FERTILITY AND STERILITY

Vol. 57, No. 4, April 1992
Printed on acid-free paper in U.S.A.

The long protocol of administration of gonadotropin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for in vitro fertilization

Seang-Lin Tan, M.R.C.O.G.*†‡
Charles Kingsland, M.R.C.O.G.*
Stuart Campbell, F.R.C.O.G.†
Carla Mills, Ph.D.*
Janet Bradfield, S.R.N.*
Neil Alexander, Ph.D.§
John Yovich, M.D.*‖
Howard S. Jacobs, F.R.C.P.¶

The Hallam Medical Centre; King's College School of Medicine and Dentistry; Royal Postgraduate Medical School, Hammersmith Hospital; and The Middlesex Hospital, London, United Kingdom

Objective: To investigate whether pituitary desensitization with the gonadotropin-releasing hormone agonist (GnRH-a), buserelin acetate, before the administration of human menopausal gonadotropin (hMG) for ovarian stimulation in in vitro fertilization (IVF) is superior to the simultaneous administration of both hormones at the beginning of the treatment cycle.

Design: Prospective randomized study.

Patients: Ninty-one patients having their first attempt at IVF.

Interventions: Patients in group 1 (long protocol) were administered subcutaneous (SC) buserelin acetate 200 μg/d from day 1 of the menstrual cycle, and hMG was started only after pituitary desensitization had been achieved at least 14 days later. Patients in group 2 (short protocol) were administered SC buserelin acetate 200 μg/d from day 2 and the same dose of hMG used in the long protocol from day 3 of the menstrual cycle.

Results: The median total amount of hMG required in both groups was comparable. There were significantly more follicles ($P = 0.0001$), oocytes ($P = 0.0008$), fertilized oocytes ($P = 0.0001$), and cleaved embryos ($P = 0.0001$), and a higher fertilization rate ($P = 0.0047$) in patients in group 1. The pregnancy rates per initiated cycle and per embryo transfer were 19.57% and 25.71% in group 1 compared with 8.89% and 16.67% in group 2.

Conclusions: The long protocol is superior in terms of significantly greater follicular recruitment, oocyte recovery and fertilization rates, and significantly greater number of embryos available for transfer. In general, it is the preferred method when GnRH-a are used for ovarian stimulation in IVF. Fertil Steril 1992;57:810–4

Key Words: Long and short gonadotropin-releasing hormone agonist, in vitro fertilization

Although the first successful pregnancy resulting from in vitro fertilization and embryo transfer (IVF-ET) was achieved in a natural, unstimulated cycle, current practice is to stimulate the ovaries to achieve multiple follicular development. This is because, in general, the larger the number of oocytes recovered, the more embryos will be generated for transfer and the higher will be the pregnancy rate (PR). The ovarian stimulation regimens used initially consisted of gonadotropins, either alone or in combination with clomiphene citrate (CC). Using this approach,

Received July 19, 1991; revised and accepted November 22, 1991.
* The Hallam Medical Centre.
† Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry.
‡ Reprint requests: Seang-Lin Tan, M.R.C.O.G., Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX, United Kingdom.
§ Department of Medical Physics, Royal Postgraduate Medical School, Hammersmith Hospital.
‖ Present address: John Yovich, M.D., Pivet Medical Centre, Perth, WA 6007, Australia.
¶ Department of Reproductive Endocrinology, The Middlesex Hospital.

however, despite close monitoring of follicular growth, 15% to 30% of patients have a premature surge of luteinizing hormone (LH) resulting in cancellation of the cycle (1), whereas others have asynchronous development of the ovarian follicles (2). To overcome these problems, many investigators have incorporated the use of gonadotropin-releasing hormone agonists (GnRH-a), which act by desensitizing the pituitary after an initial stimulatory phase (3), in their ovarian stimulation programs.

