

## Comparison of short versus long-term leuprolide acetate—human menopausal gonadotrophin hyperstimulation in in-vitro fertilization patients

J.H.Check<sup>1</sup>, K.Nowroozi and J.S.Chase

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Camden, NJ, USA

<sup>1</sup>To whom correspondence should be addressed at: 8002 E.Greentree Commons, Marlton, NJ 08053, USA

The use of leuprolide acetate for at least 10 days beginning in the mid-luteal phase prior to initiating human menopausal gonadotrophin (HMG) stimulation has been fairly successful in preventing cancellations for premature luteinization and allowing retrieval of more oocytes, which in turn provide more embryos for cryopreservation. However, it is theoretically possible that the long-term use of leuprolide may have some adverse effect on either pregnancy rates or on mean survival after cryopreservation and thawing. Recently, a short 3 day regimen of a gonadotrophin-releasing hormone agonist, buserelin effectively prevented premature luteinization during HMG stimulation. The present study indicated that substitution of a 3 day course of leuprolide acetate also effectively prevented premature luteinization but was less expensive, required statistically fewer ampoules of HMG and much less leuprolide.

**Key words:** controlled ovarian hyperstimulation/IVF—ET/leuprolide acetate/short versus long protocol

### Introduction

Luteinizing hormone (LH) surges prior to maturation of the leading follicle(s) cause premature luteinization, resulting in poor fertilization and pregnancy rates (Fleming *et al.*, 1985). Although this may also happen in natural cycles (Check *et al.*, 1991), the use of ovulation stimulating drugs increases the chance of premature luteinization (Zimmerman *et al.*, 1984; Fleming *et al.*, 1985). Besides poor fertilization related to atresia of oocytes (Lobo *et al.*, 1985), viability of the embryos may also be reduced when fertilization does occur (Stanger and Yovich, 1985).

Ovarian stimulation used for in-vitro fertilization (IVF) is even more likely to cause premature luteinization. Fleming and Coutts (1986) found this condition in 51% of patients treated exclusively with human menopausal gonadotrophin (HMG) for ovarian stimulation. Attempts to inhibit premature luteinization were made using gonadotrophin-releasing hormone agonists (GnRHa); one of the first studies combined buserelin and HMG (Porter *et al.*, 1984).

Two protocols using GnRHa with gonadotrophins have been used for ovarian stimulation: a long protocol where HMG is only used after complete pituitary suppression (Wildt *et al.*, 1986; Neveu *et al.*, 1987; Smitz *et al.*, 1987) and a short protocol where the gonadotrophins are used concomitantly with GnRHa or shortly after (Barriere *et al.*, 1987). The benefit of the latter is that the initial agonistic actions of the GnRHa helps increase the number of follicles recruited (the so-called flare-up effect). A randomized study comparing the long versus the short protocol found only small differences in the clinical outcome (Frydman *et al.*, 1988). There was no difference in the number of oocytes obtained or oestradiol level at the time of human chorionic gonadotrophin (HCG) administration. The duration of treatment and the amount of HMG employed was also similar.

An ultrashort method of combining GnRHa and HMG has also been described (Macnamee *et al.*, 1989). The GnRHa used was buserelin, which in longer protocols had been shown to down-regulate gonadotrophins (Macnamee *et al.*, 1987; Rutherford *et al.*, 1988). The randomized study found a significantly higher number of oocytes and a higher pregnancy rate with the ultrashort buserelin—HMG technique compared to a clomiphene citrate—HMG technique (Macnamee *et al.*, 1989).

The randomized prospective study presented here compared various clinical parameters using an ultrashort GnRHa—HMG protocol compared to a long protocol. The GnRHa used was leuprolide acetate.

