

The efficacy of short-term gonadotrophin-releasing hormone agonists versus human chorionic gonadotrophin to enable oocyte release in gonadotrophin stimulated cycles

J.H.Check¹, A.Nazari, E.R.Barnea, W.Weiss and B.H.Vetter

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

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

One of the reasons for failure to conceive following human menopausal gonadotrophin (HMG) therapy may be due to non-release of oocytes from the follicles. We hypothesized that by using a gonadotrophin-releasing hormone agonist (GnRHa) for a short duration, endogenous release of luteinizing hormone and follicle stimulating hormone may enable oocyte release to occur, similar or superior to the effect of human chorionic gonadotrophin (HCG). This study attempted to compare the efficacy of HCG versus the GnRHa leuprolide acetate to release oocytes and achieve pregnancies and to compare the effectiveness of leuprolide acetate versus a combination of HCG with HMG to release oocytes. Unfortunately due to lack of prior data, many patients preferred to reject leuprolide acetate in favour of HCG, resulting in three times as many patients being treated with HCG in cycle 1; 78.2% of oocytes were released following leuprolide acetate versus only 55.7% with HCG. Interestingly, 87.5% of those females in whom oocyte release failed in cycle 1 with HCG did indeed release with leuprolide acetate in cycle 2, but none of these previous failures released with HCG in cycle 2. Pregnancy rates were equal in those women releasing oocytes, whether treated with HCG or leuprolide acetate. These preliminary data justify a larger randomized study.

Key words: human chorionic gonadotrophin/human menopausal gonadotrophins/luteinized unruptured follicle/leuprolide acetate

Introduction

Pelvic sonography has been utilized to determine the release of oocytes from follicles (Hackeloer, 1978; Daly *et al.*, 1985; Liukkonen *et al.*, 1984). The use of human menopausal gonadotrophin (HMG) makes the diagnosis of failure to release any oocytes, referred to as luteinized unruptured follicles (LUF), more difficult because other follicles which have also been recruited may fill the spot vacated by the ruptured follicle (Katz, 1988). However, there is evidence that sonography can predict

oocyte release even in HMG therapy; using this treatment, a positive relationship was shown between apparent oocyte release and increased pregnancy rates whilst no such relationship occurred with LUF (Check *et al.*, 1990a). Additionally, those who failed to release an oocyte in their first cycle following a single injection of human chorionic gonadotrophin (HCG) had the tendency to fail in subsequent cycles when there was no change in therapy (Check *et al.*, 1990a).

Gonadotrophin-releasing hormone agonists (GnRHa), e.g. leuprolide acetate, generally have been developed by the substitution of two amino acids in the decapeptide gonadotrophin-releasing hormone (GnRH). Although the word 'agonist' denotes an aid in stimulating gonadotrophins, these agents by blunting the pulsatility of luteinizing hormone (LH) and follicle stimulating hormone (FSH), eventually cause their suppression. In fact, their main use in gynaecological endocrinology has been to inhibit LH and FSH secretion.

There are a few reports of leuprolide acetate being used for its short-term stimulatory effect, e.g. in the induction of ovulation in hypergonadotrophic females (Check *et al.*, 1988, 1990b). It has also been utilized in the early follicular phase to augment HMG stimulation of follicles in in-vitro fertilization (IVF) programmes (flare-up technique) (Garcia *et al.*, 1985).

We considered the possibility that the use of GnRHa, e.g. leuprolide acetate, at mid-cycle might cause oocyte release by stimulating endogenous gonadotrophins. There is also the possibility that endogenous release of LH and FSH, in proper amounts, may allow for more efficient oocyte release than HCG, which has traditionally been used in circumstances where aid to the natural release process is needed, e.g. following HMG stimulation. A study was thus initiated to compare the efficiency of HCG versus leuprolide acetate to induce oocyte release. Rates of pregnancy were also compared.

