertility. As expected, there was a significant difference in the mean day on which hCG was administered, the maximum serum E_2 concentration, the number of days of serum E_2 rise, and the mean diameter of the largest follicle. There was, however, no significant difference in the number of preovulatory or medium size follicles or the number of oocytes collected.

Interestingly, extending the duration of ovarian stimulation by 2 days did not affect the rates of oocyte recovery, fertilization, or embryo cleavage. The numbers of cases of failed fertilization and ovarian hyperstimulation syndrome were not significantly different in the three groups. The total number of embryos available for cryopreservation and the number of patients in whom this was achieved were also comparable. The mean number of embryos transferred in the three groups were virtually identical, and there were no significant differences in the PRs per initiated cycle and per ET. Indeed, it was calculated that if the same proportion of patients in the three groups achieved pregnancy in an identical but much larger study of 10,000 patients, there would still have been no significant difference in the PRs.

These results are at variance with those reported by Clark et al. (14) who found that prolongation of follicular stimulation by 1 day significantly reduced the PR, despite an increase in the number of large follicles at the time of hCG administration. They found that the reduced PR was not related to the number of oocytes recovered or embryo quality, and they suggested that it could be caused by postmaturity of the oocyte. However, their study differs from ours in one critical aspect, namely, in their use of the short protocol of GnRH-a administration in which treatment with hMG was started, together with GnRH-a, at the beginning of the menstrual cycle before pituitary desensitization had been achieved. In a recent prospective randomized study (15), we found that the long protocol of GnRH-a administration was superior to the short protocol in terms of significantly greater follicular recruitment, oocyte recovery and fertilization rates, and in the significantly greater number of embryos available for transfer. It has been shown that the degree of LH suppression is more variable when the short protocol is used (16, 17), and it has been suggested that 5% to 10% of such cycles may still be complicated by a premature LH surge (18). These fluctuations of serum LH concentrations may well explain the deleterious effects of delayed hCG administration in the study of Clark et al. (14) because a pro-

portion of the patients may have experienced a premature rise of LH levels that triggered completion of the first meiotic division. Delay in hCG administration may have prolonged the interval between completion of oocyte maturation and ovulation so that the oocytes released would have been functionally aged and less able to generate pregnancies (4).

Besides the trial of Clark and co-workers (14), there has been only one other study investigating the effects of delayed hCG administration (13). As in our study, the long protocol of GnRH-a administration was used, and no significant difference between those who had hCG administered at the standard time (47 patients) and those who had it delayed by 24 hours (96 patients) was found in the mean number of oocytes retrieved, the fertilization rates, the proportion of patients who achieved ET or the implantation rate in those who had two or three embryos transferred (13). The authors did, however, find that the PR per oocyte retrieval was significantly greater when hCG was administered 1 day later. The reason for this difference between their study and ours is not exactly clear.

What are the clinical implications of our study? Given the difficulty that patients undergoing IVF have to face to achieve pregnancy and the frequent necessity of repeated IVF attempts before pregnancy and live birth are achieved (19), there is an understandable reluctance for IVF programs to modify their management unless there is clear evidence that it is in the patients' interests to do so. It has, therefore, remained an article of faith that close monitoring of ovarian stimulation is required even when GnRH-a are used to desensitize the pituitary before ovarian stimulation for IVF. The major implication of our study is the notion that when the long protocol of GnRH-a is used, much less monitoring of ovarian stimulation is necessary. There is a window of at least 3 days during which hCG can be given with good results, and there is no significant advantage in timing hCG administration any more accurately. Because repeated US scans and hormonal assays are expensive, inconvenient, and time consuming, and add not only to the cost of running the IVF program but also to the stress experienced by patients undergoing assisted conception, the ability to reduce monitoring to an absolute minimum would be a most welcome development. Indeed, over the past year, some IVF programs have completely abandoned the use of hormonal assays when GnRH-a are used in the long protocol. For example, in one IVF program in which hormonal assays have been abandoned and patients have on average three US scans only during each treatment cycle, PRs/ET of approximately 30% have been maintained (Parsons J., personal communication).

The major clinical development in IVF practice over the past few years has been simplication of treatment procedures. From laparoscopic oocyte recovery under general anaesthesia, we have gradually moved to outpatient US-guided oocyte retrievals under sedation. Similarly, a number of recent prospective randomized studies comparing aspiration only with aspiration and flushing for transvaginal US-directed oocyte recovery have shown that aspiration alone produces comparable oocyte recovery rates as aspiration and flushing while decreasing the operating time significantly (20, 21). In the same spirit, the present study suggests that precise timing of hCG administration is unnecessary when pituitary desensitization is achieved before ovarian stimulation in IVF and supports the notion that if we cannot improve results for patients undergoing IVF, we should at least endeavor to make it as easy as possible for them.