### Materials and methods

During a 6-month interval, all infertility patients desiring IVF and embryo transfer were offered the opportunity to participate in a prospective and randomized study in order to compare the ultrashort versus the long leuprolide acetate—HMG protocols of ovarian stimulation. Participation entailed being randomly assigned to a treatment group; one group received the ultrashort leuprolide acetate stimulation protocol and the other received the long leuprolide acetate stimulation protocol. The randomization was based on the last digit of the patient's social security number, odd and even numbers receiving short and long protocols respectively. Furthermore, patients were asked to agree to alternate the stimulation regimen which they received if they were to require other IVF cycles in the 6 month period. Those patients who were willing to participate in the study received a 25% reduction in the charge for IVF. The only IVF patients excluded from this study were participants in our donor oocyte programme.

The short leuprolide acetate—HMG protocol consisted of administering GnRHa on day 2 subcutaneously at 1 mg/day for

3 days only, after which it was stopped, then starting HMG at 4 ampoules (75 IU) per day on day 3. The GnRHa for the long protocol may be administered in early follicular phase (day 1 or day 2) or in mid-luteal phase (Meldrum *et al.*, 1989). The long leuprolide protocol used here was initiated in the luteal phase on day 21 and continued for at least 10 days before starting the HMG at 4 ampoules per day. The HMG was not started unless the serum oestradiol was <30 pg/ml and the serum progesterone was <1.5 ng/ml. When the HMG was started, the leuprolide dose was reduced to 0.5 mg daily.

Monitoring of the IVF cycle involved measurement of the serum oestradiol levels using the Solid Phase Radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), measurement of the serum progesterone levels using enhanced luminescence (Amersham Corporation, Arlington Heights, IL) and measurement of LH using the double antibody radioimmunoassay (Amersham Corporation, Arlington Heights, IL). Follicular number and mean follicular diameter during the IVF cycle were monitored by transvaginal ultrasound using an ATL Ultramark 4 Unit (Advanced Technology Laboratories Bothnell, Washington) equipped with a 5 MHz endovaginal transducer. When two leading follicles reached 18 mm in diameter and the serum oestradiol was >800 pg/ml, 10 000 units of HCG were administered i.m. Premature luteinization was defined as a rise of serum progesterone above 2 ng/ml before adequate follicular number and maturation occurred.

The efficacy of the protocols was evaluated by comparing the mean serum oestradiol and progesterone levels at the time HCG was administered, the number of ampoules of HMG required, the number of failed IVF cycles due to premature luteinization and to zero fertilization, the mean number of follicles punctured, oocytes recovered, embryos transferred and embryos available for cryopreservation; the mean survival rate after thawing and finally the number of pregnancies and subsequent live births occurring. An IVF cycle was considered to result in pregnancy only if the  $\beta$  HCG level reached 5000 mIU/ml and a gestational sac was seen by ultrasound.

Statistical analysis of the data from the first IVF cycle included Student's *t*-tests to compare the mean serum oestradiol and progesterone levels, number of follicles punctured, number of eggs retrieved, fertilization rate, number of embryos transferred and the number of ampoules of HMG administered between the two treatment protocols. Chi-square analysis was used to compare the pregnancy rates. For those patients undergoing two IVF cycles, one with the short protocol and one with the long protocol, statistical comparison between the results of their two cycles was performed using paired *t*-tests and McNemar's test for related proportions. A *P*-value of <0.05 was used as the level of statistical significance.

## Results

In the 6 month interval 106 women entered this study; 56 underwent IVF using the long leuprolide acetate-HMG protocol (long) and 50 underwent IVF using the ultrashort leuprolide acetate-HMG protocol (short). Fourteen of the women underwent a second IVF cycle within the 6-month study period in which they used the alternative stimulation protocol to that used in the

first cycle. The average age for the women in the long protocol group was 33.7 ( $\pm 4.06$ ) years, compared to 34.1 ( $\pm 4.79$ ) for those in the short protocol group. The average number of previous IVF cycles where a successful pregnancy did not occur was 0.3 for those in the long protocol group, and 1.1 for those in the short protocol group.