Materials and methods

Two separate studies were conducted. In the first study patients who were treated in their first cycle with HMG for ovulatory problems were randomly offered HCG or leuprolide acetate at the time of follicular maturation. This larger study involved 93 patients. The reason for HMG therapy in these patients included 11 with hypogonadotrophic hypogonadism and oestrogen deficiency who were never offered clomiphene citrate therapy. Clomiphene therapy had been offered to the remaining 82 patients, nine of whom preferred HMG from the start because of the better quality mucus associated with the latter (seven of these had husbands with male factor problems); the other 73

patients had been previously treated with clomiphene from 1 to 11 cycles. The reasons for switching to HMG included 35 because of hostile cervical mucus and lack of desire to have intra-uterine insemination (IUI), six patients because of other side-effects of clomiphene and 32 because of failure to conceive following clomiphene for 1 to 11 months, despite good post-coital tests or IUI. The need for ovulation-inducing drugs in these 82 patients included oestrogenic anovulation ($n = 45$) and luteal phase defects with release of oocytes from immature follicles ($n = 37$).

These patients were informed of some of the theoretical advantages of leuprolide acetate over HCG and also of previous data demonstrating failed oocyte release in a significant minority of HMG-HCG cycles. The agonist was provided at no extra cost to the patient, although HCG was given in lieu if the patients desired it. Initially patient enrolment in the study consisted of 100 consecutive cycles but in seven cases the follicle number and serum oestradiol concentration were too high for oocyte releasing therapy, leaving 93 cycles for evaluation.

Treatment typically began with 1–2 ampoules of HMG (75 IU) on days 3–5 with doses adjusted according to the number and diameter of the follicles; the serum oestradiol was required to reach at least 200 pg/ml per mature follicle with a minimum diameter of 17 mm, without the serum progesterone rising above 1.5 ng/ml. The first of three leuprolide acetate injections, or the one HCG injection was given when at least one follicle attained average diameter 17 mm with serum oestradiol measuring at least 200 pg/ml for each follicle of diameter ≥ 18 mm and the serum progesterone was < 1.5 ng/ml. If the serum progesterone exceeded 1.5 ng/ml, no releasing agent was administered and the cycle was cancelled.

Patients receiving HCG were given 10 000 IU i.m. 24 h after the last HMG injection. Patients receiving leuprolide acetate were injected s.c. with 1 mg every 12 h for three doses, also beginning 24 h from the last HMG injection. Repeat sonography was obtained 2–3 days after the initial leuprolide acetate or HCG injection. Failure of the follicle to collapse by at least 5 mm in average diameter, associated with a rise in serum progesterone above 2 ng/ml and a decrease in oestradiol were considered sufficient criteria to diagnose LUF. Release was thus defined as collapse of the follicle by at least 5 mm within 2–3 days of attaining a mature follicle of 17–24 mm average diameter with an oestradiol concentration > 200 pg/ml per mature follicle, or oestradiol concentration of at least 400 pg/ml when multiple follicles were stimulated. All patients were given progesterone supplementation in the luteal phase to decrease the rate of spontaneous abortions (Check *et al.*, 1985, 1987).

In the second study, the effectiveness of leuprolide acetate was

compared with that of an HMG-HCG mix to release oocytes in cycles 3 and 4 of HMG therapy in females failing to release oocytes in cycles 1 and 2 following standard HCG therapy. The combination of 150 IU HMG and 15 000 IU HCG was known to be effective in causing oocyte release in females who failed to release with HCG alone (Check *et al.*, 1986, 1992). A different group of 10 women with ovulatory defects were evaluated in this study. Because of small numbers, it was decided not to randomize the treatments but to treat patients sequentially so that all received HMG-HCG mix in cycle 3 and those failing to conceive received leuprolide acetate in cycle 4.

Statistical evaluation was performed using Fisher's exact test and chi-square analysis, where appropriate. A probability level of 5% or less was considered statistically significant.

The oestradiol concentrations were measured by radio-immunoassay (Diagnostic Product Corporation, Los Angeles, CA, USA) and the progesterone concentrations by enzyme immunoassay [Amersham (Eastman Kodak Company), New York, NY, USA]. Transvaginal sonography was performed using an ATL Ultramark 4 Unit (Advanced Technology Laboratories, Bothell, WA, USA) equipped with a 5 MHz endovaginal transducer.

Results

The incidence of failure to release oocytes in the first HMG cycle following either HCG or leuprolide at the time of follicular maturation in study 1 is seen in Table I. Any patient failing to demonstrate the criteria of definite oocyte release was considered to have LUF. Although HCG or leuprolide for oocyte release were offered on a randomized basis, many patients were sceptical about leuprolide and chose HCG, so that only 23 received it compared with 70 treated with HCG. A statistically higher percentage of patients demonstrated oocyte release with leuprolide in cycle 1 of HMG therapy (78.2%) than with HCG (55.7%).