Because a number of studies have suggested that the use of GnRH-as leads to a lower cancellation rate (4), improved follicular response (5), and increased fertilization and implantation rates (6, 7) resulting in a net increase in the PR per cycle (7), the method has gained widespread popularity, and many programs currently use it virtually as the sole method of ovarian stimulation. The optimum protocol of treatment, however, remains contentious. Although the original and most commonly used protocol involves pituitary desensitization before ovarian stimulation (the so-called long protocol) (5), one problem associated with its use is that the treatment cycle is lengthened and a higher dose of gonadotropins is needed to stimulate the ovaries. More recently, the simultaneous commencement of administration of GnRH-a and gonadotropins at the beginning of the menstrual cycle (the so-called short protocol) has been advocated. At present, there is no agreement as to which protocol is better, and the information available is rather conflicting. The present study was therefore designed to investigate the comparative merits of the two protocols.

MATERIALS AND METHODS

Ninty-one patients who were referred to the Hallam Medical Centre in London for IVF were recruited for the trial that had Institutional Review Board Approval. None of the patients had previously been treated with IVF (either at this center or elsewhere). They were prospectively randomized into two groups by drawing serially numbered sealed envelopes, each of which contained a study group number (1 or 2) which were allocated by reference to random tables. Patients in group 1 (long protocol) were administered the GnRH-a, buserelin acetate (D-Ser(tBu)6,Pro9-NHEt), luteinizing hormone-releasing hormone (LH-RH, Suprefact; Hoechst, Hounslow, United Kingdom) starting on the 1st day of the menstrual cycle. The GnRH-a was administered by subcutaneous injection in a dose of 200 μg/d; 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 daily buserelin acetate injections were continued at the same dose until, and including, the day of human chorionic gonadotropin (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. Patients in group 2 (short protocol) received the same daily dose of buserelin acetate as those in group 1, but treatment was started on day 2 of the menstrual cycle. Human menopausal gonadotropin was started on the 3rd day of the menstrual cycle at the same dose as those in group 1.

Monitoring of follicular growth was achieved with daily us scans and serum E_2 measurements from day 6 of ovarian stimulation. The dose of hMG was increased if there was poor follicular growth. Human chorionic gonadotropin, 10,000 IU/L, was administered when the mean diameter of the largest follicle reached 18 mm and there were two other follicles > 14 mm with appropriate serum E_2 concentrations (approximately 1,000 pmol/L per follicle of at least 14 mm in diameter). Transvaginal US-directed oocyte recovery was performed 35 hours after hCG administration. Standard techniques of IVF-ET were used. Embryo transfer was scheduled approximately 48 hours after oocyte recovery, and luteal support was provided by the administration of hCG (2,000 IU on the day of ET and repeated 3 days later).

The age of each patient, total amount of hMG used, number of follicles developed, number of oocytes recovered, fertilization and cleavage rates, and PRs per initiated cycle and per ET were calculated. The results of the two protocols were compared using Mann-Whitney and χ^2 tests.

RESULTS

There were 46 patients in group 1 and 45 in group 2. The median age of the patients and indications for IVF were comparable in the two groups (Table 1).

Table 1 Patient Characteristics

	Long protocol	Short protocol	Probability
Number of cases	46	45	
Age (y)	33 (24 to 41)*	33 (26 to 41)	NS†
Tubal damage	23	30	
Endometriosis	4	1	
Male factor infertility	7	3	
Unexplained infertility	12	11	

* Values are medians with ranges in parentheses.
† NS, not significant

The median total dose of hMG used in the patients on the long protocol was 27 ampules (range, 14 to 61) compared with 24 (range, 14 to 62) in those on the short protocol. The difference was not statistically significant, suggesting that the initial agonistic flare effect of the short protocol did not reduce the hMG requirements for ovarian stimulation. A higher proportion of patients on the short protocol had their treatment canceled before oocyte recovery, usually because of inadequate follicular recruitment. The median number of follicles recruited (Table 2) was 14 (range, 0 to 27) in the long protocol compared with 9 (range, 0 to 25) in the short protocol ($P = 0.0001$), and there was also a significantly greater number of oocytes collected in those on the long protocol, median of 10 (range, 0 to 21) compared with 5 (range, 0 to 17) ($P = 0.0008$). The fertilization but not the cleavage rate (Table 2) was significantly higher in the patients on the long protocol, median of 77.78% compared with 57.14% ($P = 0.0047$), even though the proportion of patients with male factor infertility was higher in the former group (Table 1).