A summary of the results of the IVF cycles is presented in Table I. Within the long protocol group, two cycles had to be cancelled because of an inadequate number of mature follicles. In the short protocol group, two cycles had to be cancelled because of an inadequate number of mature follicles and three cycles had to be cancelled for premature luteinization. Two women who had premature luteinization were subsequently shown to have premature ovarian failure and are awaiting donor oocytes. The third completed an unsuccessful IVF cycle using the long protocol and is also awaiting donor oocytes. There were 13 cycles (24%) with zero fertilization in the long protocol group versus 6 cycles (13.3%) with zero fertilization in the short protocol group. Women in the long protocol group received significantly more (*P* < 0.01) ampoules of HMG (a mean of 42) compared to women in the short protocol group (28.4) (Table I).

**Table I.** Comparison of in-vitro fertilization (IVF) results according to the type of ovarian stimulation protocol used

	Long leuprolide acetate	Ultrashort leuprolide acetate
Number of cycles performed	56	50
Cancellations for inadequate number of mature follicles	2	2
Cancellation for premature luteinization	0	3
Cycles with zero fertilization	13	6 <sup>a</sup>
Outcome:		
Pregnancies	9	4 <sup>a</sup>
Abortions	2	0
Mean number of ampoules HMG	42.0 (14.5)	28.4 <sup>b</sup> (10.2)
Mean oestradiol level on day of HCG (pg/ml)	1377.9 (686.95)	1449.6 <sup>a</sup> (914.12)
Mean progesterone level on day of HCG (ng/ml)	0.94 (0.35)	1.04 <sup>a</sup> (0.51)
Mean number of follicles punctured	14.0 (7.45)	15.2 <sup>a</sup> (10.19)
Mean number of oocytes retrieved	11.98 (6.66)	12.68 <sup>a</sup> (11.06)
Mean fertilization rate (%)	45.96 (33.96)	45.97 <sup>a</sup> (31.16)
Mean number of embryos transferred	3.07 (1.75)	3.11 (1.87)
Cryopreservation of embryos:		
Number of cycles in which embryos frozen	18	14
Mean number of embryos frozen	4.16	7.85
Number of cycles in which embryos thawed	7	7
Mean survival rate	80%	73%

<sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05.

HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

The mean serum oestradiol and progesterone levels at the time of HCG were the same in both treatment groups. There were also no differences in the mean number of follicles punctured, oocytes retrieved, fertilization rates, or number of embryos transferred in the two groups. Neither technique provided any advantage for cryopreserving additional embryos. Eighteen of the women undergoing the long protocol had embryos frozen, with a mean number of 4.16 embryos frozen each. Only seven of these women have so far had their embryos thawed, with a mean survival rate of 80%. Similarly, 14 of the women undergoing the short protocol had their embryos frozen, with a mean number of 7.85 embryos frozen each. Seven of these women have had their embryos thawed with a mean survival rate of 73%.

There were four and nine pregnancies in the short and long protocol groups respectively. Allowing for abortions, the live birth rate was 8.8% for short versus 12.96% for long protocols.

Fourteen women returned for a second IVF cycle in the 6-month interval and agreed to use the alternative stimulation protocol for the second cycle. They had an average age of 34.4 years ( $\pm 4.5$ ) and an average of 1.07 unsuccessful IVF cycles previous to their entry into the study. Table II compares the results of their short and long cycles. As before, the only significant

difference detected was in the number of ampoules of HMG administered.

## Discussion

A prospective randomized study has been published previously comparing the long to the short flare-up protocol using leuprolide as the agonist (Frydman *et al.*, 1988). In contrast to that study, Garcia *et al.* (1990) found a reduction in the amount of pure follicle stimulating hormone (FSH) and HMG used with the short versus long protocol. They found an average difference of 11 ampoules between short (lower) and long protocols and we found a difference of somewhat over 12 ampoules. Other researchers have also found that when GnRHa is added to HMG therapy, more of the latter is required to accomplish follicle maturation (Lewinthal *et al.*, 1988). The use of the ultrashort protocol would have saved the patient at least \$500 per cycle in the United States.