REFERENCES

1. Smitz J, Devroey P, Braeckmans P, Camus M, Khan I, Staessen C, et al. Management of failed cycles in an IVF/GIFT programme with a combination of a GnRH analogue and hMG. Hum Reprod 1987;2:309–14.
2. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in vitro fertilization using buserelin and gonadotropins. Lancet 1984;2:1284–5.
3. Tan SL, Bennett S, Parsons J. Surgical techniques of oocyte collection and embryo transfer. Br Med Bull 1990;46:628–42.
4. Jacobs HS, Homburg R. The endocrinology of conception. In: Franks S, editor. Bailliere's clinical endocrinology and metabolism. London: Bailliere Tindall, 1990;4:195–205.
5. Williams RF, Hodgen GD. Disparate effects of human chorionic gonadotropin during the late follicular phase in monkeys: normal ovulation, follicular atresia, ovarian acyclicity, and hypersecretion of follicle-stimulating hormone. Fertil Steril 1980;33:64–8.
6. Tamada T, Matsumoto S. Suppression of ovulation with human chorionic gonadotropin. Fertil Steril 1969;20:840–8.
7. Punnonen R, Ashorn R, Vilja R, Heinonen PK, Kujansuu E, Tuohimaa P. Spontaneous luteinizing hormone surge and cleavage of in vitro fertilized embryos. Fertil Steril 1988;49:479–82.
8. Loumaye E. The control of endogenous secretion of LH by gonadotropin-releasing hormone agonists during ovarian hyperstimulation for in-vitro fertilization and embryo transfer. Hum Reprod 1990;5:357–76.
9. Zorn JR, Boyer P, Guichard A. Never on a Sunday: programming for IVF-ET and GIFT. Lancet 1987;1:385.
10. Vauthier D, Lefebvre G. The use of gonadotropin-releasing hormone analogs for in vitro fertilization: comparison between the standard form and long-acting formulation of D-Trp-6-luteinizing hormone-releasing hormone. Fertil Steril 1989;51:100–4.

11. Dimitry ES, Bates SA, Oskarsson T, Margara R, Winston RML. Programming in vitro fertilization for a 5- or 3-day week. Fertil Steril 1991;55:934–8.
12. Abdalla HI, Baber R, Leonard T, Kirkland A, Mitchell A, Power M, et al. Timed oocyte collection in an assisted conception programme using GnRH analogue. Hum Reprod 1989;4:927–30.
13. Dimitry ES, Oskarsson T, Conaghan J, Margara R, Winston RML. Beneficial effects of a 24 h delay in human chorionic gonadotrophin administration during in-vitro fertilization treatment cycles. Hum Reprod 1991;6:944–6.
14. Clark L, Stanger J, Brinsmead M. Prolonged follicle stimulation decreases pregnancy rates after in vitro fertilization. Fertil Steril 1991;55:1192–4.
15. Tan SL, Kingsland C, Campbell S, Mills C, Bradfield J, Alexander N, et al. The long protocol of administration of gonadotropin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for in vitro fertilization. Fertil Steril. 1992;57:810–4.
16. Loumaye E, Vankrieken L, Depreester S, Psalti I, de Cooman S, Thomas K. Hormonal changes induced by short-term administration of a gonadotropin-releasing hormone agonist during ovarian hyperstimulation for in vitro fertilization and their consequences for embryo development. Fertil Steril 1989;51:105–11.
17. Fleming R, Jamieson ME, Coutts JRT. The use of GnRH-analogs in assisted reproduction. In: Matson PL, Lieberman BA, editors. Clinical IVF forum. Current views in assisted reproduction. Manchester:Manchester University Press, 1990:1–19.
18. Ho PC, Chan YF, So WK, Yeung WSB, Chan STH. Luteinizing hormone (LH) surge in patients using buserelin spray during ovarian stimulation for assisted reproduction. Gynecol Endocrinol 1990;4 Suppl 2:112.
19. Tan SL, Royston P, Mason BA, Steer C, Jacobs HS, Campbell S, et al. Cumulative conception and live birth rates in in vitro fertilization. Lancet. In press.
20. Kingsland C, Taylor CT, Aziz N, Bickerton N. Is follicular flushing necessary for oocyte retrieval? A randomised trial. Hum Reprod 1991;6:382–3.
21. Tan SL, Waterstone J, Wren M, Parsons J. A prospective randomized study comparing aspiration only with aspiration and flushing for transvaginal ultrasound directed oocyte recovery. Fertil Steril. In press.