There were significantly more cleaved embryos available for transfer in the patients on the long protocol. The median number of embryos transferred was significantly higher in the patients on the long protocol, and the clinical PR per initiated cycle in this group was more than twice that in the short protocol, 19.57% per cycle compared with 8.89% per cycle (Table 2).

DISCUSSION

Effective superovulation is essential for IVF because PRs are related to the number of good quality oocytes recovered and fertilized and of embryos transferred to the uterus. Since the original description of the use of gonadotropins with GnRH-a for ovarian stimulation in IVF (5), the method has gained widespread popularity, and many IVF programs use the technique as the predominant, if not sole, method of ovarian stimulation. After an initial stimulatory phase, GnRH-as reduce spontaneous release of gonadotropins by the pituitary gland and protect the developing oocyte from perturbations in LH release at the critical stages of follicular development. Moreover, because spontaneous ovulation is prevented, the timing of hCG administration in GnRH-a cycles is not as critical as when CC and gonadotropins are used for ovarian stimulation. The timing of oocyte recovery can therefore be programmed for convenience, which is obviously very attractive for many IVF programs.

One problem associated with the use of GnRH-a for pituitary desensitization before ovarian stimulation is that the treatment cycle is lengthened and a higher dose of gonadotropins may be needed to stimulate the ovaries as compared with cycles in which ovarian stimulation is attempted with CC and

Table 2 Clinical Response and Outcome of Patients

	Long protocol	Short protocol	Probability
HMG (ampules)	27 (14 to 61)*	24 (14 to 62)*	NS†
Cases abandoned (%)	8.69	13.33	NS
Day of hCG administration	10 (0 to 14)	10 (0 to 13)	
No. of follicles	14 (0 to 27)	9 (0 to 25)	0.0001
No. of oocytes recovered	10 (0 to 21)	5 (0 to 17)	0.0008
Fertilized oocytes	6 (0 to 17)	2 (0 to 10)	0.0001
Cleaved embryos	4 (0 to 13)	1 (0 to 10)	0.0002
Fertilization rate (%)	77.78 (0 to 100)	57.14 (0 to 100)	0.0047
Cleavage rate (%)	91.67 (0 to 100)	75 (0 to 100)	NS
No. of embryos transferred	3 (0 to 4)	1 (0 to 4)	0.01
PR/initiated cycle (%)	19.57	8.89	NS
PR/ET (%)	25.71	16.67	NS

* Values are medians with ranges in parentheses. † NS, not significant.

gonadotropins. The rationale of the short or flare protocol is therefore to attempt to circumvent these disadvantages by augmenting the action of exogenous gonadotropins with the endogenous follicle-stimulating hormone released by the initial stimulatory effect of the agonist and thus to lower the required dosage of exogenous gonadotropins. In the present study, all factors that could affect the outcome of treatment were controlled. The patients assigned to the two protocols were comparable in terms of age and indications for IVF. Only patients having their first attempt at IVF were recruited so that the identical starting dose of gonadotropins could be used. The same GnRH-a, buserelin acetate, was used and the identical dose and mode of administration utilized. In both groups of patients, the agonist was started in the early follicular phase. The only variable therefore was that in the long protocol pituitary desensitization was achieved before hMG was started. The results of the study indicated that the so-called flare effect of the short protocol produced no advantage. The amount of hMG required was similar, and the only difference in drug cost was the extra 2 weeks of treatment with buserelin acetate required in the long protocol. Because the overwhelming cost in ovarian stimulation is that of hMG, there was essentially no significant financial saving in using the short protocol. The flare effect did not enhance folliculogenesis either, and our results were consistent with those previously reported that the long protocol results in greater follicular recruitment and oocyte recovery (8) and a higher fertilization rate (9).