The results of cycle 2 are shown in Table II. The option of HCG or leuprolide was once again offered in a randomized fashion. More patients were then willing to try after being shown the results of cycle 1. There were 54 patients available for cycle 2 (15 achieved pregnancy in cycle 1 and one dropped out of the study); 28 patients were treated with HCG in cycle 2, with 21 of those demonstrating release (75%), compared to 21 of 26 (80.7%) releasing after treatment with leuprolide in cycle 2. However, 24 of the 28 women treated with HCG had released in cycle 1 with HCG. Thus 21 of 24 (87.5%) HMG treated patients who released following HCG in cycle 1 did so again in cycle 2. However, none of the four who failed to release in

Table I. Incidence of luteinized unruptured follicles (LUF)—i.e. failure to release any oocytes—in first human menopausal gonadotrophin stimulated cycle following either human chorionic gonadotrophin (HCG) or leuprolide acetate at time of follicular maturation

Releasing agent ^a	No. patients treated	No. patients releasing oocytes ^b	Incidence of LUF ^c
HCG (10×10^3 U)	70	39	44.3%
Leuprolide	23	18	21.7%

^aRandom selection offered, experimental nature of leuprolide explained, HCG given if patient refused leuprolide.

^bInconclusive release was counted as non-release.

^c $P < 0.05$ comparing incidence of LUF following HCG versus leuprolide.

cycle 1 were successful in cycle 2 following HCG. In contrast, 83.3% (15 out of 18) of women failing to release with HCG in cycle 1 were successful with leuprolide in cycle 2, as were 100% (4/4) of those successfully treated with it in cycle 1. Patients failing to release in the first two cycles following HCG were offered leuprolide for release in cycle 3. Thus six of the seven release failures with HCG in cycle 2 were now treated with leuprolide in cycle 3, and five of six (83.3%) released oocytes.

Study 2 evaluated oocyte release in cycles 3 and 4 in 10 women failing to release in both previous HMG cycles after HCG. All patients were treated with the HCG-HMG combination for release in cycle 3; only three of 10 (30%) released oocytes. In contrast, only leuprolide was given for release in cycle 4, and five of seven (71.4%) women were successful ($P = 0.15$).

Rates of pregnancy according to type of treatment and oocyte release, are shown in Table III. No difference in pregnancy rate between cycles stimulated with HCG or leuprolide were observed. Counting all cycles in study 1, including LUF, the pregnancy rates with HCG for cycles 1 and 2 were 14.3% (10/70) and 16.1% (5/31) respectively, compared to 21.7% (5/23) and 26.1% (6/23) for women treated with leuprolide. These were not significantly different.

Discussion

There are many potential pitfalls in diagnosing LUF, especially in HMG treated patients, as have been pointed out by Katz (1988). A vacated follicle may be replaced by a contiguous one giving a false diagnosis of failed release, or a patient may have a collapsed follicle entrapping an oocyte giving a false conclusion of oocyte release. Furthermore, there is debate whether LUF is

an isolated phenomenon that can occur in a minority of cycles, or is an actual syndrome with a tendency to repeat in subsequent cycles (Katz, 1988).

The results of this study support previous conclusions that failure to demonstrate oocyte release in the first HMG treated cycle following HCG injection is associated with a high rate of failure to release oocytes in the subsequent cycle after treatment with the same releasing agent (Check *et al.*, 1990a). Furthermore there were no pregnancies in cycles where LUF were apparently repeatedly diagnosed.

These data also demonstrate that for first attempts, leuprolide acetate is at least as effective as HCG in causing the sonographic appearance of oocyte release in cycles. The GnRHa seemed to be much more effective than HCG, or a mixture of HCG and HMG, in releasing oocytes in patients who in preceding cycles had demonstrated the LUF syndrome (Check *et al.*, 1986, 1992).

The pregnancy rates were comparable with both releasing agents. However, both groups were supported with supplementary progesterone in the luteal phase; it is not known whether HCG would prove more effective in providing better luteal function than leuprolide if no supplementary hormone replacement had been given.

The numbers in this study were not large and therefore it should be considered preliminary at this time. We are continuing the study and hope that with the encouraging preliminary data concerning leuprolide acetate, more patients who are randomly assigned the option of taking leuprolide in their first cycle of HMG will now be willing to use it. These data need to be corroborated by other treatment centres; the ideal dosage schedule and appropriate luteal phase support are still to be determined. Also it would be interesting to determine if another GnRHa, e.g. buserelin, has similar or even superior effects on oocyte release.