Garcia *et al.* (1990) did not measure progesterone levels, so they did not address specifically the issue of premature luteinization. However, their data suggest that leuprolide was successful in inhibiting premature luteinization, since there was a far lower cancellation rate than controls not treated with analogues. The theory behind the follicular phase flare-up technique is that initially the GnRHa acts as a stimulus for LH and FSH release from the pituitary but as the follicles mature, by its daily use in conjunction with HMG, the GnRHa will suppress endogenous LH surges. We found a very small incidence of premature luteinization with the ultrashort protocol (not significantly different from the long protocol) using leuprolide acetate as the GnRHa, thus supporting the conclusions of Macnamee *et al.* (1989) using the ultrashort protocol with busserelin as the GnRHa. These two studies suggest that in the early follicular phase, 3 days of use of GnRHa is all that is necessary to inhibit premature luteinization. A randomized study comparing ultrashort protocols to flare-up is needed to determine whether the ultrashort protocol offers any other benefits besides reduction in frequency of GnRHa injections and resultant reduction in expenditure.

One question that needs to be answered is whether prolonged use of leuprolide has an adverse effect on subsequent cryopreservation results. Our data did not show this effect. More extensive comparative studies are needed to determine whether one of these techniques (long versus flare-up versus ultrashort protocols) will produce a more favourable live baby birth rate.

Though no statistical difference was noted in the incidence of premature luteinization following these two techniques, nevertheless, the few cases of premature luteinization that did occur happened only with the short GnRHa technique, suggesting inadequate pituitary suppression in some cases.

## Acknowledgements

The authors wish to thank Ahmad Nazari, MD for his help in patient treatment; Amy Baker, Gail Goldsmith, and Gail Murray for aid in embryo development; Pam Spirito and Charlene Fisher for their nurse coordination; and Deborah Lurie PhD for her help in statistical analysis.

**Table II.** Comparison of in-vitro fertilization (IVF) results according to type of controlled ovarian hyperstimulation protocol used, for patients undergoing two IVF cycles within 6 months, where a different protocol was used on each occasion

	Long leuprolide acetate	Ultrashort leuprolide acetate
Number of cycles performed	14	14
Cancellations for inadequate number of mature follicles	0	0
Cancellation for PML (premature luteinization)	0	1
Cycles with zero fertilization	4	2 <sup>a</sup>
Outcome:		
Pregnancies	1	0
Abortions	0	0
Mean number of ampoules HMG	46.6 (19.1)	28.8 <sup>b</sup> (8.2)
Mean oestradiol level on day of HCG (pg/ml)	1194.21 (451.2)	1234.77 <sup>a</sup> (662.65)
Mean progesterone level on day of HCG (ng/ml)	0.75 (0.27)	0.85 <sup>a</sup> (0.26)
Mean number of follicles punctured	12.3 (5.18)	12.2 <sup>a</sup> (5.18)
Mean number of oocytes retrieved	9.28 (3.87)	8.92 <sup>a</sup> (5.29)
Mean fertilization rate (%)	37.71 (34.19)	37.15 <sup>a</sup> (32.73)
Mean number of embryos transferred	2.78 (1.80)	2.61 (1.76)
Cryopreservation of embryos:		
Number of cycles in which embryos frozen	2	1
Mean number of embryos frozen	3	3
Number of cycles in which embryos thawed	0	0

<sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ .

HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

## References

- Barriere,P., Lopes,P., Boiffard,J.P., Pousset,C., Quentin,M., Sagot,P., L'hermine,A., Lerat,M.F. and Charbonnel,B. (1987) Use of GnRH analogues in ovulation induction for in vitro fertilization: benefit of a short administration regimen. *J. In Vitro Fertil. Embryo Transfer*, **4**, 64-65.
- Check,J.H., Chase,J.S., Nowroozi,K. and Dietterich,C. (1991) Premature luteinization: treatment and incidence in natural cycles. *Hum. Reprod.*, **6**, 190-193.
- Fleming,R. and Coutts,J.R.T. (1986) Induction of multiple follicular growth in normally menstruating women with endogenous gonadotropin suppression. *Fertil. Steril.*, **45**, 226-230.
- Fleming,R., Haxton,M.J., Hamilton,M.P., McCure,G.S., Black,M.P., MacNaughton,M.C. and Coutts,J.R. (1985) Successful treatment of infertile women with oligomenorrhea using a combination of LHRH agonist and exogenous gonadotrophins. *Br. J. Obstet. Gynaecol.*, **92**, 369-373.
- Frydman,R., Belaisch-Allart,J., Parneix,I., Forman,R. Hazout,A. and Testart,J. (1988) Comparison between flare up and down regulation effects of luteinizing hormone-releasing hormone agonists in an in vitro fertilization program. *Fertil. Steril.*, **50**, 471-475.
- Garcia,J.E., Padilla,S.L., Bayati,J. and Baramki,T.A. (1990) Follicular phase gonadotropin-releasing hormone agonist and human gonadotropins: a better alternative for ovulation induction in in vitro fertilization. *Fertil. Steril.*, **53**, 302-305.
- Lewinthal,D., Taylor,P.J., Pattinson,H.A. and Corenblum,B. (1988) Induction of ovulation with leuprolide acetate and human menopausal gonadotropin. *Fertil. Steril.*, **49**, 585-588.
- Lobo,R.A., diZerega,G.S. and Marrs,R.P. (1985) Follicular fluid steroid levels in dysmature and mature follicles from spontaneous and hyperstimulated cycles in normal and anovulatory women. *J. Clin. Endocrinol. Metab.*, **60**, 81-87.
- Macnamee,M.C., Howles,C.M. and Edwards,R.G. (1987) Pregnancies after IVF when high tonic LH is reduced by long-term treatment with GnRH agonists. *Hum. Reprod.*, **2**, 569-571.
- Macnamee,M.C., Howles,C.M., Edwards,R.G., Taylor,P.J. and Elder,K.T. (1989) Short-term luteinizing hormone-releasing hormone agonist treatment: prospective trial of a novel ovarian stimulation regimen for in vitro fertilization. *Fertil. Steril.*, **52**, 264-269.
- Meldrum,D.R., Wisot,A., Hamilton,F., Gutlay,A.L., Kempton,W. and Huynh,D. (1989) Routine pituitary suppression with leuprolide before ovarian stimulation for oocyte retrieval. *Fertil. Steril.*, **51**, 455-459.
- Neveu,S., Hedon,B., Bringer,J., Chinchole,M.M., Arnal,F., Humeau,C., Cristol,P. and Viala,J.L. (1987) Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist and gonadotropins for in vitro fertilization. *Fertil. Steril.*, **47**, 639-643.
- Porter,R.N., Smith,W., Craft,I.L., Abdulwahid,N.A. and Jacobs,H.S. (1984) Induction of ovulation for in vitro fertilization using buserelin and gonadotropins. *Lancet*, **2**, 1284-1285.
- Rutherford,A.J., Suback-Sharpe,R.J., Dawson,K.J., Margora,R.A., Franks,S. and Winston,R.M.L. (1988) Improvement of in vitro fertilization after treatment with buserelin, an agonist of luteinizing hormone releasing hormone. *Br. Med. J.*, **296**, 1765-1768.
- Smitz,J., Devroey,P., Braeckmans,P., Camus,M., Khan,I., Staessen,C., Van Waesberghe,L., Wisanto,A. and Van Steirteghem,A.C. (1987) Management of failed cycles in an IVF/GIFT programme with the combination of a GnRH analogue and hMG. *Hum. Reprod.*, **2**, 309-314.
- Stanger,J.D. and Yovich,J.L. (1985) Reduction in in-vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. *Br. J. Obstet. Gynaecol.*, **92**, 385-393.
- Wildt,L., Diedrich,K., Van Der Ven,H., Hasani,S.A., Hubner,H. and Klasen,R. (1986) Ovarian hyperstimulation for IVF controlled by GnRH agonist administered in combination with human menopausal gonadotropin. *Hum. Reprod.*, **1**, 15-19.
- Zimmerman,R., Buhnet,H.W., Weise,H.C. and Leidenberger,F.R. (1984) Preliminary report about a modified gonadotropin (human menopausal gonadotropin/human chorionic gonadotropin). Treatment in infertile patients with premature luteinization. *Fertil. Steril.*, **41**, 714-718.

Received on April 26, 1991; accepted on October 3, 1991