Those studies that have suggested that the short protocol is as effective as the long protocol have suffered from a number of limitations: (1) they were retrospective analysis of data (10); (2) they compared different GnRH-a for their long and short protocols (11); (3) the initiation of the GnRH-a was at a suboptimal time (12). In the latter study (12), the administration of the GnRH-a in the long protocol was commenced 3 to 5 days after follicular rupture in the preceding cycle. It has been shown that initiation of GnRH-a in the early luteal phase produces significantly fewer follicles compared with initiation in the early follicular or midluteal to late luteal phase (13).

There are a number of possible reasons why the long protocol of administration of GnRH-a is superior to the short protocol. It has been well demonstrated that the initial agonistic action of the GnRH-a results in increases in LH concentrations to preovulatory surge levels. This may lead to rescue of the corpus luteum, luteinization of immature follicles as shown by a rise in the serum P levels (14), and an increase in thecal androgen levels that may reduce folliculogenesis (15). Exposure of the developing follicle to inappropriately high levels of LH may be particularly severe in patients in whom the return to baseline levels of LH takes longer than average, for example, in those who have polycystic ovarian disease or who form cysts as a result of agonist administration (16). It has also been shown that the degree of LH suppression is more variable when the short protocol is used (9, 17). In fact, some studies have suggested that when the short protocol is used, 5% to 10% of cycles may be complicated by a premature surge of LH (18).

In one recent study in which GnRH-a was commenced on day 2 and hMG on day 5 of the cycle (19), it was found that the best results were obtained in those cases in which there was a prompt elevation of the serum E_2 concentrations followed by a fall by cycle day 4 to 6. In the 20% of cycles in which the serum E_2 concentration showed a prompt and persistent rise through cycle day 5, implantation rates and PRs were significantly lower. Little information on the fluctuations of LH in the early follicular phase was given in that study, but the results suggest that once follicular growth occurs, exposure to the agonistic phase of GnRH-a is probably inimical. The results of that study (19) also suggest that it is not so much that pituitary desensitization before administration of gonadotropins is unnecessary but rather that some patients achieve pituitary desensitization very rapidly so that by the time the active phase of follicular growth occurs, the levels of LH are already at basal levels.

There is a considerable amount of data supporting the adverse fertility effects of exposure to high LH concentrations (15, 20, 21). It is associated with an increased incidence of infertility and miscarriage (20) and failure to conceive despite ovulation (21, 22).

It has been suggested that to optimize results with short protocols, US examination of the ovaries and measurement of the serum P concentration should be performed before initiation of GnRH-a therapy so that if an ovarian cyst is visualized or the serum P concentration is elevated, gonadotropin administration can be delayed until ovarian inactivity is demonstrated (23). It has also been suggested that daily measurements of the serum E_2 concentration should be performed so that if they do not fall promptly, the long protocol could be used in subsequent cycles (19). Both these approaches negate one of the major advantages of GnRH-a therapy in

comparison with ovarian stimulation using CC and hMG, namely, the simplicity of use and the reduction of monitoring afforded; for example, cysts seen in long GnRH-a cycles can be safely ignored (24). Given the fact that the short protocol produces no significant financial savings, it would appear that the long protocol is to be preferred for use in IVF.

In conclusion, although this study does not preclude the possibility that there could be subgroups of patients who may be appropriately treated by the short protocol, our results suggest that, in general, the long protocol is the preferred method when GnRH-as are used for ovarian stimulation in IVF.