Table II. Incidence of luteinized unruptured follicles (LUF)—i.e. failure to release any oocytes—in second human menopausal gonadotrophin stimulated cycle following mid-cycle human chorionic gonadotrophin (HCG) or leuprolide acetate according to treatment and outcome of first cycle

Prior treatment and outcome	Treated with HCG			Treated with leuprolide		
	No. patients treated	No. patients releasing oocytes	% LUF	No. patients treated	No. patients releasing oocytes	% LUF
Release with HCG (n = 24)	24	21	12.5			
Non-release with HCG (n = 22)	4	0	100	18	15	16.7
Release with leuprolide (n = 4)				4	4	0
Non-release with leuprolide (n = 4)				4	2	50

Table III. Pregnancy rates according to type of therapy for oocyte release

	Cycle 1		Cycle 2	
	Total no. patients	No. of pregnancies (%)	Total no. patients	No. of pregnancies (%)
Release with HCG	39	10(25.6)	24	5(20)
Release with leuprolide	18	5(27.8)	21	6(28.6)
Non-release with HCG	31	0	7	0
Non-release with leuprolide	5	0	2	0

Acknowledgements

This research was funded in part by a grant from TAP Pharmaceuticals, Inc., Deerfield, IL, USA.

References

- Check, J.H., Wu, C.H. and Adelson, H.G. (1985) Decreased abortions in hMG-induced pregnancies with prophylactic progesterone therapy. *Int. J. Fertil.*, **30**, 45–47.
- Check, J.H., Chase, J.S., Adelson, H.G. and Dieterich, C. (1986) New approaches to the diagnosis and therapy of the luteinized unruptured follicle syndrome. *Int. J. Fertil.*, **30**, 29–32.
- Check, J.H., Chase, J.S., Wu, C.H., Adelson, H.G., Teichman, M. and Rankin, A. (1987) The efficacy of progesterone in achieving successful pregnancy: I. Prophylactic use during luteal phase in anovulatory women. *Int. J. Fertil.*, **32**, 135–138.
- Check, J.H., Wu, C.H. and Check, M.L. (1988) The effect of leuprolide acetate in aiding induction of ovulation in hypergonadotropic hypogonadism: a case report. *Fertil. Steril.*, **49**, 542–543.
- Check, J.H., Adelson, H.G., Dieterich, C. and Stern, J. (1990a) Pelvic sonography can predict ovum release in gonadotrophin-treated patients as determined by pregnancy rate. *Hum. Reprod.*, **5**, 234–236.
- Check, J.H., Wu, C.H. and Adelson, H.G. (1990b) Case report: Opposite response to the addition of leuprolide acetate to human menopausal gonadotropin therapy in two perimenopausal women. *Int. J. Fertil.*, **35**, 343–346.
- Check, J.H., Dieterich, C., Nowroozi, K. and Wu, C.H. (1992) Comparison of various therapies for the luteinized unruptured follicle syndrome. *Int. J. Fertil.*, **37**, 33–40.
- Daly, D.C., Soto-Albors, C., Walter, C., Ying, Y. and Riddick, D.H. (1985) Ultrasonographic assessment of luteinized unruptured follicle syndrome in unexplained infertility. *Fertil. Steril.*, **43**, 62–65.
- Garcia, N., Campo, S., Panetta, V., Venneri, M., Siccardi, P., Dargenio, R. and DeTomasi, F. (1985) Induction of ovulation with purified urinary follicle-stimulating hormone in patients with polycystic ovarian syndrome. *Am. J. Obstet. Gynecol.*, **151**, 635–640.
- Hackeloer, B.J. (1978) Ultrasonic demonstration of follicular development. *Lancet*, **i**, 941.
- Katz, E. (1988) The luteinized unruptured follicle and other ovulatory dysfunctions. *Fertil. Steril.*, **50**, 839–850.
- Liukkonen, S., Koshimies, A.I., Tenhunen, A. and Ylostalo, P. (1984) Diagnosis of luteinized unruptured follicle (LUF) syndrome by ultrasound. *Fertil. Steril.*, **41**, 26–30.

Received on August 13, 1992; accepted on December 10, 1992