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. Pampiglione J, Tan SL, Steer C, Wren M, Parsons J, Campbell S. Drainage of the dominant follicle in in-vitro fertilisation. Assisted Reprod Technol Androl 1991;1:363–6.
3. Tan SL, Armar NA, Adams J, Abdulwahid NA, Honour JW, Jacobs HS. The use of superactive LHRH analogue and FSH—a new method for induction of ovulation in patients with polycystic ovarian syndrome. Singapore J Obstet Gynaecol 1986;17:93–6.
4. Sathanandan M, Warnes GM, Kirby CA, Petrucco OM, Matthew CD. Adjuvant leuprolide in normal, abnormal and poor responders to controlled ovarian hyperstimulation for in-vitro fertilisation/gamete intrafallopian transfer. Fertil Steril 1989;51:998–1006.
5. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in vitro fertilisation using buserelin and gonadotropins. Lancet 1984;2:1284–5.
6. Neveu S, Hedon B, Bringer J, Chinchole J-M, Arnal F, Humeau C, et al. Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist and gonadotropins for in vitro fertilisation. Fertil Steril 1987;47:639–43.
7. Rutherford AJ, Subak-Sharpe RJ, Dawson KJ, Margara RA, Franks S, Winston RML. Improvement of in-vitro fertilisation after treatment with buserelin, an agonist of luteinising hormone releasing hormone. Br Med J 1988;296:1765–8.
8. Hedon B, Arnal F, Badoc E, Boulot P, Huet JM, Fries N, et al. Comparaison randomisee protocole long-protocole court dans les stimulations de l'ovaire en association avec un agoniste de la GnRH en vue de fecondation in vitro. Contraception Fertilite Sexualite 1988;16:624–7.
9. 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;51:105–111.
10. Zorn JR, Barata M, Brami C, Epelboin S, Nathan C, Papageorgiou G, et al. Ovarian stimulation for in-vitro fertilisation combining administration of gonadotropins and blockade of the pituitary with D-Trp6-LHRH microcapsules: pilot studies with two protocols. Hum Reprod 1988;3:235–9.
11. Frydman R, Parneix I, Belaisch-Allart J, Forman R, Hazout A, Fernandez H, et al. LHRH agonists in IVF: different methods of utilization and comparison with previous ovulation stimulation treatments. Hum Reprod 1988;3:559–61.
12. Garcia JE, Padilla SL, Bayati J, Baramki TA. Follicular phase gonadotropin-releasing hormone agonist and human gonadotropins: a better alternative for ovulation induction in in vitro fertilization. Fertil Steril 1990;53:302–5.
13. Pellicer A, Simon C, Miro F, Castellvi RM, Ruiz A, Ruiz M, et al. Ovarian response and outcome of in-vitro fertilization in patients treated with gonadotropin-releasing hormone analogues in different phases of the menstrual cycle. Hum Reprod 1989;4:285–9.
14. Fleming R, Jamieson ME, McQueen D, Hamilton MPR, Coutts JRT. Follicular phase initiation of GnRH-A therapy for ovarian stimulation. Gynecol Endocrinol 1990;4 Suppl 2: 87.
15. Stanger J, Yovich JL. Reduced in-vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. Br J Obstet Gynaecol 1985;92:385–93.
16. Salat-Baroux J, Alvarez S, Marie Antoine J, Tibi C, Cornet D, Mandelbaum J, Plachot M, Junca AM. Comparison between long and short protocols of LHRH agonist in the treatment of polycystic ovary disease by in-vitro fertilization. Hum Reprod 1988;3:535–9.
17. Fleming R, Jamieson ME, Coutts JRT. The use of GnRH-analogs in assisted reproduction. In: Mateon 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. Padilla SL, Bayati J, Garcia JE. Prognostic value of the early serum estradiol response to leuprolide acetate in in vitro fertilization. Fertil Steril 1990;53:288–94.
20. Regan L, Owen EJ, Jacobs HS. Hypersecretion of luteinising hormone, infertility and miscarriage. Lancet 1990;336: 1141–4.
21. Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS. Influence of serum luteinising hormone concentrations on ovulation, conception and early pregnancy loss in polycystic ovary syndrome. Br Med J 1988;297:1024–6.
22. Shoham (Schwartz) Z, Borenstein R, Lunenfeld B, Pariete C. Hormonal profiles following clomiphene citrate therapy in conception and non-conception cycles. Clin Endocrinol (Oxf) 1990;33:271.
23. Howles CM, Macnamee MC, Edwards RG. Short term use of LHRH agonist to treat poor responders entering an in-vitro fertilization (IVF) programme. Hum Reprod 1987;2: 655–6.
24. Rizk B, Tan SL, Kingsland C, Steer C, Mason BA, Campbell S. Ovarian cyst aspiration and the outcome of in vitro fertilization. Fertil Steril 1990;54:661–4.